

ANTIBIOTIC PROFILE OF BACTERIA ISOLATED FROM THE SKIN SURFACE FROM EXTENSIVELY RAISED SWINE

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Abstract

Antibiotic resistance of Staphylococcus is increasing worldwide and is becoming a global concern. The present report exposes the study of the antibiotic susceptibility in isolates of Staphylococcus spp obtained from farm pigs. The samples were collected from healthy pigs, from different farms, in the period between 2019-2020. Forty strains of Staphylococcus were isolated from the anterior nares of individual pigs, and from the skin behind the ears of the animals. These isolates were tested against 12 different antimicrobial agents using the Kirby-Bauer disk-diffusion method. The antibiotic susceptibility profile of different bacteria isolates was studied to detect MAR, MDR. Multiple antibiotic resistance (MAR) indices were determined and it was above 0.2 in 24 of the total of 40 bacteria studied. The highest MAR index was detected in Staphylococcus lentus (0.5) and Staphylococcus xylosus (0.41) and 26 (65%) strains was MDR.

In conclusion, the high levels of antibiotic resistance generally correlates with high antibiotic usage and antibiotics used are a risk to humans with occupational contact with livestock antibiotics.

Key words: staphylococcus, antibiotic resistance, MDR, MAR.

INTRODUCTION

Staphylococci are Gram-positive facultative anaerobic bacteria that belong to the Staphylococcaceae family and *Staphylococcus* genus. Most bacteria from this genus normally inhabit the skin and mucosae of humans and animals and are also a part of soil microbial communities (Bardasheva et al., 2021).

Staphylococci colonize the skin and mucous membranes of humans and animals and are considered commensals or opportunistic pathogens (Silva et al., 2022).

The role of commensals in the spread of resistance among bacteria around the globe cannot be overemphasized. They have been pointed out as the possible sources of antibiotic resistance genes which are now extensive among many systemic bacterial pathogens (Adegoke & Okoh, 2013; Ungureanu et al., 2019). Multidrug-resistant *Staphylococcus* spp. isolates from humans and animals have been reported in recent decades, and those pose a

challenge in both human medicine and also in veterinary medicine (Godoy et al., 2016).

Pork meat is in high demand worldwide, which demand is expected to further increase in the future. Pork is often raised in intensive conditions, which is conducive to the spread of infectious diseases. However, bacterial strains resistant to antibiotics are more and more frequently found on pig farms, in animals and their habitat (Monger et al., 2021). Antimicrobial agents are of extended use equally in veterinary and in human medicine. The intensive use of antimicrobials in animals may promote the insertion and persistence of antimicrobial resistance genes in bacteria, which may be zoonotic or capable to transfer these genes to human-adapted pathogens or to human gut microbiota directly or via food or the environment (Argudin et al., 2017).

S. sciuri is mostly recovered from skin and mucous membranes of animals and has long been considered a non-pathogenic commensal bacterium. In the past years, it has been

frequently isolated from cases of bovine mastitis, from goat infected with viruses as secondary infection agents, from canine dermatitis and from several outbreaks of fatal exudative epidermitis in piglets (Beims et al., 2016).

For the past two decades, during the continuous increase in antimicrobial resistance of bacteria, the discovery of new antibiotics to oppose the development of anti-microbial resistance has rapidly declined (Iwu et al., 2020).

The time required for conventional antibiotic susceptibility tests can result in a significant delay in the administration of an effective drug. Thus, the likelihood of antibiotic resistance to an empirically selected therapy is increasing and timely information on antibiotic susceptibility becomes of particular importance (Hombach et al., 2016).

The aim of this study was to investigate the presence and the antibiotic resistance of *Staphylococcus* spp. isolated from healthy pigs.

MATERIALS AND METHODS

Sampling. Samples from the skin surface were swabbed from extensively raised, clinically healthy pigs, kept on farms (n = 5) of different sizes and located in different years. The samples were then processed by methods of classical bacteriology for identification. A total of 40 *Staphylococcus* strains, isolated during the period 2019-2020 were collected and evaluated.

Identification. All isolates were identified by conventional methods and confirmed by means of API Staph (BioMeroeux, France).

Antimicrobial susceptibility testing. Antimicrobial susceptibilities tests were performed on all *Staphylococcus* isolates to investigate their antibiotic-resistance profile by the agar dilution and disk diffusion methods as described in the Clinical and Laboratory Standards Institute, 31st Edition.

These isolates were tested against 12 different antimicrobial agents, belonging to different antibiotic classes, using the Kirby-Bauer disk-diffusion method.

Using a sterile loop, four to five isolated colonies of the *Staphylococcus* strains were harvested and suspended in 5 ml of sterile

saline, to obtain an inoculum equivalent to 0.5 on the McFarland scale.

The entire surface of a Mueller-Hinton agar plate was flooded. After the exceeding suspension was removed, the plate was left to dry.

Disks containing 10 UI of G Penicillin (P), Tetracycline (TE) 30 µg, Marbofloxacin (MAR) 5 µg, Clindamycin (CD) 2 µg, Florfenicol (FFC) 30 µg, Imipenem (IPM) 10 µg, Colistin (CS) 30 µg, Methicillin (MET) 5 µg, Vancomycin (VA) 30 µg, Erythromycin (E) 15 µg, Sulfamethoxazole/ trimethoprim (SXT) 1.25/23.75 µg, Cefoxitin (FOX) 30 µg, were positioned on the plate and gently pressed onto the inoculated Mueller Hinton agar to ensure the contact with the surface. Plates were incubated at 37°C, for 18-24 h under aerobic conditions.

The inhibition diameters were measured and the obtained values were used to quantify the sensitivity/resistance of the tested strains. All the *Staphylococcus* isolates were classified as resistant, intermediate resistant or sensitive to a particular antibiotic.

Establishment of MDR and MAR index among *Staphylococcus* spp. isolates.

The multiple antibiotic resistance (MAR) index was calculated using the following formula:

$$\text{MAR index} = \frac{\text{number of antibiotics to which the isolate was resistant}}{\text{total number of antibiotics tested}}$$

(Raja & John, 2016).

Multidrug resistance was quantified by identifying the isolates resistant to three or more antimicrobial classes (Godoy et al., 2016).

RESULTS AND DISCUSSIONS

The 40 isolates which were identified during the study were involved as follows: 19 isolates were *S. xylosus*, 12 isolates were *S. lentus*, and 9 isolates were *S. sciuri*.

In the current study we also describe the antibiotic susceptibility level of *S. xylosus*, *S. lentus*, and *S. sciuri*, obtained from the anterior nares of individual pigs, and from the skin behind the ears of the healthy pigs (Figure 1).

The highest resistance rates of the *Staphylococcus* spp. ranked as follows: cefquinome (90%), penicillin (65%), marbofloxacin (45%), and tetracycline (37.5%), but resistance to other important antimicrobials was also observed including vancomycin (22.5%), and sulfamethoxazole/trimethoprim (10%), florfenicol (7.5%), colistin (8%), erythromycin (8%), and methicillin (8%). A low frequency of resistance was found towards clindamycin (0%), imipenem (0%) (Figure 2).

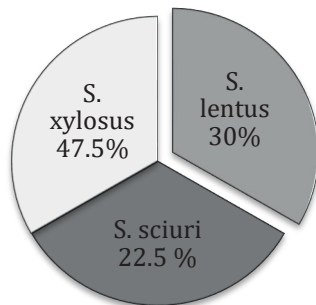


Figure 1. Incidence of *Staphylococcus* strains

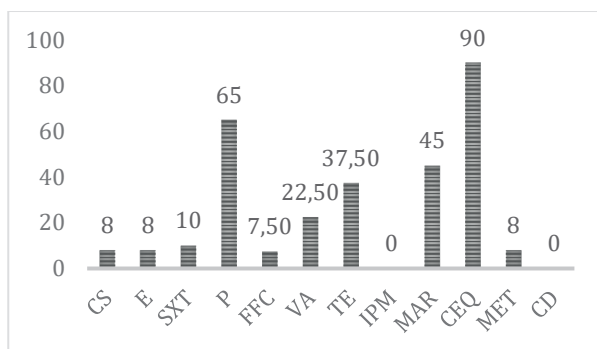


Figure 2. Occurrence of *Staphylococcus* isolate resistance to antimicrobials

Antimicrobial sensitivity testing showed that all the *Staphylococcus* strains were resistant to penicillin, vancomycin, marbofloxacin, and Cefquinome (Table 1.)

Both *Staphylococcus xylosus* and *S. lentus* strains were resistant to 9 of the total of 12 antibiotics tested, including methicillin. *S. sciuri* strains were resistant to only 4 classes of antibiotics tested (Table 1).

A MAR index higher than 0.20 implies that such bacteria originate from an environment where several antibiotics were used. The MAR indices obtained in this study were a possible indication of a very large proportion of the bacterial isolates to have been exposed to several antibiotics (Adeshina et al., 2012).

These bacteria are common environmental organisms, which act as opportunistic pathogen in clinical cases where the defense system of the patient was compromised (Raja and John, 2016).

Table 1. Resistance profile of *Staphylococcal* strains isolated from healthy pigs

| Antibiotics | <i>S. lentus</i> (n=12) | <i>S. xylosus</i> (n=19) | <i>S. sciuri</i> (n=9) |
|----------------|-------------------------|--------------------------|------------------------|
| Colistine | 0,5 | 0,5 | 0 |
| Erythromycin | 1 | 0 | 0 |
| Co-trimoxazole | 0.5 | 0.5 | 0 |
| Penicilline | 26.92% | 46.15% | 26.92% |
| Florfenicole | 66.66% | 33.33% | |
| Vancomycine | 22.22% | 22.22% | 55.55% |
| Tetraciclina | 26.66% | 73.33% | |
| Imipeneme | 0 | 0 | 0 |
| Marbofloxacin | 11.11% | 55.55% | 11.11% |
| Cefquinome | 27.77% | 47.22% | 0.25 |
| Methicillin | 0 | 1 | 0 |
| Clindamycin | 0 | 0 | 0 |

The high resistance can be explained by the fact that these antibiotics represent the antibiotics of choice for the treatment of several staphylococcal infections.

The multiple antibiotic resistance (MAR) indices were calculated with reference to the tested antibiotics.

All isolates had MAR index between 0-0.5, and none had a MAR index ≥ 1 .

Multiple antibiotic resistance (MAR) indices were above 0.2 in 24 of 40 studied bacteria strains (Table 2). The highest MAR indices detected were identified in *Staphylococcus lentus* (0.5) and *Staphylococcus xylosus* (0.41).

Table 2. MAR index of *Staphylococcus* spp.

| | MAR index | | |
|----------------------------|------------|-------------|------------|
| | ≤ 0.2 | 0.21-0.49 | ≥ 0.5 |
| <i>S. lentus</i> | 5 (41.66%) | 6 (50%) | 1 (8.33%) |
| <i>S. xylosus</i> | 5 (26.31%) | 14 (73.68%) | 0 |
| <i>S. sciuri</i> | 5 (55.55%) | 4 (44.44%) | 0 |
| <i>Staphylococcus</i> spp. | 15 (37.5%) | 24 (60%) | 1 (2.5%) |

Appropriate use of antibiotics remains the key to reduce the spread of multidrug resistant strains (Kengne et al., 2019).

Table 3 shows the frequency of MDR profiles among *Staphylococcus* spp. isolated from healthy pigs.

Table 3. MDR *Staphylococcus* spp isolated from pigs (strains)

| Resistance to number of drug classes | MDR <i>Staphylococcus</i> spp. (strains) |
|--------------------------------------|--|
| 3 | 10 |
| 4 | 11 |
| 5 | 4 |
| 6 | 1 |

Multidrug-resistance (MDR), defined as resistance to 3 or more antimicrobial classes, was common among the *Staphylococcus* isolates.

Out of total 40 strains isolated 26 (65%) was MDR. 11 (27.5%), of isolated strains, were classified in MDR 4, followed by MDR 3, in 10 (25%) of the isolated strains (Figure 3).

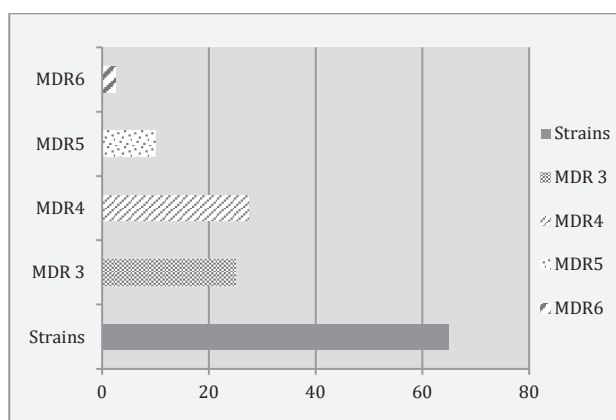


Figure 3. Percentages of MDR strains from the *Staphylococcus* isolates

This MDR phenomenon may be due to acquiring of numerous resistant genes through the R plasmid (Caraciolo et al., 2012; Couto et al., 2000; Moglad, 2020).

Although the clinical significance of *S. sciuri* may remain controversial, the capacity of this species to carry resistance determinants is well established (Couto et al., 2000). Other studies, based on genomic and plasmid encoded genes, reveal that multiresistant *S. sciuri* isolates carry resistance genes against all major classes of antibiotics; they have already been reported and support the potential of staphylococci to temporarily serve as a “bacterial shuttle” by transmitting genetic information between bacterial species (Beims et al., 2016).

CONCLUSIONS

Our study showed that the majority of isolates of all found species were sensitive to most of the antibiotics tested, including penicillin.

The MAR were high in both *Staphylococcus lentus* and *Staphylococcus xylosum*, previously considered harmless bacteria, but for which, their multiple drug resistance stands for potential pathogenicity.

The highest resistance rates found to these antibiotics in the current study can be justified, in part, by the indiscriminate and inadequate use of these drugs over the years, favored by their low cost. The use of antimicrobials select lineages of resistant bacteria, which is very likely to be the main cause of antimicrobial resistance.

Scientists should devote more attention to staphylococci not only as ubiquitous bacteria but also as potential pathogens with enhanced power to jump over species and cause disease.

The high levels of antibiotic resistance found were most probably correlated with high antibiotic usage, posing health risks to humans as occupational contacts of animals or as consumers.

REFERENCES

- Adegoke, A. A., Okoh, A. I. (2016). Species diversity and antibiotic resistance properties of *Staphylococcus* of farm animal origin in Nkonkobe Municipality, South Africa. *Folia Microbiol* 59: 133–140.
- Adeshina, G. O., Jibo, S. D., Agu, V. E. (2012). Antibacterial Susceptibility Pattern of Pathogenic Bacteria Isolates from Vegetable Salad Sold in Restaurants in Zaria, Nigeria. *Journal of Microbiology Research*, 2(2): 5-11.
- Argudín, M. A., Deplano, A., Meghraoui, A., Dodémont, M., Heinrichs, A., Denis, O., Nonhoff, C., Roisin, S. (2017). Bacteria from Animals as a Pool of Antimicrobial Resistance Genes. *Antibiotics* Belgium.
- Bardasheva, A., Tikunov, A., Kozlova, Y., Zhirakovskaia, E., Fedorets, V., Fomenko, N., Kalymbetova, T., Chretien, S., Pavlov, V., Tikunova, N., Morozova, V. (2021). Antibiotic Resistance and Pathogenomics of Staphylococci Circulating in Novosibirsk, Russia. *Microorganisms*, 9, 2487, Rusia.
- Beims, H., Overmann, A., Fulde, M., Steinert, M., Bergmann, S. (2016). Isolation of *Staphylococcus sciuri* from horse skin infection. *Open Veterinary Journal*, Vol. 6(3): 242-246;
- Caraciolo, F. B., Maciel, M. A. V., Santos, J. B., Rabelo, M. A., Magalhães, V. (2012). Antimicrobial

- resistance profile of *Staphylococcus aureus* isolates obtained from skin and soft tissue infections of outpatients from a university hospital in Recife -PE, Brazil. *Anais Brasileiros de Dermatologia*, 87(6):857-6.
- Couto, I., Sanches, I. S., Sá-leão, R., Lencastre, H. (2000). Molecular characterization of *Staphylococcus sciuri* strains isolated from humans. *Journal of clinical microbiology*, New York.
- Godoy, I., Moraes, D. F. S. D., Pitchenin, L. C., Rosa, J. M. A., Kagueyama, F. C., Silva, A. J., Dutra, V., Nakazato, L. (2016). Antimicrobial susceptibility profiles of *Staphylococcus* spp. from domestic and wild animals. *Ciência Rural*, V. 46, n.12, dez, Brasil.
- Hombach, M., Jetter, M., Blchliger, N., Kolesnik-Goldmann, N., Böttger, E. C. (2016). Fully automated disc diffusion for rapid antibiotic susceptibility test results: a proof-of-principle stud. *Journal of antimicrobial chemotherapy*, Zurich.
- Iwu, D. C, Korsten, L., Okoh, A. I. (2020). The incidence of antibiotic resistance within and beyond the agricultural ecosystem: A concern for public health. *Microbiology open*.
- Kengne, M., Fotsing, O., Ndomgue, T., Nwobegahay, J. M. (2019). Antibiotic susceptibility patterns of *Staphylococcus aureus* strains isolated at the Yaounde Central Hospital, Cameroon: a retro prospective study. *Pan African Medical Journal*.
- Monger, X. C., Gilbert, A. -A., Saucier, L., Vincent, A. T (2021). Antibiotic Resistance: From Pig to Meat. *Antibiotics*.
- Raja, M. M. M. and John, S. A. (2016). Biosynthesis of Silver Nanoparticles by Novel Isolate of Marine Micromonospora species (KU 867645) and its Antibacterial Activity against Multidrug Resistant Hospital-acquired Uropathogens in Reference with Standard Antibiotics. *Indian Journal of Pharmaceutical Sciences*, India.
- Silva, V., Caniça, M., Ferreira, E., Vieira-Pinto, M., Saraiva, C., Pereira, J. E., Capelo, J. L., Igrejas, G., Poeta, P. (2022). Multidrug-Resistant Methicillin-Resistant Coagulase-Negative Staphylococci in Healthy Poultry Slaughtered for Human Consumption. *Antibiotics* 2022, 11, 365.
- Ungureanu, V., Corcionivoschi, N., Gundogdu, O., Stef, L., Pet, I., Pacala, N., Madden, R. H. (2019). The emergence of β lactamase producing *Escherichia coli* and the problems in assessing their potential contribution to foodborne illness: a review. *Agrolife Scientific Journal*, 8(1): 248-260.