CHARACTERIZATION OF BIOFILM FORMING ABILITY OF STAPHYLOCOCCUS PSEUDINTERMEDIUS ISOLATES

M. Ananda Chitra, R. Renjith and R. Rishvanth

Department of Veterinary Microbiology,
Madras Veterinary College,
Chennai – 600 007

Received 8-8-2014 Accepted 8-9-2014

Corresponding Author : m.anandachitra@tanuvas.org.in

ABSTRACT

In this study, biofilm forming capacity of *S. pseudintermedius* isolates from canine skin infections by quantitative tissue culture plate method was carried out. A total of 53 *S.pseudintermedius* (SP) isolates were obtained from 90 samples , 24 of them were from pyoderma, 10 from demodicosis and 5 from otitis cases. Fifteen isolates (28%) were confirmed as methicillin resistant *S.pseudintermedius* (MRSP) by mecA PCR. Prevalence of MRSP in Chennai SP isolates is lower than the western countries. Majority of the SP isolates were either weak (30%) or no ability (34%) to produce biofilm, with only 17% being classified as strong and 19% as moderate biofilm producers. Biofilm formation was not different between isolates of MRSP and MSSP, which correspond to their equivalent virulence clinically. This is the first report of biofilm forming ability of *S.pseudintermedius* isolates from dogs in India.

KEYWORDS: *S.pseudintermedius*, skin infections, biofil, dog

INTRODUCTION

Staphylococcus pseudintermedius is the major skin pathogen of dog. Formation of biofilm by bacteria aid in the establishment of infection and it is an important virulence factor recognized in several staphylococcal species.

Staphylococcus pseudintermedius, a new staphylococcal species described in 2005 is a normal inhabitant of the skin and mucosa and can be isolated from the nares, mouth, pharynx, forehead, groin and anus of healthy dogs and cats. *Staphylococcus pseudintermedius* is a coagulase-positive Staphylococcus and is thought to be the most common causative agent of pyoderma and otitis externa indogs. A bacterial biofilm is a complex, sessile community of bacteria embedded within a self-produced matrix of carbohydrates, proteins and DNA (extracellular polymeric substance, EPS) (Flemming and Wingender, 2010). Biofilms are associated with many medical conditions including indwelling medical devices, dental plaque, upper respiratory tract infections, peritonitis, and urogenital infections. Biofilm formation is now recognized as an important virulence factor in several *Staphylococcus spp*. The ability to form a biofilm is likely variable between bacterial species. The objective of this study was to detectthe biofilm forming ability of *S.pseudintermedius* isolates from canine skin infections by quantitative tissue culture plate method.

MATERIALS AND METHODS

Bacterial Isolates

A sterile cotton swab was used to sample canine otitis and pyoderma cases and swabs were inoculated by streaking on mannitol salt agar plates. A single representative colony of each sample was propagated by streaking it on nutrient agar plate and subjected for Gram's staining, catalase tests and other biochemical tests.

DNA extraction

Four to five colonies were suspended in PBS and centrifuged at 6000 rpm for 10 min. The pellet was suspended in 100 µl of sterile distilled water and boiled at 100ºC for 10 min. Then the tubes were cooled immediately by placing them on ice. Later, they were centrifuged at 10000 rpm for 10 min and the supernatant was used as template in amplification reaction.

Species identification by PCR and PCR-RFLP

PCR was carried out using the designed primers targeting the nuclease (nuc) gene of *S.pseudintermedius* to differentiate SIG from *S.aureus*. PCR was performed in a reaction volume of 10 µl containing approximately 100 ng of genomic DNA, 5 pmol of each primer and 2x master mix (Ampliqon, Denmark). Cycling conditions were 94°C for 3 min, followed by 30 cycles of denaturation at 94ºC for 30 sec, annealing at 60ºC for 30 sec, extension at 72ºC for 30 sec and a final extension cycle of 5 min at 72° C. PCR products were loaded on a 1.5% agarose gel for electrophoresis, visualized with ethidium bromide and documented. All the above positive isolates were confirmed as *S.pseudintermedius* by PCR-RFLP targeting phosphoacetyltransferase gene sequence as developed by Bannoehr *et al.* (2009).

Biofilm formation

Biofilm formation was carried out as per the method described by Hassan et al. (2011) using a quantitative spectrophotometric microtiter plate assay. The isolates were classified as strong, moderate, weak or zero biofilm producers based on their OD570 (4× ODc< OD_{570} = strong biofilm producer, 2× ODc< $OD_{570} \leq$ 4× ODc = moderate biofilm producer, ODc< $OD_{570} \leq$ 2× ODc = weak biofilm producer, $OD_{570} \leq$ ODc = no biofilm producer (ODcutoff (ODc) = average OD_{570} of negative control + (3× standard deviation of negative control)).

RESULTS AND DISCUSSION

A total of 53 (59%) *S.pseudintermedius* (SP) isolates were obtained from 90 samples. Of the 53 *S.pseudintermedius* isolates, 24 were from pyoderma,10 were associated with demodicosis, 5 from otitis cases, and 13 were from others cases such as allergy, tick infestations etc. SP was the predominant isolate of canine skin infection in the present study and this is in accordance with the number of other studies (Bannoehr *et al*., 2007; Anonymous, 2009; Perreten *et al.*, 2010). Fifteen isolates (28%) were confirmed to be methicillin resistant *S.pseudintermedius* (MRSP) with the detection of mecA gene. MRSP was first reported in 2005 and since then more numbers of MRSP was isolated from various countries. In this study, the frequency of MRSP is 28% which is comparatively lesser than the prevalence of MRSP in western countries (Perreten *et al*., 2010) and in China (Feng *et al.,* 2012) but significantly higher than the occurrence of MRSP in West Indies (Hariharan *et al.*, 2014) and Croatia (Matanovic *et al.*, 2012).

The majority of *S.pseudintermedius* isolates evaluated in this study were either weak or no ability to produce biofilm, with only 17% being classified as strong and 19% as moderate biofilm producers. Biofilm formation was not different between isolates of MRSP and MSSP, which correspond to their equivalent virulence clinically; however, the number of MRSP isolates studied was low. Osland *et al.* (2012) reported that all 23 MRSP isolates from dogs in Norway were biofilm producers with isolates belonging to sequence type (ST) 71 producing significantly more biofilm compared with other STs. Similarly, all 20 MRSP isolates evaluated by Dicicco *et al.* (2012) formed biofilm and also reported that clarithromycin was ineffective in eradicating MRSP biofilm at therapeutic doses. Singh *et al.* (2013) reported that 96% of 140 SP isolates from dogs in Canada and United States were able to produce biofilm and the biofilm production was not significantly different amongst isolates from clinical infections compared with isolates obtained from colonized dogs. To best of our knowledge this is the first report of biofilm forming ability of S.pseudintermedius isolates from dogs in India.

Biofilm production by S.pseudintermedius may play an important role in the pathophysiology of disease and potentially colonization, and could be a contributing factor in the rapid, worldwide emergence of MRSP (Perretenet al 2010). Regardless of methicillin resistance, biofilm formation may play a role in clinical infection with *S.pseudintermedius* and further study into the biofilm forming ability of MRSP with a larger number of isolates is warranted. Biofilm production has been correlated with clinical infection in Staphylococcus species and so more studies on the mechanism of biofilm formation and its contribution in the pathogenesis of S.pseudintermedius skin infections are required.

ACKNOWLEDGEMENT

The authors are thankful to Dr.N.Daniel Joy Chandran, Dr.C.Jayanthy and Dr. B. Nagarajan for their help in carrying out this study.

REFERENCES :

Anonymous. (2009) . Reflection paper on meticillin-resistant Staphylococcus pseudintermedius. Committee for Medicinal Products for Veterinary Use (CVMP), European Medicines Agency, EMA/ CVMP/SAGAM/736964/2009.

Bannoehr J, Ben Zakour N L, Waller A S,Guardabassi L, Thoday K L, Broek A H M. and Fitzgerald J R. (2007). J Bacteriol**189**:8685–8692.

Bannoehr J, Franco A, Iurescia M, Battisti A and Fitzgerald J R. (2009). Journal of Clinical Microbiology**47**: 469–471.

Dicicco M, Neethirajan S, Singh A, Weese JS. (2012). BMC Vet Res, **8**:225.

Feng Y, Tian W, Lin D, Luo Q, Zhou Y, Yang T, Deng Y, Liu Y H, Liu J H. (2012). Vet. Microbiol.**160**:517–524

Flemming HC, Wingender J.(2010)..Nat Rev Microbiol, 8:623–633.

Gotz,F.(2002) . Mol.Microbiol. **43:**1367–1378.

Hariharan H, Gibson K, Peterson R, Frankie M, Matthew V, Daniels J, Martin N A, Andrews L, Paterson T and Sharma R N. (2014) . Veterinary Medicine International, http://dx.doi.org/10.1155/ 2014/850126

Hassan A, Usman J, Kaleem F, Omair M, Khalid A and Iqbal M. (2011) . Brazilian Journal of Infectious Disease **15(4)**:305-311

Matanoviæ K, Mekiæ, S l anddBrank.(2012).VETERINARSKI ARHIV **82**(5), 505-517and Onuma K, Tanabe T. and Sato H.(2011). Vet. Dermatol, **23**, 17–e5.

Osland AM, Vestby LK, Fanuelsen H, et al: (2012).JAntimicrobChemother, 67:841–848.

Perreten V, Kadlec K, Schwarz S, Andersson U G, Finn M, Greko C, Moodley A, Kania S A, Frank L A, Bemis D A, Franco A, Iurescia M, Battisti A, Duim B, Wagenaar J A, Duijkeren E, ScottWeese J, Fitzgerald R J, Rossano A and Guardabassi L. (2010) . J AntimicrobChemother**65**: 1145–1154

Singh A, Walkar M, Rousaaeau J. and Weese JS.(2013) . BMC Vet Res**9**:93.doi:10.1186/1746-6148-9-93.

❑