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Full Length Research Paper

Multiresistant *Staphylococcus intermedius* isolated from otitis externa in dogs and them human owners – A practical approach

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Staphylococcus intermedius **is a commensal bacterium isolated from the mucous of clinically healthy dogs but commonly associated with pyoderma and otitis in dogs, causing opportunistic side infections. Otic exudates samples from 34 dogs affected by recurrent otitis externa and from 15 humans who had daily contact with them as pets were collected. Samples from five individuals who had no contact with any pet were also taken (as control group). Prevalence of antimicrobial resistance of strains of *S. intermedius* isolated from cases of otitis externa in dogs and their human owners was assessed. Gram stain, haemolysis, catalase and, respectively coagulase production for identification of staphylococci tests were used. Antimicrobial resistance was evaluated using difusometric standardized technique. All strains of *S. intermedius* isolated from dogs were resistant to polymyxin B (100%), a significant number of strains to erythromycin (66.66%), kanamycin (50%), tetracycline, lincomycin (45.8%), gentamicin and amoxicillin/clavulanic acid (37.5%), but highly susceptible to cefaclor (100%). Resistance high levels were also found among the eleven *S. intermedius* strains isolated from humans (100% polymyxin B, 72.7% kanamycin amoxicillin/clavulanic acid, tetracycline 45.5%), and also against lincomycin and gentamicin (27.3%). 13 strains of methicillin-resistant *S. intermedius* (MRSI) were identified: five strains from dogs and eight strains from humans. Otic, oral, nasal and anal mucosa can serve as excellent *S. intermedius* reservoir for colonization in dogs. From these places, bacteria can be transferred easily to humans, especially if they are in contact with their pets and *vice versa*.**

Key words: *Staphylococcus intermedius*, Otic, dogs.

INTRODUCTION

Staphylococcus is a large genus consisting of species of commensal bacteria, with habitats in the skin and mucous membranes of mammals, birds, and reptiles from around the world. In this group, named *Staphylococcus intermedius* exist three distinct species (*S. intermedius*, *Staphylococcus pseudintermedius*, *Staphylococcus delphini*), all are commensal bacterium isolated most frequently from clinically healthy dogs mucous (Euzéby (1996; 2001).

Based on our earlier investigations using simple phenotypic and biochemical methodology currently used in Romanian veterinary microbiology labs, it was established

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that the most frequent species in dogs was *S. intermedius* (Cristina and Degi, 2009). Although *S. Intermedius* was considered responsible for most cases of canine pyoderma, this species being present in various inflammatory skin diseases, are commonly associated with pyoderma and otitis in pets, frequently causing opportunistic and postoperative side infections in many body tissues (Abraham et al., 2007; Guardabassi et al., 2004; Normand et al., 2000).

As opportunistic pathogens, all staphylococci species reveal with high morbidity and even mortality in pets and humans for the veterinary practician as being more important in the general management of this issue. Identification of bacterial species (through genetic sequencing) is essential, especially for epidemiological detailed studies of *Staphylococcus* species and to assess the risk of zoonotic transmission for certain research purposes. Studies have reported that this bacterium is responsible for more than 90% of clinical cases of pyoderma and 45% of cases of acute otitis externa in dogs (Goodacre et al., 1997). However, an increasing number of reports substantiate that prolonged treatment and/or incorrectly selected antibiotics may give rise to the development of certain populations of *S. intermedius* resistant to multiple classes of antibiotics (Abraham et al., 2007; Loefler et al., 2007; Morris et al., 2006).

The presence of methicillin-resistant strains of *S. intermedius* became also a serious problem for the veterinary medical practice (Griffeth et al., 2008; Jones et al., 2007; Loefler et al., 2007). The possibility of transferring zoonotic strains of *S. intermedius* from dogs to humans has also been reported, and the presence and potential transfer of resistant strains from dogs to their owners is a critical public health concern for all (Bes et al., 2002; Goodacre et al., 1997; Harvey et al., 1994; Tanner et al., 2000).

Until now, information on the prevalence of resistant strains of *Staphylococcus* including MRSI in veterinary practices in our country is absent or sparse. This study intends to be a practical approach, rather than a typing or molecular one. We tried to investigate, from a therapeutic point of view (Kirby-Bauer methods) applicable for a veterinarian, the prevalence of antimicrobial resistance of a *Staphylococcus* spp representative, identifiable by this method isolated from dogs with recurrent otitis and from their owners.

MATERIALS AND METHODS

Sampling

From 34 dogs, otic exudates samples were collected during the period October, 2010 to March, 2011. Only dogs affected by recurrent otitis externa were included in this study. Also, these animals were reportedly exposed to prolonged antibiotic topical treatments (Table 1). Human subjects were represented by 15 pet owners, clinicians and students in veterinary medicine who had daily contact with their pets in the reason to increase the chances to isolate *S. intermedius*. Knowing that *S. intermedius* is a dog specific species, samples were also taken from five individuals who had no contact with any dog or pet constituting the control group in the reason to see if this strain could be found yet (Table 2). Samples were collected from peoples' nasal cavity (known as representatives for staphylococci colonisation) (Harvey et al., 1994), but also from oral or from skin (head, hands, neck). Samples were collected into sterile tubes (sterile swabs) moistened with sterile saline solution.

Bacteria isolation and identification

For the isolation and identification of staphylococci, microbiological standard methods including Gram staining, haemolysis, catalase test and, respectively coagulase production were used. Samples were processed in the laboratory within 3 h after collection. Swabs were seeded on 5% blood agar (Biomedics, Spain), MacConkey agar (Difco Laboratories UK) and were incubated at 37°C under aerobic conditions for 24 h. After incubation on 5% blood agar, the bacterial colonies were morphologically characterized and in terms of tinctorial affinity. All Gram positive cocoids were subjected to the catalase test. Catalase positive strains and Gram positive cocoids were included in the staphylococci group and coagulase activity has been tested. Mannitol fermentation was tested on hyper chlorinated Chapman medium (Liofilchem, Italy).

To highlight bound coagulase (clumping factor), latex agglutination kit Prolex™ System - Staph Latex kit (Pro-Lab Diagnostics, UK) was used with fresh stems in contact with latex particles sensitized with fibrinogen and IgG. Free coagulase was highlighted using citrated rabbit and bovine plasma, with the help of coagulated bactident coagulase kit (Merck, USA). Coagulase in most strains of pathogenic staphylococci was present. Biochemical characterization of staphylococci isolated was done by API Staph tests (BioMérieux, France), in accordance with manufacturer's recommendations. For identification, APIweb™ V4.1 API Staph (BioMérieux, France) software was used.

Antibiotic resistance determination

Susceptibility to antimicrobials was determined after Clinical Laboratory Standard Institute (CLSI) (CLSI/NCCLS, 2004) Kirby Bauer difusometric method, using commercial impregnated discs (Bioanalyse, France) with antibiotics for aerobic bacteria. Interpretation of antibiotic sensitivity test was performed by measuring the growth inhibition zone diameter, in accordance with the standards provided by the company producing the discs. For testing, Mueller Hinton agar medium (Oxoid, UK) was used. The strains of *S. intermedius* isolated from dogs and humans were evaluated against the most frequently used antibiotics in our veterinary clinics: methicillin - 30 µg, gentamicin - 10 µg, tetracycline - 30 µg, ciprofloxacin - 30 µg, kanamycin - 30 µg, novobiocin - 30 µg, doxycycline - 30 µg, erythromycin - 15 µg, vancomycin - 30 µg, ceftriaxone - 30 µg, polymyxin B – 50 UI, rifampicine - 30 µg, lincomycin - 30 µg, cefaclor - 30 µg, pristamicine - 15 µg, ampicillin/sulbactam - 30 µg, and amoxicillin/clavulanic acid - 30 µg.

RESULTS

Strains isolated in this study did not produce pigment or had a slight white tint, were urease positive, had fermented mannitol difficult and late, were coagulase positive

Table 1. Samples taken from dogs.

Type of otitis externa	Dog breed	Age/Sex
Relapsed chronic	Chow Chow	2 years/M
Erythematous/ceruminous	German Shepherd	4 years/M
Chronic	Labrador	8 months/F
Purulent acute	Golden Retriever	1 year/M
Relapsed chronic	Cocker Spaniel	6 years/F
Erythematous/ceruminous	Mongrel	10 years/F
Relapsed chronic	Poodle	2 years/M
Relapsed chronic	Shar Pei	10 months/M
Relapsed chronic	Labrador	6 years/F
Erythematous/ceruminous	Mongrel	3 years/F
Chronic	German Shepherd	1 year/M
Relapsed chronic	Poodle	3 months/M
Relapsed chronic	Pekingese	12 years/M
Relapsed chronic	West highland white terrier	2 years/F
Relapsed chronic	German Shepherd	5 years/F
Relapsed chronic	Poodle	8 years/M
Relapsed chronic	German Shepherd	6 months/M
Chronic	Golden Retriever	9 years/M
Chronic	Mongrel	15 years/F
Chronic	Pekingese	2 years/F
Relapsed chronic	Cocker Spaniel	1 year/F
Relapsed chronic	Mongrel	4 years/M
Relapsed chronic	Shar Pei	5 months/M
Relapsed chronic	Chow Chow	10 months/F
Relapsed chronic	Labrador	5 years/M
Relapsed chronic	Mongrel	8 years/M
Erythematous/ceruminous	Poodle	6 years/F
Chronic	Westy	2 years/F
Erythematous/ceruminous	Cocker Spaniel	7 years/M
Chronic	Golden Retriever	11 months/M
Relapsed chronic	Westy	6 years/M
Relapsed chronic	Carpathian Shepherd	4 years/M
Relapsed chronic	German Shepherd	2 years/M
Relapsed chronic	Poodle	1 year/F

and produced a β-type haemolysis (incomplete at 37°C and complete at 4°C). Digital code system API *Staph multitest* for *S. intermedius* was: 6,736,153. These specific phenotypical and biochemical compartment makes us to ascertain that the most probable strain is *S. intermedius.* A number of 24 strains of *S. intermedius* were isolated from dogs and 11 strains from the humans who had daily contact with dogs. Bacteriological examination of the samples taken from people who had no contact with dogs and which formed the control group revealed no presence of the *S. intermedius*. Distribution of isolates from humans was as follows: 5 strains (5/15) from skin samples, 4 strains (4/15) from the nasal cavity and 2 strains (2/15) from the oral cavity. Results of the antimicrobial sensitivity tests of *S. intermedius* isolated from dogs and humans are given in Tables 3 and 4. 13 strains of methicillin-resistant *S. intermedius* (MRSI), 5 strains from dogs with otitis externa and 8 strains from people were isolated.

Susceptibility tests showed that *S. intermedius* isolated from dogs were fully resistant to polymyxin B (100%). High level of resistance was observed to erythromycin (66.66%), kanamycin (50%), tetracycline and lincomycin (45.83%), gentamicin and amoxicillin/clavulanic acid (37.5%), but were highly susceptible to cefaclor (100%).

Among the eleven *S. intermedius* strains isolated from humans, we have identified a high level of resistance to polymyxin B (100%), kanamycin (72.72%), amoxicillin/

Table 2. Samples taken from people.

Sample (Area where collected)	Age/Sex	Occupation	Owned dog/daily contact (species, race, sex, age)
Skin (head)	28/F	Vet. clinician	Chow Chow, (F), 2 years
Skin (neck)	21/M	Vet. student	German Shepherd, (M), 4 years
Nasal cavity	22/M	Vet. student	Shar Pei, (F), 1 year
Nasal cavity	22/F	Vet. student	Mongrel, (M), 6 years
Skin (head)	35/M	Vet. clinician	Cocker Spaniel, (F), 3 years
Skin (head)	41/F	Pet owner	Labrador, (M), 10 months
Skin (hands)	38/M	Pet owner	Labrador, (F), 8 months
Nasal cavity	37/F	Vet. clinician	German Shepherd, (F), 2 years
Nasal cavity	25/M	Vet. student	Chow Chow, (M), 5 years
Nasal cavity	23/F	Vet. student	Poodle, (F), 8 years
Skin (hands)	18/M	Pet owner	Golden Retriever, (F), 3 years
Skin (head)	40/M	Pet owner	Westy, (F), 2 years
Nasal cavity	21/F	Vet. student	Poodle, (M), 12 years
Oral cavity	39/M	Pet owner	Cocker Spaniel, (M), 4 years
Skin (hands)	37/M	Vet. clinician	Shar Pei, (M), 4 years
Skin (hands)	28/M	Sales agent	-
Nasal cavity	32/F	Accountant	-
Skin (head)	21/F	Sales agent	-
Oral cavity	35/M	Security agent	-
Nasal cavity	45/M	Driver	-

clavulanic acid and tetracycline (45.45%).

DISCUSSION

These results indicate that *S. intermedius* is certainty a specific dog's staphylococcus. Its presence in humans is being closely linked to contact with the animals. Published data suggest that staphylococci are rarely resistant to first generation cephalosporins such as cephalexin or cefadroxil (< 5% of isolates), to synthetic β-lactamase-resistant penicillins (like oxacillin, dicloxacilin, amoxicillins potentiated with clavulanic acid), gentamicin, tobramycin, enrofloxacin, bacitracin and polymyxin B (Goodacre et al., 1997; Griffeth et al., 2008; Harvey et al., 1994). Resistance to potentiated sulphonamides, chloramphenicol and tylosin is also reported with a relatively low frequency (in 6 to 19% of isolates), while higher resistance to lincomycin, clindamycin and erythromycin (20 to 37% of isolates) is reported (Guardabassi et al., 2004).

Isolates from clinical disease in dogs and cats are frequently resistant to penicillin G, amoxicillin, neomycin and tetracycline (Guardabassi et al., 2004). Antibiotic sensitivity of *S. intermedius* is often difficult to assert, given the relative frequent confusion with *S. aureus* subsp. *aureus* (Abraham et al., 2007; Yoon et al., 2010).

The phenomenon of resistance was found to the following antibiotics (in descending order): beta lactams-lactamase-sensitive, tetracycline, florfenicol, streptomycin, erythromycin, sulphonamides and associations: trimethoprim, lincosamides and floroquinolones. Resistance to chloramphenicol, tetracyclines, macrolids and lincosamides is encoded by small plasmids (Normand et al., 2000).

Lilenbaum et al. (2000) tested the sensitivity to antibiotics of staphylococci isolated from external otitis in dogs. Resistance to at least one antibiotic was observed in 90.9% of isolates. Antibiotic multi-resistance was present in most tested strains. Resistance to three different antibiotics was commonly reported, being described in 36.4% of isolates, both Staph coagulase positive and negative.

In a study performed by Pedersen et al. (1995), the antimicrobial sensitivity of a total of 60 strains of *S. intermedius* isolated from dogs was tested. Of the total isolates, 15 strains were from healthy dogs, 9 and 36 from external pyodermatitis. Of the 60 strains, 60% showed resistance to penicillin, 24% to spiramycin, 20% to tetracycline, 16.5% to chloramphenicol and 2% to ficidic acid. Yamashita et al. (2005) have also isolated staphylococci from external auditory meatus in dogs with otitis externa (48.3%) and also from dogs without ear diseases to a rate of 68.3%. Collected samples were tested against 17 different antibiotics and resistance was

Table 3. Susceptibility to antibiotics of 24 *S. intermedius* strains isolated from dogs' ear channels.

Antimicrobial substance (disk concentration)	Strains of *S. intermedius* isolated from humans N(%)		
	Susceptible	Intermediate	Resistant
Methicillin-ME-30 µg	16(66.66)	3(12.5)	5(20.83)
Gentamicin-CN-10 µg	14(58.33)	2(8.33)	9(37.5)
Tetracycline-TE-30 µg	11(45.83)	2(8.33)	11(45.83)
Ciprofloxacin-CIP-30 µg	22(91.66)	2(8.33)	0
Kanamycin-K-30 µg	5(20.83)	7(29.16)	12(50)
Novobiocin-NV-30 µg	19(79.16)	6(25)	0
Doxycycline-DO-30 µg	14(58.33)	4(16.66)	6(25)
Erythromycin-E-15 µg	7(29.16)	2(8.33)	16(66.66)
Vancomycin-VA-30 µg	18(75)	7(29.16)	0
Ceftriaxone-CRO-30 µg	20(83.33)	5(20.83)	0
Polymyxin-PB-50 UI	0	0	24(100)
Rifampicine-RA-30 µg	22(91.66)	3(12.5)	0
Lincomycin-L-30 µg	5(20.83)	8(33.33)	11(45.83)
Cefaclor-CEC-30 µg	24(100)	1(4.16)	0
Pristinamycin-PT-15 µg	21(87.5)	4(16.66)	0
Ampicillin/Sulbactam-SAM-30 µg	17(70.83)	8(33.33)	0
Amoxicillin/clavulanic acid-AMC-30 µg	11(45.83)	5(20.83)	9(37.5)

Table 4. Susceptibility to antibiotics of 11 strains of *S. intermedius* isolated from dog owners.

Antimicrobial substance (disk concentration)	Strains of *S. intermedius* isolated from humans N(%)		
	Susceptible	Intermediate	Resistant
Methicillin–ME-30 µg	3(27.27)	0	8(72.72)
Gentamicin-CN-10 µg	6(54.54)	2(18.18)	3(27.27)
Tetracycline-TE-30 µg	2(18.18)	4(36.36)	5(45.45)
Ciprofloxacin-CIP-30 µg	10(90.90)	1(9.09)	0
Kanamycin-K-30 µg	2(18.18)	1(9.09)	8(72.72)
Novobiocin-NV-30 µg	11(100)	0	0
Doxycycline-DO-30 µg	3(27.27)	4(36.36)	4(36.36)
Erythromycin-E-15 µg	5(45.45)	1(9.09)	5(45.45)
Vancomycin-VA-30 µg	7(63.63)	4(36.36)	0
Ceftriaxone-CRO-30 µg	8(72.72)	3(27.27)	0
Polymyxin B-PB-50 UI	0	0	11(100)
Rifampicine-RA-30 µg	11(100)	0	0
Lincomycin-L-30 µg	2(18.18)	5(45.45)	3(27.27)
Cefaclor-CEC-30 µg	11(100)	0	0
Pristamicine-PT-15 µg	11(100)	0	0
Ampicillin/Sulbactam-SAM-30 µg	7(63.63)	4(36.36)	0
Amoxicillin/clavulanic acid-AMC-30 µg	2(18.18)	4(36.36)	5(45.45)

reported in 59.4% of all isolates. Resistance to penicillin G and amoxicillin was the most frequently found incidence.

Cole et al. (2006) evaluated the possibility of results extrapolation of ciprofloxacin susceptibility *in vitro* testing to enrofloxacin, in the case of bacterial strains isolated from external otitis in dogs. In the case of 41.4% of dogs and of 17.1% of bacteria were reported discrepancies

between sensitivity test results for enrofloxacin and ciprofloxacin. The results of these tests were highly dependent on the concentration of antibiotic used in treatments. Therefore, according to CLSI recommendations, individual testing of floroquinolones is required. The results of our study are confirmed also by Baptiste et al. (2005), Gortel et al. (1999) and Van Duijkeren et al. (2004) who reported that most methicillin-resistant strains of staphylococci isolated from dogs with clinical infections were *S. intermedius* isolates.

Conclusions

Animals that received treatments with antibiotics constitute the category with high risk of acquiring resistant organisms, encouraging the transfer of such bacteria by antibiotics, by reducing the normal population of bacteria of the normal resident *Staphylococcus.* Resistance phenomenon of *S. intermedius* was observed in dogs in the order: polymyxin B, erythromycin, kanamycin, tetracycline, lincomycin, gentamicin and amoxicillin/clavulanic acid, respectively but was highly susceptible to cefaclor. Otic, oral, nasal and anal mucosa can serve as excellent *S. intermedius* reservoir for colonization in dogs. From these places, bacteria can be transferred easily to humans, especially if they are in contact with their pets and *vice versa*. Resistance high levels were also found among the eleven *S. intermedius* strains isolated from humans in the order: polymyxin B, kanamycin amoxicillin/clavulanic acid, tetracycline and also against lincomycin and gentamicin. Methicillin-resistant *S. intermedius* (MRSI) were identified in many cases: five strains from dogs and eight strains from humans. A rapid and correct diagnosis of MRSI strains is of great importance for the correct treatment with antibiotics in dogs. In this respect, we recommend the implementation of our methodology, as national program in veterinary practice from our country.

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