Evaluation of the Resistance of *Salmonella enterica* subsp. *enterica* Isolated from Pigs to Biocides Used in the Agri-food Industry

Summary of Dissertation for the Degree of Master in Microbiology

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ABSTRACT

Combating the persistence of *Salmonella* in the abattoir's environment has become crucial through the implementation of cleaning and disinfection programmes, especially as their susceptibility to biocides can tend to decrease. The present study aimed to evaluate the resistance of *Salmonella enterica* isolates from pigs slaughtered in abattoirs to biocides used in the agri-food industry. Forty-four *Salmonella* isolates from slaughtered pigs were used to detect the presence of both efflux pump and quaternary ammonium compound (QAC) biocide resistance genes by PCR. Susceptibility to the biocides Suma Bac D10® (QAC-based formulation) and Mida FOAM 193® (chlorine-based formulation) at three levels of organic matter (absent, low and high) was assessed in twelve selected isolates. At all levels of organic matter, the susceptibility of the isolates to Suma Bac D10® remained 10x (0.1%) lower than the concentration indicated by the manufacturer (1-4%), in terms of minimum bactericidal and inhibitory concentrations. The presence of resistance genes to QACs did not seem to induce any changes in the susceptibility of the isolates to biocide at the studied formulation concentrations. As for Mida FOAM 193®, decreased susceptibility only occurred with the presence of organic matter at high levels and for biocide concentrations <2.5%, still 4x below the concentration recommended by the manufacturer (10%). Efflux pump genes were detected in all isolates, so the decrease in susceptibility observed for this biocide in the studied conditions could be linked to the barrier effect of organic matter. In conclusion, the findings obtained revealed the importance of using biocides at concentrations that effectively eliminate *Salmonella* spp. from contaminated surfaces, including the abattoir environment, since their misuse could potentially lead to the persistence of this bacterium.

Keywords

Salmonella enterica subsp. *enterica*; Chlorine-based biocide; QAC-based biocide; Resistance genes; Minimum Inhibitory Concentration; Minimum Bactericidal Concentration.

1. INTRODUCTION

Salmonella spp. is a gram-negative rod-shaped bacterium belonging to the family *Enterobacteriaceae*[1], containing only two species, *Salmonella enterica* and *Salmonella bongori*[2], the most prominent being *S. enterica* with over 2600 different serotypes identified[3].

S. enterica causes a variety of infections, the most common of which is salmonellosis caused by non-typhoidal *Salmonella* (NTS), making it a global public health concern. In 2019, the high incidence of

NTS-derived salmonellosis in the European Union (EU) has led to it being a major cause of foodborne outbreaks, especially by "pork and products thereof"[4].

In the abattoir, as more animals are together, the more difficult it is to prevent infection by *Salmonella* spp.[5]. This kind of scenario may be prevented if there was an effective application of good hygiene practices (GHPs) and the availability of hazard analysis and critical control point (HACCP) programmes[4] to reduce the contamination and persistence of *Salmonella* spp. in abattoirs environment[6].

When facing biocide resistance, one of the most significant systems is the AcrAB-TolC complex since the expression of *acrA*, *acrB* and *tolC* genes lead to biocide exit and contributes to multidrug resistance (MDR)[7]. As for resistance to QACs, several resistance genes have been identified in gram-negative bacteria, like *qacE*, *qacEΔ1*, *qacF*, *qacG* and *sugE* genes[8].

Therefore, this study aims to investigate the resistance of *Salmonella* isolates from pigs slaughtered in a Portuguese abattoir to commercial biocides, which involves assessing the susceptibility of *Salmonella* isolates to two biocides commonly used in the agri-food industry, such as Mida FOAM 193® (chlorine-based biocide) and Suma Bac D10® (QAC-based formulation), in terms of pheno- and genotypic characteristics. Further, to assess the presence of biocide associated resistance genes, namely *acrA*, *acrB*, *tolC*, *qacE*, *qacEΔ1*, *qacF*, *qacH* and *qacI,* by molecular biology methods and to evaluate the resistance and/or susceptibility to the aforementioned biocides determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). Lastly, all of the data collected will be compared to the findings of Cota et al. (2019)[6] research in terms of phenotypic and genotypic correlations, deepening the knowledge of how *Salmonella* is found in pigs and pork in a Portuguese abattoir.

2. METHODS

2.1. Bacterial Isolates

A collection of 44 *Salmonella* spp. isolates gathered from an abattoir in 2014 for Vanessa Silva's master's thesis was analysed. In addition, five control organisms were used in this study, specifically *S. enterica* subsp. *enterica* ser. Typhimurium CECT 443, *Escherichia coli* ATCC 10536, *Pseudomonas aeruginosa* ATCC 15442, *Staphylococcus aureus* ATCC 6538, and *Enterococcus hirae* ATCC 10541. Professor Manuela Oliveira (FMV-ULisboa) kindly provided these control samples.

2.2. Polymerase Chain Reaction (PCR) amplification

PCR amplification was conducted to detect genes encoding efflux pumps belonging to the AcrAB-TolC complex (*acrA*, *acrB* and *tolC*), and for QAC resistance genes (*qacEΔ1*, *qacE* and *qacF/H/I* complex). The primers for the amplification of *acrA*, *acrB* and *tolC* were designed from their sequences provided by GenBank and using the Primer3Plus software (https://primer3plus.com/) (Table 1).

Each PCR reaction had a total volume of 25 µL and consisted of 12.5 µL of NZYTaq II 2x Green Master Mix (Nzytech®, Lisbon, Portugal), 9.5 µL of nuclease-free water filtered with a 0.2 µm filter (Nalgene®, New York, USA), 1 µL of the forward primer (STAB VIDA, Lda., Caparica, Portugal) with a final concentration of 0.4 µM, 1 µL of the reverse primer (STAB VIDA, Lda., Caparica, Portugal) with a final concentration of 0.4 µM and 1 µL of DNA template from each isolate. Each PCR program had an initial denaturation of the DNA that occurred at 95 °C for 5 min and a final extension that occurred at 72 °C for 5 min. As for the other three steps, these occurred in 30 cycles, and each one has its one specificity.

After each PCR run, electrophoresis was performed in a 2% agarose gel stained with GreenSafe Premium® (Nzytech®, Lisbon, Portugal), at 90 volts (V), and the agarose gel was visualized by ChemiDoc XRS+ (BIO-RAD Laboratories, Inc., Algés, Portugal).

Table 1. List of primers sequences used to target genes encoding efflux pumps and QACs.

Primer	Sequence (5' → 3)	Product size (bp)	Accession number
***acrA*-FW**	TGGCAAATGGTTCGCTGAAA	220	MH933961.1
***acrA*-RV**	GGTTTTGTCCCTTCCTGCAG		
***acrB*-FW**	ACGTAATCAGTTGTTCGGCG	242	NC_003197.2
***acrB*-RV**	ATTTCGCTTCGGACATCACG		
***tolC*-FW**	CCGTACTGGCGAATGAAGTG	165	NC_003197.2
***tolC*-RV**	TTTCCGCTTCCTTCAACAGC		
***qacEΔ1*-FW**	AATCCATCCCTGTCGGTGTT	175	JN596280 JN566044
***qacEΔ1*-RV**	CGCAGCGACTTCCACGATGGGGAT		
***qacE*-FW**	AAGTAATCGCAACATCCG	258	X68232
qacE-RV	CTACTACACCACTAACTATGAG		
***qacF/H/I*-FW**	GTCGTCGCAACTTCCGCACTG	229	HQ875011 FJ160769 JN596279
***qacF/H/I*-RV**	TGCCAACGAACGCCCACA		

Legend: bp – base pair.

2.3. Determination of Minimal Inhibitory Concentrations (MICs) and Minimal Bactericidal Concentrations (MBCs) of bacterial isolates

To perform MIC and MBC, twelve out of forty-four isolates of *S. enterica* were selected. Four isolates were used as control strains, according to EN 1656:2009[9], such as *E. hirae* ATCC 10541, *S. aureus* ATCC 6538 *E. coli* ATCC 10536 and *P. aeruginosa* ATCC 15442.

Before beginning any experiment and concerning the biocide Suma Bac D10®, eight solutions were created with a starting concentration that allowed for final solutions of 5%, 4%, 3%, 2%, 1%, 0.5 %, 0.25 %, and 0.1 % (the recommended in-use concentration vary between 1 and 4 %). For Mida FOAM 193®, eight solutions were also created with an initial concentration that allowed for final solutions of 15%, 12.5 %, 10% (recommended concentration), 7.5 %, 5%, 2.5 %, 1%, and 0.5 %.

Since biocides have a contact time determined by the manufacturer, standard EN 1656:2009[9] indicated which neutralisers were most suitable to use according to the biocide required, to mimic this effect in the laboratory. For Suma Bac D10®, the neutralizer was composed of 30 g/L polysorbate 80 (Merck & Co., Inc., New Jersey, USA), 30 g/L saponin (Sigma-Aldrich, St. Louis, Missouri, USA) and 3 g/L lecithin (The British Drug Houses Ltd., London), applied after 5 minutes contact with the biocide. As for Mida FOAM 193®, its neutralizer was a mixture of 3 g/L of sodium thiosulfate (Merck & Co., Inc., New Jersey, USA), 30 g/L of polysorbate 80 (Merck & Co., Inc., New Jersey, USA) and 3 g/L of lecithin (The British Drug Houses Ltd., London), after a 5-minute contact time with the biocide. Before these experiments, both neutralizers were subjected to optimization essays and controls to validate the neutralization protocol and to confirm the inexistence of toxicity to the bacterial suspension (data not shown).

Since organic matter was on all surfaces in the abattoir, it was replicated by employing low and high levels of interfering substances (LIS and HIS, respectively), which both were prepared using EN 1656:2009[9]. The LIS solution was prepared by dissolving 3 g of bovine albumin fraction V (AppliChem®, Darmstadt, Germany) in 100 mL of water, which was sterilized by a sterile syringe filter of 0.2 µm (Nalgene®, New York, USA). The HIS solution was prepared with the dissolution of 50 g of yeast extract (Oxoid, Ltd., Hampshire, England) in 250 mL of water, which was sterilized by autoclave (120 °C, 20

min) and cooled until reached 20 °C ± 1 °C. In another container, 5 g of albumin was dissolved in 25 mL of water and sterilized by a sterile syringe filter of 0.2 µm (Nalgene®, New York, USA). To this last solution, 25 mL of the previous suspension of yeast extract was added.

Because the protocol for creating MICs for Suma Bac D10® and Mida FOAM 193® is based on EN 1656:2009[9], only the particular elements differ, like the biocide and the neutralizer solution. The first stage was to prepare all of the 96-wells plates (VWR International®, Leuven, Belgium). Only the wells of columns 5 to 12 in the first plate ("Plate 1") were filled with 160 µL of biocide (Suma Bac D10® or Mida FOAM 193®), at the eight distinct concentrations previously mentioned. Columns 1 to 4 were left blank. The wells in columns 1 to 4 on the second plate ("Plate 2") were left empty, while the rest of the columns 5 to 12 were filled with 160 µL of neutralizer (each specifically for Suma Bac D10® and for Mida FOAM 193®), and 20 µL of pure sterile water. Finally, for the third plate ("Plate 3"), column 1 was filled with negative control to demonstrate that no contamination occurred in the Tryptone Soya Broth (TSB) (Oxoid, Ltd., Hampshire, England) medium employed, thus it was only filled with 200 µL of liquid TSB. Column 3's wells were filled with 180 µL of liquid TSB as a positive control to check that bacteria were present in the original suspension. Finally, columns 5 to 12 were employed for MIC testing, therefore, they were filled with 180 µL of liquid TSB. Columns 2 and 4 have been left empty.

With all plates prepared, all bacterial suspensions of the isolates were prepared in a diluent solution at a concentration corresponding to 0.5 on the McFarland scale (~1.5×10^8 CFU/mL). These suspensions were prepared from 24 h bacterial cultures grown on BHI (VWR® International, Leuven, Belgium) agar medium. All MIC tests were performed at room temperature (~20 °C). Positive controls in Plate 3 were first filled with 20 µL of this bacterial suspension.

Since there were three types of conditions in test, it was needed to adapt the volumes of the wells in Plate 1. When evaluating MICs without interfering substances, 20 µL of sterile water were added and when testing with low or high interfering substances 20 µL of LIS or of HIS were added to the wells.

In columns 5 to 12, the assays were performed in duplicate (A and B correspond to isolate 1, C and D to isolate 2, and so on), so 20 µL of bacterial suspension were added to each of the wells in Plate 1, which previously contained the mixture of biocide with the sterile water or with LIS or HIS, depending on which condition it was tested. After the wells in Plate 1 were filled, it was incubated for the contact time of 5 min ± 10 s for both biocides at room temperature, during which time it was stirred at 700 rotations per minute (rpm).

From the suspension of Plate 1, 20 µL were transferred to Plate 2. Following, it was necessary to incubate at room temperature for both neutralizers at 5 min ± 10 s, with a stirring of 700 rpm.

After Plate 2 finished its stirring time, 20 µL of suspension were removed and transferred to Plate 3, resulting in a mixture of 180 µL of liquid TSB with 20 µL of suspension (a mixture of biocide, neutralising substance, purified sterile water or with LIS/HIS, and bacterial suspension), and incubated for 24 h at 37 °C.

After 24 hours, it was possible to evaluate in which wells cell multiplication may have occurred. The MIC is the minimum concentration of Suma Bac D10® or of Mida FOAM 193® at which it was possible to visually verify that bacterial multiplication was inhibited.

Once the MICs had been determined, the values associated with the MBC were also determined. To did so, 5 µL were taken from the wells where no bacterial multiplication had been observed and transferred onto TSA plates for 24 hours at 37 °C to assess colony formation.

2.4. Data analysis

All the data analysis and the graphs shown in this study were performed using Microsoft Excel® (Microsoft Corporation®, Washington, USA).

3. RESULTS

3.1. Determination of MICs and MBCs of bacterial isolates

Table 2 shows the average values obtained for MIC and MBC for the 12 isolates of *S. enterica* for the biocides Suma Bac D10® and Mida FOAM 193®.

Table 2. Suma Bac D10® and Mida FOAM 193® medium values of MICs and MBCs for all *S. enterica* subsp. *enterica* isolates tested, divided between assays with no interfering substance (NIS), low interfering substance (LIS) and high interfering substance (HIS) (percentage, %).

Sample code	Suma Bac D10®						Mida FOAM 193®					
	MIC (%)			MBC (%)			MIC (%)			MBC (%)		
	NIS	LIS	HIS	NIS	LIS	HIS	NIS	LIS	HIS	NIS	LIS	HIS
ce37	<0.100	<0.100	<0.100	0.100	0.100	0.100	<0.500	0.750	<2.500	0.500	0.750	2.500
ci21	<0.100	<0.100	<0.100	0.100	0.100	0.100	<0.500	<0.500	<2.500	0.500	0.500	2.500
p64	<0.100	<0.100	<0.100	0.100	0.100	0.100	<0.500	<0.500	<2.500	0.500	0.500	2.500
ce21	<0.100	<0.100	<0.100	0.100	0.100	0.100	<0.500	<0.500	<2.500	0.500	0.500	2.500
p1	<0.100	<0.100	<0.100	0.100	0.100	0.100	<0.500	<0.500	<1.000	0.500	0.500	1.000
ci38	<0.100	<0.100	<0.100	0.100	0.100	0.100	<0.500	<0.500	0.500	0.500	0.500	0.750
p55	<0.100	<0.100	<0.100	0.100	0.100	0.100	<0.500	<0.500	<1.000	0.500	0.500	1.000
p56	<0.100	<0.100	<0.100	0.100	0.100	0.100	<0.500	<0.500	<1.000	0.500	0.500	1.000
ci104	<0.100	<0.100	<0.100	0.100	0.100	0.100	<0.500	<0.500	<2.500	0.500	0.500	2.500
ci117	<0.100	<0.100	<0.100	0.100	0.100	0.100	<0.500	<0.500	0.500	0.500	0.500	1.000
p109	<0.100	<0.100	<0.100	0.100	0.100	0.100	<0.500	<0.500	<1.000	0.500	0.500	1.000
ce70	<0.100	<0.100	<0.100	0.100	0.100	0.100	<0.500	<0.500	1.000	0.500	0.500	1.750
$\bar{x}$	0.100	0.100	0.100	0.100	0.100	0.100	0.500	0.521	1.542	0.500	0.521	1.667
σ	0	0	0	0	0	0	0	0.069	0.828	0	0.069	0.738

Legend: MIC – Minimum Inhibitory Concentration; MBC – Minimum Bactericidal Concentration; $\bar{x}$ – medium MIC and MBC value for each group; σ – corresponding standard deviation of each population; “p” – isolates that came from the skin before scalding; “ci” – isolates that came from the internal part of the carcass; “ce” – isolates that came from the external part of the carcass.

Regarding the Suma Bac D10® biocide, in the assays NIS, LIS and HIS, it was verified that for all isolates, the MIC value obtained was the lowest of the concentrations studied (0.100 %). For the same three conditions, as the lowest concentration tested (0.100 %) had a bactericidal effect (MBC).

As for the MIC values for the biocide Mida FOAM 193®, in the NIS assay, the results obtained were the lowest concentration used for this biocide (0.500 %) in all isolates. In the case of the value obtained for MBC, in the same condition (NIS), there were no changes compared to the MIC values (0.500 %). In the LIS assay, the result for isolate ce37 changed in comparison with the other isolates, presenting a MIC value 1.5 times higher (0.750%) than the others, which remained at concentrations of 0.500%. As for the MBCs under these conditions, the same results were observed, with 0.750 % for the isolate ce37 and 0.500 % for the others. Finally, for the HIS assays, the MIC values were variable. The lowest observable MIC value corresponded to the isolates ci38 and ci117 with a value of 0.500 %, while isolates p1, p55, p56, p109 and ce70 had a value of 1.000 % and, finally, ce37, ci21, p64, ce21 and ci104

presented a value of 2.500 %. Concerning the MBC values for this assay, the results also varied depending on the isolates. The isolates ci38 and ci117 had an MBC value greater than the MIC value, increasing 1.5 times (0.750 %) and 2 times (1.000 %), respectively, as did the isolate ce70 which increased the MBC value 1.75 times compared to the MIC value (1.750 %). The remaining isolates, ce37, ci21, p64, ce21, p1, p55, p56, ci104 and p109, maintained the same MIC values in MBCs.

3.2. Relationship between biocide susceptibility genotypes and phenotypes determined by MBCs

Table 3 gather the genotypic information previously determined by PCR for the 12 isolates of *S. enterica* subsp. *enterica* selected together with the mean values determined for the MBCs of the biocides Suma Bac D10® and Mida FOAM 193®.

Table 3. Association between the genotypic sequences determined by PCR for 12 isolates of S. *enterica* subsp. *enterica* and the medium values determined for the MBCs of Suma Bac D10® and Mida FOAM 193®, under the conditions of no interfering substance (NIS), low interfering substance (LIS) and high interfering substance (HIS) (percentage, %).

Sample code	Genotype sequences		Suma Bac D10®			Mida FOAM 193®		
			MBC (%)			MBC (%)		
	Efflux pump genes	QAC resistance genes	NIS	LIS	HIS	NIS	LIS	HIS
ce37	acrA/acrB/tolC	*qacEΔ1/qacF/H/I*	0.100	0.100	0.100	0.500	0.750	2.500
ci21	*acrA/acrB/tolC*	*qacEΔ1/qacF/H/I*	0.100	0.100	0.100	0.500	0.500	2.500
p64	*acrA/acrB/tolC*	*qacEΔ1/qacF/H/I*	0.100	0.100	0.100	0.500	0.500	2.500
ce21	*acrA/acrB/tolC*	*qacEΔ1/qacF/H/I*	0.100	0.100	0.100	0.500	0.500	2.500
p1	*acrA/acrB/tolC*	–	0.100	0.100	0.100	0.500	0.500	1.000
ci38	*acrA/acrB/tolC*	*qacF/H/I*	0.100	0.100	0.100	0.500	0.500	0.750
p55	*acrA/acrB/tolC*	*qacEΔ1*	0.100	0.100	0.100	0.500	0.500	1.000
p56	*acrA/acrB/tolC*	–	0.100	0.100	0.100	0.500	0.500	1.000
ci104	*acrA/acrB/tolC*	*qacEΔ1/qacF/H/I*	0.100	0.100	0.100	0.500	0.500	2.500
ci117	*acrA/acrB/tolC*	*qacEΔ1*	0.100	0.100	0.100	0.500	0.500	1.000
p109	*acrA/acrB/tolC*	*qacEΔ1/qacF/H/I*	0.100	0.100	0.100	0.500	0.500	1.000
ce70	*acrA/acrB/tolC*	*qacEΔ1*	0.100	0.100	0.100	0.500	0.500	1.750

Legend: MBC – Minimum Bactericidal Concentration; "p" – isolates that came from the skin before scalding; "ci" – isolates that came from the internal part of the carcass; "ce" – isolates that came from the external part of the carcass.

For the 12 isolates screened for susceptibility to the Suma Bac D10® biocide and whose susceptibility can be related to the *qac* genes, there were different genotypes present. Six of these 12 isolates contained the *qacEΔ1/qacF/H/I* genotype (ce37, ci21, p64, ce21, ci104 and p109), and three contained only the genotype associated with the *qacEΔ1* gene (p55, ci117 and ce70) and one contained only the genotype associated with the *qacF/H/I* gene complex (ci38). QAC resistance genes were not detected in isolates p1 and p56. Independently of the genotype determined for these isolates, it could be verified that the mean values determined for the MBCs, in the three conditions (NIS, LIS and HIS), did not change, maintaining the lowest value of biocide concentration (0.100 %).

The same 12 isolates tested for susceptibility to the biocide Mida FOAM 193®, which decreased susceptibility could be associated with the genes belonging to the AcrAB-TolC complex, all contained

the genotype *acrA/acrB/tolC*. However, the mean MBC values determined for the three conditions varied. In the NIS and LIS conditions, the MBC values remained at 0.500 %, which was the lowest concentration of biocide tested, except for isolate ce37 in which the MBC value was 0.750 % in the LIS condition. In the HIS condition, the mean values of associated MBCs ranged between 0.750 % and 2.500 %, being higher than in the other previous conditions.

4. DISCUSSION

The control of pathogens, such as *Salmonella* spp., within abattoirs, is of supreme importance, and this is only possible through good hygiene practices[10]. To this end, the use of biocides becomes an essential part of this process, as these are important for the reduction or elimination of pathogens[11], particularly in food processing environments such as slaughterhouses.

For the three tested conditions of organic matter, NIS, LIS and HIS, concerning the biocide Suma Bac D10®, the *S. enterica* isolates tested showed high susceptibility to that biocide. This was indicated by the MIC and MBC medium values remaining unchanged throughout the three assays at the lowest tested concentration of Suma Bac D10® (0.100 %), as can be seen in Table 2. Thus, it did not appear that as organic matter increases the susceptibility of the isolates to the biocide decreases since the results remained constant throughout the experiments.

One of the possible reasons for obtaining these results in the Suma Bac D10® biocide assays is related to the fact that the *S. enterica* isolates studied, being gram-negative bacteria, contain a layer of lipopolysaccharides on the outside of their plasma membrane[12–15]. This can lead to a higher susceptibility to Suma Bac D10®, because it contributes for the disintegration of the plasma membrane through its interaction with it, resulting in the escape of the entire cytoplasmic contents of *S. enterica* to the exterior[16]. Another reason is associated with the presence of the *qacEΔ1* gene and the *qacF/H/I* gene complex), since 10 out of 12 *S. enterica* isolates, either alone or in combination, did not seem to influence the susceptibility of the isolates to the tested biocide formulation. However, contrary to the results obtained in the present study, other studies indicate that resistance genes to QACs seemed to suggest that their presence could lead to altered susceptibility or even resistance to QAC-based formulations[8,17], such as the biocide Suma Bac D10®.

Similarly, the Mida FOAM 193® assay was made in the same three conditions of organic matter, to verify if there is low or high susceptibility to this biocide (Table 2). In the assay with HIS, it was found that organic matter may influence the results obtained in MICs and MBCs[18,19], since there was lower susceptibility to the biocide tested at concentration values <2.500 %, and on average the MIC value was relatively lower than the MBC value, with 1.542 % and 1.667 % respectively (Table 2). These results indicated that there is a possible reduction in biocide efficacy in the presence of organic matter, since several factors can contribute to the strong influence of organic matter, like the formation of a protective barrier around the bacteria, the formation of bacteria aggregates and the neutralization of the biocide, reducing its availability in the environment[20–23]. Furthermore, as it was possible to verify through the results obtained with HIS, in which only with concentrations higher than 2.500 % of Mida FOAM 193®, there was a lower susceptibility of the *S. enterica* isolates tested, in which the higher the concentration of chlorine-based biocide the more likely it was to act as an antimicrobial, because it affected the membranes of bacteria, caused damage at the DNA level, inhibited synthesizing proteins, oxidized

respiratory components of cells or even acted several of these factors at the same time[24]. Another aspect to consider is related to the presence of possible biocide resistance genes, linked to efflux pumps, such as those of the AcrAB-TolC complex. In Table 3, it can be seen that all 12 isolates tested contained the genotype *acrA/acrB/tolC*, thus indicating that it contained the efflux pumps of the AcrAB-TolC complex, which was indicative that these genes were constitutively expressed and its synthesised proteins were part of the plasma membrane itself[25]. This efflux pump mechanism has as its main objective to pump harmful components out of the cells, even if these components are not specific[26], as is Mida FOAM 193® case. When *Salmonella* is exposed to a biocide formulation an increase in *ramA* expression occurs. As the RamA protein is responsible for activating *acrAB* and *tolC*, then when its production increases there is an increase in the expression of the efflux pumps of the AcrAB-TolC complex, leading to *Salmonella* being MDR[7,27–29].

5. CONCLUSIONS

Both Suma Bac D10® (QAC-based formulation) and Mida FOAM 193® (chlorine-based formulation) appeared to be efficient in the elimination of the *S. enterica* isolates tested that were previously collected from pig carcasses in an abattoir, and no phenotypic resistances were detected. From the genotypes determined and associated with a susceptibility study to the biocide Suma Bac D10®, it was found that the presence of the *qacEΔ1* genes and the *qacF/H/I* gene complex did not appear to produce phenotypic effects on the *S. enterica* isolates tested when exposed to the previously indicated formulation under the three conditions studied in the assays (NIS, LIS and HIS), since there were no changes in the results throughout the assays concerning this biocide. The three levels of organic matter tested (NIS, LIS and HIS) did not influence the susceptibility of *S. enterica* isolates to the biocide Suma Bac D10®, maintaining the same bacterial concentration throughout the experiments, remaining below the concentration range of 1 – 4 % recommended by the manufacturer. In the case of the biocide Mida FOAM 193®, there was a lower susceptibility to the biocide on the isolates tested in the assay with high organic matter, verified by the increase of the MBC values in this assay, even though it remained below the concentration of 10% recommended by the manufacturer. In conclusion, these findings revealed that the use of the biocides Suma Bac D10® and Mida FOAM 193® in disinfection programmes and associated with good hygiene practices could help preventing the proliferation of *S. enterica* in the abattoir environment.

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