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PRELIMINARY SCREENING OF CLOXACILLIN SUSCEPTIBILITY IN STAPHYLOCOCCAL SPECIES ISOLATED FROM HEALTHY DOGS PRESENTED TO VETERINARY TEACHING HOSPITAL, UNIVERSITY OF PERADENIYA, SRI LANKA

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SUMMARY: *Staphylococcus spp* are part of the normal flora of the skin of dogs where it is also found as an opportunistic pathogen. Antimicrobial resistance is changing over time and is generally rising steadily for those antimicrobials that are frequently used. The cloxacillin susceptibility of staphylococcus isolates from 30 healthy dogs, presented to the Veterinary Teaching Hospital, University of Peradeniya, Sri Lanka was investigated in this study. Samples collected from both the ear canals and the skin of each dog were initially grown on nutrient agar *identified* using conventional biochemical methods. Out of the 64 Staphylococci isolates, 36(56.25%) were obtained from the skin surface and 28 (43.75%) isolates from the ear canals. The susceptibility of 64 isolates to cloxacillin was examined by cloxacillin-added agar medium (5µg/ml). Out of 64 isolates, 14 isolates (21.87%) of *Staphylococcus spp* were resistant to cloxacillin and 7 (50%) of them were *S. aureus* which was confirmed by biochemical tests. Out of 28 isolates of *Staphylococcus spp* from the ear canal, 2 (7.14%) isolates were resistant to cloxacillin, and out of 36 isolates from the skin, 12 (33.34%) isolates were resistant to cloxacillin. A high number of cloxacillin-resistant Staphylococci isolated from dogs reflects the emergence of cloxacillin-resistant strains of *Staphylococcus spp*. This signals the need for antibiotic susceptibility testing in order to choose antimicrobial agents to treat diseases caused by resistant *Staphylococcus spp* and also paves the way for future studies paying more attention regarding development of cross-resistance to other antibiotics by these resistant strains.

KEYWORDS: Dogs**,** *Staphylococcus spp*, antimicrobial resistance, cloxacillin-resistant, Sri Lanka.

INTRODUCTION

The awareness of the normal microbial population of the skin is important in understanding the pathophysiology of skin disease in companion animals. *Staphylococcus spp* is one of the most common pathogens isolated from skin and ear infections in dogs. Though Staphylococcus *spp* commonly occurs as a part of the normal flora, they can be an opportunistic pathogen (Rubin and Chirino-trejo, 2011). Coagulase-positive staphylococci (COPS) such as *S. aureus*, *S. intermedius* and *S. pseudintermedius* are found as primary staphylococcal pathogens in dogs. The coagulase-negative staphylococci such as *S. epidermidis*, *S. haemolyticus* and *S. hycus* are found to be less or non-pathogenic commensals (Conner *et al*., 2018). *S. intermedius* colonizes the skin and mucosal surfaces in 90% of healthy dogs (Priyantha *et al*., 2016), where *S. intermedius* is a common cause of pyoderma and otitis externa encountered in nosocomial infections in dogs (Duijkeren *et al*., 2011). In addition, *S. aureus* and *S. schleiferi* are emerging as common pathogenic species in companion animals (Kong *et al*., 2008).

Acquired multi-drug resistance to commonly used antimicrobials in COPS is increasing over time in companion animals (Pedersen *et al*., 2007). A recent study showed opportunistic *Staphylococcus spp* also has high resistance to ampicillin, amoxicillin, clindamycin and erythromycin (Bertelloni, Cagnoli, Ebani, 2021). Resistance to penicillin among staphylococci is due to the production of staphylococcal beta-lactamase enzymes that hydrolyse beta-lactam antibiotics (Lowy, 2003). Additionally, methicillin-resistant staphylococci, including methicillin-resistant *Staphylococcus aureus* (MRSA) isolates have acquired an altered penicillin-binding protein, PBP2a, which is encoded

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by the *mecA* gene (Witte *et al*., 2007). From 2006 there is a dramatic worldwide increase in the frequency of methicillin-resistant *Staphylococcus spp* (Moodley *et al*., 2014). Methicillin-Resistant *Staphylococcus aureus* (MRSA) was found in nosocomial infection (health-care-associated MRSA (HA-MRSA)) in many parts of the world (Lee *et al*., 2018). Reported zoonotic transmission of MRSP infection from dogs to humans indicates the potential occupational and health hazard of this organism (Rubin and Gaunt, 2011).

The frequent use of antimicrobials is one of the contributing factors to the development of antimicrobial resistance (Castro-Sánchez *et al*., 2016) and more studies have reported higher levels of resistance development to β-lactams, most likely due to the selection pressure resulting from a higher frequency of drug usage in companion animals (Conner *et al*., 2018). Understanding the development of antimicrobial resistance is important to minimise the increase in the incidence of resistance which needs to be developed and implemented within the clinical setting. Therefore, this preliminary study was undertaken to investigate the study on cloxacillin susceptibility of staphylococcal species isolated from the skin and ear canal of healthy dogs presented to Veterinary Teaching Hospital, University of Peradeniya. Cloxacillin is frequently used for the treatment of common bacterial infections of dogs, including impetigo, cellulitis and otitis externa. However, it is not effective for MRSA. Rapid identification of cloxacillin resistance may be used as a surrogate method for detecting MRSA.

MATERIAL AND METHODS

Samples were collected from 30 healthy male and female dogs which were presented to the vaccination unit at the Veterinary Teaching Hospital, University of Peradeniya over a period of three months. The signalment, history, and vital parameters (temperature, heart rate, pulse rate and respiratory rate) were obtained upon admission. The dogs with dermatological conditions and contact with other sick dogs were excluded from the study. Two ear swabs, one from each ear canal and one swab from the skin of each healthy dog were obtained separately. Skin samples were obtained by swabbing around the nares, axilla, inguinal and perineal region.

Isolation and identification of Staphylococcus species

For each dog sampled, a fresh pair of sterile gloves was used. Swabs were inoculated on nutrient agar and incubated aerobically at 37^0 C for 24 hours. Gram-positive and catalase-positive morphotypes were seeded on mannitol salt agar medium and incubated aerobically at 37^0 for 24 hours (Kateete *et al*., 2010). The non-mannitol fermenters were inoculated into sheep blood agar and incubated aerobically at 37^0 for 24 hours to evaluate the hemolytic ability (UK Standards for Microbiology Investigations, Identification of Staphylococcus species, Micrococcus species, and Rothia species, ID 07 (Quinn *et al., 2011*).

Detecting cloxacillin Susceptibility

The susceptibility of *Staphylococcus spp* for cloxacillin was investigated using a cloxacillin resistance screening agar method. The nutrient agar medium was autoclaved (121°C), cooled to 55°C and cloxacillin was added with 5µg /ml by using a micropipette. Briefly, all isolates were inoculated into nutrient agar containing 5µg /ml cloxacillin. Plain nutrient agar was used as a control. The plates were incubated at 37^0 C aerobically for 24 hours. This test was repeated for confirmation. Finally, cloxacillin-resistant cultures were characterized by the Voges-Proskauer (VP) test in order to differentiate cloxacillin-resistant *S. aureus* from other species.

RESULTS

Isolation of Staphylococcus species

Total 111 bacterial isolates were recovered. Of those, 64 isolates (57.65%) were *Staphylococci.* Out of the 64 staphylococcal isolates, 28 (43.75%) were originating from the ear canal samples and 36 (56.25%) from the skin samples. Of the 64 staphylococcal isolates, 41 (64.1%) were mannitol fermenters while among the 28 isolates from the ear canal, 15 (53.57%) were mannitol fermenters. Among the 36 isolates from the skin 26 (72.22%) were mannitol fermenters. (Table I and Figure II). Out of the total 23 mannitol non-fermenters, 12 isolates (52.17%) had non-haemolytic colonies whereas 7 isolates (30.43%) had alpha-haemolysis (partial haemolysis) and 4 isolates (17.39%) had a double zone of haemolysis (beta-haemolysis) in sheep blood agar.

Of the 64 *Staphylococcus* isolates, 14 (21.87%) isolates were resistant to cloxacillin. Two (7.14%) of those cloxacillin-resistant isolates were originated from ear canal samples, while the remaining 12 (33.34%) cloxacillin-resistant isolates originated from skin samples. Among cloxacillin-resistant isolates, 9 (25%) isolates were mannitol fermenters (Figure IV). Among cloxacillin resistant isolates, 7 isolates (50%) were presumptively identified as *S. aureus* and all those 07 isolates originated from the skin samples.

DISCUSSION

Staphylococcus spp are a major constitutes of the normal flora and a common opportunistic pathogen in the integumentary system of dogs. In this study, the results indicated a higher number of the Staphylococci (56.25%) isolated from the skin of healthy dogs than from the ear canal, and the majority (33.34%) of the cloxacillin resistant isolates were also originated from skin samples and presumably, 50% of those (n=7) may be MRSA. However, a previous study reported approximately equal colonization of cloxacillin-resistant *Staphylococcus* species in the skin (14.1%) as well as ear canal (15.32%) (Hoekstra and Paulton, 2002). Another study on *Staphylococcus spp* isolated from groin skin of healthy dogs reported that 12.8% (14/109) were resistant to cloxacillin (Chah *et al*., 2014). Infection with methicillin resistant *S. aureus* in dogs with wounds, pyoderma, otitis, and urinary tract infections has been recorded in many countries (Weese and van Duijkeren, 2010). *S. pseudintermedius* is commonly isolated from canine pyoderma (Ganiere *et al*., 2005).

Studies indicated disk-diffusion tests using 5µg cloxacillin disks may be insufficient for the detection of low-level cloxacillin resistance and carries the risk that low-level cloxacillin-resistant *S. aureus* isolates will remain undetected (Witte *et al*., 2007). However, studies demonstrate that cloxacillin minimum inhibitory concentration (MIC) testing methods that use the current breakpoints (5µg), reliably identify *mecA*-mediated cloxacillin resistance in *Staphylococcus spp* (Huse *et al*., 2018).

Various case reports have documented methicillin-resistant staphylococci infection in dog owners associated with colonization of genetically related strains in their dogs (Han *et al*., 2016; Petinaki Spiliopoulou, 2015). Case reports also indicated the strong association found in healthcare occupationals than close human contacts related to *S. aureus* colonization (Boost, O'donoghue, and James, 2008). These colonization rates in veterinary personnel are generally higher than in the general population and exposure to resistance stains becomes an occupational hazard. In other ways, cross-resistance to other antimicrobials is the most common in cloxacillin / methicillin-resistant species than in cloxacillin-sensitive *Staphylococcal* species (Japoni *et al*., 2010; Morris *et al*., 2017).

The results of this study highlight the existence of cloxacillin resistance S*taphylococcus spp*, particularly *S. aureus*, in healthy dogs in Sri Lanka, suggesting that MRSA prevalence may be higher among them. Developing cross-resistance to other antibiotics is common with this resistant *Staphylococcus spp* and the risk of transmission of cloxacillin-resistant *Staphylococcus spp* between animals and human is a serious public health concern. Recognizing these risks may add value for the identification of appropriate antimicrobials to treat infection with *Staphylococcus spp* and developing hygienic practices to prevent zoonotic contamination with public health benefits.

CONCLUSION

The most prevalent site of cloxacillin-resistant *Staphylococcus spp* was encountered in the skin than in the ear canal of healthy dogs in this study. The existence of a higher number of pathogenic species emphasizes the emergence of cloxacillin-resistant *Staphylococci* as a potential pathogen of skin and ear infections in dogs. The potential risk of developing multi-drug resistance provokes the selection of suitable antibiotics upon the infection and zoonotic colonization of resistant species brings consideration to proper hygiene in the public health aspect.

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List of abbreviations

SIG- *S.intermedius* group; MIC - minimum inhibitory concentration; COPS-Coagulase Positive Staphylococcus *spp*; VP test- Voges- Proskauer test; MRSA - Methicillin Resistant *Staphylococcus spp*

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