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PREVALENCE OF ZOONOTIC STAPHYLOCOCCI IN DOGS – PRELIMINARY STUDY

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Abstract

This study was conducted to investigate the prevalence of zoonotic staphylococci isolated from dogs in Western Romania. Samples were obtained from adult dogs of both sexes submitted to the University Veterinary Clinics Timisoara and private veterinary practice. Animals selected for this study had no known history of previous antibiotic treatment. Samples were identified and labeled as to source, male or female, adult and the anatomical area of harvesting. A total of 51 samples were obtained from different anatomical sites such as nose, eyes, ears, extremities reproductive and skin. After growth, staphylococcal isolates were identified according to their characteristics as outlined in Bergey's Manual of Determinative Bacteriology and the Manual of Clinical Microbiology. 35 samples were positive for staphylococci, being isolated both positive and coagulase-negative species. The species most frequently isolated were S. (pseud)intermedius, S. aureus, S. hycus, S. epidermidis and S. haemolyticus. The prevalence of staphylococcal infections in veterinary medicine is increasing worldwide. Staphylococci have shown a frequent and rapid development of nosocomial infections. Unfortunately, these studies have not been documented continuously in veterinary medicine. The present investigation has examined the clinical prevalence of zoonotic staphylococci in the dogs that may constitute a reservoir for these bacteria.

Key words: staphylococci, zoonotic, dogs, nosocomial infection

INTRODUCTION

Staphylococci are one of the most important groups of commensal bacteria that are isolated from dog skin and mucous membranes. Moreover, they are responsible for opportunistic infections acquired in hospitals and communities, affecting mostly the skin and ears and other anatomic areas. Coagulase-positive staphylococci (CoPS), *Staphylococcus aureus*, *S. intermedius*, *S. pseudintermedius*, *S. delphini* and *S. schleiferi subsp. coagulans*, are the most common cause of staph infections in dogs.

Both groups of staphylococci are characterized alarming rates increasing antibiotic resistance, the problem belongs to the most important aspects of the management of staphylococcal infections worldwide. In particular, the problem of resistance is important for multidrug-resistant strains (MDR) and methicillin-resistant (MR). Staphylococci are frequently isolated from areas porting clinically healthy dogs. It is not surprising, because the nostrils, mouth and anus are considered sources of colonization and infection with staphylococci other areas. Frequency porting staphylococcal infections in dogs, especially skin infections up to 100%.

MATERIALS AND METHODS

Methods-Animals and sample collection

Samples were obtained from 112 dogs belonging to both sexes, from September to December 2013. The dogs were from owners of Timisoara, which were submitted for routine checkup or vaccination clinics University Veterinary Faculty of Veterinary Medicine Timisoara.

Samples were collected by buffering after the animals have passed through a preliminary primary medical attention (thermometry, general clinical examination) which excludes the existence of a local or generalized

infection. All samples were collected by a veterinarian.
The study included 51 healthy dogs completely without symptoms of infection or history of antibiotic treatment prior to sampling. Three swabs were collected from each dog in this group. With one buffer pharyngeal exudate collected a sample was taken from the ear canal, the second mouth (gingival area) and third in the perineal region (buffer was introduced approximately 0.5 cm into the anus).

Phenotypic Identification of staphylococci isolated

Isolates were sub-cultured on 5% blood agar incubated at 37°C for 24 hours aerobically. Suspected staphylococcal isolates were identified based on colony characteristics, pigment production, appearance on Gram stain and hemolysis. Results were confirmed using the Vitek 2 system (bioMeriux, France) according to the manufacturer's recommendations.
To identify the prevalence and antibiotic susceptibility of pathogenic staphylococci, investigations were carried out on 51 clinically healthy dogs.
For identification of pathogenic staphylococci work using the following protocol: harvesting biological material by tapping sub gingival area or on the surface of teeth and skin areas (perianal); seeding on selective culture media, blood agar ram 5 and 10%, Chappmann agar - selective for staphylococci - examining and reading the plates was performed after 24 hours, incubated at 37°C under aerobic conditions.

Identification of strains - using GP identification card

The test bacterial suspension was adjusted to a value of 0.5 McFarland standards in 2.5 ml of 0.45% sodium chloride, with a tool VITEK 2 system (DensiChek; bioMerieux). Time between inoculum preparation and filling card was always less than 30 minutes. GP card format includes 64 wells on a plastic card, which contains 43 tests.

RESULTS AND DISCUSSION

Research has established the prevalence of zoonotic risk strains of staphylococci, in clinically healthy dogs at the same time allowed for routine examinations to isolate and identify these bacterial species.
Bacteriological examinations carried out of the 51 samples collected from clinically healthy dogs, allowed the isolation of several bacterial species, the largest share with a staphylococcus; the results are shown in table 1.

Table 1Total number of strains of staphylococci isolated according to the anatomical areas

Anatomy cal areas	Strains of staphylococci isolated				
	S. (pseud)intermedius	*S. aureus*	*S. hycus*	*S. epidermidis*	*S. haemolyticus*
External auditory canal	22	2	2	1	1
Oral cavity	9	3	3	-	-
Anus	12	1	2	2	-
Total	**18**	**6**	**7**	**3**	**1**

Bacteriological examinations carried out on a total of 51 samples from clinically healthy dogs, allowed the isolation of several strains of staphylococci belonging to the species *S. (pseudo)intermedius* and *S. aureus*, *S. hycus*, *S. epidermidis* and *S. haemolyticus*.
Strains of staphylococci unexposed to the pressure of antibiotics are sensitive to these substances; on the other hand, strains isolated from dogs with various conditions, under pressure due to antibiotic therapy, can provide multiple resistance phenomenons.
The results of antibiotic susceptibility testing of staphylococci strains isolated from clinically healthy dogs are shown in tables 2.
The 35 zoonotic risks of staphylococci strains isolated of clinically healthy dogs were tested against 20 antibiotics belonging to several classes of antibiotics against methicillin respectively.
The results of antibiotic resistance 35 staphylococcal strains isolated from clinically healthy dogs shown in Table 2.

Table 2. The results of antibiotic resistance of 18 strains of *S. (pseudo)intermedius* isolated from clinically healthy dogs

Antimicrobian agents	Number of isolates of *S. (pseudo)intemedius* (%)		
	Resistant	Intermediate	Sensitive
Benzylpenicillin	6 (33,33)	2 (11,11)	10 (55,55)
Ampicillin	12 (66,67)	6 (33,33)	0
Ampicillin/sulbactan	18 (100)	0	0
Oxacillin	10 (55,56)	2 (11,11)	6 (33,33)
Imipenem	18 (100)	0	0
Gentamicin	10 (55,55)	1 (5,56)	7 (38,88)
Kanamycin	4 (22,22)	1 (5,56)	13 (72,22)
Enrofloxacin	18 (100)	0	0
Marbofloxacin	18 (100)	0	0
Erythromycin	5 (27,78)	2 (11,11)	11 (61,11)
Clindamycin	12 (66,67)	6 (33,33)	0
Vancomycin	18 (100)	0	0
Tetracycline	5 (27,78)	3 (16,66)	10 (55,55)
Nitrofurantion	8 (44,45)	10 (55,55)	0
Fusidic acid	16 (88,89)	2 (11,11)	0
Mupirocin	14 (77,78)	4 (22,22)	0
Chloramphenicol	15 (83,34)	3 (16,66)	0
Rifampicin	17 (94,44)	1 (5,56)	0
Trimethoprim/sulfamethoxazole	5 (27,78)	3 (16,66)	10 (55,55)
Methicillin	16 (88,89)	0	2 (11,11)

If antibiotics: ampicillin, ampicillin/sulbactan, rifampicin, enrofloxacin, marbofloxacin, clindamycin, nitrofurantion, fusidic acid and chloramphenicol mucopirinul sensitivity was highest, these antibiotics are considered of choice for staphylococci. This suggests that isolates and tested from animals to which these antibiotics were not used, except lincomycin. Also, we can say that these four antibiotics for staph kit is used, usually men, lincomycin is used only in animals.

Compared used β-lactams (methicillin, benzylpenicillin, ampicillin, ampicillin/sulbactan, oxacillin, imipenem, rifampicin) was antibiosensibilitatea maximum ampicillin, ampicillin/sulbactan and rifampicilină. The strains tested were largely resistant to β-lactams other. The distribution of resistant strains was as follows: 6 strains benzylpenicillin, 10 strains oxacillin and 2 strains were resistant to methicillin (Table3).

Table 3Results of antibiotic resistance for 2 methicillin resistant strains of *S. (pseudo)intermedius*

Antimicrobian agents	Number of isolates of *S. (pseudo)intemedius* (%)		
	Resistant	Intermediate	Sensitive
Benzylpenicillin	1 (50)	1 (50)	0
Ampicillin	2 (100)	0	0
Ampicillin/sulbactan	0	0	2 (100)
Oxacillin	0	1 (50)	1 (50)
Imipenem	0	0	2 (100)
Gentamicin	0	1 (50)	1 (50)
Kanamycin	0	1 (50)	1 (50)
Enrofloxacin	0	0	2 (100)
Marbofloxacin	0	0	2 (100)
Erythromycin	1 (50)	1 (50)	0
Clindamycin	0	0	2 (100)
Vancomycin	0	0	2 (100)
Tetracycline	1 (50)	0	1 (50)
Nitrofurantion	0	0	2 (100)
Fusidic acid	0	0	2 (100)
Mupirocin	0	1 (50)	1 (50)
Chloramphenicol	0	1 (50)	1 (50)
Rifampicin	0	0	2 (100)
Trimethoprim/sulfamethoxazole	1 (50)	1 (50)	0

The phenomenon of antibiotic resistance in the case of β-lactams is based on the type of genetic determinants of plasmid and chromosomal governing the synthesis of β-lactamase, broad spectrum, which provides the resistance of staphylococci. Resistance to methicillin is transmitted by plasmids (R factor) having a pattern common to other β-lactams. For this reason, methicillin-resistant staphylococcal strains are considered zoonotic risk strains of staphylococci particularly with a complex circuit human-animal-human, respectively (Devriese et al. 2009; Dufour et al. 2002; Guardabassi et al. 2004; Lee 1994).

Compared to aminoglycosides (gentamicin, kanamycin) and macrolides (erythromycin and vancomycin), antibiotic sensitivity was different to the maximum and minimum vancomycin against kanamycin, erythromycin and gentamicin. These classes of antibiotics has been an increased resistance to antibiotics commonly used in pet therapy, as 4 strains resistant to kanamycin, 5 to erythromycin and 10 to gentamicin.

Antibiotic sensitivity to tetracyclines (tetracycline) was reduced 5 strains were resistant to this group of antibiotics, the phenomenon of resistance is plasmid and chromosomal type.

All strains tested were sensitive to enrofloxacin, marbofloxacin because these fluoroquinolones are used infrequently or not at all pets. Also to clindamycin (lincosamide) nitrofurantion, fusidic acid (other classes of antibiotics) mucopirin (class monoxycarbolic acid) and chloramphenicol (chloramphenicol) all isolates were susceptible to dogs.

The development of resistance staphylococci to different antibiotics, it is a consequence of wasteful use in the treatment of diseases in dogs and cats. Antibiotics used irrationally creates a selection pressure, are selected and transmitted genetic determinants of plasmid and chromosomal type. Consequently, the phenomenon of multiple resistance that is transmitted intra and interspecific. It is important particularly because the resistance to methicillin can be associated with resistance to β-lactams and other groups of antibiotics (Guardabassi et al. 2004).

As a result of the test strains of staphylococci isolated from clinically healthy dogs community, compared to 20 antibiotics were identified methicillin-resistant strains and several type of resistance, to β-lactams, tetracyclines, macrolides, aminoglycosides. The isolated strains were methicillin susceptible.

The data on methicillin resistance and type of resistance identified are similar to the results communicated by other authors on the phenomenon of resistance to antibiotics (Bywater et al. 2004; Bywater 2004; Fluit et al. 2006; Frank et al. 2009; Guardabassi et al. 2004; Kawakami et al. 2010).

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