Antimicrobial resistance in Pasteurella and Mannheimia: epidemiology and genetic basis

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Review article

Antimicrobial resistance in *Pasteurella* and *Mannheimia*: epidemiology and genetic basis

Corinna KEHRENBERG[a], Gundula SCHULZE-TANZIL[a], Jean-Louis MARTEL[c], Elisabeth CHASLUS-DANCLA[b], Stefan SCHWARZ[a*]

[a] Institut für Tierzucht und Tierverhalten, Bundesforschungsanstalt für Landwirtschaft (FAL), 29223 Celle, Germany
[b] Institut National de la Recherche Agronomique, Pathologie Aviaire et Parasitologie, 37380 Nouzilly, France
[c] Agence Française de Sécurité Sanitaire des Aliments, Pathologie Bovine et Hygiène des viandes, 31 avenue Tony Garnier, 69364 Lyon Cedex 07, France

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Abstract – Isolates of the genera *Pasteurella* and *Mannheimia* cause a wide variety of diseases of great economic importance in poultry, pigs, cattle and rabbits. Antimicrobial agents represent the most powerful tools to control such infections. However, increasing rates of antimicrobial resistance may dramatically reduce the efficacy of the antimicrobial agents used to control *Pasteurella* and *Mannheimia* infections. This review presents a short summary of the infections caused by *Pasteurella* and *Mannheimia* isolates in food-producing animals and the possibilities of preventing and controlling primary and secondary pasteurellosis. Particular reference is given to antimicrobial chemotherapy and the resistance properties of *Pasteurella* and *Mannheimia* isolates. The genetic basis of the most predominant resistance properties such as resistance to β-lactam antibiotics, tetracyclines, aminoglycosides, sulfonamides, and chloramphenicol is discussed. This is depicted with reference to the role of plasmids and transposons in the spread of the resistance genes among *Pasteurellaceae* and members of other bacterial families and genera.

antimicrobial resistance / *Pasteurella* / *Mannheimia* / plasmid / transposon / horizontal gene transfer

Résumé – Résistance aux antimicrobiens chez *Pasteurella* et *Mannheimia* : épidémiologie et bases génétiques. Les bactéries des genres *Pasteurella* et *Mannheimia* provoquent une grande variété de maladies ayant des conséquences économiques considérables chez les volailles, les porcs,

* Correspondence and reprints
Tel.: (49) 5141 384673; fax: (49) 5141 381849; e-mail: stefan.schwarz@fal.de

les bovins et les lapins. Les antibiotiques représentent les outils les plus efficaces pour contrôler ces infections. Cependant, l'augmentation de la résistance aux antibiotiques pourrait réduire de manière dramatique leur efficacité dans le contrôle des infections à *Pasteurella* et *Mannheimia*. Cette revue de la littérature présente brièvement les infections causées par les *Pasteurella* et *Mannheimia* chez les animaux entrant dans la chaîne alimentaire, et les possibilités de prévenir et de contrôler les pasteurelloses primaires et secondaires. Une attention particulière est donnée à la chimiothérapie antimicrobienne et aux propriétés de résistance des *Pasteurella* et de *Mannheimia*. Les bases génétiques de la résistance aux β-lactamines, tétracyclines, aminosides, sulfamides et chloramphénicol, sont décrites avec une référence au rôle des plasmides et des transposons dans la propagation des gènes de résistance parmi les *Pasteurellaceae* et les membres d'autres familles et genres bactériens.

résistance aux antimicrobiens / *Pasteurella* / *Mannheimia* / plasmide / transposon / transfert horizontal de gène

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1. INTRODUCTION

Isolates of the genera *Pasteurella* and *Mannheimia* represent pathogens which are involved in a wide variety of infections in food-producing animals. Antimicrobials are still the tools of choice for prevention and control of infections due to *Pasteurella* and *Mannheimia*. However, imprudent use of antimicrobials bears a high risk of selecting resistant bacteria, promoting the spread of resistance genes located on plasmids and transposons, and consequently, reducing the efficacy of the antimicrobial agents currently available for the treatment of food-producing animals. On the one hand, this review focuses on the possibilities to prevent and control infections due to *Pasteurella* and *Mannheimia* isolates in animals; on the other hand, data on resistance development among *Pasteurella* and *Mannheimia* are presented and the genetics of the respective resistance genes are described in detail. Most of the resistance

genes currently known to be present in *Pasteurella* and *Mannheimia* are associated with mobile genetic elements. Since only few of these resistance genes appear to be indigenous to *Pasteurella* and *Mannheimia*, isolates of these two genera may acquire resistance genes from other bacteria by horizontal gene transfer. The data presented in this review will help to understand the processes involved in the acquisition, exchange, and further emergence of resistance genes among bacteria of the genera *Pasteurella* and *Mannheimia.* This calls for prudent use of antimicrobial agents to retain the efficacy of antimicrobials in the future.

2. TAXONOMY AND PATHOGENICITY OF *PASTEURELLA* AND *MANNHEIMIA*

2.1. Taxonomy of the genera *Pasteurella* and *Mannheimia*

The family *Pasteurellaceae* which currently comprises the five genera *Pasteurella*, *Mannheimia*, *Actinobacillus*, *Haemophilus*, and *Lonepinella* has been the subject of extensive reclassification in the past. In recent years, studies based on DNA-DNA hybridisations [3, 50] and comparisons of the nucleotide sequences of 16S ribosomal RNA [3, 20] formed the basis for the current taxonomy of the genus *Pasteurella*, but also served to define the new genus *Mannheimia* with the new species *Mannheimia* (*M.*) *haemolytica* which includes bacteria formerly assigned to the biovar Arabinose within the *Pasteurella* (*P.*) *haemolytica* complex. The new species *P. trehalosi* includes bacteria previously assigned to the biovar Trehalose within the *P. haemolytica* complex [2, 3]. Thus the term "*P. haemolytica*" is used in this review when referring to isolates studied or data elaborated before the new genus *Mannheimia* was described. A continuously updated summary of the latest taxonomic developments within the five genera of the family *Pasteurellaceae* can be obtained from http://www.bacterio.cict.fr [23]. Although bacteria have been better defined at the species level, there is a growing awareness that a great variety of host-adapted serotypes or variants is present and the significance of their role in disease is now being examined.

The family *Pasteurellaceae*, including members of the genera *Mannheimia, Pasteurella* and *Haemophilus,* are natural inhabitants of the mucosal surfaces of vertebrates, particularly of ruminants, where they are able to survive in the upper part of the respiratory tract of clinically healthy animals and are occasionally involved in diseases. Table I shows the species currently assigned to the genera *Pasteurella* and *Mannheimia*, their major habitats and their association with diseases in animals and humans.

2.2. *Pasteurella* and *Mannheimia* isolates as primary and secondary pathogens

As a *primary pathogen* and in tropical countries *P. multocida* is responsible for "haemorragic septicaemia of cattle and water buffaloes": Capsular type B occurs in Africa, the Middle East and Asia, and capsular type E in West, Central and South Africa. Bacteria assigned to the new species *P. trehalosi,* capsular types T3, T4, and T10 are encountered world-wide in sheep and goats where they cause severe septicaemia in young animals. In other animal species, *P. multocida* isolates can also act as primary pathogens; the corresponding diseases include fowl cholera and snuffles in rabbits [7, 53]. As *secondary pathogens*, bacteria within the family *Pasteurellaceae* play a major role in the final progression to severe pleuropneumonia in cattle, sheep and goats where "pasteurellosis" is often synonymous with "respiratory disease", but also in enzootic pneumonia and progressive atrophic rhinitis of swine, as well as pasteurellosis in small laboratory rodents and

Table I. Species within the genera *Pasteurella* and *Mannheimia* [43], their major habitats and their association with diseases in humans and animals.

Species	Habitat	Clinical relevance
P. aerogenes	physiological flora of oropharynx and intestinal tract of pigs	abortion, stillbirth in pigs, dogs, rabbits
P. anatis	physiological flora of ducks	–
P. avium	respiratory tract of poultry	–
P. bettyae	genital tract of humans	infections of the urogenital tract
P. caballi	respiratory tract of horses	pneumonia, peritonitis
P. canis	respiratory tract of calves and dogs	infections after bites
P. dagmatis	respiratory tract of cats and dogs	infections after bites
P. gallinarum	respiratory tract of chickens	–
P. langaaensis	respiratory tract of poultry	–
P. mairii	genital tract of pigs	abortion, sepsis in piglets
P. multocida subsp. *multocida, septica* and *gallicida*	respiratory tract of mammals and birds	infections of the respiratory tract, septicaemia
P. pneumotropica	respiratory tract of guinea pigs, hamsters, rats, cats and dogs	pneumonia, conjunctivitis, ataxia
P. stomatis	respiratory tract of cats and dogs	infections after bites
P. testudinis	respiratory tract of desert tortoises	infections of the upper respiratory tract
P. trehalosi	respiratory tract of ruminants	septicaemia
P. volantium	respiratory tract of poultry	–
M. haemolytica	respiratory tract of ruminants	pneumonia, septicaemia, mastitis
M. glucosida	respiratory tract of ruminants	–
M. granulomatis	respiratory tract of domestic and wild ruminants, hares	pneumonia, conjunctivitis, panniculitis
M. ruminalis	physiological ruminal flora	–
M. varigena	respiratory tract and intestinal tract of ruminants and pigs	pneumonia, mastitis, enteritis, septicaemia

* In addition to the species recognised so far, several unnamed taxa have been described in the genus *Mannheimia* [3].

fur-bearing animals [7, 53]. It is generally recognised that these *Pasteurella* infections are multifactorial and multi-agent diseases involving viruses (Parainfluenza virus 3, Bovine Herpes virus 1, Bovine Respiratory Syncytial virus in cattle), *Mycoplasma* spp. (*M. bovis* in cattle, *M. hyopneumoniae* in pigs) and bacteria such as *Pasteurellaceae*, but also *Arcanobacterium pyogenes* in cattle, and *Bordetella bronchiseptica* in pigs. Under predisposing environmental conditions and/or management conditions which constitute stress for the animals, such as transport (shipping fever), marketing, change of feed, climate or ventilation, viruses and/or *Mycoplasma* spp. are determining in initiating a pathological process commonly accompanied by a high morbidity but low mortality. Great economic losses are a result of the retarded growth rate of the affected animals [53].

3. CONTROL OF INFECTIONS DUE TO *PASTEURELLA* AND *MANNHEIMIA*

3.1. Prophylactic measures

Most of the animal diseases involving isolates of the genera *Pasteurella* and

Mannheimia represent diseases which are mainly influenced by a wide variety of environmental and management risk factors. Thus the reduction or even elimination of such predisposing factors is of major importance. Non-immunoprophylactic measures such as optimised management, but also optimised hygienic, climatic and feeding conditions may be supported by the use of vaccines. To date, there are very few *Pasteurella* and *Mannheimia* vaccines available for the different animal species, all of which are inactivated vaccines.

Problems with vaccination arise from the observation that there are more than one serotype of a certain species, e.g. *M. haemolytica*, involved in diseases in the respective animals, and that some vaccines may lack host-adapted type capsular polysaccharides. Therefore, information on capsular serotypes must also be taken into consideration in developing effective vaccines. In the case of *M. haemolytica*, serotype A1 is the most commonly isolated serotype from pneumonic lungs of cattle and generally the unique serotype introduced into commercial vaccines. We have drawn attention to this point in order to adapt the composition of the vaccine according to the target animal species, indicating the difference of serotype distribution in cattle, sheep and goats [46]. In addition, we are now observing that isolation of serotype A6 is increasing in the UK [21], in France (Martel, unpublished results) and in North America [52]. Unfortunately, serotyping is not carried out routinely due to the lack of commercially available antisera. Therefore, inadequacy between the composition of vaccines in terms of bacterial species and capsular serotypes, and the local epidemiological situation may be the main cause of the failure to protect. As a consequence of the ineffectiveness of vaccination, clinical diseases may occur more frequently and require effective treatment of the diseased animals.

3.2. Therapeutic measures

The control of ongoing infections with *Pasteurella* and *Mannheimia* isolates is difficult for two reasons: (a) in most of these infections, the *Pasteurella* and *Mannheimia* isolates are not the only causative agents and even with the successful control of the *Pasteurella* and *Mannheimia* isolates involved, there is no complete cure for the cause of the disease, (b) bacteria of the genera *Pasteurella* and *Mannheimia* exhibit increasing resistance to a large number of antimicrobial agents available for use in the respective animal species. Because of the rapid spread of resistance, the antimicrobial sensitivity of the *Pasteurella* and *Mannheimia* isolates should be tested and a suitable antibiotic should be chosen on the basis of the *in vitro* sensitivity. However, taking a sample, sending it to a diagnostic laboratory, isolating the *Pasteurellaceae* and testing their *in vitro* sensitivity usually takes several days during which the disease can progress dramatically. Therefore, it is commonly required to start the therapy immediately. For the choice of the antibiotics, veterinary surgeons often rely on recommendations based on the resistance data obtained from the national monitoring programmes [9] or on recommendations which are given in the different textbooks. The latter recommendations include, for almost all animal species, sulfonamides, tetracyclines and streptomycin. Chloramphenicol, which was recommended in the older textbooks was banned from use in food-producing animals in 1994 and has since been replaced by florfenicol which was licensed for the treatment of bovine respiratory diseases in 1995.

Selective pressure by antimicrobial agents, which favours the emergence of resistant isolates represents a serious problem, particularly in developed countries where domestic ruminants are reared in intensive units. That is the reason why in France *Pasteurellaceae* have been included in the national network for the monitoring of

antimicrobial resistance within the main pathogenic bacteria for cattle [47]. To date, *M. haemolytica* is the most frequent pathogen isolated from bovine pneumonic lungs and is generally more resistant to antibiotics than *P. multocida*, and isolates of *H. somnus* are generally the most sensitive. *M. haemolytica* isolates from sheep and goats, that are less commonly reared in intensive conditions, are generally less resistant to antibiotics than *M. haemolytica* isolates from cattle.

4. ANTIMICROBIAL RESISTANCE OF *PASTEURELLA* AND *MANNHEIMIA* ISOLATES

4.1. Determination of antimicrobial resistance and epidemiological aspects

Antimicrobial resistance of *Pasteurella* and *Mannheimia* isolates varies according to the host animal species, time, geographical origin and antimicrobial pretreatment of the animals. It also depends on the access of the *Pasteurella* and *Mannheimia* isolates to the resistance genes present in the respective gene pools and on the horizontal gene transfer mechanisms available within these gene pools. Moreover, data on antimicrobial resistance may also vary based on differences in the methodology of resistance testing and the breakpoints for resistance as laid down in the standards used in different countries, such as Deutsche Industrienorm (DIN) 58940 (Germany), National Committee for Clinical Laboratory Standards (NCCLS) document M31-A (USA), and Comité de l'Antibiogramme de la Société Française de Microbiologie (CA-SFM) (France). Striking differences in the numbers of isolates tested and the lack of data on epidemiological relatedness of the isolates make it difficult to compare the data on antimicrobial resistance of *Pasteurella* and *Mannheimia* isolates determined in different countries. Isolates of the genera *Pasteurella* and *Mannheimia* are investigated for their antimicrobial resistance properties in the national monitoring programmes of only five European countries (see Concerted Action Fair 5-CT97-3654, Antibiotic resistance in bacteria of animal origin, http://www.fougeres.afssa.fr/arbao): France, Germany, the UK, the Netherlands, and Portugal [9, 47]. Another often underestimated problem arises from the species identification and differentiation of bacteria assigned to these two genera. In contrast to bacteria of other families and genera, there is no commercially available system that is easy to use and does not require specific expensive equipment. An additional problem in the resistance statistics of *Pasteurella* and *Mannheimia* isolates is the multiple inclusion of the same isolate in such statistics. Routinely, diagnostic laboratories do not perform a differentiation of isolates in addition to resistance testing. Thus isolates of the same clone which are spread within a certain geographical area may be mistakenly handled as unrelated isolates and their resistance data may be included several times in each set of statistics. As with many other bacterial pathogens, differentiation of single isolates is achieved most efficiently by applying molecular methods [8, 14, 41]. Various methods are available and have been used successfully for the differentiation of *Pasteurella* and *Mannheimia* isolates: plasmid profiling, ribotyping, various PCR techniques, and macrorestriction analysis (for a review see [8, 33]). While these techniques have most often been used to differentiate between pathogenic and toxigenic isolates, but also for taxonomic and epidemiological purposes, they have rarely been used in relation to antimicrobial resistance. Recently, macrorestriction analysis (Fig. 1) served to determine the relatedness of chromosomally tetracycline-resistant *P. aerogenes* isolates [35–37], but also to prove the horizontal transfer of tetracycline resistance plasmids between *P. multocida* and *P. aerogenes* isolates from pigs [36].

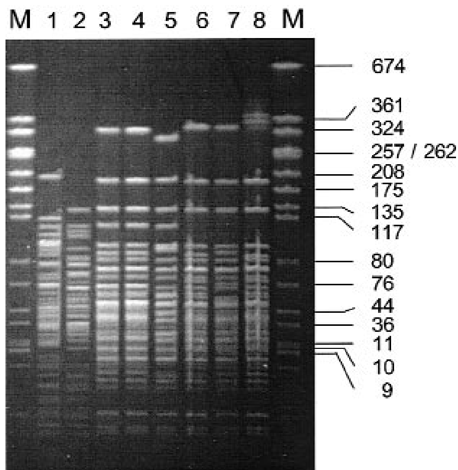

Figure 1. *Sma*I macrorestriction patterns of *P. aerogenes* (lanes 1, 2) and *P. multocida* isolates (lanes 3–8) harbouring the *tet*(H)-carrying Tc resistance plasmid pPAT1 [36]. Lanes M contain the *Sma*I fragments of *Staphylococcus aureus* NCTC 8325. Sizes of the marker fragments are given in kb on the right hand side.

4.2. Resistance data for *Pasteurella* and *Mannheimia* isolates

Data obtained from Germany and France, in 1997, are summarised in Table II.

These data illustrate that there is a high degree of variability among isolates assigned to different genera (e.g. *P. multocida* vs. "*P. haemolytica*"), but originating from the same animal source (e.g. cattle). The data obtained from the continuous monitoring of isolates from year to year from one country, for example in the case of Germany, shows that the resistance rates may also vary over time (Tab. III). Nevertheless, percentages of resistant isolates also vary between the older and newer molecules in each family. In both countries, resistance to the newer cephalosporins (ceftiofur, cefquinome) and to florfenicol has not yet been encountered. The large majority of strains are still susceptible to the fluoroquinolone enrofloxacin. For macrolides, the therapeutic breakpoints proposed by the national committees are not

Table II. Antimicrobial susceptibility (in %) among bovine *P. multocida* and "*P. haemolytica*" isolates obtained in 1997 in Germany and France.

Antimicrobial agents	Germany[a]			France[b]		
	Diameter zone (MIC, µg·mL^{-1})[c]	*P. multocida*	"*P. haemolytica*"	Diameter zone (MIC, µg·mL^{-1})[d]	*P. multocida*	"*P. haemolytica*"
Penicillin G	24 (0.125)	82 (242)[e]	69 (212)	29 (0.25)	39 (180)	10 (342)
Ampicillin	22 (2)	89 (476)	78 (461)	19 (4)	90 (678)	40 (1000)
Streptomycin	15 (4)	33 (396)	22 (396)	15 (8)	37 (604)	6 (834)
Tetracycline	22 (1)	71 (493)	68 (469)	19 (4)	64 (804)	39 (1015)
Chloramphenicol	21 (8)	88 (137)	87 (142)	23 (8)	74 (566)	65 (877)
Nalidixic acid	–	n.d.	n.d.	20 (8)	83 (423)	71 (577)
Sulfonamides	16 (16)	17 (215)	27 (184)	17 (100)	34 (608)	19 (935)
Sulfonamides/ Trimethoprim	16 (16/0.8)	74 (474)	82 (461)	16 (128/8)	85 (617)	68 (834)

[a] Data from pathological samples, collected in 1997 from Trolldenier [65] and the Concerted Action FAIR 5-CT97-3654.
[b] Data from pathological samples, cumulated in 1997 from Resabo [47].
[c] Evaluation of the diameters of the zones of growth inhibition followed DIN recommendations except for streptomycin where NCCLS guidelines were used.
[d] Evaluation of the diameters of the zones of growth inhibition followed the CA-SFM recommendations.
[e] Numbers in parentheses indicate the numbers of isolates tested.

Table III. Percentages of susceptible bovine *P. multocida* and bovine "*P. haemolytica*" isolates from Germany 1990–1996 according to [61–65].

Year	n[a]	Antimicrobial agents[b]								
		Amp	Pen	Tc	Cm	Sm	Nm	Gm	Sul-3	SxT
P. multocida										
1990	184	–[c]	81	87	82	52	86	–	36	–
1991	310	87	76	81	77	42	77	74	27	69
1992	471	90	89	70	77	39	58	71	25	85
1993	428	90	87	59	73	27	45	51	16	79
1994	488	90	81	74	80	35	64	45	26	84
1995	685	91	71	77	81	42	64	38	23	77
1996	560	90	66	70	72	38	56	51	14	70
"*P. haemolytica*"										
1990	300	–	89	91	89	62	97	–	47	–
1991	217	71	75	75	71	42	83	81	53	73
1992	536	70	71	50	68	31	68	72	43	85
1993	580	68	68	59	71	26	62	52	26	75
1994	572	71	65	62	79	23	65	41	29	82
1995	757	70	48	68	77	32	65	37	19	84
1996	535	68	45	67	70	21	54	49	17	74

[a] n = numbers of isolates investigated.
[b] Abbreviations of antimicrobial agents: Amp (ampicillin), Pen (penicillin G), Tc (tetracycline), Cm (chloramphenicol), Sm (streptomycin), Nm (neomycin), Gm (gentamicin), Sul-3 (sulfadiazin, sulfamerazin, sulfamethazin), SxT (sulfamethoxazole/trimethoprim).
[c] – = no data available.

pertinent for both therapeutic and monitoring purposes. However, we observe that erythromycin, spiramycin, tylosin, and tilmicosin are still active to date.

4.3. Genetic basis of antimicrobial resistance of *Pasteurella* and *Mannheimia*

4.3.1. Resistance to β-lactams

Resistance to β-lactams among *P. multocida* isolates is often mediated by small plasmids of 4.1–4.4 kb [44, 54]. The occurrence of similar-sized β-lactam resistance plasmids of 4.2–5.2 kb in "*P. haemolytica*" isolates has also been reported [4, 11, 12, 17, 49, 55, 57]. These resistance plasmids were commonly identified by transformation experiments using *E. coli* strains as recipients. Several of these β-lactam resistance plasmids down-regulated their copy number in some of the *E. coli* hosts, however without negative effects on the level of β-lactam resistance [4, 17, 44, 56, 68]. Whenever molecular analyses were performed to identify the type of the β-lactamase (*bla*) gene, the gene bla_{ROB1} was detected [4, 44]. The ROB1-β-lactamase is a member of the Ambler Class A (= Bush class 2b) β-lactamases which are highly sensitive to inhibition by β-lactamase inhibitors and mediate resistance to penicillins and 1st generation cephalosporins [60].

The ROB1-β-lactamase was first described in a *H. influenzae* type b meningitis isolate from a child in USA. The corresponding bla_{ROB1} gene was on a plasmid of 4.4 kb. Later on, the ROB1 enzyme was detected in porcine *Actinobacillus pleuropneumoniae* isolates throughout the USA, and

in bovine and porcine *P. multocida, P. haemolytica* and *P. aerogenes* in France [44, 45, 48, 51]. In all the bovine strains of *Pasteurella* studied at that time, the bla_{ROB1} genes were present on plasmids of 4.1 kb except in one strain of *P. haemolytica* harbouring a plasmid of 4.4 kb. Such 4.4 kb plasmids were also encountered in *H. influenzae* strains. The bla_{ROB1} encoding plasmids from *Pasteurella* and *Haemophilus* strains were compared by hybridisation and restriction endonuclease analysis resulting in the hypothesis of a close genetic relationship between the 4.1 and 4.4 kb plasmids. In *P. aerogenes,* the bla_{ROB1} gene was present on the chromosome [44].

So far, five bla_{ROB1} gene sequences are known: two from "*P. haemolytica*" (database accession numbers: X52872, Z21724), two from *Actinobacillus* (*A.*) *pleuropneumoniae* (AB034202; S51028 or M97481), and one from *Haemophilus* (*H.*) *influenzae* (AF022114). All ROB1-β-lactamase protein sequences of 305 amino acids (aa) are identical [34, 44, 45, 48]. The sequence of ROB1 protein was compared with the sequences of other class A β-lactamases and showed about 40% homology with all the known class A enzymes. The ROB1 enzyme exhibited the highest similarity to the β-lactamases from gram-positive bacteria and appeared as a possible link between the β-lactamases of gram-positive and gram-negative bacteria [45].

4.3.2. Resistance to tetracyclines

Tetracycline resistance genes of the three different hybridisation classes H, B, and M have been identified among members of the genera *Pasteurella* and *Mannheimia*. The gene *tet*(M) is the most widespread *tet* gene among gram-positive and gram-negative bacteria. It has been identified as part of a number of conjugative transposons in gram-positive cocci, but has in the meantime been observed in a wide variety of gram-positive and gram-negative bacteria [15]. So far, the gene *tet*(M) has been detected by hybridisation in the chromosomal DNA of two bovine *P. multocida* isolates, one from France [13] and the other from the USA [27]. The *tet*(B) gene is part of the non-conjugative transposon Tn*10* [10] and represents the most frequently observed *tet* gene among *Enterobacteriaceae*. To date, this gene has been detected in a single bovine "*P. haemolytica*" isolate from France [13] and in two porcine *P. multocida* isolates from the USA and Germany [35]. However, the *tet*(B) gene proved to be the predominant *tet* gene among *P. aerogenes* isolates of porcine sources [37]. Hybridisation patterns obtained from *Sfu*I-digested whole cellular DNA of the *P. multocida* and *P. aerogenes* isolates points towards the presence of complete copies of Tn*10* in the majority of the isolates investigated [37]. The gene *tet*(H) was originally detected on plasmid pVM111 from an avian *P. multocida* isolate from the USA [26], but later also in the chromosomal DNA and on plasmids of *P. multocida* and "*P. haemolytica*" from porcine and bovine sources in North America [27]. Because of its plasmid and chromosomal location, the *tet*(H) gene was assumed to be associated with a transposable element [27]. This assumption was proven by the identification of Tn*5706*, a non-conjugative composite transposon of 4378 bp (Fig. 2) which is located on plasmid pPMT1 from bovine *P. multocida* obtained in Germany [38]. The *tetR*-*tet*(H) gene region in Tn*5706* is bracketed by copies of two almost identical insertion elements, IS*1596* and IS*1597* (Fig. 2). Tn*5706* is the first and so far only known transposon originally identified among members of the genus *Pasteurella* [33]. Truncated Tn*5706* elements were also identified on the 4.4 kb plasmid pMHT1 from *M. haemolytica* [39] and the 5.5 kb plasmid pPAT1 which was observed in *P. aerogenes* and *P. multocida* [36]. All currently known TetH proteins (U00792; Y15510; Y16103; AJ245947) differ slightly from one another. The TetH protein of plasmid pPAT1, however, lacks the terminal 8 aa due to a recombination between the *tet*(H) gene and the

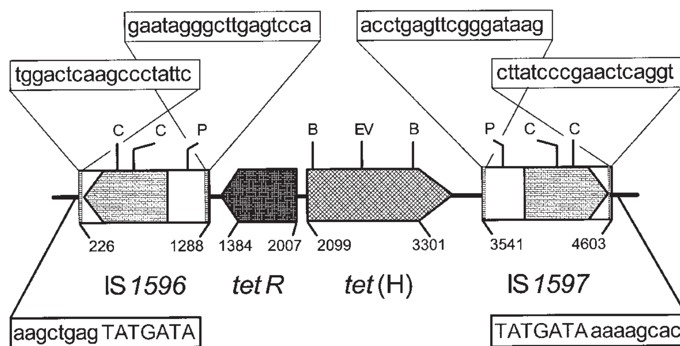

Figure 2. Organisation of transposon Tn*5706*, consisting of the *tetR-tet*(H) resistance gene region bracketed by the insertion elements IS*1596* and IS*1597*. The numbers below the respective elements and genes refer to the positions within the 4828 bp region of plasmid pPMT1 deposited in the databases (Y15510). The arrow-shaped boxes indicate the directions of transcription of the genes *tet*(H) and *tetR* as well as the genes coding for the putative transposases of IS*1596* and IS*1597*. The 18 bp perfect, invertedly repeated sequences at the ends of the insertion elements, are displayed in boxes above the IS elements. The 7 bp directly repeated sequences at the integration site are shown in capital letters, the adjacent pPMT1 sequences in lower case letters below the map of Tn*5706*. Cleavage sites for restriction endonucleases are abbreviated as follows: B (*Bcl*I), C (*Cla*I), EV (*Eco*RV) und P (*Pvu*II).

adjacent IS*1597* sequence [36]. This terminal deletion, however, had no influence on the level of Tc resistance mediated by the respective TetH efflux system.

4.3.3. Resistance to aminoglycosides

Molecular analyses of aminoglycoside resistance in *Pasteurella* and *Mannheimia* focussed on streptomycin resistance. The first reports on transferable streptomycin resistance were published in 1978 by Berman and Hirsh [6]. This resistance property was mainly associated with small plasmids of less than 10 kb in *P. multocida* from turkeys, pigs and cattle, but also in "*P. haemolytica*" from cattle [6, 12, 16, 29–31, 58, 59, 67, 68]. However, a large conjugative plasmid of approximately 113 kb which mediated resistance to streptomycin, kanamycin, tetracycline and sulfonamides has also been identified [31]. The gene mediating streptomycin resistance, *strA*, codes for an aminoglycoside- 3'-phosphotransferase (E.C. 2.7.1.-) which enzymatically inactivates streptomycin. This gene has been found to be part of transposon Tn*5393* from *Erwinia amylovora* (M95402; M96392), but virtually identical *strA* genes have also been identified on plasmids or in the chromosome of a wide range of bacteria including *P. multocida* (U57647), "*P. haemolytica*" (M83717; B56649), *Haemophilus ducreyi* (L23118), *Mannheimia* spp. (AJ249249), *Pseudomonas* (*Ps.*) *aeruginosa* (AF024602), *Ps. syringae* (M77502), *Yersinia pestis* (AJ249779), *E. coli* (M28829; JH0123), *Xanthomonas campestris* (U20588), as well as *Corynebacterium striatum* (AF024666) and uncultured eubacteria (AJ271879; AJ293027). All these phosphotransferase proteins consist of 267 aa and differ by up to 8 aa which corresponds to identities of 97–100 %.

4.3.4. Resistance to sulfonamides and trimethoprim

Sulfonamide resistance is one of the most often detected resistance properties among *Pasteurella* and *Mannheimia* isolates (Tabs. II and III). It is commonly mediated

by the gene *sul*II which codes for a type II dihydropteroate synthase (E.C. 2.5.1.15) of 271 aa. Most of the currently known *sul*II genes have been detected in members of the family *Pasteurellaceae*, such as *P. multocida* (U57647), "*P. haemolytica*" (A56649; M83717), *H. ducreyi* (L23118), but have also been detected on conjugative or broad host range plasmids, such as pGS05 (M36657) and RSF1010, as well as on plasmids obtained from *E. coli* (A34950), *Photobacterium* (*Ph.*) *damselae* subsp. *piscicida* (D37825), and uncultured eubacteria (AJ271879; AJ293027). Most of the *sul*II genes detected in bovine "*P. haemolytica*" and *P. multocida* isolates from poultry, pigs and cattle are located on plasmids which vary in size between 4.2 and 16.7 kb [6, 12, 16, 24, 29–31, 59, 67], some of which also mediate streptomycin, kanamycin and/or tetracycline resistance [31, 67].

Trimethoprim resistance is commonly due to dihydrofolate reductases. Trimethoprim resistance with MIC values of more than 16 $\mu g \cdot mL^{-1}$ is rarely encountered among *P. multocida* and "*P. haemolytica*" isolates (Tabs. II and III). Studies on trimethoprim-resistant bovine "*P. haemolytica*" isolates from France showed that trimethoprim resistance was not associated with plasmids and also was not transferable by conjugation. Hybridisation experiments with gene probes specific for the genes *dhfr* I through *dhfr*V did not yield positive results [22] suggesting that other determinants are responsible for trimethoprim resistance in *Mannheimia* isolates. These strains were simultaneously resistant to the O/129 component (2,4-diamino.6,7-diisopropyl-pteridine) used for the identification of *Pasteurella*. In such situations, resistance to the O/129 component could result in misidentification of trimethoprim-resistant *Pasteurella*.

4.3.5. Resistance to chloramphenicol and florfenicol

Chloramphenicol resistance is mainly mediated by chloramphenicol acetyltransferases (E.C. 2.3.1.28), many of which are located on plasmids or transposons. Plasmids mediating chloramphenicol resistance have been identified in porcine *P. multocida* isolates [67], but also in bovine *P. multocida* and "*P. haemolytica*" isolates [66]. The first detailed studies on the genetic basis of chloramphenicol resistance among *Pasteurella* and *Mannheimia* isolates were conducted by Vassort-Bruneau et al. [66]. They designed PCR primers specific for the three most frequently occurring *cat* genes among gram-negative bacteria, *catA*I – *catA*III, and detected *catA*I as well as *catA*III genes among bovine *P. multocida* and "*P. haemolytica*" isolates [66]. The *catA*III gene proved to be located on small plasmids of 5.1 kb while the *catA*I gene was located on plasmids of either 17.1 or 5.5 kb [66]. Plasmid-borne *catA*III genes have also been detected in porcine *P. aerogenes* and bovine *Mannheimia* isolates by PCR (Fig. 3) (Kehrenberg and Schwarz, unpublished data). Recently, a small plasmid of 4992 bp detected in a bovine *Mannheimia* isolate of unnamed taxon 10 was completely

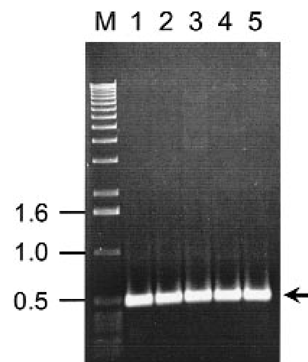

Figure 3. PCR detection of the chloramphenicol resistance gene *catA*III. The arrow indicates the position of the 473 bp amplicon seen in five unrelated *Pasteurella* and *Mannheimia* isolates (lanes 1–5). Lane M contains the DNA size standard (1 kb ladder, Gibco-BRL) of which the sizes of some fragments are given in kb on the left-hand side.

sequenced (database accession no. AJ 249249). This plasmid mediated resistance to sulfonamides by *sul*II, to streptomycin by *strA* and to chloramphenicol by *cat*AIII (Kehrenberg and Schwarz, unpublished data). The *cat*AI gene which codes for a CAT monomer of 219 aa was first identified on transposon Tn*9* [1]. Similar or identical *cat*AI genes have also been detected on plasmids or in the chromosomal DNA of *Acinetobacter* (*A.*) *baumannii* (M62822), *A. calcoaceticus* (M37690) and *Ph. damselae* subsp. *piscicida* (D16171). In comparison to *cat*AI, the *cat*AIII gene codes for a slightly smaller CAT monomer of 213 aa. Several *cat*AIII genes virtually identical to that from *Mannheimia* taxon 10 have been found on plasmid R387 in *Shigella flexneri* and *E. coli* (X07848; P00484), but also on plasmids obtained from uncultured eubacteria (AJ271879; AJ293027). None of the chloramphenicol resistance plasmids detected so far in *Pasteurella* and *Mannheimia* isolates mediated resistance to the fluorinated chloramphenicol derivative, florfenicol. Florfenicol resistance has rarely – if at all – been detected among *Pasteurella* and *Mannheimia* isolates. A gene designated *pp-flo* (D37826) was detected in the fish pathogen *Pasteurella piscicida* [40] which has recently been re-classified as *Ph. damselae* subsp. *piscicida* [25]. So far, no florfenicol resistance genes have been identified among isolates currently assigned to the genera *Pasteurella* and *Mannheimia*.

5. THE ROLE OF HORIZONTAL GENE TRANSFER IN THE SPREAD OF ANTIMICROBIAL RESISTANCE GENES TO AND FROM *PASTEURELLA* AND *MANNHEIMIA* ISOLATES

Most of the resistance genes to date encountered among *Pasteurella* and *Mannheimia* isolates are associated with either small plasmids (e.g. bla_{ROB1}, *strA*, *sul*II, *cat*AIII), or with conjugative (e.g. *tet*(M)) and non-conjugative transposons (e.g. *cat*AI, *tet*(H), *tet*(B)). For their replication, transposons depend on replication-proficient vector molecules of the host cell, such as chromosomal DNA or plasmids, in which they can integrate. Consequently, the transposon-borne *tet* genes of classes B, H, and M have been detected in the chromosomal DNA of *Pasteurella* and/or *Mannheimia* isolates [13, 35, 37, 39]. In some cases, transposon-borne *tet*(H) and *tet*(B) genes, but also *cat*AIII genes were located on small plasmids of *Pasteurella* and *Mannheimia* isolates. Most of the resistance plasmids identified so far among *Pasteurella* and *Mannheimia* isolates exhibit sizes of less than 10 kb and are thus too small to carry the *tra* gene complex which is essential for conjugative transfer. Nevertheless, some of these plasmids have been shown to harbour up to three different *mob* genes (Kehrenberg and Schwarz, unpublished data) whose products enable horizontal transfer by mobilisation in the presence of a co-resident conjugative element. Since the Mob proteins originating from one plasmid may be used for the mobilisation of all plasmids of the same bacterial cell – provided these plasmids carry an "origin of transfer" (*ori*T) –, plasmids which do not harbour *mob* genes can also be mobilized. Mobilisation is also the most reasonable explanation for the occurrence of indistinguishable type pPAT1 *tet*(H)-carrying plasmids in porcine *P. multocida* and *P. aerogenes* isolates [36], but also for the presence of identical plasmid-borne bla_{ROB1} genes in members of the genera *Pasteurella* [44, 45], *Actinobacillus* [34], and *Haemophilus* [18, 48]. Conjugative plasmids not associated with antimicrobial resistance, but promoting mobilisation of co-resident small resistance plasmids have been described in *P. multocida* from turkeys [29]. In the latter case mobilisation occurred not only into other *P. multocida* isolates, but also into *E. coli*. A conjugative plasmid which mediated resistance to tetracyclines,

streptomycin, sulfonamides and kanamycin also proved to be able to move into other *P. multocida* and *E. coli* strains and to express its resistance properties in both host bacteria [31]. Another possibility of horizontal transfer of small plasmids is by transduction. This way is however limited by the amount of DNA that can be packaged into a phage head and the requirement for specific receptors for phage attachment on the surface of the new host cells. These receptors are usually only present among cells of the same or closely related species within the same genus. Although phages have been detected in pasteurellae, very little is known about transducing phages in *Pasteurella* and *Mannheimia* [33, 42].

The fate of a resistance plasmid introduced into a new host cell is uncertain. In the most simple case, this plasmid is able to stably replicate and express its resistance properties in the new host cell. This has been observed with many resistance plasmids from *Pasteurella* and *Mannheimia* when introduced into *E. coli* host cells. However, plasmids of *Enterobacteriaceae* which harbour a ColE1 replication origin proved to be replication-deficient in *Pasteurella* or *Mannheimia* host cells [5]. There are also several known "broad host range" plasmids which have shown to be able to replicate in many different bacteria. Some of these plasmids, such as R100 (database accession no. AP000342), carry copies of the small non-conjugative resistance transposons Tn*9* (*cat*AI) or Tn*10* (*tet*(B)) and by their spread also promote the spread of these resistance transposons. Even if the plasmid itself may be replication-deficient in a new host, its transposons can excise and integrate into plasmids and the chromosomal DNA of the new host cell. This might be an explanation for the occurrence of Tn*10* copies in the chromosome of *P. aerogenes* isolates [37]. Plasmid incompatibility between the transferred resistance plasmids and those plasmids still resident in the new host cell may negatively influence the stable maintenance of the newly acquired resistance plasmids. In addition, *Pasteurella* and *Mannheimia* cells may also harbour restriction/modification systems which protect the cell from foreign DNA. Such host cell defense systems have been described to occur in *P. multocida* [32] and "*P. haemolytica*" [28].

Simple and efficient ways for transferred resistance plasmids to circumvent potential problems arising from replication deficiency, plasmid incompatibility or destruction by restriction systems are (a) the integration – in part or in toto – into the chromosomal DNA of the new host cell, but also (b) the fusion with or the integration into plasmids of the new host cell. In the latter case, novel plasmids are formed which carry additional resistance genes and are able to replicate in the new host cell. A single copy of a resistance gene in the chromosomal DNA bears the problem that this gene may be functionally deleted as the result of mutations, recombinations or insertions. However, the presence of *Pasteurella* and *Mannheimia* isolates in a polymicrobial environment offers the opportunity to acquire new resistance genes from the respective gene pool by horizontal gene transfer mechanisms [19]. Thus bacteria may acquire resistance genes which have developed in other bacteria but have been refined under selective pressure. With regard to an optimised function in the new host, the resistance genes may be subjected to slight modifications resulting in few amino acid exchanges in the respective resistance gene products as mentioned in Sections 4.3.2–4.3.5. Most of the resistance genes currently known to occur in *Pasteurella* and *Mannheimia*, such as *tet*(B), *cat*AI, *cat*AIII, *sul*II, or *strA*, are most likely of enterobacterial origin, but have gained access to bacteria living in other environmental compartments such as the respiratory tract. Others, such as bla_{ROB1}, seem to be widely distributed among respiratory pathogens and a gene like *tet*(H) seems to be limited to *Pasteurella* and *Mannheimia* isolates present in the respiratory tract. On the one hand, it is observed that *P. aerogenes* isolates from the intestinal tract mainly

harbour *tet*(B) genes – which are the most predominant *tet* genes among *Enterobacteriaceae*. On the other hand, *P. multocida* isolates from the respiratory tract almost exclusively carry *tet*(H) genes. This underlines the role of the gene pools present in certain environments, such as the intestinal or the respiratory tract, for the acquisition of resistance genes by bacteria of different species and genera [37].

6. CONCLUSION

Isolates of the genera *Pasteurella* and *Mannheimia* are still among the most economically important veterinary bacterial pathogens. Due to the often unsatisfying or even ineffective immunoprophylactic measures taken, antimicrobials are used to a large extent for prophylaxis, metaphylaxis or the therapy of diseases in which *Pasteurella* and *Mannheimia* isolates are involved. Although improved vaccines are under development, the current principles of antibiotic use in the control of infections due to *Pasteurella* and *Mannheimia* need to be refined according to prudent use guidelines. Molecular analysis has provided insight into the variety of resistance genes so far known to be present in *Pasteurella* and *Mannheimia* isolates. Most of these resistance genes are associated with mobile genetic elements and, can thus easily be exchanged between bacteria. The occurrence of these resistance genes in a wide range of bacteria implies that *Pasteurella* and *Mannheimia* isolates have access to large gene pools within which an interchange of resistance genes takes place. Recent studies confirmed that some of the resistance genes encountered in *Pasteurella* and *Mannheimia* such as *cat*AIII and *sul*II, are widely distributed in *Enterobacteriaceae* and other gram-negative bacteria, while other genes such as *tet*(M) and bla_{ROB1}, appear to demonstrate links between gram-negative and gram-positive bacteria. The current knowledge of resistance genes and their ways of spreading, as described for the resistance genes mentioned in this review, leads to the assumption that resistance development in *Pasteurella* and *Mannheimia* isolates is a continuous process in which novel resistance genes may be acquired or developed under the selective pressure imposed by the use of new drugs.

Although animal pasteurellosis has only limited consequences for public health, it would be hazardous to stop or to maintain the monitoring of resistance of *Pasteurella* and *Mannheimia* isolates at only a low level. Monitoring antimicrobial resistance is essential to assist practitioners in the rational selection of antimicrobial agents and the prudent use of these drugs. Recent results on the evolution of antibiotic resistance in *Pasteurella* and *Mannheimia* call for a reinforcement of monitoring at the national and international levels. This also implies the development of rapid and reliable tests for species identification of these bacteria and antimicrobial sensitivity testing, as well as the development of new vaccines to effectively prevent pneumonic diseases in food-producing animals and thus reduce the overall use of antimicrobial agents.

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