Development of a new infection model to analyse the heterogeneity of Salmonella infection in chicks

Pierrette Menanteau, Elisabeth Bottreau, Jérôme Trotereau, Florent Kempf, Samantha Schaeffer, Edouard Guitton, Isabelle Virlogeux-Payant, Philippe Velge

Final Program and Abstracts

ASM CONFERENCES

5th ASM Conference on Salmonella

August 29 – September 1, 2016
Potsdam, Germany

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Washington, DC 20036-2904
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ASM CONFERENCES

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ASM Conferences Committee

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To identify emerging or underrepresented topics of broad scientific significance.

To facilitate interactive exchange in meetings of 100 to 500 people.

To encourage student and postdoctoral participation.

To recruit individuals in disciplines not already involved in ASM to ASM membership.

To foster interdisciplinary and international exchange and collaboration with other scientific organizations.

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Acknowledgments

The Conference Program Committee and the American Society for Microbiology acknowledge the following for their support of the 5th ASM Conference on Salmonella. On behalf of our leadership and members, we thank them for their financial support:

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General Information

REGISTRATION AND NAME BADGES

ASM Staff will be available at the registration desk in the Foyer at the Kongresshotel Potsdam during posted registration hours. Participants may collect name badges and program materials at the registration desk. A name badge is required for entry into all sessions and meals. Each participant may purchase tickets for a guest to attend the Welcome Reception and/or Conference Dinner. Guests may not attend sessions, poster sessions, lunches or coffee breaks.

GENERAL SESSIONS

All general sessions will be held in the Congress Hall.

POSTER SESSIONS

Poster boards are located in the Foyer at the Kongresshotel Potsdam.

Posters should be put up on the assigned poster board space before 9:00 am on the day of the official presentation date (Session A posters are mounted on Tuesday, August 30, Session B posters are mounted on Wednesday, August 31, and Session C posters are mounted on Thursday, September 1). Posters should be removed the same day, immediately following the conclusion of the poster session assigned.

Official presentation of posters will occur in Poster Sessions A or B or C. Posters in each session are grouped by the topic selected by the author during submission, and are comprised of the oral session topics that have been held prior to the poster presentation.

MEALS AND SOCIAL EVENTS

Registration includes attendance at the Welcome Reception on Monday evening, Lunches on Tuesday, Wednesday and Thursday, and the Conference Dinner on Thursday. Ample time has also been scheduled for participants to network during coffee breaks.

CERTIFICATE OF ATTENDANCE

Certificates of Attendance will be available by request and will be sent by email to the requestor upon verification of attendance.
Note: Certificates of Attendance do not list session information.

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Taking photographs or video of projected images in the session rooms with any device is prohibited. Photographs of posters are only allowed after receiving the presenter's permission. Report any violations to a member of the ASM staff.

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Children are not permitted in session rooms, poster sessions, conference meals or social events. Please contact the hotel to arrange for babysitting services in your hotel room.

Travel Grants

ASM STUDENT TRAVEL GRANTS

ASM encourages the participation of graduate students and new postdocs at ASM Conferences. To support the cost of attending the conference, ASM has awarded travel grants to each of the following individuals:

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Pawan Singh
Ilana Sinuani-Fratty
Sandeep Thapa
Brian Tuinema
Camila Valenzuela
Nancy Wang

Scientific Program

Monday, August 29, 2016

3:00 pm – 4:30 pm Congress Hall	**Opening Session**
3:00 – 3:10 pm	Welcome Remarks
3:10 – 3:50 pm	OS:1 Overview of the Field ***Jorge Galan**; Yale University Sch. of Med., New Haven, CT*
3:50 – 4:30 pm	OS:2 The Pyromaniac Inside You: *Salmonella* Metabolism in the Host Gut ***Andreas Baumler**; University of California, Davis, Davis, CA*
4:30 pm – 5:00 pm Congress Hall Foyer	**Coffee Break**
5:00 pm – 7:00 pm Congress Hall	**Session 1: Single Cell Approaches/Systems Biology**
5:00 – 5:25 pm	S1:1 Comparing the Intracellular Niche Formation of Shigella and *Salmonella* within Infected Epithelial Cells ***Jost Enninga**; Institut Pasteur, Paris, FRANCE*
5:25 – 5:50 pm	S1:2 *Salmonella* Forms Intracellular Persisters with TacT ***Sophie Helaine**; Imperial College, London, UNITED KINGDOM*
5:50 – 6:05 pm	S1:3 Single Molecule Localization and Tracking of SPI2-T3SS Effector Proteins in *Salmonella enterica* Infected Cells ***Vera Göser**; University of Osnabrück, Osnabrück, GERMANY*

6:05 – 6:20 PM

S1:4
Single-cell RNA-seq Reveals Disparate Macrophage Responses to Intracellular *Salmonella*
Antoine-Emmanuel Saliba*; University of Wuerzburg, Institute for Molecular Infection Biology, Wuerzburg, GERMANY*

6:20 – 6:35 pm

S1:5
Identification and Functional Characterization of Host microRNAs Restricting *Salmonella* Infection
Ana Eulalio*; University of Würzburg, Würzburg, GERMANY*

6:35 – 7:00 pm

S1:6
Probing the *Salmonella*-host Interface with High-throughput Approaches
Athanasios Typas*; European Molecular Biology Laboratory, Heidelberg, GERMANY*

7:00 pm – 10:00 pm
Congress Hall Foyer

Welcome Reception

Tuesday, August 30, 2016

9:00 am – 12:25 pm
Congress Hall

Session 2: Pathogen Evolution and Innate Immunity

9:00 – 9:25 am

S2:1
Using Genomics to Investigate Host/*Salmonella* Interactions
Gordon Dougan*; The Wellcome Trust, Hinxton, UNITED KINGDOM*

9:25 – 9:50 am

S2:2
How Cells Defend Their Cytosol Against *Salmonella* Invasion
Felix Randow*; MRC Laboratory of Molecular Biology, Cambridge, UNITED KINGDOM*

9:50 – 10:05 am

S2:3
CycA in Both *Salmonella* and Uropathogenic *E. coli* Prevents Killing by D-amino Acid Oxidase
Brian Tuinema*; McMaster University, Hamilton, ON, CANADA*

10:05 – 10:20 am	S2:4 Genotypic and Phenotypic Diversity Arising During the Clonal Expansion of an MDR Monophasic *Salmonella Typhimurium* epidemic ***Robert Kingsley**; Institute of Food Research, Norwich, UNITED KINGDOM*
10:20 – 10:35 am	S2:5 Participation of nsSNPs in Variable Adaptation of *Salmonella* to Different Host Species ***Dieter Schifferli**; University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA*
10:35 – 11:05 am	Coffee Break
11:05 – 11:30 am	S2:6 Pyroptosis Triggers Pore-induced Intracellular Traps (PITs) that Capture Bacteria and Lead to their Clearance by Efferocytosis ***Edward Miao**; University of North Carolina, Chapel Hill, NC*
11:30 – 11:45 am	S2:7 *Salmonella Typhimurium* Undergoes Distinct Genetic Adaption During Chronic Infections of Mice: Emergence of an Intestinal Super-colonizer Clone ***Lotte Jelsbak**; Roskilde University, Roskilde, DENMARK*
11:45 – 12:00 pm	S2:8 Exquisite Adaptation of Nontyphoidal *Salmonella Typhimurium* ST313 to HIV Infected Human Macrophages ***Florence Niedergang**; Institut Cochin Inserm, Université Paris Descartes, Paris, FRANCE*
12:00 – 12:25 pm	S2:9 Commensal *E. coli* Induced Colonization Resistance Against Intestinal *Salmonella* Infection ***Chunchi Lu**; University of Washington, Seattle, WA*
12:30 pm – 2:00 pm Congress Hall Foyer	**Lunch**

2:00 pm – 4:00 pm
Congress Hall Foyer

Poster Session A
Posters 1 – 46 will be presented.

4:00 pm – 7:00 pm
Congress Hall

Session 3: Gene Regulation, Antibiotics, Vaccines

4:00 – 4:25 pm

S3:1
Comparative Transcriptomics Identifies a Single SNP Linked to Virulence of the African *S. Typhimurium* ST313
***Jay Hinton**; University of Liverpool, Liverpool, UNITED KINGDOM*

4:25 – 4:50 pm

S3:2
Super-resolution Imaging of *Salmonella* SPI-2 Regulation: A View from 30,000 Feet to 20 nm
***Linda Kenney**; Mechanobiology Institute, Singapore, SINGAPORE*

4:50 – 5:05 pm

S3:3
IscR, a New Regulator for Spi1
***Laurent Aussel**; Aix-Marseille University - CNRS, Marseille, FRANCE*

5:05 – 5:20 pm

S3:4
In vivo RNA Association Patterns Direct Discovery of Virulence Functions of RNA-binding Proteins CspC and CspE
***Charlotte Michaux**; University of Wuerzburg, Wuerzburg, GERMANY*

5:20 – 5:35 pm

S3:5
A Chemical Genetics Approach to Bacterial Pathogenesis
***Corrie Detweiler**; University of Colorado, Boulder, CO*

5:35 – 6:05 pm

Coffee Break

6:05 – 6:30 pm

S3:6
Title to be announced
***Eduardo Groisman**; Yale School of Medicine/HHMI, New Haven, CT*

6:30 – 6:45 pm

S3:7
Novel HilD-dependent Chemotaxis Contributes to Invasion of Host Cells
***Kendal Cooper**; NIH/NIAID/Rocky Mountain Labs, Hamilton, MT*

6:45 – 7:00 pm

S3:8
Regulation of the *Salmonella* SPI1 Type Three Secretion System by Inorganic Phosphate
***Yekaterina Golubeva**; University of Illinois at Urbana-Champaign, Urbana, IL*

Wednesday, August 31, 2016

9:00 am – 12:15 pm
Congress Hall

Session 4: Structure and Function of Virulence Factors

9:00 – 9:25 am

S4:1
Visualising the SPI-1 Type III Secretion System in Action
***Thomas Marlovits**; University Medical Centre Hamburg-Eppendorf, Hamburg, GERMANY*

9:25 – 9:50 am

S4:2
Title to be announced
***Natalie Strynadka**; University of British Columbia, Vancouver, BC, CANADA*

9:50 – 10:05 am

S4:3
Structural and Functional Characterization of the *Salmonella* Type III Secretion Sorting Platform
***Maria Lara-Tejero**; Yale University School of Medicine, New Haven, CT*

10:05 – 10:20 am

S4:4
Assembly and Protein Export Mechanisms of the Bacterial Flagellum of *Salmonella Typhimurium*
***Marc Erhardt**; Helmholtz Centre for Infection Research, Braunschweig, GERMANY*

10:20 – 10:35 am	S4:5 Interplay Between Chemotaxis Protein CheM and SPI-4 Components Modulates Function of the SPI-4 Encoded Adhesin SiiE ***Stefanie Hoffmann**; Robert Koch-Institut, Wernigerode, GERMANY*
10:35 – 11:05 am	Coffee Break
11:05 – 11:30 am	S4:6 Using Images to Understand Structure, Function and Dynamics of Type VI Secretion Systems ***Marek Basler**; University of Basel, Basel, SWITZERLAND*
11:30 – 11:45 am	S4:7 Structural and Functional Characterization of a VirG-like Protein in *Salmonella Typhimurium* ***Mrutyunjay Suar**; School of Biotechnology, KIIT University, Bhubaneswar, INDIA*
11:45 – 12:00 pm	S4:8 Effect of *Salmonella* Pathogenicity Island Excision in Early Stages of Infection in Mice ***C. Pardo-Roa**; Pontificia Universidad Católica de Chile, Santiago, CHILE*
12:00 – 12:15 pm	S4:9 Control of *Salmonella* Invasion by Chemical Signals of the Intestine ***Craig Altier**; Cornell University, Ithaca, NY*
12:30 pm – 2:00 pm Congress Hall Foyer	**Lunch**
2:00 pm – 4:00 pm Congress Hall Foyer	**Poster Session B** *Posters 50 – 100 will be presented.*

Thursday, September 1, 2016

9:00 am – 12:25 pm Congress Hall	**Session 5: Physiology and Metabolism**
9:00 – 9:25 am	S5:1 Metal Efflux in *Salmonella* Virulence and Resistance to Oxidative and Nitrosative Stress ***Ferric Fang**; University of Washington, Seattle, WA*
9:25 – 9:50 am	S5:2 *Salmonella* Heterogeneity during Infection: Impact on Disease Progression and Therapy ***Dirk Bumann**; University of Basel, Basel, SWITZERLAND*
9:50 – 10:05 am	S5:3 An Oxidative Central Metabolism Enables *Salmonella* to Utilize Gut Microbiota-derived Succinate as a Carbon Source During Infection ***Sebastian Winter**; UT Southwestern Medical Center, Dallas, TX*
10:05 – 10:20 am	S5:4 Genome-guided Design of a Novel Defined Mouse Microbiota That Confers Colonization Resistance Against *Salmonella enterica* serovar Typhimurium ***Bärbel Stecher**; LMU Munich, Munich, GERMANY*
10:20 – 10:35 am	S5:5 Genomic Population Structure of *Salmonella enterica* ***Zhemin Zhou**; University of Warwick, Coventry, UNITED KINGDOM*
10:35 – 11:05 am	Coffee Break
11:05 – 11:30 am	S5:6 The Battle for Metals in the Gut: How *Salmonella* Evades Nutritional Immunity ***Manuela Raffatellu**; University of California, Irvine Sch. of Med., Irvine, CA*

11:30 – 11:45 am	S5:7 A Cross-species Comparison of Bacterial Gene-drug Interactions ***Birgit Pfalz**; EMBL, Heidelberg, GERMANY*
11:45 – 12:00 pm	S5:8 Evaluation of Methionine Metabolism and Transport in *Salmonella Typhimurium* Virulence ***Asma Husna**; The University of Melbourne, Melbourne, AUSTRALIA*
12:00 – 12:25 pm	S5:9 Mechanisms of Increased Susceptibility to Invasive NTS Infection During Malaria and Malnutrition ***Renee Tsolis**; University of California, Davis, CA*
12:30 pm – 2:00 pm Congress Hall Foyer	**Lunch**
2:00 pm – 5:10 pm Congress Hall	**Session 6: Cell Biology of Infection, Trafficking**
2:00 – 2:25 pm	S6:1 *Salmonella enterica* Remodels the Host Cell Endosomal System for Efficient Intravacuolar Nutrition ***Michael Hensel**; Osnabrueck University, Osnabrueck, GERMANY*
2:25 – 2:50 pm	S6:2 Novel, Translocon-independent Secretion of Type III Secretion System 1 Effectors Promotes Cytosolic Replication of *Salmonella Typhimurium* ***Olivia Steele-Mortimer**; NIAID, Hamilton, MT*
2:50 – 3:05 pm	S6:3 One-two Punch: *Salmonella* Strategies to Counteract the BLOC-3/Rab32 Host Defense Pathway ***Stefania Spano**; University of Aberdeen, Aberdeen, UNITED KINGDOM*

3:05 – 3:20 pm	S6:4 The SseK Family of Glycosyltransferase Effectors From *Salmonella* Modify Host Cell Signalling Proteins ***Joshua Newson**; The Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, AUSTRALIA*
3:20 – 3:35 pm	S6:5 Demonstrating the Complex Interplay of SCV and SIF-Related Effectors During *Salmonella* Infection ***Katelyn Knuff**; University of British Columbia, Vancouver, BC, CANADA*
3:35 – 4:05 pm	Coffee Break
4:05 – 4:30 pm	S6:6 RNA-seq-based Identification of Cytosol-specific *Salmonella* Genes ***Leigh Knodler**; Washington State University, Pullman, WA*
4:30 – 4:45 pm	S6:7 Identification of the Rck Receptor Required for *Salmonella* Cell Invasion ***Agnes Wiedemann**; INRA Centre Val de Loire, Nouzilly, FRANCE*
4:45 – 5:10 pm	S6:8 Cytoskeleton Remodelling by Cooperating Small GTPases at the Membrane ***Vassilis Koronakis**; University of Cambridge, Cambridgeshire, UNITED KINGDOM*
5:15 pm – 7:15 pm Congress Hall Foyer	**Poster Session C** *Posters 101 – 148 will be presented.*
7:30 pm – 10:30 pm Congress Hall	**Conference Closing Dinner**
**8:45 – 9:00 pm*	*Poster Prizes and Closing Remarks will be presented.*

Speaker Abstracts

OS:1

Invited Speakers

J. E. Galan;
Yale Univ. Sch. of Med., New Haven, CT.

OS:2

THE PYROMANIAC INSIDE YOU: SALMONELLA METABOLISM IN THE HOST GUT

A. J. Baumler;
Univ. of California, Davis, Davis, CA.

The lumen of the mammalian intestine is host to a metabolically diverse microbial community that occupies all available nutrient-niches, thereby making it difficult for pathogens to establish themselves in this highly competitive environment. Salmonella serovars sidestep the competition with resident microbes by using their virulence factors to coerce the host into creating a novel nutrient-niche. Inflammation-derived nutrients available in this new niche support a bloom of Salmonella serovars in the gut lumen, thereby ensuring transmission of the pathogen to the next susceptible host by the fecal oral route. Recent insights into the 'winning metabolic strategy' Salmonella serovars use to edge out competing microbes in the inflamed intestine will be the focus of this presentation.

S1:1

COMPARING THE INTRACELLULAR NICHE FORMATION OF SHIGELLA AND SALMONELLA WITHIN INFECTED EPITHELIAL CELLS

J. Enninga;
Institut Pasteur, Paris, FRANCE.

A common strategy of bacterial pathogens is active or passive uptake into host cells. There, they can localize within a bacterial containing vacuole (BCV) or access the host cytoplasm through BCV rupture. Hence, intracellular pathogens are often classified as vacuole-bound or cytoplasmic. Recently, this definition has been challenged by the discovery that many vacuole-bound pathogens, including Mycobacterium tuberculosis and Salmonella enterica, access the host cytoplasm, and by the insight that cytoplasmic bacteria, like Shigella flexneri or Listeria monocytogenes, do not always escape the BCV. To shed light on these discrepancies, we have combined biochemical, cell biological and ultrastructural approaches with the aim to investigate key features of the intracellular niche formation during bacterial entry. Here, I will present recent findings on two relevant human pathogens, Shigella flexneri and Salmonella enterica. Interestingly, both of them exploit conserved pathways to move to their distinct intracellular locations.

S1:2

SALMONELLA FORMS INTRACELLULAR PERSISTERS WITH TACT

S. Helaine;
Imperial College, LONDON, UNITED KINGDOM.

The recalcitrance of many bacterial infections to antibiotic treatment is thought to be due to the presence of persisters that are non-growing antibiotic-insensitive cells. Eventually persisters resume growth, accounting for relapses of infection. After macrophage phagocytosis, a significant proportion of the Salmonella population forms non-growing persisters through the action of toxin-antitoxin modules. Here we reveal that one such toxin - TacT, is an acetyltransferase that blocks the primary amine group of amino acids on charged tRNA molecules, thereby inhibiting translation and promoting persister formation. As persisters retain the ability to resume growth, we identify a detoxifying mechanism in Salmonella explaining how bacterial persisters can resume growth.

S1:3

SINGLE MOLECULE LOCALIZATION AND TRACKING OF SPI2-T3SS EFFECTOR PROTEINS IN *SALMONELLA ENTERICA* INFECTED CELLS

V. Göser[1], *C. Richter*[2], *R. Kurre*[2], *M. Hensel*[1]; [1]*University of Osnabrück, Department of Microbiology, Osnabrück, GERMANY,* [2]*University of Osnabrück, Department of Biophysics, Osnabrück, GERMANY.*

Background: *S. enterica* manipulates various host cell processes. Among these, aggregation and tubulation of endosomal membrane vesicles leads to formation of *Salmonella*-induced filaments (SIFs). Responsible for this phenomenon are effector proteins translocated into the host cell via *Salmonella* pathogenicity island 2 (SPI2)-encoded type III secretion system (T3SS). These effector proteins co-localize with SCV and SIFs. So far, analyses of dynamics of translocation and intracellular trafficking of these effector proteins was hampered by lack of fluorescence tags compatible with T3SS translocation. We devised a new approach deploying genetically encoded self-labeling enzymes (HaloTag) and TIRF super-resolution microscopy (SRM) that enabled us to investigate the subcellular localization and dynamics of SPI2-T3SS effector proteins in living cells. **Methods** Effector proteins were fused to HaloTag, an enzyme tag which rapidly forms a covalent bond to its ligands. Labeling took place using 20 nM of a cell-permeable, reactive chloroalkane-based ligand for the HaloTag conjugated to tetramethylrhodamine (TMR). To first establish the functional secretion of effector proteins with HaloTag, HeLa cells were infected, labeled and fixed 8 h p.I. and the localization of effector proteins were determined using dSTORM SRM. Next, we deployed in live, infected cells tracking and localization microscopy (TALM) of selected proteins to reveal the precise localization, as well as the dynamics of a single effector protein in membranes of SIFs. **Results** We tested the translocation of various effector-HaloTag fusion proteins and for most proteins labelling suitable for SRM was observed. The HaloTag is compatible with translocation by the SPI2-T3SS, fusion proteins are functional as effectors and the HaloTag is functional as self-labeling enzyme after translocation into host cells. The localization of various SPI2 effector proteins could be determined in relation to SIFs in *Salmonella*-infected cells. Additionally, the mobility of selected effector proteins (SifA, PipB2, SseF) in SIFs was established employing live cell SRM. The effector proteins SifA and SseF show a similar mobility as the membrane protein LAMP1. In contrast PipB2 showed a distinct association to the SIF membrane. **Conclusion:** Here we present a new tool to study the localization, translocation kinetics and dynamics of *Salmonella* effector proteins in infected cells. SPI2 effector proteins fused to a HaloTag can be functionally translocated in infected cells and localize to SCV and SIFs. Moreover it is possible to monitor a single effector protein in SIFs during infection. This new technique provides an opportunity to compare mobility and localization of different SPI2 effector proteins and consequently allows an interpretation of the interaction of effector proteins with host cell membranes and further target structures.

S1:4

SINGLE-CELL RNA-SEQ REVEALS DISPARATE MACROPHAGE RESPONSES TO INTRACELLULAR *SALMONELLA*

A. E. Saliba[1], *L. Li*[1], *A. J. Westermann*[1], *S. Appenzeller*[1], *L. N. Schulte*[1], *S. Helaine*[2], *J. Vogel*[1]; [1]*University of Wuerzburg, Institute for Molecular Infection Biology, Wuerzburg, GERMANY,* [2]*Medical Research Council (MRC) Centre for Molecular Bacteriology and Infection, Imperial College London, London, UNITED KINGDOM.*

Intracellular bacterial pathogens can exhibit large heterogeneity in growth rate inside host cells with major consequences for the infec-

tion outcome. If and how the host responds to this heterogeneity remains poorly understood. Here, we combined a fluorescent reporter of bacterial cell division with single-cell RNA-seq analysis to study the macrophage response to different intracellular states of the model pathogen Salmonella enterica serovar Typhimurium. The transcriptomes of individual infected macrophages revealed a spectrum of functional host response states to growing and non-growing bacteria. Intriguingly, macrophages harboring non-growing Salmonella display hallmarks of the pro-inflammatory M1 polarization state and differ little from bystander cells, suggesting that non-growing bacteria evade recognition by intracellular immune receptors. By contrast, macrophages containing growing bacteria have turned into an anti-inflammatory, M2-like state, as if fast-growing intracellular Salmonella overcome host defense by reprogramming macrophage polarization. Additionally, our clustering approach reveals intermediate host functional states between these extremes. Altogether our data suggest that gene expression variability in infected host cells shapes different cellular environments, some of which may favor a growth arrest of Salmonella facilitating immune evasion and the establishment of a long-term niche; while others allow Salmonella to escape intracellular antimicrobial activity and proliferate.

S1:5

IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF HOST MICRORNAS RESTRICTING *SALMONELLA* INFECTION

C. Lisovski[1], C. Aguilar[1], C. Maudet[1], U. Sunkavalli[1], M. Sharan[1], K. Forstner[1], M. Mano[2], ***A. Eulalio[1];***
[1]University of Würzburg, Würzburg, GERMANY, [2]University of Coimbra, Coimbra, PORTUGAL.

Background: MicroRNAs (miRNAs) have well-established functions in physiological and pathological processes. Accumulating evidence indicates a crucial role for miRNAs in the interaction between bacterial pathogens and host cells. However, a comprehensive analysis of miRNA function during bacterial infection has not been performed previously. To tackle this question we used as a model the bacterial pathogen Salmonella Typhimurium, and applied an integrated systems biology approach. **Methods and Results:** By applying high-content fluorescence microscopy-based screenings using genome-wide libraries of miRNA mimics and inhibitors, we discovered that host miRNAs strongly determine the outcome of infection by Salmonella. Detailed time-course experiments, using wild-type and mutant Salmonella strains, showed that the identified miRNAs regulate different stages of infection (e.g. adhesion, intracellular survival, vacuole maturation and replication). Interestingly, parallel functional screenings with the closely related pathogen Shigella flexneri revealed that infection by these two pathogens is regulated by a distinct subset of host miRNAs. Among the strongest inhibitors of Salmonella infection we identified miRNAs that hinder G1/S cell cycle progression, uncovering a crucial role of the host cell cycle during infection. In particular, we demonstrated that miRNAs that induce G1 cell cycle phase arrest of host cells, including the miR-15 family, miR-26a/b and miR-744, inhibit Salmonella intracellular replication. Using various approaches, we demonstrated that maturation of the Salmonella containing vacuole is strongly impaired in the G1 phase of the cell cycle. Conversely, Salmonella replication is favoured in the G2 phase of the cell cycle. RNA-sequencing analysis of the small RNA fraction revealed that Salmonella induces dramatic changes of the host miRNome. Interestingly, Salmonella decreases expression of the miRNAs shown to inhibit G1/S cell cycle progression. Accordingly, inhibition of these miRNAs with specific miRNA inhibitors leads to an increase of Salmonella replication, further demonstrating the relevance of these miRNAs to a productive infection. **Conclusions:** Our findings uncover

a novel mechanism whereby Salmonella renders host cells more susceptible to infection by controlling cell cycle progression through the active modulation of host cell miRNAs. Overall, our work demonstrates the value of using genome-wide approaches to study miRNA function and highlights its potential to identify novel molecular players/pathways governing the complex interaction between host and bacterial pathogens.

S1:6

PROBING THE SALMONELLA-HOST INTERFACE WITH HIGH-THROUGHPUT APPROACHES

***A. Typas**;*
European Molecular BIology Laboratory, Heidelberg, GERMANY.

High-throughput quantitative approaches can provide unique insights into the overall cellular network architecture and prime novel mechanistic hypotheses. Here I will present how we use such genetic, cell biology and biochemical approaches to shed light into gene function and pathway organization in Salmonella, and map its interface with the host.

S2:1

USING GENOMICS TO INVESTIGATE HOST/*SALMONELLA* INTERACTIONS

***G. Dougan**;*
The Wellcome Trust, Hinxton, UNITED KINGDOM.

The availability of detailed genome sequence is providing a blueprint for new approaches to the design of experiments to interrogate Host/Salmonella interactions. On the 'Salmonella' side well annotated reference genomes can guide transcriptome and proteome analysis and facilitate high throughput mutagenesis and phenotyping experiments. Acquiring genome information from microbial populations facilitates transmission tracking, evolution studies and can help identify emerging trends such as the evolution of antibiotic resistance. Such approaches can also help define the microbial communities living in and on the host. The availability of draft genomes for vertebrate hosts including humans, mouse and zebrafish are helping, again in terms of transcriptome, proteome and RNAi-type inhibition studies. They are also facilitating high throughput mutagenesis and phenotyping programmes that can provide valuable data to the community as open access. As we sequence human populations we can use this data to identify loci under selection in different human populations and even investigate rare mutations with extreme phenotypes. Here, specific examples built around clinical studies and infection models will be used to illustrate the power of some of these approaches.

S2:2

HOW CELLS DEFEND THEIR CYTOSOL AGAINST SALMONELLA INVASION

***F. Randow**;*
MRC Laboratory of Molecular Biology, Cambridge, UNITED KINGDOM.

Intracellular pathogens inhabit specific cellular niches determined by the degree of compartment-specific immune surveillance and the pathogen's need for host cell activities and nutrients. Most intracellular bacteria dwell in vacuoles while only few have conquered the cytosol, a perhaps counterintuitive situation considering the abundant energy sources available in the cytosol for bacterial growth. Potent cytosolic defense mechanisms must therefore exist. I will discuss the role of cell-autonomous immunity in defending the cytosol from Salmonella invasion, in particular how 'eat-me' signals including galectins and ubiquitin become associated with cytosol-invading bacteria, how a novel E3 ubiquitin ligase transforms the bacterial surface into an anti-bacterial and pro-inflammatory signaling platform, and how cargo-selecting autophagy receptors target cytosolic bacteria for destruction.

S2:3

CYCA IN BOTH *SALMONELLA* AND UROPATHOGENIC *E. COLI* PREVENTS KILLING BY D-AMINO ACID OXIDASE

***B. Tuinema**, V. Lau, B. Ilyas, B. Coombes; McMaster University, Hamilton, ON, CANADA.*

Neutrophils act as the first line of defense during bacterial infections. Using multiple antimicrobial pathways, including D-amino acid oxidase (DAO), neutrophils limit pathogen replication and systemic spread, however they fail to fully restrict Salmonella growth at early times after infection. Previously we showed that Salmonella limits its exposure to neutrophil reactive oxygen species by sequestering D-alanine, a key substrate for DAO-mediated oxidative killing. This sequestration mechanism involved an ABC transporter DalSTUV that has been coregulated with intracellular virulence factors via cis-regulatory evolution. We considered whether a second importer of D-alanine and D-serine , CycA, might be involved in a similar host-pathogen interaction in Salmonella and other CycA-containing bacteria. We show that Salmonella lacking cycA are sensitized to the killing activity of purified DAO and to purified human neutrophils in vitro. During mouse infections, Salmonella cycA mutants are out-competed by wild type bacteria but can be rescued by in vivo inhibition of DAO. In vivo imaging of mice infected with a Salmonella strain that reports on ROS stress showed that this killing correlated with an increased exposure to ROS during infection. As DAO expression is also high in the kidney, we show that CycA in uropathogenic E. coli (UPEC) is important for UPEC survival during kidney infection but is dispensable in the absence of DAO activity. This work sheds light on a novel virulence pathway common to two distinct pathogens and further demonstrates the importance of DAO subversion to support a range of bacterial lifestyles.

S2:4

GENOTYPIC AND PHENOTYPIC DIVERSITY ARISING DURING THE CLONAL EXPANSION OF AN MDR MONOPHASIC *SALMONELLA* TYPHIMURIUM EPIDEMIC

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Background: Bacteria are constantly evolving, and selection of beneficial mutation / gene acquisition can modify the outcome of host-pathogen, host-environment interactions and transmission dynamics of the pathogen. Epidemics of bacterial pathogens are generally considered clonal, although we have report a remarkable degree of microevolution in the genomes of a clonally expanding epidemic strain of Salmonella Typhimurium. **Methods:** The phylogenetic relationship of isolates was determined using variation in the core genome sequence. Genome sequence determined by Illumina or Pacbio was assembled and genome evolution determined in the phylogenetic context of the isolates. The impact of SGI-3 on copper homeostasis was determined using allelic exchange and determination of MIC. **Results:** We describe a comparative whole genome sequence and phylogenomic analysis of monophasic *Salmonella* Typhimurium isolates from the UK and Italy from 2005-2012. Monophasic isolates from this time formed a single clade distinct from recent monophasic epidemic clones described previously from North America and Spain. The current UK monophasic epidemic clones encode a novel genomic island encoding resistance to heavy metals (SGI-3), and composite transposon encoding antibiotic resistance genes not present in other Typhimurium isolates, that may have contributed to the epidemiological success. We also report a remarkable degree of genotypic variation that accumulated during clonal expansion of a UK epidemic including multiple independent acquisitions of a novel prophage

carrying the *sopE* gene and multiple deletion events affecting the phase II flagellin locus. **Conclusions:** New clones of Salmonella may be selected by changes in the environment by acquisition genes by horizontal gene transfer. Once emerged, *Salmonella* clones may evolve rapidly within 5-10 years during an epidemic impacting antigenicity, resistance and virulence characteristics.

S2:5

PARTICIPATION OF NSSNPS IN VARIABLE ADAPTATION OF *SALMONELLA* TO DIFFERENT HOST SPECIES

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Salmonella enterica serovar Newport, a frequent human and livestock pathogen, carries 11-12 chaperone-usher fimbrial gene clusters. A comparative analysis of the FimH tip-adhesin and the predicted tip-adhesins BcfD and StfH of two other fimbrial gene clusters for 261 Salmonella Newport strains identified two alleles for each protein, designated A and B, that were each further divided into two variants for StfH. There was an uneven distribution of the A and B variants, as bovine and porcine isolates had mainly allele A, environmental (such as plant or water) isolates had mainly allele B, whereas avian, equine and human strains had similar numbers of each allele, except StfH B alleles were predominant in human isolates. The bcf and stf gene clusters were shown to be capable of producing fimbrial structures on the surface of recombinant bacteria. Both BcfD alleles mediated strong bacterial binding to human colonic RKO and porcine jejunal IPEC-J2 cell lines, with a significant preference by allele A for porcine cells and allele B for human cells. Bacteria with either BcfD allele adhered also to the bovine jejunal cell line J8, albeit less efficiently. The StfH A1 allele bound best to bovine jejunocytes and RKO cells. In contrast to StfH A1, bacteria with other StfH alleles weakly attached to the bovine colonic cell line CMS, and the B2 allele bound best to the human RKO cells. The most frequent combinations of FimH/StfH/BcfD alleles in one strain were A/A1/A and B/B2/B, with the former set being the most frequently found in bovine and porcine strains and best suited for binding to their enterocytes. The latter combination was most frequently found in environmental and human isolates and expanded bacterial binding range to the human and various animal hosts. These findings indicated that allelic variation in multiple adhesins can contribute to either bacterial adaptation to certain preferential hosts or maintenance of a broad host range.

S2:6

PYROPTOSIS TRIGGERS PORE-INDUCED INTRACELLULAR TRAPS (PITS) THAT CAPTURE BACTERIA AND LEAD TO THEIR CLEARANCE BY EFFEROCYTOSIS

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Inflammasomes activate caspase-1 in response to cytosolic contamination or perturbation. This inflammatory caspase triggers the opening of the GSDMD pore in the plasma membrane, resulting in lytic cell death called pyroptosis. We had previously assumed that pyroptosis releases intracellular bacteria to the extracellular space. Here, we find that viable bacteria instead remain trapped within the cellular debris of pyroptotic macrophages. This trapping appears to be an inevitable consequence of how osmotic lysis ruptures the plasma membrane, and may also apply to necroptosis and some forms of non-programmed necrosis. Although membrane tears release soluble cytosolic contents, they are small enough to retain organelles and bacteria. We call this structure the pore-induced intracellular trap (PIT),

which is conceptually parallel to the neutrophil extracellular trap (NET). The PIT coordinates innate immune responses via complement and scavenger receptors to drive recruitment of and efferocytosis by neutrophils. Ultimately, this secondary phagocyte kills the bacteria. Hence, caspase-1 driven pore-induced cell death triggers a multifaceted defense against intracellular bacteria facilitated by trapping the pathogen within the cellular debris. Bona fide intracellular bacterial pathogens, such as Salmonella, must prevent or delay pyroptosis in order to avoid being trapped in the PIT and subsequently killed by neutrophils.

S2:7

SALMONELLA TYPHIMURIUM UNDERGOES DISTINCT GENETIC ADAPTION DURING CHRONIC INFECTIONS OF MICE: EMERGENCE OF AN INTESTINAL SUPER-COLONIZER CLONE

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Background: Typhoid fever caused by Salmonella enterica serovar Typhi (S. Typhi) is a severe systemic human disease and endemic in regions of the world with poor drinking water quality and sewage treatment facilities. A significant number of patients become asymptomatic life-long carriers of S. Typhi and serve as the reservoir for the disease. The specific mechanisms and adaptive strategies enabling S. Typhi to survive inside the host for extended periods are incompletely understood. Yet, elucidation of these processes is of major importance for improvement of therapeutic strategies. In the current study genetic adaptation during experimental chronic S. Typhimurium infections of mice, an established model of chronic typhoid fever, was probed as an approach for studying the molecular mechanisms of host-adaptation during long-term host-association. **Results:** Individually sequence-tagged wild type strains of S. Typhimurium 4/74 were used to establish chronic infections of 129X1/SvJ mice. Over the course of infections, S. Typhimurium bacteria were isolated from feces and from livers and spleens upon termination of the experiment. In all samples dominant clones were identified and select clones were subjected to whole genome sequencing. Dominant clones isolated from either systemic organs or fecal samples exhibited distinct single nucleotide polymorphisms (SNPs). One mouse appeared to have distinct adapted clones in the spleen and liver, respectively. Three mice were colonized in the intestines by the same clone containing the same non-synonymous SNP in a transcriptional regulator, kdgR, of metabolic genes. This likely indicates transmission of this clone between mice. The mutation was tracked to have occurred prior to 2 weeks post infection in one of the three mice and had subsequently been transmitted to the other two mice. Re-infection with this clone confirmed that it is superior to the wild type for intestinal colonization. **Conclusions:** During 4 to 6 weeks of chronic infections, S. Typhimurium acquired distinct SNPs in known regulators of metabolic and virulence genes. One SNP, the kdgR-SNP was confirmed to confer selective advantage during chronic infections, in particular for colonization of the mouse intestines, and constitute a true patho-adaptive mutation. I am presently investigating the molecular basis for the enhanced intestinal colonization of this adapted clone and will present these data at the conference. Together, the results provide evidence for rapid genetic adaptation to the host of S. Typhimurium and validate experimental evolution in the context of host infection as a strategy for elucidating pathogen host interactions at the molecular level.

S2:8

EXQUISITE ADAPTATION OF NONTYPHOIDAL *SALMONELLA* TYPHIMURIUM ST313 TO HIV INFECTED HUMAN MACROPHAGES

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Macrophages are a main target for the Human Immunodeficiency Virus (HIV)-1. Because of their resistance to cytopathic effects, they can be considered as persistent viral reservoirs. HIV-infected macrophages exhibit defective functions that contribute to the development of opportunistic diseases. In particular, severe invasive nontyphoidal Salmonella (iNTS) disease caused by Salmonella Typhimurium ST313 has been prevalent in the last three decades in sub-Saharan African children or adults with HIV.We have shown that internalization of large particles, bacteria and fungi, is impaired in HIV-infected primary human macrophages due to the virulence factor Nef. We also demonstrated that HIV-1 infection also perturbs phagosomes maturation into phagolysosomes, leading to impaired bacterial clearance. Our data revealed that the viral protein Vpr was sufficient to critically alter localization of microtubule plus ends binding proteins EB1 and p150Glued, hence altering the centripetal movement of phagosomes and phagolysosome biogenesis. Interestingly, HIV-1 infected macrophages exhibited a higher basal level of activation but appeared unable to respond efficiently to phagocytic triggers, and their cytokine response was modified. To get further insight into the development of iNTS, we analyzed the gene expression of human macrophages infected with HIV-1 or not, and co-infected with iNTS bacteria. Our experiments show that the genetic divergence of Salmonella Typhimurium ST313 appears to provide an additional advantage to the invasive ST313 strain, which shows enhanced intracellular survival and proliferation, and triggers a higher pro-inflammatory response in human macrophages. The Salmonella containing vacuole and the HIV containing compartments in macrophages were analyzed with FIB-SEM tomography. These phenotypes could support invasive systemic disease.

S2:9

COMMENSAL *E. COLI* INDUCED COLONIZATION RESISTANCE AGAINST INTESTINAL *SALMONELLA* INFECTION

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Salmonella is the causative agent in Salmonellosis and is a leading cause of gastrointestinal bacterial infections worldwide. During mucosal *Salmonella* infection, Salmonella must first establish a niche in the gastrointestinal tract in order to initiate infection. Colonization resistance (CR), defined as the ability of intestinal microbiota and host defenses to protect against pathogens, is the first line defense against intestinal infection. The complex intestinal microbiota is capable of constraining the extracellular growth of *Salmonella* in the gut lumen and mucus layers to prevent the SPI-1/2 type three secretion systems (TTSS) dependent intracellular growth phase of the infection. The mechanism for commensal bacteria mediated CR has been studied for a long time; however, only a few studies have explored how microbiota can induce CR against *Salmonella* infection. We have identified a murine commensal *E. coli* strain *(E. coli XZ*) that mediates colonization resistance against mucosal *Salmonella Typhimurium* infection, which is independent of the inflammasome, TLR5, MyD88, and

adaptive immunity. We demonstrate that *E. coli* XZ does not directly compete with *Salmonella* growth *in vitro*, but is fully capable of inducing colonization resistance *in vivo*. *E. coli* XZ limits the early phase of *Salmonella*'s invasion into the murine gut ecosystem. *Salmonella Typhimurium* requires the SPI-2 TTSS to overcome *E. coli XZ* mediated colonization resistance in the presence of complex intestinal microbiota but not in germ free mice. Our data indicate that murine commensal *E. coli* limit the early phase of gut colonization by *Salmonella*, and that in the presence of *E. coli* XZ and a more complex microbiota, *Salmonella* requires SPI-2 to overcome *E. coli XZ* mediated colonization resistance and efficiently colonize the gut. Understanding how commensal microbes prevent intestinal infections will help design new strategies to prevent and treat enteric infections.

S3:1

COMPARATIVE TRANSCRIPTOMICS IDENTIFIES A SINGLE SNP LINKED TO VIRULENCE OF THE AFRICAN *S. TYPHIMURIUM* ST313

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S3:2

SUPER-RESOLUTION IMAGING OF SALMONELLA SPI-2 REGULATION: A VIEW FROM 30,000 FEET TO 20 NM

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S3:3

ISCR, A NEW REGULATOR FOR SPI1

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Background: Iron-sulfur (Fe-S)-containing proteins contribute to various biological processes, including redox reactions or regulation of gene expression. Living organisms have evolved by developing distinct biosynthetic pathways to assemble these clusters, among which ISC (Iron Sulfur Cluster) and SUF (Sulfur Utilisation). Salmonella possesses all known prokaryotic systems to assemble Fe-S clusters, including ISC and SUF. Because iron starvation and oxidative stress are detrimental for Fe-S enzyme biogenesis and because such environments are often met by Salmonella during its intracellular life, we investigated the role of the ISC and SUF machineries during the course of the infection. **Methods and Results:** The iscU mutant, which is predicted to have no ISC system functioning, was found to be defective for epithelial cell invasion and for mice infection, whereas the sufBC mutant, which is predicted to have no SUF system functioning, did not present any defect. Moreover, the iscU mutant was highly impaired in the expression of Spi1 TTSS. IscR, a transcriptional regulator carrying a [2Fe-2S] cluster, is matured by the ISC machinery. Combining bioinformatic and biochemical approaches, two IscR-binding sites were identified on the promoter region of hilD, a gene encoding a master regulator of Spi1. Using transcriptional fusions and qPCR, we showed that IscR represses hilD expression, leading to the down-regulation of Spi1 gene expression and consistent with the observation that an iscR mutant is hyperinvasive in epithelial cells. **Conclusions:** Collectively, our findings indicate that the ISC machinery plays a central role in Salmonella virulence through the ability of IscR to down-regulate Spi1 gene expression. At a broader level, this model represents a new adaptive mechanism used by bacterial pathogens to modulate their infectivity according to iron and oxygen availability.

S3:4

IN VIVO RNA ASSOCIATION PATTERNS DIRECT DISCOVERY OF VIRULENCE FUNCTIONS OF RNA-BINDING PROTEINS CSPC AND CSPE

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Bacterial pathogens express many predicted RNA-binding proteins whose potential roles in physiology and virulence remain little understood for lack of knowledge of their cellular ligands. In enteric bacteria including the gastrointestinal pathogen, *Salmonella typhimurium,* members of the cold-shock protein A (CspA) family constitute the largest group of non-ribosomal RNA-binding proteins. While some of them are indeed induced and ensure gene expression at low temperature, other CspA family members of largely unknown physiological function are expressed at high levels under conditions relevant for host infection. Here, to identify biological processes controlled by the six members of the CspA family in *S. typhimurium*, we have systematically profiled their cellular target transcripts by RNA co-immunoprecipitation and sequencing. This ligand-centric approach predicted a distinct yet overlapping association of the constitutively expressed CspC and CspE proteins with multiple pathways of high relevance for *Salmonella* pathogenesis. Phenotypic assays *in vitro* confirmed these predictions, demonstrating that the two proteins compensate for each other's function in the survival of bile and oxidative stress as well as in bacterial motility and biofilm formation. Moreover, double deletion of the *cspC* and *cspE* genes fully attenuates *Salmonella* in a mouse model of bacterial virulence. Our results highlight the importance of RNA-binding proteins as regulators of bacterial pathogenicity and potential targets of antimicrobial therapy and identify functional redundancy as a viable reason for why the essential virulence function of these two CspA-like proteins remained undetected in previous phenotypic screens.

S3:5

A CHEMICAL GENETICS APPROACH TO BACTERIAL PATHOGENESIS

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Much remains unknown regarding how Salmonella enterica survives within macrophages. To identify probes of the host-pathogen interface, we developed a quantitative, high throughput, phenotypic screen for small molecules that kill intracellular Salmonella (Typhimurium) with minimal toxicity to mammalian cells. We screened the 14,400 compounds of the Maybridge HitFinder Library, a collection of compounds with drug-like properties. This approach identified 77 compounds that reduce bacterial load and were verified in a secondary screen that quantified bacterial colony forming units in macrophages. Only three of these hits inhibited bacterial growth in standard microbiological media, indicating the remainder function specifically in the context of an infected macrophage. We are finding that some of these probes appear to target the bacterium and others the host cell. We will describe the screening platform and present the results of our studies characterizing top hits.

S3:6

Invited Speakers

E. A. Groisman*; Yale School of Medicine/HHMI, New Haven, CT.*

S3:7

NOVEL HILD-DEPENDENT CHEMOTAXIS CONTRIBUTES TO INVASION OF HOST CELLS

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Background: Invasion into host cells is mediated by the *Salmonella* pathogenicity island 1 (SPI1)- encoded type III secretion system (T3SS1). HilD is the SPI1-encoded master regulator that controls the expression of the SPI1 regulon, which includes the T3SS1 as well as other target genes outside SPI1, some of which have unknown functions. This study evaluated T3SS1-independent contributions of HilD to invasion of host cells. **Methods:** *Salmonella enterica* serovar Typhimurium, strain SL1344 was used in this study. Invasion was measured using the gentamicin protection assay. Manipulation of gene expression and contributions to invasion were analyzed using bacterial gene deletion mutants and arabinose-inducible expression plasmids. RNAseq and transcriptional GFP reporters were used to evaluate factors involved in gene expression. **Results:** Initial observations revealed that various T3SS1 mutants entered primary human monocyte-derived macrophages (hMDM) much better than *hilD* deletion mutants. Furthermore, induced expression of *hilD* in a SPI1 deletion background resulted in increased invasion. To identify HilD-regulated genes involved in SPI1-independent invasion, we performed RNAseq and identified a chemoreceptor (*cheM*/*mcpC*) with HilD-dependent expression under our growth conditions. Deletion of *cheM* resulted in reduced levels of invasion in hMDM and cultured epithelial cells. In order to determine the mechanism of HilD regulation of *cheM*, we constructed a series of transcriptional reporters that revealed expression of *cheM* is dependent on both HilD and the master regulator of flagella, FlhDC. **Conclusions:** Our results reveal a novel mechanism of regulatory crosstalk between SPI1 and the motility/chemotaxis system that contributes to efficient invasion of host cells.

S3:8

REGULATION OF THE *SALMONELLA* SPI1 TYPE THREE SECRETION SYSTEM BY INORGANIC PHOSPHATE

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Invasion of the intestinal epithelial cells mediated by the SPI1 Type Three Secretion System (T3SS) is a critical step for *Salmonella* to establish disease in the host. The expression of *hilA*, encoding the transcriptional activator of the T3SS structural genes, is directly controlled by three AraC-like regulators, HilD, HilC and RtsA, each of which can activate *hilD*, *hilC*, *rtsA*, and *hilA* genes, forming a complex feed-forward regulatory loop. Expression of the SPI1 genes is tightly controlled by numerous regulatory inputs to ensure appropriate production of the T3SS apparatus. Growth in high salt LB medium is known to increase SPI1 gene expression, and serves as the standard *in vitro* inducing conditions. We report that inorganic phosphate starvation serves as a far greater activating signal for SPI1 genes. Using a *hilA-lac* transcriptional fusion as a readout for the SPI1 system, we show that *hilA* expression is greatly increased in low phosphate conditions compared to high phosphate medium, with HilD playing a major role in this regulation. The observed 50-fold increase in *hilA* transcription in low phosphate conditions is largely independent of the two-component regulatory system PhoBR, which controls a large number of genes in response to inorganic phosphate limitation. Loss of PhoBR results in only a 2-fold reduction in *hilA* expression under these conditions. Strikingly, *hilA* induction in low phosphate also requires supplementation with a specific set of 7 amino acids. The observed phenomena suggest that inorganic phosphate and particular amino acids serve as additional independent environmental cues to control SPI1 gene expression.

S4:1

VISUALISING THE SPI-1 TYPE III SECRETION SYSTEM IN ACTION.

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S4:2

Invited Speakers

N. Strynadka;
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S4:3

STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF THE *SALMONELLA* TYPE III SECRETION SORTING PLATFORM

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We have been using a multidisciplinary approach to determine the structural organization, topology and function of the different components of the Salmonella Typhimurium type III secretion sorting platform encoded in the Salmonella pathogenicity island 1 (SPI-1). During my presentation I will discuss the methodology as well as all that we have learned about the organization, topology and function of this essential component of the type III secretion system.

S4:4

ASSEMBLY AND PROTEIN EXPORT MECHANISMS OF THE BACTERIAL FLAGELLUM OF *SALMONELLA* TYPHIMURIUM

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Gastrointestinal infections by Enterobacteriaceae pose a serious health risk in developing and developed countries. One causative agent of gastrointestinal diseases is the gram-negative, food-borne pathogen Salmonella enterica serovar Typhimurium. Salmonella uses rotation of a helical organelle, the flagellum, for directed movement in various environments. The bacterial flagellum is closely related to virulence-associated injectisome systems of many pathogenic bacteria. Both nanomachines utilize a type-III secretion system (T3SS) to export proteins across the inner membrane in a proton motive force (pmf)-dependent manner. Type-III protein secretion is essential for the assembly of the flagellum, the injectisome, as well as for secretion of effector proteins. The filament of the flagellum is made of several thousand subunits of a single protein, flagellin, and extends several micrometer beyond the cell surface. The T3SS-dependent export of flagellar building blocks is a remarkable fast process and more than 1500 amino acids per second are transported during filament growth. A fundamental problem concerns the molecular mechanism of how the long, external filament grows at a rapid rate in the absence of any cellular energy sources. Here, we present a molecular mechanism to explain the growth of flagellar filaments based on simple biophysical parameters. We provide experimental evidence to demonstrate that growth of flagella follows a saturated diffusion mechanism and decreases with length. We determined the growth rate of single flagella using in situ labelling and real-time immunostaining of growing flagellar filaments. The growth rate data revealed a negative correlation between the rate of filament polymerization and the length of the flagellum. Addition of uncoupling agent that disrupted the pmf prevented filament elongation. Growth was resumed after removal of uncoupler, indicating a major contribution of the pmf in driving flagellin export. Competitive export of flagellin mutant proteins deficient in head-to-tail chain linkage did not impair the flagellum growth rate. While inter-subunit interactions between flagellin monomers might be important during substrate docking, these results suggest that the pulling force of chain

of flagellin molecules does not contribute substantially to the filament elongation dynamics. In summary, we propose a flagellum growth model based on simple biophysical parameters where the filament growth rate is driven by both hindered diffusion and proton motive force-dependent secretion of subunits.

S4:5

INTERPLAY BETWEEN CHEMOTAXIS PROTEIN CHEM AND SPI-4 COMPONENTS MODULATES FUNCTION OF THE SPI-4 ENCODED ADHESIN SIIE

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Background: During the infection process *Salmonella enterica* has to overcome the intestinal barrier formed by polarized epithelial cells. For that functional cooperation between the SPI-1 encoded type three secretion system and the type one secretion system (T1SS) encoded on SPI-4 is required. The SPI-4 T1SS secretes the giant non-fimbrial adhesin SiiE, which mediates intimate contact of *Salmonella* to microvilli on the apical membrane of the host cell. SiiE has to be retained on the bacterial surface to allow proper adhesion and subsequent invasion into polarized cells. Previously we demonstrated that the SPI-4 encoded SiiAB form a MotAB-like proton channel which is essential for this process. Given this similarity we asked whether the chemotaxis system is involved in controlling the switch between secretion and retention of the adhesin SiiE. **Methods:** Several mutants lacking up to 7 methyl-accepting chemotaxis proteins (MCP) were constructed using scarless mutagenesis and tested for invasion in polarized and non-polarized epithelial cell infection models. Possible interactions of SPI-4 components SiiA, SiiB, SiiD and SiiF with CheM were investigated in a Bacterial-Two-Hybrid system (BacTH). The ability to retain SiiE on its surface was tested in the absence and presence of α-methyl-D, L-aspartate (MeAsp), a non-metabolizable chemoattractant sensed by CheM, using the SIMPLE (screening with immunomagnetic particles for ligand expression) assay. The amounts of secreted SiiE were quantified under the same conditions with a SiiE-specific ELISA. The capability to invade polarized (MDCK) epithelial cells was assessed in the presence of MeAsp. **Results:** CheM mutants showed attenuation for invasion of polarized epithelial cells comparable to a *cheY* mutant strain. In BacTH direct interactions of CheM with itself and the SPI-4 components SiiA, SiiB, SiiD, but not SiiF, were detected. Using the SIMPLE-assay we observed increased surface retention of SiiE in the presence of MeAsp. Correspondingly, the amounts of secreted SiiE were decreased in the presence of the chemoattractant. Stimulation of CheM signaling resulted also in increased invasion of polarized MDCK cells. **Conclusion:** The direct interaction of CheM with SPI-4 components and modulation of SiiE surface expression by the CheM ligand MeAsp reveals a surprising functional link between a chemotaxis component and SPI-4 mediated adhesion. Our findings contribute not only to the understanding how host cells can be sensed by *Salmonella* but also how virulence functions can be controlled in a precise spatio-temporal fashion.

S4:6

USING IMAGES TO UNDERSTAND STRUCTURE, FUNCTION AND DYNAMICS OF TYPE VI SECRETION SYSTEMS

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The bacterial Type VI secretion system (T6SS) is a large dynamic organelle that is functionally analogous to contractile tails of bacteriophages. T6SS is used by Gram-negative bacteria to inhibit adjacent cells via translocation of toxic effector proteins and thus plays an important role in bacterial pathogenesis and ecology. Time-lapse fluorescence light microscopy revealed that T6SS sheath,

which powers the secretion, cycles between assembly, quick contraction, disassembly and re-assembly. Single cell analysis of subcellular localization of T6SS assembly revealed that T6SS organelle encoded by Pseudomonas aeruginosa H1-T6SS cluster is assembled and aimed to specifically retaliate against attack by other bacteria. I will present latest update on the structure, function and dynamics of T6SS as well on mechanisms of effector delivery and function in various T6SS+ organisms. I will also focus on the structure of the T6SS sheath solved by cryo-electron microscopy and the implications for T6SS dynamics and assembly. I will show examples of how various imaging techniques helped us to understand many aspects of T6SS.

S4:7

STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF A VIRG-LIKE PROTEIN IN *SALMONELLA* TYPHIMURIUM

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Bacterial secretion systems are the weapons for injecting pathogenic effectors into the host cytosol for establishment of bacterial pathogenesis. Salmonella enterica serovar Typhimurium is a facultative intracellular pathogen which infects non-phagocytic cells for the establishment of their pathogenic cycle. The two most essential virulence determinants of Salmonella spp. are SPI-1 and SPI-2 encoded T3SS responsible for the pathogen entry into non-phagocytic cells and infection establishment in macrophages. Other than the T3SS; the Type VI secretion system in Salmonella is still unexplored with respect to host modulatory effects during infection. It comprises of 13 core set of genes essential for T6SS assembly, secretion and coordinated eukaryotic functioning. Salmonella Typhimurium VirG-like protein (STV) is a non-core protein of T6SS encoded within the S. enterica centisome 7 genomic island (SCI). The in-vitro characterization of Stv revealed this protein to be an essential virulence determinant during the invasion and survival of pathogen in epithelial and murine macrophage cell-lines respectively. With an upregulation of IL-1 and IL-8 pro-inflammatory cytokines; stv gene was known to play a role in Salmonella invasion. The major regulators of SPI-1 like hilA, hns, hilD showed two-fold increase in the mutant (STM Δstv) strain. Being periplasmic; STV was constitutively expressed in the pathogen at all growth phases; however being more inducible under nutrient deprived conditions during stationary phase. This led to the study of STV expression under inducible stress conditions of pH, temperature and osmolarity. STV showed a significant increased expression profile under the stress parameters of pH, higher temperature as well as greater salt concentration. Additionally, the expression of STV in-vitro after 6h of infection contributes to the persistence of Salmonella infection at later stages in RAW264.7 macrophage cell-line. To this effect, the X-ray crystal structure of STV was solved by the SAD method using the iodine derivative data sets processed to 2.19 Å resolution. This study has also investigated the contribution of S. Tyhpimurium STV for cecal colonization and inflammation kinetics profile in-vivo. The mutant strain didn't cause colitis with absence of inflammation in mice due to the minimized zinc availability in the host system (STV being a Zn2+ bound protein) that can contribute Salmonella's virulence in murine models.

S4:8

EFFECT OF *SALMONELLA* PATHOGENICITY ISLAND EXCISION IN EARLY STAGES OF INFECTION IN MICE

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ROD21 is a Pathogenicity Island present in the chromosome of S. Enteriditis (SEN), S. Gallinarum, S. Dublin, and S. Typhi that harbors several genes encoding virulence associated proteins. ROD21 has a gene coding an integrase (sen1970) and an excisionase (sen1998). We have demonstrated that ROD21 is an excisable PAI, whose excision could be influenced by environmental conditions. For instance, excision increased when S. Enteritidis was infecting dendritic cells and macrophages in vitro. Also, conditions that mimic the lysosome environment, such as the presence of peroxide or lower pH, increase the ROD21 excision in vitro. In mice, strains unable to excise ROD21 show reduced ability to colonize liver and spleen. Furthermore, strains unable to excise ROD21 show higher expression of the genes within the PAI. These results suggest that the excision of ROD21 plays a role in virulence of this serovar. In this work, we determined the effect of ROD21 excision in vivo during the early stages of the infective cycle, using a mouse model. Methods: Ten C57BL/6 mice were infected intragastrically with 1x106 CFU of SEN PT4 or SEN PT4 Δsen1970:Δsen1998 (unable to excise ROD21); they were euthanized in groups of 2 individuals at 1, 3, 6, 24 and 48 hpi. Gastric content, ileum, cecum, colon, mesenteric nodes, peripheral blood, spleen, liver, gallbladder and feces was recovered and CFU count was performed by serial dilution in plate. gDNA was extracted using Phenol:Chloroform method and ROD21 excision frequency was determined by qPCR, being expressed as Number of copies of attB-1/ Number of copies of rpoD. Results: We confirmed that deletion of sen1970 and sen1998 abrogates the ability of ROD21 to excise. After different times post infection, CFU count was performed and we detected Salmonella presence in all times, both for WT and mutant strains, in at least two different sites of digestive tract. We observed that transit of S. Enteritidis across small intestine occurs rapidly during the first 6h. No significant differences between strains were found on caecum where a high bacterial load was maintained until 24h and before decreases drastically. A similar behavior was observed for WT strain at colon however, mutant strain showed an initial high load but was undetectable after 24h suggesting a dramatic decrease in bacterial population coincident with a significant decrease of fecal bacterial load at those times. Importantly, our results show that mutant strain unable to excise ROD21 showed a severe defect to colonize systemic tissues. Conclusions: Excision of ROD21 is a process that affect S. Enteritidis infection, specifically associated with the ability to colonize internal organs. Probably Salmonella ability to modulate ROD21 excision plays a key role on regulation of gene expression and it gives the bacteria a comparative advantage to develop the systemic phase of disease.

S4:9

CONTROL OF *SALMONELLA* INVASION BY CHEMICAL SIGNALS OF THE INTESTINE

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Salmonella invasion of the intestinal epithelium, requiring the type three secretion system encoded within Salmonella Pathogenicity Island 1 (SPI1), dictates pathogen virulence. Salmonellae coordinate SPI1 expression with anatomical location via chemical cues within the intestinal environment, including bile and fatty acids. We have shown that both of these function through post-translational effects on HilD, a central transcriptional activator of invasion, and that synergistic activity between specific bile acids contributes to the repressive potency of bile. Fatty acids and bile repress invasion gene expression by reducing the proportion of the bistable population expressing SPI1 genes, an effect that requires the autoregulatory function of hilD expression. Strains with hilD expression exogenously controlled continue to be repressed by these chemicals, but effects on message levels vary among the specific chemicals, suggesting the existence of

multiple mechanisms of control. One means by which this diversity of control is achieved is through the post-transcriptional regulator CsrA, known to control a host of metabolic functions, as mutagenesis studies indicate that message secondary structure within the CsrA-binding region mediates hilD repression. These studies indicate common mechanisms by which diverse environmental cues inhibit SPI1 expression and inform the development of therapeutics to inhibit invasion as a means of repressing Salmonella pathogenicity.

S5:1

METAL EFFLUX IN SALMONELLA VIRULENCE AND RESISTANCE TO OXIDATIVE AND NITROSATIVE STRESS

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Metals are essential for all living organisms; one-third of proteins and nearly half of structurally-characterized enzymes contain transition metal ions. Bacteria use multiple systems to obtain scarce metals from their environment, but less attention has been paid to the mechanisms by which they export metals to avoid their toxic effects. Metal efflux can ameliorate the toxicity of reactive oxygen or nitrogen species, which target iron-sulfur cluster- and zinc finger-containing metalloproteins to release metals. Damage from mobilized free ferrous iron results from Fenton-mediated modification of DNA, proteins and lipids. Mechanisms of zinc toxicity are less well understood but thought to result from irreversible mismetallation of proteins by free zinc. At least four putative iron export pumps have recently been identified in *Salmonella enterica*: IceT (MdtD), YieO, FetAB and STM3944. For at least some of these pumps, iron is exported in complex with the chelator citrate. The expression of IceT is regulated by the BaeSR two-component system, but regulation of other iron exporters has not yet been characterized. At least three pumps designated ZntA, ZntB and ZitB are responsible for the export of zinc. Expression of ZntA is controlled by the Zn-responsive transcriptional activator ZntR. Functional redundancy is observed, as inactivation of the genes encoding some of the iron or zinc efflux pumps exhibit additive effects on bacterial susceptibility to hydrogen peroxide or nitric oxide, and overexpression of individual iron pumps confers tolerance to antibiotics such as ciprofloxacin. Mutants deficient in zinc efflux are attenuated for virulence in C57BL/6 mice but not in congenic *iNOS phox* ko mice incapable of producing high levels of reactive oxygen and nitrogen species, and preliminary studies of iron efflux mutants also suggest a role in virulence, supporting an important role of metal efflux in *Salmonella* stress resistance and pathogenesis.

S5:2

SALMONELLA HETEROGENEITY DURING INFECTION: IMPACT ON DISEASE PROGRESSION AND THERAPY

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During infections, host-pathogen interactions occur in complex, dynamically changing host tissues. Our goal is to characterize relevant microenvironments in the mouse typhoid fever model, identify microenvironment-specific host-*Salmonella* interactions, and to determine their impact on disease progression and therapy. Our results show that after oral infection, individual *Salmonella* arrive at both the spleen marginal zone and the red pulp, but proliferation occurs predominantly in the red pulp. Infection foci consist of loosely connected infected cells with several non-infected cells in between. Most infected cells are resident macrophages. Within these cells, Salmonella reside largely in complex vacuoles that also contain heterogeneous debris. Massive numbers of neutrophils accumulate in infected spleen, largely in the marginal sinuses and veins where they form clusters around thrombi. Thrombosis is associated with greatly diminished overall blood flow, which negatively correlates with

splenomegaly. Local *Salmonella* subsets in the various spleen microenvironments have differential access to host nutrients and disparate exposures to nitric oxide, reactive oxygen species, and drugs, which affects their survival and growth rates. FACSorting and proteome analysis of *Salmonella* subsets reveal distinct local adaptations involving just a few, or up to hundreds of proteins. During treatment with antibiotics, the various subsets differ in their survival with both synergistic and antagonistic interactions between immune effectors and antibiotics. These results show that this salmonellosis consists of strikingly heterogeneous and dynamic *Salmonella*-host encounters involving diverse tissue regions, cell types, and molecular mechanisms. Overall disease progression results from failures in host control or antibiotic therapy at some tissue sites, despite successful simultaneous eradication in others. Disparate *Salmonella*-host encounters thus make the difference between lethal disease and successful control.

S5:3

AN OXIDATIVE CENTRAL METABOLISM ENABLES *SALMONELLA* TO UTILIZE GUT MICROBIOTA-DERIVED SUCCINATE AS A CARBON SOURCE DURING INFECTION

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The enteric pathogen Salmonella enterica serovar Typhimurium (S. Tm) employs its virulence factors to elicit an acute inflammatory host response in the gastrointestinal tract. This inflammatory response creates a nutritional niche in the anaerobic gut lumen that favors the growth of the pathogen over the native microbiota. The metabolic pathways underlying the adaptation to this peculiar niche remain poorly characterized. Here, we investigate the central metabolism of S. Tm in animal models of Salmonella-induced colitis. Conventional wisdom holds that under anaerobic conditions, the TCA cycle in Enterobacteriaceae is divided into an oxidative and reductive branch. Curiously, succinate dehydrogenase and 2-oxoglutarate dehydrogenase, key enzymes for a complete TCA cycle, conferred a significant growth advantage for S. Tm in the inflamed gut. These enzymes were dispensable in the absence of inflammation or when production of iNOS-derived nitrate was abolished, indicating that the electron acceptor nitrate enables S. Tm to run a complete TCA cycle under anaerobic conditions. Obligate anaerobic commensals such as Bacteroides spp. produce succinate as a major end product of glycan fermentation. Consistent with the idea that a complete TCA cycle allows S. Tm to utilize microbiota-derived succinate as a carbon source, succinate uptake and utilization genes did not provide a growth advantage in germ-free mice. Monoassociation of germ-free mice with Bacteroides thetaiotaomicron was sufficient to restore succinate uptake and metabolism in S. Tm during infection. We conclude that the switch from a branched to a complete TCA cycle is a critical metabolic adaption for S. Tm to compete with the fermenting gut microbiota in the inflamed gut. Furthermore, our work identifies microbiota-derived succinate as a major carbon source for S. Tm during infection, establishing succinate metabolism as a novel interaction of S. Tm with the gut microbiota.

S5:4

GENOME-GUIDED DESIGN OF A NOVEL DEFINED MOUSE MICROBIOTA THAT CONFERS COLONIZATION RESISTANCE AGAINST *SALMONELLA ENTERICA* SEROVAR TYPHIMURIUM

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Protection against enteric infections, also termed colonization resistance (CR), results from mutualistic interactions of the host and its indigenous microbes. The gut microbiota of humans and mice is highly diverse and it is therefore challenging to assign specific properties to its individual members. Here, we used a collection of murine bacterial strains and a

modular design approach to create a minimal bacterial community that, once established in germ-free mice, provided CR against the human enteric pathogen Salmonella enterica serovar Typhimurium (S. Tm). Initially, a community of twelve strains, termed Oligo-Mouse Microbiota (Oligo-MM12) representing members of the major bacterial phyla in the murine gut was selected. This community was stable over consecutive mouse generations, and provided CR against S. Tm infection, albeit not to the degree of a conventional complex microbiota. Comparative (meta)genome analyses identified functions represented in a conventional microbiome but absent from the Oligo-MM12. By genome-informed design, we created an improved version of the Oligo-MM community harboring three additional strains from a culture collection that provided conventional-like CR. In conclusion, we established a highly versatile experimental system that showed efficacy in an enteric infection model. Thus, in combination with exhaustive bacterial strain collections and systems-based approaches, genome-guided design can be used to generate novel insights into microbe-microbe and microbe-host interactions for the investigation of ecological and disease-relevant mechanisms in the intestine.

S5:5

GENOMIC POPULATION STRUCTURE OF *SALMONELLA ENTERICA*

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Background: High-throughput sequencing is now being applied for routine typing of many pathogens, including *S. enterica*. There are over 50,000 sets of *Salmonella* reads in public sequence repositories. These data could provide a basis for a global perspective of this species. However, such analyses are confounded by a paucity of standardized typing approaches. In 2012 we applied 7-gene Multilocus Sequence Typing (MLST) to group the majority of the typed *S. enterica* isolates into 138 independent genetic clusters - eBurstGroups (eBGs) - of closely related sequence types (STs). However, MLST lacks resolution below the ST level, and cannot reliably identify deep evolutionary history. To effectively manage and compare genomic data in a scalable way, advanced frameworks for describing the global population as well as local variation are required. **Methods:** To address these issues, we have developed automatic pipelines within EnteroBase to assemble genomes from public sequence repositories or registered users. These pipelines not only derive classical MLST eBGs from all assembled genomes of adequate quality, but also extend to more discriminant schemes such as ribosomal MLST (rMLST; 51 genes), core genome MLST (cgMLST; 3,002) and whole-genome MLST (wgMLST; 21,065). We have also calculated species trees for core genes in *S. enterica* with two independent strategies, in order to reconstruct its evolutionary history. **Results:** The *Salmonella* database in EnteroBase serves genotyping data for >47,000 genome assemblies along with 7,000 records from the legacy MLST database. The genomic data defines >3,000 rSTs and 381 reBGs, which are consistent with legacy eBGs but more discriminant. >90% of the genomes have been assigned to an reBG. Strains within each reBG are uniform for serovar. A species tree of one genome per rST was consistent with the reBGs, and contains clear signals of deep phylogenetic structure. Furthermore, fine-grained genetic structures within reBGs were largely resolved with a novel cgMLST scheme. This scheme gives a comparable resolution as SNPs, whereas much more standardized and portable, and is being increasingly used for epidemiological analyses. Using online tools in Enterobase, users can easily map any isolate onto global, high-definition perspectives of reBGs that were previously unresolvable, such as reBG1 (Typhimurium) or reBG4.1 (Enteritidis). **Conclusion:** EnteroBase provides access

to high-resolution genotyping data (MLST, rMLST and cgMLST) and visualization tools, allowing microbiologists to investigate the genomic relationships between all *Salmonella* serovars of clinical significance through an easy to use web interface. We anticipate that it will result in a transformational change in genotypic designations and global communication. Enterobase is available at http://enterobase.warwick.ac.uk.

S5:6

THE BATTLE FOR METALS IN THE GUT: HOW SALMONELLA EVADES NUTRITIONAL IMMUNITY

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Mucosal surfaces are often the first interface between the host, the commensal microbiota and pathogenic microorganisms. Among the most complex of these environments is the gut mucosa, where trillions of bacteria (the commensal microbiota) coexist with the host in a mutually beneficial equilibrium. Infection with enteric pathogens like *Salmonella* Typhimurium disrupts this equilibrium by causing intestinal inflammation, a response that suppresses the growth of the commensal microbiota and favors the growth of *S*. Typhimurium by several mechanisms. Infection with *S*. Typhimurium results in the upregulation of antimicrobial proteins that inhibit bacterial growth by limiting the availability of essential nutrients, including metal ions, in a process termed "nutritional immunity". In my talk, I will discuss how nutritional immunity is orchestrated in the host, as well as some of the mechanisms by which *S*. Typhimurium evades nutritional immunity and acquires metal ions in the inflamed gut, allowing this pathogen to successfully compete with the microbiota for these essential nutrients.

S5:7

A CROSS-SPECIES COMPARISON OF BACTERIAL GENE-DRUG INTERACTIONS

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Novel genes are being discovered at constantly increasing rates by sequencing bacterial genomes and bacterial communities. Gene function discovery, however, has been lagging behind. Our lab uses robotic platforms and arrayed mutant libraries to move traditional reverse genetic approaches to a genome-wide scale. Here we profiled the growth of two single-gene deletion libraries of the pathogen Salmonella Typhimurium in ~600 perturbations including physical stresses, antibiotics, human targeted drugs and host defense molecules. Analysis of gene-drug interaction scores revealed significant phenotypes for 75% of the mutants including many genes of unknown function (orphans). The data set provides a number of novel biological inferences, linking orphans to known pathways and providing insights into drug mode-of-action, uptake and efflux. Using similar high-throughput data available for E. coli we furthermore provide the first comprehensive cross-species comparison of genetic networks in bacteria. Correlation analysis and detection of functional modules reveals broad conservation of cellular pathways and drug responses between Salmonella and E. coli. However, we also find intriguing cases of network rewiring and investigate how species-specific and orphan genes connect to conserved modules. The presented chemical genomics data set was also used to investigate the impact of a type 2 diabetes drug on bacterial growth. The gene-drug interactions revealed primary target preference as well as the mechanism of efflux leading to a high resistance level in Salmonella.

S5:8

EVALUATION OF METHIONINE METABOLISM AND TRANSPORT IN *SALMONELLA* TYPHIMURIUM VIRULENCE

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Background: *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) is a common cause of gastroenteritis and diarrhoea, contributing to significant morbidity and mortality worldwide. In recent years, the prevalence of antibiotic resistance in *S.* Typhimurium has, like many other pathogens, increased, highlighting the need for new drug targets. *Salmonella*, like most other prokaryotes, is capable of *de novo* methionine biosynthesis, a process which is absent from higher eukaryotes. We aimed to investigate the role of methionine biosynthesis, transport and metabolism in the virulence of *S.* Typhimurium strain SL1344. **Methods:** A series of single knockouts were generated in a) the *de novo* biosynthesis pathway, 2) the high-affinity transporter, and 3) downstream pathways that use methionine including the activated methyl cycle. All mutants were tested in M9 minimal media to study growth characteristics *in vitro.* The intracellular survival and replication of the mutants was determined in HeLa cells. *In vivo* studies in murine model were conducted to determine the "essentiality" of the pathways in *Salmonella* virulence. **Results:** The growth data showed that the *de novo* biosynthesis mutants could not replicate in M9 media without methionine. Some growth attenuation, generally after 5 hours, was also observed in mutants unable to synthesize methionine *de novo*, in HeLa cells. None of the mutants unable to synthesize methionine were attenuated murine model; all mutants caused lethal infections in BL6 mice 5-7 days after infection with 200 bacteria. *Salmonella* import methionine from the environment, via at least two mechanisms, MetNIQ (MetD) the high affinity transporter, and MetP, a cryptic methionine transporter. Mutants that were deficient in both high affinity transporter MetD (Δ*metNIQ*) and *de novo* methionine biosynthesis (Δ*metB*) were attenuated in mice. This double mutant, Δ*metNIQ*Δ*metB,* exhibited 1000-fold growth attenuation compared with the wild-type SL1344 in the murine model, suggesting functional cooperation between methionine biosynthesis and transport in virulence of *S.* Typhimurium. **Conclusion:** Methionine biosynthesis in *S.* Typhimurium is effectively replaced by methionine transport in the murine animal host. Work is progressing to define the importance of downstream processing of methionine through the Activated Methyl Cycle and via a quasi salvage pathway, in the virulence of *S.* Typhimurium.

S5:9

MECHANISMS OF INCREASED SUSCEPTIBILITY TO INVASIVE NTS INFECTION DURING MALARIA AND MALNUTRITION

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The very young and elderly, as well as individuals with compromised immunity, are at risk of developing disseminated infections with non-typhoidal Salmonella (NTS) that can manifest as bacteremia or focal infections at systemic sites. Disseminated NTS infections can be fatal and are responsible for over 600,000 deaths annually. Most of these deaths are in sub-Saharan Africa, where multi-drug resistant NTS clones are currently circulating in a population with a high proportion of individuals that are susceptible to disseminated disease. This talk will discuss results from mouse infection models that shed light on host mechanisms promoting susceptibility to invasive infection with NTS in individuals with immunocompromising conditions, specifically malaria and malnutrition.

S6:1

SALMONELLA ENTERICA REMODELS THE HOST CELL ENDOSOMAL SYSTEM FOR EFFICIENT INTRAVACUOLAR NUTRITION

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Salmonella enterica is a facultative intracellular pathogen that survives and proliferates in the *Salmonella*-containing vacuole (SCV). The nutritional basis for vacuolar replication remains to be determined. Intracellular *Salmonella* converts the host endosomal system into an extensivenet work of interconnected tubules. *Salmonella*-induced filaments (SIF) are the most prominent compartments. We detected a continuum of membranes and lumen of SIF and SCV and demonstrate that vacuolar *Salmonella* have access to various types of endocytosed material. Membrane proteins and luminal content are rapidly diffusing between SIF and SCV. *Salmonella* in SCV without connection to SIF have highly reduced access to endocytosed components. On single-cell level, *Salmonella* within the SCV/SIF continuum exhibit higher metabolic activity than vacuolar bacteria lacking SIF. We conclude that formation of the SCV/SIF continuum allows *Salmonella* to bypass nutritional restriction in the intracellular environment by acquiring nutrients from endosomal system of the host cell.

S6:2

NOVEL, TRANSLOCON-INDEPENDENT SECRETION OF TYPE III SECRETION SYSTEM 1 EFFECTORS PROMOTES CYTOSOLIC REPLICATION OF *SALMONELLA TYPHIMURIUM*

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Type III secretion systems (T3SSs) are critical virulence factors that translocate effector proteins directly into eukaryotic cells. The translocation process is initiated upon contact with, and formation of a translocon pore in, host cell membranes. Salmonella Typhimurium relies on the Salmonella Pathogenicity Island 1 (SPI1)-encoded T3SS1 to invade epithelial cells. Here, we investigated the post invasion role of two T3SS1 effectors, SipA and SopB. We found that these effectors are delivered by cytosolic bacteria and contribute to optimal bacterial replication in the host cell cytosol. Strikingly, the T3SS1 translocon pore is not required for the delivery of SopB and SipA by cytosolic bacteria, thus, revealing a non-canonical, contact-independent secretion activity of a T3SS within eukaryotic cells.

S6:3

ONE-TWO PUNCH: *SALMONELLA* STRATEGIES TO COUNTERACT THE BLOC-3/RAB32 HOST DEFENSE PATHWAY

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Host defense mechanisms protect complex organisms against the attack of microorganisms, including invasive pathogens. The mechanisms underlying pathogen clearance are still mostly unknown. We identified a host trafficking pathway that prevents the human-restricted bacterial pathogen *Salmonella* Typhi from surviving in mouse macrophages and therefore infecting mice. This pathway depends on the host GTPase Rab32 and its guanine nucleotide exchange factor BLOC-3. Rab32-dependent clearance of *Salmonella* Typhi does not require known antimicrobial mechanisms suggesting that this trafficking pathway delivers novel antimicrobial factors. We also showed that in contrast to *Salmonella* Typhi, the broad host pathogen *Salmonella* Typhimurium infect mice by counteracting the BLOC-3/Rab32 trafficking pathway. Neutralization of this pathway occurs through the delivery of two type-III-secretion effectors: GtgE, which is a specific protease cleaving Rab32; and SopD2, which is a Rab GTPase activating protein (GAP).

Each one of these effectors confers *Salmonella* Typhimurium the ability to infect mice, while a *Salmonella* Typhimurium strain deficient for both these effectors is unable to infect mice, yet it is fully virulent in BLOC-3 deficient mice. These results indicate that the *Salmonella* Typhimurium effectors GtgE and SopD2 act redundantly to counteract a powerful host defense pathway that can prevent bacterial infections.

S6:4

THE SSEK FAMILY OF GLYCOSYLTRANSFERASE EFFECTORS FROM *SALMONELLA* MODIFY HOST CELL SIGNALLING PROTEINS

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Background: Pathogenic serovars of Salmonella utilise two type-three secretion systems to deliver distinct cohorts of effector proteins into host cells during infection. These effector proteins interact with specific human proteins to subvert normal cellular processes, thus impairing the ability of host cells to respond to the invading bacteria. Our research focuses on the SseK family of Salmonella effectors, which have unknown function and play an unknown role in Salmonella infection. The SseK effectors show strong sequence similarity to NleB1, a unique glycosyltransferase effector of enteropathogenic E. coli, and therefore we predict the SseK effectors may also function as glycosyltransferases. NleB1 catalyses a novel post-translational modification in which the sugar N-acetylglucosamine is transferred to arginine residues of several host immune signalling proteins. We predict the SseK effectors have a similar biochemical activity, and therefore the aim of our work is to identify the host substrates of the SseK family, and to determine the contribution of this activity to Salmonella infection. **Methods:** We have used an antibody raised against N-acetylglucosamine-modified arginine to explore the potential glycosyltransferase activity of the SseK family. We have combined immunofluorescence microscopy and western blotting to observe glycosylation of host protein during infection. Further, we have developed an immunoprecipitation method that takes advantage of the unique nature of this glycosylation, and we have used mass spectrometry analysis to identify and characterise modified host proteins. **Results:** We have found that SseK1 and SseK3 are active glycosyltransferases that mediate the modification of several mammalian proteins during infection. By immunofluorescence, we have observed a co-localisation of these effector proteins with their uniquely glycosylated substrates. By immunoprecipitation, we have identified the mammalian death-domain protein TRADD as a substrate of SseK1. Further, we have discovered that while NleB1 and SseK1 both modify TRADD, the site of modification is not conserved. Unexpectedly, we also observed self-glycosylation of several arginine residues of SseK1 and SseK3. Catalytically-inactivated mutants were unable to modify themselves or TRADD. These findings have been further validated through mass spectrometric analysis of recombinant protein. **Conclusions:** We have discovered that SseK1 is a glycosyltransferase that modifies the human death domain protein TRADD, implicating a possible means for Salmonella to manipulate host cell signalling and cell death during infection. Further, the discovery that arginine residues of SseK1 and SseK3 are glycosylated suggests that Salmonella engages in self-glycosylation of bacterial proteins. We are currently exploring the contribution of these modifications to pathogenesis.

S6:5

DEMONSTRATING THE COMPLEX INTERPLAY OF SCV AND SIF-RELATED EFFECTORS DURING *SALMONELLA* INFECTION

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As an intracellular pathogen, *Salmonella* Typhimurium must survive and replicate within host cells. The type III secretion system 2 (T3SS2) functions to translocate bacterial effectors from intracellular *Salmonella* residing within the *Salmonella*-containing vacuole (SCV) into the host-cell cytosol. We are interested in a subset of T3SS2-secreted effectors that are implicated in both maintenance of the SCV membrane during infection, and formation of *Salmonella*-induced filaments (SIFs); these effectors include SifA, SseJ, PipB2, SopD2, SseF, SseG, and SteA. The functions of these seven effectors fall into at least one, if not several, of the following categories during infection: promotion of SIF biogenesis, perinuclear positioning of the SCV, maintaining SCV membrane stability, modification of the SCV membrane, and recruitment and/or regulation of microtubule motor activity at the SCV membrane. We hypothesize that these seven T3SS2-secreted effectors form a network of interactions, with each other and with host proteins, to act in a coordinated manner in order to facilitate SIF biogenesis and maintain the SCV membrane during infection.

As these effectors have overlapping and seemingly redundant roles during infection, we constructed a *Salmonella* Typhimurium strain SL1344 that does not secrete any of the seven effectors, as well as deletion of functional effector subsets therein. These deletion mutants enabled examination of certain effectors in the absence of others to determine effector subset phenotypes and potential interaction partners. We assessed the intracellular phenotype of each strain by evaluating presence of SIFs and integrity of SCVs, intracellular localization of *Salmonella*, and intramacrophage replication. The deletion mutant library confirmed the phenotype of some effectors and found new functions for others. We show that SifA and SseJ are the minimum subset of effectors required to maintain the SCV membrane and form SIFs, and that PipB2 appears to play an important role in the centrifugal movement of the SCV away from the Golgi apparatus at 8 hours post infection. We also show that subsets of effectors interact with one another to promote replication within macrophages and that the formation of SIFs seems to play a crucial role. Together, these data demonstrate the complex interplay of these seven effectors and the importance of these interactions to *Salmonella* during infection.

S6:6

RNA-SEQ-BASED IDENTIFICATION OF CYTOSOL-SPECIFIC SALMONELLA GENES

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In epithelial cells *Salmonella enterica* resides within two distinct compartments, the *Salmonella* containing vacuole (SCV) and the cytosol. We have previously shown that cytosolic and vacuolar bacteria express a distinct set of virulence genes at later times post-infection (≥ 8 h p.i.); vacuolar bacteria are induced for type III secretion system 2 (T3SS2), whereas cytosolic bacteria express T3SS1 and flagellar genes. We predict that *Salmonella* expresses vacuolar- and cytosolic-specific virulence factors in order to survive and replicate within these diverse intracellular niches, and here we sought to identify *Salmonella* genes that are specifically induced in the cytosolic environment. By modulating the levels of cellular autophagy, a host cell innate defense mechanism that limits nascent SCV damage and cytosolic proliferation of *Salmonella*, we have identified conditions that either decrease or increase bacterial hyper-proliferation in the cytosol of epithelial cells at 8 h p.i., compared to untreated cells. We optimized extraction conditions for

the selective recovery of bacterial RNA from infected epithelial cells and compared the absolute levels of gene expression (TPM) under the two conditions by RNA-seq-based transcriptomic analysis. 308 *Salmonella* genes and ncRNAs showed a significant increase in TPM under conditions of increased cytosolic proliferation, including genes encoding for T3SS1, which demonstrates the feasibility of our approach. Gene promoter transcriptional fusions to green fluorescent protein (GFP) were constructed to verify by fluorescence microscopy whether or not candidate genes/ncRNAs were up-regulated in the cytosol of epithelial cells. Here I will present an overview of the cytosol-specific *Salmonella* genes/ncRNAs that we have identified in our study.

S6:7

IDENTIFICATION OF THE RCK RECEPTOR REQUIRED FOR *SALMONELLA* CELL INVASION

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Salmonella enterica are invasive Gram-negative bacterial pathogens causing a wide spectrum of diseases from gastro-enteritis to typhoid fever in humans and many animal species. An essential feature of the pathogenicity of Salmonella is its capacity to cross a number of barriers requiring invasion of a large variety of phagocytic and non-phagocytic cells. To invade non-phagocytic host cells, this bacterium has developed different mechanisms to induce its own internalization into the cells via a cellular cytoskeleton remodelling. The main entry mechanism of Salmonella requires a type three secretion system (T3SS-1), encoded by the Salmonella Pathogenicity Island-1. However, recent evidences show that Salmonella can cause infection in a T3SS-1-independent manner. An outer membrane protein, called Rck, has been clearly identified as Salmonella invasion, mediating a Zipper entry process. In our group, we demonstrated that Rck-mediated invasion requires induction of cellular signals, which is initiated by the interaction with a host cell surface receptor. More recently, the cell signalling receptor required for Rck-mediated adhesion and invasion has been identified as the Epidermal Growth Factor Receptor (EGF Receptor). First, an alteration in Rck-mediated adhesion and invasion were observed when EGF Receptor activity and expression were modulated. Then, the Rck-EGF Receptor association was demonstrated by pull down and immuno-precipitation assays. To demonstrate the direct interaction of Rck with the extracellular domain of the human EGF Receptor, surface homogeneous time-resolved fluorescence and plasmon resonance biosensor technologies were used. Finally, our study strongly suggests a non-competitive binding of EGF and Rck to EGF Receptor. These data change our classic view of Salmonella invasion. The identification of the EGF Receptor as the Rck receptor should help to determine the Rck role in Salmonella pathogenicity. In addition, the characterization of the Rck-mediated entry pathway will help to better understand the virulence mechanisms used by Salmonella to infect their hosts.

S6:8

CYTOSKELETON REMODELLING BY COOPERATING SMALL GTPASES AT THE MEMBRANE

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Rho small GTPases are master regulators of cell function and they control actin filament polymerisation from the membrane where they are anchored by acylation. The Rho GTPase Rac1 is known to mediate actin polymerisation through the WAVE regulatory complex (WRC)

which is pivotal to cell motility, macropinocytosis and phagocytosis. The membrane is critical to Rac1 function but studying small GTPase signalling at the membrane is challenging and often neglected. Our group has addressed this challenge by developing microspheres coated in defined phospholipid bilayers that enable the reconstitution of signalling pathways at the membrane in cell-free extract. To our surprise, membrane-anchored Rac1 was found to be insufficient for WRC activation. Instead, WRC activity required an unexpected collaboration between Rac1 and Arf GTPase, revealing a novel crosstalk between small GTPase families. Unravelling WRC regulation continues to resolve how cytoskeletal networks are controlled and how the virulence effectors of bacterial pathogens like Salmonella Typhimurium and enteropathogenic E.coli subvert small GTPase crosstalk to establish mammalian cell infections.

Poster Abstracts

1

GROWTH INHIBITION OF NON-TYPHOIDAL *SALMONELLA* SEROVARS BY A CRANBERRY POMACE EXTRACT AGAINST IN BROTH AND A MEAT MODEL

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Background: Non-typhoidal Salmonella enterica serovars are common causes of bacterial gastroenteritis worldwide. Research on exploring alternative approaches to limit contamination of food for human consumption is urgently needed. This study investigated the potential of 95% ethanol cranberry (Vaccinium macrocarpon) fruits pressed-cake (pomace) extracts (FC111) against different Salmonella enterica serovars. **Methods:** The composition of FC111 was determined by HPLC. The minimum inhibitory concentrations (MICs) against eight isolates of antibiotics resistant S. enterica belonged to serovars Typhimurium (3), Thompson (1), Hadar (2) and Heidelberg (2) were obtained using broth microdilution method in cation adjusted Mueller Hinton broth (CAMHB) according to the Clinical Laboratory Standard Institute's guidelines. Growth curve were generated by a spectrophotometric method using BioScreen. Time-kill assays were performed in CAMHB supplemented with 0 to 32 mg/ml of CF111 and viable cell counts were made over a 24h incubation period at 37°C. Salt tolerance was measured using a plate infusion method and survival on meat for 7 days at 4°C was evaluated using a cooked chicken breast meat model. **Results:** The HPLC-Diode Array Detector results showed that the principal compounds in FC-111 were anthocyanins (24%), flavonols (18%) and other phenolics (58%). The primary anthocyanins in FC111 were peonidin-3-O-galactoside (33%) and cyanidin-3-O-galactoside (25% abundance), but cyanidin and peonidin-3-O-arabinosides were also present. The dominant flavonol was quercetin-3-O-galactoside (34%), followed by myricetin-3-O-galactoside (20%) and three different quercetin-3-O-pentosides (19%). The MIC was 32 mg/ml against all tested S. enterica isolates however 16 mg/ml induced a delay and a strong inhibition of growth. In general, 32 mg/ml of FC111 induced a strong bactericidal effect (5 to 6 Log10 CFU/mL reduction) against serovars Typhimurium and Heidelberg after 2 to 4 hours of incubation in broth. Treatment of salt increased the susceptibility of Salmonella to FC111. At a concentration of 8%, CF111 deceased Salmonella number under detectable level after one day at 4°C suggested a significant effect on survival of this pathogen in our meat model. **Conclusion:** Results from this study demonstrate that CF111 exerts antibacterial effects against S. enterica in broth and on meat, indicating that further examination of the potential of cranberry extracts against Salmonella are warranted.

2

IS REPTILE-EXOTIC-PET-ASSOCIATED SALMONELLOSIS (REPAS) A PUBLIC HEALTH PROBLEM? REPORT OF A FATAL CASE FROM AUSTRIA

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Reptiles are suspected to be an often unrecognized source for salmonellosis in humans,

and estimates state that reptile-exotic-pet-associated salmonellosis (REPAS) might be responsible for 3 to 11% of all human salmonellosis cases (1-3). Several publications in the past decade indicate that although infections attributed to exposure to reptiles represent only a small proportion of all human salmonellosis cases, nevertheless it is likely to be an underestimate and growing problem that deserves closer attention (4,5). A study of transmission of Salmonella from reptile to children in Germany showed that almost 50% of the reporting households kept at least one reptile (6). Here we describe the probable transmission of Salmonella Kintambo and S. Poano from animal to human based on next generation sequencing (NGS) data. The strains were isolated from a fatal infection of a child from Austria and different reptiles living in the household of the family. **Materials and Methods:** Pulsed field gel electrophoresis (PFGE) was carried out according to the standardized NRC protocol for subtyping of Salmonella. The Salmonella strains of the fatal case and the reptiles were analysed at the National Reference Centre for Salmonella in Graz, Austria. Control strains of S. Kintambo and Poano were included from the RKI Strain Culture Collection For Library preparation the Nextera XT Kit was used and sequencing was performed on an MiSeq sequencer to create 2 x 300 bp paired end reads. The genomes were assembled into contigs using the A5 assembler.For subsequent data analyses, a customised pipeline based on publicly available tools was used. Core genome(cg)MLST was performed on SeqSphere+ (Ridom® GmbH, Münster, Germany). **Conclusion:** S. Kintambo and S. Poano were isolated from the body of a deceased boy. While S. Kintambo isolates are closely related, differing in just one locus from the reptile isolate, S. Poano shows 21 loci difference. We assume that differences in S. Poano isolated from monitor lizard and from human heard blood are due to different populations colonizing the animals. Therefore population studies are necessary for epidemiological connections. Reptiles often carry Salmonella on the surface of their bodies or shed populations of them even when they appear healthy and clean. The bacteria then contaminate potentially any object the animal had contact with. So people can become infected either directly by handling the animals or indirectly via contaminated surfaces. It is important to keep reptiles out of homes with children younger than 5 years or immunocompromised people. 1. Mermin J et al. (2004) CID 38 Suppl 3:S253 2. Woodward DL et al. (1997) JCM 35:2786 3. Kaibu H et al. (2006) Jap. JID 59:281 4. Cyriac J and Wozniak, ER (2000) Com. Dis. and Public Health 3, 66. 5. Ward L. (2000) Salmonella perils of pet reptiles. Com. Dis. and Public Health 3, 2 6. Pees M et al. Euro Surveill. 2013 Nov 14;18 (46).

3

BISTABILITY IN FLAGELLAR GENE EXPRESSION

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Flagellar gene expression is bistable in Salmonella enterica. Under certain growth conditions, some cells express the flagellar genes whereas others do not. This results in mixed populations of motile and non-motile cells. We previously found that nutrients specify the fraction of motile cells within the population. In the present study, we used a combination of single-cell analysis and computational modeling to investigate the mechanisms governing the bistable expression of the flagellar genes in S. enterica. We found that two independent mechanisms induce the bistable expression of the flagellar genes. One induces the bistable expression of the class 2 genes in response to nutrient concentrations. This was previously found to result from a double-negative feedback loop involving FliZ and YdiV. The other induces the bistable expression of the class 3 genes. The key contribution of the present work is to demonstrate that class 3 bistability results from a positive feedback loop involving

σ28. In particular, we found that σ28 increases expression from the class 1 flhDC promoter, independent of FliZ. This means that σ28 can indirectly increase its own expression, giving rise to a positive feedback loop. Likely, this positive feedback loop triggers the expression of the class 3 genes when free σ28 concentrations exceed some threshold. Our results suggest that FlgM sets this threshold for activation. In addition, we were able to eliminate class 3 bistability by removing this positive feedback loop. Collectively, these results further our understanding of this complex gene network in S. enterica.

4

SALMONET, AN INTEGRATED NETWORK OF TEN *SALMONELLA ENTERICA* STRAINS REVEALS COMMON AND DISTINCT PATHWAYS TO HOST ADAPTATION

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Salmonella enterica is a prominent bacterial pathogen with implications on human and animal health. Salmonella serotypes could be classified as gastro-intestinal or extra- intestinal. Genome-wide comparisons revealed that extra-intestinal strains are closer relatives of gastro-intestinal strains than to each other indicating a parallel evolution of this trait. Given the complexity of the differences, a systems-level comparison could reveal key mechanisms enabling extra-intestinal serotypes to cause systemic infections. Accordingly, in this work, we introduce a unique resource, SalmoNet, which combines manual curation, high-throughput data and computational predictions to provide an integrated network for Salmonella at the metabolic, transcriptional regulatory and protein-protein interaction levels. SalmoNet provides the networks separately for five gastro-intestinal and five extra- intestinal strains. As a multi-layered, multi-strain database containing experimental data, SalmoNet is the first dedicated network resource for Salmonella. It comprehensively contains interactions between proteins encoded in Salmonella pathogenicity islands as well as regulatory mechanisms of metabolic processes with the option to zoom-in and analyse the interactions at specific loci in more detail. Application of SalmoNet is not limited to strain comparisons as it also provides a Salmonella resource for biochemical network modelling, host-pathogen interaction studies, drug discovery, experimental validation of novel interactions, uncovering new pathological mechanisms from emergent properties and epidemiological studies.

5

DISTINCT TRANSCRIPTOMIC PROFILE REVEALED ADAPTATION CHANGE AMONG TOMATO ASSOCIATED *SALMONELLA* NEWPORT STRAINS

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Background: The consumption of fresh tomatoes has been linked to numerous foodborne outbreaks involving various serovars of *Salmonella enterica*, specifically *S.* Newport. Moreover, *S.* Newport strains associated with tomato and tomato growing environment mostly belong to lineage III. The purpose of this study is to understand the adaptation of lineage III Newport in tomato using transcriptomic approach. **Methods:** Total RNAs of different lineages of *S.* Newport strains grown to

mid-log phase in hand-expressed tomato cellular fluid from four different tomato varieties including red and round, roma, and grape, and those in Luria-Bertani (LB) broth at pH4.1 in three biological replicates were extracted and subjected to RNA-seq. RNA-Seq paired-end reads were aligned to the bacterial strain reference set of genes. RNA-Seq transcript abundance was estimated using the method RSEM (RNA-Seq by Expectation Maximization). Differential gene expression was calculated from the read counts produced by the RSEM method using the statistical method *edgeR* and reported with a log2 fold cutoff threshold greater than or equal to 2.0 and a false discovery rate (FDR) probability threshold of less than or equal to 0.05. **Results:** Transcriptomic profiles 1) between *S.* Newport strain in a variety of tomato cellular fluid and LB broth with pH 4.1; 2) between different lineage Newport strains in same variety of tomato cellular fluid; 3) between *Salmonella* serovar Newport and Typhimurium, and Enteritidis in same variety of tomato cellular fluid were compared. Not only tomato cellular fluid, but also the variety of tomato affects the transcriptional profiles of *S.* Newport strains. Compared to growth medium, *S.* Newport in tomato cellular fluid showed a distinct transcriptional profile with genes coding for the type III secretion system, *Salmonella* pathogenicity island I (SPI1), SPI2, and genes in the nitrate and sulfate metabolism pathways highly enriched. Significantly decreased expression was shown in genes coding for the flagellar system. Principle component analyses (PCAs) showed different lineages of *S.* Newport strains responded differently to the same variety of tomato. Most importantly, *rtcR*, a sigma N-dependent regulator (negative regulation) of *rtcBA* expression, was highly expressed across the tomato varieties among all the lineage III Newport strains while significantly suppressed in the lineage I and II Newport strains in the study. Also interesting to note, the relationship of the transcriptomic profiles of these *S.* Newport strains from different lineages shown in PCA agrees with their phylogenetic relationship among the lineages. **Conclusions:** The differing response of *S.* Newport lineages may indicate the fitness of lineage III in tomato and the tomato growing environment. This study provided insights into how *S.* Newport adapts to this unique niche and acquires increased virulence.

6

CELL SIZE AND EXPRESSION OF VIRULENCE FACTORS PREDETERMINE *SALMONELLA* TYPHIMURIUM SURVIVAL AFTER HEAT SHOCK

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Even under homogeneous environmental conditions isogenic cells within a clonal bacterial population tend to display intercellular differences that can result in differential behavior. An important example of such phenotypic heterogeneity is the typical observation that not all cells within a clonal population are equally resistant to a stressful encounter, with the underlying reason or mechanism often being elusive. However, when using time-lapse microscopy to scrutinize individual cellular survival odds in an isogenic population of Salmonella Typhimurium after heat treatment, we surprisingly found the size of S. Typhimurium cells to be variable and closely correlated with cellular fate after heat shock. In fact, smaller cells within the clonal population displayed a lower probability of heat survival, and we were able to link both phenotypes to the bistable expression of Salmonella virulence factors. As such, while heterogeneity in stress resistance is often thought to stem from the unpredictable stochastic variation of cellular attributes, our results underscore the impact of more deterministic biological differentiation events on individual cellular survival within clonal bacterial populations.

7

SALMONELLA-HOST INTERPLAY MEDIATED BY PROTEIN PHOSPHORYLATION MODIFICATIONS

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Background: Salmonella enterica is a bacterial pathogen that causes gastroenteritis and typhoid fever. During infection, it secrets numerous proteins called "effectors" which subvert the host's cells via several mechanisms including changes in post-translational modifications of the host's proteins. In this study, we aimed to understand how the host's proteins and signaling systems are manipulated by pathogen-mediated phosphorylations, using salmonella enterica serovar typhimurium and mouse macrophage cell line as a model system. **Methods:** Proteins from Raw cells at 0, 4, 8 and 20 hours post-infection, and those with mock treatment, were collected. LC-MS/MS analysis was performed to quantitatively compare the global dynamics change of phosphorylations on host proteins in the course of infection. Finally, informatics analysis was used to estimate the change in host kinase activity from experimentally obtained phosphorylations on the basis of known kinase-substrate relationships. **Results:** The dynamics of about 5,000 host phosphosites were identified and quantified per biological replicate at all four time points after infection. Informatics analysis predated that AKT1 and MAPK kinases are activated at the beginning of the infection, which is consistent with prior knowledge. We also found that the activity of ATM, a kinase responding to DNA damage, was increased toward the later time points while cell cycle-related kinases (CDK family, PKMYT1, and PLK1) were repressed. **Conclusions:** Global and temporal phosphoproteome in host cells revealed extensive changes of host signaling events leaded by salmonella infection, providing a broad landscape of host responses from initial to the late stage of infection.

8

A POWERFUL SYSTEMS BIOLOGY APPROACH TO IDENTIFY NEW *SALMONELLA*-HOST AUTOPHAGY PATHWAY INTERACTIONS

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Background: Autophagy is instrumental to stress responses, regulation of inflammation, intestinal homeostasis, as well as eliminating intracellular pathogens. Interestingly, autophagy is often hijacked by intestinal pathogens such as Salmonella to modulate their intracellular population and mediate their spreading. Understanding the interactions of certain bacteria on the regulation of intestinal autophagy could help identify inflammatory bowel disease and colon cancer prognosis markers, contributing to developing much needed alternative therapies. **Methods and Results:** The lack of systems-level autophagy-related information prompted the development of an online integrated database resource, Autophagy Regulatory Network (ARN; http://autophagy-regulation.org). ARN contains manually curated, imported and predicted interactions of autophagy components in humans. We listed transcription factors, miRNAs

regulating autophagy components or their protein regulators. To investigate how Salmonella is modulating autophagy we developed the first large-scale network resource for Salmonella enterica, called SalmoNet, integrating known and predicted regulatory, metabolic and signalling interactions. We then integrated previously identified Salmonella-host interactions and data from ARN to list novel genes that are likely to modulate autophagy in the gut. We validated our approach with the example of the SPI1 virulence effector SopE. This Guanine Exchange Factor-like protein is required for inducing host membrane ruffles through Rho-GTPases such as Rac1. The Rho pathway is also involved in autophagy regulation. Combining epithelial cell invasion assays, qPCR, heterologous protein expression and microscopy, we confirmed that SopE does modulate the autophagy pathway inside intestinal epithelial cells. **Conclusion:** This study illustrates how powerful and promising tools the SalmoNet and ARN networks are for identifying new interactions between pathogen virulence factors or proteins of yet unknown function and specific host cellular pathways.

9

COMMUNITY PROFILING THE INFLAMED INTESTINE

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Salmonella enterica serovar Typhimurium is one of the leading causes of food-borne illness. This bacterium initiates an inflammatory response that disrupts the healthy gut microbiota, enabling Salmonella pathogenesis. To further understand the impact of Salmonella on the host gut microbiome, we used the CBA/J mouse model to examine the microbial community responses to three inducers of inflammation: chemically-treated (Dextran Sulfate Sodium, DSS), streptomycin-treated, and Salmonella-treated. Over a 16-day period, 5 mice were administered 4% DSS in their drinking water, 5 mice were administered streptomycin in their drinking water, 30 mice were inoculated with 109 CFU Salmonella enterica serovar Typhimurium strain 14028, and 5 control mice received no treatment. The Salmonella treatment was variable in effect, so 10 of the 30 mice that had the most inflammation were chosen for further analysis. The cecum microbiome of each mouse was analyzed using Illumina amplicon sequencing of the 16S rRNA gene (V4 region). Comparison of mice cecal microbial communities revealed significant differences within the Salmonella treatment, as three of the ten mice were enriched with Salmonella (52±4.3%), denoted as high responders, while in the remaining mice, Salmonella constituted a small portion (<0.1%) of the community. Microbial communities between treatment groups were statistically different, with the high responding Salmonella communities being the most altered from control. Salmonella high responders had a significantly lower Shannon diversity (1.5±0.35) compared to the control (4.2±0.20). In the control, DSS, and streptomycin treatment groups, the cecal microbial communities were dominated by the family Lachnospiraceae (68±5.3%, 50±8.3%, and 39±9.2%, respectively), while the high responding Salmonella group had significantly less (10±5.2%). Alistipes showed resistance to inflammation being detected in Salmonella (13±10.2%) and DSS treatment groups (5±3.2%). Some taxa were co-enriched only in the Salmonella treatment group, including members of the Proteobacteria, (e.g. genus Enterobacter). Together our findings demonstrate that microbial membership and abundance is impacted by the source of inflammation and we posit that Salmonella infection reshapes the chemical environment of the gut favoring selection of closely related Proteobacteria. Ongoing metagenomic and metabolomics findings from these samples will identify the mechanisms by which Salmonella infection restructures the host microbiome.

10

A NOVEL, TRANSLOCON-INDEPENDENT FUNCTION OF *SALMONELLA* TYPHIMURIUM TYPE III SECRETION SYSTEM 1

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Background: Type III Secretion Systems (T3SS) deliver effector proteins across the Gram-negative envelope and eukaryotic cell membrane to the host cell. Translocation is triggered upon contact with the host cell membrane and formation of a 'translocon' pore. *Salmonella* Typhimurium employs two T3SSs during infection of mammalian epithelial cells. The extracellularly-induced T3SS1 is essential for invasion and early vacuole biogenesis. Post-invasion, T3SS1 is down-regulated and T3SS2 is induced to establish the modified intracellular compartment known as the '*Salmonella*-Containing Vacuole' (SCV). In epithelial cells, *Salmonella* can replicate in the SCV or in the cytosol. Cytosolic *Salmonellae* hyper-replicate between 4 and 8 hours post-infection, resulting in a sub-population of infected cells containing >50 bacteria/cell. The cytosolic, hyper-replicating *Salmonellae* express T3SS1, whereas vacuolar bacteria express T3SS2. The function of T3SS1 and its effectors 'early' during infection are well characterized. However, the functional state of T3SS1 in the cytosol is not known. In this study we examined the functionality of T3SS1 in the epithelial cell cytosol by looking at delivery of effectors and their contribution to cytosolic replication. **Methods:** We used immunofluorescence microscopy and single-cell analysis to determine whether expression of T3SS1 by cytosolic bacteria establishes a functional protein delivery system in HeLa and C2BBe1 epithelial cell lines. **Results:** Cytosolic, hyper-replicating *Salmonellae* express functionally active T3SS1. During the peak of hyper-replication, SopB is delivered exclusively in cells containing cytosolic *Salmonellae*. Effector delivery is dependent on the T3SS1 apparatus but does not require the T3SS1 translocon. **Conclusions:** T3SS1 expression by cytosolic *Salmonellae* establishes a functionally active secretion system. Effector delivery does not require the T3SS1 translocon and thus constitutes a non-canonical, novel form of type III secretion.

11

SINGLE CELL ANALYSIS REVEALS NOVEL ASPECTS OF *SALMONELLA* TYPHIMURIUM REPLICATION IN HUMAN AND MOUSE MACROPHAGES

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Background: *Salmonella enterica* serovar Typhimurium can infect and replicate in a variety of mammalian cell types, including epithelial cells and macrophages. In vitro, the classical method for quantification of intracellular bacteria is the gentamicin protection assay. Two limitations of this population-based approach are: (1) It measures net replication (a combination of replication, survival and death), and; (2) It does not provide any information on the heterogeneity of the system. Even when invasion is highly synchronized, there is diversity, both within the cell (*i.e.* vacuolar versus cytoplasmic bacteria) and within the population as a whole. Intracellular *Salmonella* can die, replicate or persist. Infected host cells can die via several mechanisms and with different kinetics. Live cell imaging can be used to study dynamic cellular systems at the single cell level and, combined with semi-quantitative analysis, has been used to obtain a more detailed picture of *Salmonella* Typhimurium replication in cultured epithelial cells. Here we use this approach to characterize and compare growth and survival in human and mouse macrophages. **Methods:** Live cell imaging using a spinning disc confocal microscope, laser scanning confocal microscopy, gentamicin

protection assays, immunofluorescence, and quantitative single cell analysis. **Results:** In macrophages, *Salmonella* Typhimurium has a number of functional phenotypes. Replication was observed in both human and mouse macrophages, although the percentage of infected cells containing replicating bacteria can vary and is dependent on multiple factors, including how the bacteria are grown prior to infection and the cell type. In some cells, the presence of elongated bacteria indicates that they are subject to metabolic stress. Mutants lacking either of the two Type III Secretion systems (T3SS1 and T3SS2) had distinct phenotypes. As expected, T3SS2 mutants were replication defective compared to the wild type, however, early replication was indistinguishable. We also observed novel T3SS1-dependent replication in macrophages. **Conclusion:** In cultured macrophages, *Salmonella* populations are highly heterogeneous. Intracellular replication may occur in subsets of infected cells and may not be apparent in population-based assays.

12

REDUCED BIOFILM FORMING ABILITY AND LONG-TERM SURVIVAL OF INVASIVE *SALMONELLA TYPHIMURIUM* ST313

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Salmonella enterica serovar Typhimurium is an enteric pathogen that causes a self-limiting gastroenteritis and forms biofilms on different biotic and abiotic surfaces. In sub-Saharan Africa, Salmonella Typhimurium of a novel sequence type (ST) 313 was identified and was shown to produce septicemia in the absence of gastroenteritis. So far no animal reservoir has been identified and it is hypothesized that transmission occurs via human-to-human. In this study, we show that invasive strains of Salmonella Typhimurium ST313 from Mali produce poor biofilm compared to Salmonella Typhimurium ST19 strains, which are found worldwide and are known to be associated with gastroenteritis, using several in vitro biofilm assays including crystal violet staining, examination of the rdar morphotype and pellicle formation and a continuous flow system of biofilm formation. Colonies of one month-old Salmonella Typhimurium ST19 strains also survived in the absence of exogenous nutrients and were highly resistant to sodium hypochlorite treatment compared to Salmonella Typhimurium ST313 strains. This study for the first time demonstrates the comparative biofilm forming ability and long-term survival of clinical Salmonella Typhimurium ST19 and ST313 isolates. We furthermore show that reduced biofilm formation in Salmonella Typhimurium ST313 is attributed, in part, to reduced expression of flagellin in this genotype that are important in the initial steps of biofilm formation. Salmonella Typhimurium ST19 strains are strong biofilm producers and can survive desiccation compared to Salmonella Typhimurium ST313 that form weak biofilms and survive poorly following desiccation. Our data suggests that like Salmonella Typhi, Salmonella Typhimurium ST313 lacks mechanisms that allow it to persist in the environment.

13

SALMONELLA INFECTION TRIGGER AUTOIMMUNITY VIA THE EXPRESSION OF BACTERIAL AMYLOID, CURLI

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Systemic lupus erythematosus (SLE) is an autoimmune disease that has a multifactorial pathogenesis. Among the environmental triggers, infection is a major cause of morbidity and mortality in SLE patients. Some bacterial species, such as *Salmonella*, behave more aggressively in SLE patients, causing bacteremia and complications in soft tissues, rather than the expected gastroenteritis. It has been dem-

onstrated that protein/DNA complexes lead to the production of Type I interferons as well as generation of autoantibodies including anti-ds-DNA and anti-chromatin antibodies, hallmarks of SLE, in patients. Amyloids are complex proteins with a conserved beta sheet structure. Bacteria use amyloids to decorate the extracellular matrix of their biofilms, highly structured multicellular communities. Curli are amyloids expressed by enteric bacteria, including *Salmonella* Typhimurium and *Escherichia coli*. Recently, a study demonstrated that an artificially formed amyloid protein can bind DNA *in vitro* and this complex elicits an autoimmune response in mice triggering autoantibodies to dsDNA and chromatin. Since the biofilm extracellular matrix naturally harbors amyloids and DNA, we explored the interactions between these two molecules and determined that bacterial DNA released during biofilm formation is incorporated into curli fibers accelerating amyloid polymerization. DNA that integrates into the amyloid fibers becomes resistant to enzymatic degradation. Curli and DNA synergistically activate immune cells, *in vitro* and *in vivo* via Toll like receptor (TLR) 2 and TLR9, respectively, leading to a Type I interferon response. Furthermore, infection with curli-expressing *S.* Typhimurium or E. coli as well as systemic exposure to purified curli/DNA complexes trigger autoimmunity and accelerate lupus leading to the generation of anti-dsDNA/anti-chromatin autoantibodies in lupus prone mice. Intriguingly, sera from lupus patients that are experiencing a flare show elevated levels of anti-curli antibodies, suggesting that the infections with curli-expressing bacteria are major environmental triggers for SLE.

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TRANSCRIPTION FACTORS ARCA AND SLYA HAVE A ROLE IN *SALMONELLA* TYPHIMURIUM SURVIVAL INSIDE NEUTROPHILS

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Salmonella enterica serovar Typhimurium (S. Typhimurium) is a Gram-negative and intracellular pathogen that is able to survive inside both phagocytic and non-phagocytic cells during its infection process. Once S. Typhimurium colonizes the first portion of the small intestine and overcomes this physical barrier they confront the host's innate immune response which includes diverse defense mechanism. Inside phagocytic cells, it faces several different kinds of stress, like highly toxic Reactive Oxygen Species (ROS), specifically inside neutrophils due to the myeloperoxidase action the bacteria must face great amounts of hypochlorous acid (HOCl) which is capable of successfully withstand due to the up-regulation of several key genes. In this context we have found that the the ArcAB two-component system, is also involved in ROS resistance to both hydrogen peroxide and hypochlorous acid. Additionally, SlyA a global regulator that has been associated with bacterial virulence in mice, plays an important role in the bacterial resistance to phagosomal environment conditions, specially inside murine neutrophils. We proposed to determine the transcriptional levels of several relevant genes such as Phosphofructokinase (fruK), Secreted Effector Proteins (sopD and sopE2), Glycerol Phophatase Dehydrogenase (glpA), Ketoglutarate Permease (kgtP), and the Transcriptional Regulator hilA, during the bacterial infection from Salmonella Typhimurium 14028s, S. Typhimurium arcA and S. Typhimurium slyA strains harvested from infected murine neutrophils in order to determine the involvent that these transcriptions factors may have in this stage of bacterial invasion. We have found that in the S. Typhimurium slyA strain that glpA and fruK are significally up-regulated in the presence of 3 mM of sodium hypoclorite NaOCl an analogous to HOCl as a consequence the bacteria, as other genes not required for this invasion stage are negatively regulated namely effector pro-

teins (sopE2 and sopD), this dinamic allows this patogen to survive in this toxic conditions. FONDECYT 1160315

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COMPARATIVE WHOLE GENOME ANALYSIS OF THREE CONSECUTIVE *SALMONELLA* DIARIZONAE ISOLATES CAUSING DIARRHEA AND SEPSIS WITH FATAL OUTCOME

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Background: Most cases of human salmonellosis are caused by *S. enterica* subsp. *enterica*, whereas infections of humans caused by other subspecies are very rare. Strains of *S. enterica* subsp. *diarizonae* (*S. diarizonae*) are often isolated from cold-blooded animals. Little is known about the virulence capabilities of *S. diarizonae* and underlying molecular mechanisms. Although *S. diarizonae* possess the *Salmonella* Pathogenicity Island 2 (SPI-2) required for intracellular survival and systemic infection they are attenuated in a mouse infection model. We herein describe the molecular characterization of three *S. diarizonae* isolated consecutively from a hospital patient having developed diarrhea and sepsis with fatal outcome. **Methods:** The first *S. diarizonae* isolate was subjected to *de novo* genome sequencing using PacBio technology. All strains were sequenced using the Illumina MiSeq platform and reads were mapped to the PacBio-derived reference. BLAST was used for genome comparisons with other *Salmonella* strains and to determine the presence of genomic islands. To assess their virulence capacity *in vitro* intracellular replication of all strains was quantified in RAW264.7 macrophages. Furthermore, activity of a fluorescent SPI-2 reporter was determined *in vitro* and in bacteria isolated from macrophages using flow cytometry. **Results:** Whole genome sequencing revealed 3 single nucleotide polymorphisms (SNPs) and an insertion mutation in the first isolate. One SNP was found within the gene coding for the RNA chaperone Hfq, two SNPs within *purK* and the insertion within *gpt.* The *purK* and *gpt* genes encode both for enzymes involved in purine synthesis. BLAST-based comparison of 78 *S. diarizonae* genomes showed a characteristic pattern of SPI content and revealed the presence of 5 uncharacterized genomic islands in the patient isolates. One of the genomic islands harbored fimbrial genes and genes for structural components of a type three secretion system. All isolates were attenuated for replication within mouse macrophages and SPI-2 activation was significantly lower in *S. diarizonae* compared to *S.* Typhimurium. **Conclusion:** The presence of only a few SNPs in the first isolate and the lack thereof in the other isolates points towards variability within a bacterial population rather than host-directed evolution. Concordantly, no impact of these mutations on virulence in a macrophage infection model was detected. Quantification of SPI-2 activation revealed a defect in SsrAB-dependent gene expression in *S. diarizonae* which might explain its reduced capability for intracellular replication. Thus, immunosuppression and multimorbidity of the patient likely favored systemic *S. diarizonae* infection leading to a fatal outcome.

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SALMONELLA SENSES HOST REACTIVE OXYGEN SPECIES TO LAUNCH AN INTRACELLULAR SURVIVAL PROGRAM

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Niche-adapted gene expression in bacteria is coordinated with environmental sensing systems that relay signals at the membrane to cytosolic response regulators for a directed

transcriptional output. Enteric pathogens face a range of antimicrobial stresses during their passage within a host. For bacteria that colonize immune cells with microbe-killing properties, their adaptation must be rapid and is likely coordinated with early intracellular cues. While innate host responses such as pH changes, antimicrobial peptides and nutrient limitation can serve to limit pathogen colonization, these signals can also provide cues to the bacteria for adaptive gene expression needed for host exploitation. However, the chemical signals that may serve to activate intracellular virulence genes in Salmonella is not fully understood. Using a chemical-biology screen with a collection of small molecules with known mode of action, we found that reactive oxygen triggers expression of intracellular virulence genes in Salmonella. Follow up experiments using a combination of transcriptional reporters and quantitative RT-PCR confirmed a reactive oxygen species-triggered activation of SPI-2 gene expression. In vivo, this programming also responded to host NOX2 activity and required the SsrA-SsrB two-component regulatory system that controls the type III secretion system required for bacterial adaptation to the host environment. Together, these data indicate that the generation of reactive oxygen species by the host is an innate immune signal sensed by Salmonella to cue its intracellular survival program.

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EFFECT OF CELL-WALL-DEGRADING ENZYMES IN SURVIVAL OF *"SALMONELLA"* TYPHIMURIUM ON LEAFY GREENS

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Background: Consumption of fresh fruit, vegetables and leafy greens may be associated with pathogen-related outbreaks, especially Salmonella enterica serovars. Mechanisms of attachment and growth/survival of Salmonella on plants are under extensive investigation, but it is not clear how enteric pathogens invade the plant host. Plant pathogens facilitate invasion to the host by the activity of depolymerases that degrade plant cell-wall. Therefore, Cell Wall Degrading Enzymes (CWDE) play a dominant role in plant's infection and improve microorganism's survival on plants. This study set out to assess the importance of CWDE in survival and invasion of Salmonella Typhimurium on plants. **Methods and Results:** Bioinformatics search of the S. Typhimurium genome resulted in a list of putative enzymes capable of degrading polysaccharides found in plants. From this list, two genes, celC and bcsG encode to putative cellulases. We propose that CelC and BcsG degrade polysaccharides in the plant, thus having a significant role in Salmonella's invasion and survival in plants. Results of activity assays demonstrate that wild type (wt) S. Typhimurium has a weak cell-associated cellulase activity. A stronger cellulase activity was observed with CelC overexpressing S. Typhimurium and a knockout mutant of both, CelC and BcsG, lost the ability to degrade cellulose. Furthermore, expression studies indicated that bcsG, but not celC, is significantly induced in the presence of different polysaccharides or in extracts of parsley leaves. These results reveal that CelC and BcsG may function as cellulases. To study the role of cellulases in survival on leaves, we irrigated parsley plants (overhead irrigation) with water contaminated with S. Typhimurium wt, and Salmonella-overexpressing-CelC or BcsG (pCelC and pBcsG). S. Typhimurium pBcsG, survived in higher levels compared with the other strains after 7 days. pCelC and the wt showed similar survival after overhead irrigation, but when water contaminated with pCelC strain was injected to leaves, S. Typhimurium pCelC had larger number of bacteria in the leaf after 7 and 14 days compared with the wt. Moreover, pCelC resulted in necrotic areas in the leaves, symptoms that have never been described before, neither with the wt strain nor with the other mutant strains. **Conclusions:** CelC and BcsG are cellulases involved in the complicated parsley-Salmonella interactions. BcsG, which may play a role in Salmonella

survival on parsley, is triggered when Salmonella is exposed to plants components, and CelC plays a role in triggering the plant immune response. This study reveals for the first time, that cellulases of S. enterica are important for the plant-bacteria interactions.

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THE BIOFILM PHENOTYPE IS IMPAIRED IN INVASIVE NONTYPHOIDAL *SALMONELLA* TYPHIMURIUM STRAIN D23580, BUT IS LOST IN *SALMONELLA ENTERITIDIS* STRAIN D7795

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Conservation of the biofilm phenotype in nontyphoidal *Salmonella* species is thought to be important for the environmental persistence of these pathogens. However, some nontyphoidal *Salmonella* emerging from sub-Saharan Africa have no apparent environmental reservoir and are responsible for an increase in systemic bloodstream infections. This switch in behaviour, combined with extensive genome degradation in sequenced isolates, prompted our investigation into the biofilm phenotype of these unique nontyphoidal *Salmonella*. We focused our study on two invasive nontyphoidal (iNTS) strains emerging from Malawi, *S.* Typhimurium D23580 and *S.* Enteritidis D7795, and compared their biofilm phenotype to nontyphoidal *Salmonella* associated with gastroenteritis as well as *S.* Typhi of the H1 and H58 haplotypes. While both iNTS strains appeared biofilm-negative on tryptone agar, flask cultures of *S.* Typhimurium D23580 showed that it is still partially capable of biofilm formation. In agreement with this, cellulose and curli fimbriae were measurable in cultures of *S.* Typhimurium D23580. In contrast, all biofilm phenotypes were absent in *S.* Enteritidis D7795. We identified a potential promoter mutation in D7795 that shuts off production of CsgD, the main transcriptional activator for the biofilm phenotype. Using homologous recombination, introduction of the *csgD* promoter region from *S.* Typhimurium 14028 into *S.* Enteritidis D7795 partially rescued both cellulose production and the biofilm phenotype. Overall, our findings contradict published reports about loss of the biofilm phenotype in *S.* Typhimurium D23580, but show that other invasive nontyphoidal *Salmonella* associated with bloodstream infections have indeed lost this important physiology. We hypothesize that iNTS strains exist on an evolutionary spectrum, where variability in the biofilm phenotype is indicative of decreased selection pressure to persist in the environment and a greater bias towards chronic shedding from the host.

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IMPACT OF THE ONSET OF EGG-LAY ON INNATE IMMUNITY IN HEALTHY OR *SALMONELLA* PULLORUM-INFECTED HENS

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Pullorum disease caused by *Salmonella enterica* serovar Gallinarum biovar Pullorum (*S.* Pullorum) is a lethal infection in chicks. The infected hen is known to become asymptomatic carrier transmitting the bacteria to their offspring via trans-ovarian infection. It has been reported that the acquired immune responses against *Salmonella* are depressed in the hens at the onset of egg-lay, which is possibly associated with the subsequent carrier state. The aim of this study is to investigate the impact of the onset of lay on the innate immune responses against *S.* Pullorum in a short-term infection. The layer chickens were prepared for 3 different stages such as pre-laying, onset of laying and peak laying. Twelve chickens each of the stages were inoculated orally with *S.* Pullo-

rum strain S'-1, isolated from infected broiler breeders, and another 12 chickens with PBS served as the uninfected control. The expression of IL-1, IL-6, IL-12, CXCLi1, CXCLi2 and IL-10 in the ileal peyer's patch and the ovary of 4 chickens each at 6, 24 and 48 hours after inoculation was analyzed by qRT-PCR. In addition, the bacterial counts in the cecal contents and the ovarian tissue in the infected groups were also determined. In uninfected controls, the base-line expression of CXCLi1 and CXCLi2 in the ovary decreased at onset of lay compared to those at the other stages. The bacterial counts in the ovary at 6 hours after infection was higher in the hens at onset of laying than those at pre-laying. At 24 hours post inoculation, the hens at onset of laying showed differential expression profiles compared to those at pre-laying: increased expression of IL-10 and decreased expression of IL-12 and CXCLi2 in the peyer's patch, and decreased expression of IL-6, IL-10, IL-12 and CXCLi2 in the ovary. The expression of these cytokines at peak laying substantially reverted to the pre-laying level. These results suggest that the onset of laying affects the cytokine expression profile, which leads to the increased bacterial counts in the ovary and the lowered innate responses in the payer's patch and the ovary during *S.* Pullorum infection, and possibly in turn, to the reported suppression of acquired responses as well.

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GENOME-BASED ANALYSES OF *SALMONELLA* ASSOCIATED WITH A COLLECTION OF VENOMOUS SNAKES

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Background: A colony of venomous snakes is housed at the Liverpool School of Tropical Medicine (LSTM); the majority of snakes were caught in Africa, and some were bred in captivity. The colony contained cobras, mambas, vipers and puff-adders, snakes responsible for bites that are frequently life-threatening for human victims. The association of *Salmonella* with the gastrointestinal tract of reptiles has been widely reported in the literature. We conducted an in-depth survey of the carriage of *Salmonella* by 11 species of venomous snakes. To identify *Salmonella* serovars, and to assess the evolutionary diversity and phylogenetics of the snake-associated *Salmonella,* we did a genome sequence-based analysis of 38 isolates. **Methods:** Standard microbiological methods were used to enrich and isolate *Salmonella* bacteria from snake faeces, and from the infrastructure of the herpetarium. Enumeration of *Salmonella* colonies from snake faeces was determined by viable counts. Strains were tested for antibiotic susceptibility against 12 clinically-relevant antibiotics using a disk-diffusion method. *Salmonella* isolates were sequenced using Illumina short read technology. **Results:** *Salmonella* was isolated from 84% of 31 venomous snakes tested. The levels of *Salmonella* bacteria in snake faeces ranged from 1.1×10^5 to 6.0×10^9 CFU/g. Of those tested, 17 *Salmonella* isolates were antibiotic-resistant and 5 had a multiple drug-resistant phenotype. Genome sequence analysis identified two *Salmonella* serovars capable of causing human infection that were snake-associated, namely Enteritidis and Hadar. These two pathogenic serovars were found in two monocled cobras and three puff adders, respectively. The serovars Arizonae, Sundvall and Saugus were also carried in the gastrointestinal tract of two Egyptian cobras and two saw-scaled vipers. Several of the *Salmonella* strains had previously unreported sequence types. **Conclusions:** This study highlights the high levels of *Salmonella* colonization of the gastrointestinal tract of venomous snakes, and identifies the potential risk posed to humans by *Salmonella* from wild venomous reptiles. The

results of a comparative genomic approach for the determination of the evolutionary diversity and phylogenetics associated with snake-derived *Salmonella* strains will be shown. The relatedness of snake-associated *Salmonella* to African environmental isolates of *Salmonella* will be discussed.

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VARIATION IN BIOFILM FORMATION OF *SALMONELLA* TYPHIMURIUM AND MONOPHASIC VARIANTS IN THE REPUBLIC OF IRELAND FROM PRE-HARVEST PORK CHAIN

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Background: The ability to survive in the extra-host environment in biofilms may contribute to the success of foodborne pathogen variants as they play a key role in the persistence and transmission. A monophasic variant of Salmonella Typhimurium lacking the second-phase flagellin, fljB, has rapidly spread across Europe, accounting for an increasing number of human cases and becoming the dominant MDR epidemic clones. Currently, it represents the third most common Salmonella serovar in Europe. The primary reservoir of monophasic Typhimurium is pigs, with pork products as the main vehicle of the transmission. In the present study, variation in RDAR phenotype and the the ability to form biofilm on plastic surfaces or at the air liquid interface were investigated in the context of genotypic and phylogenetic relationship of 108 Salmonella Typhimurium pre-harvest pork chain. **Methods:** The phylogenetic relationship of isolates was determined using variation in the core genome sequence. The ability of the Typhimurium isolates to produce biofilms was qualitatively evaluated through pellicle formation assay and colony morphology after culture on congo red agar, and quantitatively measured by crystal violet staining in micro titre plate assay. **Results:** We describe the population structure of 108 S. Typhimurium isolates and their ability to form biofilms on plastic surfaces and at the air - liquid interface. The majority of the isolates produced strong and rigid pellicle (59%) and exhibited RDAR phenotype on congo red agar plates (92.4%). In addition, the isolates produced biofilms on plastic at both temperatures, with greater efficiency at 22°C than at 37°C. Despite monophasic and biphasic S. Typhimurium displayed differences in pellicle formation and colony phenotype on congo red agar plates, they formed biofilms on plastic to the same extent. Pellicle formation was seen to correlate with the ability of S. Typhimurium to produce biofilm on plastic, but not for the monophasic. **Conclusions:** S. Typhimurium isolates from the preharvest pork chain exhibited considerable variation in their ability to form biofilms on plastic surfaces. Monophasic isolates exhibited less variability than biphasic isolates reflecting the genotypic diversity and phylogenetic relationship. The pellicle formation assay may be used as preliminary analysis to evaluate the ability of S. Typhimurium isolates to produce biofilms on plastics. The lack of this correlation for the monophasic suggests the involvement of different pathways in the production of biofilms on the two types of surfaces, water and plastic.

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VACCINATION PREVENTS INFLAMMATION-MEDIATED BACTERIOPHAGE-TRANSFER BETWEEN *SALMONELLA* SPP.

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Horizontal gene transfers (HGT) by temperate bacteriophages play a key role in the evolution of bacteria. However, little is known about the dynamics of bacteriophage transfer in vivo (lysogenic conversion). Using a murine model

of Salmonella diarrhea, we have quantitatively assessed the transfer rate of the temperate bacteriophage SopEΦ between two strains of Salmonella enterica Typhimurium. This rate is highly dependent on the host immune status. The stressful conditions in the lumen of the inflamed gut stimulate lysogenic conversion by eliciting the bacterial SOS response. This SOS response triggers the lytic cycle of the bacteriophage and phage transfer. More than 60% of lysogenic conversion of the recipient strain was observed within 3 days post-infection. On the other hand, in absence of inflammation the rate of lysogenic conversion was reduced by 10^5-fold. This was observed upon infection with avirulent S. Typhimurium mutants and in wild-type infections of vaccinated mice which were protected from enteropathy. Thus, enteric disease is a key stimulus of phage transfer in the host's gut and vaccination not only prevents disease but could also provide a practical means for limiting entero-pathogen evolution via bacteriophage-borne HGT.

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T3SS-2 FUNCTIONS IN EVASION OF NEUTROPHIL-MEDIATED KILLING OF *SALMONELLA ENTERICA* SEROVAR TYPHIMURIUM IN THE MOUSE

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Background: Non-typhoidal Salmonella serovars, such as S. enterica serovar Typhimurium cause a self limited gastroenteritis in immunocompetent individuals, but patients with underlying immunodeficiency can develop a life-threatening disseminated infection. The Salmonella pathogenicity island 2 (SPI2)-encoded type III secretion system (T3SS-2) is an essential virulence factor required for survival in host tissue, but its role during host pathogen interaction has not been fully resolved. While the T3SS-2 enhances survival of S. Typhimurium in cultured macrophages, in vivo analysis suggests inactivation of this virulence factor does not prevent multiplication in this cell type, but mediates evasion of phagocyte NADPH oxidase. Here we investigated the role of T3SS-2 in evading inflammatory macrophages and neutrophils, the main cells types mediating NADPH oxidase-dependent killing. **Method:** We investigated whether neutrophil depletion, using anti-Ly-6G antibody, or depletion of inflammatory monocytes, using anti-CCR-2 antibody, altered recovery of the S. Typhimurium wild type or a T3SS-2-deficient mutant (spiB mutant) from internal organs of mice infected with a 1:1 mixture of both strains. **Result:** Depletion of neutrophils and inflammatory monocytes both resulted in recovery of increased numbers of S. Typhimurium from the liver and spleen of mice. However, only neutrophil depletion significantly reduced the fitness advantage conferred by T3SS-2 during S. Typhimurium infection. Neutrophil depletion resulted in an accumulation of immature neutrophils (CD11b+ Gr-1int cells) in the spleen, which produced little reactive oxygen species and harbored S. Typhimurium during infection. **Conclusion:** Our results suggest that T3SS-2 functions in evading neutrophil mediated killing activities against S. Typhimurium in vivo.

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REDUCTION OF BONE MARROW IMMUNOGLOBULIN G-SECRETING PLASMA CELLS BY *SALMONELLA* INFECTION

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Background: Host innate and adaptive immune responses confer crucial protection against Salmonella infection. On the other hand, *Salmonella* can escape from the host immune system by producing virulence factors, leading to establishment of systemic

infection. To understand the mechanism by which *Salmonella* escapes the adaptive immune response, we focused on the influence of *Salmonella* infection on the production of immunoglobulin (Ig)-secreting plasma cells. **Methods:** C57BL/6 mice were infected intraperitoneally with 1 x 10^4 CFU of live Lon-deficient attenuated *Salmonella*. Alternatively, 200 µL of culture supernatant prepared from *Salmonella* was inoculated. Total amount of IgM or IgG in serum was determined by ELISA. The numbers of plasma cells and B cells were analyzed by ELISpot assay or flow cytometry. **Results:** We showed that the great majority of bone marrow IgG-secreting plasma cells was diminished within 4 days after intraperitoneal infection of live Lon-deficient attenuated *Salmonella*, whereas bone marrow IgM-secreting and splenic IgG-secreting plasma cells were unaffected. The infection also reduced total IgG titers in serum. This selective diminishment was also induced by culture supernatants from *Salmonella* but not by those from *Escherichia coli* within 24 hours after intraperitoneal injection. The culture supernatant depleted of ligands for Toll-like receptors, lipopolysaccharide and flagellin, also reduced the plasma cells. **Conclusions:** This study revealed that *Salmonella* reduces bone marrow IgG-secreting plasma cells which are the main source of serum IgG, leading to escape from the host humoral immunity. It is suggested that *Salmonella*-specific component is involved in the reduction.

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GENOTYPIC AND PHENOTYPIC VARIATION OF *SALMONELLA* TYPHIMURIUM IMPACTING RISK TO FOOD SAFETY

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Background: *Salmonella enterica* serovar Typhimurium is the most prevalent serotype of *Salmonella* associated with disease. It is the most common serovar in zoonotic reservoirs for human infection and the environment and contains a number of variants able to infect multiple livestock hosts and humans, as well as others that have become highly restricted to a single host. There is also a variation in levels of antimicrobial resistance and virulence often facilitated through horizontally acquired or mobile elements leading to differential impact on food safety from different lineages. These factors make Typhimurium an excellent focus for the study of the evolution of pathogenesis. The whole genome sequence of strains representing much of the genotypic diversity of Typhimurium were investigated to identify microevolution associated with distinct risk to food safety. **Methods:** Whole genome sequences were analysed with an array of bioinformatics and computational biological techniques to determine the phylogenies at the whole genome and multiple phenotypic levels. Genome degradation in terms of hypothetically disrupted coding DNA sequences (HDCs) and the loss and acquisition via transposable elements of genes associated with virulence and resistance were compared to differences in phenotypic traits. Variation at the gene level caused by single nucleotide polymorphisms and insertions and deletions were orthogonally investigated using molecular biological techniques to directly link genotype with phenotype. **Results:** We report on the full repertoire of genotypic variation within Typhimurium and describe the history of horizontal gene transfer within the clade. Adaptation to a single host is often associated with genome degradation in the form of HDCs and indeed this was also seen within the representative isolates. Phenotypic traits such as catalase activity, biofilm formation, motility and invasion are discussed. **Conclusions:** Whole genome sequencing combined with a macroscopic analysis of *Salmonella* serovar Typhimurium is able to detect genome differences at a range of scales from SNPs to the presence or absence of transposable elements such as phages or plasmids. Many on these differences can be directly linked to phenotype and can be used

to inform hypotheses regarding adaptation and evolution.

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WGS ANALYSIS OF THE *SALMONELLA* ENTERITIDIS POPULATION ISOLATED FROM DANISH RETURNING TRAVELERS

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Salmonella Enteritidis is the most prevalent serovar isolated from Danish patients. The majority of the cases are travelers returning from holidays all over the world. We selected a subset of the isolated strains from both patients with known travel history and patients whom acquired their infection in Denmark. A total of 87 strains were sequenced by WGS, of which 19 were isolated from patients infected in Denmark. Based on a SNP analysis we made a core-SNP tree that included 1726 SNPs called in the 87 selected strains. Surprisingly, the tree showed two separated populations divided by a branch of 250 core SNPs and both of the two groups had a clear starburst-like structure. Further analysis of accessory genome is in progress. We looked into the travel destination of the cases and there were some suggestive patterns of one group having an overrepresentation of travel to Eastern Europe and the other group included the majority of cases infected in Denmark. Furthermore, all cases from travelers to Asia were located in one of the major groups. Additionally, smaller groups showed geographical correlation but none with statistical significance. In the data, there are an overrepresentation of cases infected in Turkey as 25 of the cases (29% of total cases) were infected there. The strains isolated from these cases clustered in two tight branches, distributed in each of the two major groups of the tree. Salmonella Enteritidis isolated from Danish travelers seems to be divided in to major groups, a correspondence to travel geography is present, however it is somewhat fussy in this dataset.

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WHOLE GENOME SEQUENCING ANALYSES OF *SALMONELLA* LUBBOCK ISOLATED FROM LIVER ABSCESSES OF FEEDLOT CATTLE

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Liver abscesses in feedlot cattle are a common sequel to an aggressive grain feeding program in the United States. Fusobacterium necrophorum, a Gram negative anaerobe that originates from the rumen, is the primary etiologic agent. Recently, we reported for the first time, the occurrence of a novel serotype, Salmonella Lubbock, in liver abscesses of feedlot cattle. Because of the finding of a novel serotype, we conducted a study to determine the prevalence and characteristics of Salmonella Lubbock associated with liver abscesses. The study included a total of 383 abscessed livers collected in USDA-inspected abattoirs from eight different feedlots. The abscess material was used to isolate Salmonella by direct plating on selective medium as well as by enrichment procedure. The genus identification of the putative isolates were determined by PCR detection of invA gene, agglutination by polyvalent sera, and MALDI-TOF. The isolates were submitted to the National Veterinary Services Laboratory for serotyping. The overall prevalence of Salmonella was 25.3% (97/383). The predominant serotype was Lubbock (59/97; 60.8%), followed by Agona (26/97; 26.8%), Cerro (10/97; 10.3%), Give (1/97; 1%), and Muenster (1/97; 1%). Whole genome sequencing of Lubbock strains was performed with NextEra XT library preparation to identify the serotype, sequence type, and analyze virulence potential of Salmonella isolates from liver abscesses. Genome assembly was carried out using CLC Genom-

ics Workbench followed by annotation using NCBI's prokaryotic genome analysis pipeline. Serotype Lubbock was confirmed by uploading raw paired end sequence reads to SeqSero, a web based tool. The Jaccard Index was calculated to measure similarity of the k-mer content between pairs of Salmonella strains. The Neighbor joining trees were generated from the Jaccard index distance matrix using MEGA6 software. MLST analyses revealed that all Lubbock strains belonged to ST413. In addition, our results show that Salmonella was a secondary pathogen in liver abscesses of cattle and that Lubbock, a novel serotype, was predominant. Currently, we are analyzing the sequence data to assess virulence potential and antimicrobial resistance determinants of the Lubbock serotypes.

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HOST COSTS AND BENEFITS OF ENDEMISM OF INFECTION WITH HOST ADAPTED *SALMONELLA* TYPHIMURIUM

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Background: Host-adapted strains of Salmonella enterica such as serovar Typhi require mechanisms that allow sustaining endemic pathogen populations in a narrow host spectrum. Mechanisms used by these strains and costs and benefits for the host are poorly understood. We here use infections with a host-adapted Salmonella Typhimurium strain in pigeons as a model to study mechanisms of pathogen endemism and the associated costs and benefits on various host reproductive and individual traits by comparing infection and disease dynamics between an infected and non-infected pigeon flock. **Methods:** Forty clinically healthy one-month-old pigeons were obtained from a Salmonella-free breeding colony. In one flock, an endemic Salmonella Typhimurium infection was established by inoculation with 108 CFU of Salmonella Typhimurium strain DAB69 and subsequently followed during 66 weeks. The second flock served as negative control. Mechanisms and costs/benefits of Salmonella endemism in the flock were examined by 1) determining Salmonella infection dynamics with focus on identifying routes of transmission, both horizontal (faecal shedding, crop feeding) and vertical (egg and/or semen contamination), 2) quantifying the effect of a Salmonella infection on pigeon health and reproductive parameters and 3), estimating the role of population immunity in protection against clinical disease. **Results:** In the endemically infected pigeons, Salmonella was shed intermittently at low levels in the faeces during the entire experimental trial and also in the crop of crop-feeding parents. Salmonella was only found in 5/111 eggs and at least 4 of these were found to be non-fertilized, but in none of the semen samples. At day of hatch, none of 35 faecal and cloacal swabs from hatchlings were positive for Salmonella. During the nesting period, Salmonella was detected in the cloacal swabs of 53.3% of the chicks only during the second breeding period. The endemic Salmonella infection reduced the flock's reproductive fitness by significantly reducing egg and birth weight, weight gain, delayed fledging and causing Salmonella induced chick mortality in the second breeding period. The host immune response to Salmonella infection was evidenced by a pronounced humoral response in the endemically infected pigeons and marked maternal antibody titres in their offspring. **Conclusions:** We have demonstrated that pigeon-adapted Salmonella Typhimurium exploits host characteristics associated with reproduction (e.g. crop feeding) to establish and maintain endemism in the pigeon population. This entails a reproductive cost for the host population, which is outweighed by the development of immunity that protects the birds against clinical disease, yet allowing the pathogen to maintain a stable reservoir.

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MOLECULAR BASIS OF FOODBORNE DISEASE RISK OF VARIANTS OF *SALMONELLA* TYPHIMURIUM DT193 AND U288

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Background: Multidrug resistant (MDR) *Salmonella enterica* Serovar Typhimurium epidemiology is characterised by successive epidemics of variants exhibiting a specific phage type. In humans, phage type DT104 emerged as the dominant clone in Europe and the UK during the 1990s, and was replaced by DT193 in 2006. This prevalence mirrors that in cattle and pigs, due to their role as a zoonotic reservoir, and facilitated by the broad host range of *S.* Typhimurium. A third phage type, U288, emerged in pig herds simultaneously to DT193, but interestingly, this is rarely associated with the disease in humans. A phylogenetic analysis of 128 *S.* Typhimurium isolates identified several distinct lineages. The U288 isolates were clustered in a sub-clade of samples collected from pigs, and many of the DT193 isolates were located in a separate lineage of samples collected from pigs, poultry and cattle, and represent an emerging epidemic of "monophasic" *S.* Typhimurium. Here we test the hypothesis that genotypes associated with distinct risk to food safety are associated with lineage specific phenotypic variation impacting transmission, colonisation or pathogenesis. **Methods:** Phenotypic variation of the U288 sub-clade and 3 representative DT193 monophasic isolates was investigated in relation to environmental survival. Isolates were tested for resistance to desiccation, osmotic stress, and tolerance to oxidising disinfectant. Biofilm formation is an indicator of resistance to many environmental stresses. Biofilm production was assessed by crystal violet staining following incubation at 25°C, colony morphology on LB agar stained with congo red, and pellicle formation in LB without salt. **Results:** There was considerable heterogeneity between U288 and DT193 isolates tested. Biofilm formation was significantly different between the U288 and DT193 groups, as was salt tolerance and resistance to desiccation. The monophasic representatives were overall more resistant to the tested environmental stresses than the U288 isolates. Variation within the U288 sub-clade was also noticeable, with some isolates behaving similarly to the monophasic strains. **Conclusion:** The increased hardiness of the monophasic isolates to environmental stresses may improve transmission rates from livestock to humans *via* the food processing chain and represent a significant risk to food safety. Also, the reduced resilience of certain U288 isolates to environmental stress may imply an evolution towards a host-adapted life cycle (in this case, pigs).

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FERAL PIGEONS: A NOVEL RESERVOIR OF ZOONOTIC *SALMONELLA* ENTERITIDIS STRAINS

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Background: Salmonella is the second most important foodborne human pathogen within the European Union (EU) with Salmonella enterica subspecies enterica serovar Enteritidis the most frequently isolated serovar. Poultry eggs, egg products and meat are well known to be a primary source of human salmonellosis caused by serovar Enteritidis. The implementation of various Salmonella control programs in the primary production of the food chain has led to a decline in reported human salmonellosis cases and led to a decline of positive poultry flocks, increasing the relative impor-

tance of other potential Salmonella sources We here report a relatively high prevalence of Salmonella Enteritidis in feral pigeons (Columba livia) in urban environments, rendering pigeons a potential source of salmonellosis for humans. **Methods:** Live feral pigeons were sampled in 4 urban populations across Flanders, Belgium (Antwerp (n = 35), Bruges (n = 39), Brussels (n = 30) and Louvain (n = 29) (different locations within 1 city) for the presence of Salmonella sp.. All pigeons underwent a physical examination and a cloacal swab was collected and plated on Brilliant Green Agar (BGA) plates (LabM, Lancashire, UK). If negative after direct plating, the samples were pre-enriched overnight in buffered peptone water (Oxoid, Basingstoke, UK), then enriched overnight in tetrathionate brilliant green broth (Merck KGaG, Darmstadt, Germany) and plated on BGA. Salmonella isolates were Phage typed at the Salmonella and Escherichia coli reference lab of the Animal & Plant Health Agency (APHA), Weybridge, England and Pulsed Field Gel Electrophoresis (PFGE) was done according to the PulseNet protocol (www.cdc/gov/pulsenet/). **Results:** Ten out of 30 (33%) pigeons from the Brussels population but none from the other cities were found to shed Salmonella Enteritidis belonging to phage type 4 (PT4). All 10 isolates had and identical PFGE banding pattern and they could not be discriminated from human Salmonella Enteritidis PT4 isolates, and from a Salmonella Enteritidis strain isolated from pigeons in 2001. **Conclusions:** Salmonella infections in pigeons have typically been associated with host adapted serovar Typhimurium strains, being of little concern to public health. The discovery of phage type 4, a dominant phage type in human cases and similar PFGE patterns with previously isolated human derived strains suggest a possible role for feral pigeons in human salmonellosis or in spreading these serovars to other animals. Therefore, feral pigeons, especially in cities with a high density may be a novel reservoir for zoonotic Salmonella strains and thus may pose a threat to human health.

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DEVELOPMENT OF A NEW INFECTION MODEL TO ANALYZE THE HETEROGENEITY OF *SALMONELLA* INFECTION IN CHICKS

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Host-to-host transmission of a pathogen ensures its successful propagation and maintenance within a host population. One hallmark of *Salmonella* pathogenicity is its ability to establish an asymptomatic carrier state in numerous animals, which is characterized in poultries by a high *Salmonella* excretion in feces and persistence in caeca. Although this is an essential stage of the *Salmonella's* life cycle, we do not know whether this carrier state corresponds in chicken to a continuous recontamination or to a true persistence in caeca. To address this question we have developed a new infection model in isolator where only few cross contaminations occur. In these conditions, we observed a heterogeneous infection of chicks: some chicks excreting no or very few bacteria (Resistant) whereas others continuously excreted more than $1x10^5$ bacteria/g feces (Super-Shedder). Heterogeneity of infection and excretion has been observed when 1, 10, or 30 chicks were reared per isolators of different sizes, whereas levels of excretion and caeca contamination were homogeneous between all chicks and superior to $1x10^5$ bacteria/g feces in large cages containing 10, 30 or even 200 chicks.The presence of Super Shedder chicks in the isolator model shows that *Salmonella* can persist within one chick for a long time in caeca even without recontaminations between chicks. The presence of resistant chicks indicates that recontaminations play a crucial role in the establishment of the carrier state at the flock level by inducing a homogeneous level of infection. Moreover, when we infected chicks in a large cage to

obtain a homogeneous level of excretion and then transferred them in isolators, all chicks remain contaminated with a continuous high excretion level. This result shows that heterogeneity of *Salmonella* infection is not related to the ability of chicks to discard *Salmonella* but rather on their ability to block the first *Salmonella* colonization. Finally, by introducing non-inoculated chicks in isolator or in another cage close to a cage containing infected chicks, we demonstrated that recontaminations occur mainly through contaminated dust particles present in air. In conclusion, we demonstrate that cross contaminations between chicks are crucial for the establishment of the carrier state at the flock level, but are not required for *Salmonella* persistence in caeca of susceptible animals. The *Salmonella* carrier state does not thus require constant fecal-oral recontaminations. This transmission is probably related to the super-shedders, functioning as a reservoir for the pathogen. This novel model of infection in isolator will facilitate the study of host, microbiota and pathogen factors that contribute to colonization of chicks, persistence in caeca and transmission between animals.

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CONVERGENT EVOLUTION OF COMPLEMENT EVASION BY TYPHOIDAL *SALMONELLA* SEROVARS

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Salmonella enterica serovar Paratyphi A is the causative agent of paratyphoid fever, which is indistinguishable in its presentation from typhoid fever caused by *S. enterica* serovar Typhi, but differs markedly from gastroenteritis caused by *S. enterica* serovar Typhimurium. Differences in the clinical presentation of typhoid fever and gastroenteritis have been attributed to the *S.* Typhi Vi capsular polysaccharide, which inhibits human complement component C3 deposition on the bacterial surface. However, Vi capsular polysaccharide is absent in *S.* Paratyphi A, which raises the question as to why host responses elicited by this pathogen are similar to those elicited by *S.* Typhi. Here we show that the *S.* Paratyphi O-antigen fulfills similar function during host pathogen interaction as the *S.* Typhi Vi capsular polysaccharide. The *S.* Typhi Vi capsular polysaccharide inhibited human complement component C3 deposition and a complement-dependent generation of a oxidative burst in human neutrophil-like (HL60) cells. Similar to *S.* Typhi, *S.* Paratyphi A inhibited C3 deposition and an oxidative burst in HL60 cells. In contrast, C3 was deposited on the surface of *S.* Typhimurium, which triggered a robust oxidative burst in HL60 cells. To investigate how *S.* Paratyphi A inhibits the neutrophil oxidative burst, we genetically engineered a strain in which paratose (O2-antigen) in the O-antigen was replaced with abequose (O4-antigen), a sugar present in the O-antigen of *S.* Typhimurium. *S.* Paratyphi A expressing the O4-antigen triggered a robust oxidative burst in HL60 cells. Collectively, our results suggest that the *S.* Paratyphi A O2-antigen and the *S.* Typhi Vi capsular polysaccharide serve corresponding functions for immune evasion, which represents a striking example of convergent evolution of typhoidal *Salmonella* serovars.

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SALMONELLA TYPHI LIPOPOLYSACCHARIDE O-ANTIGEN MODIFICATION IMPACTS ON HOST-PATHOGEN INTERACTIONS

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The O-antigen side chain of lipopolysaccharide acts as a physical barrier to protect the surface of Gram-negative bacteria from components of the innate immune system, is an immunodominant antigen and phage receptor. It is also considered for vaccine development for a range of bacterial infections, including for the human-adapted, invasive serovar *Salmonella enterica* Typhi. Previously, we reported that *Salmonella enterica* genomes contain variable repertoires of O-antigen modifying *gtr* operons, and determined that *S. Typhi* encodes two *gtr* operons. Here we examine these *gtr* operons, as they suggested the existence of undocumented O-antigen variation which may impact on host-pathogen interactions . We identified the modifications that these two operons confer to the *S.* Typhi O-antigen using a combination of molecular and chemical approaches. We identified the genes responsible for glucosylation of the O-antigen and a novel modification that confirmed that one *gtr* family has acyltransferase activity. Expression of the latter is controlled by phase variation in line with many other *gtr* operons, indicating heterogeneity exists in O-antigen phenotype within clonal *S.* typhi populations. This could contribute to immune evasion. In contrast, the glycosylating operon has acquired a regulatory mutation leading to abrogation of phase variation. Data will be presented that indicate that this modification enhances survival in human serum. The O-antigen recognition repertoire of serum derived from murine immunization, and from vaccine volunteers and typhoid patients serum samples will be presented. The murine model indicates antigenic dominance of the modification but there are disparities between the model system and human serum that will be discussed. Together, the findings suggest that the repertoires of O-antigen modification should be considered in vaccine design to facilitate efficacy against a broad range of *S.* Typhi strains.

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NOVEL PROPHAGE BTP1 IS ASSOCIATED WITH EPIDEMIC AFRICAN *SALMONELLA* TYPHIMURIUM ST313 BUT NOT PRESENT IN DIVERSE UK ST313 LINEAGES

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In the past 30 years, *Salmonella* bloodstream infections have become a significant health problem in sub-Saharan Africa and are responsible for the deaths of ~390,000 people each year. The disease is largely caused by a recently described sequence type of Salmonella Typhimurium: ST313. Comparative genomic analysis showed that the ST313 lineage is closely-related to the ancestral gastroenteritis-associated Typhimurium sequence type ST19, but carries two novel prophages. Until recently, Salmonella ST313 had only been identified in sub-Saharan Africa, but Public Health England has recently identified diverse lineages of ST313 that cause human gastrointestinal disease in the UK and lack the BTP1 prophage. Genomic and TEM characterisation showed that BTP1 is a lambdoid podoviridae which closely resembles *Salmonella* phage P22. We found that the abundance of infective BTP1 virions equals the number of bacterial cells in non-induced cultures, indicating a high rate of spontaneous prophage induction. The level of prophage induction was 1000-fold greater than for isogenic strains lysogenized with phage P22. Spontaneous prophage induction is a

well-documented phenomenon in bacteria, but its impact on bacterial pathogens and disease is not well understood. Our data pose interesting questions about the potential fitness costs and benefits of novel prophages in epidemic *S.* Typhimurium ST313.

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ALTERED COPPER HOMEOSTASIS IN THE MULTIDRUG RESISTANT *MONOPHASIC S.* TYPHIMURIUM EPIDEMIC CLONE

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Background: Salmonella Typhimurium epidemiology is characterized by successive waves of dominant clones. Since around 2005, a monophasic variant has emerged as the dominant MDR clone associated with zoonosis and diseases in human, replacing the previously dominant clone DT104. The monophasic clone encodes a novel genetic island designated SGI-3 that encodes genes with sequence similarity to those previously implicated in copper homeostasis in some enteric bacteria. SGI-3 was acquired immediately prior to clonal expansion of the monophasic clade and therefore may have been important for the epidemiological success of this clone. In this study, we investigated the role of two loci encoded on SGI-3 and that exhibited homology with copper homeostasis genes and the role of environmental factors like oxygen tension to investigate the potential role of SGI-3 in the biology of the monophasic epidemic clone. These loci encode orthologues designated Cus, an efflux pump (cusCFBA) and a cupredoxin detoxification system (pcoABC). **Methods:** We selected representative strains from previous variants (DT204 and DT104) and U288 dominant in pigs. For the genetic manipulation, we used a representative monophasic clade reference strain SO4698-09. We knocked-out the cus and/or pco locus by Datsenko and Wanner method. The CuSO4 MIC has been performed in 24 h liquid cultures with CuSO4 concentrations from 0 (control) to 15 mM (step 1 mM) in LB. The OD600nm has been measured and we arbitrarily chose OD600nm = 0.1 as a cut-off for the MIC. The aerobic, microaerophilic and anaerobic conditions were defined as 20, 5 and 0 % oxygen respectively. **Results:** SGI-3 confers increased copper resistance in monophasic strains carrying it especially in anaerobic condition. We showed that the two main loci homologous to copper resistance systems present in other bacteria species present homologies with a copper efflux pump (CusABC) and a cupredoxin system (PcoABCD). Experiments performed in the strains disrupted for one or both locus showed that the CusABC system enhances copper resistance in anaerobic, microaerophilic and aerobic conditions and the PcoABCD system is active only under anaerobic condition when the Cus system is absent. **Conclusions:** Our results strongly suggest that the monophasic clade acquired SGI-3 that confers a strong growth advantage in anaerobic and high concentration of copper. Because copper sulfate is a micronutrient added in piglets' husbandry, we can speculate that acquisition of SGI-3 gave a selective advantage to the monophasic variant in porcine gut particularly. Then, because the copper is disseminated in the close environment of porcine farms, SGI-3 also helped for dissemination of the monophasic that became the dominant variant.

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EMERGENT *SALMONELLA ENTERICA* SEROVAR INFANTIS TRIGGERS A PRO-INFLAMMATORY HOST RESPONSE THAT IS DEPENDENT ON THE PRESENCE OF THE PESI VIRULENCE PLASMID

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Salmonella enterica serovar Infantis is one of the most commonly isolated non-typhoidal Salmonella serovars. We set out to understand the recent worldwide emergence of S. Infantis and analyzed the host response to pre-emergent and emergent strains of S. Infantis. We have previously shown that emergent strains carry a virulence plasmid (pESI) that provides resistance to several antibiotics and to environmental stress, encodes for two fimbrial genes as well as other virulence factors such as the Yersinia high pathogenicity island. Using in vitro tissue culture and in vivo mouse infections, we demonstrate that tight junction disruption is higher for the emergent strain than the pre-emergent strain and that this phenotype is dependent on the fimbriae encoded on the virulence plasmid. In addition, although expression of the plasmid-encoded fimbriae does not influence intestinal colonization levels, fimbriae are responsible for systemic translocation of the bacteria. Emergent S. Infantis colonizes the intestine of mice to higher levels than pre-emergent S. Infantis or S. Typhimurium at early time points post infection. Furthermore, emergent S. Infantis triggers potent intestinal inflammation which is much stronger compared to pre-emergent S. Infantis but weaker compared to the serovar S. Typhimurium. In addition, pro-inflammatory cytokine response is dependent on the presence of the virulence plasmid. These data show that acquisition of the virulence plasmid plays a key role for the interaction with host cells and inducing pathological damage to the host.

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COMPARATIVE GENOMIC ANALYSIS OF A SUB-LINEAGE OF MULTI-DRUG RESISTANT NON-TYPHOIDAL *SALMONELLA* TYPHIMURIUM ST313 THAT HAS RECENTLY EMERGED IN BLANTYRE, MALAWI

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Background: *Salmonella enterica* serovar Typhimurium is a major cause of bacteremia among immune-compromised individuals in sub-Saharan Africa. Invasive non-typhoidal *Salmonella* Typhimurium accounts for 388,000 deaths annually in Africa. Since 2002, multi-drug resistant S. Typhimurium ST313 lineage II strains have been isolated from patients in Malawi. Surveillance data have revealed the emergence of a distinct phylogenetic group of strains after 2006, designated sub-lineage IIa, that shares the same multi-drug resistance profile as lineage II. The sub-lineage IIa isolates are now causing increasing levels of bloodstream infections by non-typhoidal Salmonella in Malawi. This work focused on two representative isolates of *S.* Typhimurium ST313, namely D23580 (lineage II) and D37712 (sub-lineage IIa). **Methods:** We used finished PacBio-generated genomes to investigate whether genetic differences account for the emergence of sub-lineage IIa strains in Malawi. The presence/ absence of particular plasmids in D37712 were confirmed with information from the draft sequences of the D23580 pBT2 and pBT3 plasmids provided by Rob Kingsley. **Results:** Comparative genome analysis between strains D23580 and D37712, representing lineage II and sub-lineage IIa respectively, revealed differences in plasmid profile, gene composition and SNPs. There were 27 SNPs identified, including 15 non-synonymous SNPs in genes responsible for metabolism, energy production, cell membrane and transport. Both D23580 and D37712 strains share a similar prophage profile and two plasmids, pSLT-BT and pBT3. However, the D23580-associated plasmids pBT1 and pBT2 were absent from the sub-lineage IIa strain

D37712, and this strain had acquired two novel plasmids, pBT4 and pBT5. The new plasmids share high levels of homology with *Salmonella* Weltevreden and *E. coli* plasmids. **Conclusions:** We have identified genetic differences between lineage II and sub-lineage IIa. Further work is in progress to relate genetic differences to the transcriptome, and shed light on the emergence of sub-lineage IIa.

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DETECTION OF ANTIMICROBIAL EFFECT OF SILVER NANOWIRES EMBEDDED IN FILTER PAPER ON *E.COLI*, *SALMONELLA* AND *S.AUREUS*

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Silver nanowires (AgNWs), newly developed materials, have been used in our daily life such as solar cells, clothes, touch screens and coating. Although silver nanoparticles have been used as an antimicrobial agent due to its disrupting effect on proliferation of microorganisms examining silver nanowire in this field is the virgin side of the food packaging design. In this study filter paper (FP) was used as bases of AgNWs. To determine the antimicrobial effects of silver nanowires were operated with Disk Diffusion method (DD) on both gram negative (*Salmonella* Enteritidis*., E.coli* ATCC 25922) and gram positive bacteria (*S.aureus* ATCC 29213). DD methods were conducted and 1.1 cm clear zone on gram negative, 1.4 cm on gram positive bacteria were observed by using silver nanowire with FP with AgNWs. These results gave a deeper understanding of the antimicrobial effect of silver nanowires on environmental pathogens instead of sanitizing agents as well as it can be used as a contact packaging material in food industry. AgNWs might be a promising packaging material in food industry due to its significant antimicrobial activity.

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PROTEOMIC COMPARISON AMONG ISOLATES OF SEROVAR DUBLIN AND ENTERITIDIS: LOOKING FOR TRAITS INVOLVED IN THEIR DIFFERENTIAL PATHOGENICITY IN HUMANS

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"Salmonella" infections are among the most common foodborne diseases worldwide. The Enteritidis and Dublin serovars of "Salmonella enterica" are closely related yet they differ significantly in pathogenicity and epidemiology. Enteritidis is a broad-host-range serovar that commonly causes gastroenteritis and infrequently causes invasive disease in humans. Dublin mainly colonizes cattle but upon infecting humans often results in invasive disease. The aim of this work was to elucidate the molecular factors responsible for the differential pathogenic behavior between both serovars. We performed a comparative proteomic analysis between two isolates of each serovar grown under gut- mimicking conditions, by 2-dimensional gel electrophoresis. 9 proteins over-represented in Dublin and 7 over-represented in Enteritidis were identified by mass spectrometry. We also carried out RTqPCR analysis that revealed significant differences in mRNA levels in 12 of the 16 genes analyzed, supporting the proteomic results. Interestingly, 6 out of 9 proteins over-represented in Dublin are reported as involved in the response to acid or oxidative stress conditions, and regulated by stress response RpoS sigma factor. "Salmonella" is exposed to such conditions when internalized into phagocytic cells, and survival inside the phagosome is relevant for spreading to internal organs. To have a deeper insight into the putative roles of the proteins identified, we selected three stress-response proteins over-represented in Dublin (Dps, YciF and YgaU), as well as

one protein over-represented in Enteritidis that participates in the metabolism of amino acids (carbamate kinase), to inactivate the corresponding genes in one strain of each serovar. We constructed single chromosomal deletion mutants in the genes coding for the selected proteins, as well as double mutants. The invasive ability of the mutants was evaluated in the murine model of salmonellosis, using co-infection experiments with the corresponding wild type strain. Four days after oral infection, the counts of mutants or wild type bacteria in spleens were quantified. No significant differences were found in the invasion ability of the single or double mutant strains compared to the respective wild type strain. In conclusion, in this work we show that, in infection-relevant growth conditions, serovar Dublin produces higher levels of proteins involved in stress responses compared to Enteritidis. As no differences were found between the mutants and the wild type strains in the mouse model, and to discard possible redundant roles for the genes inactivated, we plan to construct a triple mutant ("dps,yciF,ygaU") to evaluate it in the same model. In addition, we plan to evaluate the mutant strains in "in vitro" models of persistence, including macrophage infection assays and survival in oxidative and acid growth conditions.

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MODIFICATION OF LPS BY EPTB INHIBITS INTELECTIN BINDING AND INCREASES SYSTEMIC INFLAMMATION DURING *SALMONELLA* INFECTION

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Salmonella enterica is a highly diverse species of Gram-negative bacteria that can be grouped into typhoidal and non-typhoidal serovars. Non-typhoidal serovars, such as S. Typhimurium, cause gastroenteritis and inflammatory diarrhea, whereas typhoidal serovars, such as S. Typhi, cause systemic disease with a comparatively decreased inflammatory response. However, the properties that distinguish these two closely related groups remain poorly understood. Previously, comparative analysis of Salmonella genomes revealed that typhoidal serovars contain a higher number of pseudogenes than non-typhoidal serovars. One such pseudogene is eptB, which codes for a phosphoethanolamine transferase that can specifically modify the outer keto-deoxyoctulosonate (KDO) residue of lipopolysaccharide (LPS). Human intelectin-1 is known to bind to and recognize multiple microbial glycan epitopes, including KDO. Interestingly, previous studies have shown that S. Typhimurium LPS is not bound by intelectin, despite possessing KDO residues. Here, we show that EptB modification of LPS may lead to increased systemic inflammation during infection by S. Typhimurium by preventing intelectin binding and subsequent detoxification of LPS. We found that LPS isolated from typhoidal serovars possessing an eptB pseudogene is bound by intelectin and furthermore that loss of EptB function in S. Typhimurium allows binding of intelectin to S. Typhimurium LPS. Additionally, there is a decrease in expression of inflammatory cytokines in the spleen in mice infected with an eptB mutant S. Typhimurium compared to mice infected with the wild-type strain. Together, these results suggest that in the absence of EptB, intelectin is able to bind to and detoxify S. Typhimurium LPS, leading to decreased systemic inflammation during infection. These results have broad implications for how pathogens such as S. Typhimurium induce systemic shock during infection and may also help to explain a mechanism for how S. Typhi is able to evade immune detection and enhance dissemination to systemic sites.

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PHENOTYPIC COMPARISON AND WHOLE GENOME SNP ANALYSIS AMONG *SALMONELLA* ENTERITIDIS STRAINS ISOLATED IN THE PRE AND POST-PANDEMIC PERIOD IN BRAZIL

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Background: Salmonella Enteritidis emerged since the 80's as the most isolated serovar from gastroenteritis cases worldwide. In Brazil it is believed that an epidemic and more virulente clone of this serovar has been inserted in the country in the mid-90 through the exchange of chickens with other countries. This study comparatively analyzed by phenotypical tests and whole genome SNP analysis (wgSNP), S. Enteritidis strains isolated in the pre and post-pandemic period in Brazil. **Methods:** For this, 27 S. Enteritidis strains isolated in the pre-pandemic period (1968-1993) and 20 in the post-pandemic period (1994-2013) in Brazil were studied. The strains were compared regarding survival to the acid and oxidative stress, ability to grow in the egg albumen, invasion to human epithelial cells (CACO-2), survival and multiplication capacity in human (U937) and chicken (HD-11) macrophages. Moreover, four strains had their whole genome sequenced and a SNP phylogenetic tree was constructed using the genome of S. Enteritidis P125109 strain as reference. **Results:** Pre-pandemic strains were less resistant than post-pandemic strains to the exposition of 10^8 CFU of each strain to acid stress by 10 minutes, with mean of survival of 0.255% and 2.159% respectively ($P \leq 0.05$). Moreover, pre-pandemic strains were less capable of surviving and multiplying in human macrophages (U937) with mean of multiplication of 104% and 406% respectively ($P \leq 0.05$). The same pattern was observed in some of the other tests such as survival to acid stress by 1 hour, with mean of survival of 0% for pre-pandemic and 0.000065% for post-pandemic, survival to oxidative stress by 10 minutes where pre-pandemic strains were less resistant (21.96%) than post-pandemic (25.89%) by 1 hour, with mean of survival of 5.76% and 7.01% respectively. Also, pre-pandemic strains were less invasive to CACO-2 cells than post-pandemic strains, with mean of invasion of 0.34% and 0.56% respectively. However, the results above were not statistically significant ($P \geq 0.05$). The wgSNP analysis showed the two post-pandemic strains isolated in 1999 and 2013 and the strain isolated in 1993 with 21, 20 and 33 SNPs of difference from the reference strain, while the pre-pandemic strain isolated in 1986 showed 280 SNPs of difference. **Conclusions:** Post-pandemic strains showed better capability of surviving in acid stress conditions and to invade and multiply in human macrophages than pre-pandemic strains, which might had contributed to the increase of S. Enteritidis isolation from gastroenteritis cases in Brazil. The sequenced S. Enteritidis strains isolated after the 90's were more similar to the reference strain P125109 of phagetype 4 than the strain isolated in 1986, reinforcing the insertion of a new clone in Brazil in mid-90.

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A HIGH-THROUGHPUT *IN VITRO* SYSTEM TO STUDY *SALMONELLA*-NEUTROPHIL INTERACTIONS

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Background: *Salmonella* benefits from the neutrophilic inflammatory response that occurs during acute enteric infection. Although this response is critical for the pathogenesis of enteric salmonellosis, no unbiased strategy has been pursued to identify the complement of *Salmonella* Typhimurium (STm) mechanisms required to induce neutrophil antimicrobial defenses. We hypothesize that STm actively

induces neutrophils to produce reactive oxygen species (ROS). **Methods:** We developed a neutrophil-STm co-culture method to characterize genes essential for induction of the neutrophil oxidative burst and adapted this for high-throughput assays. Neutrophils were isolated from the blood of healthy human donors by Ficoll-gradient centrifugation, suspended in media containing human serum, and primed with granulocyte-monocyte colony stimulating factor. STm was added to neutrophil suspensions and H_2O_2 production measured using dihydrorhodamine fluorescence. Culture conditions were optimized to maximize the neutrophil oxidative burst by altering the multiplicity of infection and serum components. Finally, we screened a multi-gene deletion (MGD) library for mutants that alter the neutrophil oxidative burst (Porwollik 2014). **Results:** We found that neutrophils generated more ROS in response to live as compared with killed STm, confirming that stimulation of the oxidative burst requires live STm. We show the optimal ratio of STm:neutrophil to stimulate the oxidative burst is between 50-75:1. Confirming their importance in host-cell interaction, we found that maximal ROS production in response to STm requires both type-3 secretion systems. Finally, we tested 192 MGD mutants in our co-culture system and found that 31 mutants, deleted for 377 genes, stimulate an altered neutrophil oxidative burst as compared to wild-type. Six of the 31 mutants are deleted for known virulence determinants including flagellar apparatus, fimbrial proteins, and the type-3 secretion system-1. Of the 31 mutants identified in this screen, 26 contain genes with predicted importance in bovine and 20 in porcine enteric disease (Chaudhuri 2013, Elfenbein 2013). **Conclusions:** Our STm-neutrophil co-culture system provides a unique opportunity to evaluate the interaction between STm and neutrophils. We have found 31 genomic regions that are important for STm-neutrophil interaction, many of which have predicted functions during enterocolitis. Further work to characterize the STm mechanisms that influence the neutrophilic antimicrobial responses will improve our understanding of how *Salmonella* interacts with this critical component of the innate immune response.

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MOLECULAR TYPING OF *SALMONELLA* DUBLIN STRAINS ISOLATED FROM HUMANS AND ANIMALS OVER 32 YEAR-PERIOD IN BRAZIL

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Background: Salmonella Dublin is a serovar strongly adapted to cattle causing enteritis and/or systemic disease with high rates of mortality. However, sporadically it can be isolated from humans, usually causing serious systemic infection, specially in patients with underlying chronic diseases. The aim of this study was to molecularly type S. Dublin strains isolated from humans and animals in Brazil, verify the presence of the capsular antigen (Vi) and the virulence plasmid pSDL2 and access its antimicrobial resistance profiles. **Methods:** A total of 59 S. Dublin strains isolated in Brazil from humans (30) and animals (29) between 1983 and 2015 were studied. The genotypic diversity was accessed by PFGE using the enzyme XbaI and the presence of the virulence plasmid pSDL2 and of the tviB gene that codify the capsular antigen (Vi) were searched by PCR. The capsular antigen also was searched serologically. The strains were tested against the following antimicrobials ampicillin, chloramphenicol, tetracycline, cefoxitin, ceftriaxone, ceftazidime, cefepime, piperacillin, amoxicillin- clavulanic acid, imipenem, gentamicin, streptomycin, ciprofloxacin, nalidixic acid, sulfamethoxazole-trimethoprim and nitrofurantoin. **Results:** PFGE grouped the 56 strains studied into two groups. Group A clustered

52 strains isolated between 1983 and 2015 with a similarity >80.6%, while group B presented only four strains isolated in 1997 and 2004 with a similarity >85.2%. The similarity between the two groups were >77.4%. Three strains were nontypeable by PFGE. None of the strains presented the capsular antigen (Vi) neither the tviB gene. The pSDL2 plasmid was present in 55 of the 59 strains studied. Seven strains isolated from humans presented resistance to ampicillin (6.6%), tetracycline (10.0%) and nalidixic acid (6.6%) while two strains isolated from animals showed resistance only to nalidixic acid (6.9%). **Conclusions:** Molecular typing may suggest that S. Dublin isolated in Brazil descend from a common ancestor that differed little over 32 years and have been contaminating humans and animals in Brazil. The high frequency of the plasmid may indicate that it is essential to S. Dublin to cause disease, a fact not observed for the capsular antigen (Vi). The antimicrobial resistance results indicates that the S. Dublin human isolates may be more resistant than the animal strains in Brazil.

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TRICHOMONAD ASSOCIATED PROTECTION AGAINST *SALMONELLA* INFECTION

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Complex interactions between the host and commensal microbiota create a barrier against infection by pathogenic microbes, which is termed colonization resistance (CR). CR can be the result of direct competition for vital nutrients between the commensals and pathogens (direct CR), or the result of indirect mechanisms whereby commensal organisms induce host defenses that prevent pathogen colonization and infection. In our studies of innate immune recognition of *Salmonella* during intestinal infection, we discovered that *Naip5*$^{-/-}$ and *Rag1*$^{-/-}$ mice harbor microbiota that protect mice against *Salmonella* infection. This protection is associated with intestinal colonization by *Tritrichomonas muris* (*Tm*). *Tm* is a protozoon commonly found in laboratory, pet shop and wild mice, which has no definitive disease association. Until recently, *Tm* also had no definite impact on research and was an ignored component of the murine gut microbiota. Recent studies have demonstrated that *Tm* and other intestinal parasites are sensed by tastant receptors expressed by specialized tuft cells in the intestine. This sensing leads to tuft cell secretion of IL-25, inducing lamina propria iLC2 to secrete IL-13, which acts on intestinal epithelial progenitor cells to induce tuft cell and goblet cell hyperlasia. Helminths and protozoa have also been associated with changes in the intestinal microbiota, suggesting that intestinal protozoa may also produce biologically significant alterations in the composition of gut bacteria. We demonstrate that *Tm* colonization of mice is associated with decreased inflammation in the intestine during *Salmonella* infection of streptomycin pretreated mice. *Tm* colonization is also associated with increased resistance to *Salmonella* infection and colonization resistance. Finally, we demonstrate that *Tm* colonized mice have an altered gut microbiome, suggesting that *Tm* cultivates a protective microbiome that helps prevent *Salmonella* colonization and infection. Our studies indicate that intestinal protozoa may influence the complex environment within the gut to help limit *Salmonella* invasion of the gut ecosystem and *Salmonella* induced intestinal inflammation, and provide insight into novel strategies that may help prevent *Salmonella* infection and transmission.

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HOST PROTECTION AGAINST ORAL *SALMONELLA* INFECTION IS MEDIATED BY THE NLRC4 INFLAMMASOME

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Salmonella enterica serovar Typhimurium is a leading cause of gastroenteritis worldwide and a deadly pathogen in children, immunocompromised and elderly. *Salmonella* induces innate immune responses through the Nlrc4 inflammasome, and both the Nlrp3 and Nlrc4 inflammasome have been implicated in protecting mice against oral infection. We used wild type, flagellin-deficient and flagellin-overproducing *Salmonella* to assess the contribution of the Nlrp3 and Nlrc4 inflammasome components during systemic and mucosal infection. During oral infection, the Nlrc4 inflammasome is required for host protection. In contrast, the Nlrp3 inflammasome had mostly no significant influence on systemic or mucosal *Salmonella* infection. Asc provided partial protection during mucosal infection. Tissue injury and inflammation induced by oral *Salmonella* infection was limited by Nlrc4 and Casp/11, but not Nlrp3 or Asc, suggesting that Nlrc4 initiated pyroptosis prevents excessive tissue injury during infection of the intestine. Using *Salmonella* mutants we demonstrate that the Nlrc4 inflammasome recognizes both flagellin and the SPI-1 type three secretory system during oral infection. Our data indicate that Nlrc4 is the dominant inflammasome mediating protective innate immunity during *Salmonella* infection.

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GUT MICROBIOTA DYSBIOSIS ENHANCES *SALMONELLA* GROWTH IN THE INTESTINE THROUGH FORMATE METABOLISM

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Intestinal inflammation is frequently associated with an alteration of the gut microbiota, termed dysbiosis. This phenomenon is exploited by enteric pathogens to enhance gut colonization and transmission success. Non-typhoidal Salmonella serovars such as S. Typhimurium induce a potent, acute inflammatory response in the intestinal tract that results in dysbiosis and a bloom of the pathogen population in the gut lumen. The molecular mechanisms of how the dysbiotic microbiota interacts with S. Typhimurium during infection are poorly understood. To investigate which changes in the metabolic landscape are associated with dysbiosis, we performed a metagenomics analysis on the murine gut microbiome in a simplified model in which inflammation is induced chemically (dextran sulfate sodium-induced colitis model). In the metagenomics analysis, one of the most overrepresented metabolic pathways during inflammation was linked to bacterial formate utilization, suggesting a pivotal role for this metabolism. Formate dehydrogenase genes provided a fitness advantage to S. Typhimurium in murine models of Salmonella-induced colitis. Complimentary metabolite profiling and targeted metabolomics indicated formate levels in the intestine are increased during episodes of intestinal inflammation. Formate was of microbial origin since no formate was detected in germ-free mice and association of germ-free animals with obligate anaerobic bacteria, such as B. thetaiotaomicron, partially restored intestinal formate levels. We conclude that gut microbiota dysbiosis is accompanied by increased availability of formate, which in return enhances S.

Typhimurium growth in the gut lumen by formate oxidation. This work identifies formate metabolism as a novel metabolic pathway that is perturbed during dysbiosis.

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WHOLE-GENOME SEQUENCING OF DRUG-RESISTANT *SALMONELLA ENTERICA* ISOLATED FROM DAIRY CATTLE AND HUMANS IN NEW YORK AND WASHINGTON STATES REVEALS SOURCE AND GEOGRAPHIC ASSOCIATIONS

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Salmonella enterica is a pathogen of concern for both humans and cattle. It can be spread from cattle to human populations through direct contact with animals shedding *Salmonella*, as well as through the food chain. Infections caused by multidrug-resistant (MDR) isolates can be challenging to treat, making MDR *Salmonella* a relevant human health hazard. The objective of this study was to use whole genome sequencing to compare antimicrobial-resistant (AMR) *S.* Typhimurium, Newport, and Dublin isolated from dairy cattle and humans in Washington state and New York state at a genotypic and phenotypic level. A total of 90 drug-resistant *Salmonella* isolates were selected for this study, 45 of which were from Washington state (WA state; 20 from dairy cattle and 25 from humans) and 45 from New York state (NY state; 21 from dairy cattle and 24 from humans). Isolates were selected based on location, source, and serotype stratified by year. All isolates were tested for phenotypic AMR to 12 drugs using Kirby-Bauer disk diffusion. Genomes of all isolates were sequenced using the Illumina HiSeq platform and assembled de novo using SPAdes. SRST2 and ARG-ANNOT were used to detect AMR genes in each isolate, while Plasmid Finder was used to detect the presence of plasmid replicons. BEAST was used to construct phylogenetic trees based on variant sites, and the genotypic AMR, phenotypic AMR, and plasmid replicon profiles of isolates from different sources and geographic locations were compared. For *S.* Typhimurium, sulfamethoxazole-trimethoprim resistance was only observed in human isolates ($P < 0.05$). In addition, the IncI1 replicon was more commonly detected in *S.* Typhimurium from NY state ($P < 0.05$). *S.* Newport bovine and human isolates formed distinct clusters based on AMR gene presence-absence patterns ($P < 0.05$). *CMY-33* and *CMY-44* genes, which play a role in resistance to cephalosporins, were associated with *S.* Newport isolates obtained from bovine and human sources, respectively ($P < 0.05$). *S.* Dublin isolates from NY state differed from those from WA state based on the presence and absence of plasmid replicons, as well as phenotypic AMR resistance-susceptibility patterns ($P < 0.05$). Genes *aadB* and *cmlA5* were only found in the sequences of *S.* Dublin isolates from WA state, while resistance to streptomycin was more common in *S.* Dublin isolates from NY state ($P < 0.05$). In addition, the IncFII(S) replicon was more commonly detected in the sequences of *S.* Dublin isolates from NY state ($P < 0.05$). The results of this study indicate that, for some antimicrobials, MDR *Salmonella* isolated from different sources and geographic locations harbor different AMR genes, suggesting that distinct events can contribute to emergence of AMR in humans and farm animals and in different regions.

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POSSIBLE TRANSMISSION OF DRUG-RESISTANT *'SALMONELLA TYPHIMURIUM'* AND *'LISTERIA MONOCYTOGENES'* WITHIN THE BEEF INDUSTRY IN ABUJA, NIGERIA

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Background: Widespread use of antibiotics in large-scale across the globe in livestock production has become of public and veterinary health importance because of its implication in antibiotic resistance. Adequate data in this area of research is not readily available in Nigeria; this study was undertaken in view of the possible link between antimicrobial resistance in farm animals and humans. **Methods:** We collected fifty samples of raw beef from different vendors and slaughters houses within Abuja and screened them for the presence of Listeria monocytogenes and Salmonella typhimurium using standard microbiological methods. The total bacterial and fungal counts were determined. The susceptibility of the isolates to ten different antibiotics and heat sensitivity at 55, 60 and 65 oC for 15 minutes was also determined. **Results:** Our results show that ten isolates of Listeria monocytogenes and eighteen isolates of Salmonella typhimurium were isolated from the samples. The total viable bacteria count range was 1x 109 - 8x 109 cfu/g while the fungal count was 1x 103 - 9x 109 cfu/g. One (10 %) of the Listeria monocytogenes isolates was resistant to all antibiotics tested while all the Listeria monocytogenes isolates were resistant to cefuroxime. Eight (44.4%) of the Salmonella typhi isolates were resistant to at least three antibiotics. All the Listeria monocytogenes and Salmonella typhi isolates did not survive beyond 60 oC upon heat treatment. **Conclusions:** Our results indicate high prevalence of Salmonella typhi and Listeria monocytogenes in selected beef in Abuja. Beef therefore may represent a large reservoir for antimicrobial-resistant Salmonella typhi and Listeria monocytogenes.

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DUAL RNA-SEQ OF PATHOGEN AND HOST

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A comprehensive understanding of host-pathogen interactions requires knowledge of gene expression changes in both the pathogen and the host. While traditional, probe-dependent approaches like microarrays are insufficient to analyze the full (i.e. coding and non-coding) transcriptome at high resolution, the probe-independent RNA-sequencing (RNA-seq) has begun to revolutionize transcriptomics. We took transcriptomics a step further and utilized "Dual RNA-seq" to investigate the interplay of Salmonella Typhimurium, an important facultative intracellular model pathogen, with human host cells during infection. Infections of cell culture models were carried out with constitutively GFP-expressing Salmonella and invaded cells were selected by cell-sorting. Application of Dual RNA-seq led us to the detection of all major (coding and non-coding) transcript classes of an infected cell. On the pathogen's side, one of the highest induced genes upon host cell entry was a previously uncharacterized small non-coding RNA (sRNA). Classical genetics and biochemical assays placed this sRNA in the center of a complex regulatory network of virulence gene expression in vivo and using a comparative Dual RNA-seq approach, we revealed effects of this sRNA on the host immune response, including expression changes in a set of human long non-coding RNAs. Together, Dual

RNA-seq proofs to be an invaluable technique for genome-wide host-pathogen interaction studies.

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AN EPIDEMIOLOGICAL STUDY OF ANTIBIOTIC RESISTANCE OF *SALMONELLA TYPHI* AND *SALMONELLA PARATYPHI* ISOLATED FROM PATIENTS ATTENDING DIFFERENT HOSPITAL IN DHAKA CITY, BANGLADESH

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The current study aimed to determine the prevalence and multidrug resistance of Salmonella typhi and Salmonella paratyphi in patients with Typhoid fever at Dhaka, Bangladesh.A hospital based cross sectional study was performed on Salmonella typhi and Salmonella paratyphi cases attending IPD (In-patients) and OPDs (outpatients departments) at Microbiology research laboratory,Primeasia University, Dhaka, Bangladesh.The antibiotic susceptibility of clinical isolates collected from blood samples were identified. The patients were divided into 9 age groups. Susceptibility and resistance was also tested by Kirby-Bauer disc diffusion method using 8 regularly used antibiotics. Total of 1178 blood samples were analyzed in this study. Among which 106 showed positive result, only 9% patients were ill, the causative agents-Salmonella typhi was found to be most prevalent (73.58%) follow by Salmonella paratyphi (26.42%), both of them showed 100% resistance to Nalidixic Acid and Azithromycin. Both were sensitive to Amoxicillin 80% and Cotrimoxazole (90%) respectively. This study revealed high prevalence of MDR Salmonella isolates, this represents is a serious health problem in the region under study that should be prevented by some effective measure such as eating safely prepared foods and get vaccinated (Ty21a). Ceftriaxone, Ciprofloxacin, Cefixime & Levofloxacin are drugs of choice for the treatment of typhoid fever in the community.

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DISCRIMINATION AMONG *SALMONELLA ENTERICA* SEROTYPES BY MULTI-LOCUS SEQUENCE TYPING (MLST) SCHEME AS A GLOBAL PROSPECTIVE STRATEGA

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Background: Salmonella as a major food-borne disease has imposed significant public health and economic loss along the globe and claimed an important zoonotic illness among developing countries. Based on 1956 Pasteur Institute annual Report, Iran has a long history of research on this pathogen, by representing Salmonella Hessarek, as an uncommon serotype, isolated for the first time by Professor Kaveh et al. from a raven (Corvus corax). Traditionally, Serotyping depends on immunological typing methods, which is reliable and accepted in a global scale, but has lots of shortcomings, including low throughput, expensive antisera and considerable expertise. Unfortunately Results acquired by most of molecular methods as an alternative approaches can't be compared among different laboratories. Also these techniques should possess the ability that in a long-term epidemiological study, the origin of a single globally distributed clonal lineage be thoroughly determined. To solve all similar shortcomings, based on sequences of multiple housekeeping genes, MLST scheme has been implemented as a generic, repeatable, robust and portable technique. **Methods:** Having a previous history of clonal population structure among 76 Salmonella Enteritidis by using MLST technique, this study was designed to evaluate the discrimination ability of this method among different Salmonalla serotypes. 4 DNA templates belonging to 3 different serogroup D1, B and C1and One standard salmonella Enteritidis were exploited in this study. Three out of seven MLST house-

keeping genes including hisD, thrA, sucA which stand for 3 conserved genes encoding enzymes of "histidinol dehydrogenase", "aspartokinase+homoserine dehydrogenase" and "alpha ketoglutarate dehydrogenase", were considered to be amplified. The PCR products were gel-electrophoresed using agarose gels and amplicons were sent to be sequenced in both directions and were gone under comparison alignment evaluation with full genome standard fragments and MLST web data base information. **Results:** Embarking on this technique, while we observed a clonal population group among our Salmonella Enteritidis isolates, we successfully differentiated 3 most frequent Iranian serotype including Enteritidis, Typhimurium and Infantis, in different clustal groups. **Conclusions:** Generally, molecular typing methods are intended to tackle two different levels of epidemiological problems, which reflect different insights toward solving a local or global epidemiology in different timeframes. In one hand localized outbreak of disease in a short period of time should be assessed and on the other, relation between strains causing a disease in one geographic area with those observed around the world during a longer period. This study proves MLST method is a satisfactory platform to provide all these needs and can be regarded as a desirable prospective global typing strategy.

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PREDOMINANT SEROTYPES OF *SALMONELLA ENTERICA* IN BREEDING FARMS IN THE ALBORZ PROVINCE

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Introduction: Foodborne diseases caused by nontyphoidal Salmonella (NTS) represent an emerging public health problem that are mostly derived from the consumption of foods of animal origin, particularly poultry products. This microorganism is responsible for tens of millions of illnesses and more than hundred thousand deaths annually worldwide and has become a major concern due to the emergence of resistant to antimicrobials. The aim of this study is survey on predominant serotype of salmonella in breeding farms in Alborz province. **Materials & Methods:** A total of 5540 swab cloacae samples were collected from 2010 to 2012 in Alborz province. Then cultured on selective media and biochemical identification following the protocol described by WHO. Isolated Salmonella were serotyped at the Microbiology Department, Razi Vaccine & Serum Research Institute, Karaj, Alborz. The isolates stored with 20 % glycerol in TSB at -80◦C. **Results:** The serotypes of Salmonella in Alborz province were Salmonella enteritidis(O9) (32), typhimurium(O4)(22), infantis(O7) (4), thompson(O7)(1), montevideo(O7) (1), sandow(O8)(1), newport(O8)(1) and arizona(O35)(1). The results of this study showed that Salmonella enteritidis and typhimurium existed as an predominant serotypes in Alborz province. Besides these serotypes, serotype O7, O8 and O35 was seen. **Conclusions:** This observation draws serious attention as poultry serves as an important source of transmission Salmonella serovars to humans. Also it must be more attention to multidrug resistant serotypes that can be transmitted via this way to human.

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MOLECULAR CHARACTERIZATION OF *SALMONELLA ENTERICA* ISOLATES BY PULSED-FIELD GEL ELECTROPHORESIS

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Introduction: *Salmonella* infections are the second leading cause of zoonotic bacterial foodborne illness. Main source of infection in human is contaminated food products. The aim of this study was molecular characterization of *Salmonella enterica* isolates by Pulsed Field

Gel Electrophoresis (PFGE) technique. **Methods:** All 56 *Salmonella* isolates were serotyped and then subjected to PFGE. Total isolates were analyzed by means of the molecular technique *XbaI* PFGE. In this study, PFGE and serotyping were used to subtype 56 *Salmonella* isolates belonging to 35 different serovars and derived from human and different food origins. **Results:** Among these isolates, *S. Typhimurium* and *S.Enteritidis* were found to be the most predominant serovars. 50 PFGE patterns out of 56 isolates were obtained. The Discrimination Index obtained by serotyping (DI =0.88) was lower than PFGE (DI = 0.99). **Conclusions:** Molecular characterization of *Salmonella enterica* is very important and shows that animal origin can be one of a reservoir that potentially could be transferred to human through the food chain. In addition, results of this study also revealed that is a golden standard for genotyping of such *Salmonella* serotypes. **Keywords:** PFGE, *Salmonella enterica,* Serotyping, Molecular characterization, Genotyping

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ISOLATION, IDENTIFICATION AND ANTIMICROBIAL RESISTANCE PROFILING OF *SALMONELLA ENTERICA* COLLECTED FROM BREEDER FARMS OF TEHRAN PROVINCE OF IRAN

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Background: Considering the contribution and role of Salmonella as one of the most important zoonotic diseases in public health and role of poultries as a whole in transmission of this food-borne disease, this study was conducted in Tehran province, along with other provinces under a national surveillance frame work. **Material/Methods:** During two-year project, 4160 samples from 24 poultry houses including 11 breeder farms,13 layer farms, 1 hatchery center and 2 abattoir were taken. All samples were gone under isolation and serotyping process based on Kauffman-White scheme. To reveal antimicrobial resistance of serotyped isolates, disc diffusion method was applied according to CLSI guideline. **Results:** From the total of 30 isolates 22 Salmonella Enteritidis, 4 Salmonella Typhimurium, 2 Salmonella Arizona, and 2 non-typable isolates were identified. From hatchery center, two different serotypes including Salmonella Typhimurium and Salmonella Enteritidis were detected. Serotyped isolates were then gone under antimicrobial resistance typing against 29 antimicrobial drugs by disc diffusion method. Based on multi-drug resistance typing results, 9 patterns in Salmonella Enteritidis, 4 patterns in Salmonella Typhimurium, and 1 pattern for each Salmonella Arizona and non-typable isolates were recorded. Among multi-drug resistance patterns, interestingly, a Salmonella Arizona isolate with a resistant panel of 16 antimicrobial drugs demonstrated the greatest profile pattern among others. **Conclusions:** In Conclusion, in spite of the low rate and non-significant salmonella infection prevalence and considering the detected isolates with different serotypes in selected farms, implementing more controlling policies and periodic monitoring systems are advised. Evidence of antimicrobial resistance in some strains emphasizes that drug resistance phenomena is expanding and national surveillance programs should be continued to ensure that it won't become a health problem in the country.

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EVALUATION OF FLAGELLAR GENE EXPRESSION IN *SALMONELLA ENTERICA* SEROVARS IDENTIFIES SPECIES WIDE ADAPTATION OF FLHD4C2 ACTIVITY

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Salmonella enterica colonizes plants, animals, reptiles and humans. Whole genome sequence analysis of *S. enterica* generates a phylogentic tree comprising of three clades: A1, A2 and B. *S. enterica* exploits the bacterial flagellum to be motile in liquid environments and over surfaces. Motility across the species is a very robust phenotype, with the majority of isolates being motile. A recent study of sv. Typhimurium isolates suggests that their motility adapts to their host source with strains being more motile when isolated from a clinical setting.The flagellar system of *S. enterica* is organized into a transcriptional hierarchy of three promoter classes. Our understanding of flagellar gene regulation in *S. enterica* stems from seminal work conducted over the last 30-40 years in the sv. Typhimurium. We asked how does activation of flagellar gene expression across the species *S. enterica* compare to sv. Typhimurium? We will show a comparison of motility phenotypes, flagellar gene expression and bioinformatics analysis of flagellar genes in serovars representative of the 3 clades of *S. enterica*. Our data suggests that the timing of flagellar gene expression for all serovars is similar, but the magnitude of flagellar gene expression varies significantly. The changes in the magnitude of flagellar gene expression across serovars was attributed to the regulation of $FlhD_4C_2$ activity. The expression and protein levels of $FlhD_4C_2$ are controlled by a wide variety of factors, dictated by internal cellular and environmental signals. We asked what was the impact in changes to $FlhD_4C_2$ itself upon the output of the flagellar system? We have analyzed how switching *S. enterica flhDC* with *flhDC* from *E. coli* impacted motility and flagellar gene expression. Our data suggests that even though strongly conserved, differences in each protein, FlhD and FlhC, can significantly impact the output of the flagellar system. Together these data have shown that the robust phenotype of motility in *S. enterica*, as a species, is subject to an adaptable response via the regulation of *flhDC* gene expression and $FlhD_4C_2$ activity.

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ACETYLATION OF LYSINE 201 INHIBITS THE DNA-BINDING ABILITY OF PHOP TO REGULATE *SALMONELLA* VIRULENCE

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Salmonella Typhimurium is a facultative intracellular pathogen capable of survival within host phagocytic cells. The two-component system PhoP-PhoQ is highly conserved in bacteria and regulates virulence in response to various signals for bacteria within the mammalian host. Here, we demonstrate that PhoP could be acetylated by Pat and deacetylated by deacetylase CobB enzymatically in vitro and in vivo in Salmonella Typhimurium. Specifically, the conserved lysine residue 201(K201) in winged helix-turn-helix motif at C-terminal DNA-binding domain of PhoP could be acetylated, and its acetylation level decreases dramatically when bacteria encounter low magnesium, acid stress or phagocytosis of macrophages. PhoP has a decreased acetylation and increased DNA-binding ability in the deletion mutant of pat. However, acetylation of K201 does not counteract PhoP phosphorylation, which is essential for PhoP activity. In addition, acetylation of K201 (mimicked by glutamine substitute) in S. Typhimurium causes significantly attenuated intestinal inflammation as well as systemic infection in mouse model, suggesting that deacetylation of PhoP K201 is essential for Salmonella pathogenesis. Therefore, we propose that the reversible acetylation of PhoP K201 may ensure Salmonella promptly respond to different stresses in host cells. These findings suggest that reversible lysine acetylation in the DNA-binding domain, as a novel regulatory mechanism of gene expression, is involved in bacterial virulence across microorganisms.

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DIFFERENCES IN HOST CELL INVASION AND SPI-1 EXPRESSION BETWEEN *SALMONELLA ENTERICA* SEROVAR PARATYPHI A AND THE NON-TYPHOIDAL SEROVAR TYPHIMURIUM

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Active invasion into non-phagocytic host cells is central to Salmonella enterica pathogenicity and dependent on multiple genes encoded within the Salmonella Pathogenicity Island-1 (SPI-1). To better understand the unique pathogenicity of typhoidal salmonellae, we explored the invasion phenotype and the expression of SPI-1 genes in the typhoidal serovar S. Paratyphi A, in comparison to the non-typhoidal serovar S. Typhimurium. We found that in response to temperatures equivalent to fever (39^0C and above), S. Paratyphi A, but not S. Typhimurium attenuates its motility, epithelial cell invasion and uptake by macrophages. Under these fever-like conditions, the impaired motility and invasion are associated with downregulation of T3SS-1 genes and classes II and III, (but not I) of the flagella-chemotaxis regulon. Similarly, we demonstrated that while S. Typhimurium is equally invasive under both aerobic and microaerobic conditions, S. Paratyphi A invades only following growth under microaerobic conditions. RNA-Seq, RT-PCR, western blot and secretome analyses established that S. Paratyphi A expresses much lower levels of SPI-1 genes and secretes lesser amounts of SPI-1 effector proteins compared to S. Typhimurium, especially under aerobic growth. Bypassing the native SPI-1 regulation by inducible expression of the SPI-1 activator, HilA, considerably elevated SPI-1 gene expression, host cell invasion, disruption of epithelial integrity, and induction of pro-inflammatory cytokine secretion by S. Paratyphi A, but not by S. Typhimurium, suggesting that SPI-1 expression is naturally down-regulated in S. Paratyphi A. Using streptomycin-treated mice, we were able to establish substantial intestinal colonization by S. Paratyphi A and showed moderately higher pathology and intestinal inflammation in mice infected with S. Paratyphi A, overexpressing hilA. Collectively, our results reveal unexpected differences in SPI-1 expression between S. Paratyphi A and S. Typhimurium and indicate that host cell invasion by S. Paratyphi A is suppressed under aerobic conditions and at elevated physiological temperature. We propose that lower invasion and suppressed expression of immunogenic SPI-1 components under these physiologically relevant conditions contribute to the restrained-inflammatory infection elicited by S. Paratyphi A.

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CHARACTERIZATION OF SRNAS IN THE ACID TOLERANCE RESPONSE AND VIRULENCE OF *SALMONELLA* TYPHIMURIUM

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Background: Salmonella enterica serovar Typhimurium is a Gram-negative, neutralophilic enteropathogen that is disseminated through contaminated food and water. Globally, it is responsible for 80.3 million cases of food-borne gastroenteritis and about 1.5 million deaths. During pathogenesis of the host, it encounters a number of stress conditions namely, pH, oxygen limitation, bile salts and antimicrobial peptides of which, low pH serves as one of the first lines of defense presented by the host. The phenomenon by which Salmonellae are able to sense low pH environments and mount an adaptive counter response is termed the acid tolerance response (ATR). It is relatively recently that small RNAs have

been investigated for their roles in stress and virulence networks. These non-coding RNA molecules serve as activators or repressors by binding to target mRNAs or proteins, causing either degradation of their targets or melting of inhibitory structures at the ribosome binding site. Previous work from our laboratory on the global transcriptome of Salmonella under the ATR revealed a large number of sRNAs in addition to several protein coding genes, to be differentially expressed under this response. A subsequent study on the sRNA DsrA in Salmonella revealed its influence on the acid tolerance response of this pathogen. Additionally, an isogenic mutant was unable to cause inflammation in a streptomycin pre-treated mouse model, both results highlighting the important role of sRNAs in stress response and virulence. **Methods:** RNA-seq, acid tolerance response assays, in-vitro and in-vivo virulence assays, bioinformatic predictions, dual reporter systems. **Results:** RNA-seq data analysis revealed six candidate sRNAs significantly up-regulated under the ATR. One such sRNA RyeC was shown to have minimal influence on the ATR, however, isogenic mutants of the same were shown to possess increased motility, higher adhesion and invasion of epithelial cell lines (HCT116 and HeLa) as well as increased ability to replicate within mouse macrophages (RAW 264.7). Both, promoter analysis using GFP reporter assays, as well as qRT-PCR of sRNA levels revealed RyeC to be constitutively expressed with highest levels observed during the late exponential phase in rich medium. Target prediction of this sRNA using three algorithms (CopraRNA, RNApredator and TargetRNA2) identified several targets, of which ptsI was predicted by all three to be regulated through complementary base-pairing of an 11 nucleotide stretch between RyeC and ptsI. **Conclusion:** The sRNA RyeC of Salmonella Typhimurium plays a role in virulence of this pathogen through regulation of its target ptsI.

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IDENTIFYING THE VIRULENCE AND BIOFILM-FORMING TARGETS OF CSGD USING CHROMATIN IMMUNOPRECIPITATION (CHIP) AND NEXT-GEN SEQUENCING

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Salmonella species cause 94 million cases of gastroenteritis annually, and despite decades of research, many aspects of Salmonella pathogenicity and transmission still remain unclear. We have recently demonstrated that a pure culture of Salmonella enterica serovar Typhimurium (S. Typhimurium) exposed to environmental stress differentiates into two specialized cell types: planktonic cells and multicellular aggregates. This population divergence may promote transmission under variable conditions; aggregates can resist harsh environmental conditions until an opportunity for infection arises, whereas planktonic cells express virulence factors and are immediately able to infect a host. Previous RNA-seq data revealed differential expression of 34% of genes between the two cell types, which is directed in part by bistable expression of CsgD, the Salmonella central biofilm regulator. We are using Chromatin immunoprecipitation (ChIP), coupled with Next-Gen DNA Sequencing (ChIP-seq) to aid the global identification of genes that are directly controlled by CsgD. ChIP-seq DNA samples were prepared from S. Typhimurium 14028s and a CsgD overexpressor strain for comparison to maximal CsgD binding, at time points significant for biofilm production and population maturity, and with two different monoclonal antibodies for epitope binding. Identification of the genetic targets bound by CsgD will illuminate our understanding of population divergence and the bistable switch

and potential differences in transmission or virulence between the two cell types. This information will become part of new strategies to reduce transmission of S. Typhimurium and other gastroenteritis-causing serovars.

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DETECTION OF HILA GENE OF *SALMONELLA ENTERICA* SEROVAR TYPHI AND ANTIBIOGRAM

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Salmonella enteric serovar Typhi is a gram-negative, rod-shaped facultative anaerobe that only infects humans which is major causative agent for typhoid fever. Enteric fever is a systemic infection of reticuloendothelial system caused by the human adapted pathogens Salmonella enterica serotype Typhi and Salmonella enterica serotype paratyphi A, B. However, the prevalence of Salmonella enterica B and C infection has not been reported in Nepal so for. These are important causes of febrile illness in crowed and impoverished population with poor sanitation and food habits.A total of 141 blood samples were collected after inform consent form. Salmonella enteric serovar Typhi was isolated from patients with typhoid fever and identification of the isolates was confirmed through microbial examination like biochemical and antibiotic susceptibility test using different antibiotics like chloramphenicol, tetracycline, ampicillin, cotrimoxazole, ofloxacin and nitrofurantoin through Kirbybauer technique. Moreover, PCR was employed in which hilA gene was amplified for the molecular confirmation. Among them 21 (14.9%) were culture positive and 66(46.9%) were PCR positive. Out of 141 suspected cases 78 were male patients and 63 were from female patients. PCR positivity cases were higher in male patients then female patients. PCR positivity cases were higher in age groups 0-15 which was followed by age groups 15-30 and age groups 30-45. This study confirmed the association of virulent strains of S. enteric serovar Typhi from typhoid fever among human population and suggested that PCR based diagnostic could be very useful for the early detection of S. Typhi isolates. This study is a baseline study which can be further implemented in proper diagnosis, screening, immunological assay and drug testing.

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THE EFFECTS OF ENVIRONMENTAL STRESS ON THE ANTIBIOTIC RESISTANCE PHENOTYPE OF *SALMONELLA* TYPHIMURIUM

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Bacteria work to reduce the effects of sub-lethal stress by making phenotypic and genotypic changes. Phenotypic changes may provide resistance to further stress events and may also lead to cross-protection against a number of environmental challenges including resistance to antibiotics which bacteria see as a form of environmental stress. The effects of stress (temperature, pH, osmotic effects and starvation) on the antibiotic resistance phenotypes of S. Typhimurium DT104 were investigated in vitro. The antibiotics; tetracycline (T), streptomycin (S) and trimethoprim and sulfamethoxazole (SXT) were selected as resistance to T, S and SXT has been frequently observed in Salmonella isolated from farm animals in Northern Ireland. The investigation was carried out using standard antibiotic disk diffusion methodologies adapted to incorporate a stress inducing step. In terms of outcomes, starvation stress had the most effect on the susceptibility of Salmonella to the three antibiotics tested. Of the three antibiotics tested, Streptomycin was

the least effective under starvation, osmotic and low temperature stresses. These results indicate that some types of sub-lethal stress may contribute to the expression of antibiotic resistance in Salmonella.

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INVESTIGATION OF THE DIVERSITY OF ANTIBIOTIC RESISTANCE GENES AND MOBILE GENETIC ELEMENTS IN *SALMONELLA* ASSOCIATED WITH U.S. FOOD ANIMALS

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With the emergence of antibiotic resistance (AR), multidrug resistance (MDR), and carbapenem resistant Enterobacteriaceae (CRE), the specter of widespread untreatable bacterial infections threatens human and animal health. The ability of these emerging resistances to transfer between bacteria on mobile genetic elements (MGEs) could cause the rapid establishment of MDR bacteria in animals leading to a foodborne risk to humans. To investigate this, we identified AR genes, plasmids, and integrons in the genomes of Salmonella enterica isolated from animal sources.To obtain the greatest variety of AR genes and MGEs, Salmonella enterica isolates (n=193) from beef and dairy cattle, chicken, swine, turkey, and their meat products were selected based on their resistance phenotypes, serovar, and PFGE patterns, resulting in 75 serovars and diverse PFGE patterns within a serovar being selected. Isolates were sequenced using an Illumina HiSeq. Draft genomes were assembled using A5miseq pipeline with an average of 115 contigs per isolate and annotated with Prokka. Resistance genes were identified using ARG-ANNOT and integrons were identified using INTEGRALL. Plasmid sequences were identified by the presence of replicon and relaxase genes or homology to known plasmids. A total of 913 AR genes were detected in the 193 isolates. Class I integrons were detected in 65/193 isolates. Forty-four integrons contained a single gene cassette (aadA of various alleles n=38, dfrA of various alleles n=5, and carb n=1). Nine integrons had multiple genes (five with aadB and cmlA, three with aadA and dfrA, and one with aadA and estX-3), two had no AR gene, six had incomplete AR gene sequences, and another six were novel AR gene cassettes. Plasmids were detected in 155/193 isolates. The most prevalent replicons detected in the isolates were A/C (n=32), colE (76), F (43), HI1 (4), HI2 (21), I (62), N (4), Q (7), and X (35). AR genes were found on most of the plasmids, with many of them encoding MDR. For example, 27 of the 32 A/C plasmids contained AR genes with 16 different patterns of AR genes and as many as ten AR genes detected (e.g. tetAR, strAB, sulII, cmy, floR, aadB, cmlA, and aph3). AR genes on the other plasmid replicons were also highly variable representing a diversity of AR and MDR genotypes resulting in their observed antibiotic resistances. Most isolates contained plasmids, integrons, or both encoding AR or MDR. Many of the mobile elements and AR genes have been previously found in Salmonella; however, they are arranged differently and have not previously been found in animal associated isolates or in the serovars analyzed. The identification of AR genes, plasmids, and integrons demonstrates linkage of MGEs and AR in these food animal associated Salmonella. This begins to reveal the complexity of AR and MGE assembly and spread among Salmonella in the food animal environment.

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COMPARATIVE ANALYSIS OF CUEP AND CUS FOR *SALMONELLA* PERIPLASMIC COPPER HOMEOSTASIS AND VIRULENCE

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Copper is an essential ion that participates in enzymatic reactions carried out by bacterial periplasmic cuproproteins such as cytochrome oxidases, NADH dehydrogenases, Cu,Zn-superoxide dismutases, laccases and multicopper oxidases, among others. It is, at the same time, extremely reactive causing damage to proteins, lipids and other cellular components. Most enterobacterial species harbor a copper-responsive two-component system, CusR/CusS, to control the ion levels in the cell envelope. CusR/CusS responds to the surplus of periplasmic copper inducing the expression of the CBA-type efflux complex CusC(F)BA that pumps out of the cell the excess of the metal ion. Most *Salmonella* serotypes lack both the genes coding for CusR/CusS and the operon encoding the CusC(F)BA efflux complex, and different lines of evidence suggest that envelope protection against copper overload depends on CueP, a major copper-binding protein in the periplasm required for macrophage survival and virulence. The *Salmonella*-specific CueP-coding gene was originally identified as part of the Cue regulon under the transcriptional control of the cytoplasmic copper sensor CueR, but its expression differs from the rest of CueR-regulated genes. We now show that *cueP* expression is controlled by the concerted action of CueR, which detects the presence of copper in the cytoplasm, and by CpxR/CpxA that monitors envelope stress. The integration of two ancestral sensory systems -CueR, which provides a signal-specificity, and CpxR/CpxA that detects stress in the bacterial envelope- restricts the expression of this periplasmic copper resistance protein only to cells encountering surplus copper that disturbs envelope homeostasis, emulating the role of the CusR/CusS regulatory system present in other enteric bacteria. Furthermore, we show that coordinated regulation of *cueP* by CueR and CpxR/CpxA is required for optimal growth. CueP expression from a CpxR-independent promoter impairs growth during exponential phase. In addition, this strain shows a marked growth defect in presence of H_2O_2. Finally, a strain with the *Escherichia coli cus* locus in place of *cueP* displayed wild-type resistance to the metal ion but showed a deficiency in macrophage survival, suggesting that *cueP* acquisition by *Salmonella* was necessary to establish its intracellular lifestyle.

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EVALUATION OF THE *SALMONELLA ENTERICA* SEROVAR PULLORUM PATHOGENICITY ISLAND 2 MUTANT AS A LIVE ATTENUATED VACCINE CANDIDATE

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Background: Salmonella enterica serovar Pullorum (S. Pullorum) causes Pullorum disease (PD), a severe systemic disease of poultry and results in considerable economic losses in developing countries. In order to develop a safe and immunogenic vaccine, the immunogenicity and protective efficacy of S06004ΔSPI2, a Salmonella pathogenicity island 2 (SPI2) deleted mutant of S. Pullorum was evaluated in 2-day old chickens. **Results:** S06004ΔSPI2 was severely less virulent than the parental wild-type strain S06004 as determined by the 50% lethal dose (LD50) for 3-day-old chickens when injected intramuscularly. Single intramuscular vaccination with S06004ΔSPI2 (2 × 107 CFU) of chickens revealed no differences in body weight or clinical symptoms compared to control group. Two-day-old chickens immunized with a single oral dose of S06004ΔSPI2 showed no differences in body weight or

clinical symptoms compared with those in the negative-control group. S06004ΔSPI2 bacteria can colonize and persistent in liver and spleen of vaccinated chickens approximately 14 days, and specific humoral and cellular immune responses were significantly induced. Immunized chickens were challenged with S. Pullorum strain S06004 and Salmonella enterica serovar Gallinarum (S. Gallinarum) strain SG9 at 10 days postimmunization (dpi), and efficient protection against the challenges was observed. None of the immunized chickens died, the clinical symptoms were slight and temporary following challenge in immunized chickens compared with those in the control group, and these chickens recovered by 3 to 5 dpi. **Conclusion:** These findings suggest that S06004ΔSPI2 appears to be a highly immunogenic and efficient live attenuated vaccine candidate.

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OPTIMIZATION OF A *SALMONELLA ENTERICA* SEROVAR PARATYPHI C MURINE INFECTION MODEL FOR USE IN VACCINE DEVELOPMENT

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Background: Non-typhoidal *Salmonella* (NTS) are responsible for 94 million cases and 155,000 deaths worldwide annually. More than 50 serogroups have been described based on surface OPS antigens, however only a handful are associated with disease in animals and humans. In the United States, *Salmonella* serogroups B, D, C_1 and C_2-C_3 cause the majority of human infections. Multiple serogroup B (e.g., *S.* Typhimurium) and D (e.g., *S.* Enteritidis) vaccines have been evaluated in animal models. However, very little work has been performed to date to develop vaccines that can target serogroups C_1 and C_2-C_3. In this study, our goal was to develop a murine model that can be used to evaluate candidate vaccines that target *Salmonella* serogroup C_1. **Methods:** Sixteen *S.* Paratyphi C (6,7:c:1,5) strains were isolated from blood of febrile patients in Bamako, Mali. Identification of the strains was confirmed by serum agglutination and Multi-Locus Sequence Typing (MLST). Oral (p.o.) and intraperitoneal (i.p.) routes of infection of BALB/c and CD-1 mice were used to assess lethality produced by eight of these strains. We also developed a colonization model in which mice were administered 10^6 CFU (BALB/c, p.o.) or 10^5 to 10^8 CFU (CD-1, i.p.) of *S.* Paratyphi C I8, and spleen and liver were harvested at various time-points ranging between 6 h and 5 days. Bacterial loads in these organs were then measured by plating dilutions of homogenized organs. **Results:** Single-dose infection as well as LD_{50} experiments showed that four strains (I8, I30, Q87 and R77) are highly lethal in BALB/c mice (LD_{50} i.p. $<10^4$ CFU and LD_{50} p.o. = 10^3 CFU) but not in CD-1 mice (LD_{50} i.p. and p.o. $> 10^7$ CFU). The other four strains (P53, P114, P134 and J82) were not lethal in BALB/c mice at a dose of 10^8 CFU i.p. In addition, there was high variability in the dose required to consistently cause 100 % mortality. Since using lethality as an endpoint proved inconsistent, we developed an organ colonization model, whereby we assessed bacterial load in the spleen and liver at different time-points following infection. In our optimized model, all BALB/c mice infected orally with 10^6 CFU of I8 had no detectable counts in the spleen or liver at days 1 and 3 post-infection. All mice had detectable loads at day 5 p.i. in the spleen. In contrast, CD-1 mice infected i.p. with I8 had detectable bacterial loads as early as 6 hours post-infection, with higher bacterial counts found in mice given higher challenge doses up to 10^7 CFU. **Conclusions:** *S.* Paratyphi C infection of mice caused an unusual, highly variable lethality pattern. Here we describe an optimized organ colonization model whereby BALB/c mice were preferred for their ability to be infected orally, a more relevant route of administration for a *Salmonella* infection model. We are currently using this model to evaluate vaccines that target *Salmonella* serogroup C1.

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OPTIMIZATION OF A *SALMONELLA ENTERICA* SEROVAR TYPHIMURIUM LIVE-ATTENUATED VACCINE CANDIDATE

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Background: Non-typhoidal *Salmonella* (NTS) are a common cause of gastroenteritis. In the United States, 1.2 million cases of NTS occur each year, costing the economy $3.3 billion. The most common causes of Salmonellosis in the United States are serovars Typhimurium, Enteritidis and Newport. To protect against NTS disease, we have been optimizing live-attenuated vaccine candidates against these three serovars. This approach was chosen as it has previously been used successfully for *Salmonella* Typhi. In early experiments, we showed that a live-attenuated *Salmonella* Typhimurium vaccine candidate, CVD 1921 (I77 Δ*guaBA* Δ*clpP*), is safe and immunogenic in rhesus macaques. While promising, the vaccine was shed for up to 10 days post-immunization. In mice, vaccinemia was also noted at low levels (1/15 mice). This study describes improvements to the safety of this vaccine. **Methods:** Mutations were created in two genes, *pipA* and *htrA*, by lambda red recombination. The *htrA* mutation is known to be attenuating, and has previously been used in *Salmonella* live-attenuated vaccines. The *pipA* gene has been shown to be required for fluid accumulation, which is used as a proxy for diarrhea, in *Salmonella* Dublin. Rabbit ileal loops were infected with 10^8 CFU of I77, or an I77 Δ*pipA* mutant, and assessed for fluid accumulation after 18 hours of incubation. To assess vaccine efficacy, 6-8 week old mice were given three doses of live-attenuated vaccine (10^9 CFU/dose) by oral gavage. At one or three months post immunization, mice were challenged perorally with 100-700 × LD_{50} (50% lethal dose) of the wild-type strain, I77. Survival rates were used to calculate vaccine efficacy, and significance was determined using Fisher's exact test. **Results:** We confirmed that *pipA* is required for fluid accumulation in *Salmonella* Typhimurium by using a rabbit ileal loop model of gastroenteritis. Inoculation of loops with an I77 Δ*pipA* mutant induced 50% less fluid accumulation than the wild-type strain, I77. We also added a *htrA* mutation to further attenuate the vaccine strain. This new strain, CVD 1926 (I77 Δ*guaBA* Δ*clpP* Δ*pipA* Δ*htrA*), was 100% safe in mice (n=30) after three immunizations. Anti-LPS serum IgG titers elicited by CVD 1921 Δ*pipA* and CVD 1926 were similar. Upon lethal challenge with 100 × LD_{50} of wild-type *Salmonella* Typhimurium I77, both vaccine candidates showed equal levels of protective efficacy (64% and 60%, respectively). **Conclusions:** The revised vaccine candidate, CVD 1926, incorporates two additional mutations which decrease fluid secretion and increase vaccine safety. Despite being less reactogenic than its precursors, this new vaccine strain is equally as immunogenic and protective as previous iterations in a mouse model.

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GENOMIC ANALYSIS OF *SALMONELLA ENTERICA* SEROVAR HADAR ISOLATED FROM BROILER CHICKEN

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Background: Hadar is among the top Salmonella enterica serovars associated with poultry that cause Salmonellosis in humans. The colonization of the chicken gut by this serovar could result in contamination of the environment and the food chain. The objective of this study was to compare the genomes of diverse isolates of S. enterica serovars Hadar to better understand their virulence and antibiotic resistance potential. **Methods:** The

genomes of three previously sequenced S. Hadar isolated from chickens [#ABB1048-1 (resistant to streptomycin and tetracycline); #ABBSB1020-2 (resistant to amoxicillin-calvulanic acid, ceftiofur, ceftriaxone, ampicillin, cefoxitin, streptomycin, sulfisoxazole and tetracycline) and #ABBSB1121-1 (resistant to ampicillin, trimethoprim/sulphamethoxazole and sulfisoxazole)] were used. These genomes were compared to those of 28 other Hadar isolates from the PATRIC database. The CD-HIT program was used to build clusters of protein sequences with a minimum of 97% sequence identity. The distribution of proteins across the 31 isolates was then examined. **Results:** A total of 147,728 protein sequences were assigned to 6531 clusters; of these, 3957 were encoded by a single gene in all 31 genomes. Several of these universal proteins confer resistance to multiple drugs, metal and organic solvents or have virulence-associated functions including adhesion and anaerobic survival. CRISPR-associated proteins (cas and cse) were identified in all 31 Hadar isolates which also harbour iron-enterobactin and -ferrichrome as well as ferrous iron uptake operons. The efflux pump AcrD and the aminoglycoside n(6')-acetyltransferase genes were detected in all isolates in addition, six isolates including one of the chicken isolates also carried the aminoglycoside n(3')-acetyltransferase gene cluster among which five also carried the dihydropteroate synthase type-2 conferring sulfonamide resistance. In these isolates, a transposon Tn21 protein of unknown and an integron integrase associated with conjugative DNA transfer elements were also found. Isolates #ABBSB1020-2 and #ABBSB1121-1 carried the mobile element transposon Tn21 resolvase. Six isolates including #ABBSB1020-2 harboured the Inc1 type plasmid. **Conclusion:** This whole-genome sequencing analysis showed that despite conservation of most of the core genome proteins across Hadar isolates, dissimilarities that could reflect their origin exist between them. This study also showed that chicken Hadar isolates could be a reservoir of antimicrobial resistance genes that could be disseminated to other bacteria.

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INACTIVATED ORAL VACCINES AND WITHIN-HOST DYNAMICS REVEAL THE PROTECTIVE MECHANISM OF BACTERIAL SURFACE-SPECIFIC IGA

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Bacterial enteropathogens remain a major socio-economic burden in both humans and domestic animals. The only known adaptive immune components capable of influencing enteropathogenic colonization of the intestinal tract prior to tissue invasion are secretory antibodies, particularly IgA. However, the functioning of this isotype is poorly understood, hampering the development of effective oral vaccines. We have developed an efficient method to generate high-dose inactivated oral vaccines from a broad range of species/serovars. When 1010 particles of these vaccines are administered weekly over 3 weeks, a robust specific IgA response is induced in the absence of any inflammatory or pathological sequalae. A Salmonella Typhimurium vaccine constructed in this way provided dose-dependent protection from non-typhoidal Salmonellosis. Concordant with the "extracorporeal" function of secretory IgA, this vaccine was also protective against non-Typhoidal Salmonellosis in models of severe innate immune deficiency. We could subsequently demonstrate that IgA-mediated protection from non-Typhoidal Salmonellosis was attributable to massive aggregation of the lumenal Salmonella population, resulting in a 100-fold decrease in invasive bacteria. Critically, IgA-mediated cross-linking produced aggregates predominantly by non-segregation during bacterial replication, and not by classical agglutination. Thus aggregates have a clonal/oligoclonal structure which has striking conse-

quences on the effective pathogen population size, the infection kinetics and the pathogen's within host evolution. In conclusion, pathogen specific sIgA interferes with segregation and this overlooked function has profound effects for the pathogen-host interaction.

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LINALOOL-INDUCED AGGREGATION IN *SALMONELLA* SENFTENBERG

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Background: Basil is not normally associated with foodborne pathogens and infections, probably due to its production of various essential oils. However, following the 2007 basil outbreak in Europe, caused by *Salmonella enterica* serovar Senftenberg (*S.* Senftenberg), it was important to comprehend the antimicrobial activity of basil essential oils and the mechanisms in which *Salmonella* may overcome them. It was found that linalool, one of the major constituents of basil oil, perforates bacterial membranes, inhibits cell motility and induces cell aggregation. Interestingly, the aggregation is observed much faster with a linalool-adapted mutant of *S.* Senftenberg (termed as LASS) and larger aggregates are formed. Herein, we aim to reveal the mechanism(s) that lead to the linalool-induced aggregation. **Methods and Results**: Components in the medium that may have resulted from cell response to linalool and serve as aggregation factors were investigated by a conditioned medium assay, in which fresh planktonic cells of *S.* Senftenberg and LASS were incubated with the supernatant of such cells that had been incubated with linalool. Particle size distribution of the cultures demonstrated that bacterial factors were involved in the aggregation process. By using physical and chemical perforating methods (e.g. sonication and eugenol addition, respectively) it was shown that the aggregation is apparently induced from treatments that cause pores in the cell and leakage of intracellular components. Since an increase of protein concentration was observed during incubation with linalool, aggregation measurements were next performed after the proteins in the conditioned medium were degraded by proteinase K preceding incubation with the planktonic cells. As a consequence, the aggregation was inhibited, indicating that the proteins are involved in the aggregation process. Incubating planktonic cells of *Salmonella* with other proteins (e.g. bovine serum albumin) further supported this hypothesis. **Conclusions:** Linalool-induced aggregation of *S.* Senftenberg is mediated by leakage of intracellular proteins caused by linalool perforating effect. A further study could assess this aggregation potential role to resist membrane-targeted treatments.

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PREPARATION AND EVALUATION OF O-SPECIFIC POLYSACCHARIDE BASED CONJUGATES WITH DIPHTHERIA TOXOID AGAINST HUMAN SALMONELLOSIS

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Background: *Salmonella enterica* serovars Typhi (*S.* Typhi) and Paratyphi A (*S.* Paratyphi A) are main bacterial pathogens causing typhoidal diseases in humans. Typhoidal infections cause mild to moderate gastroenteritis and in some cases lead to sever systemic infections including typhoid and paratyphoid fever. Currently licensed typhoid vaccines are based on capsular polysaccharide (Vi) of *S.* Typhi and have certain limitations regarding their efficacy and inability to induce an immune response especially in individuals under 2 years of age. Conjugate vaccines which consist of a bacteria-specific polysaccharide (OSP) chemi-

cally bound to a carrier protein overcome these problems by inducing a T-cell dependent immune response characterized by enhanced immunogenicity in all ages. **Methods:** We have extracted and purified lipopolysaccharides (LPS) of *S.* Typhi and *S.* Paratyphi A followed by their acid hydrolysis resulting in purified OSP of these pathogens. The antigenicity of the purified LPS and OSP was found adequate by double immunodiffusion assay against indigenously generated hyper immune mice sera. The OSP of *S.* Typhi and *S.* Paratyphi A were conjugated to diphtheria toxoid (DT) using adipic acid dihydrazide (ADH) as linker and purified using size exclusion chromatography. High performance liquid chromatography (HPLC) analyses of *S.* Typhi and *S.* Paratyphi A samples including OSP, DT, OSP-ADH and OSP-ADH-DT conjugates were performed. Immunogenicity of the synthesized conjugates was evaluated in mice and anti-LPS IgG titers were determined by ELISA. Use of DT as carrier protein for *Salmonellae* has not been reported before. **Results:** *S.* Typhi OSP-AH-DT conjugate 1 and 2 elicited significantly higher ELISA antibody titer (P = 0.0241 and 0.0245 respectively) than polysaccharide alone. Three-dose injection schedule-B (weeks 0-4-8) was found better than schedule-A (weeks 0-2-4). The conjugate of *S.* Paratyphi A OSP with DT without linker molecule did not elicit sufficient immune response to be used as conjugate vaccine candidate while antibody response against *S.* Paratyphi A OSP-AH-DT conjugate was found significantly higher (P = 0.0446) than polysaccharide alone. **Conclusions:** We demonstrated diphtheria toxoid as a potential carrier protein for conjugate vaccine candidates using OSP from *S.* Typhi and *S.* Paratyphi A. Conjugates of OSP from human *Salmonella* with DT induce superior immune response than LPS alone.

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A NOVEL BINDING MODE BY H-NS DRIVES CHROMOSOME COMPACTION AND GENE SILENCING

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H-NS, is a nuclear-associated histone-like protein that regulates DNA condensation. In addition, H-NS can selectively repress laterally acquired genes through binding AT-rich regions of the genome. Despite the fundamental nature of H-NS-DNA interactions, the molecular details of how H-NS interacts with DNA are lacking, which in turn precludes an understanding of the molecular basis of gene silencing and regulation. A C-terminal DNA binding domain construct lacking the flexible linker displayed a substantially lower affinity for DNA compared to the full-length protein. We reasoned that the linker might be involved in promoting H-NS/DNA binding functions. We therefore set out to examine the role of the linker connecting the two H-NS domains (oligomerization and DNA binding). We observed that a linker deletion of H-NS led to a significant decrease in gene repression that was not relieved by replacement with a dummy linker. Hence, the H-NS linker does not function as a completely passive tether connecting two functional domains, and some amino acids in the linker must contribute to its function. Mutant proteins were over-expressed and purified and their binding behavior was analyzed by Atomic Force Microscopy (AFM), using the csgD promoter region as a DNA target. We also tracked the interaction of H-NS:DNA in vivo by monitoring the movement of PAmCherry-tagged H-NS using single-particle tracking and have localized H-NS in E. coli using super-resolution PALM imaging. Our study reveals that the H-NS linker promotes DNA

binding and therefore plays an important role in gene regulation and chromosome condensation. Since hns is widely distributed in the enterobacteriaceae, we compared 8 representative H-NS proteins from different genus including Salmonella, Citrobacter, Enterobacter, Shigella, Klebsiella, Serratia, and Yersina. H-NS proteins are highly conserved, the similarity between proteins was >92%. Thus, it seems likely that this mechanism is also conserved. Supported by RCE in Mechanobiology from MOE, Singapore, VA 5IO1BX-00372 and AIR21-123640 to LJK.

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RNA CHAPERONS, CSPC AND CSPE REGULATE MRNAS INDUCED IN *SALMONELLA* SURVIVING IN MACROPHAGES AFTER PHAGOCYTOSIS

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Background: Cold shock proteins (Csps), an RNA chaperone family widely conserved in bacteria, regulate cell functions through direct binding to their target mRNAs and thereby modulate the mRNA stability. Of the six Csps contained in *S. enteirca* serovar Typhimurium, CspC and CspE share 84% identity and are constitutively expressed under physiological condition. Whereas the involvement of CspC and CspE in adaptation to environmental stress is well-investigated, the role on virulence remains unknown. In this study, we studied the regulation of mRNAs by CspC and CspE in *Salmonella* surviving in macrophages after phagocytosis because the intracellular survival is a key strategy of *Salmonella* infection. **Methods:** RNA-seq was applied to globally analyze the quantity of mRNA prepared from the wild type strain and the *cspC* and *cspE*-disrupted strain at 2 hours after phagocytosis by RAW264.7. To identify the target mRNA molecules of CspC and CspE, *in vivo* mRNA stabilities were examined. To reveal the binding region of CspC and CspE, the prediction of secondary structure in mRNA molecules was performed by Vienne RNA fold. The putative binding structures were destroyed by introducing a *gfp* gene into the target gene. **Results:** First, we examined the effect of *cspC* and *cspE* disruption on the ability of *Salmonella* to survive intracellularly after phagocytosis by RAW264.7. The result indicated that CspC and CspE are involved in the early intracellular survival of *Salmonella*. Our RNA-seq approach obtained 58 genes as candidates of those regulated by CspC and CspE. Out of those 58 genes, *STM1630*, *STM3132*, *STM3133*, and *pagK* were observed to be specifically upregulated in response to phagocytosis by macrophages. Moreover, the presence of CspC and CspE stabilized *STM1630* mRNA. The analysis with the translational *STM1630-gfp*-fusions suggested that CspE binds to multiple stem-loops within the coding region. **Conclusions:** Our findings suggest that CspC and CspE play a critical role in the intracellular survival of *Salmonella* by stabilizing several mRNA molecules specifically upregulated in response to phagosomal environment.

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COMPARATIVE MULTI-CONDITION INTERSTRA*IN TRANS*CRIPTOMICS IDENTIFIES UNEXPECTED DIFFERENCES BETWEEN GLOBAL AND AFRICAN SEQUENCE TYPES OF *SALMONELLA* TYPHIMURIUM

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Background: Salmonella Typhimurium infects a wide range of animal hosts, and generally causes a self-limiting gastroenteritis in humans. However, some variants of this serovar, sequence-type ST313, are causing an emerging invasive Salmonella disease in sub-Saharan Africa that targets susceptible HIV+, malarial and malnourished individuals. A genomic comparison between an ST313 isolate, D23580, and the well-characterized gastroenteritis isolate 4/74 (sequence-type ST19) showed that the two strains share 96% of coding genes. Genetic differences include 1000 SNPs, two D23580-specific prophages and the presence of pseudogenes. **Methods:** To investigate the hypothesis that altered gene expression patterns reveal distinct virulence mechanisms between ST19 and ST313, RNA-seq-based transcriptomic data were obtained for strains 4/74 [1,2] and D23580, grown under sixteen infection-relevant in vitro conditions and during infection of murine macrophages. Key transcriptomic data were validated with a proteomic approach. **Results:** Comparative transcriptomics of the two bacterial strains showed that between 1 to 6% of all ST19 and ST313 genes were differentially-expressed in individual stress conditions. Early stationary phase (ESP) is a condition that induces the SPI1-encoded invasion system, and 2% of genes showed differential expression. Proteomics confirmed that 16% of the differentially-expressed genes also showed altered expression at the protein level in the ESP condition. **Conclusions:** We are investigating whether the differences observed in gene expression of virulence-associated genes under specific environmental conditions reflect the distinct pathogenic mechanisms of these two S. Typhimurium strains. **References:** [1] Kröger et al (2013) Cell Host Microbe 14:683-95. [2] Srikumar et al (2015) PLoS Pathog 11:e1005262.

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USE OF ISOGENIC TAGGED STRAINS TO STUDY THE IMPACT OF ANTIMICROBIALS ON THE WITHIN-HOST DYNAMICS OF *SALMONELLA* BACTERIAL INFECTIONS

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Background: Antimicrobials do not always determine the rapid and complete resolution of acute infections, resulting in carrier states or in the relapse of infections upon cessation of the treatment. More effective treatments and eradication of infections will benefit from a better understanding of how bacterial growth dynamics are modified under antimicrobial pressure. **Methods:** We have studied in a murine infection model, the effects of two widely used classes of antimicrobials (the β-lactam ampicillin and the fluoroquinolone ciprofloxacin) on the early stages of treatment using Isogenic Tagged Strains (ITS) of S. enterica serovar Typhimurium. We used a wild-type fast-growing strain, an ΔaroC slow-growing strain, and a ΔsseB strain with reduced growth and ability to spread from cell to cell. We have followed the dynamics of bacterial populations before, during, and upon the cessation of antimicrobial treatment. We have determined total bacterial counts in the infected organs (spleen, liver, mesenteric lymph nodes (MLN), and blood), and analysed numerical and spatial fluctuations of ITS subpopulations using a sequencing-based approach combined with a novel method for Bayesian bottle-neck analysis. **Results:** Both antibiotics reduced (up to ~95%, with ciprofloxacin producing the highest reduction) bacterial loads of the wild type bacteria in spleen, liver and blood, with a marked and constant drop during the first days of treatment followed by a phase of more moderate effect. Cessation of the treatment resulted in an immediate relapse of the infection. The antimicrobials had smaller effect on CFU counts in MLN during treatment, but a

strong increase in bacterial numbers was still observed upon cessation of antibiotic therapy. Treatment of infections with the ΔaroC strain and ΔsseB strains showed a smaller but continuous reduction in CFU counts in spleens and livers; in both infections cessation of the treatment resulted in a carrier state. Both antimicrobials acted homogeneously on all the bacterial populations within various organs and at all the time-points. Relapse of the infection with the wild type strain was not due to the amplification of a restricted number of ITS subpopulation, similarly to what observed also for the carrier states observed after the stop of antimicrobials in ΔaroC and ΔsseB infections. **Conclusions:** The efficacy of ampicillin or ciprofloxacin treatment is more pronounced in infections with fast-growing strains and more marked in the early stages of treatment. Antibiotic pressure does not select for ITS subpopulation. Chronic and relapsing infections do not appear to be caused by the persistence or amplification of selected subpopulations of Salmonella.

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RNA-SEQ BRINGS NEW INSIGHTS TO THE INTRA-MACROPHAGE TRANSCRIPTOME OF *SALMONELLA* TYPHIMURIUM

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Background: The burden of Salmonellosis remains unacceptably high throughout the world and control measures have had limited success. Because Salmonella bacteria can be transmitted from the wider environment to animals and humans, the bacteria encounter diverse conditions that include food, water, plant surfaces and the extracellular and intracellular phases of infection of eukaryotic hosts. Although Salmonella enterica serovar Typhimurium is arguably the world's best-understood bacterial pathogen, crucial details about the genetic programs used by the bacterium to survive and replicate in macrophages have remained obscure due to the challenge of studying gene expression of intracellular pathogens during infection. **Methods:** We used RNA-seq to reveal the transcriptional architecture of Salmonella during infection of murine macrophages. Here, we report the coding genes, sRNAs & transcriptional start sites that are expressed within macrophages at 8 hours after infection, and use these to infer gene function. **Results:** We characterized 3583 transcriptional start sites that are active within macrophages, and highlight 11 of these as candidates for the delivery of heterologous antigens from Salmonella vaccine strains. Salmonella Pathogenicity Islands SPI13 and SPI2 were the most highly expressed pathogenicity islands during Salmonella intra-macrophage survival. We identified 31 S. Typhimurium genes that were strongly up-regulated inside macrophages but expressed at low levels during in vitro growth. A majority (88%) of the 280 S. Typhimurium sRNAs were expressed inside macrophages. The SalComMac online resource [http://tinyurl.com/SalComMac] allows the visualisation of every transcript expressed during bacterial replication within mammalian cells. **Conclusions:** This primary transcriptome of intra-macrophage S.-Typhimurium describes the transcriptional start sites and the transcripts responsible for virulence traits, and catalogues the sRNAs that may play a role in the regulation of gene expression during infection.

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SALCOMREGULON ADDS REGULATORY INSIGHTS TO THE ONLINE SALCOM *SALMONELLA* GENE EXPRESSION COMPENDIA

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RNA-sequencing (RNA-seq) has become the tool of choice to analyse bacterial transcriptomes, but the comprehensive visualisation of these large datasets remains a challenge. In 2013, we launched the free online Salmonella expression compendium (SalCom) to make our S. Typhimurium transcriptomic data easily accessible for the Salmonella community. The website contains gene expression data of all Salmonella enterica serovar Typhimurium (S. Typhimurium) genes expressed in 22 infection-relevant growth conditions (Kröger et al., Cell Host Microbe. 2013;14(6):683-95). We have recently extended our collection to create the SalComMac website which adds Salmonella expression data from infected RAW 264.7 macrophages to the original SalCom database (Srikumar, Kröger et al., PLoS Pathog 2015;11(11):e1005262). Here, we present the newest addition to the SalCom compendia: SalComRegulon which contains gene expression data of 18 S. Typhimurium mutant strains lacking key virulence-associated regulatory proteins. Using infection-relevant growth conditions, we identified a total of 1257 genes that are controlled by one or more regulatory system, including a sub-class of genes that reflect a new level of cross-talk between SPI1 and SPI2. The SalCom, SalComMac and SalComRegulon databases are easy to interrogate and interpret using heatmaps. The flexible search function allows the identification of gene expression patterns of custom gene lists, and the expression of Salmonella pathogenicity islands or flagella genes can be viewed conveniently by clicking on pre-assembled gene lists in the header of the website. To explore the transcriptome architecture in greater detail, the data are also viewed in the online browser Jbrowse (Skinner et al., Genome Res. 2009; 19, 1630-1638). This fast and easy-to-use, zoom- and scrollable online browser allows the identification of new transcriptomic features, such as antisense transcripts and small RNAs. We welcome feedback about the SalCom compendia during the Potsdam conference. The three websites can be reached at: SalCom: http://tinyurl.com/HintonLab, SalCom SalComMac: http://tinyurl/SalComMac, SalComRegulon: http://tinyurl.com/SalComRegulon

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MULTIDRUG-RESISTANT D-TARTRATE FERMENTING VARIANT *SALMONELLA ENTERICA* SEROVAR PARATYPHI B ACCUMULATED EXTENDED-SPECTRUM SS-LACTAMASE OR AMPC SS-LACTAMASE GENES IN GERMANY EARLY IN THE 2000S

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Background: In the last decades occurrence of multidrug-resistant Enterobacteriaceae has increased worldwide. Resistance against 3rd generation cephalosporins encoded by extended-spectrum ß-lactamase (ESBL)- and AmpC ß-lactamase genes is considered as a major public health concern and is commonly associated with plasmids belonging to different incompatibility groups. D-tartrate-fermenting Salmonella enterica serovar Paratyphi B (S. Paratyphi B dT+) is a common cause of gastroenteritis in humans. A multidrug-resistant

lineage of this serovar has been established in poultry since the end of the 1990s, especially in Germany, the Netherlands and Belgium. In Germany, 3rd generation cephalosporins and fluoroquinolone resistance D tartrate fermenting (dT+) Salmonella enterica serovar Paratyphi B has been isolated for the first time in the early 2000s from poultry and poultry meat. In this study, we carried out a molecular characterization of 3rd generation cephalosporin resistant S. Paratyphi B dT+ isolates from food and animals in Germany. **Methods:** Thirty-four epidemiologically unrelated S. Paratyphi B dT+ isolates, showing a MIC for ceftiofur/cefotaxime/cefatzime ≥ 4 mg/L, were selected from the collection of the National Salmonella Reference Laboratory. Antimicrobial susceptibility against 15 β-lactams/β-lactam inhibitors was tested by disc-diffusion. PCR amplification-sequencing, XbaI- and S1-PFGE, mating experiments, plasmid replicon typing, and Southern-blot-hybridization were used to characterize the resistance determinants and the epidemiological relationship between the isolates. **Results:** All S. Paratyphi B (dT+) isolates were multidrug-resistant, harboring a 2300bp/dfrA1-sat1-aadA1 class 2 integron. Their XbaI-PFGE patterns were highly similar although various resistance phenotypes and plasmid profiles were observed. Fourteen isolates carried a blaCTX M 1 on self-transferable IncI1 plasmids (85-100 kb) and four isolates showed blaCTX M 2 on conjugative IncHI2 plasmids (240-300 kb). TEM variants blaTEM-52 (six isolates) and blaTEM-20 (two isolates) were located on IncI1 (80-100 kb) plasmids. AmpC ß-lactamase encoding blaCMY-2 gene was observed in nine isolates, in one case in combination with blaCTX M 1 and blaTEM-52. None of ESBL-producing S. Paratyphi B (dT+) isolates were positive for the colistin resistance mediating gene mcr-1. **Conclusion:** In conclusion, the multidrug-resistant, poultry-associated S. Paratyphi B dT+ lineage has evolved to cephalosporin resistance through acquisition of ESBL- or AmpC- gene encoding plasmids by several independent events.

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IN VITRO CONJUGAL TRANSFER FREQUENCY OF MULTIDRUG RESISTANT NDM-1 CARBAPENEMASE HARBORING PLASMID TO DIFFERENT *SALMONELLA* SEROVARS MAINLY IMPLICATED IN HUMAN SALMONELLOSIS

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Background: Carbapenems are critically important antimicrobials considered as drugs of last choice in clinical settings. During the last few years, the prevalence of resistance to these antimicrobial agents in Enterobacteriaceae from human origin has increased worldwide. Detection of carbapenemase producing Salmonella and E. coli isolated from animal and environmental samples in Germany revealed the genus Salmonella as potential new emerging reservoir and source of spreading carbapenemase-encoding genes among other Enterobacteriaceae. The objective of this study was to analyse whether transmission of resistance genes through horizontal gene transfer varies among the most common serovars implicated in human Salmonellosis (S. Enteritidis, S. Infantis and S. Paratyphi B) at different temperatures and whether this is influenced by the presence of the pSEV virulence plasmid in S. Enteritidis isolates in vitro. **Methods:** A S. Corvallis isolate carrying a 180 kb Inc A/C plasmid "pRH-R1738" harboring the blaNDM-1 (and a further AmpC blaCMY-16) gene isolated 2012 from a black kite in Germany was used as a donor for conjugation experiments. Four Salmonella isolates resistant to nalidixic acid (S. Paratyphi B, S. Infantis, S. Enteritidis without the 60 kb IncFIIA/FIA pSEV and another S. Enteritidis harboring the pSEV) selected from the strain collection of the National Reference Laboratory for Salmonella in Germany were used

as recipients. Filter mating was carried out at room temperature (RT), 37°C and 42°C for four hours. Transconjugants were selected using XLD agar supplemented with 50 mg/l nalidixic acid, 0.125 mg/l meropenem and 1 mg/l cefotaxime and subsequently confirmed by PCR-, S1- PFGE and serological analysis. **Results:** Conjugal transfer frequency (CTF) varied between 9.3 x 10-6 (in S. Enteritidis without pSEV, RT) to 1.1 x 10 2 (in S. Paratyphi B, 42°C). In all recipients highest CTF was observed at 42°C (8.2 x 10-3 - 1.1 x 10-2), followed by 37°C (3.7 x 10-3 - 8.1 x 10-3) and a lowest CTF at RT (4.9 x 10-5 - 3.5 x 10-4) was observed. **Conclusion:** Although CTF of the NDM-1 plasmid "pRH-R1738" was just slightly higher in S. Paratyphi B at RT and 42°C than in other serovars, CTF seems not to differ significantly among the tested serovars and might not be hampered by the presence of additional Salmonella virulence plasmids like pSEV in S. Enteritidis. However, similar variation of CTFs at different temperatures among the four tested serovars gives evidence that the horizontal resistance gene transfer of "pRH-R1738" is temperature depended and most efficient at 42°C.

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WHOLE GENOME SEQUENCING FOR ROUTINE IDENTIFICATION, DRUG RESISTANCE, DETECTION AND EPIDEMIOLOGY OF *SALMONELLA*: A REVOLUTION IN PUBLIC HEALTH MICROBIOLOGY

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Salmonella is a major human pathogen and a global public health burden. There is also genuine concern from the threat of emerging multidrug resistant (MDR) Salmonella. As part of the public health action to control Salmonella, all isolates (c.10,000 a year) from human infections in England and Wales are sent to the Salmonella Reference Service(SRS), Gatrointestinal Bacteria Reference Unit (GBRU) at Public Health England(PHE). GBRU has an on-going programme of evaluating emerging whole genome sequencing (WGS) technologies to assess their potential value in improving routine microbiology and as of 1st April 2015 WGS has been adopted for routine use in SRS for: 1. Salmonella identification: A WGS approach using a bioinformatics pipeline has been developed to extract MLST directly from the sequence data such that a serotype can be inferred. Here we provide an insight into the genetic population structure of all Salmonella serovars in England and Wales during a 12 month period. 2. Detection of antimicrobial (AMR) resistance: The use of an in-house AMR pipeline for drug resistance detection and characterisation of resistance mechanisms/ regions that were previously challenging to define. WGS was used to determine the prevalence of azithromycin in a U.K population of non-typhoidal Salmonella (NTS) and the detection of a novel Salmonella Azithromycin Resistance Genomic Island in Salmonella Blockley. A cause of concern as azithromycin is being used as the drug of choice for enteric fever and invasive NTS treatment in many parts of the world. 3. Typing: High resolution typing based on single nucleotide polymorphisms (SNP) typing for detection and surveillance of outbreaks as well as the detection of emerging pathogens. WGS is revolutionising and transforming public health microbiology. Rapid advances in WGS methodologies have resulted in the ability to perform robust high throughput sequencing of bacterial genomes at low cost making WGS an economically viable alternative to traditional typing methods for public health surveillance, outbreak and AMR detection.

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ANTISENSE RNAS REGULATE THE REPLICATION OF GIFSY BACTERIOPHAGES AND THE CARBON METABOLISM IN *SALMONELLA* TYPHIMURIUM

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In bacteriophages (phages), cis-encoded antisense RNAs (asRNAs) play a key role in the control of diverse biological functions. asRNAs are regulatory non-coding transcripts encoded at the same locus of their target gene, but on the opposite DNA strand. The short asRNAs transcripts have a perfect complementarity with the cis-encoded mRNA molecules and use base-pairing to modulate the stability and/or the translational rate of the target mRNA. In the temperate phage λ of Escherichia coli, the OOP asRNA is encoded on the opposite strand of the cII gene and overlaps with the 3' end of the cII mRNA coding sequence. OOP interacts by base-pairing to facilitate the degradation of the λ CII-repressor mRNA and reduces the level of the CII protein, which is a pivotal regulator of the "lysis vs. lysogeny" decision of λ in E. coli. In the enteropathogenic bacterium Salmonella Typhimurium strain 4/74, we used RNA-seq technology to reveal high expression of STnc1390 and STnc1080, two asRNAs encoded within the lambdoid prophages Gifsy-1 and Gifsy-2, respectively. Like the λ OOP asRNA, STnc1390 and STnc1080 are encoded tail-to-tail to cII and head-to-head to the O gene, which encodes for the Gifsy O replication proteins. Unlike OOP, the antisense transcription of these RNAs does not overlap with the 3' coding region of the cII mRNA, suggesting a novel function in Gifsy phage biology. During lysogeny, prophage-encoded regulatory RNAs have been recently reported to regulate core-genome genes in enterobacteria, demonstrating the prophage-driven manipulation of bacterial metabolism by regulatory RNAs. Therefore, we investigated the role of the asRNAs STnc1390 and STnc1080 in the regulation of the Gifsy phage functions, and in the modulation of Salmonella core-genome genes expression. We examined the role of STnc1390 during the infection of Salmonella by Gifsy-1 and discovered that the overexpression of this asRNA in naive bacteria prevents the proliferation of this phage. Moreover, we discovered that the overexpression of STnc1080 severely impairs the growth of S. Typhimurium 4/74 on C4-dicarboxylic acids, suggesting a novel asRNA-based mechanism for controlling the Salmonella carbon metabolism. In this presentation we will discuss the role of STnc1390 and STnc1080 in the control of both Gifsy phage replication and Salmonella central metabolism, highlighting a dual role of prophage-encoded antisense regulatory RNAs.

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USE OF ATTENUATED BUT METABOLICALLY COMPETENT *SALMONELLA* AS A PROBIOTIC TO PREVENT OR TREAT *SALMONELLA* INFECTION

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Salmonella enterica is among the most burdensome of foodborne diseases. There are over 2600 serovars that cause a range of disease manifestations ranging from enterocolitis to Typhoid Fever. While there are two vaccines in use in humans to protect against Typhoid Fever, there are none that prevent enterocolitis. If vaccines preventing enterocolitis were to be developed, they would likely protect against only one, or a few, serovars. In this report, we tested the hypothesis that probiotic organisms could compete for the preferred nutrient sources of Salmonella and thus prevent or treat infection. To this end, we added the fra locus, which encodes a utilization pathway for the Salmonella-specific nutrient source, fructose-asparagine (F-Asn), to the probiotic bacterium,

E. coli Nissle 1917 (Nissle) to increase its ability to compete with Salmonella in mouse models. We also tested a metabolically competent, but avirulent, Salmonella serovar Typhimurium mutant for its ability to compete with wild-type Salmonella. The modified Nissle strain became more virulent and less able to protect against Salmonella in some instances. On the other hand, the modified Salmonella strain was safe and effective in preventing infection with wild-type Salmonella. While we only tested for efficacy against serovar Typhimurium, the modified Salmonella strain may be able to compete metabolically with most, if not all, Salmonella serovars, representing a novel approach to control of this pathogen.

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ANTIMICROBIAL RESISTANCE OF *SALMONELLA* SPP. ISOLATED FROM TABLE EGGS IN RASHT, IRAN

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Foodborne disease is a major health and economic problem in the world and Salmonellosis is the most common type of food poisoning in developed and developing countries that is caused by Salmonella serotype. Eggs are early digestive and nutritious food that can affects family health. The main contaminants of this product are presence of pathogenic bacteria. The aim of this study was to determine the Prevalence rate and the level of antibiotic resistance patterns in Salmonella spp. isolated from table eggs. A total of 500 eggs were randomly collected. After culturing and isolation processes, Salmonella spp. bacteria were isolated from shell and Contents (albumen + yolk) of eggs. Then, antibiotic resistance was determined by PCR and agar disk diffusion method. Among the 500 samples, 9.2 % of the egg shell and 12.2% of contents samples were infected with Salmonella spp. In evaluation of antibiotic resistance patterns of bacteria, Salmonella isolates showed resistance to all the antibiotics disks used. The relatively high prevalence of resistance to antimicrobial agents amongst isolates of Salmonella may pose therapeutic implications, particularly in egg-borne salmonellosis. We recommend use of antibiotics in chicken food must be control to avoid the antibiotic resistance.

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AN ASSESSMENT OF HOUSEHOLD DRINKING WATER AS A RESERVOIR OF ANTIMICROBIAL RESISTANT-*SALMONELLA* SPECIES

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Antimicrobial resistance is a growing problem across the world and is becoming a major threat to global public health. It threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria and some other microorganisms. This has led to the discovery of antibiotic resistance bacteria in clinical environment but not only confined to this environment. It can also be found in aquatic environment especially in water used for domestic purposes such as drinking water which might be an important contributory factor in the spread of resistance because of the unavoidable use and exposure of every mankind to water. The objective of this study was to investigate household drinking water as a reservoir of antibiotic resistant - *Salmonella* species. Source and stored drinking water of some selected households and sources such as wells, spring, streams and boreholes in three States of Nigeria with filter on sterile absorbent pad soaked with m-Endo broth for

total coliform then subcultured on prepoured sterile Salmonella-Shigella agar plate. *Salmonella* sp. were screened for, confirmed using standard methods, subjected to antibiotics susceptibility tests using disk diffusion and double disk test for phenotypic detection of extended spectrum beta lactamase. Extended spectrum beta lactamase (ESBL) - resistant genes were detected using polymerase chain reaction. One hundred and eighty water samples were collected in all out of which twenty *Salmonella* sp. were isolated. Antibiotic susceptibility profile showed that all the 20 (100%) *Salmonella* sp. showed resistant to augmentin (aug), ceftazidime (cez), cefixime (cef), tetracycline (tet), nalidixic acid (nal) and co-trimoxazole (cot) whereas 30% and 20% of the *Salmonella* sp. were sensitive to ciprofloxacin (cro) and ofloxacin (ofl) respectively while 5% were sensitive to gentamicin (gen) and cefuroxime (cefu). None of the isolates expressed ESBL activity both phenotypically and genotypically. Although, none of the *Salmonella* sp. screened for ESBL resistant genes (Bla $_{TEM}$, Bla $_{SHV}$ and Bla $_{CTX-M}$) were positive for the genes but there could be other factors responsible for the observed multiple resistance. Household drinking water can be contaminated as a result of lack of personal hygiene by people and sanitation of the environment which could serve as a vehicle of transmission of resistant pathogens especially *Salmonella* sp. Keywords: Water, *Salmonella* species, Antibiotics profile, Antimicrobial resistant

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SSRB AS A DRIVER OF LIFESTYLE CHANGES IN *SALMONELLAE*

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SsrA/B is a two-component signaling system in Salmonella enterica that is encoded on one of the horizontally acquired AT-rich segments of the genome called Salmonella Pathogenecity Island-2 (SPI-2). It is essential for the successful existence of serovars Typhi and Typhimurium inside host cells and is absent in the nearest phylogenetic neighbor, S. bongori. In response to environmental stimuli such as changes in pH and osmolality, transcriptional activation of SPI-2 by SsrB~P regulates the intracellular lifestyle of Salmonella. However, for successful pathogenesis in terms of carriage and persistence, Salmonella exists as multicellular communities. We recently found that this sessile lifestyle was also regulated by SsrB. SsrB activated the expression of the master regulator of biofilm formation, csgD (agfD), in the absence of any phosphate donors, including SsrA. This was achieved by relieving transcriptional silencing by H-NS at the csgD regulatory region. Atomic force microscopy revealed that the full-length unphosphorylated SsrB was bound to the upstream regulatory region of csgD, in agreement with our genetic and biochemical results. This binding and subsequent changes in the local DNA topology was sufficient to partially drive off H-NS and activate csgD expression. In contrast, SsrB~P regulates expression of the SPI-2 regulon by both direct transcriptional activation and anti-silencing. Our findings unravelled a novel role for unphosphorylated SsrB in regulating gene expression and established the mechanism by which anti-silencing occured. Therefore, depending on its phosphorylation state, SsrB, assists Salmonella Typhimurium to decide its lifestyle choice: intracellular versus the carrier state or biofilms. Building on this paradigm, we are now studying the larger role of SsrB and SsrB~P in regulating enivronmentally sensitive genes as Salmonella alternates between the two lifestyles. Precise mechanisms of biofilm formation in the human-restricted serovar, Typhi, are still not clear, and thus the new insights we obtain from the SsrB versus SsrB~P regulons in Typhimurium will help understand the carrier state of Typhi. Supported by RCE in Mechanobiology, NUS Ministry of Education, Singapore, NIHR21-AI123640 and VA 5IO1BX000372 to LJK.

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A NOVEL APPROACH TO ELUCIDATING ANTIGEN SPECIFICITY OF CD4+ T CELLS IN *SALMONELLA* INFECTION

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The critical role of $CD4^+$ T cells in mediating protective immunity against *Salmonella* is well established in human diseases as well as in mouse models. Endogenous $CD4^+$ T cells respond to a diversity of *Salmonella*-derived antigens, and are highly heterogeneous in their *in vivo* abundance, tissue localisation, immune signature and potential for protective immunity. On the other hand, *Salmonella* continuously manipulate protein expression to suit their lifestyle in the host, generating a shifting landscape of antigenic output that the repertoire of *Salmonella*-specific $CD4^+$ T cells must adapt to in order to remain effective as the infection ensues. To date, few $CD4^+$ T cell epitopes are known for *Salmonella* and none can confer full protection against lethal infection, suggesting that other as yet unidentified $CD4^+$ T cell epitopes also make significant contributions to protective immunity. Elucidating the hierarchy of $CD4^+$ T cell antigen specificity in *Salmonella* infection is vital to the development of prophylactic and intervention strategies that are much needed in the face of rapidly rising antibiotic resistance, but this objective remains unresolved so far. We hypothesise that effective immunity to *Salmonella* depends on concurrent targeting of multiple *Salmonella*-derived antigens by $CD4^+$ T cells, with each antigen making an incremental contribution to the overall immune response. To test this, we have employed a global approach to identify naturally presented, *Salmonella*-derived $CD4^+$ T cell epitopes *in vitro*. MHC-II-bound peptides were isolated from *Salmonella*-infected dendritic cells and sequenced using liquid chromatography coupled with high-resolution tandem spectrometry (LC-MS/MS). So far we have successfully identified an immunopeptidome that consists of two previously reported FliC epitopes and additional peptides that clustered around 85 unique epitopes derived from 61 *Salmonella* proteins. We synthesised one "consensus" peptide for each epitope, and a number of these peptides were able to re-stimulate IFN-γ production *ex vivo* from $CD4^+$ T cells of mice immunised with *Salmonella* live-attenuated vaccine strains, confirming their immunogenicity. Ongoing work aims to determine the relative contribution of each immunogenic epitopes to overall protective immunity. While a number of epitopes may be revealed as protective, a subset of these epitopes may act as "decoys" to misdirect host immunity and $CD4^+$ T cell responses. We anticipate establishing bacterial expression profiles or "signatures" that distinguish protective from non-protective $CD4^+$ T cell epitopes. This will provide vital information on how $CD4^+$ T cell select antigens from a complex bacterial pathogen, and bears profound implications for dissecting polyclonal immunity in infection with *Salmonella* and possibly other bacterial pathogens.

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PROTEIN ACETYLATION IS INVOLVED IN *SALMONELLA ENTERICA* SEROVAR TYPHIMURIUM VIRULENCE BY REGULATING HILD

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Salmonella causes a range of diseases in different hosts, including enterocolitis and systemic

infection. Lysine acetylation regulates many eukaryotic cellular processes, but its function in bacteria is largely unexplored. The acetyltransferase Pat and nicotinamide adenine dinucleotide (NAD^+)-dependent deacetylase CobB are involved in the reversible protein acetylation in *Salmonella* Typhimurium. In this study, we used cell and animal models to evaluate the virulence of *pat* and *cobB* deletion mutants in *S.* Typhimurium, and found that *pat* is critical for bacterial intestinal colonization and systemic infection. Next, to understand the underlying mechanism, genome-wide transcriptome was analyzed. RNA-seq data show the expression of *Salmonella* pathogenicity islands 1 (SPI-1) is partially dependent on *pat*. In addition, HilD, a key transcriptional regulator of SPI-1, is a substrate of Pat. The acetylation of HilD by Pat maintained HilD stability. We further show that Lys 297 (K297) located in the helix–turn– helix motif of HilD, is acetylated by Pat. Acetylated K297 elevates HilD stability, but reduces the DNA-binding affinity of HilD. These findings demonstrate that lysine acetylation can regulate both protein stability and DNA-binding ability of HilD, which balances the protein amount and transcriptional activity of HilD to regulate SPI-1 expression and mediate *S.* Typhimurium virulence.

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TRANSCRIPTIONAL REGULATION OF "*SALMONELLA*" TYPHIMURIUM PEF FIMBRIAE BY H-NS, HHA AND YDGT NUCLEOPROTEINS

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Background: Gastroenteritis caused by "Salmonella" Typhimurium is triggered by bacterial adherence to intestinal epithelium cells that leads to invasion and destruction of the mucosal surface. The "pef" operon of "S". Typhimurium is responsible for the biosynthesis of plasmid-encoded fimbriae (Pef), which mediate adhesion to mouse intestinal epithelium. As most of the 13 fimbriae of "S". Typhimurium, expression of Pef fimbriae is tightly regulated. In vitro, their expression was previously detected only in standing cultures grown in rich acidic medium. Previous work and microarray studies suggested a role of the nucleoproteins H-NS and Hha-YdgT in this negative regulation of Pef fimbriae expression. In this study, our objective was to demonstrate this repression and to characterize the underlying mechanism. **Methods:** Due to instability of "hns" mutants, strains carrying a deletion of the "hns" gene were freshly constructed by P22 transduction before each experiment. Promoters activities were quantified using plasmid-based transcriptional fusions carried by wild-type, "hns" and/or "hha-ydgT" mutants. Expression of Pef fimbriae was measured by RT-PCR and Western blot by measuring "pefA"/PefA expression, which encodes the major subunit. **Results:** We demonstrate that H-NS and Hha-YdgT negatively regulate "pef" operon transcription by acting on the promoter located upstream of pefB, the first gene of the operon. The effect of H-NS was much more pronounced than that of Hha-YdgT. Moreover, we observed that Hha and YdgT can repress pef expression independently of H-NS when bacteria were cultivated in acidic medium under standing conditions, but not after culture in neutral pH medium. **Conclusions:** This work demonstrates that the weak expression of Pef fimbriae in vitro is partly due to the combined action of H-NS and Hha-YdgT nucleoproteins on the transcriptional activity of the promoter region located upstream of the pef operon. A debate still exists in the literature concerning the exact mode of action of these nucleoproteins. Experimental evidence and a mechanistic model recently described indicate that Hha and YdgT act primarily through H-NS to modulate gene expression. On the contrary, few reports show that Hha can bind to specific regulatory sequences independently of H-NS. Our results on "pef" operon transcriptional regulation are in favor of the existence of these two models. Indeed, Hha and YdgT can act through H-NS to modulate pef expression. Nevertheless, ac-

cording to the culture conditions used in our experiments, it appears that Hha and YdgT can also act independently of H-NS.

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ANALYSIS OF THE INTERACTIONS BETWEEN *SALMONELLA* GENOMIC ISLAND 1 AND INCA/C HELPER PLASMIDS

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The Salmonella Genomic Island 1 (SGI1) was first detected in the 80's in *Salmonella enterica* serovar Typhimurium DT104. Since then numerous SGI1 variants have been found in *Salmonella* serovars and recently in a few *Proteus* and *Acinetobacter* strains. SGI1 contains a complex In104 integron region encoding resistance for ampicillin, chloramphenicol, florphenicol, streptomycin, spectinomycin, sulphonamides and tetracycline. Most of the variants share common backbone, although different variants encode diverse resistance patterns, which imply serious health risk for humans and livestocks. Some ORFs of the conserved backbone of SGI1 have been proved to participate in the site-specific excision and integration of the genomic island, however majority of ORFs have unknown functions. The large conjugative plasmids of Incompatibility group A/C (IncA/C) take part exclusively in the conjugal transfer of SGI1 as particular helper. SGI1 utilizes the relaxase, the pilus and pilus assembly proteins of IncA/C plasmids. In addition, the plasmid-encoded *flhDC*-family regulator,*acaCD* have been identified as an activator of SGI1 excision. Aims of this work are to identify and characterize the essential ORFs and non-coding sequences involved in the horizontal transfer of SGI1, and in co-habitation of SGI1 and IncA/C plasmids. Furthermore, we would like to create the fully transfer competent and also simplified SGI1 model system for further investigations. A set of SGI1 deletion mutants enables us to study the role of SGI1 regions in excision, conjugal transfer and stability. We could determine SGI1 regions that have no significant effect on the transfer frequency of the island, thus can be omitted in a minimal SGI1 model. We found an *acaCD* homolog, $flhDC_{SGI1}$, encoded on SGI1 backbone, which may have a role in the conjugal transfer. The $FlhDC_{SGI1}$ also induces SGI1 excision and has activating effect on other predicted AcaCD binding sites in SGI1 promoter regions, furthermore, can complement the transfer deficiency of the *acaCD* mutant helper plasmid. ß-gal assays showed that $FlhDC_{SGI1}$ has ca. 1/3 of the activity of AcaCD when triggering expression from promoters containing predicted AcaCD binding sites on SGI1. Stability assays showed that several SGI1 ORFs influence the co-habitation of the IncA/C plasmids and SGI1, especially the region encoding S003-S005 ORFs appears to be involved in this issue.

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ADOPTIVE CELL TRANSFER OF IFNγ-PRODUCING CD4+ T CELLS PLUS B CELLS REVEALS INTERDEPENDENCE IN IMMUNITY AGAINST WILD-TYPE *SALMONELLA* TYPHIMURIUM

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Background: Cellular immunity and cytokine production are essential in immunity to *Salmonella enterica* var Typhimurium (*S.* Typhimurium) in mice and mice that are *nramp1*$^{se/se}$ (e.g. C57Bl/6, (B6)) can provide a model for human invasive non-typhoidal salmonellosis (iNTS). While naïve B6 animals die approximately one week after wild-type (wt, SL1344) infection, vaccinated animals are protected from wt challenge. Although B cells, and interferon gamma (IFNγ)-producing T cells and NK cells, are known to be important in immunity against *S.* Typhimurium wt re-infection, the mechanisms behind immunity remain poorly resolved. **Methods:** B6 mice were vaccinated intravenously (iv) with 200 CFU of attenuated

S. Typhimurium strains (Δ*aroA* (BRD509) or Δ*edd*Δ*pfkA*Δ*pfkB* (TAS2010)) and splenocytes were analysed by FACS and cell transfer into lymphocyte-deficient RAG2$^{-/-}$ common gamma Chain$^{-/-}$ (RAG2γC) recipients. Recipient animals were challenged with 200CFU wt iv, 20-24h post-transfer. Protection was measured by survival and CFU counts. *In vivo* and *in vitro* antibody-depletion and neutralization, as well as cell enrichment, were performed to identify those factors most important to protection. **Results:** Recipients receiving splenocytes from mice vaccinated with TAS2010 showed superior protection against wt challenge. Transfer of splenocytes from B6 donors, 2 weeks after vaccination, but not later, with TAS2010 into RAG2γC demonstrated maximal protection. Analyses of IFNγ gene activity (using IFNγ eYFP reporter mice) was used to define IFNγ production. As expected, CD4$^+$ T cells showed the highest IFNγ expression, followed by CD8$^+$ T and NK1.1$^+$ CD3$^-$ cells. Post-transfer treatment *in vivo* using antibodies in adoptively-transferred recipients (ie. in RAG2γC) revealed functional redundancy between IFNγ and Thy1.2$^+$ cells in cell transfers, as well as the importance of CD4$^+$ T cells, in this model. However, CD4$^+$ T cells alone from animals vaccinated for 2 weeks failed to protect lymphocyte-deficient recipients against wt challenge. *In vitro* B cell depletion from vaccinated donors followed by transfer of the remaining subsets also impaired protection in both B6 and RAG2γC recipients, suggesting an 'interdependence' between IFNγ-producing CD4$^+$ T cells and B cells in protection against challenge. This 'interdependence' hypothesis was supported by recapitulating data showing a lack of vaccine protection in B-cell-deficient (ie. μMT) animals. **Conclusion:** Splenocyte-mediated protection against wt *S.* Typhimurium is optimally transferable at week 2 post-vaccination and interaction between IFNγ-producing CD4$^+$ T cells and B cells plays a pivotal role in protection seen in this novel transfer model.

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THE DETECTION OF *SALMONELLA* TYPHIMURIUM USING MOLECULAR SEROTYPING METHOD IN CHICKEN PASTA

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Deboned chicken meat is used as a primary substance to produce sausage in Iran. Deboned chicken meat obtained from mechanical separation of meat pieces from bone. The quality of this primary substance plays an important role in the production of different food products in the view of microbial load and especially, Salmonella contamination.in this research 45 deboned chicken meat were collected under sterile condition and transferred to laboratory and surveyed for Salmonella contamination. Among 16 identified Salmonella isolates using conventional culture and biochemical tests, 3 of them were positive for Salmonella Typhimurium with m-PCR method. Results showed that 16 samples (35.5%) out of 45 samples were contaminated with Salmonella and 3 of them (6.6%) with Salmonella Typhimurium. one of the most important ways of chicken paste contamination by Salmonella is the use of combinated carcasses. For this reason, accuracy in the use of carcasses without contamination and compliance the principles of sanitation could have a key role in lowering the amount of germs in chicken paste especially its contamination with Salmonella.

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IDENTIFICATION AND ANALYSIS OF SYNERGISTIC DRUG INTERACTIONS IN *SALMONELLA ENTERICA* STRAINS

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Background: In food-born outbreaks, one of the most common pathogens of gastrointestinal tract is Salmonella in humans and animals. In some patients with immune defficiency or underlying diseases, it may cause severe results including death. Anti-bacterial resistance is an emerging and considerable problem which needs to have serious consideration. This study relates to novel antibacterial combinations comprising two different antibacterial agents which exhibit synergistic effect on both susceptable and resistant Salmonella strains isolated from clinical specimens. **Methods:** Previously isolated and automatically identified 2 susceptable and 2 resistant Salmonella strains were used for the study. Six different antimicrobial drugs were evaluated including Chloramphenicol (CHO), Clarithromycin (CLA), Erythromycin (ERY), Ciprofloksasin (CIP), Tetracycline (TET) and Kanamycin (KAN). For each drug, logarithmic and lineer dilutions were done for determination of 90% MIC values. 90% MIC values for the drugs used as the maximum concentration applied to each strains in 96-well plate interaction assay. Overnight bacterial growth were measured by using spectrophotometer at 600nm wavelength. **Results:** 15 pairwise anti-microbial drug combinations were evaluated in this study. As expected, different strains showed slightly different responses to drug treatments. Resistant strains showed more resistance to pairwise drug combinations compared to susceptable strains. But synergistic drug combinations were found effective on both susceptable and resistant strains which indicate conserved effects of synergistic drug interactions. According to results, KAN-AMK, PIP-KAN, CIP-PIP and KAN-SPEC drug pairs were found synergistic especially for multidrug resistant Salmonella strains. **Conclusion:** Results indicate that susceptable or multi-drug resistant Salmonella strains show similar response to synergistic drug combinations. In this study, clinically relevant synergistic drug combination were found. Antibiotic resistance is a serious and emerging problem. Therefore, investigations of effective drug combinations for combinatorial antibacterial therapies are crucial.

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IN VIVO SALMONELLA ENVELOPE STRESS RESPONSES

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The Salmonella envelope is involved in uptake of nutrients, excretion of waste products, sensing of external stimuli, and secretion of virulence factors. During infection, it is attacked by host effector mechanisms. Envelope stress is sensed by several different pathways, and among these the extracytoplasmic stress response sigma factor E (σE, encoded by rpoE) seems to be particularly relevant for systemic virulence in mice based on the strongly attenuated phenotype of an rpoE mutant (Humphreys et al, IAI 67, 1560). σE regulates expression of more than 300 genes including well-known virulence factors such as HtrA. However, it remains unclear which of these genes are particularly relevant for envelope maintenance and what kind of physiological problem they solve. To address these questions, we generated a transposon library in a Salmonella enterica serovar Typhimurium SL1344 rpoE mutant. We used a transposon that carries two outward facing promoters with different strengths (Pbla, Ptac) which could activate expression of genes around the transposon insertion site, in addition to disrupting genes hit by insertion. We intravenously infected susceptible BALB/c mice with this library and prepared spleen and liver 5 days post infection. Whereas the parental rpoE mutant was completely cleared from infected mice within 5 days, several clones of the library could be

isolated demonstrating partial rescue of virulence. Interestingly, one region carrying genes associated with outer membrane porins were hit in different dominating clones. Individual virulence tests revealed that these clones were able to survive for at least 5 days at high levels but did not show net in vivo growth. We used proteomics and clean mutations to demonstrate that the lack of one major porin enabled Salmonella rpoE to survive in mouse spleen. By contrast, inactivation of another closely related major porin had no impact suggesting divergent properties of these two major porins. We currently investigate the role of various potential differences in in vivo abundance and small molecule permeation. We also performed another round of transposon mutagenesis on the rescued Salmonella strain to test if we can further rescue virulence. After two in vivo passages, we obtained four different clones that regained considerable in vivo growth capabilities. Intriguingly, some of the affected genes have also links to outer membrane porins. We are currently further analyzing these clones but the data already suggest that regulation and/or repair of outer membrane permeability determinants might be central to in vivo envelope stresses and associated virulence requirements. Together, our results indicate that surprisingly just a few genetic alterations can partially rescue the very strong attenuation caused be a defective major sigma factor with a large regulon.

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BACTERICIDAL ACTIVITIES AND POST-ANTIBIOTIC EFFECTS OF OFLOXACIN AND CEFTRIAXONE AGAINST DRUG RESISTANT *SALMONELLA ENTERICA* SEROVAR TYPHI

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Typhoid fever responds rapidly to fluoroquinolone treatment when the infecting isolate is fully susceptible (MIC $\leq$ 0.06 µg/mL), but slowly if the isolate has intermediate susceptibility (MIC 0.25-1.0 µg/mL). The clinical response to treatment with ceftriaxone in typhoid fever is also slow despite its low MIC (0.03-0.12 µg/mL). The mean time to reach a 99.9% reduction in log10 counts (bactericidal activity) and the post antibiotic effects (PAE) of ofloxacin and ceftriaxone were determined for 18 clinical isolates of S.Typhi with different susceptibility patterns in time-kill experiments (MIC range for ofloxacin 0.06 to 1 µg/mL and for ceftriaxone 0.03 to 0.125 µg/mL). Analysis was performed using a logistic regression model. The mean (SD) bactericidal activity of ofloxacin was 35.6 (19.8) minutes compared with 383 (82) minutes for ceftriaxone. After a 30 minute exposure to ofloxacin, the mean (SD) duration of PAE was 157.7 (11.2) minutes. There was no detectable PAE after one-hour exposure to ceftriaxone. Bactericidal activity, AUC, PAE and model parameters did not significantly differ between isolates with full susceptibility or intermediate susceptibility to ofloxacin provided ofloxacin concentrations were maintained at 4 x MIC. The slow bactericidal activity of ceftriaxone and lack of PAE may explain the slow clinical response to ceftriaxone in typhoid. Infections with partially fluoroquinolone resistant S. Typhi may respond to higher doses that maintain drug concentrations at 4 x MIC at the site of infection

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MULTICOPY SINGLE-STRANDED DNA REGULATES TRANSLATION AND METABOLISM IN *SALMONELLA*

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Background: Non-typhoidal *Salmonella* must replicate within the low-oxygen conditions present in the lumen of the intestine during enteritis. *STM3846*, a reverse transcriptase, produces an unusual single stranded DNA-RNA hybrid called multicopy single-stranded DNA (msDNA). msDNA is both important during growth of *Salmonella* in the intestine and is critical for its growth in anaerobic conditions *in vitro*. We hypothesized that msDNA may be a regulator of gene expression. **Methods:** To determine what proteins and transcripts were altered in abundance in msDNA mutants during anaerobic growth, we used both proteomic and transcriptomic approaches. We compared protein abundance during aerobic and anaerobic growth between mutants deficient in msDNA and the isogenic wild-type. We also compared transcript abundance after shift from aerobic to anaerobic conditions. **Results:** Pathways known to be important for anaerobic growth and virulence, including ethanolamine and 1,2-propanediol utilization, vitamin B12 biosynthesis, anaerobic carbon metabolism, and utilization of anaerobic electron acceptors were among the proteins of altered abundance in msDNA mutants exclusively in anaerobic conditions (Elfenbein 2015). In our transcriptomic analyses, 37 genes appeared to have altered expression in mutants unable to produce msDNA. Functional predictions for these genes revealed that 25% have putative functions in transport and metabolism, 17% are involved in translation, and 42% are of unknown function. More than half of the msDNA-regulated genes have a GC content less than 52%, the average for the *Salmonella* genome. One of the msDNA-repressed genes alters translation of a transcriptional regulator in the aerobic regulatory pathway governing CsrA, a global regulator of metabolism and virulence. Together these results suggest that msDNA regulates the anaerobic mRNA abundance of genes that function in metabolism, transport, and translation. **Conclusions:** Our data suggest that msDNA is involved in the regulation of genes needed for anaerobic growth. Further characterization of the regulatory targets of msDNA should improve our understanding of how *Salmonella* thrives in conditions of sparse oxygen, including the intestinal lumen.

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"*IN SILICO*" APPROACH TO DEVELOP A POLYVALENT VACCINE AGAINST "*SALMONELLA*" SEROVARS INVOLVED IN FOOD BORNE DISEASES

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Non-typhoidal salmonellosis is among the most frequent cause of food borne diseases worldwide and food of animal origin are the main source. Most cases of salmonellosis are caused by a few serovars that are highly prevalent. We aim at developing a live vaccine that could broadly cross-protect food-producing animals against the main serovars involved in food borne disease in humans. For this, we propose to use a live attenuated serovar Typhimirum strain (LVR01) to express a set of surface proteins representative of the most prevalent "Salmonella" serovars as a vaccine strain. Based on global epidemiological data we selected serovars Typhimurium, Enteritidis, Newport, Montevideo and Infantis. The genomes of 49 "S. enterica" strains covering

these five serovars where used to define the set of surface proteins rendering a total of 1177 candidates. Blast-p was used to predict surface proteins that vary among serovars, finding 269 proteins shared between all of them and 908 that were variable in at least one genome. We proposed as candidates to be included in the vaccine those proteins that differed to its orthologue in LVR01 but that also showed high intraserovar aminoacidic identity. Candidates with the highest chances to induce an immune response, were selected using an "in silico" approach to predict B and T epitopes. Using these combined approaches we selected FlgK, IroE and RcsF. Assuming that common surface proteins are going to be present in LVR01, we hypothesized that these 3 proteins co-expressed at the surface of the vaccine strain could elicit a protective immune response against the 5 serovars. We have already cloned this proteins fused to the FLAG epitope, in a plasmid under the control of the anaerobically inducible promoter nirB. Currently we are assessing the "in vitro" expression by western blot using antibodies against the FLAG epitope. The potential of this recombinant "Salmonella" strain to elicit cross-reactive immune responses is going to be first evaluated in the murine model. We expect to obtain a candidate vaccine strain available for further studies in target veterinarian species.

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CHLORTETRACYCLINE AND FLORFENICOL INDUCE EXPRESSION OF GENES INVOLVED IN TYPE III SECRETION SYSTEMS AND ATTACHMENT IN MULTIDRUG-RESISTANT *SALMONELLA ENTERICA* SEROVAR TYPHIMURIUM

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Background: Multidrug-resistant (MDR) *Salmonella* is a serious threat to public health, resulting in infections that are more difficult and costly to treat in both humans and animals. Previously, we demonstrated that both tetracycline and chloramphenicol induce invasion of certain DT104 and DT193 *S.* Typhimurium isolates *in vitro* and that genes associated with attachment and invasion, as well as intracellular survival and replication, are upregulated. Our objective in the current work was to determine if chlortetracycline (CTC) and florfenicol would also affect MDR *S.* Typhimurium strains in a similar way. Unlike tetracycline and chloramphenicol, these two antibiotics are frequently used in food-producing animals to treat and prevent infections. **Methods:** In this study we investigated the effect that CTC at 16 µg ml^{-1} or 32 µg ml^{-1} and florfenicol at 16 µg ml^{-1} have on the transcriptome of one DT104 and three DT193 *S.* Typhimurium isolates using RNA-Seq. The four MDR *S.* Typhimurium isolates were grown to early-log phase and exposed to one of the two antibiotics for 30 min prior to RNA extraction; a no antibiotic control was also employed. Additionally, we examined the effect of these antibiotics on the invasion of these isolates in HEp-2 cells. **Results:** Approximately 3,800 and 3,600 genes were significantly differentially expressed in all four isolates following exposure to CTC and florfenicol compared to the no-antibiotic control, respectively. Both antibiotics resulted in the increased expression of a number of genes associated with pathogenesis. Type III secretion system genes, namely *invE*, *invG*, *invI*, *pipB*, *prgK*, *spaR*, *sseA*, *ssaC, ssaD*, *ssaJ*, and *ssaU,* as well as attachment-related genes such as *safA*, *stcA*, *stcB,* were upregulated. Genes linked with replication and protein synthesis were downregulated and motility and SOS genes were largely unaffected. Invasion of HEp-2 cells was enhanced in *S.* Typhimurium that had been exposed to CTC, however, florfenicol exposure resulted in decreased invasion. **Conclusions:** Our results indicate that MDR *S.* Typhimurium strains that are exposed to CTC or florfenicol for 30 min differentially express 71 to 74% of the total genes in the genome, including a large number of genes associated with pathogenesis and attachment. However, this is not a result of an SOS response. Cells

treated with CTC were also more invasive *in vitro*. These findings have implications for public health and livestock industries, as animals that are treated with CTC or florfenicol for other indications and are asymptomatically colonized with MDR S. Typhimurium, may be more likely to develop salmonellosis marked by increased colonization and shedding.

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BIOCHEMICAL AND BIOPHYSICAL ANALYSIS OF CSGBA REGULATION BY H-NS AND THE ALTERNATIVE SIGMA FACTOR σS IN *SALMONELLA ENTERICA*

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Background: Some horizontally-acquired genes silenced by the nucleoid-associated protein H-NS can be transcribed by RNA polymerase (RNAP) associated with σ^S but not with the general housekeeping sigma factor σ^{70}, suggesting that σ^S may serve as a counter-silencer. Regulation of the *csg* operon required for curli fimbrial expression was analyzed to determine whether σ^S directly counters H-NS-mediated silencing of the *csg* genes, and whether co-regulation by H-NS can account for σ^S selectivity of certain promoters. **Methods:** Regulated knock-down of H-NS expression and mutational inactivation of other regulatory loci were used to assess the effects of specific regulatory proteins on *csg* gene expression *in vivo* during logarithmic and stationary phase growth. Gene expression was correlated with function by the assessment of biofilm formation with crystal violet assays and assessment of RDAR colonial morphology. *In vitro* transcription assays directly examined the roles of purified regulatory proteins on *csg* transcription, and DNaseI footprinting correlated transcriptional output with specific changes in DNA binding and nucleoprotein structure. **Results:** An analysis of published datasets found that only 1.3% of *Salmonella enterica* sv. Typhimurium genes are transcriptionally co-regulated by σ^S and H-NS. The *csgBA* genes encoding the curli nucleator protein and major curli subunit were among this group and chosen for in-depth analysis. In accord with previous studies, we found that H-NS silencing of the *csgB* promoter is relieved in the presence of σ^S. However, an *rpoS* mutation abrogated the expression of both *csgB* and *csgD*, which encodes the upstream transcriptional regulator of *csgBA*. Furthermore, we observed that σ^S is no longer required for *csgB* expression when *csgD* is constitutively expressed, suggesting that CsgD is able to overcome H-NS silencing and that σ^S acts upstream of *csgD* and does not directly regulate the *csgB* promoter. *In vitro*, CsgD is able to counter H-NS silencing of the *csgB* promoter independent of σ-factor. Furthermore, DNaseI footprinting indicates that CsgD creates a sharp bend at nucleotide -33 in the *csgB* promoter region, which is predicted to disrupt H-NS nucleoprotein filament formation. **Conclusions:** Stationary phase expression of *csgBA* results from counter-silencing of H-NS by the transcriptional regulator CsgD in association with bending of the *csgB* promoter region. The σ^S-dependence of curli expression appears to result from the σ^S-dependence of CsgD expression rather than from a direct interaction of σ^S and H-NS at the *csgB* promoter. Our observations do not provide support for the hypothesis that σ^S is able to counter transcriptional silencing by H-NS.

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FUNCTIONAL RELATEDNESS IN THE INV/MXI-SPA TYPE III SECRETION SYSTEM FAMILY

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Background: The type III secretion system (T3SS) is a common virulence factor among Gram-negative pathogens. These structurally conserved nanomachines act as molecular syringes for translocation of type III effector proteins to modulate host cell signal transduction pathways. T3SSs are phylogenetically grouped by basal body proteins spanning the inner and outer bacterial membrane, from which a needle protrudes to contact the eukaryotic host via the tip complex and translocon pore. *Salmonella enterica* possesses two T3SSs, one belonging to the Inv/Mxi-Spa family (T3SS1) and one to the Esc family (T3SS2). Inv/Mxi-Spa T3SSs, including *S. enterica* serovar Typhimurium T3SS1, are often involved in facilitating bacterial invasion of eukaryotic hosts, a process largely dependent on four products of the Inv/Mxi-Spa translocator operon. In *S.* Typhimurium, these are SicA, a cytosolic chaperone that stabilizes translocators prior to their export, SipB and SipC translocator proteins forming a heteroligomeric translocon pore within host membranes, and SipD, the tip complex scaffold for the translocon. SipB, SipC and SipD are the only components that directly interface with eukaryotes, and though comparisons of basal body components provide detail on T3SS evolutionary rate, they may overlook pathogenesis-driven diversification of T3SSs. Thus, here we have studied the evolution and functional conservation of the T3SS1 translocator proteins and their dedicated chaperone. **Methods:** *S.* Typhimurium *sicA*, *sipB*, *sipC* and *sipD* deletion mutants were used as surrogates for expression of orthologs from six human pathogens with syntenic translocator operons, identified from an extensive phylogenetic analysis of Inv/Mxi-Spa translocator and chaperone proteins. Functional interchangeability of these orthologs was assessed by restoration of bacterial entry into non-phagocytic cells and type III effector translocation of a T3SS1-dependent substrate (SipA). **Results:** Functional complementation correlated with amino acid sequence identity between orthologs, with SicA the most permissive to heterologous replacement and SipC the least. Interestingly, SipC could not be complemented with orthologs provided *in trans*, yet was amenable to limited complementation upon chromosomal replacement. This may implicate SipC, and/or its corresponding interactions, as the limiting determinant of functional interchangeability in the translocator operon. **Conclusions:** This analysis is the first in-depth study of functional relatedness of Inv/Mxi-Spa proteins acting directly at the host-pathogen interface, and via sequence comparisons, suggests structural regions that contribute to translocator protein functionality. In the future, we envisage that generation of translocator hybrid proteins will allow us to pinpoint key domains that dictate the specificity of their functional conservation.

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IDENTIFICATION OF NOVEL GENES WITH ROLES IN *SALMONELLA* VIRULENCE AND PERSISTENCE

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Non-typhoidal *Salmonella* (NTS) strains are the causative agents of human gastroenteritis. In general, NTS isolates are thought to have a broad host range and are exposed to the environment each time they pass out of an infected host. The formation of biofilm is predicted to be essential for environmental survival and is controlled by CsgD, a transcription factor that controls the expression of many biofilm components, including curili fimbriae and cellulose. Previous research has shown that *Salmonella* serovar Typhimurium cells exposed to environmental stress in liquid culture (28ºC, limiting nutrients) differentiate into two distinct cell types within the fluid phase, multicellular aggregates and non-aggregated (planktonic) cells. Our lab is focused on understanding how differentiation into two distinct cell types is connected to the broad host range of NTS (*S.* Typhimurium). Previously we used RNAseq to show that 1856 genes (34% of the

genome) were differentially expressed between multicellular aggregates and planktonic cells. Multicellular aggregates had higher expression of genes associated with biofilm formation, while planktonic cells had higher expression of virulence pathways. 43% of the 1856 genes that were differentially expressed between multicellular aggregates and planktonic cells represent function unknown (FUN) genes. We hypothesize that a proportion of FUN genes highly expressed in planktonic and aggregates cells have roles in *Salmonella* virulence or persistence respectively and represent an untapped resource for understanding the broad host range of *Salmonella* isolates. In our RNAseq study we identified numerous transcriptional start and termination sites, which enabled us to map the FUN genes to their native transcriptional units. We used the Kyoto encyclopedia of genes and genomes (KEGG) and gene ontology (GO) databases to screen through and select 23 FUN gene operons for deletion mutagenesis. We are currently in the process of performing these deletions and screening strains in a variety of experiments to assess their virulence and persistence characteristics.

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SPIC MUTANT AS A CANDIDATE LIVE ATTENUATED VACCINE OF *SALMONELLA* PULLORUM IDENTIFIED BY PCR SIGNATURE-TAGGED MUTAGENESIS IN A CHICKEN

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Salmonella Gallinarum biovar Pullorum (S. Gallinarum biovar Pullorum) is the causative agent of pullorum disease (PD) in chickens which results in considerable economic losses to the poultry industries in developing countries. PCR-Signature Tagged Mutagenesis was used to identify virulence determinants of S. Gallinarum biovar Pullorum and novel attenuated live vaccine candidates for use against this disease. A library of 1800 signaturetagged S. Gallinarum biovar Pullorum mutants was constructed and screened for virulence-associated genes in chickens. The attenuation of 10 mutants was confirmed by in vivo and in vitro competitive index (CI) studies. The transposons were found to be located in SPI-1 (2/10 mutants), SPI-2 (3/10), the virulence plasmid (1/10) and non-SPI genes (4/10). One highly attenuated spiC mutant persisted in spleen and liver for less than 10 days and induced high levels of circulating antibody and protective immunity against oral challenge in young broiler chickens. The spiC mutant is a potential new vaccine candidate for use with chickens against this disease

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INACTIVATION OF THE GENE ST313-TD IN *SALMONELLA* DUBLIN INCREASES VIRULENCE IN THE MOUSE INFECTION MODEL

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Background: Salmonella enterica (S. enterica) serovar Dublin is a host-restricted serovar associated with typhoidal disease in cattle which can occasionally infect humans causing invasive disease. In previous studies a new virulent gene discovered in the pathogenic and multidrug-resistant lineage S. Typhimurium ST313, st313-td, of unknown function, was found to be conserved in the S. Dublin genomes. In ST313, the gene is harbored is a new potential pathogenicity island (ST313-GI of ca. 17,7 kb) while in Dublin, it is contained in a region of ca. 6.8 kb matching a part of ST313-GI (99% identity). **Material/Methods:** In order to analyze the role of st313-td in the virulence of S. Dublin, several mutants, including the knocked-out and the complemented strains (αSDst313-td and SD3246-C) were constructed by using mutagenesis techniques. The wild type S. Dublin 3246 and isogenic strains were tested in different experiments, includ-

ing; infection of cultured cell lines (human gut epithelial, murine and cattle macrophages), mice mixed infections, as well as growth competition in LB and cattle blood. **Results:** In contrast to the results obtained for S. Typhimrium ST313, the lack of st313-td is associated to an increase in virulence in S. Dublin; the inactivation of the gene leads to an increased uptake by macrophages and the mutant strain (αSDst313-td) outcompetes the wild type isolate in the mouse model of infection. **Conclusions:** st313-td also affects pathogenicity of this serovar and since it plays opposite roles in both S. Typhimurium ST313 and S. Dublin, it may act in a serovar specific-manner and might mediate regulatory functions. This study also contributes to better understand the relevance of a particular gene on host-pathogen interactions allowing the increase of knowledge about serovar differences on host adaptation.

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INVESTIGATION OF VIRULENCE FACTORS AND ANTIBIOTIC RESISTANCE OF *SALMONELLA ENTERICA* STRAINS BY USING MOLECULAR TECHNIQUES AND IDENTIFICATION OF CLONAL RELATIONSHIPS AMONG THE STRAINS

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Background: Salmonella is one of the most common pathogens of the gastrointestinal tract for both humans and animals leads to foodborne outbreaks. Also it may cause systemic infections and death in patients with underlying diseases and immunosuppressive drug users. Moreover, emerging and transferable antimicrobial resistance raises difficulties in treatment. In this study we aimed to reveal the factors that effect virulence of Salmonella, to identify the antibiotic resistance genes and plasmids that confer to resistance to several antibiotics in multidrug resistant strains and also to determine clonal relationship between the strains. **Methods:** InvA, sipA, sipD, sopB, sopD, sopE2 virulence genes that are located in Salmonella Pathogenicity Island-1, ssaR, sifA genes that are located in Salmonella Pathogenicity Island-2 and spvB, prot6E genes that are carried on plasmids are investigated with polymerase chain reaction. According to antibiotic susceptibility results seven strains that are resistant or show intermediate resistance to ampicillin were analyzed for the presence of TEM-1, SHV-1, CTX-M-1 beta lactam resistance genes and qnrA1, qnrB1, qnrS1 plasmid mediated quinolone resistance genes. Also one strain which is resistant to trimetoprim-sulfometoxazol combination was assayed for the presence of sulI and sulII sulfonamide resistance genes. Distribution and frequency of the plasmids among the strains that are responsible from the antibiotic resistance were investigated with plasmid based replicon typing. Moreover pulsed field gel electrophoresis assays were conducted in order to reveal the clonal relationships between the strains. **Results:** Among the seven strains blaTEM-1 found in three, blaSHV-1 found in six, blaCTX-M-1 found one, qnrS1 found in five and qnrB1 found in one strain. Trimetoprim-sulfometoxazol resistant strain found as positivite to presence of sulI whereas negative to sulII. Plasmid based replicon typing assays demonstrated that among the 50 strains all carried FIIS (100%) 13 (26%) carried I1, one (2%) carried I2, four (8%) carried P, one (2%) carried A/C and four (8%) carried X1 replicon. 46 out of 50 strains were typed with pulsed field gel electrophoresis. 46 strains were classified into eight major clusters, 33 pulsotypes and eight different clusters. **Conclusion:** Experiments revealed that all carried all virulence genes located on both Salmonella Pathogenicity Island 1 and 2 also plasmids which shows the high pathogenic potencial of the strains. Also all antibiotic resistant starins carried at

least one of the resistance genes of interest which indicates phenotype-genotype relations in antibiotic resistance.

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EXPRESSION AND CHARACTERIZATION OF THE ADHESIOME OF *SALMONELLA ENTERICA* SEROVAR TYPHIMURIUM

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Background: Adhesins play a crucial role in pathogenicity and dissemination of *Salmonella* and other pathogens. *Salmonella enterica* serovar Typhimurium encodes for a large repertoire of adhesins including 13 fimbrial, three autotransported (ShdA, SadA and MisL) and two T1SS-secreted adhesins (SiiE and BapA)[1]. While most fimbrial and non-fimbrial adhesins are expressed in the intestinal tract in animal models[2], only a few of them can be successfully expressed under in vitro conditions[3]. Therefore only a small subset of adhesins in *S.* Typhimurium have been characterized yet. **Methods:** To overcome this burden, we constructed a set of strains where we exchanged the natural promotors of all known adhesins in *S.* Typhimurium with a tetracycline-inducible expression cassette. For heterologous expression these constructs were then assembled on the low-copy plasmid pWSK29. Afterwards, novel fimbrial and non-fimbrial adhesins were visualized by atomic force and transmission electron microscopy. **Results:** We could successfully express all fimbrial and non-fimbrial adhesins of *S.* Typhimurium both in *S.* Typhimurium and heterologously in a non-fimbriated *E. coli* strain. We then verified by a flow cytometry-based approach that the adhesins were assembled on the surface of the bacterium. Afterwards novel adhesins were visualized by atomic force microscopy and transmission electron microscopy. Thereby fimbrial adhesins divided into clearly distinguishable groups with distinct expression levels and morphologies. **Conclusions:** This collection of strains allows for the first time the study of the complete adhesiome of *S.* Typhimurium. This offers the opportunity to specifically express and characterize novel, yet uncharacterized adhesins. The long term goal of this study is the functional characterization of the complete adhesiome of *Salmonella* and to answer the question why *Salmonella spp.* encode for such a large repertoire of fimbrial and non-fimbrial adhesins. 1 Wagner, C. & Hensel, M. Adhesive mechanisms of *Salmonella enterica*. Adv Exp Med Biol 715, 17-34 (2011). 2 Humphries, A. D. et al. The use of flow cytometry to detect expression of subunits encoded by 11 *Salmonella enterica* serotype Typhimurium fimbrial operons. Mol Microbiol 48, 1357-1376 (2003). 3 Sterzenbach T. et al. Hierarchical regulation of fimbriae in *Salmonella enterica* serovar Typhimurium via a novel CsrA titration mechanism. EMBO J 32: 2872-2883 (2013).

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LOW-OXYGEN TENSIONS FOUND IN SALMONELLA-INFECTED GUT TISSUE BOOST *SALMONELLA* REPLICATION IN MACROPHAGES BY IMPAIRING ANTIMICROBIAL ACTIVITY AND AUGMENTING *SALMONELLA* VIRULENCE

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Background: The *Salmonella* pathogenicity island-2 (SPI-2)-encoded type three secretion system (T3SS2) is of key importance for systemic disease and survival in host cells. *In vivo* SPI-2-dependent *Salmonella* replica-

tion in lamina propria monocytic phagocytes/macrophages (MΦ) is required for the development of colitis. Containment of intracellular *Salmonella* in the gut critically depends on the antimicrobial effects of the phagocyte NADPH oxidase (PHOX), and possibly type 2 nitric oxide synthase (NOS2). For both antimicrobial enzyme complexes, oxygen is an essential substrate. Based on previous findings, we hypothesized that upon *Salmonella* infection inflamed gastrointestinal tissue displays low oxygen tensions which in turn might promote replication within. In this project, we studied the *Salmonella* replication and survival within MΦ, T3SS2 activation as well as hypoxia-induced modulation of host innate immune defense. **Methods:** To determine whether hypoxia promotes *Salmonella* survival and replication in host cells, bone marrow-derived, peritoneal- and RAW264.7 MΦ were infected with *S.* Typhimurium (STM) under different oxygen levels. Besides quantifying intracellular bacteria using plating assays their ability to replicate was assessed with the help of a fluorescence dilution reporter and subsequent analysis by flow cytometry. We assessed production of reactive nitrogen- and oxygen species (RNS, ROS) by the host cells quantifying nitrite and using CellRox DeepRed staining, respectively. ROS perception by *Salmonella* could be detected using a H_2O_2-responsive fluorescence reporter. To gain further insight into the impact of hypoxia on SPI2 activity a T3SS2-dependent fluorescence reporter plasmid and immunoprecipitation of translocated SPI-2 effector protein SseJ were applied under normoxic and hypoxic conditions. **Results:** Our experiments revealed an increased intracellular replication and survival of wild-type and T3SS2 deficient STM under hypoxia. These findings were paralleled by blunted RNS and ROS production and consequently reduced ROS perception by STM. In addition, hypoxia enhanced SPI-2 transcription and translocation of the SPI-2-encoded effector protein SseJ. Neither pharmacological blockade of PHOX and NOS2 nor impairment of T3SS2 virulence function alone was able to mimick the effect of hypoxia on *Salmonella* replication under normoxic conditions. **Conclusion:** In summary, we demonstrate that a hypoxic microenvironment as present in the gut promoted SPI-2-dependent *Salmonella* virulence and impaired oxygen-dependent host cell defense mechanisms. These data imply that under hypoxia both effects cooperate in a synergistic way to allow for enhanced *Salmonella* replication in MΦ. Hence, analysis of *Salmonella*-host interaction under physiologically relevant low oxygen conditions can unravel novel aspects of *Salmonella* pathogenesis.

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EXPRESSION AND PRODUCTION OF FIMBRIAL GENE CLUSTERS FROM *SALMONELLA ENTERICA* SEROVAR TYPHI

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Salmonella enterica serovar Typhi is a human-specific pathogen mostly found in developing countries. It is the etiologic agent of typhoid fever, causing more than 20 million cases and 400 000 deaths annually. Moreover, multi-resistant S. Typhi strains have recently spread, provoking an increase in the number of cases that can eventually lead to many more deaths worldwide. To fight this pathogen, we need to gain a better understanding of its mechanisms of virulence and infection. Salmonella serovars possess a variety of virulence factors that can be targeted. One of the virulence factors of S. Typhi is the presence of multiple adhesion systems called fimbriae. Fourteen putative fimbriae are detected in the S. Typhi genome and are categorized in three classes: the Chaperone-Usher Pathway (CUP) fimbriae, the curli fimbriae and the type IV pili. None of them have been characterized so far, mainly due to their poor expression under laboratory conditions. However, in other Salmonella serovars, fimbriae are involved in biofilm formation, serocon-

version, cellular invasion, and interactions with macrophages. Therefore our hypothesis is that the S. Typhi fimbriae are functional and could be implicated in diverse steps of pathogenesis in humans. To confirm the role of fimbriae, individual fimbrial mutants as well as a mutant strain lacking all fimbrial gene clusters were constructed. In order to determine the expression of the 14 fimbrial systems, each fimbrial promoter was cloned in fusion with the lacZ reporter gene. Several culture conditions (rich, poor, SPI-1, SPI-2, etc.) were tested and each fimbria had a specific expression pattern that was conserved within these conditions. Among all conditions, fimbrial expression was always higher when grown in minimal medium (M63) with glucose as carbon source. For the production of fimbrial proteins and visualization of the bacterial surface appendage structure, each fimbrial gene cluster was cloned in an inducible vector and transformed in the afimbrial strain. We have already confirmed the presence of the Tcf, Saf, Stb and Fim fimbriae on the bacterial surface by electron microscopy. In conclusion, the characterization of S. Typhi fimbriae will lead to a better understanding of S. Typhi pathogenesis. The actual experiments will be completed by human cell infection assays to demonstrate effects of each fimbria in the different steps of pathogenesis. This research can bring insight on the development of new antimicrobial therapies that will be more accurate and specific to Salmonella Typhi and other serovars.

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DISSEMINATION OF "*SALMONELLA ENTERICA*" SEROVAR TYPHIMURIUM TO THE MOUSE BRAIN IS INDEPENDENT OF "*SALMONELLA*" PATHOGENICITY ISLAND 1

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Background: *Salmonella enterica* serovar Typhimurium is a common cause of foodborne gastroenteritis in immunocompetent humans and of bacteremia in immunocompromised populations. In sub-Saharan Africa, where HIV/AIDS, malaria and malnutrition are common, invasive non-typhoidal *Salmonella* infections have become a major public health problem. These systemic infections can lead to bacterial meningitis, with poor survival outcomes. How *Salmonella* disseminates to the brain is not understood. In mouse models of systemic Salmonellosis, dissemination from the gut depends upon the *Salmonella* Pathogenicity Island 1 (SPI1)-encoded Type III Secretion System (T3SS1). T3SS1 is also required for infection of cultured enterocytes; however, the role of SPI-1 in brain infections and crossing the blood-brain barrier is unclear. Our goal was to evaluate whether SPI-1 has a significant role in the dissemination of *Salmonella* to the central nervous system. **Methods:** Groups of C57BL/6 and NRAMP1 reconstituted C57BL/6 (NRAMP1$^{+/+}$) mice were infected intravenously with wild type, ΔSPI-1, or ΔSPI-2 *Salmonella enterica* serovar Typhimurium (SL1344). To follow the course of infection animals were euthanized when they exhibited clinical signs of systemic disease and cerebrospinal fluid, brain, blood and spleen were harvested. Colony forming units (CFUs) were assessed by plating. **Results:** C57BL/6 mice infected with the WT or succumbed to invasive *Salmonella* disease within 4-6 days post infection, and the bacterial loads in tissues were comparable. C57BL/6 NRAMP1$^{+/+}$ survived longer but again there was no apparent difference between the ability of WT and the ΔSPI-1 mutant to disseminate to the brain. More importantly, we did not detect a significant difference in the bacterial load of the cerebrospinal fluid. As expected, the ΔSPI-2 mutant failed to colonize the mouse organs, including the brain. **Conclusions:** Our data show that dissemination of *Salmonella enterica* serovar Typhimurium to the brain is not mediated by SPI-1 encoded functions.

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DEPLETION OF BUTYRATE-PRODUCING CLOSTRIDIA FROM THE GUT MICROBIOTA DRIVES AN AEROBIC LUMINAL EXPANSION OF *SALMONELLA*

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The mammalian intestine is host to a microbial community that prevents pathogen expansion through unknown mechanisms, while antibiotic treatment can increase susceptibility to enteric pathogens. Here we show that streptomycin treatment depleted commensal, butyrate-producing *Clostridia* from the mouse intestinal lumen, leading to decreased butyrate levels, increased epithelial oxygenation, and aerobic expansion of *Salmonella enterica* serovar Typhimurium. Epithelial hypoxia and *Salmonella* restriction could be restored by tributyrin treatment. *Clostridia* depletion and aerobic *Salmonella* expansion were also observed in the absence of streptomycin treatment in genetically resistant mice but proceeded with slower kinetics and required the presence of functional *Salmonella* type III secretion systems. The *Salmonella* cytochrome *bd*-II oxidase synergized with nitrate reductases to drive luminal expansion, and both were required for fecal-oral transmission. We conclude that *Salmonella* virulence factors and antibiotic treatment promote pathogen expansion through the same mechanism: depletion of butyrate-producing *Clostridia* to elevate epithelial oxygenation, allowing aerobic *Salmonella* growth.

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THE NICHE SPECIFIC REQUIREMENT FOR THE ETHANOLAMINE TRANSPORTER EUTH DURING *SALMONELLA* INFECTION

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Background: Ethanolamine (EA) is found throughout mammalian hosts, and pathogens, including Salmonella, metabolize EA to enhance growth in vivo. Additionally, Salmonella uses EA as a signal to recognize the intracellular environment and promote survival and dissemination. Genes encoding EA sensing and regulation, metabolism, and transport are located within the EA utilization (eut) operon. Previous studies showed that the eut-encoded EA transporter EutH is not required for EA metabolism in vitro but may be important in the presence of low EA concentrations and a slightly acidic pH (Penrod, et al. 2004). These conditions are suggestive of the Salmonella containing vacuole within macrophages. Thus, we hypothesize that EutH is required for Salmonella to survive within macrophages during infection. The goal of this study is to examine the importance of EutH in vivo. **Methods:** To determine the role of EutH in vivo, we performed colitis infections with orally infected mice and systemic infections with mice infected via intraperitoneal injection. Additionally, macrophages were infected in vitro. At indicated times post-infection, RNA was extracted and bacterial gene expression was quantified. Alternatively, bacterial survival was determined by lysing macrophages and enumerating colony forming units. **Results:** The eutH mutant had no intestinal colonization defect during colitis, where EA metabolism provides Salmonella with a growth advantage over the resident microbiota. These data indicate that EutH is dispensable for EA metabolism in vivo and are in agreement with previous in vitro studies. In contrast, the eutH mutant had a significant dissemination defect during systemic infection. The ability of Salmonella to adapt to and survive within macrophages

is vital for systemic infection. We measured a significant induction of eutH expression during macrophage infection in vitro, and the eutH mutant was significantly attenuated for intramacrophage survival. These data suggest that EutH is required for Salmonella to sense and adapt to the slightly acidic and low EA containing intramacrophage environment. **Conclusions:** Salmonella encounters a wide variety of environments throughout a mammalian host and the ability of Salmonella to adapt to these distinct niches is critical for infection. Our data suggest that EutH is required for Salmonella to sense and respond to EA within macrophages, a critical aspect of Salmonella pathogenesis. Our findings provide an ecological context for EutH and identify its requirement for survival within macrophages. EutH is highly conserved across Gram-negative and Gram-positive bacteria and thus may represent a broadly applicable target for therapeutic intervention.

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METABOLIC NETWORKS REQUIRED FOR PLANT COLONIZATION BY *SALMONELLA ENTERICA* REVEAL CROSS-KINGDOM COLONIZATION STRATEGIES OF A GENERALIST

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Plants are now a well-established vector of Salmonella enterica to humans. Numerous salmonellosis outbreaks linked to S. enterica-contaminated fresh produce have occurred in recent years; produce now ranks as the riskiest commodity for foodborne illness. Salmonella's success or failure to colonize the food source of a primary host is fundamental to the pathogen's cross-kingdom life cycle. However, knowledge of the metabolic networks essential to thrive on plants is minimal. In order to reduce foodborne illness, we need to understand the biology of S. enterica on plants, before the pathogen reaches humans and causes disease. We used bacterial proteomics and genetics and competition assays to obtain a comprehensive overview of Salmonella nutrition, growth, and colonization in an alfalfa seedling model. The alfalfa seedling model offers an understanding of the most common plant vehicle of current salmonellosis infection in the United States. We found that Salmonella used at least 28 nutrients in germinating alfalfa root exudates that serve as sole C- or N-sources or vitamins. By comparing our metabolic mutants plant colonization results to those reported by Steeb, et al [doi:10.1371/journal.ppat.1003301], we tested our hypothesis that different metabolic networks are required for the distinct plant- and animal-associated lifestyles of S. enterica due to differences in host physiologies and niches colonized. We found that the presence of individual nutrients and the utilization of particular pathways differed between the two host but similar metabolic networks were required for S. enterica colonization of both. Though root exudates support rapid replication of S. enterica, 1 million-fold within 48 h, Salmonella requires biosynthesis of at least 15 amino acids, as well as nucleosides, fatty acids, and vitamins. Some metabolic networks are required for both environments but differentially; fatty acid biosynthesis in plants and in contrast, fatty acid degradation in animals. A manA mutant was also non-competitive for alfalfa seedling colonization, defective in both attachment and growth. S. enterica uses factors required for animal virulence to colonize plants. Our data indicate that O-antigen, but not colanic acid, contributed to S. enterica plant colonization. In plants, mannose metabolism is important for host-microbe interactions. Our data suggests that mannose-6-phosphate, produced endogenously by ManA or by transport and phosphorylation by ManXYZ, influences S. enterica nutrient utilization and is thus a previously unknown metabolic regulator. The primary role of this network in animals is to facilitate virulence by immune evasion, whereas in plants it contributes to attachment, growth, and nutrient utilization.

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HOST DERIVED LACTATE SUPPORTS *SALMONELLA* OUTGROWTH DURING INFLAMMATION

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The induction of inflammation by *Salmonella enterica* Typhimurium (*S.* Tm) is essential for colonization, as it dramatically changes the nutritional environment of the gut and allows *S.* Tm to outcompete the microbiota. Little is currently known about the metabolic interaction between the host and *S.* Tm. To address this question, we performed untargeted metabolic profiling experiments to identify potential carbon sources of *S.* Tm in a murine model. We found that lactate is one of the most abundant metabolites in the intestinal lumen during *S.* Tm infection. *L*-lactate dehydrogenase (LldD) activity provided a fitness advantage in colonizing the intestinal tract, while utilization of D-lactate was dispensable. Bacterial LldD activity still conferred a fitness advantage in the absence of a gut microbiota, i.e. in germ-free mice, suggesting that the lactate utilized by *S.* Tm was host derived. Furthermore, administration of oxamate, an inhibitor of the mammalian lactate-producing LDH-5 enzyme, reduced luminal lactate levels and lactate utilization by *S.* Tm. A decrease in commensal *Clostridia* populations and a concomitant drop in microbially-produced butyrate have been shown previously to induce a switch in colonocyte metabolism from beta-oxidation to lactate fermentation. Consistent with the idea that a disruption of the gut microbiota during *S.* Tm infection alters host metabolism, *S.* Tm infection in conventional mice decreased butyrate concentrations by 10-fold while increasing luminal lactate concentrations by 4-fold. Additionally, mono-association of gnotobiotic mice with butyrate-producing *Clostridium symbiosum* decreased the fitness advantage associated with lactate utilization. The picture emerging from this study is that *S.* Tm-induced mucosal inflammation significantly impacts microbiota-derived butyrate production, which in turn affects host metabolism. Ultimately, host-derived lactate accumulates in the gut lumen and is utilized by *S.* Tm as a primary carbon source. Collectively, this study elucidates a novel metabolic between the host, the gut microbiota, and *S.* Tm in the context of a natural infection.

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CATALASE AND PYRUVATE PROMOTE RESUSCITATION OF THE VIABLE BUT NON-CULTURABLE (VBNC) *SALMONELLA* VIA DIFFERENT PATHWAYS

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Background: VBNC is a state of the bacterial cells that endows them with dormant but persistent phenotypes, which seem to be related to early stages of biofilm formation. Many microorganisms, including *Salmonella*, are considered to be in the VBNC state in natural environments. It is also considered that experimentally stressed microorganisms turn readily into VBNC state. The VBNC cells of pathogenic bacteria could be a problem in the hygienic field because of its non-culturability by colony-formation assay but its resuscitation to the culturable state. We report that *Salmonella* Enteritidis (SE) enters into the VBNC state by exposure of oxidative stress and low-humid stress but can be resuscitated differently by incubation with sodium pyruvate or catalase.
Methods: An environmental isolate of virulent SE cells from a poultry in Japan were cultured in LB medium until they reached at the mid-logarithmic growing phase. The cells were treated either with 3 mM hydrogen peroxide (H_2O_2) in Luria-Bertani (LB) medium at 37°C for 60 min for oxidative stress exposure, or were suspended in saline and incubated under 10-20% relative humidity in room temperature overnight for low-humid stress exposure. Then

the cells were suspended in glucose-free M9 minimal medium with catalase or sodium pyruvate, incubated at 37°C for up to 6 h to resuscitate. Culturability of the cells was assessed by colony-formation assay on LB-agar plate, and metabolic activities of cells were assessed by flow cytometery. Expression of *katE* was measured by qRT-PCR and endogenous catalase activity was assayed with a catalase assay kit. **Results:** Almost all H_2O_2-treated cells were not culturable but maintained high activities of respiration, DNA synthesis and glucose uptake, showing that they were entered into the VBNC state. A certain population of them could regain their culturability by the treatment with 0.3 mM pyruvate or 2,000 U catalase for 6 h, without multiplication of culturable cells. The resuscitation induced by pyruvate showed a biphasic curve, that by catalase, monophasic one which resembled the latter part of the curve with pyruvate. The expression of *katE* and the endogenous catalase activity seemed to be increased at 1 h of resuscitation. These results suggest that pyruvate might trigger the resuscitation of VBNC cells by enhancing their endogenous catalase activity and gene expression. We also confirmed that dehydrated SE cells showed very low culturability but maintained relatively high metabolic activities, suggesting that they were entered the VBNC state. These cells also regained their culturability by subsequent treatment not with pyruvate but with catalase. **Conclusion:** Catalase and pyruvate might act as a key molecule on the resuscitation of the VBNC *Salmonella* via different pathways.

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ANTIBIOTIC TREATMENT REDUCES PPARγ SIGNALING AND REGULATORY T CELLS NUMBERS TO DRIVE POST-ANTIBIOTIC PATHOGEN EXPANSION

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An antibiotic-mediated disruption of the gut microbiota lowers colonization resistance against Salmonella enterica serovar Typhimurium by lowering the concentration of microbiota-derived short-chain fatty acids (SCFAs), which in turn increases oxygenation of enterocytes in the colon (colonocytes), thereby driving an aerobic pathogen expansion. However, the mechanism by which depletion of SCFAs alters the metabolism of colonocytes remains elusive. Here we show that a streptomycin-mediated depletion of butyrate-producing microbes diminished signaling of the intracellular butyrate sensor PPARγ (peroxisome proliferator-activated receptor gamma) in colonocytes, which in turn drove an aerobic respiration-dependent expansion of S. Typhimurium by increasing colonocyte oxygenation. However, genetic ablation of Pparg expression in the intestinal epithelium was not sufficient to increase colonocyte oxygenation. By depleting SCFAs, streptomycin treatment reduces the numbers of regulatory T cells (Tregs) in the colonic mucosa, resulting in low level inflammation. Interestingly, mice lacking Pparg expression in the intestinal epithelium supported an aerobic expansion of S. Typhimurium after depletion of Tregs, while depletion of Tregs in wild-type littermate control mice did not. Similarly, induction of low-level inflammation by treatment with 1% sodium sulfate sodium (DSS) increased colonocytes oxygenation in mice lacking Pparg expression in the intestinal epithelium but not in littermate controls. We conclude that reduced PPARγ-signaling in combination with a contraction of the colonic Treg pool are sufficient for elevating colonocytes oxygenation, thereby driving an aerobic post-antibiotic pathogen expansion in the gut.

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THE *IN VITRO* REDUNDANT ENZYMES PURN AND PURT ARE NOT REDUNDANT FOR INFECTION IN *SALMONELLA* SEROVAR TYPHIMURIUM

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The understanding of the metabolism of Salmonella during infection has caught increased attention, mainly to search for novel targets for antibiotics. Metabolic enzymes show a high degree of redundancy, and for that reason it is generally difficult to identify essential reaction by standard genetic approaches. Instead metabolic modelling has been applied. Such modelling, as well as in vitro growth characterization, suggests that the enzymes PurN and PurT are redundant in Salmonella enterica serovar Typhimurium (S. Typhimurium), where they perform the third step in the purine synthesis. Surprisingly the results of the current study demonstrated that single gene deletions of each of the genes encoding these enzymes caused attenuation (competitive infection index < 0.03) in mouse infections. While the ΔpurT mutant multiplied as fast as the wild type strain in cultured J774A.1 macrophages, net multiplication of the ΔpurN mutant was reduced by approximately 50 % over 20 hours. The attenuation of the ΔpurT mutant was abolished by simultaneous removal of the enzyme PurU, responsible for formation of formate for the PurT catalysed reaction, indicating that the attenuation was related to formate accumulation. Purine and carbon-1 metabolism are linked exactly at this step in the purine synthesis. In the process of further characterization, we discovered that the enzyme-complex GCV was the most important for formation of C1 units in vivo (CI: 0.03 ± 0.03). In contrast, GlyA was the only important enzyme for the formation of C1 units during growth in vitro. The results with the ΔgcvT mutant further revealed that formation of serine by SerA and further conversion of serine into C1 units and glycine by GlyA was not sufficient to ensure successful infection. The study calls for re-investigations of the concept of metabolic redundancy in S. Typhimurium in vivo.

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EFFECTS OF ANTHRANILATE ON BIOFILM FORMATION OF PATHOGENIC BACTERIA

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A group of bacteria harboring tnaA gene encoding tryptophanase that converts tryptophan into indole, pyruvate, and ammonia produce indole from tryptophan degradation (for examples, Salmonella enterica, Escherichia coli, Haemophilus influenzae, and Vibrio vulnificus), while other bacteria, including P. aeruginosa, degrade tryptophan to anthranilate through a kynurenine pathway using the kynBAU genes. Therefore, cells in mixed bacterial population including both types of bacteria necessarily encounter anthranilate and indole in tryptophan-rich environments. It has been recently reported that anthranilate deteriorates the biofilm structure by reducing the level of intracellular c-di-GMP and modulating the expression of Psl, Pel, and alginate in P. aeruginosa. We investigated the anthranilate effect on biofilm formation of various bacteria and the underlying mechanism of the anthranilate effect. Static and flow-cell systems were used for biofilm formation and direct confocal microscopic observation was carried out to monitor the anthranilate effect on biofilm formation. We also measured bacterial motility and intracellular c-di-GMP levels with the anthranilate treatment. Anthranilate has the similar deteriorating effect on biofilms of many bacteria including Vibrio vulnificus, Bacillus subtilis, and Staphylococcus aureus, but it has distinct effect on some enteric bacteria such as E. coli and S. enterica Typhimurium. Since anthranilate significantly enhanced swimming

and swarming motility of V. vulnificus and B. subtilis, we suggest that the biofilm inhibition by anthranilate may be caused by the enhanced motility. Anthranilate has an effect on the biofilm formation in wide range of bacterial species.

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ENZYME IIANTR REGULATES THE PROPIONATE METABOLISM WHICH IS INVOLVED IN *SALMONELLA* INVASION

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Background: Nitrogen-metabolic phosphotransferase system (PTS^{Ntr}) is composed of enzyme I^{Ntr}, NPr, and enzyme IIA^{Ntr} ($EIIA^{Ntr}$) encoded by *ptsP*, *ptsO*, and *ptsN,* respectively. $EIIA^{Ntr}$ has been reported to demonstrate varieties of regulations associated with metabolism of carbon and nitrogen, potassium homeostasis, and virulence of several pathogens. *Salmonella* Typhimurium is an intracellular pathogen and its invasion is mediated by a number of environmental signals within the intestine. Among them, the intestinal short chain fatty acids (SCFAs) such as propionate are produced as fermentation products by microbiota and present in high concentrations in the animal intestine. It has been suggested that *Salmonella* uses propionate as an environmental cue as well as a source of carbon and energy. **Methods:** In order to understand roles of $EIIA^{Ntr}$ further, we analyzed transcriptome of wild-type and a mutant *Salmonella* strain lacking *ptsN* by RNA sequencing. We selected one of the highly down-regulated genes in the *ptsN* mutant and compared the transcriptional expressions using quantitative Real-time PCR and β-galactosidase assay. Translational expressions of the regulator PrpR were assessed by western blots. Invasion assay using caco-2 was performed under the presence of propionate to compare the *Salmonella* invasion ability. **Results:** One of the highly down-regulated genes in the *ptsN* mutant was the propionate catabolism operon (*prpBCDE*). We verified that the expression levels of the *prpBCDE* operon decreased more than 3-fold in the *ptsN* mutant compared to wild-type. To elucidate the mechanism, we determined transcriptional levels and protein levels of *prpR* in the wild-type and *ptsN* mutant. Although there is no difference in transcriptional levels of *prpR*, the half-life of PrpR protein in the wild-type was 10-fold longer than that of *ptsN* mutant, indicating post-translational regulation of PrpR by *ptsN.* Moreover, *Salmonella* invasion increased in the *ptsN* mutant in the presence of propionate. This may be due to the downregulation of propionate metabolism considering the existing fact that propionyl-CoA, intermediate of propionate metabolism, reduces *Salmonella* invasion. **Conclusions:** In this study we provide first evidence that $EIIA^{Ntr}$ encoded by *ptsN* regulate the propionate metabolism. Since the intermediate propionyl-CoA inhibits *Salmonella* invasion, the *ptsN* mutation results in the increase of *Salmonella* invasiveness in the presence of propionate. These result indicate that $EIIA^{Ntr}$ is involved in *Salmonella* invasion via propionate metabolism in the intestine.
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MAINTENANCE OF AMINO SUGAR HOMEOSTASIS BY DIRECT INTERACTION BETWEEN EIIANTR AND GLMS IN *SALMONELLA* TYPHIMURIUM

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Background: The bacterial phosphotransferase system (PTS) is a translocation system in which a phosphate moiety of phosphoenolpyruvate is transferred to a specific substrate via a cascade of enzymes in sequence, and the phosphorylated substrate is translocated into the cytoplasm. However, the nitrogen phosphotransferase system (PTS^{Ntr}), which is composed of EI^{Ntr}, NPr, and $EIIA^{Ntr}$, lacks specific substrate information. Instead, the location of PTS^{Ntr} genes in close proximity to *rpoN* suggests that PTS^{Ntr} is involved in nitrogen-associated regulation. To define the correlation between PTS^{Ntr} and nitrogen metabolism and its primary roles, we performed ligand fishing with $EIIA^{Ntr}$ as a bait. **Methods:** In order to search for target proteins interacting with $EIIA^{Ntr}$, $EIIA^{Ntr}$ tagged with His_6 ($EIIA^{Ntr}$-His_6) was isolated and used as bait in a ligand-fishing strategy. $EIIA^{Ntr}$-His_6 was incubated with a crude protein extract of *Salmonella* Typhimurium SL1344, and the proteins bound to $EIIA^{Ntr}$-His_6 were pulled down with a metal-affinity resin. The SDS-PAGE analysis of isolated protein complexes from ligand-fishing experiments revealed a protein band of approximately 70 kDa that specifically co-precipitated with $EIIA^{Ntr}$-His_6. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) peptide mapping identified this protein as GlmS. **Results:** We found out that D-glucosamine-6-phosphate synthase (GlmS) directly interacted with IIA^{Ntr} in a heterotrimeric form. GlmS, which converts D-fructose-6-phosphate into D-glucosamine-6-phosphate, is a key enzyme producing amino sugars through glutamine hydrolysis. Amino sugar is an essential structural building block for bacterial peptidoglycan and LPS. We further verified that $EIIA^{Ntr}$ inhibited GlmS activity by direct interaction in a phosphorylation-state-dependent manner. Therefore $EIIA^{Ntr}$-mediated GlmS inhibition might lead to a growth defect attributable to the impaired integrity of the cell wall and further imply a role for $EIIA^{Ntr}$ controlling the production rate of amino sugars depending on nitrogen accessibility. $EIIA^{Ntr}$ was dephosphorylated in response to excessive nitrogen sources and was rapidly degraded by Lon protease upon amino sugar depletion. **Conclusions:** $EIIA^{Ntr}$, which is phosphorylated under nitrogen-limiting conditions, compromises amino sugar biosynthesis by inhibiting GlmS and decelerates production of peptidoglycans and LPS. The regulation of GlmS activity by $EIIA^{Ntr}$ and the modulation of *glmS* translation by RapZ (RNase adaptor protein encoded immediately downstream of *ptsN*) suggest that the genes comprising the *rpoN* operon play a key role in maintaining amino sugar homeostasis in response to nitrogen availability and the amino sugar concentration in the bacterial cytoplasm. **Funding:** This research was supported by a grant (14162MFDS972) from the Ministry of Food and Drug Safety, Korea in 2016.

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THE ROLE OF HISTO-BLOOD GROUP GLYCOSYLTRANSFERASES IN SUSCEPTIBILITY TO *SALMONELLA* INFECTION

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Histo-blood group antigens play important roles in host-microbe interactions. Host-derived glycans present in mucosal secretions can shape the intestinal microbiota composition. Interestingly, variation in host glycosylation can also influence the susceptibility to enteric pathogens. Indeed, changes in the glycosylation profile of the GI tract is often mediated by variation in histo-blood group glycosyltransferases. Using a Salmonella Typhimurium infection model in mice, we investigated the role of two histo-blood group glycosyltransferase genes, B4galnt2 and Fut2, in the susceptibility to bacterial-induced inflammation and their influence on microbiota diversity. The glycosyltransferase gene b4galnt2 encodes a beta-1,4-N-acetylgalactosaminyltransferase known to catalyze the last step in the biosynthesis of the Sd(a) and Cad blood group antigens and is expressed in the GI tract of most mammals, including humans. Loss of B4galnt2 expression is associated with altered intestinal microbiota composition. In our study, we found B4galnt2 intestinal expression was strongly associated with increased susceptibility to Salmonella infection as evidenced by increased histopathological changes, intestinal inflammatory cytokines and infiltrating immune cells. Fecal transfer experiments demonstrated a crucial role of the B4galnt2 dependent microbiota in conferring susceptibility to Salmonella infection. The fut2 gene encodes a α-1,2-fucosyltransferase responsible for the expression of ABO histo-blood group antigens on the gastrointestinal mucosa and bodily secretions. In Fut2 deficient mice, we observed significantly lower Salmonella colonization in the colon and cecum at day 7 and 14 post infection. Furthermore, decreased histopathological changes were observed in the colon tissue of Fut2 deficient mice. Stronger infiltration of immune cells in Fut2 wildtype mice compared to Fut2 deficient mice was detected by immunofluorescence staining. Thus, our data demonstrate that histo-blood group glycosyltransferases influence the susceptibility to Salmonella induced inflammation which is most likely due to differences in microbiota composition.

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A METABOLIC INTERMEDIATE OF THE FRUCTOSE-ASPARAGINE UTILIZATION PATHWAY INHIBITS GROWTH OF A *SALMONELLA* FRAB MUTANT

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Insertions in the Salmonella enterica fra locus, which encodes the fructose-asparagine (F-Asn) utilization pathway, are highly attenuated in mouse models of inflammation (>1000-fold competitive index). Here, we report that F-Asn is bacteriostatic to a fraB mutant (IC50 19 μM), but not to wild-type or fra island deletion mutant. We hypothesized that the presence of FraD kinase and absence of FraB deglycase causes build-up of a toxic metabolite: 6-phosphofructose-aspartate (6-P-F-Asp). We used biochemical assays to assess FraB and FraD activities, and mass spectrometry to confirm that the fraB mutant accumulates 6-P-F-Asp. These results, together with our finding that mutants lacking fraD or the fra island are not attenuated in mice, suggest that the extreme attenuation of a fraB mutant stems from 6-P-F-

Asp toxicity. Salmonella FraB is therefore an excellent drug target, a prospect strengthened by the absence of the fra island in most of the gut microbiota.

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SALMONELLA ANAEROBIC β-OXIDATION OPERON (YDIQRSTD) CONFERS A FITNESS ADVANTAGE *IN VIVO*

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Maintaining the gut microbial ecosystem is essential for human health. The gut flora produces short-chain fatty acids (SCFAs) that are utilized by colonocytes for energy while conferring colonization resistance to invading enteric pathogens (e.g., *Salmonella*). SCFAs can serve as a carbon source as well as regulate *Salmonella* virulence gene expression that aid in invading the intestinal epithelium. Although SCFAs are present in the intestinal lumen, utilizing SCFAs by an aerobic pathway would be futile due to the hypoxic intestinal environment. An anaerobic β-oxidation pathway, encoded by ydiQRSTD, was discovered in *E. coli* and is highly conserved in *Salmonella* strains. Although ydiQRSTD was shown to be essential for *E. coli* growth under anaerobic conditions, it remains unclear what role ydiQRSTD plays in *Salmonella* growth and pathogenesis. We hypothesized that ydiQRSTD deficiency would render *Salmonella* less effective in colonizing the inflamed gut. To assess the contribution of ydiQRSTD to growth within the host intestinal environment, CBA/J mice were orally gavage with a 1:1 mixture of wild type and ydiQRSTD (competitive infection). 17 days post infection, wild type was recovered in significantly higher numbers (4 fold) from the colon than ydiQRSTD. In gnotobiotic or Streptomycin treated C57BL/6J mice, WT and ydiQRSTD grew to similar levels due to the depletion of SCFAs. Conversely, if the gnotobiotic mice were pre-colonized with *Clostridia* strains (butyrate producers), wild type grew 2.5 times more than ydiQRSTD in both the colon and cecum. Furthermore, 150 mM acetate or butyrate treatment prior to infection with the 1:1 mixture in gnotobiotic mice (WT:ydiQRSTD) yield 3 to 5 times more wild type than ydiQRSTD in colonic and cecal contents. SCFA are known to drive the invasive nature of *Salmonella*, therefore, the ydiQRSTD mutant may be defective in invading the intestinal epithelium - further investigation is ongoing. Taken together, our data demonstrate that ydiQRSTD contributes to the survival of *Salmonella* within the host intestinal environment.

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MALARIA REDUCES COLONIZATION RESISTANCE TO NON-TYPHOIDAL *SALMONELLA* THROUGH ALTERATIONS TO THE INTESTINAL MICROBIOTA

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Background: Malaria is a substantial risk factor in childhood cases of disseminating non-typhoidal *Salmonella* (NTS) infections. While changes to host immune function resulting from malaria have been implicated in increased systemic dissemination capability by NTS, little is known as to whether malaria has an effect on host resistance to NTS intestinal colonization. **Methods:** Using a murine co-infection model, we assessed the effects of malaria on intestinal inflammatory tone, host microbiota composition, and resistance to concurrent colonization by non-typhoidal *Salmonella enterica* serotype Typhimurium (*S.* Typhimurium). Changes to the intestinal microbiota of mice during infection with the malaria parasite *Plasmodium yoelii* were characterized by 16S rRNA sequence analysis. Short chain fatty acid (SCFA) levels were measured by HPLC, and colonocyte metabolic gene expression was assessed by qRT-PCR.

Infectious dose (ID_{50}) of *S.* Typhimurium was determined by oral inoculation over a range of doses. Germ-free (GF) mice reconstituted with the cecal microbiota from *P. yoelii*- or mock-infected mice were also challenged with *S.* Typhimurium to determine the contribution of microbiota changes to colonization resistance. **Results:** Based on histopathology, qRT-PCR for inflammatory markers, and flow cytometric analyses, *P. yoelii* infection induced intestinal inflammation in mice. Acute malaria was also associated with shifts in intestinal microbiota compositions, including a reduction in members of the class *Clostridia* and increased abundance of the class *Bacteroides*. Further, infection was linked with a drop in intestinal SFCA levels, along with a decrease in expression of genes relevant to beta-oxidation and an increase in anaerobic glycolysis gene transcripts by colonocytes. Remarkably, malaria substantially reduced the ID_{50} for intestinal colonization by *S.* Typhimurium, and GF mice reconstituted with cecal contents from parasite-infected mice exhibited lower colonization resistance than animals getting a transfer from mock-infected mice. **Conclusions:** *P. yoelii*-infected mice display reduced colonization resistance to intestinal infection with *S.* Typhimurium. Additionally, although the inflammation induced by *P. yoelii* could provide a niche for increased NTS growth in co-infected mice, the microbiota transfer experiments suggest the indirect effects of malaria on the microbiota and/or metabolite abundance are also relevant to reduced resistance.

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SALMONELLA TYPHIMURIUM CONSUMES GABA IN THE INFLAMED MOUSE GUT

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Salmonella *enterica* encodes an impressive array of metabolic pathways, however the significance of many of these pathways during infection remains to be determined. Interestingly, a recent *in silico* analysis revealed *Salmonella* serovars that cause intestinal disease, but not those that cause extra-intestinal disease, are able to uptake and catabolize gamma-aminobutyric acid (GABA). GABA is a major inhibitory neurotransmitter in the mammalian nervous system that may play a role in gastrointestinal function via involvement in the enteric nervous system. To explore the consequences of GABA utilization during infection, we first measured GABA concentration in the cecal contents of CBA/J mice infected with *S.* Typhimurium. Mice infected with a mutant in the GABA utilization operon (gabTP), had significantly more GABA compared to uninfected and wild-type infected mice, confirming that *S.* Typhimurium consumes GABA during infection. As GABA can be both host and microbiota derived, we next wanted to find the source of GABA. We conducted the same experiment in germ-free (GF) Swiss Webster mice, and intriguingly, we found no difference in GABA levels between the wild-type and gabTP-infected GF mice. Additionally, cecal contents of GF mice contained approximately 100 times less GABA than those of conventional mice, indicating GABA in the cecal contents of conventional mice is primarily microbiota-derived. While GABA levels changed, the wild-type had no growth advantage over the mutant in CBA/J mice in single or competitive infections. The significance of GABA to host gut physiology is still unclear, but could contribute to GI motor activity as well as modulation of immune cells. We therefore reasoned that *S.* Typhimurium GABA consumption could alter host physiology or immune responses during infection. To test this idea, we first measured whole gut transit time of infected mice using the Evans blue dye method. Gut transit time was decreased in mutant infected mice compared to wild-type and mock, suggesting that GABA concentrations alone could affect gut motility. We next used qPCR to determine differences in inflammatory status in the cecal tissue of infected mice. At ten and fourteen days post infection in CBA/J mice, we found

a trend towards decreasing pro-inflammatory cytokines in the mutant infected mice compared to the wild-type, including Kc, a murine neutrophil chemoattractant. Our results indicate that *S.* Typhimurium consumes GABA during infection, resulting in a slower gut transit time and increased inflammatory tone. This work helps further illustrate the effect of the gut microbiota-derived metabolic profile on the host, and further work will explore the mechanisms of GABA modulation on the host during infection.

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AEROBIC AND ANAEROBIC RESPIRATION SYNERGIZE TO PROMOTE INTESTINAL GROWTH OF *SALMONELLA* ON 1,2-PROPANEDIOL

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Analysis of the pseudogene evolution in S. enterica suggests that genes within the 1,2-propanediol utilization (pduA-X) operon are conserved in serovars causing gastrointestinal disease, such as S. Typhimurium, but are often disrupted in closely related serovars associated with extraintestinal disease, such as S. Typhi (Nuccio, MBio, 2014). 1,2-propanediol (1,2-PD) is a product of fucose and rhamnose fermentation and can be catabolized by S. Typhimurium using a pathway that requires oxygen or tetrathionate as electron acceptors for respiration (Price-Carter, J Bac, 2001). Tetrathionate is generated as a by-product of the inflammatory response induced by Salmonella during intestinal colonization (Winter, Nature, 2010). This inflammatory response also increases epithelial oxygenation, which drives an aerobic expansion of S. Typhimurium through cytochrome bd-II oxidase (cyxA) in synergy with anaerobic respiration (Rivera-Chavez, CHM, 2016). Based on these observations we hypothesized that Salmonella can take advantage of 1,2-PD utilization during growth in the inflamed intestine by using either tetrathionate or oxygen as electron acceptors to respire this otherwise non-fermentable carbon source. Using the CBA/J mouse model of colitis, we show that utilization of 1,2-PD promotes intestinal growth of S. Typhimurium but only during pathogen-induced inflammation. Salmonella infections of germ-free mice or mice mono-colonized with B. thetaiotaomicron suggest that this growth benefit is dependent on microbiota-liberation of fucose or rhamnose from complex carbohydrates. Surprisingly, tetrathionate respiration was dispensable for intestinal growth on 1,2-PD since deletion of the gene encoding tetrathionate reductase (ttrA) did not abrogate the fitness advantage of a propanediol-proficient strain over a propanediol-deficient strain in a competitive infection. There is a variety of electron acceptors generated during intestinal inflammation that could promote intestinal growth on 1,2-PD in addition to tetrathionate, such as nitrate, TMAO or DMSO. Therefore, we tested whether anaerobic respiration contributes to 1,2-PD utilization by deleting the moaA gene required for synthesis of molybdopterin, a cofactor for the enzymes used to respire these alternative electron acceptors. Deletion of moaA did not abrogate the fitness advantage conferred by 1,2-PD utilization. Similarly, taking away the ability to respire oxygen by deleting the cyxA gene was not sufficient to prevent 1,2-PD utilization. Remarkably, only genetic ablation of both cyxA and moaA abrogated the fitness advantage conferred by 1,2-propanediol-utilization. Collectively, our data suggest that aerobic and anaerobic respiration synergize to promote S. Typhimurium growth on 1,2-PD allowing Salmonella to sidestep competition with the microbiota that cannot use this non-fermentable carbon source.

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MODULATION OF SNARES BY *SALMONELLA* DURING ITS TRAFFICKING IN THE HOST CELLS

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Intracellular trafficking pathways are controlled by a series of vesicle fusion events regulated by Rab and SNARE (soluble N-ethylmaleimide-sensitive factor attachment receptor) proteins. Because of their indispensible roles, Rabs and SNAREs appear to be possible targets for intracellular pathogens, which often rely on the subversion of the host trafficking machinery. *Salmonella*, a well-known intracellular pathogen, uses diverse survival strategies to combat host defence mechanisms. One of the key strategies observed is to survive and replicate within specialized intracellular compartment (known as Salmonella-Containing Vacuole or Phagosomes) inside the infected host cells by manipulating host vesicular transport processes. This evasive maneuvering requires a repertoire of virulence effecter proteins secreted by its specialized type three secretion system (T3SS); however the role of many effectors still remains elusive. Previous works from our laboratory have shown that SopE, a *Salmonella* effector protein specifically binds and recruits Rab5 from host cells on the Salmonella-containing phagosomes (SCP). This promotes the fusion of SCP with early endosomal compartments thereby inhibiting its maturation into phagolysosomes (Mukherjee et al., J. Cell Biol. 2000; Hashim et al., J. Biol. Chem. 2000; Mukherjee et al., J. Biol. Chem. 2001; Mukherjee et al., J. Cell Sci. 2002). These studies have also shown that the recruitment of Rab5 on SCP helps in the acquisition of N-ethylmaleimide sensitive fusion protein (NSF), suggesting a role of SNARE proteins in the maturation of SCP. In the present investigation, we have found that *Salmonella* actively recruits SNAREs like Syntaxin 6, Syntaxin 7, Syntaxin 8 and Syntaxin 13 on the SCP during its trafficking inside the host cells. This recruitment of host proteins led us to speculate that the pathogen might be modulating these proteins to regulate the maturation of SCP. Subsequently, studies have been carried out to understand the functional basis of host SNAREs recruitment in survival strategies of *Salmonella*. Here, I will discuss our results which suggest that recruitment of SNAREs help *Salmonella* to restrict the maturation of SCP possibly to avoid its fusion with lysosomes.

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THE ACTIVE ROLE OF THE SPI-1 OFF SUBPOPULATION IN *SALMONELLA ENTERICA* SPI-1 BISTABILITY

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Salmonella enterica pathogenicity island 1 (SPI-1) is a gene cluster that encodes a type 3 secretion apparatus and effectors involved in invasion of epithelial cells. A well known trait of SPI-1 is bistable expression, which generates SPI-1 ON and SPI-1 OFF subpopulations. The biological significance of SPI-1 bistability has been adressed by previous, insightful studies. Bistability has been viewed, for instance, as a division of labour involving self-destructive altruism by the SPI-1 OFF subpopulation (Ackermann et al. Nature 454, 987-90, 2008). Another study, however, has envisaged that the SPI-1 OFF subpopulation might benefit from inflammation triggered by the SPI-1 subpopulation (Stecher et al. PLOS Biology 5:2177-89, 2007). Furthermore, enhanced tolerance to antibiotics has been detected in slow-growing SPI-1 ON cells (Arnoldini et al. PLOS Biology e1001928, 2014). In this communication we describe an additional, unsuspected feature of SPI-1 bistability: we show that a pure SPI-1 ON population obtained by bacterial cell sorting is non invasive, suggesting that the SPI-1 OFF subpopulation plays an active role in invasion. In support of this view, we also show that the invasion defect associated to unimodal

expression of SPI-1 can be suppressed by mutations that permit formation of a SPI-1 OFF subpopulation.

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IDENTIFICATION OF GENES PREFERENTIALLY EXPRESSED BY *SALMONELLA ENTERICA* SEROVAR ENTERITIDIS IN INFECTED MACROPHAGES

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Background: Salmonella enterica serovar Enteritidis (S. Enteritidis) is a communicable zoonotic bacterium. Consumption of contaminated food by S. Enteritidis results in an acute self-limiting gastroenteritidis in human. Residing in macrophage is an essential process for Salmonella survival, transmission, and infection. So identification of genes expressed in Salmonella-infected macrophages will help us to understand the pathogenesis of Salmonella. **Methods:** Selective capture of transcribed sequences (SCOTS) was used to screen the S. Enteritidis genes preferentially expressed during the contact with macrophages. The avian macrophage HD-11 and murine macrophage RAW264.7 were both used for Salmonella infection. The expression of these genes in both macrophages were then confirmed and compared by real-time PCR analysis. **Results:** A total of 61 predicted genes and 54 expressed genes were identified from Salmonella-infected HD-11 cells and RAW264.7 cells, respectively. These expressed genes were involved in various functional groups including virulence, metabolism, stress response, transport, regulator, others and unknown functions. Interestingly, most of the captured sequences were obvious different in two different Salmonella-infected macrophages except two genes, cbiK and dnaG. However, lots of genes were involved in the same pathway of metabolism or virulence. This implied although different genes were expressed in both macrophages, the same survival or metabolic pathways were needed during the Salmonella infection. To confirm the differentially expressed genes by S. Enteritidis in infected macrophages, 12 genes (invJ, ycaM, bigA, SEN4299, SEN3610, SEN0988, nlpB, SEN2555, SEN0815, SEN2967, stdB and yiaG) from infected HD-11cells and 12 genes (dnaG, cbiK, rbfA, copS, rtcR, rfaQ, rseB, secG, spvB, flgK, lpfC and phoB) from RAW264.7 were selected randomly and verified using quantitative real-time RT-PCR. The results showed that all these genes were expressed in both infected macrophages, and most of these genes were up-regulated apparently during the infection process compared to cultivation in vitro. According to the real-time PCR results, 5 genes (rtcR, rfaQ, ycaM, SEN2967, SEN4299) including rfaQ which is related to LPS synthesis were randomly selected for mutation, and competitive assay in vitro and in vivo confirmed mutation of the genes lead to the attenuation of S. Enteritidis. **Conclusion:** The differentially genes expressed between Salmonella-infected avian and murine macrophages by S. Enteritidis revealed that the pathogen showed various pathogenicity to different hosts.

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SALMONELLA TYPHIMURIUM REQUIRES SOPB AND SIFA TO SURVIVE INTRACELLULARLY IN A VACUOLAR COMPARTMENT IN DICTYOSTELIUM DISCOIDEUM

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The ability of Salmonella to survive intracellularly in eukaryotic cells is explained, in part, by the modification of the intracellular niche within the cell in a compartment called the Salmonella containing vacuole (SCV). For this purpose, Salmonella employs a number of effector proteins secreted by the type-three secretion systems encoded in Salmonella

pathogenicity island 1 (T3SS SPI 1) and 2 (T3SS SPI 2). Previously, we reported a role for these T3SS in the survival of Salmonella Typhimurium in the social amoeba Dictyostelium discoideum. In this work, we evaluated the role played by two effector proteins in this process. SopB (secreted by T3SS SPI 1) is a phosphoinositide phosphatase that acts increasing the PI(3)P species in the newly formed SCV in other models. SifA (secreted by the T3SS SPI 2) is involved in the formation of Salmonella-induced filaments (Sifs), a membrane network that extends from the SCV. We performed phagocytosis assays using axenic cultures of D. discoideum strain AX2 infected with S. Typhimurium wild-type strain 14028s, derived mutants ΔsopB and ΔsifA and complemented strains. Viable bacterial cells were recovered from D. discoideum at 0, 3 and 6 hours post-infection and titrated by serial dilution and plating in selective media. Our results show that ΔsopB and ΔsifA mutants are recovered ~5-fold less than the wild-type strain at 6 hours post-infection, indicating that these mutants are defective in intracellular survival in the amoeba. This phenotype was complemented using plasmids carrying wild-type copies of the deleted genes and these strains showed wild-type levels of intracellular survival. Next, we assessed whether Salmonella was contained in a vacuole containing a well-known SCV marker in other models. For this, we performed infections of D. discoideum cells expressing a GFP fusion of the vacuolar ATPase (VatM-GFP) using the wild-type strain expressing the red fluorescent protein mCherry, and analyzed the infected cells by confocal laser microscopy for up to 4 hours post-infection. We found viable intracellular bacteria at all times studied and determined the presence of the VatM-GFP label surrounding the vacuoles containing Salmonella. Altogether, our results suggest that S. Typhimurium resides in a SCV-like compartment in D. discoideum and that requires effectors SopB and SifA to survive within this amoeba. Additional studies to determine other cellular factors present in this vacuolar compartment are currently being performed in our laboratory.

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SALMONELLA MANIPULATION OF HOST SIGNALLING PATHWAYS PROVOKES CELLULAR TRANSFORMATION

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Background: Cancer is a multistep process fuelled by deregulation of signalling pathways in control of cellular growth and proliferation. These pathways are also targeted by infectious pathogens during infection. Galbladder carcinoma (GBC) is frequent in the Indian subcontinent, with chronic Salmonella Typhi infection as a risk factor. Direct association and causal mechanisms between Salmonella Typhi infection and GBC have not been established. **Methods:** Soft agar colony formation assays; Chemical inhibitors; Organoid culture; in vivo salmonella infections in mice; PCR on patient derived material. **Results:** We deconstruct the epidemiological association between GBC and Salmonella Typhi infection by showing that Salmonella enterica induces malignant transformation in predisposed mice, murine gallbladder organoids and fibroblasts with TP53 mutations and c-MYC amplification. Mechanistically, activation of MAPK and AKT pathways, mediated by Salmonella enterica effectors secreted during infection, is critical to both ignite and sustain transformation, consistent with observations in GBC patients from India. We report the results of a nation-wide epidemiological study on the relationship between

Salmonella typhimurium infection and colon cancer. **Conclusions:** Salmonella promotes, as collateral damage of its infection cycle, transformation in genetically predisposed cells and cancer in infected tissues. We present the first report on association between food poisoning by Salmonella and colon cancer.

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RNAI SCREEN ESTABLISHMENT FOR HOST FACTOR IDENTIFICATION IN *SALMONELLA*-SPECIFIC MEMBRANE COMPARTMENT MANIPULATION

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Background: Key virulence traits of "Salmonella enterica" serovar Typhimurium are invasion of epithelial cells and formation of a replicative niche inside host cells termed "Salmonella"-containing vacuole (SCV). Another hallmark of the intracellular lifestyle of "Salmonella" is the formation of different tubular structures, one type being "Salmonella"-induced filaments (SIFs). Several bacterial factors were identified that are important for the formation of SCVs and SIFs, i.e the "Salmonella" pathogenicity island 2-encoded type 3 secretion system and several of its effector proteins. However, little is known about corresponding host factors required for biogenesis of those structures. **Methods & Results:** RNA interference (RNAi) is a valuable method to probe host-pathogen interactions. Therefore, we set out to establish a novel RNAi screen to determine new host factors involved in SIF biogenesis in a sizable format. Using HeLa cells stably transfected for LAMP1-GFP expression, we defined biological, technical and methodological prerequisites and parameters necessary for high resolution automated microscopy of "Salmonella" infection on a spinning disk confocal microscope system. The absence of SIFs served as primary readout. Due to the dynamic nature of SIFs, live cell imaging (LCI) was implemented in our setup to avoid loss of information. Scoring of SIF phenotypes was performed by visual inspection of image series. For the entry and collection of data we therefore developed a software utility named SifScreen. **Conclusions:** The establishment of our LCI-based RNAi approach now allows the execution of a screen to identify host factors involved in SIF formation. Potentially, this unravels new host factors so far uncharacterized in context of "Salmonella" infection. Furthermore, this setup can be readily applied to interrogate other "Salmonella"-induced tubules as well as pathogen-unrelated tubular structures of general cellular biological interest.

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STRUCTURAL INSIGHTS IN THE REMODELING OF THE HOST ENDOSOMAL SYSTEM BY *SALMONELLA ENTERICA*

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Background: Salmonella enterica is a facultative intracellular enteropathogen. After invasion of epithelial cells, S. enterica is located inside a membrane-bound compartment called Salmonella-containing vacuole (SCV). By means of T3SS-translocated effector proteins, intracellular Salmonella is able to massively remodel the host cell endosomal system. This remodeling results in the formation of extensive, highly dynamic membrane tubules growing from the SCV termed Salmonella-induced filaments (SIF). Importantly, the ability to induce SIF formation is strictly correlated with an efficient intracellular replication of Salmonella and virulence in a mouse model. However, the ultrastructure and biogenesis of SIF are largely unknown. **Methods:** We used high resolution microscopy to resolve the ultrastructure of SIF. Currently, correla-

tive light and electron microscopy (CLEM) of the same structure is the best tool to combine authentic live cell imaging with the resolution power of EM. **Results:** By applying CLEM for Salmonella-infected host cells we could show that a subset of SIF is composed of a double membrane. These double membrane SIF enclose host cell cytosol and cytoskeletal filaments within the inner lumen, and various types of endocytosed material between the two SIF membranes, a space continual with endosomal lumen and SCV lumen. We found that the formation of SIF double membranes requires the function of the Salmonella T3SS effector proteins SseF and SseG. In addition, we determined the luminal volume of SIF that is accessible to Salmonella within the SCV. **Conclusions:** Our work shows how an intracellular pathogen manipulates the endosomal membrane system of its host cell in order to generate a unique tubular compartment that is permissive for intracellular proliferation. T3SS effector proteins mediate formation of tubular double membrane compartments in a novel mechanism independent from autophagy.

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INFLUENCE OF *SALMONELLA* FIMH SEQUENCE VARIATION ON BINDING TO PANCREATIC SECRETORY GRANULE MEMBRANE MAJOR GLYCOPROTEIN GP2 OF HUMAN AND PORCINE ORIGIN

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Background: Pancreatic secretory granule membrane major glycoprotein 2 (GP2) is specifically expressed on the surface of M cells and takes part in uptake of type 1 fimbriae (T1F)-positive bacteria and transport to underlying mucosal immune tissues. This process continues with local and systemic dissemination of bacteria. T1F are one of the most common adhesive organelles in the family of Enterobacteriaceae and important adhesion factors in Salmonella intestinal pathogenicity. The FimH protein is located on top of the T1F shaft and directly interacts with receptors. Several studies have shown, that serotype-associated FimH variants can differ significantly in receptor recognition and this can lead to change in course of infection. Therefore, our aim was to investigate the role of FimH sequence variation on binding to GP2 isoforms from various hosts. **Methods:** Human and porcine GP2 isoforms were expressed in SF9 cells. fimH gene sequences from 128 Salmonella isolates from five serotypes (Typhimurium (Tym), Enteritidis, Choleraesuis, Dublin and Gallinarum) of human, cattle, swine and chicken origin were determined. Expression of FimH protein in these isolates was tested with a static anti-FimH antibody adhesion assay. Bacteria were cultivated for 48h under static conditions. 96-well plates were coated with rabbit polyclonal anti-FimH antibody. After incubation of bacteria with anti-FimH, washing to remove non-adherent bacteria, and propidium iodide staining of bacteria, plates were automatically analyzed for bound bacteria (bacteria/mm2) by fluorescence microscopy. An isogenic system with one Tym strain was generated. A fimH deletion mutant was created and ten plasmids containing fimH variants were transformed into this mutant. Static adhesion assays with four human and two porcine GP2 isoforms, Horse Radish Peroxidase, RNase B and anti-FimH antibody was performed. **Results:** Comparison of fimH gene sequences from Salmonella isolates revealed 12 DNA sequence alleles including 40 variable sites. Translation of fimH gene to protein sequences revealed 11

sequence variants with 17 variable sites. T1F expression was dependent on serotype and isolation source. Cluster analysis revealed, that T1F expression is higher in strains from non-host-restricted compared to host-associated or host-restricted serotypes ($p<0.001$). In our isogenic model, binding to GP2 isoforms and standard proteins was FimH-variant dependent. The high biding phenotype was observed in case of four FimH variants, low in case of three variants and no binding in case of three variants. **Conclusions:** T1F expression varies among serotypes. T1F-expressing Salmonella can adhere to GP2 in FimH-variant dependent manner. Adhesion of FimH-positive bacteria to GP2 might be an additional entry route for Salmonella invasion. Future in vitro and in vivo studies can help to elucidate the role of FimH-GP2 interaction.

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SALMONELLA DECREASES LET-7I-3P EXPRESSION TO FAVOR MULTIPLE STEPS OF INFECTION

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Background: MicroRNAs are a class of small non-coding RNAs that regulate gene expression at the post-transcriptional level. In addition to their functions in physiological and pathological processes, microRNAs play crucial roles during infection by diverse pathogens. Bacterial pathogens have developed mechanisms to subvert the microRNA pathway for their own benefit. Here, we characterized the regulatory role of the microRNA let-7i-3p in the context of Salmonella enterica serovar Typhimurium infection. **Methods and Results:** Recently we have identified a set of microRNAs that inhibit Salmonella infection, using a microscopy-based high-throughput screening approach. Let-7i-3p was the only microRNA, among the 17 identified, to inhibit early stages of Salmonella infection. To further characterize the role of let-7i-3p during infection, HeLa cells were transfected with let-7i-3p miRNA mimic and infected with Salmonella. Bacterial quantitation by qRT-PCR, cfu and microscopy assays showed an inhibitory effect of let-7i-3p on Salmonella adhesion and replication. In particular, we show that let-7i-3p inhibits Salmonella adhesion through the induction of actin stress fibers in host cells. Moreover, to identify let-7i-3p mRNA targets responsible for the inhibition of Salmonella replication, we used a RNA-seq approach based on comparative analysis of the transcriptome of Salmonella infected cells and let-7i-3p-transfected cells. RGS2 (regulator of G-protein signaling 2) protein was identified as a let-7i-3p target responsible for the inhibition of bacterial replication. Specifically, we demonstrate that let-7i-3p and repression of its target RGS2 leads to increased lysosomal activity, consequently inhibiting Salmonella replication within the Salmonella containing vacuoles. The relevance of let-7i-3p levels to the outcome of infection is further demonstrated by the observation that decreasing let-7i-3p expression, using a let-7i-3p miRNA inhibitor, favors both bacterial adhesion and replication. Importantly, Salmonella overcomes the inhibitory effect of let-7i-3p by down-regulating its expression, through a mechanism dependent on invasion of host cells that occurs in both infected and bystander cells. **Conclusions:** Our results demonstrate that let-7i-3p plays a dual regulatory role in the context of Salmonella infection, inhibiting both early and late steps of infection. Let-7i-3p induces actin stress fibers formation, thus inhibiting Salmonella adhesion to the host cells and concomitantly, through down-regulation of RGS2, let-7i-3p inhibits bacterial replication by increasing lysosomal

activity. Importantly, Salmonella counteracts the inhibition imposed by this miRNA by decreasing let-7i-3p expression, which ultimately favors infection.

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AUTOMATED ENUMERATION OF *SALMONELLA SPP.* IN CELL LINE INFECTION ASSAYS AND SCREENING OF CELL LINE INFECTIVITY AMONG VARIOUS *SALMONELLA* ISOLATES

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Background: Adhesion and invasion to epithelial cells is one of the first steps during the pathogenesis of Salmonella infections. Salmonella has evolved various strategies to adhesion and invasion and different serovars have developed a specific combination of different adhesins. To study these interactions in vitro adhesion/invasion models are often in use. The reference assay to determine the number of bacteria adhering/infecting cell lines is the colony forming unit (CFU) determination by plating serial dilutions of bacteria suspensions on LB agar. Other methods include staining of bacteria (GFP, antibody) and manual bacteria counting with fluorescence microscopy or enumeration with flow cytometry. Therefore, our aim was to develop an automated microscopy-based system for bacterial infection assays. **Methods:** Salmonella Typhimurium (Tym) strain SL1344 was transformed with the pFPV25.1Kan plasmid resulting in Tym SL1344 GFP. Tym SL1344 GFP was used as a standard strain in infection assays on three cell lines- IPEC-J2 (porcine), Caco-2 (human) and CHIC8E11 (avian). To check the linear range of assays various dilutions of bacteria were used (2x104-3x108). The bacteria were incubated with cells for 1h, unbound bacteria were washed, cells and bound bacteria were fixed with PFA and nuclei were stained with DAPI. Automated bacteria counting was done with an automated fluorescence microscope ("VideoScan"). VideoScan software module works with a 20x magnification objective and first focuses in a well on DAPI stained nuclei. Afterwards the software takes z-stacks of images for bacteria detection, makes composition images from the z-stack and counts bacteria detected on the image. Next, 88 Salmonella strains were transformed with the pFPV25.1Kan plasmid and used in infection assays for 1h and 4h on cell lines, counted with the VideoScan module, and fitted to the appropriate statistical model. **Results:** The quantification of Salmonella was able with wide range of bacteria dilutions. For IPEC-J2 cells the linear range of assays was 2x104-2.5x108 (R2=0.96) and a maximum of 14.000 bacteria/mm2. In case of Caco-2 cells, the linear range of assays was from 2x104-3x108 (R2=0.96) and maximum of 10.400 bacteria/mm2. For CHIC8-E11 cells the linear range of assay was 2x104-3x108 (R2=0.92) and maximum of 12.350 bacteria/mm2. Multiple pairwise Wilcoxon tests for infection assay revealed different infection behavior in three cell lines discriminating S. Typhimurium from S. Enteritidis and S. Dublin. Using cluster analysis we identified three main infection patterns that were highly associated with serotype and source isolation of the strain. **Conclusions:** The new VideoScan module enables quantification of bacteria in a wide range of bacterial starting inoculum. Analysis of a preliminary screen with 88 Salmonella strains with VideoScan provides valuable information that needs further investigation.

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SPATIAL AND TEMPORAL PROTEOME DYNAMICS DURING *SALMONELLA ENTERICA* TYPHIMURIUM INFECTION

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Mammalian cells require a fast and effective response to an invading bacterial pathogen. This response relies heavily on the rapid synthesis and efficient delivery of proteins to where they can coordinate effective suppression of the invading pathogen. However, an understanding of how the proteome is spatio-temporarilly rewired in response to an intracellular pathogen is lacking. Combining quantitative proteomics and biochemistry, we have uncovered a rich and dynamic host response program to Salmonella enterica Typhimurium (STm) infection. Biochemical validation revealed that the STm SPI-2 secretion system is required to drive protease activity into the nucleus at the late stages of infection. We are currently probing the impact of this phenomenon on both host and microbe.

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PROBIOTIC *ESCHERICHIA COLI* NISSLE 1917 USES ZINC TRANSPORTERS AND THE SIDEROPHORE YERSINIABACTIN TO ACQUIRE ZINC IN THE INFLAMED GUT AND OUTCOMPETE *SALMONELLA* TYPHIMURIUM

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Background: *Salmonella enterica* serovar Typhimurium is a leading cause of foodborne illness worldwide. The pathogen can thrive in the gut, where it acquires essential nutrients, and outcompete the microbiota. We have previously shown that the probiotic *Escherichia coli* Nissle 1917 ameliorates *S.* Typhimurium infection, partly by competing with *S.* Typhimurium for iron, an essential metal nutrient. Here we sought to determine whether zinc acquisition also contributes to the probiotic activity of *E. coli* Nissle during *S.* Typhimurium infection. **Methods:** To assess whether *E. coli* Nissle can effectively outcompete *S.* Typhimurium for zinc in the gut, we first generated strains deficient in zinc uptake systems and we compared their growth in zinc-limited media. Subsequently, we infected streptomycin pre-treated mice with *S.* Typhimurium and co-administered either *E. coli* Nissle wild-type, or *E. coli* Nissle strains in which one or multiple zinc transporters were mutated. To further investigate the mechanism by which *E. coli* Nissle reduces *S.* Typhimurium colonization by competing for zinc, we used mice lacking the zinc-sequestering protein calprotectin. **Results:** We found that both *S.* Typhimurium and *E. coli* Nissle express the *znuABC* and *zupT* zinc transporters in zinc-limiting conditions. We also found that *E. coli* Nissle can additionally use the siderophore yersiniabactin to acquire zinc, as previously shown for the pathogen *Yersinia pestis*. *In vitro*, yersinabactin-mediated zinc uptake renders *E. coli* Nissle more resistant to zinc sequestration by calprotectin. In *in vivo* competition between *S.* Typhimurium and *E. coli* Nissle, the latter's expression of multiple zinc transporters gives the probiotic an edge over *S.* Typhimurium. Additionally, the host zinc sequestering protein calprotectin is necessary for the reduction of *S.* Typhimurium colonization, as *E. coli* Nissle 1917 is unable to reduce *S.* Typhimurium colonization in calprotectin-deficient mice. **Conclusions:** Our findings demonstrate that zinc acquisition plays an important role in the competition between *E. coli* Nissle 1917 and *S.* Typhimurium. Three zinc transport systems, ZnuABC, ZupT, and yersiniabactin, as well as the expression of the host protein calprotectin contribute to *E. coli* Nissle's beneficial effects and to the reduction of *S.* Typhimurium colonization of the inflamed gut.

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DOES THE MUCUS ARCHITECTURE AFFECT *SALMONELLA* TYPHIMURIUM GUT INVASION?

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In the Streptomycin mouse infection model, *Salmonella* Typhimurium (*S.* Tm) usually grows up to carrying capacity in the gut before the first tissue invasion events are observed. Within 6-10h post infection, luminal bacterial loads rise as high as 10^9 cfu/g both in the cecum as well as in the colon. Despite equal luminal loads, *S.* Tm predominantly invades the cecum whereas a significantly smaller fraction of the bacteria invades the colonic tissue. Similarly, the disease symptoms are much more pronounced in the cecum than in the colon at day 1 post infection. We hypothesized that the architecture of the intestinal mucus might contribute to the altered invasion rates in the two gut compartments. We used Carnoy's fixative in combination with immunofluorescence microscopy to visualize mucus in the colon and cecum. As a second assay, we directly imaged explanted gut tissue from transgenic mice which produce red fluorescent (mCherry tagged) mucus. With these assays we demonstrate that the cecal mucus lacks the dense inner layer which is typically observed in the distal colon. Using live microscopy, we also analyzed *S.* Tm swimming along the surface of the dense colonic mucus layer. Our data suggests that the mucus architecture is a key factor that can limit pathogen access to the gut epithelium. This may explain the differing disease susceptibility of the cecal and colonic mucosa.

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PROTEOMIC ANALYSES REVEAL AN EXTENSIVE IMPACT OF *SALMONELLA*-INDUCED FILAMENTS ON THE NUTRITIONAL SUPPLY INSIDE THE SCV

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Background: Salmonella enterica serovar Typhimurium (STM) has the unique ability to transform the host cell endosomal system into a tubular network known as Salmonella-induced filaments (SIF). Translocation of SPI2-T3SS effector proteins mediates the formation of this network. Recent work demonstrated that SIF formation is prerequisite for intracellular proliferation and that SIF are composed of double membrane tubules. Mutant strains deficient in effector protein SseF or T3SS subunit SsaV display moderate or highly reduced intracellular replication, respectively. The sseF strain induces only single membrane SIF, while the ssaV strain is unable to induce SIF formation. Here, we applied proteomic analyses of intracellular STM to elucidate the physiological consequence of normal, aberrant or absent SIF formation. **Methods:** To analyze the impact of SIF formation on the intracellular lifestyle of STM, we infected RAW264.7 macrophages with various strains and isolated Salmonella cells between 12 to 16 h p.i. For quantitative proteomics we used LC-MSE on a Waters-Synapt G2-S HDMS to detect the changes in protein abundance between the strains. **Results:** Around 1,200 STM proteins were identified and quantified for each strain, of which about 150 proteins were significantly altered in their abundance between two compared strains. In comparison to STM wild type, both mutant strains showed reduced protein biosynthesis, suggesting an overall attenuated metabolism. Furthermore, proteins involved in cell defense were up-regulated in both mutant strains. The analysis revealed that the sseF

mutant strain showed an increased capacity in uptake of iron, as well as of amino acid, suggesting an increased metabolic activity compared to ssaV-deficient strain. Moreover, these requirements indicate a limitation of amino acids and metals inside single membrane SIF in contrast to double membrane SIF induced by wild-type STM. **Conclusion:** Proteomics of intracellular STM SPI2 mutant strains provide insights into the role of SIF for the intracellular survival and proliferation. Changes in the metabolism of the sseF mutant strain compared to STM wild type and the ssaV mutant strain indicate an altered nutritional supply inside the in single membrane SIF compared to double membrane SIFs or SCV. Further proteome profiles of other mutant strains with defects in SIF biogenesis will help to elucidate the function of SIF on STM pathogenesis.

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A GENOME-WIDE *SALMONELLA* TYPHIMURIUM INFECTION SCREEN USING HIGH-THROUGHPUT MICROSCOPY

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We have developed a high-throughput microscopy platform for monitoring Salmonella typhimurium infection in epithelial cells and macrophages. ~4,000 S. typhimurium single-gene knockouts were used to infect both cell types and images were acquired at 4 different stages of the infection. Using different host cell markers, we could extract and quantify hundreds of single cell features from both infected and non-infected cells. A number of known and unknown S. typhimurium genes were thereby associated with infection at different stages and cellular levels. Here, we will present the results of both screens, highlighting novel S. typhimurium genes involved in different key steps of infection, from invasion to intracellular proliferation and cytoskeleton remodelling.

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A NEONATAL MOUSE MODEL TO STUDY INVASIVE NON- TYPHOIDAL *SALMONELLA* TYPHIMURIUM INFECTIONS: INSIGHTS INTO THE ROLE OF SALMONELLA PATHOGENICITY ISLAND 2 (SPI2)

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Non-typhoidal *Salmonella* (NTS) are among the most prevalent causative agents of infectious diarrheal disease in humans and pigs worldwide but, as zoonotic agents, also contribute to invasive infections in human infants. The pathogenicity of *Salmonella* is conferred by horizontally acquired chromosomal regions, called Salmonella pathogenicity islands (SPIs), encoding sets of effector proteins, which are delivered into the host cell cytosol via SPI-specific type-three secretion systems. The role of SPI1 and SPI2 effectors in interaction with the host cell has been extensively studied *in vitro*: while SPI1 facilitates bacterial uptake into non-phagocytic cells, SPI2 is crucial for the establishment of the intracellular *Salmonella* containing vacuole (SCV), allowing survival and replication inside the host cell [1, 2]. Since our knowledge on the role of individual SPI2 effector proteins in the interaction of *Salmonella* with the intestinal epithelial cell is limited, we used our newly established neonatal mouse model [3] to study the contribution of SPI2 to the establishment and progression of systemic *Salmonella* infections.

Oral infection of neonate mice with wildtype and SPI2-deficient *Salmonella* resulted in similar bacterial loads of the gastrointestinal tract, but re-isolation rates of SPI2 mutants from systemic organs, such as liver and spleen, were significantly decreased. Interestingly, in contrast to the general understanding of SPI2 as prerequisite for SCV formation *in vitro*, mutants were able to establish and maintain SCVs *in vivo*. In fact, SPI2 deficient bacteria grow to high numbers inside SCVs without harming their respective host cell. By evaluating in total 15 isogenic SPI2 effector protein deficient Salmonella strains, we demonstrate that SifA, a SPI2 effector, which is anchored to the SCV´s membrane, significantly contributes to the SPI2-dependent phenotype *in vivo*. Our results suggest that its lack prevents SCV transmigration from the apical to the basolateral site of the neonate enterocytes and, finally, systemic spread of *Salmonella in vivo*.

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INVASION ABILITIES OF "*SALMONELLA*" STRAINS UNABLE TO EXPRESS ONE OR MORE KNOWN INVASION FACTORS

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Salmonelloses are one of the most common and widely distributed food-borne diseases. To establish an infection of their hosts, "Salmonella" has to invade several phagocytic or non-phagocytic eukaryotic cells. This step is considered as one of the most important for "Salmonella" pathogenesis. It is now well established that "Salmonella" is able to invade cells not only by a Trigger mechanism mediated by the Type Three Secretion System number 1 (T3SS-1) but also by a Zipper mechanism using the Rck and PagN invasins. Results obtained in our lab showed that "Salmonella" strains unable to express a functional T3SS-1 were still able to enter several cell lines. Discrete and large rearrangements of the eukaryotic membrane were observed during this T3SS-1-independent invasion using scanning electron microscopy. Moreover, the fact that a "Salmonella" strain, unable to express a functional T3SS-1 and the two currently known invasins Rck and PagN, remains as invasive as the wild type strain for some cell lines, suggests the existence of unidentified invasion factors responsible either for Trigger- or Zipper-like entry. Their identification and the subsequent characterization of their entry mechanism are under investigation.

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THE ROLE OF *IN SITU* FORMED MACROPINOSOMES FOR THE INTRACELLULAR LIFESTYLE OF *SALMONELLA*

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Salmonella Typhimurium colonizes the intestinal epithelium of infected host. Eventually, it enters inside epithelial cells, where it survives and replicates inside a *Salmonella* Containing Vacuole (SCV). The intracellular infection process is based on the subversion of host membrane trafficking. *Salmonella* enters into host cells starting with an adhesion tep at the plasma membrane followed by the injection of bacterial effectors into the host cytosol through a Type 3 Secretion System. Injected bacterial factors induce a strong remodeling of the actin cytoskeleton leading to ruffle formation and to *Salmonella* engulfment into SCV. During this process, it has been proposed that the pathogen enters through *in situ* formed macropinosomes, however the underlying molecular processes remain debated. Furthermore, our team and others have shown that empty macropinosomes formed during the ruffling process and called Infection Associated Macropinosome (IAM) fuse with the SCV few minutes after bacterial internalization. The factors which drive the IAM-SCV fusions are still unknown as well as the impact of these events. Recently, we have revealed that a small percentage of SCVs do

not fuse with IAMs resulting in SCV destabilization, vacuolar escape and *Salmonella* hyperreplication within the host cytosol. Therefore, we propose that SCV-IAM communication determines the intracellular fate of *Salmonella*. Here, we present results from functional studies that identify the involved molecular regulators driving the underlying events. *In fine*, we intend to reveal the mechanism of a new key step of SCV maturation after *Salmonella* invasion of host cell that determines its intracellular fate.

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CALRETICULIN - A NOVEL TYPE 1 FIMBRIAE FIMH RECEPTOR, PLAYS A ROLE IN *SALMONELLA CHOLERAESUIS* HOST SPECIFICITY

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Background: It was suggested that minor differences in the structure of FimH are most likely associated with differences in adhesion specificities, and may determine the tropism of various Salmonella serovars to different species and tissues. We have recently shown, that FimH adhesins from host-restricted serovars, e.g. S. Choleraesuis (SCh) binds to other glycoprotein receptors expressed by enterocytes from sheep, pig and cattle, than FimH proteins from host-unrestricted S. Enteritidis (SEn). The first ones bound to membrane protein of about 55 kDa, the second one interacted with glycoprotein of about 130 kDa. Therefore, to further study the role of FimH adhesins in host-specificity of Salmonella, we isolated and characterized 55 kDa FimH receptor expressed by swine intestinal cells and analyzed its interaction with FimH adhesin from SCh. **Methods:** Glycoprotein fraction containing 55 kDa FimH receptor was purified by ConA-affinity chromatography from lysate of swine intestinal IPEC-J2 cells and analyzed by 2D electrophoresis and Mass Spectrometry. The presence of specific protein was detected by far-Western blotting using recombinant SCh FimH. The cDNA of identified receptor was obtained by RT-PCR, and overexpressed in IPEC-J2 cell line using lentiviral system. Several SCh mutant strains expressing active and non-active FimH variants were obtain using isogenic system. Interaction of Salmonella strains with IPEC-J2 and calreticulin-over-expressing IPEC-J2 cells (IPEC-J2/CALR) were analyzed by CFU adhesion and invasion assays. **Results:** Calreticulin was identified by 2D electrophoresis and Mass Spectrometry as a 55 kDa glycoprotein bound specifically by recombinant SCh FimH protein, but not by FimH from SEn. The functionality of calreticulin as specific receptor of SCh FimH adhesin was further confirmed by adhesion and invasion of mutated strains of Salmonella carrying active and non-active allelic variants of FimH proteins to IPEC-J2/CALR cells. It was found that SCh carrying active variant of FimH adhered and invaded IPEC- J2/CALR cells in significantly higher numbers than SCh expressing non-active variant of FimH, SCh expressing SEn variant of FimH and wild type SEn. Interestingly, during bacterial invasion, expression of calreticulin in IPEC-J2 cells was increased by SCh and not by SEn. **Conclusions:** The specific receptor for SCh type 1 fimbriae FimH adhesin was identified in swine intestinal cells as calreticulin, suggesting that such interaction may contribute to SCh host specificity. So far, calreticulin was known mainly as chaperon protein present in endoplasmic reticulum, however our results revealed its function in adhesion and internalization processes. Moreover, increased expression of calreticulin in intestinal cells during SCh infection suggests the involvement of FimH in intracellular signal transduction affecting the expression of specific genes.

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A FLUORESCENT REPORTER TO DIFFERENTIATE VACUOLAR AND CYTOSOLIC SALMONELLA

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Background: Salmonella enterica serovar Typhimurium can colonize both vacuolar and cytosolic compartments of epithelial cells. Most techniques to assess the location of intracellular bacteria are microscopy based and rely on determining the presence or absence of membrane markers, which do not always accurately reflect vacuole integrity given the dynamic processes that govern membrane damage and repair. Here, we exploited an intrinsic Salmonella two-component system coupled to a GFP reporter to monitor the integrity of Salmonella-containing vacuoles and the intracellular localization of Salmonella. With this reporter, intracellular Salmonella populations can be tracked based on the bacterial response to its environment within the host cell. **Methods:** We made a GFP reporter strain that responds to a metabolite found exclusively in the host cell cytosol. The fidelity of this reporter was evaluated in HeLa cells by fluorescence microscopy. **Results:** GFP expression by Salmonella harboring the **c**ytosol **a**ctivated **r**eporter (CAR) correlated with ubiquitin labelling, an autophagy marker for cytosolic bacteria, whereas GFP-negative bacteria were surrounded by the vacuolar marker, LAMP1. Fluorescence activated cell sorting analysis confirmed that infected cells with high GFP intensity contained high numbers of bacteria, infected cells with low GFP intensity had 10-30 bacteria/cell, and infected cells with no GFP signal contained few to no bacteria. **Conclusions:** The CAR accurately reflects exposure of Salmonella to the host cell cytosol, which can be used to differentiate distinct intracellular Salmonella populations.

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GLUTATHIONE REDUCTASE PLAYS A ROLE IN GUT COLONIZATION IN *SALMONELLA ENTERICA* SEROVAR TYPHIMURIUM

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Gut colonization is a central strep of the Salmonella Typhimurium infection cycle. While enteric pathogens can profit from inflammation through the alteration of the microbiota and metabolites in the intestine, they also have to adapt to reactive oxidative species (ROS) by host immune responses. In earlier work, we found that such host defenses can strongly diminish gut luminal pathogen loads. The underlying mechanisms are still not well understood. Work on ex vivo experiments and on systemic infection models has established that oxidative stress is a key factor limiting host colonization. Bacteria respond to oxidative stresses through the production of antioxidants, including thiol containing compounds, such as glutathione, and the redox enzymes, theoredoxin, superoxide dismutases and catalases. Superoxide dismutases and glutathione provide phagocytosed Salmonella Typhimurium protection against host induced oxidative and nitrosative damage. However, Salmonella responses to oxidative stress in the gut have not yet been fully characterized. We expect that antioxidants would be vital for S. Typhimurium in the intestinal lumen during the later stages of infection when ROS generating NADPH oxidases are induced by granulocytes upon inflammation. Surprisingly, we observed that genes encoding for glutathione reductase and redox sensors are essential not only during gut inflammation, but also at the initial stages of infection when overt inflammation is not yet mounted. Our current studies now focus on the cellular redox homeostasis and the redox environment during gut colonization.

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IRON ACQUISITION IN *SALMONELLA* TYPHI-HOST INTERACTIONS

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Background: Iron is sequestered by the mammalian host to limit its availability to invading pathogens. Macrophages play an important role in regulating iron compartmentalization in response to inflammatory signals. *Salmonella enterica* serovar Typhi (STy), the causative agent of typhoid fever, persists within host macrophages, whereas non-typhoidal *Salmonella* serovars such as *S.* Typhimurium (STm) induce macrophage cell death. The contrasting interactions between STy and STm and macrophages suggest that these serovars may differ in their strategies for obtaining iron during infection. **Methods:** Humanized mice engrafted with human hematopoietic stem cells (hu-SRC-SCID mice) were used as a small animal model of lethal typhoid fever. A transposon-directed insertion-site sequencing (TraDIS) screen using a high-complexity transposon mutant library in STy was used to identify STy genetic loci required for virulence in hu-SRC-SCID mice. In addition, wild-type and mutant STy and STm strains lacking specific iron-acquisition systems were compared for their ability to withstand iron-limited conditions *in vitro*. Cultured human THP-1 macrophages and primary PBMC-derived macrophages were used to determine intracellular bacterial survival and macrophage iron metabolism during infection with STy or STm. **Results:** The TraDIS screen in hu-SRC-SCID mice revealed both known and novel virulence loci required for STy virulence in this model. Mutant strains lacking genes required for enterobactin and salmochelin biosynthesis, export or uptake were strongly counter-selected during infection. *In vitro* characterization of STm and STy mutant strains lacking these iron acquisition systems demonstrated greater sensitivity of STy to iron limitation relative to STm. Studies to measure the iron content of macrophages infected with STy or STm and to determine the basis for the greater ability of STm to grow under iron-limited conditions are in progress. **Conclusions:** The hydroxamate siderophores enterobactin and salmochelin are essential for STy virulence in a hu-SRC-SCID humanized mouse model, and enterobactin is required for *in vitro* growth under iron-limited conditions. STy is more sensitive to iron limitation than STm *in vitro*, suggesting that iron may be more abundant in STy-infected macrophages than in STm-infected macrophages. These observations may relate to the enhanced susceptibility of patients with secondary iron overload to non-typhoidal but not to typhoidal *Salmonella* infection.

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GENOMIC POPULATION STRUCTURE OF *SALMONELLA ENTERICA*

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Background: High-throughput sequencing is now being applied for routine typing of many pathogens, including *S. enterica*. There are over 50,000 sets of *Salmonella* reads in public sequence repositories. These data could provide a basis for a global perspective of this species. However, such analyses are confounded by a paucity of standardized typing approaches. In 2012 we applied 7-gene Multilocus Sequence Typing (MLST) to group the majority of the typed *S. enterica* isolates into 138 independent genetic clusters - eBurstGroups (eBGs) - of closely related sequence types (STs). However, MLST lacks resolution below the ST level, and cannot reliably identify deep evolutionary history. To effectively manage and compare genomic data in a scalable way, advanced frameworks for describing the global population as well as local variation are required. **Methods:** To address these issues, we have developed automatic pipelines within En-

teroBase to assemble genomes from public sequence repositories or registered users. These pipelines not only derive classical MLST eBGs from all assembled genomes of adequate quality, but also extend to more discriminant schemes such as ribosomal MLST (rMLST; 51 genes), core genome MLST (cgMLST; 3,002) and whole-genome MLST (wgMLST; 21,065). We have also calculated species trees for core genes in *S. enterica* with two independent strategies, in order to reconstruct its evolutionary history. **Results:** The *Salmonella* database in EnteroBase serves genotyping data for >47,000 genome assemblies along with 7,000 records from the legacy MLST database. The genomic data defines >3,000 rSTs and 381 reBGs, which are consistent with legacy eBGs but more discriminant. >90% of the genomes have been assigned to an reBG. Strains within each reBG are uniform for serovar. A species tree of one genome per rST was consistent with the reBGs, and contains clear signals of deep phylogenetic structure. Furthermore, fine-grained genetic structures within reBGs were largely resolved with a novel cgMLST scheme. This scheme gives a comparable resolution as SNPs, whereas much more standardized and portable, and is being increasingly used for epidemiological analyses. Using online tools in Enterobase, users can easily map any isolate onto global, high-definition perspectives of reBGs that were previously unresolvable, such as reBG1 (Typhimurium) or reBG4.1 (Enteritidis). **Conclusion:** EnteroBase provides access to high-resolution genotyping data (MLST, rMLST and cgMLST) and visualization tools, allowing microbiologists to investigate the genomic relationships between all *Salmonella* serovars of clinical significance through an easy to use web interface. We anticipate that it will result in a transformational change in genotypic designations and global communication. Enterobase is available at http://enterobase.warwick.ac.uk.

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Bold indicates presenter.

Map of Facilities

Location

The hotel is located at the former airship station in the western part of Potsdam. You can reach the center of town in 10 minutes and Berlin in about half an hour. Berlin Tegel and Berlin Schönefeld airports are about 40 km from the hotel. A shuttle service can be organized for you at an additional cost.

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