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Antimicrobial resistance patterns of *Staphylococcus* spp. isolated from clinical specimens of companion animals in Northern Portugal, 2021–2023

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ABSTRACT

Staphylococcus spp. are growing pathogens in humans and companion animals. The emergence of multidrug-resistant bacterial infections, such as methicillin-resistant *Staphylococcus*-associated infections, due to zoonotic transmission, is a major public health concern. Domestic animals, such as dogs and cats, are possible reservoirs of multi-resistant bacterial species, which makes it relevant to monitor them due to their proximity to humans. However, there is a lack of information on the real scenario in Europe, especially in Portugal, particularly for animal infections caused by *Staphylococcus* spp. Therefore, this study aimed to investigate the antimicrobial resistance profile of *Staphylococcus* spp. isolated from cats and dogs diagnosed with infection in Northern Portugal.

During 2021–2023, 96 *Staphylococcus* isolates from dogs and cats with symptoms of bacterial infection, including animals being treated in veterinary clinics/hospitals and cadavers submitted for necropsy at INIAV were included in the study collection. Of the 96 isolates, 63 were from dogs and 33 were *Staphylococcus* spp. from cats, most of which were isolated from ear (57% and 18%, respectively), skin (19 % and 27 %, respectively) and respiratory tract infections (6 % and 27 %, respectively). Among all the isolates, 12 different *Staphylococcus* spp. were identified, with *Staphylococcus pseudintermedius* being the most identified (61 % from dogs and 30 % from cats). It is noteworthy that 36 % of the isolates were multi-drug resistant and 25 % of the isolates showed a methicillin-resistant phenotype, with the *mecA* gene having been identified in all these isolates. This study highlights a high occurrence of multidrug-resistant *Staphylococcus* spp. in companion animals in Northern Portugal. This underlines the potential for cats and dogs to act as reservoirs of antimicrobial resistance, that can be transmitted to humans, posing a serious threat to public health.

Introduction

Staphylococcus spp. are Gram-positive, facultatively anaerobic bacteria, commonly found in the microbiota of mammalian skin and mucous membranes (Achi et al., 2022). However, the genus also includes clinically relevant opportunistic pathogens in both human and veterinary medicine, that frequently colonize a variety of biotic surfaces (Sigudu et al., 2023).

In companion animals, *Staphylococcus* spp. are commonly isolated from various infection sites and can manifest in several ways. These may include skin, ear, and urinary tract infections (Garcês et al., 2022). Among *Staphylococcus* spp., *Staphylococcus pseudintermedius* and *Staphylococcus aureus* have been reported to be the most commonly associated with these infections (Afshar et al., 2023). *S. pseudintermedius* is the most common commensal microorganism on the skin of dogs, but, also a common cause of skin, ear, and soft tissue infections in cats (Assouma et al., 2023). In Portugal, there are several studies evaluating the antimicrobial resistance profile of *Staphylococcus* spp. isolated from

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production animals, but few studies have been carried out in companion animals (Andrade et al., 2022; Costa et al., 2022).

The indiscriminate use of antibiotics leads to an increase of antimicrobial resistance and poses a significant challenge to human and animal health (Garcês et al., 2022), limiting the available clinical treatments. In veterinary medicine, β-lactam antibiotics are widely used to treat bacterial infections caused by *Staphylococcus* spp. (Burke and Santoro, 2023). Among the genetic determinants of resistance, the methicillin-resistance genes, *mecA* and *mecC*, stand out, as they can confer resistance to virtually all antibiotics in this class (Saputra et al., 2017).

Nevertheless, epidemiological and etiological information on *Staphylococcus* spp. isolated from sick companion animals from Portugal is very scarce (Garcês et al., 2022). Therefore, the current study aimed to investigate the antimicrobial resistance profile of *Staphylococcus* spp. isolated from cats and dogs diagnosed with an infection and treated in veterinary clinics in Northern Portugal.

Materials and methods

Staphylococcus spp. isolation and identification

A prospective analysis was conducted between May 2021 and May 2023 on dogs and cats with symptoms of bacterial infection, including animals being treated in veterinary clinics/hospitals and cadavers submitted for necropsy at INIAV. The clinics/hospitals were selected based on their location (Northern Portugal) and had a protocol in place between INIAV and the clinics/hospitals. Sterile swabs (Tube BDVacutainer EDTA, Frilabo, Portugal) were used to collect clinical samples from wounds, nasal cavities, ears, urine, eyes, genital tracts, and the organs of animal cadavers. To isolate *Staphylococcus* strains, clinical samples were initially inoculated onto Mannitol Salt Agar (MSA, Liofilchem, Italy) and incubated for 24 h at 37 °C. Subsequently, typical colonies were selected and re-inoculated onto Tryptic soy agar (TSA, Biolife, USA) for an additional 24 h at 37 °C. Species identification was conducted through DNA Sanger-sequencing, outsourced to an external company, based on the sequencing of *rpoB* gene. The following primers were employed for the sequencing process: *rpoB*-fw (5'-CAATTCATGGACCAAGC-3'), and *rpoB*-rev (5'- CCGTCCCAAGTCATGAAAC-3') [12]. The DNA-sequencing results were compared with the Standard Nucleotide BLAST database of the National Centre for Biotechnology Information (NCBI), to identify species-specific DNA sequences.

Staphylococcus spp. antimicrobial susceptibility determination

A total of 96 *Staphylococcus* isolates were selected for antimicrobial susceptibility analysis. The tests were conducted using the disc diffusion method according to the Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals, 7th Edition (CLSI VET01S-ED7:2024) (Clinical and Laboratory Standards Institute, 2024). The following antibiotics were selected based on the antimicrobial agents recommended to be tested on isolates from dogs and cats and which contain breakpoint values for disk diffusion testing: penicillin G (P), oxacillin (OX) of the penicillin antibiotic class; cefoxitin (FOX) of the cephalosporins antibiotic class; linezolid (LZD) of the oxazolidinones antibiotics; erythromycin (E), clindamycin (DA) of the glycosamides antibiotic class; enrofloxacin (ENR) and marbofloxacin (MBX) of the fluoroquinolones antibiotics; gentamicin (GEN) of the aminoglycosides antibiotic class; doxycycline (DOX), minocycline (MH), and tetracycline (TET) of the tetracyclines antibiotic class; chloramphenicol (CHL) and trimethoprim-sulfamethoxazole (SMX) of the combined agents antibiotic class.

The tests were conducted according the manufacturer's instructions, using a colony suspension with turbidity equivalent to that of 0.5 McFarland standard. These suspensions were streaked onto Muller-Hinton Agar (Oxoid, UK), and then incubated for 16–18 h at 37 °C. Subsequently, the diameter of the inhibition zones was measured. Quality control (QC) procedures were carried out using *S. aureus* ATCC 25923. For the assessment of antibiotic susceptibility, veterinary CLSI breakpoints (VET01S-ED7:2024) (Clinical and Laboratory Standards Institute, 2024) were employed to interpret the results of the disc diffusion method.

Methicillin-resistant *Staphylococcus* spp. (MRS) were identified based on resistance to oxacillin (for *S. pseudintermedius* and *S. schleiferi*) or cefoxitin (for *S. aureus* and other *Staphylococcus* spp.). In addition, *Staphylococcus* isolates were considered multi-drug resistant (MDR) when resistant to three or more antibiotic classes (Magiorakos et al., 2012).

Staphylococcus spp. detection of resistance and virulence genes

For the presence of methicillin resistance genes (*mecA* and *mecC*), virulence genes (toxic shock syndrome toxin-1 (*tsst-1*) and Panton-Valentine leukocidin (*pvl*), a random set of 57 *Staphylococcus* isolates were screened. For that, a multiplex PCR methodology was performed using the primers described in Table 1 (Blaiotta et al., 2004; Stegger et al., 2012). Briefly, a 1 μL loop of each bacterial culture was mixed with 500 μL of ultrapure water and boiled for 15 min. Following this, the suspension was centrifuged at 12,000 g for 5 min and the resulting supernatant (2 μL) was used as the DNA template. All PCR reactions were performed in 20 μL reaction mixtures containing 1X Supreme NZYTaq II 2x Green Master Mix (NZYtech, Portugal) and 0.2 μM of each primer for *mecA*, *mecC*, *pvl* and *tsst-1* (Table 1). The thermal cycling conditions used were the same for both PCR, with an initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 sec, annealing for 30 sec at 56 °C and extension at 72 °C for 30 sec. A final extension was performed at 72 °C for 5 min. The PCR products were submitted to electrophoresis at 100 V for 1 h in a 1.5 % (w/v) agarose gel, which had been pre-stained with GreenSafe Premium (NzyTech). Subsequently, the gel was analysed under UV light to visualize the DNA bands.

Statistical methods

The GraphPad Prism version 7 (La Jolla, CA) was used to analyse all data.

Results

Prevalence of Staphylococcus spp.

Between 2021 and 2023, a total of 89 *Staphylococcus* strains were

Table 1
Sequence of primers and conditions used for multiplex PCR to detect resistance and virulence genes in the study.

Target Gene	Primers (5'-3')	Amplification product size (bp)	Reference
mecA	Forward: TCCAGATTACAACTTCACCAGG Reverse: CCACTTCATATCTTGTAACG	162	(Stegger et al., 2012)
mecC	Forward: GAAAAAAAGGCTTAGAACGCCTC Reverse: GAAGATCTTTTCCGTTTTCAGC	138	(Stegger et al., 2012)
pvl	Forward: GCTGGACAAAACTTCTTGGAATAT Reverse: GATAGGACACCAATAAATTCTGGATTG	83	(Stegger et al., 2012)
tsst-1	Forward: ATGGCAGCATCAGCTTGATA Reverse: TTTCCAATAACCACCCGTTT	350	(Blaiotta et al., 2004)

isolated from sick dogs (n=58) and cats (n=31), all with symptoms of bacterial infection and being treated in veterinary clinics/hospitals in Northern Portugal. A further 7 isolates were obtained from animal cadavers (5 dogs and 2 cats) submitted for necropsy at INIAV.

Overall, of the 12 species identified, *S. pseudintermedius* was the most commonly isolated (51.0 %), followed by *S. aureus* (16.7%) and *S. felis* (9.4 %). The remaining *Staphylococcus* spp. included *S. chromogenes* (6.3 %), *S. schleiferi* (5.2 %), *S. hominis* (3.1 %), *S. haemolyticus* (3.1 %), *S. saprophyticus* (1.0 %), *S. simulans* (1.0 %), *S. condimenti* (1.0 %), *S. xylosus* (1.0 %) and *S. arlettae* (1.0 %). This study also showed that *Staphylococcus* spp. were predominantly isolated from ear infections (43.8 %) followed by skin (21.9 %) and respiratory tract infections (13.5 %).

Of the 63 *Staphylococcus* isolates from dog samples, *S. pseudintermedius* was the most common with 61.9 % (n=39), followed by *S. aureus* with 14.2 % (n=9) and *S. schleiferi* with 7.9 % (n=5) (Fig. 1A). The remaining 16 % were identified as other *Staphylococcus* spp., including *S. haemolyticus* (3.2 %), *S. hominis* (3.2 %), *S. chromogenes* (3.2 %), *S. saprophyticus* (1.6 %), *S. simulans* (1.6%), *S. codimenti* (1.6 %) and *S. felis* (1.6 %). Regarding the source of isolation, ear infections (57.1 %) had the highest prevalence, followed by skin infections (19.0 %) and respiratory and urinary infection (6.3 % each) (Fig. 1B).

Of the 33 *Staphylococcus* isolates from cat samples, *S. pseudintermedius* was also the most prevalent with 30.4 % (n=10), followed by *S. felis* with 24.3 % (n=8) and *S. aureus* with 21.2 % (n=7) (Fig. 1C). The remaining 24.1 % were identified as other *Staphylococcus* spp., including *S. chromogenes* (12.1 %), *S. arlettae* (3.0 %), *S. hominis* (3.0 %), *S. haemolyticus* (3.0 %) and *S. xylosus* (3.0 %). Regarding the source of isolation, there was a higher prevalence of skin infections (27.3 %) and respiratory tract infections (27.3 %), followed by ear infections (18.2 %) (Fig. 1d).

Antimicrobial resistance profile of Staphylococcus spp.

The antimicrobial resistance profile of the identified *Staphylococcus* isolates is shown in Figs. 2 and 3. Fig. 2A-C shows the resistance profiles for species *S. pseudintermedius*, *S. aureus,* and *S. schleiferi* isolated from dogs, respectively. The resistance profiles shown in Fig. 2D are for the other *Staphylococcus* spp. identified. For *S. pseudintermedius* (Fig. 2A), 50 % or more isolates showed a resistant profile for P and TET, while for OX, DA, DOX, MH, E, CHL, GEN, LZD and SMX, there was a resistance profile for less than 50% of the isolates. To note, that all *S. pseudintermedius* isolates were susceptible to LZD antibiotic. For *S. aureus* isolates (Fig. 2B), 50 % or more isolates showed a resistant profile for P and E. None of the strains tested had intermediate resistance to the antibiotics tested. The *S. aureus* isolates were complete susceptible to MH, CHL, GEN, and LZD. *S. schleiferi* isolates were completely susceptible to all antibiotics tested except for P, OX, DA, TET, and E (Fig. 2C). The other *Staphylococcus* spp. (Fig. 2D) were completely susceptible to the antibiotics MH, CHL, and LZD, while 50 % or more isolates showed a resistant profile for P, DA, and E.

Fig. 3A-C shows the resistance profiles for the species *S. pseudintermedius*, *S. felis,* and *S. aureus* isolated from cats, respectively. The resistance profiles shown in Fig. 3D are for the other *Staphylococcus* spp. identified. For *S. pseudintermedius* (Fig. 3A), 50 % or more isolates showed a resistant profile for P, OX, DA, TET, ENR, MBX, E, CHL, and SMX. Furthermore, *S. pseudintermedius* isolates tested were completely susceptible to CPT, AK, TGC, LZD, and QD. For the *S. felis* isolates (Fig. 3b), all isolates were totally susceptible to P, FOX, DOX, TET, MH, ENR, MBX, CHL, LZD and SMX, and some showed an intermediate profile to DA, E and GEN. Among the *S. aureus* isolates (Fig. 3c), 50 % or more isolates showed a resistant profile for P, and were completely susceptible to MH, CHL and LZD. The other *Staphylococcus* spp (Fig. 3d) were totally susceptible to the antibiotics MH, CHL, and LZD, and

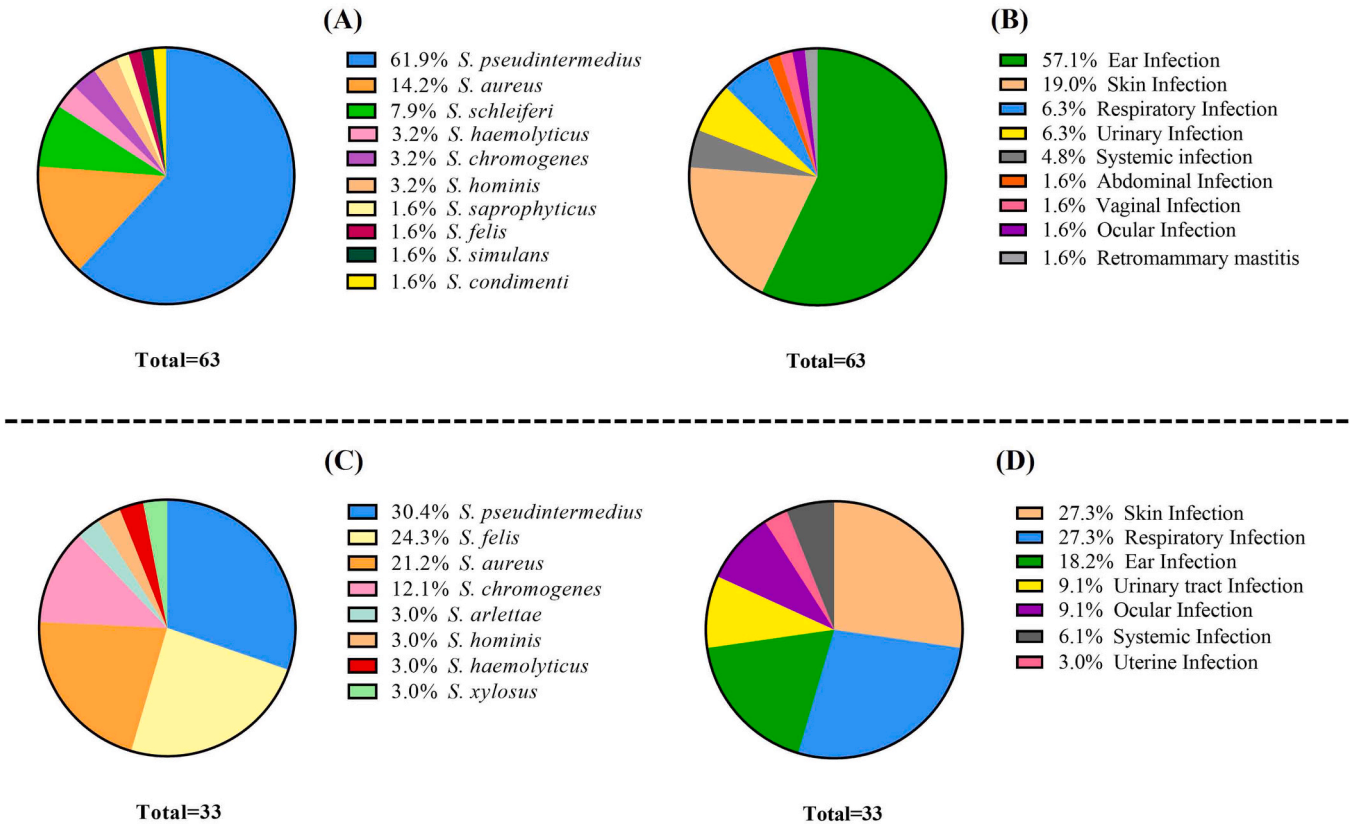

Fig. 1. Prevalence and distribution of *Staphylococcus* spp. according to the source of the sample's isolation in dogs (A and B, respectively) and cats (C and D), with symptoms of bacterial infection, including animals being treated in veterinary clinics/hospitals and cadavers submitted for necropsy at INIAV.

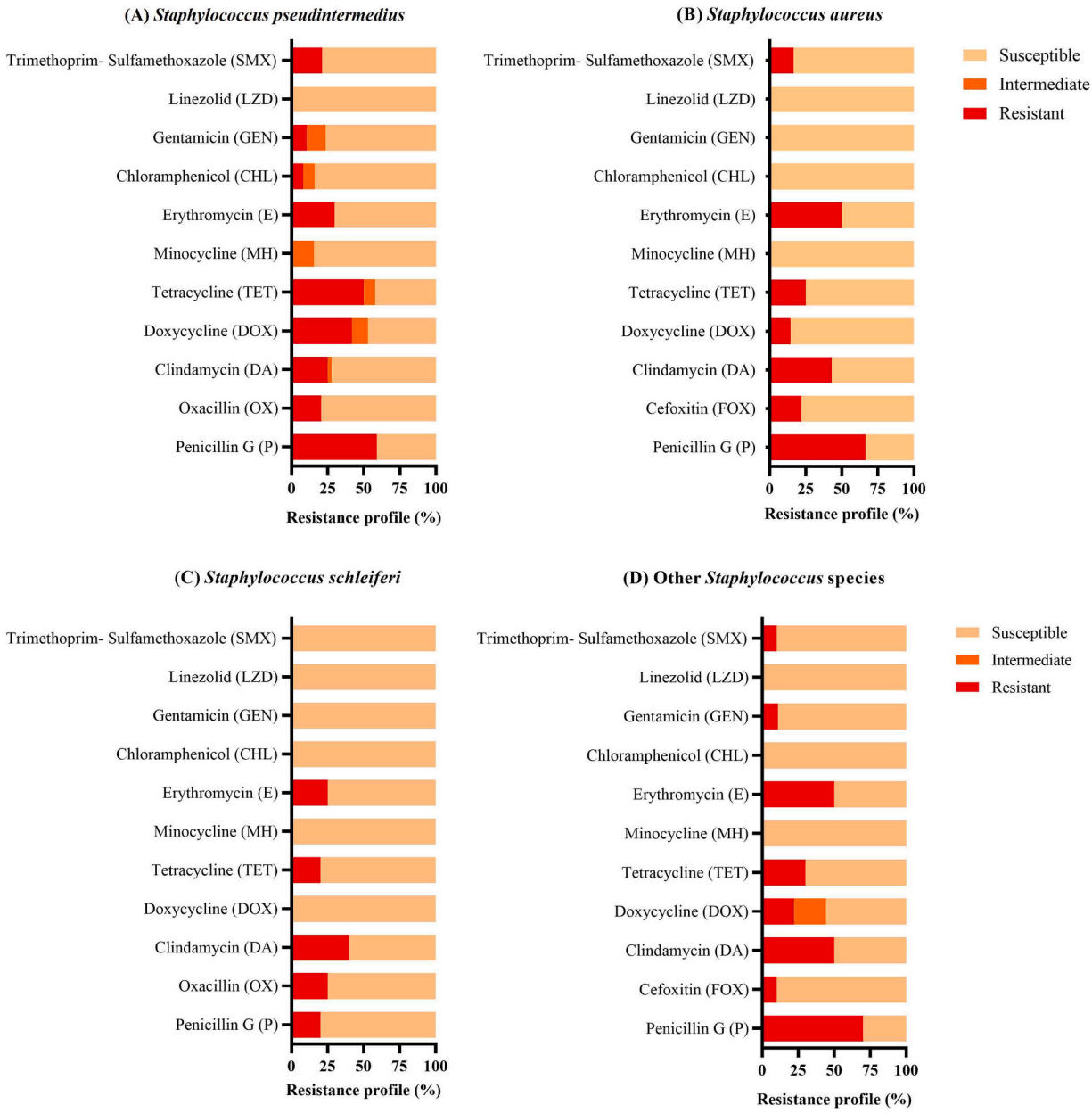

Fig. 2. Antimicrobial resistance profiles of *Staphylococcus* spp. isolated from dogs with symptoms of bacterial infection: (A) *S. pseudintermedius*; (B) *S. aureus*; (C) *S. schielferi and (D)* other *Staphylococcus* spp. (*S. chromogenes, S. haemolyticus, S. hominis, S. felis, S. simulans, S. condimenti and S. saprophyticus*). The antimicrobial resistance profile of *Staphylococcus* isolates was determined according to CLSI criteria.

showed a resistance profile lower than 50 % for all the other antibiotics tested.

Of the 96 *Staphylococcus* isolates analysed, 35 (36 %) were considered MDR due to having a resistance profile against antibiotics from 3 or more classes. Of these, 22 were isolated from dogs and included 12 (12 %) *S. pseudintermedius*, 4 (4 %) *S. aureus*, 2 (2 %) *S. haemolyticus*, 2 (2 %) *S. hominis*, 1 (1 %) *S. schleiferi* and 1 (1 %) *S. saprophyticus*. On the other hand, 13 MDR isolates came from cats and included 9 (9 %) *S. pseudintermedius*, 2 (2 %) *S. aureus*, 1 (1 %) *S. arlettae* and 1 (1 %) *S. haemolyticus*.

In addition, 24 isolates (25 %) showed an MRS phenotype (resistance to oxacillin for *S. pseudintermedius/S. schleiferi* or to cefoxitin for *S. aureus*/other *Staphylococcus* spp.), all of them classified as MDR isolates. Of these, 12 MRS isolates were from dogs, including 8 *S. pseudintermedius,* 2 *S. aureus,* 1 *S. schleiferi,* and 1 *S. haemolyticus*, and 12 MRS isolates were from cats, including 8 *S. pseudintermedius,* 2 *S. aureus,* 1 *S. haemolyticus,* and 1 *S. hominis.*

Screening of Staphylococcus spp. for acquired resistance and other virulence genes

The presence of methicillin-resistance genes (*mecA* and *mecC*) and two other virulence genes (*pvl* and *tsst-1*) was analysed for a random set of *Staphylococcus* isolates (n=57). This subgroup consisted of 34 isolates from dogs (including 26 *S. pseudintermedius,* 3 *S. schleiferi,* 1 *S. aureus,* 1 *S. simulans,* 1 *S. condiment*, 1 *S. felis* and 1 *S. haemolyticus*) and 23 isolates from cats (including 8 *S. felis,* 6 *S. pseudintermedius,* 6 *S. aureus,* 1 *S. arlettae,* 1 *S. hominis* and 1 *S. haemolyticus*), of which 15 isolates had an MRS phenotype. The genetic profile of the *Staphylococcus* isolates is shown in Table 2. Of the methicillin-resistance genes tested, the *mecA* was identified in 15 *Staphylococcus* isolates tested, including 5 *S. pseudintermedius* and 1 *S. haemolyticus* from dog samples, and

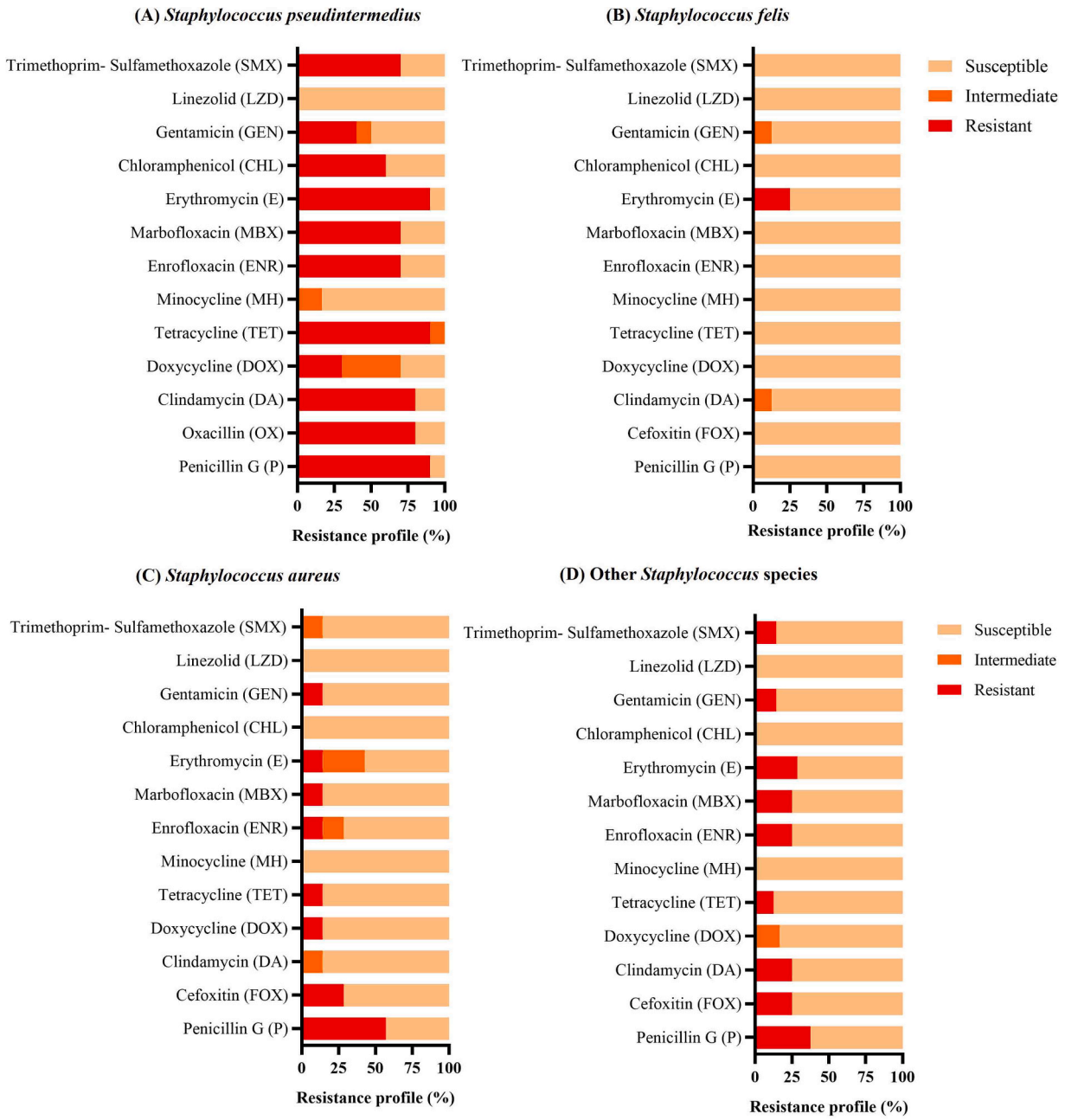

Fig. 3. Antimicrobial resistance profiles of *Staphylococcus* spp isolated from cats with symptoms of bacterial infection: (A) *S. pseudintermedius*; (B) *S. felis*; (C) *S. aureus* and (D) other *Staphylococcus* spp. *(S. chromogenes*, *S. arlettae*, *S. hominis*, *S. haemolyticus,* and *S. xylosus*). Antimicrobial resistance portfolio of *Staphylococcus* isolates was determined according to CLSI criteria.

Table 2
Percentages of antibiotic resistance genes and virulence genes of 57 *Staphylococcus* isolates selected. ND: not detected.

Function	Genes	Dog isolates (n=34)		Cat isolates (n=23)		% Total of isolates
Methicillin resistance genes	*mecA*	6 out of 57 (10.5 %)	5 *S. pseudintermedius* out of 57 (8.8 %) 1 *S. haemolyticus* out of 57 (1.8 %)	9 out of 57 (15.8 %)	5 *S. pseudintermedius* out of 57 (8.8 %) 2 *S. aureus* out of 57 (3.5 %) 1 *S. hominis* out of 57 (1.8 %) 1 *S. haemolyticus* out of 57 (1.8 %)	26.3 %
	mecC	ND		ND		0 %
Toxin production	*pvl*	ND		3.5 %	2 *S. felis* out of 57 (3.5 %)	3.5 %
	tsst-1	ND		ND		0 %

5 *S. pseudintermedius,* 2 *S. aureus* and 1 *S. hominis,* 1 *S. haemolyticus* from cat samples (Table 2). It is noteworthy that all *mecA*-positive isolates correspond to the MRS isolates previously identified phenotypically. Furthermore, the *pvl* virulence gene was identified in 2 *S. felis* isolates recovered from feline samples. The *mecC* and *tsst-1* genes were not detected in any of the isolates tested.

Discussion

Staphylococcal infections in companion animals, such as dogs and cats, are a widespread problem in veterinary medicine (Sigudu et al., 2023) and *Staphylococcus* spp., particularly *S. pseudintermedius* and *S. aureus*, are commonly associated with a variety of infections, including skin, ear, and respiratory problems (Garcês et al., 2022). Increasingly, *Staphylococcus* strains are resistant to a wide range of antibiotics, which poses a significant challenge to veterinarians. Monitoring and understanding *Staphylococcus* infections is also essential, as companion animals may serve as potential reservoirs of drug-resistant bacteria, highlighting the importance of responsible antibiotic use and surveillance in both veterinary and public health contexts (Garcês et al., 2022). Further research is needed to understand the characteristics of *Staphylococcus* strains involved and their potential antibiotic resistance patterns, which could guide treatment decisions for these infections.

This study investigated the antimicrobial resistance profiles of *Staphylococcus* spp. isolated from dogs and cats with symptoms of bacterial infection, including animals being treated in veterinary clinics/hospitals and cadavers submitted for necropsy at INIAV between 2021 and 2023.

Of the 96 *Staphylococcus* isolates, it was observed that *S. pseudintermedius* was the most common species in dogs and cats, with 62% and 30 %, respectively (Fig. 1). Similarly, a study conducted in Colombia between 2016 and 2019, also found that of 771 *Staphylococcus* isolates from dogs and cats, 53 % were identified as *S. pseudintermedius* (Gómez-Beltrán et al., 2020a). In the same study, 13 % of the isolates were positive for *S. aureus*, a result that is very similar to that found in this study in dogs (13.6 %). In a study conducted in the central region of Portugal, Andrade and colleagues (Andrade et al., 2022) found similar results for *Staphylococcus* isolated from the skin of companion animals. Of 237 isolates, 65 % were identified as *S. pseudintermedius* and 23 % as *S. aureus*.

It is worth noting that the clinical samples were collected from different sites of infection, and in both canine and feline samples, *Staphylococcus* isolates were most isolated from ear, skin, and respiratory infections (Fig. 1A and D). The study by Gómez-Beltrán *et al.* concluded that ear and urinary tract infections are among the most common niches for *Staphylococcus* isolation in companion animals (Gómez-Beltrán et al., 2020).

The evaluation of resistance profiles in bacterial isolates is vital in addressing the growing problem of antimicrobial resistance. By studying isolates obtained from infections and assessing their resistance to previously administered antibiotics, researchers and healthcare providers can better understand the mechanisms of resistance and identify effective treatment options. Additionally, monitoring resistance patterns in animals is essential to control the spread of drug-resistant bacteria and protect public health.

In this way, the resistance profile of *Staphylococcus* isolates to a group of antibiotics that are commonly used in veterinary clinical practice was assessed. It should be noted, that 36% of *Staphylococcus* isolates are considered MDR (resistant to 3 or more classes of antibiotics), and approximately 25 % are methicillin-resistant *Staphylococcus* (MRS) (Rynhoud et al., 2021). *S. pseudintermedius* isolates from cats were all resistant to tetracycline, an antibiotic widely used in veterinary medicine. This suggests that tetracycline is not a viable option for treating *Staphylococcus* infections, supporting several evidence of the high percentages of resistance of these species in animal isolates (Rao et al., 2022; Schwarz et al., 1998). *S. pseudintermedius* isolates from dogs were more resistant to antibiotics of the β-lactams and tetracycline classes (Fig. 2a). On the other hand, *S. aureus* isolates were more resistant to antibiotics belonging to the β-lactam classes. This result was expected as these antibiotics are commonly used to treat these infections, which could lead to an increase in bacterial resistance to these classes (Khairullah et al., 2023). Similar studies have shown that *S. aureus* isolates generally have high levels of resistance to β-lactam antibiotics, as reported in a recent study by Costa and colleagues that showed a high level of resistance to penicillin (approximately 80 %) (Costa et al., 2022). Among the isolates from the cat samples, isolates of *S. pseudintermedius* were quite resistant to all the classes of antibiotics that were tested (Fig. 3A). Thus, in our study, comparing the results obtained from dogs and cats, it can be concluded that *S. pseudintermedius* isolates from cats are generally more resistant to the antibiotics tested than isolates from dogs (Figs. 2A and 3A). Additional studies would be needed to further investigate the reasons behind this difference and determine if it is specific to certain regions or populations.

It is also important to note that resistance to linezolid was not detected in either canine or feline samples. This result was expected, as this antibiotic is typically used as a last resort for treating persistent infections caused by Gram-positive pathogens (Lienen et al., 2022).

As mentioned, MRS strains are particularly important, which are the most commonly used in human and veterinary medicine (Caneschi et al., 2023; Jung et al., 2023). Resistance to β-lactam antibiotics among *Staphylococcus* strains is usually conferred by the presence of the *mecA* and *mecC* resistance genes (Ballhausen et al., 2014). In addition, MRS strains can be transmitted bidirectionally between companion animals and their owners, increasing their spread and representing a zoonotic risk and public health concern (Saputra et al., 2017).

By recognizing and addressing this threat, we can work towards safeguarding public health from the dangers posed by these resistant bacteria. This study found a higher proportion of MRS isolates from cats (36 %) compared to dogs (17 %). The genetic factor responsible for the MRS phenotype detected in all the isolates tested was *mecA*, supporting the fact that this remains the most prevalent resistance gene (Platenik et al., 2022; Silva et al., 2022) (Table 2). The *mecA* gene was most frequently detected in *S. pseudintermedius*, followed by *S. aureus* and *S. haemolyticus*. In a study conducted by Lord *et al.* in the United States of America between 2006 and 2017, the prevalence of the *mecA* gene in *S. pseudintermedius* and *S. aureus* was higher than 90 % in strains resistant to oxacillin and cefoxitin (Lord et al., 2022).

The *pvl* and *tsst*-1 are genes that codified for virulence toxins, responsible for severe infections, particularly in the skin and soft tissues (Asanin et al., 2019). Therefore, the presence of both the *pvl* and *tsst-1* genes in *Staphylococcus* strains indicates a higher potential for causing more severe infections in both animals and humans (Shallcross et al., 2013). Healthcare professionals and veterinarians need to be aware of the presence of these genes in *Staphylococcus* strains as it can help guide treatment decisions and infection control measures. In a study conducted by Liu et al. in China, only 2.8 % of 143 *S. aureus* isolated from animals and humans tested positive for this toxin (Liu et al., 2018). In another study in Serbia, out of 36 MRSA isolates, only three human isolates showed *tsst-1* gene (Asanin et al., 2019). The present study analysed the prevalence of these genes in 57 *Staphylococcus* isolates and it is revealed a low prevalence of these genes in the genome of the *Staphylococcus* strains, with only two *S. felis* strains isolated from cats containing the *pvl* gene. These findings suggest that the presence of the *pvl* gene in the *Staphylococcus* strains is relatively rare. This rarity may indicate that the *S. felis* strains with the *pvl* gene are more virulent than other strains, as the *pvl* gene is known to be associated with increased virulence in *Staphylococcus*. Even so, *pvl*-positive *S. felis* isolates did not show an MDR and/or MRS phenotype, demonstrating antimicrobial treatment options for severe infections.

Conclusions

In conclusion, cats and dogs can harbor various *Staphylococcus* spp. including MDR and MRS strains that are of medical and public health concern. Our data confirms a high occurrence of antibiotic-resistant *Staphylococcus* strains, mostly to the β-lactam class, which could guide decisions for treating *Staphylococcus* infections. In addition, 36 % of all isolates were found to be MDR and 25 % of the isolates had an MRS phenotype, and the genetic determinant detected in all the isolates in the subgroup tested was the *mecA* resistance gene. Given the proximity of companion animals to humans, the identification of multidrug-resistant isolates is a serious concern. Transmission of these strains between companion animals and humans is possible, highlighting the need for more responsible use of antibiotics in veterinary medicine and the implementation of surveillance programs to monitor the emergence of these species.

Declaration of Competing Interest

The authors declare no conflict of interest.

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References

Achi, C., Thomson, P., García, P., Miles, J., Isla, D., Yáñez, C., Santibáñez, R., Núñez, A., Flores-Yáñez, C., Río, C., Del, Cuadra, F., 2022. Isolation and identification of *Staphylococcus* species obtained from healthy companion animals and humans. Veterinary Sciences 9 (2), 79.

Afshar, M.F., Zakaria, Z., Cheng, C.H., Ahmad, N.I., 2023. Prevalence and multidrug-resistant profile of methicillin-resistant *Staphylococcus aureus* and methicillin-resistant *Staphylococcus pseudintermedius* in dogs, cats, and pet owners in Malaysia. Veterinary World 16, 536–545.

Andrade, M., Oliveira, K., Morais, C., Abrantes, P., Pomba, C., Rosato, A.E., Couto, I., Costa, S.S., 2022. Virulence potential of biofilm-producing *Staphylococcus pseudintermedius*, *Staphylococcus aureus* and *Staphylococcus coagulans* causing skin infections in companion animals. Antibiotics 11 (10), 1339.

Asanin, J., Misic, D., Aksentijevic, K., Tambur, Z., Rakonjac, B., Kovacevic, I., Spergser, J., Loncaric, I., 2019. Genetic profiling and comparison of human and animal methicillin-resistant *Staphylococcus aureus* (Mrsa) isolates from Serbia. Antibiotics 8 (1), 26.

Assouma, F.F., Sina, H., Dossou, A.D., Socohou, A., Hounsou, M.C., Avogbe, P.H., Boya, B., Mousse, W., Adjanohoun, A., Baba-Moussa, L., 2023. Antibiotic resistance profiling of pathogenic *Staphylococcus* species from urinary tract infection patients in Benin. BioMed Research International 2023, 6364128.

Ballhausen, B., Kriegeskorte, A., Schleimer, N., Peters, G., Becker, K., 2014. The *mecA* homolog *mecC* confers resistance against β-lactams in *Staphylococcus aureus* irrespective of the genetic strain background. Antimicrobial Agents and Chemotherapy 58, 3791–3798.

Blaiotta, G., Ercolini, D., Pennacchia, C., Fusco, V., Casaburi, A., Pepe, O., Villani, F., 2004. PCR detection of staphylococcal enterotoxin genes in *Staphylococcus* spp. strains isolated from meat and dairy products. Evidence for new variants of seG and seI in *S. aureus* AB-8802. Journal of Applied Microbiology 97, 719–730.

Burke, M., Santoro, D., 2023. Prevalence of multidrug-resistant coagulase-positive staphylococci in canine and feline dermatological patients over a 10-year period: a retrospective study. Microbiology 169 (2), 001300.

Caneschi, A., Bardhi, A., Barbarossa, A., Zaghini, A., 2023. The use of antibiotics and antimicrobial resistance in veterinary medicine, a complex phenomenon: a narrative review. Antibiotics 12 (3), 487.

Clinical and Laboratory Standards Institute, 2024. CLSI VET01S ED7:2024 Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals, 7th Edition. http://vet01s.edaptivedocs.net/GetDoc.aspx?doc=CLSI%20VET01S%20ED7:2024&scope=user (accessed 13 May, 2024).

Costa, S.S., Ribeiro, R., Serrano, M., Oliveira, K., Ferreira, C., Leal, M., Pomba, C., Couto, I., 2022. *Staphylococcus aureus* causing skin and soft tissue infections in companion animals: antimicrobial resistance profiles and clonal lineages. Antibiotics 11 (5), 599.

Garcês, A., Lopes, R., Silva, A., Sampaio, F., Duque, D., Brilhante-Simões, P., 2022. Bacterial isolates from urinary tract infection in dogs and cats in Portugal, and their antibiotic susceptibility pattern: A retrospective study of 5 years (2017–2021). Antibiotics 11 (1520), 1–10.

Gómez-Beltrán, D.A., Villar, D., López-Osorio, S., Ferguson, D., Monsalve, L.K., Chaparro-Gutiérrez, J.J., 2020a. Prevalence of antimicrobial resistance in bacterial isolates from dogs and cats in a veterinary diagnostic laboratory in Colombia from 2016–2019. Veterinary Sciences 7, 1–11.

Jung, H.R., Lee, Yu. Jin, Hong, S., Yoon, S., Lim, S.K., Lee, Young Ju, 2023. Current status of β-lactam antibiotic use and characterization of β-lactam-resistant *Escherichia coli* from commercial farms by integrated broiler chicken operations in Korea. Poultry Science 102 (12), 103091.

Khairullah, A.R., Sudjarwo, S.A., Effendi, M.H., Ramandinianto, S.C., Gelolodo, M.A., Widodo, A., Riwu, K.H.P., Kurniawati, D.A., 2023. Pet animals as reservoirs for spreading methicillin-resistant *Staphylococcus aureus* to human health. Journal of Advanced Veterinary and Animal Research 10 (1), 1–13.

Lienen, T., Grobbel, M., Tenhagen, B.A., Maurischat, S., 2022. Plasmid-coded linezolid resistance in methicillin-resistant *Staphylococcus aureus* from food and livestock in germany. Antibiotics 11 (12), 1802.

Liu, B., Sun, H., Pan, Y., Zhai, Y., Cai, T., Yuan, X., Gao, Y., He, D., Liu, J., Yuan, L., Hu, G., 2018. Prevalence, resistance pattern, and molecular characterization of *Staphylococcus aureus* isolates from healthy animals and sick populations in Henan Province, China. Gut Pathogens 10, 31.

Lord, J., Millis, N., Jones, R.D., Johnson, B., Kania, S.A., Odoi, A., 2022. Patterns of antimicrobial, multidrug and methicillin resistance among *Staphylococcus* spp. isolated from canine specimens submitted to a diagnostic laboratory in Tennessee, USA: a descriptive study. BMC Veterinary Research 18 (1), 91.

Magiorakos, A.P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M.E., Giske, C.G., Harbarth, S., Hindler, J.F., Kahlmeter, G., Olsson-Liljequist, B., et al., 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clinical Microbiology and Infection 18, 268–281.

Platenik, M.O., Archer, L., Kher, L., Santoro, D., 2022. Prevalence of *mecA*, *mecC* and Panton-valentine-leukocidin genes in clinical isolates of coagulase positive staphylococci from dermatological canine patients. Microorganisms 10 (11), 2239.

Rao, S., Linke, L., Magnuson, R., Jaunch, L., Hyatt, D.R., 2022. Antimicrobial resistance and genetic diversity of *Staphylococcus aureus* collected from livestock, poultry and humans. One Health 15, 100407.

Rynhoud, H., Forde, B.M., Beatson, S.A., Abraham, S., Meler, E., Soares Magalhães, R.J., Gibson, J.S., 2021. Molecular Epidemiology of Clinical and Colonizing Methicillin-Resistant *Staphylococcus* Isolates in Companion Animals. Front Vet Sci 8, 620491.

Saputra, S., Jordan, D., Worthing, K.A., Norris, J.M., Wong, H.S., Abraham, R., Trott, D. J., Abraham, S., 2017. Antimicrobial resistance in coagulase-positive staphylococci isolated from companion animals in Australia: a one year study. PLOS One 12 (4), e0176379.

Schwarz, S., Roberts, M.C., Werckenthin, C., Pang, Y., Lange, C., 1998. Tetracycline resistance in *Staphylococcus* spp. from domestic animals. Veterinary Microbiology 63, 217–227.

Shallcross, L.J., Fragaszy, E., Johnson, A.M., Hayward, A.C., 2013. The role of the Panton-Valentine leucocidin toxin in staphylococcal disease: a systematic review and meta-analysis. The Lancet Infectious Diseases 13, 43.

Sigudu, T.T., Oguttu, J.W., Qekwana, D.N., 2023. Prevalence of *Staphylococcus* spp. from human specimens submitted to diagnostic laboratories in South Africa, 2012–2017. Southern African Journal of Infectious Diseases 38 (1), 477.

Silva, V., Monteiro, A., Pereira, J.E., Maltez, L., Igrejas, G., Poeta, P., 2022. MRSA in Humans, Pets and Livestock in Portugal: Where we came from and where we are going. Pathogens 11 (10), 1110.

Stegger, M., Andersen, P.S., Kearns, A., Pichon, B., Holmes, M.A., Edwards, G., Laurent, F., Teale, C., Skov, R., Larsen, A.R., 2012. Rapid detection, differentiation and typing of methicillin-resistant *Staphylococcus aureus* harbouring either *mecA* or the new *mecA* homologue mecALGA251. Clinical Microbiology and Infection 18, 395–400.