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Antimicrobial resistance of staphylococci and streptococci isolated from dogs

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Abstract. A study was conducted for the examination of bacterial species isolated in dogs from Animal Clinics of Nanjing Agricultural University, China. Forty nasal swabs were taken from dogs having respiratory signs. *Staphylococcus pseudintermedius* was the most frequently isolated pathogen (37.50 %) followed by *Staphylococcus aureus* (18.75%), *Streptococcus pluranimalium* (10.93%), *Streptococcus canis* (9.37%), *Staphylococcus schleiferi* (9.37%), *Staphylococcus intermedius* (6.25%), *Staphylococcus cohnii* (4.71%) and *Staphylococcus hominis* (3.12%). *S. pseudintermedius* and *S. pluranimalium* were subjected to commonly used antibiotics for determination of resistant drugs. Antimicrobial resistance in *S. pseudintermedius* was common in gentamicin (70.83%) and tetracycline (50%) while in *S. pluranimalium* was common in enrofloxacin (71.42%) and gentamicin (57.14%).

INTRODUCTION

Respiratory problems are common in dogs. A varying flora of bacterial pathogens is normally present in the respiratory tract of the canines without causing any clinical signs. Opportunistic infections by *Pasteurella multocida*, *Bordetella bronchiseptica*, streptococci, staphylococci, *Pseudomonas* and coliform bacteria can damage the respiratory system leading to disease conditions in dogs. Infections usually occurred in kennels, pet shops, boarding facilities and humane shelters (Kahn *et al.*, 2005). Staphylococcal infections are very common, and the bacteria are normally habitants of skin and mucus membranes of human and animals. They are differentiated by coagulase production, and coagulase positive are more pathogenic than coagulase negative staphylococci. *Staphylococcus pseudintermedius* is a zoonotic coagulase positive *Staphylococcus* first identified in 2005 (Devriese *et al.*, 2005) and is the most important opportunistic pathogen in companion animals subject to the different sites of the body. Biochemical tests arginine dihydrolase production (*Staphylococcus intermedius* is negative), D-mannitol anaerobically and β-gentiobiose fermentation gentobiose in aerobic conditions (*S. intermedius* is positive) can be used to distinguish *S. pseudintermedius* from other members of *Staphylococcus intermedius* group (SIG) especially *S. intermedius*. However, phenotypic tests cannot clearly differentiate *S. pseudintermedius* and *Staphylococcus delphini* (Sasaki *et al.*, 2007b).

After first case of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) reported in 2005, worldwide more cases of MRSP has been reported from companion animals (Van Duijkeren *et al.*, 2011). It's a new challenge in veterinary medicine and almost resistant to all antimicrobial agents available for companion animals (Loeffler *et al.*, 2007; Ruscher *et al.*, 2009; Perreten *et al.*, 2010). However, in China it is only isolated from dogs with pyoderma (Wang *et al.*, 2012). Studies on prevalence and characterization of the staphylococci have been reported from veterinary hospitals in Asia, Europe and America (Moon *et al.*, 2012). Antimicrobial resistance of bacteria originating in companion animals is of great concern, it's because of very close contact of companion animals with humans (Jackson *et al.*, 2009). It represents a major threat to veterinary medicine and as well as human health. Bacterial genes that mediate resistance are quickly evolving and diversifying (Munoz-Price *et al.*, 2013). The detection as well as control of antimicrobial resistance are important for continuous effective treatment against pathogenic microorganisms (Adams *et al.*, 2018). The continuous usage of antimicrobial drugs provides selection pressure favoring the evolution and dissemination of antimicrobial resistant bacteria in human and animal populations (Chantziaras *et al.*, 2013). The present study was designed to determine prevalence of respiratory infections and evaluate the antimicrobial resistance of bacterial species isolated from respiratory infections in dogs from Nanjing, China, in order to update data and evaluate best therapeutic indications for the antimicrobial drugs.

MATERIALS AND METHODS

A total of 40 dogs were investigated for the isolation and antimicrobial resistance of staphylococcal and streptococcal species. Sterile cotton swabs were used to collect nasopharyngeal samples from Animal Clinics of Nanjing Agricultural University in Jiangsu Province of China. The samples were taken from individual dogs showing respiratory symptoms such as coughing, sneezing and copious nasal discharge. Bacterial samples were cultured overnight on tryptic soy agar, MacConkey's agar and 5% sheep blood agar. Isolates were repeatedly sub-cultured at least four to five times to ensure similarity of the tested colonies and to avoid the risk of mixed cultures. If there was no growth in the plates after incubation, they were considered as negative while positive ones were sub-cultured to obtain pure culture. Sub-culture was done on mannitol salt agar, tryptic soy agar, MacConkey's agar and blood agar, plates were incubated at 37°C for 24 hours. Viable plate count method was used to know the number of bacteria present in the sample. Bacterial colonies that did grow were quantified by standard plate count. Colonies between 30-300 were taken into consideration to calculate the colony-forming units (CFU). Pure isolates were identified on the basis of colony characteristics, Gram stain, pigment production, hemolytic characteristics on 5% sheep blood agar. Swabs were placed in phosphate buffered saline (PBS, 1x), and kept in refrigerator at 4°C. Biochemical tests such as catalase, oxidase, coagulase, urease, mannitol fermentation etc were also performed according to Bergey's Manual of Determinative Bacteriology (Holt, 1994). Isolated bacterial strains were stored at -80°C in 20% (v/v) glycerol. In addition to above mentioned tests, PCR was performed for the confirmation of the bacteria. PCR amplification of 16S rRNA gene was performed for the confirmation of the bacteria using a pair of oligonucleotide primer (5'AGAGTTTGATCMTGGCTCA/ TACGGYTACCTTGTTACG ACTT-3'). PCR products were sequenced from Invitrogen Company (Shanghai, China). Antimicrobial testing was carried out on 12 antimicrobial agents by standard broth dilution method for *S. pseudintermedius* and *S. pluranimalium*. Plates were inoculated by streaking the agar surface with a swab on Muller Hinton (MH) medium (Oxoid, UK). Culture was incubated for 2–6 hours and was adjusted to 0.5 Mac Farland turbidity

standards before being used as an inoculum. The inhibitory zone diameters gained around the discs were measured after incubation for 24h at 37°C and evaluated according to the Clinical Laboratory Standards Institute (Wikler 2006). The strains were categorized as susceptible or resistant to the drug. The following antimicrobials were tested: Amikacin, Amoxicillin, Ampicillin, Ciprofloxacin, Enrofloxacin, Erythromycin, Gentamicin, Kanamycin, Lincomycin, Oxacillin, Tetracycline and Trimethoprim-sulfamethoxazole.

RESULTS AND DISCUSSION

In the present study, fifty-one staphylococcal spp. and thirteen streptococcal spp. were isolated from forty dogs having respiratory infection. Results of bacterial isolations are summarized in Table 1. During present study prevalence of *S. pseudintermedius* was 37.50%, followed by *S. aureus* (18.75%), *S. pluranimalium* (10.93%), *S. canis* (9.37%), *S. schleiferi* (9.37%), *S. intermedius* (6.25%), *S. cohnii* (4.71%) and *S. hominis* (3.12%). Results of present study are in accordance with the results obtained by other authors who recorded (46.2%) *S. pseudintermedius* from inpatient dogs and (19.4%) from outpatient dogs kept in a Japanese veterinary teaching hospital (Sasaki *et al.*, 2007a). Prevalence *S. aureus* and *S. pseudintermedius* in dogs documented in urban and rural areas showed 43.8% and 20.7% prevalence of *S. aureus* in urban and rural areas respectively, while prevalence of 25% and 22% of *S. pseudintermedius* was observed in urban and rural areas respectively (Gómez *et al.*, 2013b). In addition isolation of MRSP (4.5%) and *S. schleiferi* (0.8%) has also been reported from dogs in Canada (Hanselman *et al.*, 2009). However, our results are not in agreement with their results who reported higher *S. pseudintermedius* isolation from 153 (87.4%) of 175 dogs (Rubin and Chirino-Trejo 2011a). Also, higher prevalence of *S. pseudintermedius* 76.1% in 1999-2000 and 76.4% in 2009 was reported by Onuma *et al.* (2012) in dogs with pyoderma infection, while low MRSP (2.1%) and methicillin-resistant *Staphylococcus schleiferi* subsp. *coagulans* (MRSS) (0.5%) was reported in Canada by Hanselman *et al.* (2008). Concerning total bacteria quantification in CFU, 8 of the 64 isolates (12.5%) contained 1-30 CFU, 16 (25%) contained 31-60 CFU, and 40 (62.5%) contained more than 60 CFU per plate. Table 1 summarizes the CFU results according to the bacterial species isolated from dogs. Biochemical tests were performed for the identification of *S. pseudintermedius* and *S. pluranimalium* are presented in Table 2.

Antibiotic resistance of staphylococci is an alarming situation due to increased incidence of isolation of methicillin-resistant strains of *S. pseudintermedius* (Schwarz *et al.*, 2008). The antimicrobial resistance to twelve antimicrobial agents

Table 1. Number and percentage of bacteria isolated from dogs

Bacteria isolated from dogs (n-40)	Numbers	Percentage (%)	Cfu/plate		
			1-30	31-60	>60
Staphylococcus pseudintermedius	24	37.50	4	6	14
Staphylococcus aureus	12	18.75	2	3	7
Staphylococcus schleiferi	6	9.37	1	3	2
Staphylococcus intermedius	4	6.25	0	1	3
Staphylococcus cohnii	3	4.71	0	0	3
Staphylococcus hominis	2	3.12	0	0	2
Streptococcus pluranimalium	7	10.93	1	2	4
Streptococcus canis	6	9.37	0	1	5
Total	64	100	8	16	40

Table 2. The biochemical characteristics of *S. pseudintermedius* and *S. pluranimalium*

Characteristics	*S. pseudintermedius* (n=24)	*S. pluranimalium* (n=7)
Arginine dihydrolase	11 (45.83%)	2 (28.51%)
Catalase	24 (100%)	0 (0%)
Coagulase	24 (100%)	1 (14.28%)
D-arabinose	8 (33.33%)	2 (28.51%)
Glucose	20 (83.33%)	3 (42.58%)
Glycogen	12 (50%)	1 (14.28%)
Hydrolysis of Aesculin	7 (29.16%)	4 (57.14%)
Inulin	8 (33.33%)	1 (14.28%)
Lactose	20 (83.33%)	4 (57.14%)
Maltose	12 (50%)	5 (71.28%)
Mannitol	11 (45.83%)	1 (14.28%)
Ribose	17 (70.83%)	3 (42.85%)
Sorbitol	7 (29.16%)	2 (28.57%)
Starch	3 (12.5%)	0 (0%)
Sucrose	19 (79.16%)	6 (85.71%)
Urease	14 (58.33%)	0 (0%)
Voges-Proskauer	15 (62.5%)	0 (0%)
Xylose	2 (8.33%)	1 (14.28%)

Table 3. Resistance of *S. pseudintermedius* and *S. pluranimalium* isolates recovered from respiratory cases in dogs

Antimicrobial drugs	*S. pseudintermedius* (n=24)	*S. pluranimalium* (n=7)
Amikacin	2 (8.33%)	0 (0%)
Amoxicillin	3 (12.5%)	1 (14.28%)
Ampicillin	5 (20.83%)	1 (14.28%)
Ciprofloxacin	2 (8.33%)	2 (28.57%)
Enrofloxacin	9 (37.5%)	5 (71.42%)
Erythromycin	11 (45.83%)	1 (14.28%)
Gentamicin	17 (70.83%)	4 (57.14%)
Kanamycin	6 (25%)	3 (42.85%)
Lincomycin	5 (20.83%)	2 (28.57%)
Oxacillin	11 (45.83%)	2 (28.57%)
Tetracycline	12 (50%)	2 (28.57%)
Trimethoprim-sulfamethoxazole	9 (37.5%)	3 (42.85%)

was determined for *S. pseudintermedius* and *S. pluranimalium*. The results of antimicrobial resistance of bacterial isolates are presented in Table 3. The prevalence of methicillin resistant staphylococci was reported to be higher in veterinary healthcare environments and antimicrobial therapy (Huerta *et al.*, 2011) and treatment of these bacteria was difficult due to their resistant to several antibiotics including the β-lactam antibiotics (Meucci *et al.*, 2010). *S. pseudintermedius* showed higher resistance to Gentamicin (70.83%), tetracycline (50%) and oxacillin (45.83%), while antimicrobial resistance in *S. pluranimalium* was common to enrofloxacin (71.42%), gentamicin (57.14%) and trimethoprim-sulfamethoxazole (42.85%). *S. aureus* and *S. pseudintermedius* are frequently isolated as main opportunistic pathogens in companion animals. High resistance to tetracycline (56.3%) is common in *S. pseudintermedius* (Gómez *et al.*, 2013a). Higher resistance of *S. pseudintermedius* isolated from Saskatoon to tetracycline, erythromycin and

trimethoprim-sulfamethoxazole and lower resistance to penicillin have been observed (Rubin *et al.*, 2011b). Higher susceptibility or low resistance of *S. pseudintermedius* to gentamicin is consistent with our study (Rubin and Chirino-Trejo 2011a). Our results are in agreement with other authors who reported antimicrobial resistance of *S. pneumoniae* in the United States and Canada to amoxicillin (18.1% and 10.5%); erythromycin (11.7%–14.3% and 5.0%–7.4%); tetracycline (10.2% and 10.9%) and trimethoprim-sulfamethoxazole (19.8% and 15.8%) respectively (Doern *et al.*, 1998). High resistance of streptococcal species to gentamicin and enrofloxacin has been observed by Windahl *et al.* (2015) which is also in agreement with our results.

CONCLUSION

It was concluded from the present study that *S. pseudintermedius* and *S. pluranimalium* are major species in genus *Staphylococcus* and *Streptococcus*, responsible for respiratory infections in dogs. Antimicrobial resistance revealed gentamicin and tetracycline as most resistant drugs against *S. pseudintermedius* while enrofloxacin and gentamicin were most resistant drugs against *S. pluranimalium* isolates.

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