Using disinfection products less and better in the poultry and fish sectors

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Abstract

Disinfectants are essential for sanitary control. Nevertheless, their handling can be the cause of one-off accidents or chronic effects on human health if prevention and/or protection measures are not taken. The survey carried out in the ADAPT project among 108 companies (hatcheries, slaughterhouses, chicken and duck farms, fish farms) emphasises that a significant proportion of situations involves a high health risk, particularly for poultry and fish farmers. The implementation of prevention strategies should therefore be prioritised for the livestock sector, especially as awareness, information and training are less sustained there (smaller group of workers per company, more difficult to mobilise farmers on these subjects, etc.). The professionalization of farmers in terms of good practices for handling equipment (especially foam guns, which are most commonly used) and products (protection of the eyes, skin, respiratory tract, ventilation of the premises, etc.) must be reinforced in order to protect their health, the health of consumers and the environment, but also to guarantee the efficacy of disinfection. The use of alternatives or complementary methods making possible to use less disinfectant has been explored in the project: the use of barrier flora or enzymatic detergents seems to be a promising complementary method.

Keywords: biocide, poultry production, fish production, barrier flora, enzymatic detergent

1 Introduction

The avian influenza crises have demonstrated the importance of rigorous, long-term compliance with decontamination of infrastructures and equipment used for breeding and animal transport (Huneau-

Salaün et al. 2017; Scoizec et al., 2017). Optimising cleaning and disinfection protocols is an essential point. The presence of organic soiling and the organisation of microorganisms into biofilms (communities of microorganisms adhering to surfaces and producing an extracellular matrix that plays a protective role against physico-chemical aggression, Bridier et al., 2011), require the regular and massive application of biocides. Nevertheless, the handling of disinfectants can be the cause of one-off accidents if protective measures are not taken. Many substances are likely to be corrosive or irritant to the skin, the oro-rhino-laryngeal and bronchial mucous membranes (e.g. quaternary ammoniums, chlorinated agents, peracetic acid), to cause respiratory pathologies (e.g. aldehydes) or skin allergies (e.g. glutaraldehyde, quaternary ammoniums) (Abadia et al., 2003; Sanderson et al., 1995; Massin et al., 2007).

Disinfectants are among the biocides subject to European regulations (EU Regulation 528/2012), which aim to ensure a high level of protection for human and animal health, and the environment. Currently, products containing these substances are subject to a full assessment for each dedicated use. Many of the products used in poultry farming are currently subject to a "transitional" authorisation scheme, and it is highly likely that the variety of products placed on the market will be reduced as a result of stricter regulatory requirements and the limited development of new products.

In this context, complementary methods such as the application of barrier flora or the use of enzymatic detergents may be considered. Barrier flora can be used in the food industry (production and processing plants) but also in livestock farming to control the ecology of surfaces and thus limit the establishment of pathogens. They can be administered directly to animals in the form of probiotics via their feed or drinking water. Barrier flora can act in different ways: i) by colonising a target surface with so-called positive bacteria, thereby preventing pathogens from taking hold. This physical barrier creates competition for space and nutritional resources; ii) by producing inhibiting compounds (organic acids or bacteriocins). The barrier flora can also be sprayed onto the farm to prevent contamination of the animals through contact with surfaces. This voluntarily added flora then grows in the form of a biofilm in the animals' direct environment, limiting the establishment and subsequent development of pathogenic biofilms. Unlike the curative action of biocide, the biofilm formed by the barrier flora can represent a preventive strategy, by preventing the establishment of pathogens. Enzymatic detergents are associated with green chemistry because they have a neutral pH and are made from natural, biodegradable and low-toxicity compounds (Tsiaprazi-Stamou et al., 2019). They generally contain surfactants and a mixture of enzymes whose activity targets the extracellular matrix of biofilms: proteases, lipases, amylases but also cellulases, polysaccharide depolymerases, alginate lyases, dispersins and DNAses (Bridier et al., 2015). Various medical and agri-food studies have shown that the use of enzymatic detergents partially eliminates biofilms during the cleaning phase and weakens them during the subsequent disinfection stage (Delhalle et al., 2020; Tsiaprazi-Stamou et al., 2019).

An increasing number of commercial barrier flora are available on the market and sold in various forms. They are made up of microbial consortia, mainly including lactic acid bacteria and/or bacteria of the *Bacillus* genus. Their use is tending to develop in the poultry industry, although their actual effectiveness remains poorly evaluated and their mechanism of action poorly understood. As for enzymatic detergents, although these new formulations are being used more and more successfully in the agri-food industry (on open or closed surfaces), their effectiveness in a poultry farming context remains to be demonstrated.

The aDAPt project therefore addressed the issue of preventing the risks associated with exposure to disinfectant-type biocides among poultry and fish workers in France, by meeting the following objectives: (i) to gain a better knowledge and understanding of the attitudes of users of disinfectant-type biocides through a systemic approach to their practices, (ii) to identify the situations in which use practices give rise to the greatest concern in terms of risks to human health, (iii) to acquire new knowledge and provide tools for raising awareness so that use practices can evolve towards methods that make better and lesser use of these chemical inputs. The rest of this article presents the most significant results obtained from the project.

1 Materials and methods

1.1 Review of disinfectant use practices and assessment of risks to human health

For the poultry industry, 78 surveys were carried out at 12 hatcheries (Brittany, Centre-Val de Loire, New Aquitaine, Pays-de-la-Loire), 25 broiler farms (Brittany), 19 foie gras farms (Brittany and New Aquitaine), 6 duck farms ready for fattening (New Aquitaine) and 16 slaughterhouses(Bourgogne-Franche Comté, Brittany, Centre-Val de Loire, Grand-Est, New Aquitaine, Occitanie, Pays-de-la-Loire). At the same time, 30 fish farms were surveyed.

The "Système d'évaluation et d'information sur les risques chimiques en milieu professionnel" software (Seirich, http://www.seirich.fr/seirich-web/index.xhtml) was used to carry out assessments of so-called "residual" risks through inhalation and skin or eye contact. It should be noted that the wearing of personal protective equipment (PPE) is not taken into account in these assessments. This assessment is carried out at the level of the daily work task and therefore makes it possible to assess a level of risk each time a product is handled. The results are classified into 3 levels: low, moderate and high.

The company's activity is divided into work areas, in which the operator has the material resources to perform different tasks. In order to compare the results obtained in each company, the work zone was defined in this study as being an operation in the production process requiring the handling of a disinfectant product (e.g. "disinfecting lorries"). Information was collected to characterise air renewal in the working environment in these areas and any capture devices present (e.g. extraction hoods).

Several tasks can then be identified within each zone (e.g. applying a product, dosing, filling the tank). The equipment used (e.g. metering pump, foam gun) and the process (e.g. filling by pouring a product directly by hand from the canister, activation of a remote device to trigger the appliance) were modelled in the software by "process types" based on the European guide to risk assessment of new chemical substances (European Commission - Joint Research Centre - Institute for Health and Consumer Protection - European Chemicals Bureau (ECB), 2003) and "types of skin scenario and surface exposed" (Bertrand et al., 2020).

Finally, a complete inventory of the commercial specialities used for each task was drawn up (several products may be used for the same task), by collecting the physicochemical characteristics, hazard and warning statements, precautionary advice, pictograms using safety data sheets (SDS) and product consumption.

1.2 Ability of barrier flora to limit the establishment and development of *Salmonella Typhimurium*

Three commercial barrier flora (F1, F2, F3) consisting of *Bacillus sp*. and lactic acid bacteria, but with different formulations, were studied. In powder form, they were resuspended in mains water and then counted on TSAYE media (Trypticase-Soya agar supplemented with yeast extract).

Salmonella enterica serotype *Typhimurium* ATCC14028 (*S. Typhimurium* isolated from chicken liver) was chosen as the model pathogenic bacterium for the poultry industry. Suspensions were prepared by 3 successive subcultures in TSBYE broth (Trypticase-Soya Broth supplemented with yeast extract) before enumeration on TSAYE medium.

Five commercial products representative of the main families of chemical products most commonly used according to the data collected as part of action 1 of the aDAPt project (see §1.1 of this article) were selected. They belong to the family of quaternary ammoniums associated with aldehydes (A), chlorines (B), peracids (C), phenolics (D) and monopersulphate (E).

The polyethylene coupons (PE, GoodFellow, Lille, dimensions: 20 x10 x1 mm), a material present in the various links of the poultry industry, were soaked for degreasing under agitation in pure ethanol (30 min)

then rinsed 5 times with osmosed water. They are then disinfected by soaking in 70% ethanol (5 min) then in osmosis water (10 min) before being dried. They are stored sterile in a Petri dish before use.

The implantation of barrier flora on PE coupons was evaluated:

- before treatment with the disinfectant to characterise their development and
- after treatment to assess the potential residual effect of the disinfectant on the barrier flora.

The barrier flora are first prepared in sterilised tap water. They are then deposited on PE coupons (10^4 to 10^5 Colony Forming Units (CFU) per cm²). The concentrations (0.8-1%) and contact times (20-30 min) applied for the biocidal treatment are in line with the supplier's recommendations. For the residual effect, the coupons treated with the biocide were air-dried for 18 to 72 hours before being brought into contact with the barrier flora. The barrier flora were counted on TSAYE agar. In order to test the potential of the flora, at higher concentrations, to inhibit the establishment and development of the pathogen, the culture conditions of the flora on coupons were modified, by testing the effect of the addition of organic matter on the load per cm² of barrier flora obtained.

To do this, the flora were grown either in the presence of TSBYE, or in mains water supplemented with freeze-dried droppings (rich organic substrate found on the rearing site, 20 g per 100 g of water, pH: 5.3) or TSBYE**.**

Barrier flora were then used to inhibit *S. Typhimurium.* Barrier flora were deposited on PE coupons (~4-5 $\log_{10}$ (CFU/cm²)) and incubated for 24 to 48 hours at 25°C. Next, a suspension of *S. Typhimurium* (~4 $\log_{10}$ (CFU/cm²)) mixed with droppings to simulate real contamination conditions, was added to the coupon, which was incubated again for 24-48 h at 25°C. After rinsing the coupons with mains water, the bacteria were removed from the coupons using ultrasound before being counted. To assess the inhibition of *Salmonella* by the barrier flora, *Salmonella were* counted on Mc Conkey selective agar (24h 37°C) in comparison with a coupon conditioned with mains water (control without flora) instead of the barrier flora.

1.3 Ability of enzymatic detergents to combat *Escherichia coli* biofilms

On the basis of a survey of poultry industry professionals, suppliers and vets, 4 commercial products described as enzymatic detergents were selected. According to their technical data sheets, all these detergents contain surfactants with 2 or 3 types of enzymes added (amylases and proteases for product D; amylases, proteases and lipases for products A, B and C). Mains water was used as a control detergent.

The *E. coli* DSM682 strain, widely used to validate the efficacy of disinfectants, was selected to establish biofilms. This strain was grown in TSBYE broth and enumerated on TSAYE agar medium. The coupons (20 x 10 x 1 mm), made of 304L stainless steel (EML, La Hague) and polyethylene (GoodFellow, Lille), were degreased in pure absolute ethanol for 30 min and then rinsed 5 times in 50 ml sterile osmosed water. They were then disinfected in 70% ethanol for 5 min and rinsed in sterile osmosis water for 10 min. All these steps were carried out with agitation. The coupons were then dried and stored sterile until use. To prepare the biofilms, chicken droppings were reconstituted by hydrating 20 g of lyophilisate with 100 g of sterile distilled water (pH = 5.96) and then sterilised at 121°C for 15 minutes. Forty microlitres of rehydrated droppings were applied to the coupons. After one hour's incubation at 25°C, 40 µl of an *E. coli* solution calibrated to $1x10^8$ CFU/ml was added to the coupon (preliminary tests were used to define the level of dilution required to obtain a concentration of approximately $1x10^8$ CFU/ml from a 16-hour pre-culture in TSBYE). The coupons were then placed in sterile Petri dishes in an oven at 25°C for 5 days under humid conditions. Before analysis or treatment, the biofilms were rinsed twice with physiological water and then placed in glass tubes containing 10 ml of sterile physiological water. Adherent bacterial cells were removed from the supports using ultrasound at 35 kHz for 2 minutes (Elma S120H sonicator, Elmasonic) before being counted by plating a series of decimal dilutions on the surface of TSAYE agar plates.

The detergents were applied to the biofilms, which had previously been dried at 37°C for 1 hour. This drying step resulted in a reduction of around 0.5 $\log_{10}$ *of the E. coli* population on the support (data not shown). The coupons were coated with detergent at the concentration used. The coupons were then removed and rinsed twice in sterile mains water. The products were used at the concentrations and contact times indicated in the supplier's data sheet. All the enzymatic detergents were used at 2% after dilution in sterile mains water. The contact time was 20 min for detergent B and 30 min for products A, C and D.

A quaternary ammonium and glutaraldehyde-based disinfectant was applied to the residual biofilms downstream of the detergent treatment. The disinfectant was used at a sub-lethal concentration (dose divided by 5) so that the synergistic effect of an enzymatic detergent in a protocol combining detergency and disinfection could be quantified. Each experimental procedure was repeated at least 3 times. The results presented (cf. 3.7 and 3.8) are the means of the different replicas and their standard deviations calculated in Excel spreadsheets. The Kruskal-Wallis test was applied with the help of XLSTAT software to determine the significance of the differences between the modalities, with an alpha risk of 0.05.

2 Main results obtained in the aDAPt project

2.1 Families of disinfectant products used in different types of business

For the poultry industry, the family of quaternary ammoniums combined with aldehydes is the most widely used, whatever the type of business (Table 1). These are exclusively products containing glutaraldehyde. Some farmers explained *afterwards* that they might occasionally (once a year) use a product containing formaldehyde (classified as carcinogenic, mutagenic and toxic to reproduction (CMR) category 1B). In this case, they use a service company, which may explain why they didn't think to mention it when making the inventory for the survey.

For fish farms, the family of chlorinated products is widely used (27% of products listed), as are quaternary ammoniums combined with aldehydes (22% of products listed).

Table 1: Main families of disinfectant products for each type of business

Product family	Slaughterhouses	Hatcheries	Duck producers	Broiler farms	Fish farms
Quaternary ammoniums associated with an aldehyde	56 %	44 %	74 %	50 %	22%
Bases	0 %	0 %	17 %	26 %	3 %
Quaternary ammoniums	7 %	19 %	0 %	1 %	0 %
Phenols	0 %	6 %	3 %	9 %	1 %
Glycolic acid	0 %	3 %	0 %	12 %	1 %
Peracetic acid	5 %	8 %	0 %	0 %	7 %
Chlorine	14 %	3 %	0 %	0 %	27 %
Amines	14 %	2 %	0 %	0 %	0 %
Hydrogen peroxide	0 %	6 %	0 %	0 %	16 %
Aldehydes	0 %	0 %	0 %	0 %	14 %

2.2 Assessment of residual risks in different types of business

Duck producers and broiler farms account for a large proportion of tasks with a high level of residual risk through inhalation (76% and 55% respectively of the tasks assessed for these types of company). This can be explained by the more frequent use of quaternary ammoniums combined with aldehydes (especially for duck producers), combined with dispersive processes that are very common on farms (foam cannons or spray lances, fertiliser spreaders for disinfecting surroundings and runs, fogging, nebulisation and fumigation). Duck producers also have a high proportion of tasks with a high level of residual risk through skin or eye contact (79% of tasks assessed for this type of company), which is linked to the very high proportion of tasks involving processes that may generate splashes or aerosols. Processes without possible contact are more common in broiler farms than in duck farms, which translates into a lower proportion of tasks with a high level of residual risk through skin or eye contact (42% of tasks assessed for this type of company).

For fish farms, the level of residual risk through inhalation was high in 45% of the tasks assessed, and for skin/ocular risk, it was high in 51% of the tasks assessed.

In hatcheries and slaughterhouses, the proportion of tasks presenting a high level of residual risk through inhalation is more modest than in livestock farms (29% and 35% respectively of the tasks assessed for these types of enterprise). This can be explained in part by the use of closed and/or closed but regularly open processes (use of dosing pumps, or machines such as crate washers), which are more frequent in these companies than in livestock farms, and the more frequent use of less-exposing product families (particularly in hatcheries).

Closed processes are more common in hatcheries and slaughterhouses than among duck and broiler producers, which contributes to a lower proportion of tasks with a high level of residual risk through skin or eye contact (28% and 40% respectively of the tasks assessed for these types of company). Note, however, the relatively frequent use of chlorinated agents in slaughterhouses, which generates a high residual skin and eye risk.

2.3 Effectiveness of inhibition of *S. Typhimurium* by barrier flora

Figure 1 shows the quantities of *S. Typhimurium* counted according to the different barrier flora prepared first in sterilised mains water (or on the control without flora) 24 or 48 hours after inoculation with the pathogen.

The results obtained in these trials show that at these concentration levels, i.e. between 4.50 and 5.13 $\log_{10}$ (CFU/cm²), the barrier flora do not reduce the quantity of *S. Typhimurium* counted for the 3 flora tested in comparison with the control coupon without flora. On the contrary, *S. Typhimurium* managed to grow to around 7 $\log_{10}$ (CFU/cm²) 24 hours after inoculation, with no significant change in these quantities after 48 hours.

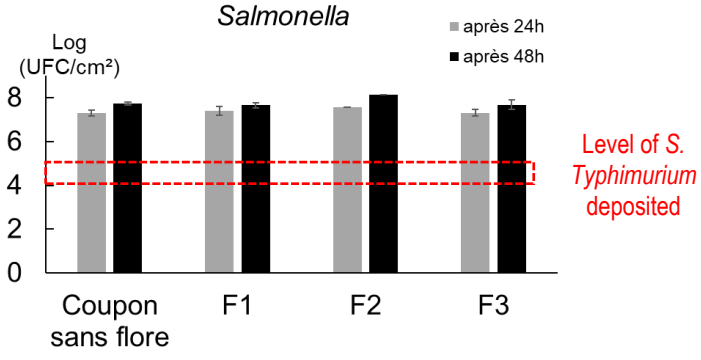

Figure 1: Quantities of *S. Typhimurium* (in log (CFU/cm²)) counted 24 or 48 hours after inoculation of the pathogen on coupons colonised by F1, F2 and F3 barrier flora developed in mains water, compared with a PE coupon without flora (control).

Trials carried out using flora grown in the presence of TSBYE showed that the deposition of an inoculum of 4 log_{10} (CFU/cm²) for the different barrier flora F1, F2 and F3 resulted in levels of 7.07, 7.56 and 5.22 log_{10} (CFU/cm²) respectively on PE coupons after 24 hours, demonstrating the ability of the flora to grow under these conditions, despite quantitative differences between the flora.

In the presence of droppings in mains water, the flora levels after 24 hours were 7.76, 4.06 and 8.00 log_{10} (CFU/cm²) respectively for the F1, F2 and F3 flora. This result reveals a heterogeneous flora response under these conditions, with the F1 and F3 flora able to grow strongly in the presence of organic matter (droppings). Conversely, the F2 flora remained at a quantity of CFU/cm² comparable to that of the inoculum (around 4 log_{10} CFU/cm²), and did not seem capable of growing in the presence of droppings, contrary to what was observed in the presence of TSBYE.

Figure 2 shows the quantities of *S. Typhimurium* counted 24 or 48 hours after inoculation with the pathogen on coupons colonised with F1, F2 and F3 barrier flora developed in the presence of droppings (A) or TSBYE (B). The results show a disparity in the effectiveness of the flora in inhibiting pathogen implantation, which is partly dependent on the CFU/cm² levels of the barrier flora. The F1 and F3 flora (7 to 8 log_{10} (CFU/cm²)) are capable of significantly inhibiting *S. Typhimurium*. The F1 flora demonstrated total efficacy within 24 hours, while comparable levels of inhibition were achieved by the F3 flora within 48 hours. The coupons with F2 flora, on the other hand, showed quantities of *S. Typhimurium* comparable to those counted on the coupons without flora. This is related to the low levels of F2 flora obtained under these conditions in the presence of droppings (~4 log_{10} (CFU/cm²)), similar to those obtained without the addition of organic matter to the network water.

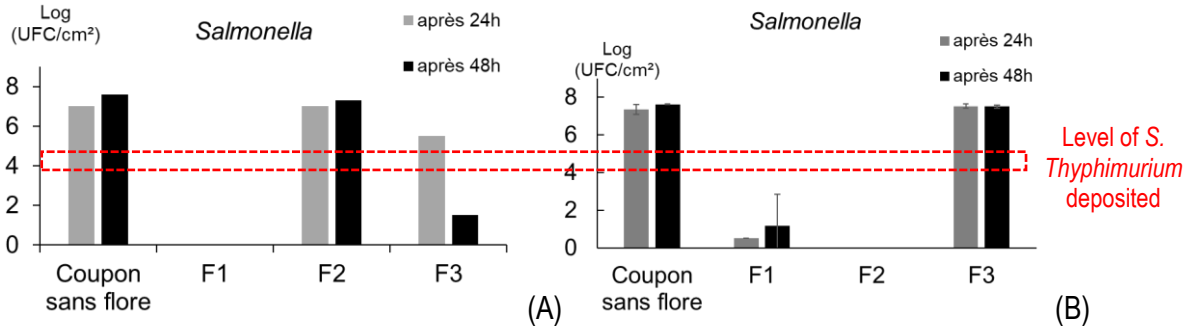

Figure 2: Quantities of *S. Typhimurium* (in log_{10} (CFU/cm²)) counted 24 or 48 hours after inoculation of the pathogen on coupons colonised with F1, F2 and F3 barrier flora developed in the presence of droppings (A) or TSBYE (B) compared with a PE coupon without flora (control).

2.4 Impact of the application of a mechanical action on the measurement of enzymatic detergents' effectiveness on stainless steel

The bacterial suspension obtained after unhooking the *E. coli* biofilms was concentrated to 7.5 ± 0.1 log_{10} CFU/ml. The density of biofilms on stainless steel coupons after 5 days of incubation at 25°C therefore reached an average of 8.5 ± 0.1 log_{10} UFC/support. These *E. coli* biofilms prepared in the presence of chicken droppings were then subjected to various enzymatic detergents.

Initially, the enzymatic detergents were applied by simply immersing the biofilms in the product (Figure 4 (A)). In the absence of mechanical action, the measurable impact of the enzymatic detergents on the biofilms, compared with a water treatment, was low. At 20°C, only detergent A showed significant activity,

with biofilm density reduced by 1 log_{10} on average compared with the "water" control ($p<0.05$). At 30°C, only treatment with detergent C significantly reduced biofilm density compared with the control (-2.75 log_{10} ; $p<0.05$).

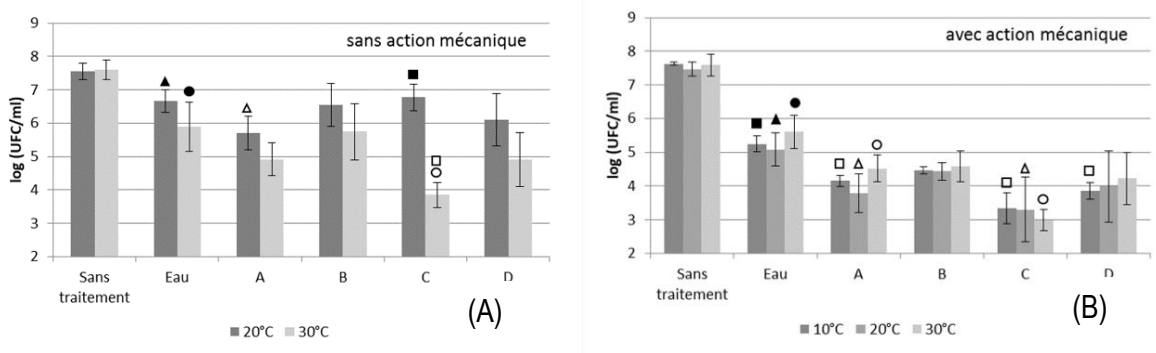

Figure 4: Impact of enzymatic detergency on the quantity of residual bacteria (log_{10} CFU/ml) on stainless steel coupons after treatment at different temperatures with or without the application of mechanical action. The presence of identical symbols shows that there is a significant difference between the treatment (empty symbol) and the reference condition (solid symbol) (Kruskal-Wallis test, $p<0.05$).

The absence of mechanical action during or after the application of detergents can have an effect on the measurement of enzyme efficiency. This was confirmed when the treatments were carried out under slow agitation. Under these conditions, the simple application of water strongly affected the structure of the biofilms, with a reduction in the concentration of residual *E. coli of* more than 2 log_{10} (Figure 4 (B)). Nevertheless, the activity of enzymatic detergents is more easily demonstrated under these conditions, since detergents A and C are both effective compared to the control ($p<0.05$) whatever the application temperature (-1 to -1.3 log_{10} reduction for A; -1.7 to -2.6 log_{10} for C). Detergent D was also effective at 10°C, with residual biofilm reduced by 1.5 log_{10} on average compared to treatment with water ($p<0.05$).

Suppliers generally recommend applying enzymatic detergents with a spray gun or foam unit. At the end of the contact time, the surfaces can be rinsed with a high-pressure cleaner. The results described here seem to show that this high-pressure rinsing stage can be decisive for the optimum effectiveness of enzymatic detergents. In fact, a simple application under static conditions significantly reduces the impact of the detergent on the biofilm.

2.5 Contribution of enzymatic detergents to a combined detergent and disinfection treatment

Enzymatic detergents destabilise the extracellular matrix of biofilms, which could enable disinfectants to reach the bacteria more effectively. This synergistic effect between enzymatic detergents and biocide treatment was assessed by applying a detergent with commercial product C (the most effective on average in previous trials) followed by moderate disinfection to *E. coli* biofilms (Table 2).

Table 2: Residual *E. coli* concentration (log_{10} CFU/ml) measured after application of treatments at 20°C to stainless steel coupons, combining detergents (water or enzymes, with or without mechanical action) and disinfection.

modality	enzymatic detergent	mechanical action	Biocide	E. coli concentration (log10 CFU/mL)
1	No	-	No	7,9 ± 0,1
2	**Water**	**Yes**	No	6,3 ± 0,2
3	**Water**	**Yes**	**Yes**	5,1 ± 0,3
4	**Yes (detergent C)**	**Yes**	No	4,1 ± 0,3
5	**Yes (detergent C)**	**Yes**	**Yes**	2,9 ± 0,1
6	**Yes (detergent C)**	No	No	4,4 ± 0,2
7	**Yes (detergent C)**	No	**Yes**	3,8 ± 0,2

These latest results confirm the benefits of mechanical action on the effectiveness of enzymatic detergency (-0.3 log_{10} of residual biofilm under agitation) and the benefits of using enzymes compared with simple cleaning with water (-2.2 log_{10} of residual biofilm). This effect of enzyme treatment is also visible when it is followed by the application of a quaternary ammonium and glutaraldehyde-based biocide. Indeed, the residual *E. coli* concentration after application of the enzymes and then the disinfectant is 3.9 log_{10} CFU/support compared with 6.1 log_{10} CFU/support when water is used as a detergent. However, it is interesting to note that application of the biocide resulted in a 1.2 log_{10} reduction in *E. coli* concentration, whether or not an enzymatic detergent was used prior to disinfection. These results therefore did not demonstrate any "sensitisation" of the biofilm to the disinfectant treatment after application of detergent C. Under the test conditions described here, the greater efficacy of an "enzymatic detergent + biocide" treatment, compared with the "cleaning with water + biocide" protocol, lies in the elimination of a greater proportion of the biofilm during the detergency phase, but does not result from a synergy between the action of the enzymes and the disinfection. A simple cumulative effect between the two stages of the cleaning and disinfection protocol was observed.

3 Conclusion

The inventory of product families produced in this study represents a snapshot at a given point in time. Products may in fact change in the future as active substances are assessed. There is a risk that a number of molecules that present the greatest risks to human health will not be approved, and will therefore no longer be used in commercial formulations.

Nevertheless, this study highlights a significant number of situations where the residual risk through inhalation or skin or eye contact remains high, particularly in poultry and fish farms. The implementation of prevention strategies should therefore be a particular priority for this sector, especially as awareness-raising, information and training are less well supported there (smaller workgroups per company, more difficult to mobilise farmers on these subjects, etc.).

Under conditions favourable to their development, and when applied in sufficient quantities (~6-7 log_{10} UFC per cm^2), barrier flora very significantly limit the formation of *S. Typhimurium* biofilms. A low impact of disinfection on the development of barrier flora for the majority of biocidal products from different chemical families was also demonstrated in these laboratory experiments (results not shown). Enzymatic detergents have also been shown to improve the effectiveness of cleaning and disinfection procedures. The application of mechanical force improves the effectiveness of these products, regardless of the temperature at which they are applied. Although these trials were carried out taking into account the specific characteristics of the sector (biofilm growth in the presence of chicken droppings, choice of

realistic application temperatures, etc.), the value of these two complementary methods tested as part of the aDAPt project can only be clearly confirmed once cross-studies on application methods and in the field have been carried out.

The aDAPt project (https://www.itavi.asso.fr/projets/projet-adapt) has developed information and recommendation tools to help raise awareness among farmers of good practices for handling equipment and products, in order to protect their health, that of consumers and the environment, and to guarantee the effectiveness of disinfection.

Ethics

The authors declare that the experiments were carried out in compliance with the applicable national regulations.

Declaration on the availability of data and models

The data supporting the results presented in this article are available on request from the author of the article.

Declaration on Generative Artificial Intelligence and Artificial Intelligence Assisted Technologies in the Drafting Process.

The authors used artificial intelligence in the translation process from French to English.

Authors' contributions

Rousset Nathalie: data collection, carrying out risk assessments, analysing results, writing.

Griveau Gildas, Riolland Claire: risk assessment using SEIRICH software, data collection

Balaine Loïc, Battaglia Antoine, Berlizo Benoît, Dumas Victor, Galliot Pascal, Kot Lucile, Pertusa Marion, Ruch Marion, Thomas Rodolphe, Van Cuick Claire : data collection

Carpentier Patrice, Deffreix Laurent, Huneau Adeline, Le Bouquin-Leneveu Sophie, Travel Angélique: expert use of disinfectants

Bridier Arnaud, Ait Ahmed Azziza, Le Grandois Patricia, Soumet Christophe: carrying out trials on barrier flora, analysing the results, writing the report

Hanin Aurélie, Levert Delphine: conducting trials on enzymatic detergents, analysing results, writing up.

Regulation (EU) No. 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products

Declaration of interest

The authors declare that they do not work for, advise, own shares in, or receive funds from any organisation that could benefit from this article, and declare no affiliation other than those listed at the beginning of the article.

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