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Challenges in predicting *Staphylococcus* spp. β-lactamic resistance in pet animals

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The present work evaluated the species distribution associated with the antimicrobial susceptibility pattern by phenotypic analysis and *mec* and *bla* gene detection in 100 *Staphylococcus* strains from 219 clinical samples from cats and dogs in the state of Rio de Janeiro, Brazil. Oxacillin susceptibility profiles were detected by the Clinical and Laboratory Standards Institute (CLSI) recommended tests. The amplification of the *mecA* gene was positive in 25% of the *Staphylococcus* spp. The whole *mec* complex (*mecA-mecI-mecR1*) was detected in four phenotypically oxacillin-resistant isolates. The CLSI recommended nitrocefin-based test detected 38% (38/100) β-lactamase producers *Staphylococcus* strains. Also, 32% (32/100) *Staphylococcus* spp. strains tested positive for *bla* genes. The whole *bla* gene complex, *blaZ-blaI-blaR1*, was detected in 7.8% (3/38) of the nitrocefinase-positive isolates. β-Lactamases was well spread among the samples and it seems to be a prevalent mechanism in resistant staphylococci strains from pet animals. The *mec* and *bla* gene regulatory systems can interfere in expression of resistance mediated by PBPs and β-lactamases, conferring the heterogeneous oxacillin-resistance in *Staphylococcus* spp. detected by phenotypic tests.

Key words: *Staphylococcus* spp., oxacillin, β-lactamase, *mec*A, pet animals.

INTRODUCTION

In veterinary medicine, *Staphylococcus* spp. are important agents of infectious diseases of several animal species. Besides *Staphylococcus aureus*, other coagulase-positive staphylococci (CoPS) have been reported to be important pathogens. The reclassification of *Staphylococcus intermedius,* a coagulase-positive species firstly described in 1976, was proposed by Devriese et al. (2005), creating the *S. intermedius* group (SIG) including *S. intermedius,* the new species *S. pseudintermedius* and *S. delphini*. This proposal was based in the high genotypic diversity observed in the formerly considered *S. intermedius* strains (Bannoehr and Guardabassi, 2012).

Like *S. aureus*, the *S. intermedius* strains isolated from

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animals have been reported to produce an array of virulence factors, including leukotoxin, enterotoxin, and hemolysins, together with elements essential for biofilm formation (Hanselmann et al., 2008). Nowadays, several CoPS species, such as *S. aureus*, *S. pseudintermedius, S. schleiferi* subsp. *coagulans,* and also coagulase-negative *Staphylococcus* spp. (CoNS), are implicated in the etiology of animal diseases, such as suppurative disease, mastitis, arthritis and urinary tract infection, due to their virulence factors (Futagawa-Saito et al., 2006; Silva et al., 2003). *Staphylococcus* species are usually resistant to ß-lactams, aminoglycosides and macrolides. The emergence and dissemination of antimicrobial resistance among staphylococci is an important problem in human and veterinary medicine worldwide because therapeutic options are becoming limited. Dogs and cats are an important source of the spread of this resistance due to the extensive use of antimicrobials and the contact with their owners (Frank and Loeffler, 2012; McCarthy et al., 2012; Morgan, 2008).

Oxacillin resistance is of particular relevance because it is conferred by different mechanisms and acts as a resistance marker for overall beta-lactamic resistance. The most-studied mechanism is related to the presence of the *mecA* gene, located on a staphylococcal chromosomal cassette (SCCmec) (Ito et al., 2001). Jansen et al. (2009) sequenced and typed SCCmec of animal origin (Jansen et al., 2009). This resistance is conferred by the production of an altered penicillin binding protein (PBP2a) with low affinity for all β-lactamic antimicrobials. The *mecA* gene expression and therefore PBP2a production is regulated by the *mecR1-mecI* gene system. The *mecI* gene codifies a repressive protein and *mecR1* a signal transmembrane protein inducible by β-lactamic antimicrobials (Petinaki et al, 2001). Whether these genes are expressed or not, they can confer heterogeneous phenotypes, possibly leading to misidentification by laboratory practitioners. Usually the SCCmec contains additional genetic material, such as *Tn554*, pUB110 and pT181, which encode resistance to multiple classes of antimicrobials frequently applied in hospitals (Hanselmann et al., 2008; Katayama et al., 2001).

Also, oxacillin-resistant *Staphylococcus* may constitute or inductively produce ß-lactamases enzymes that cleave the ß-lactam ring and inactivate the antibiotic (Li et al., 2007). ß-lactamase enzymes interfere in oxacillin resistance by the action of the *blaZ* gene complex, which includes a regulatory system composed of *blaZ*, *blaR1* and *blaI* genes (Rosato et al., 2003). These genes are located on Tn552, a transposon completely sequenced and inserted in SCC*mec* (Rowland and Dyke, 1990). More than 90% of staphylococcal isolates that produce ß-lactamase codified by the *blaZ* gene contain a *blaZ* regulatory system (*blaI* and *blaR1*) similar in sequence and function to *mecA* regulators (*mecA-mecR1-mecI*, promoter-operator-repressor system) (Mckinney et al., 2001).

The Clinical and Laboratory Standards Institute (CLSI) has standardized phenotypic testing for detection of oxacillin resistance considering the use of the cefoxitin/oxacillin disk diffusion test according to *Staphylococcus* species. In its latest version (CLSI, 2013), *mecA* gene detection is not considered a gold standard anymore, since the multiplicity of oxacillin-resistance factors requires careful investigation including detection of different resistance genetic markers for correct interpretation of heterogeneous phenotypes expressed by *Staphylococcus* spp. strains. The present study evaluated staphylococci species distribution in 100 strains from cats and dogs obtained from veterinary clinics in the state of Rio de Janeiro, Brazil. Also, their antimicrobial resistance pattern was established based on phenotypic characteristics and *mec*A and *bla* gene detection.

MATERIALS AND METHODS

Sampling

Clinical specimens from 185 dogs and 34 cats were harvested from distinct infectious sites, during routine care in a small animal veterinary clinic of Federal Rural University of Rio de Janeiro (HVPA-UFRRJ) and veterinary care units from different regions of Rio de Janeiro state, Brazil, between 2006 and 2010. The samples were obtained from canine external otitis, skin lesions, urinary and respiratory tract infections, pyometra, periodontitis and conjunctivitis. Bacterial identification and antimicrobial susceptibility assays were performed at the Veterinary Bacteriology Laboratory of Federal Rural University of Rio de Janeiro (LABAC-VET/UFRRJ). Results were sent back to the attending veterinarians to help in diagnosis and therapeutic procedures.

Staphylococcus spp. identification

Samples were inoculated primarily in blood agar (blood agar base enriched with 5% sheep blood) and incubated at 35°C for 24 h. Then the isolates were submitted to the routine microbiological diagnostics, including inoculation in selective medium for analysis of cultural properties, catalase and coagulase production, hemolysis pattern, maltose and D-mannitol fermentation, acetoin production and nitrate reduction (Winn et al., 2006). After phenoltypic identification, isolates were submitted to polymerase chain reaction for 16S rRNA to confirm the presence of *Staphylococcus* spp. (Zhang et al., 2004). Furthermore, PCR amplification of endonuclease genes (*nuc1* and *nuc2*) was performed to identify *S. hyicus.* Strains of *S. pseudintermedius* and *S. aureus* were characterized by the amplification of *nuc3* and *nuc4* genes and 23S rDNA, respectively (Sazaki et al., 2010; Silva et al., 2003). The following standard strains were used as controls: ATCC 29213 *S. aureus*, ATCC 29663 *S. intermedius*, *S. hyicus* 5368 and *S. schleiferi* 3975.

Disk diffusion test

Assays were performed using the method and interpretation criteria according to CLSI standards (CLSI, 2011), after overnight incubation at 35°C followed by measurement of inhibition zone diameters. *Staphylococcus* spp. antimicrobial susceptibility was

Table 1. Distribution of Staphylococcus species per sites of infection.

Staphylococcus species	Sites of infection* (number of isolates)										
	CO	SK	UTI	RTI	PY	OMI	CMI	GI	OM	Total	Isolates
Total species											100
S. intermedius	13	11	5	-	1	1	2	-	2		35
S. aureus spp. *aureus*	11	6	1	-	2	1	3	-	-		24
S. hyicus	11	1	1	-	-	-	-	-	-		13
S. aureus spp*. anaerobius*	-	-	-	-	1	-	-	-	-		1
S. schleiferi spp. *coagulans*	2	4	-	-	-	-	-	-	-		6
CPS**	1	4	3	-	1	-	-	-	-		9
S. xylosus	4	2	-	-		1	-	-	-		7
S. hominis	-	2	-	-	1	-	-	-	-		3
S. epidermidis	-	-	-	2	-	-	-	-	-		2

*CO, Canine otitis; SI, skin infection; UTI, urinary tract infection; RTI, respiratory tract infection; PY, pyometra; OMI, oral mucosal infection; CMI, conjunctive mucosal infection; GI, gastrointestinal infection; OM, osteomyelitis. **CPS, coagulase-positive *Staphylococcus* spp. not genetic defined.

evaluated according to the antimicrobial class clinical recommendation for each infectious site, including β-lactamic, macrolide, lincosamide, streptogramin, quinolone, tetracycline and aminoglicosyde. *S. aureus* ATCC25923 and *Escherichia coli* ATCC25922 were used as quality controls.

Oxacillin susceptibility tests

Resistance to oxacillin was determined according to phenotypic tests recommended by the CLSI (2013). The disk diffusion test was applied using oxacillin (1 µg) and cefoxitin (30 µg) disks (Sensifar-Cefar®), in an agar screen plate containing 6 µg /ml of oxacillin with Müller Hinton agar supplemented with NaCl (4% w/v; 0.68 mol/L). *S.aureus* ATCC29213 was used as quality control.

β-lactamase production

The nitrocefin disk test was applied to detect *Staphylococcus* spp. strains that produce chromogenic β-lactamase, in accordance with the CLSI standard (CLSI, 2013). *S.aureus* ATCC29213 was used as quality control.

DNA extraction and PCR analysis

A 1.5-ml overnight culture of a single *Staphylococcus* colony was centrifuged for 30 s at 14,000 rpm, washed twice in 1 mL TE buffer (10 m*M* Tris HCl, pH 8.0; 1 m*M* EDTA; 100 m*M* NaCl). The resulting pellet was resuspended in 400 µL of TE buffer including 5 µL of lysostaphin (stock concentration 1 µg/mL; Sigma-Aldrich) and incubated for 30 min at 37°C. Lysis was completed by 10 min of water incubation at 100°C. PCR assays of *mec* and *bla* gene complexes were performed using the primers and respective program previously described (Petinaki et al., 2001). The reaction was performed in a final volume of 20 µL of mixture containing PCR buffer (10 mM TrisHCl, pH 9.0; 50 mM KCl, and 0.1% Triton X-100), 3.5 mM $MgCl_2$, 250 µM of each of the deoxynucleoside triphosphates, 3.0 µM of each gene-specific primer, 2.5 U of *Taq* DNA polymerase (Promega, Madison, WI) and 5 µL of template. Amplicons were detected by 1.5% agarose gel, stained with ethidium bromide solution (0.5 mg/mL) and examined under a UV transilluminator (UvTrans). *S.aureus* ATCC43300 was used as quality control.

RESULTS AND DISCUSSION

A total of 100 *Staphylococcus* spp. isolates were phenotypically characterized, as the following species: *S. aureus* spp. *aureus* (n=24), *S. pseudintermedius* (n=44), *S. aureus* spp. *anaerobius*, (n=1), *Staphylococcus schleiferi* spp. *coagulans* (n=6) and coagulase-negative *Staphylococcus* spp. (CoNS) (n=12), represented by *S. xylosus*, *S. epidermidis* and *S. hominis*. PCR amplification of endonuclease genes (*nuc1* and *nuc2*) identified 13% (13/100) *S. hyicus*. Also 35% *S. pseudintermedius* (35/100) and 24% *S. aureus* (24/100) were genetically characterized by PCR amplification of *nuc3* and *nuc4* genes and 23S rDNA, respectively. Table 1 presents *Staphylococcus* species distribution considering the different sites of infection.

These results suggest widespread distribution of staphylococci species in pet animals' infectious sites and corroborate the importance of correct microbiological identification. In veterinary medicine, other CoPS have frequently been misidentified as *S. aureus* strains, due to their common phenotypic traits.

Unfortunately, there has been no reliable phenotypic method to distinguish among CoPS species in veterinary clinical laboratories. In the present study, we used PCR of the thermonuclease (*nuc*) genes to improve the identification of staphylococcal species, as recommended by Sasaki et al. (2010).

Empirical treatment based on *Staphylococcus aureus* as the traditional staphylococci pathogen leads to therapeutic failures and antimicrobial resistance development. As a matter of fact, the latest report from CLSI (2013) established different criteria for oxacillin-

resistance evaluation for *S. aureus* and S. *pseudintermedius* due to their importance in clinical therapy.

In the present study, the disk diffusion test was performed to form a resistance panel for the most used antimicrobial class in staphylococci infections. We detected a low level of resistance to the associations between ampicillin and sulbactam (2%) and amoxicillin and clavulanic acid (5%). This was also true for the antibiotics with restricted use due to high cost, such as imipenen (2%) and linezolide (7%) (Table 2). Tenover et al. (2007) comparing the most commonly used susceptibility testing methods challenged with linezolid-non susceptible staphylococci, concluded that generally the problem was much greater in the non-detection of resistance rather than a possible overcalling of resistance. For this study, the criterium adopted to report linezolide resistance followed CLSI standards that consider any discernible growth within the zone of inhibition as indicative of resistance to linezolide. The highest level of resistance was recorded for β-lactamic antimicrobials, such as penicillin (78%), ampicillin (66%) and ceftriaxone (64%). For the other compounds tested, the resistance was intermediate.

Despite the well-known reduction in β-lactamic efficacy, this class of antimicrobials remains widely used, mainly because of the low treatment cost. Most β-lactamic bacterial resistance mechanisms, such as β-lactamases production and transmembrane permeability reduction can interfere in the activity of other antimicrobial classes when staphylococci species are involved in the infection's etiology, justifying the importance of adopting a susceptibility test before prescribing the drug treatment regimen. Table 2 presents an antimicrobial resistance panel for the antimicrobial classes used to treat staphylococcus infections.

All *Staphylococcus* spp. isolates were submitted to oxacillin and cefoxitin disk diffusion tests. Resistances of 37% (37/100) and 53% (53/10) were detected, respectively. The oxacillin agar screen test detected 57% (57/100) resistance. The evaluation of phenotypic tests linked to *mec*A gene complex detection yielded 15 different oxacillin-susceptibility profiles (Table 3), confirming *Staphylococcus* spp. as a heterogeneous resistance phenotype. Because of this disparity and the difficulty to establish reliable parameters, the CLSI standard procedures are under constant revision. For the prediction of phenotypic oxacillin resistance, some important new recommendations were recently published (CLSI, 2013). The cefoxitin disk test should be performed for *S. aureus* and CoNS and diffusion zones larger than 21 and 24 mm, respectively, should be reported as oxacillin-resistant. Oxacillin disk diffusion is considered the best way to detect *mec*A mediated resistance in *S. pseudintermedius* and should be performed instead of cefoxitin diffusion, which is not considered predictive for *mec*A mediated resistance in this species anymore (CLSI, 2013).

The detection of the *mecA* gene used to be considered a gold standard for the prediction of oxacillin resistance in *Staphylococcus* spp. Recently, the CLSI (2013) changed this criterion, considering the different mechanisms underlying this resistance. In this study, from all isolates tested by PCR assay, just 25% (25/100) were positive for this gene. Among *Staphylococcus* species, a total of 36% (8/22) were *Staphylococcus aureus mecA* +. Other *mecA*+ isolates corresponded to 23% (8/35) *S. intermedius* and 23% (3/13) *S. hyicus*. Coagulase-negative *Staphylococcus* spp. presented a total of 50% (6/12) *mecA*-positive isolates, and the species were: *S. xylosus* (3/6), *S. epidermidis* (2/6) and *S. hominis* (1/6). Results of the *mec* regulatory system assays are presented in Table 2. It was possible to detect the whole *mec* genic complex, *mecA-mecI-mecR1*, in 16% (4/25) of *mec*A+ staphylococcus spp. In this study, the strains presenting the whole regulatory complex were phenoltypically oxacillin-resistant in all performed assays. In contrast, 16% (4/25) of *Staphylococcus* isolates that tested positive to *mecA-mecI* genes presented an oxacillin-susceptible pattern. This might be related to the strong repressive activity exerted by *mecI* in the *mecA* gene. Seven isolates (28%) tested positive to *mecA-mecR1* genes and were also phenotypically oxacillin-resistant, probably due to *mecRI* repression in the *mecI* gene. Mckinney et al. (2001) remarked that the *mecR1* gene is correlated to a membrane signal transduction system which recognizes the extracellular presence of a β-lactamic antimicrobial and induces the transcription of the *mecA* gene. Ten (10/25) *Staphylococcus* spp. isolates tested positive only for the *mecA* gene and were phenotypically oxacillin resistant, as expected by the expression of constitutive PBP2A (Li et al., 2007). These divergent results in the detection of the mec complex can be related to gene mutation or deletion, as described by Katayma and Hiramatsu (2001). It is possible that rare but significant differences in primer annealing sites causes impairment of whole *mec* gene complex detection as observed by Melo et al. (2014) in bovine isolates.

The *mecA* gene is also associated with the multidrug resistance phenotype. In this study, we considered as multidrug resistant the strains that presented resistance to at least three different antimicrobial classes, such as beta-lactamics, quinolones, cephalosporins, macrolides, lincosamide and aminoglicosyde. This profile was detected in 68% (17/25) of mecA + isolates. Among these multiresistant isolates, 41% (7/17) were *S. aureus,* 35% (6/17) *S. intermedius* and 24% (4/17) CNS (Table 3). The spread of multiresistant *Staphylococcus* spp. strains among animals has been investigated worldwide in recent decades and has been blamed on selective pressure exerted by indiscriminate antimicrobial use in veterinary medicine. This resistance provides a selective advantage for *Staphylococcus* spp. infection and colonization, limiting the efficacy of the antimicrobials

Table 2. Resistance panel to antimicrobial classes used in staphylococcus infections.

Antimicrobial class	% (n) Resistant isolates
β-lactamics	
Ampicillin (10 μg)	66% (66/100)
Penicillin (10 UI)	78%(78/100)
Oxacillin (1 μg)	37%(37/100)
β-lactamics + β-lactamase inhibitor	
Ampicillin + Sulbactam (10/10 μg)	2%(2/100)
Amoxicillin + Clavulanic (20/10 μg)	5%(5/100)
Cephalosporins	
Cefoxitin (30 μg)	53%(53/100)
Cefalotin (30 μg)	28%(28/100)
Ceftriaxone (30 μg)	64%(64/100)
Carbapenem	
Imipenem (10 μg)	2%(2/100)
Macrolides	
Azithromycin (15 μg)	53%(53/100)
Erythromycin (15 μg)	48%(48/100)
Lincosamide	
Clyndamicin (2 μg)	57%(57/100)
Quinolones	
Ciprofloxacin (5 μg)	18%(18/100)
Enrofloxacin (10 μg)	15%(15/100)
Norfloxacin (10 μg)	12%(12/100)
Aminoglicosyde	
Tobramicin (10 μg)	45%(45/100)
Gentamycin (10 μg)	22%(22/100)
Folate Pathway Inhibitor	
Sulfamethoxazole+trimetoprim (1,25 μg/23,75 μg)	37%(37/100)
Oxazolidinone	
Linezolide (30 μg)	7%(7/100)
Tetracyclines	
Tetracycline (30 μg)	30%(30/100)
Streptogramin	
Quinupristin/dalfopristin (10 μg/10 μg)	57%(57/100)

available for therapeutic procedures (Souza et al., 2012). Table 4 displays the antimicrobial resistance profile of 25 the *mecA*-positive *Staphylococcus* strains.

Interpretative antimicrobial susceptibility tests can provide clues to the mechanism underlying resistance. Beta-lactamic resistance due to beta-lactamases

Table 3. Oxacillin resistance and *mec* genes detection profiles among 100 *Staphylococcus* isolates.

Profile (n isolates)		ODD	AS	CFO	*mecA*	*mecI*	*mecR1*
Staphylococcus mec⁻ isolates							
1(18)		S	S	S	-	-	-
2(14)		S	S	R	-	-	-
3(14)		R	R	S	-	-	-
4(20)		S	R	S	-	-	-
5(6)		R	S	R	-	-	-
Staphylococcus mec + isolates							
6(5)	SCN (n=3), *S.int.* (n=1), *S.hy.*(n=1)	R	R	R	+	-	-
7(5)	*S.int.* (n=4), *S.hy.*(n=1)	S	R	R	+	-	-
8(5)	*S.int.* (n=3), *S.hy.*(n=1), SCN (n=1)	S	R	R	+	-	+
9(4)	*S. au.*(n=4)	S	S	S	+	+	-
10(2)	*S. au.*(n=1),SCN (n=1)	R	R	R	+	-	+
11(2)	*S.int.* (n=2)	S	S	S	-	+	-
12(1)	SCN (n=1)	S	S	S	-	-	+
13(1)	*S. au.*(n=1)	S	R	S	+	+	+
14(1)	*S. au.*(n=1)	S	R	R	+	+	+
15(2)	*S. au.*(n=1), SCN (n=1)	R	R	R	+	+	+

ODD, Oxacillin disk diffusion; AS, Agar screen; CFO, Cefoxitin disk diffusion; SCN, Coagulase-negative *Staphylococcus* spp.; *S. au.*, *Staphylococcus aureus*; *S.int.*, *Staphylococcus intermedius*; *S.hy.*, *Staphylococcus hyicus*.

Table 4. Antimicrobial resistance profile of 25 *mecA*-positive *Staphylococcus* spp.

Profile (n)	*mecA*	OXA	CFO	PENG	ASB	AMP	CIP	ENO	ERI	AZI	CLI	GEN	LNZ
1(6)	+	R	R	R	S	R	S	R	R	R	R	S	S
2(4)	+	R	R	R	S	R	R	R	R	R	R	S	S
3(2)	+	S	S	R	S	S	S	S	R	S	S	S	S
4(2)	+	S	R	R	R	R	S	S	R	R	R	S	S
5(1)	+	R	R	R	S	R	S	S	R	S	S	S	S
6(1)	+	R	R	R	S	R	S	S	S	R	R	S	S
7(1)	+	R	R	R	S	R	R	S	S	R	R	S	S
8(1)	+	S	S	R	S	R	S	S	R	R	R	R	S
9(1)	+	R	R	R	S	R	S	S	S	S	S	S	S
10(1)	+	S	R	R	S	R	S	S	S	S	S	S	S
11(1)	+	R	R	S	S	S	S	S	R	R	R	S	S
12(1)	+	S	S	R	S	R	S	S	S	S	S	S	S
13(1)	+	S	R	R	S	R	R	S	S	S	S	S	S
14(1)	+	S	R	R	S	R	S	R	S	S	S	S	S
15(1)	+	S	R	R	S	S	S	S	S	S	S	S	S

OXA, oxacillin; CFO, cefoxitin; PENG, penicillin G; ASB, ampicillin+sulbactam; AMP, ampicillin; CIP, ciprofloxacin; ENO, enrofloxacin; ERI, eritrhomycin; AZI, azithromicyn; CLI, clyndamicin;; GEN, gentamycin; LNZ, linezolide; n= nunber of isolates.

production is easily noticed when the resistant strain presents a susceptible pattern to oxacillin and to the association of antimicrobial plus beta-lactamase inhibitors, such as clavulanic acid or sulbactam. The CLSI (2011) recommends the nitrocefin-based test, which detected that 38% (38/100) of *Staphylococcus* spp. were

Table 5. Pheno and genotypic profile of 32 *Staphylococcus* spp. positive to *bla* genes.

Profile (n isolates)	Nitrocefin test	*blaZ*	*blaI*	*blaRI*	*mecA*
1(7)	+	+	-	-	-
3(2)	+	+	-	-	+
4(7)	-	+	+	-	+
8(7)	-	+	+	-	-
11(5)	+	+	+	-	-
12(3)	+	+	+	+	-
13(1)	-	-	+	-	-

β-lactamase producers. As expected, these isolates were resistant to β-lactamic antibiotics, such as penicillin, ampicillin and amoxicillin, whereas they were sensitive to the β-lactamic plus β-lactamase inhibitor associations, such as ampicillin+sulbactam and amoxicillin+clavulanate. Also, 32% (32/100) *Staphylococcus* spp. tested positive for *bla* genes (Table 5). β-lactamases is widespread among animals and it seems to be a prevalent mechanism in resistant staphylococci strains (Mckinney et al., 2001).

Among nitrocefinase producing isolates, nine (9/38) *Staphylococcus* spp. were positive only for the *blaZ* operator gene. Seven (7/9) were *blaZ*-positive-*mecA*-negative *Staphylococcus* spp. and phenotypically oxacillin resistant, suggesting that β-lactamase production was responsible for the observed phenotype. Two isolates tested *blaZ-mecA*-+, being able to express both β-lactamase and PBP2a. The whole *bla* gene complex, *blaZ-blaI-blaR1*, was detected in 7.8% (3/38) of the nitrocefinase-positive isolates. These isolates were oxacillin resistant but *mecA* negative, pointing to the involvement of β-lactamase in this resistance. The extracellular presence of β-lactamic antimicrobial triggers a transduction signal system constituted by BlaR1 transmembrane protein, which signals removal of the *blaI* repressive component that is located between *blaRI* and *blaZ* genes, starting *blaZ* transcription and consequently β-lactamase production, meaning a resistant phenotype (Mckinney et al., 2001). The *blaZ-blaI* genes were detected in 36.8% (14/38) of *Staphylococcus* spp. isolates. From these 14 isolates, 28.5% (4/14) tested negative for *mecA* and nitrocefinase, and presented an oxacillin-susceptible profile, confirming the inhibitory activity of the *blaI* gene in β-lactamase production. In contrast, 42.8% (6/14) tested positive for *mecA* and were also oxacillin susceptible. Mckinney et al. (2001) reported homology of the *mecA* gene to the upstream sequence of the *blaZ* gene. So, distinct mechanisms of oxacillin resistance can also be controlled by the *blaI-blaR1* regulatory system and the *blaI* gene regulates the BlaI membrane system as well as β-lactamase and PBP2a synthesis inhibition. Nine *Staphylococcus* spp. isolates that were *blaZ-blaI*-positive tested negative for *mecA* and presented resistance to oxacillin. This suggests the existence of other resistance mechanisms, such as different classes of PBPs (PBP3 and PBP4). Because of this heterogeneity, the most recent CLSI revision (CLSI, 2013) established new parameters and recommended oxacillin and cefoxitin disk diffusion plus oxacillin agar screen tests to evaluate mecA-mediated resistance. Also, tests should be performed to detect beta-lactamase production. It is no longer possible to consider *mec*A as a gold standard test in beta-lactamic resistance detection.

Conclusion

The widespread distribution of staphylococcus species in pet animal infection sites indicates the importance of correct microbiological identification. *Staphylococcus* strains have potential ability to develop different mechanisms of oxacillin resistance, resulting in a heterogeneous phenotype profile. The oxacillin resistance detected in this study is associated with the existence of different regulatory systems, such as *mec* and *bla* genic complexes, which can either be present or absent in the *Staphylococcus* spp. chromosome and can act in amplified (synergetic) or divergent (non-cumulative) ways. These resistance mechanisms were detected among *Staphylococcus* spp. strains isolated from pet animal infection sites, contributing to reduction of antimicrobial therapeutic efficacy and spread of resistance. Nevertheless, it seems that the action of β-lactamases is a prevalent mechanism in the development of resistant staphylococcus strains.

Conflict of Interest

The author(s) have not declared any conflict of interests.

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