



Communication

[Comunicação]

Detection of the *mecA* gene in *Staphylococcus* spp. isolated from the nasal cavity of pigs - Preliminary data

[Detecção do gene *mecA* em *Staphylococcus* spp. isolados da cavidade nasal de suínos - dados preliminares]

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Brazil is the fourth largest producer of pork in the world, producing approximately four million tons of pork in 2017. The State of Paraná contributed 21.01% of this product whereas the State of Santa Catarina constituted 28.38% (Relatório..., 2018).

To maintain a high production and export of pork, there are some very important variables that need to be considered; main amongst them is the sanitary status, one of the most important factors affecting the optimization of zootechnical performance, the epidemiological disease situation as the primary cause, bacterial agents, and consequently the use of antibiotics as a treatment (Aguilar *et al.*, 2015). The use of antimicrobials as therapeutics or as growth promoters, even in low doses in the diet, contribute to the selection of drug-resistant microorganisms in the microbiota of these animals (Morés and Morés, 2017).

Regarding One health, the emergence of resistant bacteria is extremely important, especially when dealing with *Staphylococcus* spp. that are responsible for diseases in different hosts, such as cattle, birds, sheep, pigs, companion animals and humans (Coelho *et al.*, 2007; Masson *et al.*, 2012). These microorganisms have different modes of virulence, such as toxins, enzymes, and the presence of resistance genes, such as the *mecA* gene, which may be responsible for resistance to the beta-lactam class of antibiotics and has been a concern in hospital and out-of-hospital

environments for decades (Blodkamp *et al.*, 2015).

Known as methicillin-resistant *Staphylococcus* (MRS), they have characteristics of penicillin binding protein production, which could be due to the expression of the *mecA* gene. They are, thus, resistant to the whole spectrum of beta-lactam antibiotics, and are responsible for problematic infections, prolonged hospitalizations, and significant contributions to mortality rates in humans, becoming an endemic problem throughout the world (Humphreys, 2012). The objective of this study was, therefore, to carry out the genetic characterization of *Staphylococcus* spp. resistance to methicillin in pigs slaughtered in a slaughterhouse inspected by the Federal Inspection Service (SIF) in the northwestern region of Paraná, Brazil.

Thirty nasal swab samples were collected from adult pigs slaughtered in a SIF-inspected slaughterhouse from the northwestern region of Paraná. The 30 samples of nasal swab were collected between March and June of 2016 from adult pigs slaughtered in a SIF refrigerator in northwestern Paraná. The nasal samples were collected at the slaughter line shortly after stunning and before bleeding using a sterile swab containing Stuart transport medium (SP Labor, Presidente Prudente, São Paulo). To cover all batches slaughtered on the day, a systematic collection standard was adopted. On the day of

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collection, it was possible to collect nasal swab from ten animals of three distinct properties.

The samples were then stored in isothermal boxes and immediately sent to the Laboratory of Preventive Veterinary Medicine and Public Health of the Graduate Program in Animal Science with Emphasis on Bioactive Products of the Paranaense University, Umuarama, Paraná, Brazil. Each sample was cultured in Brain Heart Infusion (BHI) medium for 24 hours at 37°C and then seeded in Baird Parker (BP) medium for 24 to 48 hours for the isolation of *Staphylococcus* spp. After this period, the macroscopic and microscopic characteristics of the isolates were verified and biochemical tests (catalase and coagulase) were performed for the identification of *Staphylococcus* spp. based on coagulase positivity and negativity (Quinn *et al.*, 1994).

To determine the bacterial resistance profile, the agar diffusion method was used according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2013). For this test, ampicillin (10µg), cefotaxime (30µg), ceftiofur (30µg), cephalothin (30µg), streptomycin (10µg), gentamicin (10µg), lincomycin (2µg), oxacillin (1µg), penicillin (10µg), sulfonamides (300µg), and trimethoprim (5µg) were used.

DNA was extracted from the isolates that were resistant to oxacillin (1µg) using the PureLink™ Genomic DNA Mini Kit (Invitrogen, USA) according to the manufacturer's instructions. Subsequently, the DNA was subjected to PCR using 5µM *mecA1* (AAAATCGATGGTAAAGGTTGG) and *mecA2* (AGTTCTGCAGTACCGGATTTG) primers, and the reaction was performed according to the parameters previously described by Murakami *et al.* (1991). Negative (*S. aureus* ATCC 25923) and positive (*S. aureus* ATCC 33591) controls were used for the reaction. DNA

was amplified using a thermocycler (Thermo model Px2 Thermal Cycler) and the final PCR product was subjected to electrophoresis on a 2% agarose gel stained with GelRed (Uniscience). Visualization was performed using a UV transilluminator wherein the positive sample showed a band at 533bp.

Of the 30 swabs collected, it was possible to isolate *Staphylococcus* spp. from all samples (100%). Biochemical tests yielded 12 (40%) coagulase-positive isolates and 18 (60%) coagulase-negative isolates. The four antimicrobials that had the highest resistance index were ampicillin (with 30 (100%) resistant isolates), penicillin (with 29 (96.66%) resistant isolates), lincomycin and trimethoprim (with 28 (93.33%) resistant isolates each); out of the 26 (86.66 %) isolates had oxacillin resistance (Table 1). Of the 26 isolates of oxacillin resistant *Staphylococcus* spp., 6 (23%) were positive for *mecA* (Figure 1).

Table 1. Antimicrobial resistance of 30 isolates of *Staphylococcus* spp. from 30 animals belonging to three distinct properties slaughtered in a federally inspected slaughterhouse in the northwestern region of Parana, Brazil, from March to June of 2016

Antimicrobials	Nasal Cavity	
	R	% Positive
Ampicillin (10µg)	30	100
Penicillin (10µg)	29	96.66
Lincomycin (2µg)	28	93.33
Trimethoprim (5µg)	28	93.33
Sulfonamide (300µg)	27	90.00
Cefotaxime (30µg)	27	90.00
Oxacillin (1µg)	26	86.66
Gentamicin (10µg)	25	83.33
Ceftiofur (30µg)	22	73.33
Streptomycin (10µg)	21	70.00
Cephalothin(10µg)	21	70.00

Legend: R= Resistant. %= Percentage



Figure 1. Samples of *Staphylococcus* spp. positive for the *mecA* gene obtained from 30 animals belonging to three distinct properties slaughtered in a federally inspected slaughterhouse in the northwestern region of Parana, Brazil, from March to June of 2016. Legend: M= Molecular weight marker, CN= Negative Control, CP= Positive Control, Numbers = tested samples.

Pork is the most commonly consumed animal meat in the world because it has a unique taste and excellent nutritional quality. To ensure its efficient production and export to several continents, swine herds in Brazil have been growing every year (Aguilar *et al.*, 2015). However, pig production is susceptible to microbiological contamination from different sources, and *Staphylococcus* spp. is a major pathogen associated with skin and pulmonary infections in humans and animals (Morgan, 2008). This microorganism is found in the nasal region of swine species and is one of the key factors responsible for infectious diseases in pigs (Aguilar *et al.*, 2015; Blodkamp *et al.*, 2015).

Due to the importance of detection of the *mecA* gene in swine that will be marketed for human consumption, this study shows preliminary molecular data from a large phenotypic, molecular and epidemiological study that is still underway. *Staphylococcus* spp. were isolated in all the 30 (100%) samples analyzed, however a study by Lima *et al.* (2004), investigating pig carcasses in a slaughterhouse located in the state of Minas Gerais, detected these bacteria in only 14 samples (11.7 %). The frequency of *Staphylococcus* spp. in this work can be explained by the choice of collection site, because *Staphylococcus* spp. are one of the main microorganisms present in the microbiota of pigs (Blodkamp *et al.*, 2015).

Moreover, in the present study, resistance to different antimicrobials was observed in all the samples, with 56.66% of the isolates showing resistance to 8 or more antimicrobials; these findings corroborate those of Masson *et al.* (2012), wherein the antimicrobial resistance rate was also significant and 34% of the samples showed resistance to 4 or more antimicrobials tested.

In the present study it was also found that 86.66% of *Staphylococcus* spp. isolated were resistant to oxacillin. Similar results were obtained by Masson *et al.*, (2012) in a slaughterhouse in the state of Minas Gerais, wherein oxacillin resistance was found in 3.88% isolates from nasal samples of pigs.

Pertaining to the *mecA* gene, in this work, positive samples accounted for 23 % of the studied samples, deviating from results obtained by Masson *et al.* (2012) who did not detect the gene in analyzed samples. The antibiotic resistance seen in our study may not necessarily be associated with the *mecA* gene itself, as the gene is known to have homologues. In addition to displaying similar resistance, these homologues may be present in phenotypically sensitive samples, making the search for such genes in *mecA* negative samples a good option to justify the lack of *mecA* detection (Pichon *et al.*, 2012). The transmission of *Staphylococcus* spp., between

humans and animals, particularly in swine, can be caused by different risk factors such as: handling and/or ingestion of contaminated food, poor personal hygiene, failure to use PPE for safety, health of the affected herd, and disregarding sanitary voids among other measures (Aguilar *et al.*, 2015).

Resistance to oxacillin was found in 86.66% of the *Staphylococcus* spp. samples analyzed in the present study. The presence of the *mecA* gene was observed in 23.07% of these samples, showing that pigs are carriers of MRS, possibly mediated by the *mecA* gene, and that the misuse of antimicrobials in the management of the animals may be one of the major risk factors contributing to the situation. Therefore, the search for alternative therapies for MRS infections is of paramount importance in pig production because in addition to providing protection to the animals

from bacterial infections during different stages of their growth, it also reduces the environmental exposure of carriers, veterinarians, and consumers to antibiotic-resistant bacteria and resistance-conferring genes, especially through airway transmission of these microorganisms. Therefore, in future research on such isolates, we will focus on the identifying the resistance genes that may confer possible antibiotic multi-resistance to bacteria in animals used for meat production and their origins. This project was approved on 17/08/2018 by the Ethics Committee in Research Involving Animal Experiments (CEPEEA) from the State University of Londrina under protocol No. 31128/2017.

Keywords: staphylococci, slaughterhouse, genes of resistance, pcr, sus scroffa domesticus, one health

RESUMO

O objetivo deste estudo foi realizar a caracterização genética de Staphylococcus spp. resistentes a meticilina isolados de suínos. Foram coletadas 30 amostras de swab nasal de suínos, abatidos em um frigorífico com Serviço de Inspeção Federal. Os isolados foram submetidos a análises macro e microscópicas que, em seguida, para detectar a resistência bacteriana, foram submetidos a ensaios fenotípicos da sensibilidade aos antimicrobianos. Posteriormente, as amostras resistentes a oxacilina, foram submetidas à reação em cadeia pela polimerase (PCR) para verificar a presença do gene mecA. Das 30 amostras analisadas, foram isolados 12 (40%) Staphylococcus spp. coagulase positiva, e 18 (60%) coagulase negativa, e, dentre os isolados, 26 (86,66%) foram resistentes a oxacilina sendo possível detectar o gene mecA em seis (23%) amostras. Este estudo evidencia a presença de genes de resistência em microrganismos comuns a microbiota de animais de produção que podem ser transmitidos ao homem. Além de chamar a atenção para a frequência e quantidade de antimicrobianos aos quais estes animais são expostos durante toda sua vida, podendo ser considerado um problema para a saúde única.

Palavras-chave: estafilococos, frigorífico, genes de resistência, pcr, sus scroffa domesticus, saúde única

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