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Research in Veterinary Science 143 (2022) 28–32

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Pet dogs as reservoir of oxacillin and vancomycin-resistant *Staphylococcus* spp

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ARTICLE INFO

Keywords:
Bacteria
Canine
mecA gene
Multiresistance
One health

ABSTRACT

The aim of this study was to verify the bacterial resistance profile and detect the presence of *mecA* gene in *Staphylococcus* spp. isolated from the nasal microbiota of domiciled dogs. For this purpose 100 nasal swabs from 100 domiciled dogs were collected from the central area of the city of Umuarama (PR), along with a questionnaire answered by their owners. After the isolation all *Staphylococcus* spp. isolates were submitted to the diffusion disc test by the Kirby-Bauer method, and only oxacillin-resistant samples were submitted to the PCR technique to search for the *mecA* gene and the results were then submitted to statistical analysis to verify possible risk variables. The 100 *Staphylococcus* spp. and coagulase negative, among which 41 isolates were resistant to oxacillin, no samples were positive for the *mecA* gene presence, however, 12 resistant to vancomycin were found. It can be concluded that the domiciled dogs are carriers of *Staphylococcus* spp. multiresistant, being these a possible source of human contamination.

1. Introduction

The bacterial microbiota is present in several places, including in skin and mucous of humans and animals, these microbiota are composed by different types of microorganisms, such as yeast fungi and bacteria, such microorganisms tend not to be pathogenic, however, opportunistic bacteria also colonize the mucous, as an example, the nasal cavity of healthy domesticated dogs is colonized with several important microorganisms (Banks et al., 2020; Černáková et al., 2018; Rey et al., 2020; Tress et al., 2017; Vangrinsven et al., 2020; Wipler et al., 2017).

The pet dogs are considered reservoir of a multi-resistant bacteria range, mainly considering their life habits, such as sniffing, which can be an extrinsic factor, since have been shown that extrinsic and intrinsic factors have influence on the nasal microbiota (Isaiah et al., 2017; Tress et al., 2017; Vangrinsven et al., 2020).

One of the most important characteristics of the nasal bacterial microbiota of domestic animals is their ability to transfer and share between them and humans (Bhat, 2021; Dalton et al., 2021; Wipler et al., 2017). The biggest problem encountered in this situation is the danger related to the transfer of microorganisms that are resistant to antibiotics (Awosile et al., 2018; Bhat, 2021; Joosten et al., 2020; Teixeira et al., 2019).

Among these microorganisms that are resistant to antibiotics are *Staphylococcus* spp., such bacteria are present in many cases, in humans and animals, related to bacterial infection by multi-resistant microorganisms (Almeida et al., 2017; Boswihi and Udo, 2018; Loewen et al., 2017; Sisti, 2017).

One of the biggest problems involving bacteria today is the creation of a resistance profile, and the situation is aggravated when the resistance is by last generation antimicrobials, as these should be the last choice in most treatments (Moraes et al., 2016). In this context, methicillin-resistant *Staphylococcus* spp. (MRS), usually linked to the *mecA* gene, are important microorganisms resistant to important antibiotics such as β-lactams, carbapenems and quinolones (Siddiqui and

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https://doi.org/10.1016/j.rvsc.2021.12.005
Received 23 August 2021; Received in revised form 7 December 2021; Accepted 16 December 2021
Available online 21 December 2021

Koirala, 2020), another important antimicrobial agent is the vancomycin, which has been the drug of choice in cases of MRS infections, but since 2002 there have been reports of isolates of vancomycin-resistant *Staphylococcus* spp. (VRS) that can cause a major One Health problem (Gardete and Tomasz, 2014; Hasan et al., 2016; Mello et al., 2017).

Even though those antimicrobials are not so frequently administered to dogs, this animal species can also carry the MRS and transmit them to their owners and/or other animals. Due to the importance of multiresistant microorganisms such as *Staphylococcus* spp., the aim of this study was to verify the bacterial resistance profile and detect the presence of the *mecA* gene in *Staphylococcus* spp. isolated from the nasal cavity of domiciled dogs.

2. Material and methods

2.1. Ethical aspects

This project was approved by the Research Ethics Committee Involving Animal Experimentation of UNIPAR (CEPEEA/UNIPAR) under the protocol number 32112/2016. Each owner signed a free and informed consent form and answered a questionnaire that included information about proximity to their owners, such as access to the interior of the house and furniture, access to the street, feeding, number and the species of animals in contact with dogs, the use of antimicrobials by owners and animals, frequency of disease of the animal, and the habit of hunting.

2.2. Study site and sampling

For the definition of dog sampling, the following information was taken into account: number of households occupied in the urban area (31.295 residences) of Umuarama-PR (IPARDES, 2017), and the estimation of dogs per household according to Brazilian Institute of Geography and Statistics data who were, respectively, 1.8 dogs/household.

Therefore, an estimate of 56.331 dogs was obtained. Considering a sampling error of 10%, the n was determined for the collection, according to the formula described by Barbetta (1999), where was obtained the number 99.82, and this result was rounded up to 100.

From July to October 2017, a nasal cavity swab of 100 domiciled dogs that lived as close as possible to their owners and resided in different randomly chosen neighborhoods of Umuarama (PR), Brazil, were collected aseptically. These dogs were of no defined breed, both sexes, ranging in age from 30 days to 360 months., excluding animals that were sick or submitted to antimicrobial therapy in the time of the collection.

2.3. Bacterial isolation

Each swab was inserted into Brain Heart Infusion (BHI) medium (Kasvi, São José do Pinhais, PR, Brazil), and incubated at 37 °C for 24 h, after this time, each sample was seeded in a plate with Manittol Salt Agar (Kasvi, São José do Pinhais, PR, Brazil) and incubated at 37 °C for 48 h for isolation of *Staphylococcus* spp. Each colony was submitted to analysis of the macroscopic, microscopic, catalase and coagulase tests, allowing classifying coagulase positive *Staphylococcus* (CoPS) and coagulase negative *Staphylococcus* (CoNS) (Quinn et al., 1994).

2.4. Antimicrobial susceptibility test

Antimicrobial susceptibility test were performed according to Brazilian Committee on Antimicrobial Susceptibility Testing (BrCAST) (2020). The agar diffusion method by Kirby–Bauer (1966), was performed after BHI standardization of the inoculum according to the MacFarland 0.5 scale, using the most commonly used antimicrobial discs in small animal medical clinics: amoxicillin (10 μg), azithromycin (15 μg), cefoxitin (30 μg), enrofloxacin (5 μg), erythromycin (15 μg), gentamicin (10 μg), oxacillin (1 μg), penicillin (10 U), sulfazotrim (25 μg) and, as screening test, vancomycin (30 μg). The results obtained were recorded considering the interpretation of the BrCAST (2020) and the measurement of the size of inhibition halos, in millimeters.

Isolates resistant to vancomycin in the screening test were submitted to vancomycin resistance evaluation according to BrCAST (2020) using *E*-test® strips (bioMérieux, Marcy-l'Étoile, France), which consists in a predefined antibiotic concentration gradient placed on a strips, it is used to determine the Minimum Inhibitory Concentration (MIC) of antibiotic.

2.5. Detection of mecA gene

DNA of *Staphylococcus* spp. classified as oxacillin-resistant was extracted using the Purelink Genomic DNA Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions, and PCR was performed using the mecA1 primer (AAAATCGATGGTAAAGGTTGG) and mecA2 primer (AGTTCTGCAGTACCGGTTG) according to Murakami et al. (1991).

The amplification products were visualized by electrophoresis on a 2% agarose gel stained with GelRed (Uniscience, Osasco, São Paulo, BR) using a 100-bp molecular marker, and a single band of 533 bp was obtained.

2.6. Statistical analysis

Samples that showed resistance to oxacillin were submitted to a statistical analysis program for comparison with the owners' answers regarding the questionnaire, trying to understand these isolates risk to the environment. Statistical analysis was performed using the IBM SPSS v. 21.0 and the test adopted was the Chi-Square test. The association analyzes between resistance number and the questionnaire answer were determined by the chi-square test or Fischer's exact test, at a significance level of 5%.

3. Results

In this work, of the 100 samples of nasal swab, it was possible to isolate 100 *Staphylococcus* spp. with 46 (46%) identified as coagulase positive (CoPS), 54 (54%) as coagulase negative (CoNS), the number of antibiotic resistances and of each of the samples is described in Tables 1 and 2, from these, and among these 28 CoPS (65.22%) and 11 CoNS (20.37%) were identified phenotypically as oxacillin resistant.

The 41 (41.00%) samples phenotypically resistant to oxacillin were submitted to PCR for the identification of the *mecA* gene, however no positive for the presence of the *mecA* gene.

Among the MRS found in this study, 12 (29.26%) were resistant to vancomycin, and all were confirmed as vancomycin resistant (VRS) with the aid of the *E*-test, such test showed values equal to 0 μg/ml and 2 μg/ml in all the isolates.

Table 1
Total number and percentage of resistance isolates antimicrobials from nasal swab of domiciled dogs in the urban area of the city of Umuarama (PR), 2017.

Antibotic	Number of resistance
	n
Amoxicilin	22/100
Azithromycin	26/100
Cefoxitin	27/100
Enrofloxacin	24/100
Erythromycin	38/100
Gentamicin	15/100
Oxacilin	41/100
Penicilin	79/100
Sulfazotrim	45/100
Vancomycin	12/100

All. C al Verbale n. 2 del 1 Febbraio 2022

ACCERTAMENTO CONOSCENZE INFORMATICHE DI BASE

1. **Quali tra Questi è un file excel?**
   a) profilo.xls
   b) profilo.doc
   c) profilo.zip
   d) profilo.rtf

2. **Se volessi presentare i risultati di un tuo studio ad una platea che applicazione useresti?**
   a) Access
   b) AutoCad
   c) Paint
   d) Powerpoint

3. **Che cosa vuol dire "fare il backup"?**
   a) Creare una copia di sicurezza dei dati
   b) Forzare il caricamento di un file su Internet
   c) Sostenere il computer nei momenti di massimo sforzo computazionale
   d) Operazione che non sempre è utile nei sistemi sanitari

4. **Cos'è la "PEC"?**
   a) Una raccomandata semplice
   b) Un indirizzo di posta elettronica sicuro
   c) La posta elettronica certifica
   d) Un programma di video scrittura

5. **Cos'è quella che viene definita comunemente penna usb?**
   a) Un dispositivo di archiviazione dati
   b) Un floppy disk di ultima generazione
   c) Un sistema operativo
   d) Un dispositivo di scannerizzazione per immagini

6. **Come si spegne un computer in modo corretto?**
   a) Premendo il pulsante on/off posto sul Case
   b) Cliccando sul pulsante Chiudi(X) posto in alto a destra.
   c) Premendo il pulsante on/off posto sotto lo schermo
   d) Cliccando sul pulsante Start, poi su Arresta e Arresta Sistema