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Evaluation of the active concentration of two disinfectants based on glutaraldehyde and benzalkonium chloride and antimicrobial activity *in vitro* against *Salmonella* Heidelberg and *Salmonella* Typhimurium

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ABSTRACT
Biosecurity, cleaning and disinfection of swine and poultry facilities are fundamental for the reduction of pathogenic microorganisms of importance for public and animal health. The objective of this work was to compare the levels of active ingredient described on the label and the real levels detected in high-performance liquid chromatography (HPLC) of two disinfectants., then evaluate the antimicrobial activity since, following the Germicidal Sanitizing Action and Disinfectant Detergent (Official Method AOAC 960.09) in four different dilutions with the presence of 3% organic matter during 15 min of contact, against *Salmonella* Heidelberg and *Salmonella* Typhimurium (ST). The product “A” presents active levels of agreement according to the label. The content of quantified assets for product “B” was lower than that recorded on the label. The disinfectant “A” was effective in microbiological evaluation while the disinfectant “B” had microbiocidal activity compromised by the deficit of assets.

Keywords: biosecurity; cleaning and disinfection, cross-resistance; *Salmonella*.

Salmonella infection in poultry and swine production can result from the vertical transmission, from the breeding stock, or from the horizontal transmission, and considering the environmental conditions and the health status of the flock, the bacteria can survive for more than six years in the environment (GONZALEZ et al., 2015).

According to SESTI (2005), in order to keep commercial herds free or controlled from public health agents that cause illnesses with economic impact and/or health of the flock, it is advisable to adopt biosecurity programs. In addition to enabling the reduction of disease outbreaks in the production chain, it generates benefits for animal welfare, greater productivity, and appreciation of the final product (LUYCKX et al., 2015a).

Biosecurity can be defined as all procedures implemented to reduce the risk and consequence of the occurrence of some disease-causing agents (COLLET, 2016). Among the procedures is the periodic execution of hygiene plans (GEHAN et al., 2009; SESTI, 2005).

Usually, a hygiene plan should include safe, easy-to-execute procedures, describe the correct way of applying detergents and disinfectants, proper use of application equipment, and an effective monitoring system (GEHAN et al., 2009). It is also essential to choose an effective disinfectant for disease control (SCUR et al., 2014).

The present study aimed to quantify the active level of two disinfectants and evaluate their antimicrobial activities at different dosages against *Salmonella* Heidelberg (SH) and *Salmonella* Typhimurium (ST).

In the first stage, the active concentration of two commercial disinfectants, A and B, was evaluated and quantified in high-performance liquid chromatography (HPLC). Both disinfectants tested were associations of active ingredients based on glutaraldehyde at 42.5% and benzalkonium chloride at 7.5%. The methodology applied was adapted from Resolution - RE 899/03, validated by the Agência Nacional de Vigilância Sanitária (ANVISA). In the second stage, the

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action of the same disinfectants A and B was evaluated in SH and ST in the presence of 3% skimmed-milk powder to simulate the action of organic matter, in 15 min of contact, as per Ordinance 101 (BRAZIL, 1993). There is no specific legislation for disinfectants and the accepted values for variation of assets are referenced in the Technical Regulation for Stability Tests of Pharmaceutical Products for Veterinary Use, of the Normative Instruction (IN) 15 (BRAZIL, 2005). According to IN 15, the variation in assets can reach ± 5%. In this way, "product A" is in accordance with what appears on the label of the two active ingredients. The concentration of "product B" assets was 33.5% lower than the values indicated on the label, not complying with the determination of IN 15 (Table 1).

Table 1. Percentages of concentration of glutaraldehyde, benzalkonium chloride, variation contained on the label, and HPLC result of two commercial products.

Product	Glutaraldehyde concentration (%)			Benzalkonium chloride concentration (%)		
	Label	HPLC	Variation	Label	HPLC	Variation
A	42.5%	45.7%	+ 3.2%	7.5%	7.2%	−0.3%
B	42.5%	30.4%	−12.1%	7.5%	2.8%	−4.7%

+ =Value above specification. − = Value below specification.

In the second stage of the study, disinfectant "A" was efficient in all dilutions proposed in the test with 3% organic matter for SH and ST. The disinfectant "B" proved to be inefficient in the 1/3,000 dilution with 3% of organic matter for the matter in question. The results of the second stage are available in Table 2.

Table 2. Antimicrobial activity of disinfectants "A" and "B" against SH and ST in different dilutions, with 3% organic matter in 15 minutes of contact.

Dilution	Logarithmic reduction (log)	SH		ST	
		Product A	Product B	Product A	Product B
1:500	4 log	S	S	S	S
	5 log	S	S	S	S
1:1,000	4 log	S	S	S	S
	5 log	S	S	S	S
1:2,000	4 log	S	S	S	S
	5 log	S	S	S	S
1:3,000	4 log	S	R	S	R
	5 log	S	R	S	R

SH (*Salmonella* Heidelberg); ST (*Salmonella* Typhimurium); S – Sensitive: inoculum that showed a log reduction of 4 logs (99.99% reduction) or 5 logs (99.999% reduction) compared to the disinfectant and conditions tested; R – Resistant: inoculum that did not show a log reduction of 4 logs (99.99% reduction) or 5 logs (99.999% reduction) compared to the disinfectant and conditions tested.

Cleaning and disinfection of facilities is essential to reduce the risk of introduction and permanence of animal diseases and zoonoses (LUYCKX et al., 2015a). When selecting a disinfectant for the execution of a cleaning and disinfection protocol, it is essential to consider the specific characteristics of the active principle, the target microorganisms, and environmental issues, in addition to the health of the operators (DVORAK et al., 2008).

The factors that affect the action of disinfectants, inherent to product chemistry, application conditions in the field, and to microorganisms are well described (DAVIES, 2003; DVORAK et al., 2008; GREZZI, 2008; LUYCKX et al., 2015b; MAILLARD, 2013; RUTALA; WEBER, 2008). Some laboratory tests simulate possible conditions of use against specific microorganisms, substances that interfere with the action, and contact time for action (STANIFORTH, 2013).

MAILLARD (2013) says that organic matter compromises the action of disinfectants in three ways: (i) reduction of the available concentration of the disinfectant; (ii) protection of microorganisms against external damage; (iii) formation of microbial aggregates surrounded by a layer of exopolysaccharides. However, it is worth mentioning that the deficit in active ingredients of product "B" may have compromised the result.

In contrast to antimicrobials that act on specific sites, disinfectants generally act on the structure and function of various structural macromolecules, such as carbohydrates, lipids, nucleic acids, and various essential components that in combination form cell walls of bacteria, membranes and viral envelopes (MCDONNELL, 2007; NHUNG et al., 2015).

There is growing evidence that exposure to some disinfectants can induce cross-resistance with antimicrobial agents. This occurs mainly in situations of dilution errors, in sublethal doses, chemical degradation of the molecule or reaction with other organic or inorganic compounds. The main mechanism responsible for cross-resistance is mediated by efflux pumps found in Gram-negative bacteria (MAILLARD, 2013; NHUNG et al., 2015; SILVA et al., 2015).

According to NHUNG et al. (2015), 12 strains of enterobacteria, six *Escherichia coli* and six nontyphoidal *Salmonella*, analyzed before and after *in vitro* exposure to a commercial disinfectant based on glutaraldehyde and benzalkonium chloride, showed an increase in the minimum inhibitory concentration (MIC) ranging from 0 to 100% (median of 31%). MICs for some antimicrobials tested before and after exposure to disinfectant also increased. The biggest changes were for tetracycline with an average variation of 776%, followed by ciprofloxacin with 316% and chloramphenicol with 106%, which supports the theory of cross-resistance. Still in the same work, as strains were treated with a generic efflux pump inhibitor, phenyl-arginine beta-naphthylamide (PAβN) after adaptation and resulted in an average reduction of 18% of the MIC for the disinfectant. For antimicrobials, treatment with PAβN did not result in changes in the exception of chloramphenicol, with an average MIC reduction of 24%, concluding that only a small fraction of the resistance can be per generic efflux pump.

However, through the HPLC analysis, it was possible to verify that the disinfectant "B" had an active content lower than that described on the label and this difference may characterize manufacturing error and or low instability of the formulation. This deficit in the active principle compromised the antimicrobial activity against the studied bacteria, as shown in Table 2, and can result in inefficient disinfection and still induce bacterial resistance by exposing the same underdoses.

AUTHORS' CONTRIBUTIONS

Conceptualization: Frozza, R.; Brum, J.S. **Data curation:** Frozza, R.; Almeida, L.M; Brum, J.S. **Formal analysis:** Frozza, R.; Almeida, L.M; Brum, J.S. **Funding acquisition:** Frozza, R; Brum, J.S. **Investigation:** Frozza, R.; Almeida, L.M; Brum, J.S. **Methodology:** Frozza, R.; Almeida, L.M; Brum, J.S. **Project administration:** Frozza, R.; Brum, J.S. **Resources:** Frozza, R.; Almeida, L.M; Brum, J.S. **Supervision:** Frozza, R.; Brum, J.S. **Validation:** Frozza, R.; Brum, J.S. **Visualization:** Frozza, R.; Almeida, L.M; Brum, J.S. **Writing – original draft:** Frozza, R.; Almeida, L.M. **Writing – review & editing:** Frozza, R.; Almeida, L.M; Brum, J.S.

AVAILABILITY OF DATA AND MATERIAL

The datasets generated and analyzed during the current study are available in the Acervo Digital UFPR, persistent web link to datasets: https://acervodigital.ufpr.br/handle/1884/56198.

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CONFLICTS OF INTEREST

The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

ETHICAL APPROVAL

Not applicable.

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