

## BETA-LACTAMASE PRODUCTION AND ANTIMICROBIAL RESISTANCE OF COAGULASE-POSITIVE STAPHYLOCOCCI STRAINS ISOLATED FROM DOGS AND THEIR OWNERS

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**Abstract.** Staphylococcal infections are common to veterinary and human medicine. B-lactam antibiotics are among frequently prescribed antibiotics worldwide to treat staphylococcal infections. Antimicrobial susceptibility is changing over time and is generally rising steadily for those antimicrobials that are often used. The aim of this study to find out the antimicrobial susceptibility patterns of  $\beta$ -lactamase producing coagulase-positive staphylococci isolated from dogs and their owners.

This study characterized the antimicrobial susceptibility and  $\beta$ -lactamase producing of *Staphylococcus aureus* and *Staphylococcus pseudintermedius* isolated from dogs and their owners. The susceptibility was determined by the disk-diffusion method. Polymerase chain reaction was used to detect *blaZ* gene, which encodes resistance to penicillin.

The prevalence of *Staphylococcus aureus* was identified in 4 (6.6%) dogs and in 26 (36.1%) owners. *Staphylococcus pseudintermedius* was isolated from 28 dogs (45.9%) and from 3 (4.2%) humans.

*Staphylococcus aureus* strains isolated from dogs were resistant to ampicillin (75.0%) and penicillin G (75.0%); *Staphylococcus pseudintermedius* strains isolated from dogs showed high resistance to penicillin G (43.3%), ampicillin (43.3%) and amoxicillin (26.7%) as well. Resistance of *Staphylococcus aureus* isolated from dogs' owners was most common to ampicillin (57.7%), penicillin G (50.0%) and amoxicillin (42.3%); resistance of *Staphylococcus pseudintermedius* to these antibiotics was present in 66.7%. All of the isolates were susceptible to oxacillin.

The prevalence of  $\beta$ -lactamase producing *Staphylococcus aureus* strains isolated from dogs and their owners were 75% and 46.15% respectively. *BlaZ* gene was detected in 36.67% strains of *Staphylococcus pseudintermedius* isolated from canine and in 66.67% strains isolated from humans.

**Keywords:** coagulase-positive staphylococci, antimicrobial, resistance, beta-lactamase, *blaZ*

**Introduction.** *S. aureus* and *S. pseudintermedius* are two major pathogenic staphylococci, which are frequently implicated with opportunistic infections in human and dogs (Markey et al., 2013). Pyoderma, otitis externa and others infections in dogs caused by *S. pseudintermedius* usually occurs when skin or mucosal barriers are affected by determining factors such as atopic dermatitis, medical and surgical procedures or immunosuppressive disorders (Bannoehr et al., 2009; 2012). Clinical infections including septicemia, pneumonia, wound sepsis, septic arthritis, osteomyelitis and post-surgical toxic shock syndrome with substantial rates of morbidity are the most common types of diseases in human caused by *S. aureus* (Akindede et al., 2010). The introduction of antibiotics into human and veterinary medicine has had a major impact on both human and animal health. All of these human and canine staphylococcal infections are treated using antimicrobial therapy.

B-lactam antibiotics are among the most frequently prescribed antibiotics worldwide in the control of staphylococcal infection. They act on peptidoglycan synthesis by molecularly acting on transpeptidases and carboxypeptidases thereby disrupting cell wall formation of the pathogen (Torimiro et al., 2013).

Studies show that antimicrobial resistance to  $\beta$ -lactam and other antibiotics is growing among pet infection-causing bacteria. Transmission of such organisms,

particularly pathogenic staphylococci occurs among pets and owners; pets can act as reservoirs of these bacteria. There is required to collect data about the levels of carriage of such bacteria in companion animals and risk factors associated with the transmission of bacteria to humans who contact with infected pets (Lloyd, 2007). Information about the most common isolate associated with the skin, ears, wounds and other diseases caused by bacteria, their susceptibility patterns to the most commonly used antimicrobial drugs are very useful for empirical treatment (Hariharan et al., 2014).

Due to extensive usage of beta-lactam antibiotics, bacteria have developed resistance to these antibiotics via different mechanisms. Production of  $\beta$ -lactamase is the greatest source of resistance to beta-lactams. B-lactamase is the predominant extracellular enzyme synthesized after exposure of *Staphylococcus* spp. strains to  $\beta$ -lactam antibiotics (Torimiro et al., 2013). Staphylococcal  $\beta$ -lactamase breaks open the  $\beta$ -lactam ring rendering the antibiotic inactive, in this way  $\beta$ -lactamase production prevents staphylococci to penicillin (Devapriya et al., 2013, Samant et al., 2012).

This study was designed to find out the antimicrobial susceptibility patterns of  $\beta$ -lactamase producing coagulase-positive staphylococci isolated from dogs and their owners.

### Material and methods

Our study work was performed in compliance with Lithuanian animal welfare regulations (No. B1-866, 2012; Nr. XI-2271, 2012) and was approved by the Lithuanian Committee of the Veterinary Medicine and Zootechnics Sciences (Protocol No.09/2012).

Swabs were taken from human nasal cavity and from dogs' nares and rectum for staphylococci isolation. All swabs were placed in Amies medium „TRANSWAB®“ (Medical wire, UK) and stored at 4°C until processing.

Staphylococci strains isolated from dogs and humans were characterized and identified using standard established microbiological methods, which include colonial morphology, Gram staining (Diagnostica Merck, German), and biochemical characteristics. Coagulase tests with rabbit plasma (Coagulase Plasma EDTA, Biolife, Italy), DNase activity test (Sifin, Germany), Integral system staphylococci (Liofilchem, Italy) were performed in order to identify coagulase-positive staphylococci.

Coagulase-positive staphylococci isolates were genotypically confirmed by multiplex polymerase chain reaction (M-PCR) according Sasaki recommendations with minor modifications (Sasaki et al., 2007). Oligonucleotide primers were used for *S. aureus* identification (359 bp) au-F3: 5' TCG CTT GCT ATG ATT GTG G 3'; au-nucR: 5' GCC AAT GTT CTA CCA TAG C 3'; and for *S. pseudintermedius* identification (926 bp) psc-F2; 5' TRG GCA GTA GGA TTC GTT AA 3' psc-R5; 5' CTT TTG TGC TYC MTT TTG G 3'.

After the confirmation of staphylococci species, all the isolates were further analyzed for the *blaZ* gene by using primers (*blaZ* 1: 5' TTA AAG TCT TAC CGA AAG CAG 3'; *blaZ* 2: 5' TAA GAG ATT TGC CTA TGC TT-3') designed by Olsen et al (2006).

DNA was extracted from coagulase-positive staphylococci using 5% solution of Chelex-100 (Sigma, USA). Several bacterial colonies were suspended in 500 µl of Chelex-100 solution. After thermal lysis at 56°C for 30 min and 96 °C for 10 min suspensions were chilled on ice, centrifuged at 10000 rpm for 2.5 min. twice. Supernatant was used as DNA template.

The reaction mixture for PCR consisted of 3 µl of DNA extract in a total volume of 25 µl composed of 0.3 µl (500 U) DreamTaq™ Green DNA polymerase (MBI, Fermentas), 0.5 µl each primer (Grida Lab, Lithuania), 2 µl (2mM) dNTP mixture (MBI, Fermentas), 2.5 µl (1.25mL) 10 × DraemTaq Green Buffer with (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (MBI, Fermentas), and 13.7 µl bidistilled water. Reaction mixtures were thermally cycled once at 95 °C for 2 min; 35 times at 94 °C for 1 min, 54 °C for 1 min, and 72 °C for 1 min; and then once at 72 °C for 10 min in thermocycler (G-STORM GS1, UK). DNA fragments were analyzed by electrophoresis in 1 × Tris-acetate-EDTA on a 1.2% UltraPure agarose gel (Invitrogen, UK) stained with ethidium bromide. Images of gel were taken using documentation system (Molecular Imager® Gel Doc™ XR, BioRad).

Antimicrobial susceptibility of the bacteria was determined by disc diffusion method using the Kirby-

Bauer technique (Bauer et al. 1966) and according to the recommendations reported by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Pure cultures of coagulase-positive staphylococci were transferred to a test tube with sterile 0.9% Sodium Chloride (Liofilchem, Italy). McFarland densitometer 1 (BIOS, Netherlands) were used for measurement of bacterial concentration. The turbidity was equivalent to 0.5 McFarland standards. Bacterial suspension was applied on surface of Mueller-Hinton agar medium (Liofilchem, Italy). Oxoid's Antimicrobial Susceptibility Test Discs were placed on the agar and incubated at 37°C for 24 hours. The antimicrobials included: ampicillin (10µg), amoxicillin/clavulanic acid (20µg+10µg), penicillin G (1IU), amoxicillin (30µg), oxacillin (1µg).

The SPSS 13.0 statistical packet was used to analyze the data (Version 15, SPSS Inc., Chicago, IL). Comparisons were performed using chi-squared analysis, Fisher's exact and McNemars test using exact *P*-values. A *P*-value of < 0.05 was considered significant for all comparisons.

### Results

Staphylococci strains isolated from dogs and humans were characterized and identified using biochemical tests. Two species of coagulase-positive staphylococci *S. aureus* and *S. pseudintermedius* were confirmed by using multiplex PCR method. *S. aureus* was identified in 26 (36.1%) dog owners and in 4 (6.6%) dogs. *S. pseudintermedius* was isolated from 3 (4.2%) humans, and from 28 dogs (45.9%).

Antimicrobial resistance phenotype characteristics to beta-lactam antibiotics of coagulase-positive staphylococci were investigated and shown in Table 1.

Coagulase-positive staphylococci showed a successful amplification of internal fragments with the expected size 377 bp with the primer pairs specific for *blaZ* gene, which encoding producing of β-lactamase (Fig. 1)

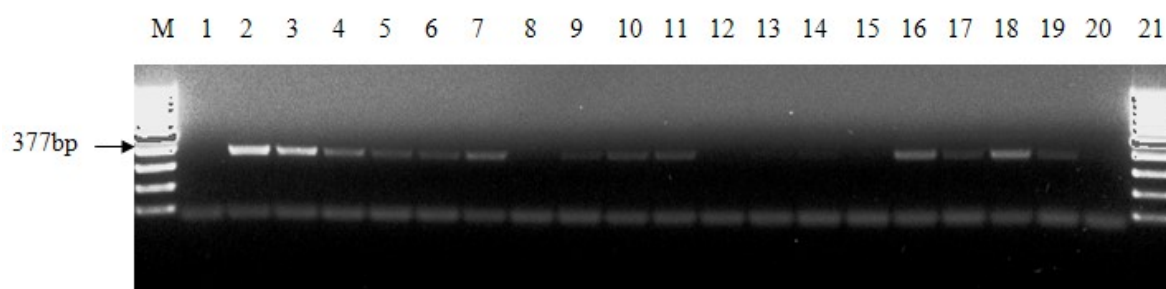
The prevalence of β-lactamase producing *S. aureus* strains isolated from humans and dogs were 12 (46.15%) and 3 (75%) respectively.

*blaZ* gene was identified in 2 (66.67%) strains of *S. pseudintermedius* isolated from humans and in 11 (36.67%) strains isolated from canine. Results of antimicrobial susceptibility patterns of β-lactamase and non β-lactamase producing strains are shown in Table 2 and Table 3.

In this stage of the investigation phenotypic resistance were compared with genotypic resistance. Among the 26 *S. aureus* strains isolated from humans, 13 (50.0%) isolates were resistant to both antibiotics – ampicillin and penicillin G, of which in 11 strains were found *blaZ* genes. We detected 9 (34.62%) *S. aureus* strains resistant to three antibiotics: amoxicillin, penicillin G and ampicillin. In all of these isolates have been identified *blaZ* genes. We found that staphylococci producing beta-lactamase were more resistant to amoxicillin (*P*=0.002), penicillin G (*P*<0.001) and ampicillin (*P*=0.001) compared to staphylococci non-producing penicillinase, the results were statistically significant.

Table 1. Antimicrobial susceptibility of *S. aureus* and *S. pseudintermedius* to beta-lactam antibiotics determined by disk-diffusion method

ANTIMICROBIALS	Number of resistant isolates (%)			
	<i>Staphylococcus aureus</i>		<i>Staphylococcus pseudintermedius</i>	
	Isolates from humans (n=26)	Isolates from dogs (n=4)	Isolates from humans (n=3)	Isolates from dogs (n=30)
Ampicillin (10µg)	15 (57.7)	3 (75.0)	2 (66.7)	13 (43.3)
Amoxicillin / clavulanic acid (20µg+10µg)	0	1 (25.0)	0	1 (3.3)
Amoxicillin (30µg)	11 (42.3)	1 (25.0)	2 (66.7)	8 (26.7)
Penicillin G (1IU)	13 (50.0)	3 (75.0)	2 (66.7)	13 (43.3)
Oxacillin (1µg)	0	0	0	0

Figure 1. Agarose gel electrophoresis of PCR product of *blaZ* gene on a 1.2 % agarose gel. Lane M = GeneRuler™ 1000 bp DNA Ladder (MBI, Fermentas). Lane 1 = negative control (reaction mixture without DNA); Positive control of amplified 377-bp DNA: lanes 2 = *Staphylococcus aureus* (ATCC 9144); 3-7; 9-11; 13-14; 16-19 = representative coagulase-positive staphylococci strains positive for the *blaZ* gene; 8; 12; 15; 20 = negative for the *blaZ* gene strains.Table 2. Antimicrobial susceptibility profile of  $\beta$ -lactamase and non  $\beta$ -lactamase producing *S. aureus* strains

ANTIMICROBIALS	<i>S. aureus</i> from humans (n=26)				<i>S. aureus</i> from dogs (n=4)			
	$\beta$ -lactamase producers (n=12)		Non $\beta$ -lactamase producers (n=14)		$\beta$ -lactamase producers (n=3)		Non $\beta$ -lactamase producers (n=1)	
	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
Ampicillin (10µg)	1 (8.3)	11 (91.7)	10 (71.4)	4 (28.6)	0	3 (100)	1 (100)	0
Amoxicillin / clavulanic acid (20µg+10µg)	12 (100)	0	14 (100)	0	2 (66.7)	1 (33.3)	1 (100)	0
Amoxicillin (30µg)	3 (25)	9 (75)	12 (85.7)	2 (14.3)	2 (66.7)	1 (33.3)	1 (100)	0
Penicillin G (1IU)	1 (8.3)	11 (91.7)	12 (85.7)	2 (14.3)	0	3 (100)	1 (100)	0
Oxacillin (1µg)	12 (100)	0	14 (100)	0	3 (100)	0	1 (100)	0

S: Sensitive, R: Resistant

*S. aureus* strains isolated from dogs rectum showed high resistance (75%) to ampicillin and penicillin G, all these resistant strains of staphylococci had *blaZ* genes as well. *S. aureus* strains isolated from dog's nasal cavity were sensitive for all antibiotics.

Among the *S. pseudintermedius* isolated from humans 2 (66.67%) strains were resistant to ampicillin, amoxicillin and penicillin G, furthermore in these strains were detected *blaZ* genes also, however, the difference was not statistically significant. All *S. pseudintermedius*

strains isolated from humans nasal cavity were sensitive for oxacillin and amoxicillin/clavulanic acid.

Most of the *S. pseudintermedius* strains isolated from canine were susceptible to amoxicillin/clavulanic acid. We found that 13 (43.33%) strains of *S. pseudintermedius* isolated from dogs were resistant to ampicillin and penicillin G, 10 (76.92%) strains were positive for *blaZ* gene. Penicillinase-producing *S. pseudintermedius* was significantly more resistant to ampicillin and penicillin G compared with other strains ( $P < 0.001$ ).

Table 3. Antimicrobial susceptibility profile of  $\beta$ -lactamase and non  $\beta$ -lactamase producing *S. pseudintermedius* strains

ANTIMICROBIALS	<i>S. pseudintermedius</i> from humans (n=3)				<i>S. pseudintermedius</i> from dogs (n=30)			
	$\beta$ -lactamase producers (n=2)		Non $\beta$ -lactamase producers (n=1)		$\beta$ -lactamase producers (n=11)		Non $\beta$ -lactamase producers (n=19)	
	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
Ampicillin (10 $\mu$ g)	0	2 (100)	1 (100)	0	0	11 (100)	16 (84.2)	3 (15.8)
Amoxicillin / clavulanic acid (20 $\mu$ g+10 $\mu$ g)	2 (100)	0	1 (100)	0	11 (100)	0	18 (94.7)	1 (5.3)
Amoxicillin (30 $\mu$ g)	0	2 (100)	1 (100)	0	5 (45.5)	6 (54.5)	17 (89.5)	2 (10.5)
Penicillin G (1IU)	0	2 (100)	1 (100)	0	0	11 (100)	16 (84.2)	3 (15.8)
Oxacillin (1 $\mu$ g)	2 (100)	0	1 (100)	0	11 (100)	0	19 (100)	0

S: Sensitive, R: Resistant

All coagulase-positive staphylococci isolated from humans and dogs were sensitive for oxacillin.

Our study showed that *S. aureus* and *S. pseudintermedius* strains producing penicillinase isolated from humans were most susceptible to amoxicillin with clavulanic acid compared with penicillin G and ampicillin. Values were statistically significant  $P=0.02$  and  $P=0.046$  respectively.

*S. pseudintermedius* strains with *blaZ* gene isolated from dogs' nasal cavity were significant more sensitive to amoxicillin clavulanic acid compared with penicillin G ( $P=0.002$ ), ampicillin ( $P=0.002$ ) and amoxicillin ( $P=0.04$ ). However isolates from dogs' rectum were most susceptible to amoxicillin clavulanic acid than to penicillin G ( $P=0.01$ ) and ampicillin ( $P=0.01$ ).

#### Discussion

This study was designed to determine the prevalence and antimicrobial susceptibility of staphylococci producing  $\beta$ -lactamase of human and their dogs.

Resistance to  $\beta$ -lactamase sensitive penicillins is widespread among staphylococci of human and dogs. Prevalence of 46.15% of  $\beta$ -lactamase producers was recorded for *S. aureus* strains isolated from healthy humans. This value is lower with the previously reported prevalence of 80% by Akindele et al. (Akindele et al., 2010) and 70.1% by Torimiro et al. (Torimiro et al., 2013). The high prevalence of penicilase production by isolated *S. aureus* explains the high resistance to penicillin G, amoxicillin and ampicillin obtained in this study. However our study showed 50.0% resistance to penicillin G is also slightly lower than other researchers published 96% (Akindele et al., 2010) and 86% (Torimiro et al., 2013). All *S. aureus* strains producing and non producing  $\beta$ -lactamase were sensitive to amoxicillin with clavulanic acid, which is  $\beta$ -lactamase inhibitor and acts by breaking the beta-lactam ring that allows amoxicillin like antibiotics to work.

Boost et al. (Boost et al., 2008) reported that *S. aureus* strains isolated from dogs were 62% resistant to penicillin G, 6.2% to oxacillin. We found the similar results 75% strains of *S. aureus* were resistant to penicillin G and ampicillin, furthermore all of them were detect *blaZ* gene. All isolates were sensitive for oxacillin in our study.

Priyantha et al. (Priyantha et al., 2016) of the 78 dogs isolates *S. pseudintermedius* resistant to penicillin G were found in 78%, to ampicillin in 61% in Canada. Norstromb et al. (Norstromb et al., 2009) reported prevalence of 70% resistance to penicillin G in Norway. Hariharan et al. (Hariharan et al., 2013) from India detected 11.6% of *S. pseudintermedius* strains resistance to penicillin G, whereas resistance to ampicillin was only 2.3%. Our results 43.33% resistance to ampicillin and penicillin G were medium compared with the previously reported prevalence. Differences in locality and investigation period might be among the possible reasons for the variations in the reported resistance rates (Matanović et al., 2012).

Norstromb et al. (Norstromb et al., 2009) made the assumption that the occurrence of antimicrobial resistance is common among *S. pseudintermedius* from dogs unexposed to antimicrobial treatment before sampling, and that there is a high genetic polymorphism among *S. pseudintermedius*.

There are not many reports about *S. pseudintermedius* isolated from human susceptibility to antimicrobial agents. Our found 66.7% *S. pseudintermedius* strains resistance to penicillin G was similar to 76.5% result reported by Humphries et al. (Humphries et al., 2016). All strains of *S. pseudintermedius* resistant to penicillin G had *blaZ* gene as well.

Our findings show that  $\beta$ -lactamase-producing staphylococci are common between dogs and their owners. High prevalence of  $\beta$ -lactamase production by isolated *S. aureus* and *S. pseudintermedius* explains the high resistance to penicillin G, amoxicillin and ampicillin obtained in this study.

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