



Multi Drug Resistant *Staphylococcus pseudintermedius* isolates from Canine Pyoderma

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ABSTRACT

Staphylococcus pseudintermedius is an important opportunistic pathogen of companion animals, especially dogs. Four dogs with a long-term inflammatory skin disorder due to infection with methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) are described. Bacteria was isolated from the skin swab samples from diseased animal and characterized with the different biochemical test and identification strips. Antibiotic resistance pattern has been examined. All strains were shown multidrug resistance pattern and found sensitive only for Amikacin. *Staphylococcus pseudintermedius* was also found coagulase positive and methicillin resistant strain.

Keywords: Canine pyoderma, multi drug Resistant, *staphylococcus pseudintermedius*

Canine pyoderma is the most common type of bacterial skin infection in the dog. Varieties include superficial pyoderma, characterized by pustules, papules, erythema, focal crusting, and pruritus and deep pyoderma with furuncles and draining tracts. Although Gram-positive bacteria, such as staphylococci are commonly involved in superficial pyoderma gram negative bacteria can cause secondary infection, particularly in cases of deep pyoderma (Rosser, 2000). The primary skin pathogens of dogs are the staphylococci species, of which the coagulase-positive species *Staphylococcus pseudintermedius*, previously referred as *S. intermedius* is most frequently isolated (Guardabassi *et al.* 2004). *S. pseudintermedius* is present in the healthy dog's skin and besides pyoderma can also cause urinary infection, corneal ulcer and endocarditis. It also causes otitis externa and otitis media which is common problems among dogs (Kumar *et al.* 2014). Treatment usually involves antimicrobial drug

therapy. Culture and antibiotic susceptibility testing are indicated in cases which do not respond to preliminary treatment and mandatory when treating deep pyoderma. In addition, information on the principal organisms associated with pyoderma and their susceptibility patterns against commonly used antimicrobial drugs is highly useful in empirical treatment.

Methicillin Resistant Staphylococci (MRS) are important pathogens in human and veterinary healthcare and are often multi-drug resistant (MDR; resistant to three or more classes of antimicrobial) extremely limiting therapeutic options (Weese *et al.* 2010; Perreten *et al.* 2010). The aim of this study was to find out the causal agents of pyoderma infections in canines and their drug sensitivity.

MATERIAL AND METHOD

All four dogs with a two or three month history of



pruritus and inflammatory skin disease were referred to TVCC of college of veterinary science and animal husbandry Mhow. Clinical examinations revealed generalized pruritic dermatitis with epidermal collaret's and focal crusty lesions. Superficial and deep skin scrapings and skin swab sample sent to the laboratory of department of microbiology of college of veterinary science and animal husbandry Mhow for fungal and bacterial examination, respectively. For fungal examination skin scraping was examined under microscope with 10% KOH mounting. Sample was collected from dog with sterile cotton swab from the skin lesion. Swab was inoculated into brain heart infusion broth and incubated aerobically for 24 hrs at 37°C. After that a loop full of broth sub cultured on nutrient agar plate and was again incubated for overnight at 37°C. Typical colonies were picked and bacteria were identified by standard laboratory procedure. *Staphylococcus* identified with the help of morphology, gram staining, and growth on selective media mannitol salt agar, catalase test, oxidase test and slide coagulase test. Further identification of the strain was done by using Hi25 identification kit KB004 (Hi-media) for *staphylococcus* with having following test: VP test, ONPG, urease, arginine utilization test and different sugars mannitol, sucrose, lactose, arabinose raffinose trehalose and maltose for sugar fermentation test. The standard strain *S. aureus* ATCC was used as control in all tests.

The susceptibilities of the isolate to 25 antibiotics (Hi-media) commonly used in veterinary and human medicine penicillin G 10 µg, oxacillin 5 µg, amoxycylav 30 µg gentamicin 30 µg, Sparfloxacin 5 µg, ceftriaxone 30 µg, azithromycin 5 µg, clindamycin 2 µg, cefoparazone 75 µg, cefuroxime 30 µg, ampicillin-cloxacillin 10 µg, Nalidixic acid 30 µg, ampicillin/sulbactam 10 µg, cephalothin 30 µg, enrofloxacin 10 µg, cephotoxime 10 µg, cefalexine 30 µg, streptomycin 10 µg, norfloxacin 10 µg, amikacin 30 µg, tetracycline 30 µg, ciprofloxacin 5 µg, ofloxacin 5 µg, cephalosporin 30 µg and levofloxacin 30 µg were performed by the disk diffusion method observing the recommendation of the Clinical and Laboratory Standards Institute (CLSI, 2007).

Although references to methicillin resistance are commonplace in the medical literature, oxacillin is often used by veterinary microbiology laboratories as the correlate for testing antimicrobial resistance. Bemis *et al.* (2009) were reported that both methicillin and oxacillin are semisynthetic penicillinase-resistant penicillins, but oxacillin is more stable *in vitro*. We also revealed one methicillin-resistance strain using the agar diffusion method (Oxacillin 5 µg, breakpoint ≤ 17mm).

The standard strain *S. aureus* ATCC was used as control in all tests.

RESULT AND DISCUSSION

Gram positive bacteria *Staphylococcus* from all samples and gram negative bacteria *Enterobacter* from one sample were recovered. Species *S. pseudintermedius* was confirmed by using Histaph identification strips and found Coagulase positive (CoPS). In reports, most of the canine strains of *Staphylococcus* group have been identified as *S. pseudintermedius* (Sasaki *et al.* 2007; Bannoehr *et al.* 2009). Presence of *Enterobacter* may be due to secondary infection through environment. *S. pseudintermedius* was found to be the primary cause in pyoderma infection. This is in agreement with previous reports. (Samanta, 2013; Scott *et al.* 2006)

In fungal examination some *Alternaria spp.* were suspected only on the basis of microscopic morphology. In most of the cases they are considered as environmental contaminants (Ileana and Adrian, 2010). But some of them are reported to be associated with lesions in buccal mucosa of dogs and skin lesions of cats (Samanta, 2015).

In antibiotic sensitivity test all *S. pseudintermedius* strains were found resistant to all tested antibiotic drugs including Oxacillin but found highly sensitive to Amikacin while Vincze *et al.* (2010) reported the same strain but found resistant to Amikacin in case of canine pyoderma. In contradictory of our results, Mondal and Sahoo (2014) reported a staphylococcal strain found sensitive to Azithromycin, Gentamycin, Enrofloxacin and Ciprofloxacin. The multi-drug resistant strains emergence is a phenomenon that is occurring worldwide and that has hindered the treatment of human and animal staphylococcal infections. Different mechanisms, such as efflux pumps capable of causing the extrusion of several types of antibiotics out of the bacterial cell, encoded by genes located on plasmids (Cohn and Middleton, 2010).

Here we report about a long-term skin disease in dogs caused by a multidrug-resistant methicillin-resistant staphylococcus *S. pseudintermedius* (MDR-MRSP) strain. Although methicillin-resistant staphylococci are not necessarily more virulent than methicillin-susceptible staphylococci, treatment options are often severely limited by multidrug resistance. This is particularly true for infections caused by methicillin-resistant *S. pseudintermedius* (MRSP). MRSP isolates are increasingly multidrug resistant. (Ruscher *et al.* 2010; Kadlec *et al.* 2010). In other words MRSP infections with this organism are going to be extremely difficult and sometimes impossible to treat.

MRSP infections in small animals and horses seem to be on the rise in general (Black *et al.*, 2009; Moodley *et al.* 2009; Ruscher *et al.* 2010), and isolates resistant to five or more classes of antibiotics have been reported recently (Loeffler *et al.* 2005; Sasaki *et al.* 2007; Ruscher *et al.* 2010).

This study has concluded that successful treatment choices should be based on culture and susceptibility testing. There has been a sudden emergence of multi resistant characteristics of these bacteria. Occurrence of resistant staphylococci on canine pyoderma and that canine host may contribute to the maintenance and dissemination of drug resistant staphylococci in our midst.

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