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Multiresistance and virulence factors of *Staphylococcus aureus* isolated from pigs

[*Multirresistência e fatores de virulência de* Staphylococcus aureus *isolados de suínos*]

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ABSTRACT

The emergence of livestock-associated methicillin-resistant *Staphylococcus aureus* strains (LA-MRSA) and the potential role of pigs in the evolution of these strains has led to increased interest in research of these microorganisms. However, this has contributed to a lack of research in the isolation and characterization of methicillin-susceptible *S. aureus* strains (MSSA). In this study, the prevalence of *S. aureus* in pigs in the nursery and finishing stages were analyzed. The susceptibility profiles to antibiotics, tolerance to heavy metals, and biofilm production of the isolates were evaluated using phenotypic and genotypic techniques. A total of 1,250 colonies suggestive of *Staphylococcus* spp. were isolated from 128 pigs, of which 63.6% (n = 795) belonged to this microbial genus. Sixty-seven colonies isolated from 34 animals (26.5%) were confirmed as *S. aureus* (8.4%). No strains resistant to copper, zinc, or methicillin were detected; however, all strains presented a resistance profile to at least three different classes of antimicrobials and 21 produced biofilms. These data are of concern, as they indicate the need for increased surveillance in the use of antimicrobials as well as reinforce the importance of studies on MSSA strains.

Keywords: multidrug resistance; MSSA, pig farming, biofilm

RESUMO

A emergência de cepas de Staphylococcus aureus *resistentes à meticilina associadas à pecuária (LA-MRSA) e o papel potencial dos suínos na evolução dessas cepas têm levado ao aumento do interesse na pesquisa desses microrganismos. No entanto, isso tem contribuído para a falta de estudos sobre o isolamento e a caracterização de cepas de* S. aureus *sensíveis à meticilina (MSSA). Neste estudo, foi analisada a prevalência de* S. aureus *em suínos nas fases de creche e terminação. Os perfis de suscetibilidade aos antibióticos, a tolerância a metais pesados e a produção de biofilme dos isolados foram avaliados por meio de técnicas fenotípicas e genotípicas. Um total de 1.250 colônias sugestivas de* Staphylococcus *spp. foi isolado de 128 suínos, das quais 63,6% (n = 795) pertenciam a esse gênero microbiano. Sessenta e sete colônias isoladas de 34 animais (26,5%) foram confirmadas como* S. aureus *(8,4%). Nenhuma cepa resistente ao cobre, ao zinco ou à meticilina foi detectada; entretanto, todas as cepas apresentaram perfil de resistência a pelo menos três classes diferentes de antimicrobianos e 21 produziam biofilme. Esses dados são preocupantes, pois indicam a necessidade de maior vigilância no uso de antimicrobianos, bem como reforçam a importância de estudos com cepas de MSSA.*

Palavras-chave: multidroga resistente, MSSA, suinocultura, biofilme

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INTRODUCTION

The *Staphylococcus* genus comprises more than 50 species described as commensal microorganisms of mammals and birds, and/or opportunists that cause various clinical manifestations (Schleifer and Bell, 2015). Several species have significant importance in human and veterinary medicine; however, *Staphylococcus aureus* is especially distinguished from the others. The presence of this microorganism colonizing different ecological niches of the human body and diverse farm, companion, or wild animals varies among studies (Haag *et al.*, 2019).

Such adaptability is also reflected in the number of different infectious clinical conditions that *S. aureus* can cause in humans and animals; these include simple infections, such as those located in the skin, and severe and disseminated diseases, such as bacteremia, endocarditis, pneumonia, meningitis, osteomyelitis, mastitis, arthritis, and toxemia, including food-borne staphylococcal intoxication (Schmidt *et al.*, 2015). This ability is due to the surprising genomic plasticity of this microorganism to acquire and express different mechanisms of virulence. This is represented by adhesins and enzymes, which act as aggressins, invasins, and modulins in the immune response (Schmidt *et al.*, 2015; Dweba *et al.*, 2018).

The resistance of *S. aureus* strains to antimicrobials, disinfectants, heavy metals, dyes, and other substances also characterizes the diversity of genetic determinants of resistance that this bacterium can possess and spread. These attributes contributed significantly to the adaptation, dissemination, and evolution of this microorganism. Antibiotic resistance is one of the most important public health concerns worldwide due to the emergence of more resistant strains, which have survived each new antibiotic used in therapy (Jensen and Lyon, 2009; Argudín *et al*., 2017; Lakhundi and Zhang, 2018).

Among the different resistance mechanisms acquired by *S. aureus*, the mobile genetic element staphylococcal cassette chromosome *mec* (SCC*mec*), which contains the *mec*A or *mec*C genes and confers resistance to all beta-lactam antimicrobials, is the most important. Its acquisition has revolutionized the epidemiology of this microorganism worldwide. Strains of this species that harbor this resistance determinant are referred to as methicillin-resistant *Staphylococcus aureus* (MRSA). From the 1960s to the present, the epidemiology of MRSA has drastically changed, and since 2005, LA-MRSA (livestock-associated methicillin-resistant *S. aureus*) has emerged in a wide variety of animal species, causing colonization and/or infections that also affect humans (Dweba *et al*., 2018; Lakhundi and Zhang, 2018; Haag *et al.*, 2019).

During the last 30 years, most studies on *S. aureus* have focused on the resistance profiles, virulence, and genomic characterizations of MRSA strains (Crombé *et al*., 2013). However, this approach has contributed to a lack of knowledge about changes in the susceptibility and virulence profiles of *S. aureus* strains that do not present this resistance phenotype: the so-called methicillin-susceptible *S. aureus* (MSSA). MSSA strains may harbor other resistance mechanisms that also limit the success of therapy, whether in human or veterinary medicine. These may also express virulence attributes, such as toxins, enzymes, and biofilm production, which are important for either colonization or development of infections (Soares *et al*., 1997; Buyukcangaz *et al*., 2013; Yılmaz and Aslantas, 2017).

Among farm animals, swine have been indicated as important reservoirs for the evolution of *S. aureus* strains, including LA-MRSA ST398 (Masson *et al*., 2012; Schmidt *et al.*, 2015). However, before the recognition of pigs and other production animals as reservoirs of MRSA strains, little interest has been shown regarding *S. aureus* in these animals. There are relatively few studies investigating the susceptibility profile and/or virulence factors of MSSA strains isolated from this species (Linhares *et al*., 2015). Due to the limited surveillance of MSSA and their potential as reservoirs for the emergence of MRSA through SCC*mec* introduction, the present work sought to analyze the epidemiology of MSSA strains by evaluating the presence of this microorganism in pigs raised in small farms that supply the consumer market from Teresina, Piauí, state located in the Northeast of Brazil. The MSSA strains identified were characterized on the basis of their resistance to antibiotics, tolerance to heavy metals, and the presence of virulence factors, such as biofilm production.

MATERIAL AND METHODS

In this study, a total of 128 pigs were randomly selected from three full-cycle farms that supply the consumer market of Teresina-PI. Eighty-two pigs were in the nursery phase (between six and eight weeks of life; approximately 15kg) and 46 were in the finisher phase (between 11 and 12 weeks of life; approximately 70kg). Clinical specimens were collected from the nasal cavity and anal region of each animal with the aid of sterilized swabs (256 samples total). All collections were carried out according to the ethical principles in animal experimentation and were approved by the Animal Experimentation Ethics Committee of the Federal University of Piauí (AEEC/UFPI), on January 6, 2016 under protocol no. 115/2015.

The nasal and anal swabs were placed in sterile test tubes containing 2.0mL TNaCl enrichment broth (10.0g tryptone/L, 75g NaCl/L, 10g mannitol/L, and 2.5g of yeast extract/L; pH 7.2 ± 0.2) and incubated at 35 ± 2 ºC in a bacteriological incubator for 24 h. Aliquots of the cultures were streaked on mannitol salt agar (Difco™, BD, USA) and incubated at 35 ± 2 °C for 48-72 h in aerobiosis. Based on macroscopic morphological characteristics and mannitol fermentation, on average, five colonies suggestive of *Staphylococcus* spp. per sample were subjected to phenotypic identification according to the protocols described by Markey *et al.* (2013). The genus, species, and presence of genes related to methicillin resistance and biofilm production were confirmed by polymerase chain reaction (PCR).

Total DNA from *S. aureus* isolates was extracted by boiling the samples in 200µL of TE buffer (10mM Tris HCl, 1 mM EDTA, pH 8). The oligonucleotides used were synthesized by Exxtend Biotecnologia LTDA (Brazil). The PCR assay targeting 16S rRNA (*Staphylococcus g*enus-specific), *nuc* (*S. aureus* species-specific), and *mec*A (a determinant of methicillin resistance) genes followed the procedures described by Zhang *et al.* (2004). For amplification of *ica*A and *ica*D genes (determinants of biofilm production), PCR was performed according to Arciola *et al*. (2001) and Mariana *et al.* (2009). Antimicrobial susceptibility tests were performed using the disc diffusion method according to the M2 document of the Clinical and Laboratory Standards Institute (CLSI, 2018a). The impregnated antibiotic discs (CEFAR, Brazil) used included: penicillin G (10 U), trimethoprim-sulfamethoxazole (25 µg), tetracycline (30 µg), tigecycline (15 µg), linezolid (30 µg), enrofloxacin (5 µg), tilmicosin (15 µg), ceftiofur (30 µg), and gentamycin (10 µg).

To determine the inducible clindamycin resistance phenotype, the D-test was carried out using erythromycin (15 µg) and clindamycin discs (2 µg). Oxacillin (1 µg) and cefoxitin (30 µg) disks were used to detect methicillin-resistant *Staphylococcus aureus* (MRSA). The susceptibility to glycopeptides was assessed using the vancomycin agar screen test as standardized by CLSI (2018a). Standard strains were included in each trial as controls for quality assessment of antimicrobial susceptibility. After incubation at 35 ± 2 °C for 24 h, susceptibility of the *S. aureus* isolates to each antimicrobial agent was assessed, and the results were interpreted in accordance with the standard criteria VET08 (CLSI, 2018b) and M100-S29 (CLSI, 2019).

To confirm methicillin-resistant strains of *S. aureus* (MRSA) and identify possible strains that heterogeneously expressed resistance to semi-synthetic beta-lactams, a screening test for methicillin resistance was performed using Mueller-Hinton agar supplemented with 25µg/mL this antibiotic (AMet test) as described by Soares *et al.* (1997). Biofilm formation was detected using the adhesion test in 96-well microplates with *S. aureus* isolates in logarithmic growth phase in TSB broth (100µL) supplemented with 0.5% glucose, according to Stepanovic *et al*. (2000) with modifications. The microplates were incubated at 35 ± 2 °C for 24 h under aerobic conditions. After incubation, the wells were washed three times with sterile distilled water. The biofilm formed was fixed with 100µL of methanol (PA) for 15 min, stained with a 0.1% crystal violet solution (v/v) for 5 min, and rinsed with sterile distilled water until complete removal dye from the negative controls (wells without bacterial inoculum). The dye was resuspended in 100µL of ethanol (95.0%), and the plates were spectrophotometrically read at 492nm. *Staphylococcus epidermidis* 70D was used as a positive control strain.

The optical density (OD) was calculated from the average of four readings for each bacterial isolate. Samples were considered as being biofilm producers when the average OD reading for each isolate was higher than the cut-off point (ODc) as defined by the following formula: $ODc = [ANC + 3s]$, where ANC is the average OD of the negative control and *s* is the standard deviation of the readings of the negative control. The bacteria were classified as strong (OD ≥ 4 ODc), moderate (2 ODc ≤ OD≤ 4 ODc), weak (ODc ≤ OD ≤ 2 ODc), or non-biofilm producers (OD ≤ ODc). The tolerance to heavy metals was determined using the Mueller-Hinton agar dilution method, with concentrations ranging from 0.25–4.0 mM of zinc chloride (final pH 5.5 ± 0.2) and copper sulfate (pH 7.0 ± 0.2), following Aarestrup and Hasman (2004).

The plates were incubated for 20 h at 37°C in an aerobic atmosphere. The minimal inhibitory concentration (MIC) was defined as the lowest concentration at which there was no visible growth. Statistical analysis was performed with R 3.6.0 software, using the chi-square of proportions test and the *odds ratio* (OR) as a measure of association, with a confidence interval of 95%, and significance level when P<0.05.

RESULTS AND DISCUSSION

A total of 1,250 colonies were isolated from 128 animals, of which 63.6% (*n* = 795) were identified with the genus *Staphylococcus* (Table 1). Among the animals tested, 126 (98.5%) had *Staphylococcus* spp. colonizing the assessed anatomical sites and 34 (26.5%) were carriers of the *S. aureus* species.

Table 1. Distribution of the strains of *Staphylococcus* spp. by growth phase age and collection site isolated from swine

Isolated colonies	Nursery		Finisher		
	Anal Nº (%)	Nasal Nº (%)	Anal Nº (%)	Nasal Nº (%)	Total Nº (%)
Non-aureus Staphylococcus	209 (26.3)	313 (39.4)	59 (7.4)	147 (18.5)	728 (91.6)
Staphylococcus aureus	6 (0.7)	23 (2.9)	7 (0.9)	31 (3.9)	67 (8.4)
Total	215	336	66	178	795

The high prevalence of this bacterial genus in the animals evaluated in this study corroborates that *Staphylococcus* spp. constitutes part of the normal microbiota of pigs (Linhares *et al*., 2015). The high isolation frequency of coagulase-negative *non-aureus Staphylococcus* strains was an expected result, as these microorganisms are part of the normal microbiota of these anatomical sites. This result was also observed in other studies on nasal colonization of this agent in pigs (Masson *et al*., 2012; Linhares *et al*., 2015).

The fact that *S. aureus* is frequently considered a member of the microbiota of mammals and classified as an opportunist pathogen, owing to its ecological importance, has resulted in only a few studies seeking to analyze the prevalence of this microorganism as normal microbiota in these monogastric animals. This makes it difficult to compare the data on the presence of these bacteria. The great majority of research has been dedicated to the isolation and identification of MRSA strains, neglecting the simple epidemiology of non-MRSA strains (Crombé *et al*., 2013; Linhares *et al*., 2015).

Based on phenotypic and genotypic assays, 67 isolates from 34 animals (26.5%) were identified as *S. aureus* (Table 2). Studies on *S. aureus* colonization conducted by Buyukcangaz *et al*. (2013), Linhares *et al*. (2015), and Zehra *et al.* (2017) reported a higher prevalence of pigs colonized by this microorganism as compared to the present work. In contrast, other studies have reported isolation rates of *S. aureus* strains similar to or even lower than that observed in this study (Fall *et al*., 2012; Guo *et al*., 2018).

Table 2. Phenotypic and genotypic characterization of *S. aureus* isolated from pigs regarding the determinants of virulence and methicillin-resistance

Samples	Gene identification			Biofilm production						
				Microplates			*ica*A/*ica*D			
	16S rRNA	*nuc*	*mec*A	NP/WP	MO	S	-/-	+/-	-/+	+/+
Nursery	29	29	0	24	5	0	0	2	1	26
Finisher	38	38	0	22	16	0	0	0	0	38
Total	67	67	0	46	21	0	0	2	1	64

NP/WP: no production/weak; MO: moderate; S: Strong; +: Positive; -: Negative.

The proportion of animal carriers of *S. aureus* varied significantly between the anatomical sites ($X^2 = 19.75$; $P<0.0001$), with a greater probability of involvement of the nasal cavity (OR = 6.5). The variation between ages was also significant ($X^2 = 5.814$; $P<0.0159$), with a greater probability of animals in the finisher phase being affected (OR=0.38). The prevalence of pigs colonized by *S. aureus* was 19.5% (*n* = 16) for nursery animals and 39.1% (*n* = 18) in finisher phase animals. Regarding the anatomical site, 24.2% (*n* = 31) of the animals were found to have this isolate in the nasal cavities and 4.7% (*n* = 6) in the anal region. The data obtained corroborate those described by Linhares *et al*. (2015), although their frequencies of isolation were higher. It is important to note that the present study was conducted at a small number of farms, and there was evidence of variation between locations. Studies on the prevalence of colonization are often influenced by environmental factors, such as animal density, ventilation rates used on the premises, time of year, and hygiene-sanitary management (Linhares *et al*., 2015).

Both *ica*A and *ica*D genes were found to be present in 95.5% (n = 64) of the *S. aureus* isolates. These data did not correlate with the results found in the adhesion assay, as there was only moderate adhesion in 21 strains (31.3%). Five strains were classified as non-biofilm producers in the phenotypic test; however, they were shown to be carriers of the two genes tested. Other studies have also demonstrated inconsistency between the phenotypic and genotypic results. Such divergence may be related to the phenotypic tests used, which may be influenced by various factors, including the composition of the culture medium and/or the type of microplate polymer (Arciola *et al*., 2012).

Screening tests for the detection of MRSA strains in methicillin agar did not show growth for any of the isolated *S. aureus* strains. Corroborating susceptibility and the specificity of this screening test, the presence of the *mec*A gene was also not detected in the samples (Table 2). Similar results for the absence of MRSA strains have been described by Buyukcangaz *et al*. (2013), Linhares *et al*. (2015), and Zehra *et al*. (2017), who researched the nasal swine microbiota. In contrast, other studies carried out in swine herds in Brazil reported the isolation of MRSA strains, including LA-MRSA ST398 strains, ranging from 5.1% to 68% (Takeuti *et al*., 2016; Dutra, 2017).

In order not to overestimate the results due to the possibility of clonal strains within the same animal, a unique isolate was randomly selected by individual and subjected to antimicrobial susceptibility tests, where the susceptibility profile of the 34 strains of *S. aureus* is presented in Table 3. It was observed that all the strains presented total susceptibility to the antimicrobials oxacillin and cefoxitin, corroborating the results presented in Table 2 (absence of the *mec*A gene).

The highest resistance rates were obtained for penicillin, tetracycline, erythromycin, and clindamycin, which corroborate the findings of other studies (Ho *et al.*, 2012; Masson *et al*., 2012; Guo *et al*., 2018). None of the samples were positive in the D-test of inducible resistance, suggesting the constitutive resistance mechanism MLSBc (macrolides, lincosamide, and streptogramin B) encoded by the *erm* gene (Jensen and Lyon, 2009). These antibiotics are frequently used in pig farming for the treatment of diseases, and the capacity of *S. aureus* to acquire resistance to these drugs has already been demonstrated (Argudín *et al*., 2017; Lekagul *et al.*, 2019). In addition, high frequencies of strains resistant to gentamicin (91.2%), enrofloxacin (70.6%), and tilmicosin (47.1%) were also observed, corroborating the results of other studies (Ho *et al*., 2012; Guo *et al*., 2018). These

results are worrisome, because these antimicrobials belong to classes that have the highest priority among those that are critically important for human medicine and should be used prudently in both humans and animals (WHO, 2019).

Table 3. Antimicrobial susceptibility profile of the 34 strains of *S. aureus* isolated from pigs in full-cycle farms

Antimicrobials	Resistant Nº (%)	Intermediate Nº (%)	Sensitive N (%)
Penicillin G (10 U)	34 (100)	0 (0.0)	0 (0.0)
Oxacillin (1 µg)	0 (0.0)	0 (0.0)	34 (100)
Cefoxitin (30 µg)	0 (0.0)	0 (0.0)	34 (100)
Tetracycline (30 µg)	34 (100)	0 (0.0)	0 (00)
Sulphamethoxazole-trimethoprim (25 µg)	0 (0.0)	3 (8.8)	31 (91.2)
Gentamicin (10 µg)	31 (91.2)	2 (5.9)	1 (2.9)
Enrofloxacin (5 µg)	24 (70.6)	7 (20.6)	3 (8.8)
Ceftiofur (30 µg)	0 (0.0)	0 (0.0)	34 (100)
Tilmicosin (15 µg)	18 (52.9)	0 (0.0)	16 (47.1)
Erythromycin (15 µg)	34 (100)	0 (0.0)	0 (0.0)
Clindamycin (2 µg)	27 (79.4)	7 (20.6)	0 (0.0)
Tigecycline (15 µg)	0 (0.0)	0 (0.0)	34 (100)
Linezolid (30 µg)	0 (0.0)	0 (0.0)	34 (100)

Similar to other studies, none of the strains showed resistance to vancomycin and the broad-spectrum veterinary drug ceftiofur. None of the strains showed resistance to either to tigecycline or linezolid; both of which are restricted to hospital use for humans (Fall *et al*., 2012; Buyukcangaz *et al*., 2013; Zehra *et al*., 2017). Unlike these results, Guo *et al.* (2018) described the presence of strains resistant to linezolid. Multiresistance is an extremely relevant phenomenon, as it compromises the effectiveness of the drugs used for the treatment of diseases caused by microorganisms such as *S. aureus.* According to Magiorakos *et al*. (2012), multiresistant bacteria are those that present resistance to three or more classes or sub-classes of antimicrobial agents in *in vitro* tests.

Despite the absence of MRSA strains, the data obtained demonstrates an alarming fact: all 34 analyzed *S. aureus* strains presented resistance to at least three classes of antimicrobials for which they should be sensitive, and that there is a therapeutic indication for pigs. For 23 of these strains, resistance to 6 classes of drugs was observed. These phenotypes characterize all *S. aureus* isolates studied as multiresistant (Magiorakos *et al*., 2012). Unlike other countries, the use of antimicrobial agents as prophylactics and growth promoters in Brazil is not prohibited. However, the Brazilian Ministry of Agriculture, Livestock, and Supply (MAPA), considering the history of international concerns (Codex Alimentarius, FAO-WHO) regarding the increasing resistance to antimicrobial agents, vetoed the use of amphenicols, tetracyclines, β-lactams (penicillin and cephalosporins), quinolones and systemic sulfonamides, spiramycin, erythromycin, colistin, tylosin, lincomycin, virginiamycin, bacitracin, and tiamulin as growth promoters and food preservatives for performance improvement in animals used for food production (Nobre *et al*., 2019).

Another method used for the detection of multiple resistance was the Multiple Antibiotic Resistance Index (MAR) described by Krumperman (1983), where values higher than 0.2 determine the phenomenon of multiresistance (Table 4). The *S. aureus* strains analyzed presented an MAR between 0.25 and 0.58, characterizing them as multiresistant and confirming the classification already mentioned by Magiorakos *et al*. (2012).

Table 4. Distribution of the resistance pattern and multiple antibiotic resistance (MAR) of the *S. aureus* isolated from pigs in full-cycle farms

Antibiotic resistance profile	No. of classes resistant	N. of samples	MAR
PEN, TET, GEN, ENO, TMC, ERY, CLI	6	17	0.58
PEN, TET, GEN, ENO, ERY, CLI	6	6	0.50
PEN, TET, ENO, TMC, ERY, CLI	5	1	0.50
PEN, TET, GEN, CLI, ERY	5	3	0.42
PEN, TET, GEN, ERY	4	5	0.33
PEN, TET, ERY	3	2	0.25

PEN: Penicillin; TET: Tetracycline; GEN:gentamicin; ENO: Enrofloxacin; TMC: Tilmicosin; SUT: Sulphamethoxazole-trimethoprim; ERY: Erytromycin; CLI: Clindamycin.

Despite the use of only one colony of *S. aureus* for animal, the possibility of the strains being clonal is real. However, clonal or not, the results showed that the production animals in question served as a reservoir for resistant multidrug bacteria, and the capacity of dissemination to other animals and even humans is a cause for concern and challenge for one health. Even so, considering the antibiotic resistance profile and the presence of identified virulence factor genes, it was possible to characterize at least eight different profiles (Table 5). Studies show that for the clonal identify of the strains to be confirmed, at least more than one of the most modern molecular techniques should be used, not possible in this study. Examples of techniques used to determine clonal samples are: PFGE (pulsed field gel electrophoresis), PCR-RFLP (PCR-restriction fragment length polymorphisms), MLST (Multilocus sequence typing), *spa* typing (sequencing of short sequence repeat (*ssr*) regions of the polymorphic X region of the protein A gene (*spa*) of *S. aureus*), or even whole-genome sequencing (Guo *et al*., 2018).

Table 5. Distribution of possible *S. aureus* profiles isolated from pigs in full-cycle farms

Profiles	Antibiotic resistance profile	*ica*A/*ica*D	N. of samples
I	PEN, TET, GEN, ENO, TMC, ERY, CLI	+/+	17
II	PEN, TET, GEN, ENO, ERY, CLI	+/+	5
III	PEN, TET, GEN, ENO, ERY, CLI	+/-	1
IV	PEN, TET, ENO, TMC, ERY, CLI	+/+	1
V	PEN, TET, GEN, CLI, ERY	+/+	3
VI	PEN, TET, GEN, ERY	+/+	5
VII	PEN, TET, ERY	+/+	1
VIII	PEN, TET, ERY	-/+	1

PEN: Penicillin; TET: Tetracycline; GEN:gentamicin; ENO: Enrofloxacin; TMC: Tilmicosin; SUT: Sulphamethoxazole-trimethoprim; ERY: Erytromycin; CLI: Clindamycin.

In addition to antimicrobial agents used in the control of infectious diseases, the contact of animals with chemical substances occurs via the use of disinfectants for the reduction or elimination of environmental contamination, as well as the use of food preservatives. Therefore, the use of substances such as copper sulfate and zinc chloride in environmental sanitization and in supplementation of animal feeds to inhibit bacterial populations has led to the selection of strains resistant to heavy metals (Aarestrup and Hasman, 2004). Currently, there are no approved interpretative criteria for the classification of *S. aureus* as susceptible or resistant to zinc chloride or copper sulfate. In this work, the resistance threshold values of MIC> 2 mM to zinc chloride and MIC> 12 mM to copper sulfate were used, as adopted by Aarestrup and Hasman (2004). Therefore, all samples were considered sensitive to the heavy metals tested (Table 6).

It is important to highlight that there is evidence showing a relationship between the use of heavy metals and the development of antibiotic resistance, as the genetic elements for both resistance mechanisms can be found on the same mobile DNA molecule (Dweba *et al.*, 2018).

Table 6. Minimum Inhibitory Concentration (MIC) of heavy metals against *Staphylococcus aureus* isolated from pigs

Heavy Metal	Minimum Inhibitory Concentration (mM)				
	0.25	0.5	1	2	4
Copper Sulfate	31	3	0	0	0
Zinc Chloride	15	14	5	0	0

CONCLUSIONS

Staphylococcus aureus have been shown to be important microorganisms present in the microbiota of pigs in the nursery and finisher phases and have been isolated from both the nasal cavity and the anal region. In this study, MSSA isolates with various profiles of multiresistance were detected. This is a reason for concern, either by compromising the success of antibiotic therapy and/or due to the possibility of dissemination in the herd. In addition, the propagation of these strains to human beings, by means of handling of the meat or direct contact with the animal, is a significant issue.

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