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STAPHYLO COCCUS PSEUDINTERMEDIUS ISOLATION FROM CANINE, BACTERIAL COLONIZATION AND CLINICAL PICTURE IN BALB/C MOUSE MODEL

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ABSTRACT

Staphylococcus pseudintermedius is an emerging pathogen causing respiratory infection in dogs. In this experiment pathogenicity characteristic of *S. pseudintermedius* were determined. The research was mainly focused on three aspects including bacterial load, histopathological changes and virulence factors. BALB/c mice were intranasaly inoculated with *S. pseudintermedius*. The results showed that the bacteria can cause serious neurologic symptoms including walking in circles, bending of neck and produces obvious histopathological changes in lung and brain of the mice. In lungs and brain higher levels of bacterial load was observed during present study. Of all 11 oxacillin-resistant isolates of *S. pseudintermedius*, 2 carried SCCmec types II–III, 2 carried SCCmec I and 3 carried SCCmec types III according to PCR-based SCCmec typing. Exfoliative toxin (*siet*), *lukS* and *luk F* genes were also identified from the isolates.

Keywords: *Staphylococcus pseudintermedius*, Mouse model, Bacterial load, Histopathology, Virulence factors.

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. Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) is coagulase positive staphylococci identified by Devriese *et al.*, (2005) has emerged globally in companion animals in the last decade (Damborg*et al.*, 2016) and is the most important opportunistic pathogen which belongs to the normal microbiota of companion animals andhumans (Bardiu *et al*., 2013;Van Duijkeren *et al*., 2011). Organism is isolated from groin and forehead, nares, mouth, anus of healthy dogs and cats (Abraham *et al.*, 2007; Griffeth *et al.*, 2008),mostly associated with skin, wound infection, otitis, pyoderma and urinary tract infections (Bannoehr and Guardabassi, 2012; Weese *et al.*, 2009). MRSP infections are generally linked with outbreaks (Weese and Van Duijkeren, 2010), colonization of healthy animals and contact of the persons working with the animals so they become carriers (Boost *et al.*, 2011; Hanselman *et al.*, 2008). It was reported from PFGE analysis, that pet owners suffering from deep pyoderma can carry the organism (Guardabassi *et al.*, 2004), and recent reports confirmed transmission of MRSP from pets to their owners (Frank *et al.*, 2009; Soedarmanto *et al.*, 2011) and to veterinary staff working in the Veterinary clinics and staff owned healthy dogs carried MRSP of same resistance pattern and PFGE profile as the patients (Van Duijkeren *et al.*, 2008). Methicillin resistance of *S. pseudintermedius* is mediated by *mecA* gene located on the chromosome of the bacterium on a mobile element called the 'staphylococcal chromosomal cassette' (SCCmec) (Weese and Van Duijkeren, 2010). These elements can be transferred between different staphylococcal species (Wielders *et al.*, 2002). *S. pseudintermedius* (previously *S. intermedius*) carry several virulence factors i.e coagulase, clumping factors, haemolysin and leukotoxin, pyogenic toxins comprising staphylococcal enterotoxins, toxic shock syndrome toxin 1, and *S. intermedius*exfoliative toxin (Futagawa-Saito *et al.*, 2004). Another *S. pseudintermedius*exfoliative toxin (SIET) showed a rounding effect on cultured epithelial cells. SIET Animal models were used to observe the effect of exfoliative in day old chickens, hamsters and dogs, but not in rats and mice. Dogs injected with SIET showed symptoms of erythema, exfoliation and crusting, which were similar to human staphylococcal scalded skin syndrome and porcine exudative epidermitis, canine pyoderma(Terauchi *et al.*, 2003). Like Panton Valentine leucocidinleukotoxin of *S. aureus*, *S. (pseud) intermedius* also produces a bicomponentleukotoxin, Luk-I encoded by two cotranscribed genes, lukS and lukF(Futagawa-Saito *et al.*, 2004; Prevost *et al.*, 1995) a leukotoxin known as Luk-I having leucotoxicity towards polymorphonuclear cells (Futagawa-Saito *et al.*, 2004) but only a little haemolytic activity on rabbit erythrocytes (Prevost *et al.*, 1995). *S. pseudintermedius* produces an immunoglobulin-binding staphylococcal protein A (spa) that binds to the IgG from the fc portion, thereby affecting its ability of opsoninsation(Moodley *et al.*, 2009) and form biofilms like *S. aureus*(Futagawa-Saito *et al.*, 2006). Complete genome sequence of *S. pseudintermedius* was published recently for better understanding of the pathogenesis of S.*pseudintermedius*(Zakour *et al.*, 2011).The aim of this

study was toinvestigate the occurrence and characteristics of S. *pseudintermedius*isolates and develop a mouse model to examine the factors associated with infection.

MATERIALS AND METHODS

Ethics statement: Before conducting the study, approval for conducting the experiments was obtained from Ethical Committee for Animal Experiments of Nanjing Agricultural University, China**.**

Isolation and Identification: Forty nasal swabs were taken from dogs having respiratory symptoms, such as coughing, sneezing and copious nasal discharge. Samples were collected from Animal Clinics of Nanjing Agricultural University in Jiangsu Province of China to isolate *S. pseudintermedius*.PCR amplification of 16S rRNA gene was performed for the confirmation of the bacteria using a pair of oligonucleotide primer (5'AGAGTTTGATCMTGGCTCA/TACGGYTACCTT GTTACGACTT-3').

Detection of virulence factors: PCR assay was used and tested for the presence of *mecA* gene. Antibiotic oxacillin was used to detect resistant isolates of *S. pseudintermedius* for SCC mectypingas described by some reports (Baron *et al.,* 2004, Zhang *et al.*, 2005). Leukocid in genes viz *lukS, lukF* and *siet* were also identified (Becker *et al.,* 1998; Futagawa-Saito *et al.*, 2004; Lautz *et al.,* 2006). The information of primers is shown in Table 1.

Table1. Primers used for PCR analysis.

Primer	Oligonucleotide sequence (5'–3')	Amplicon size (bp)	References
SEC	GGCGGCAATATTGGCGCTCG	271	(Becker *et al.*, 1998)
	TTACTGTCAATGCTCTGACC		
Sea	GCGAAACACACAATGCTTGC	127	(Becke*r et al.*, 1998)
	GGAGGAATATAACCACGCGC		
Seb	GTGGCTGGCGGTGAGTCACG	477	(Becker *et al.*, 1998)
	GAGTGAAGGTAGCCGTGGC		
SIET	CCTAAATGAATAATAACTGTAATTACGG	359	(Lautz *et al.*, 2006)
	TGGCAATATCATGAGCAGCGTTGCTG		
LUK F	CCTGTCTATGCCGCTAATCAA	572	(Futagawa-Saito *et al.*,
	AGGTCATGGAAGCTATCTCGA		2004)
LUK S	TGTAAGCAGCAGAAAATGGGG	503	(Futagawa-Saito *et al.*,
	GCCCGATAGGACTTCTTACAA		2004)
CIF2 F2	TTCGAGTTGCTGATGAAGAAGG	495	(Oliveira and de
CIF2 R2	ATTTACCACAAGGACTACCAGC		Lencastre, 2002)
RIF4 F3	GTGATTGTTCGAGATATGTGG	243	(Oliveira and de
RIF4 R9	CGCTTTATCTGTATCTATCGC		Lencastre, 2002)
DCS F2	CATCCTATGATAGCTTGGTC	342	(Oliveira and de
DCS R1	CTAAATCATAGCCATGACCG		Lencastre, 2002)
MECA P4	TCCAGATTACAACTTCACCAGG	162	(Oliveira and de
MECA P7	CCACTTCATATCTTGTAACG		Lencastre, 2002)
MECI P2	ATCAAGACTTGCATTCAGGC	209	(Oliveira and de
MECI P3	GCGGTTTCAATTCACTTGTC		Lencastre, 2002)
Type I-F	GCTTTAAAGAGTGTCGTTACAGG	613	(Zhang *et al.*, 2005)
Type I-R	GTTCTCTCATAGTATGACGTCC		
Type III-F	CCATATTGTGTACGATGCG	280	(Zhang *et al.*, 2005)
Type III-R	CCTTAGTTGTCGTAACAGATCG		
Type V-F	GAACATTGTTACTTAAATGAGCG	325	(Zhang *et al.*, 2005)
Type V-R	TGAAAGTTGTACCCTTGACACC		

The amplification procedures were as follows:

***mec A* gene, SCC mectyping:** an initial denaturation step at 94°C for 4min, followed by 30 cycles with denaturation at 94°C for 30s, annealing at 53°C for 30s and extension at 72°C for 1 min. A final extension step was done at 72°C for 4 min.

***siet*gene:** an initial denaturation step at 94°C for 2min, followed by 30 cycles with denaturation at 95°C for 1min, annealing at 55°C for 1min and extension at 72°C for 2min. A final extension step was done at 72°C for 4 min.

***lukF* and *lukS* genes:** an initial denaturation step at 94°C for 5min, followed by 35 cycles with denaturation at 94°C for 1min, annealing at 57°C for 1min and extension at 72°C for 1min. A final extension step was done at 72°C for 5 min.

Aliquots from amplification reactions were analyzed by 1.5% agarose gel electrophoresis and viewed under UV light.

Experimental infection of mice: The *S. pseudintermedius* (Sp) NJ-1 strain isolated from a dog with respiratory syndrome was used. The bacterial strain was cultured on LB and incubated at 37°C. The bacterium was identified on the basis of its biochemical characteristics and through sequencing of the 16S rRNA gene. BALB/c female mice (6 weeks of age, 18-20 g) were purchased from the Animal Experiment Centre, Yangzhou University, China. Mice were divided into Sp and PBS groups with 25 mice in each group. Mice in Sp group were intranasally inoculated with Sp ($1x10^9$ CFU/ml, 50 µl/mouse) and PBS group was inoculated with PBS intranasally as control. Five mice from each group were sacrificed humanely according to a pre-designated schedule at indicated time points of 1, 2, 3, 4 and 5 days post-infection (d.p.i.).

Bacterial loads: The lung and brain from each mouse were weighed and homogenized individually in PBS to obtain a 10% weight-to-volume suspension. The number of CFUs of Sp was determined by plating serial 10-fold dilutions of homogenates on LB agar in duplicate. The plates were incubated overnight at 37^0C. Results were counted and expressed as CFU/g for brain and lung.

Histopathological examination: At 2 d.p.i. mice were euthanized; brain and lung tissues were harvested and fixed in 10% neutral buffered formalin. After fixation, the tissues were embedded in paraffin wax. Tissue sections with a thickness of 4µm were stained with haematoxylin and eosin and examined under microscope.

RESULTS

During present study *S. pseudintermedius*were isolated and confirmed by morphology, culture and biochemical characteristics. In addition PCR was performed for the confirmation of the bacteria.

Clinical symptoms: After 24 h of *S. pseudintermedius*inoculation, clinical signs were appeared in the mice. Clinical signs included coarse hair, not shiny, apathetic appearance of distinct neurological symptoms. Abnormal reactions were observed having sensitive to touching and paralyzed on one side has lost the ability to walk (Fig 1), when the tail was lift, quick circling movement was observed, this phenomena was more pronounced on 2d.p.i.

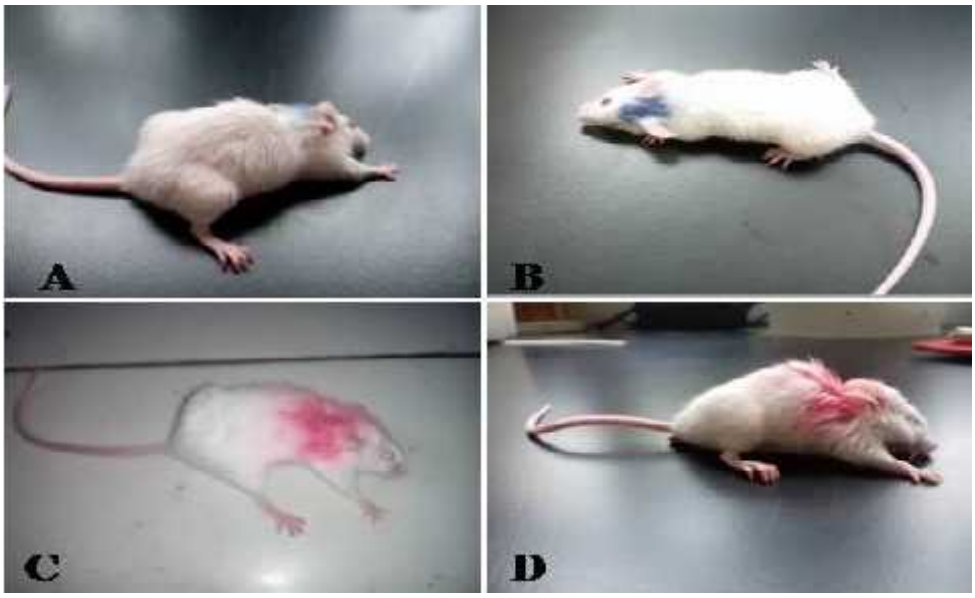


Fig 1. Clinical symptoms of infected mice at 2 d.p.i

Bacterial loads: The bacterial load in the brain and lungs of mice infected with bacteria is presented in (Fig. 2). Twenty-five mice were intranasaly inoculated with $1x10^9$ CFU/ml, 50 µl/mouse. Bacterial load was carried out from lung and brain. At 1 d.p.i bacterial load in lung and brain was more than 10^3 and 10^2 CFU/g respectively. At 2d.p.i bacterial load was more than 10^5 CFU/g in lung and more than 10^4 CFU/g in brain. Bacterial number continued to be slightly increased in brain and decreased in lung with the course of the infection. At day 2 bacterial load in lungs reached its maximum, whereas in brain

bacterial load slightly decreased, while At 5 d.p.ibacterial load becomes 10^5 CFU/g in brain and lung.

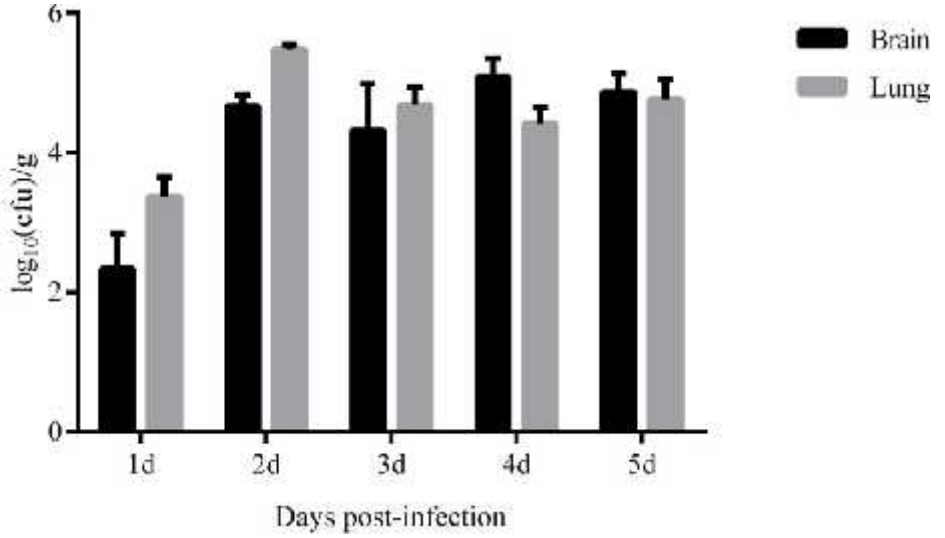


Fig 2 Bacteria loads in the brain and lung of mice infected with *S. pseudintermedius*. The bacterial loads were calculated on days 1, 2, 3, 4 and 5 post-challenge. The results are expressed as the mean log10 cfu/g.

Histopathological changes: In brain microglial cell numbers were increased and aggregated to form microglial nodules andneurophagia phenomena was observed (Fig 3A). Nerve fibres become dissolved as well as appearance of vascular cuff was noticeable (Fig 3B). In lung widening of alveolar septa and increased infiltration of the inflammatory cells including macrophages were observed. (Fig.3D, E). Brain and lungs from control group mice did not showed histopathological lesions (Fig. 3C, F).

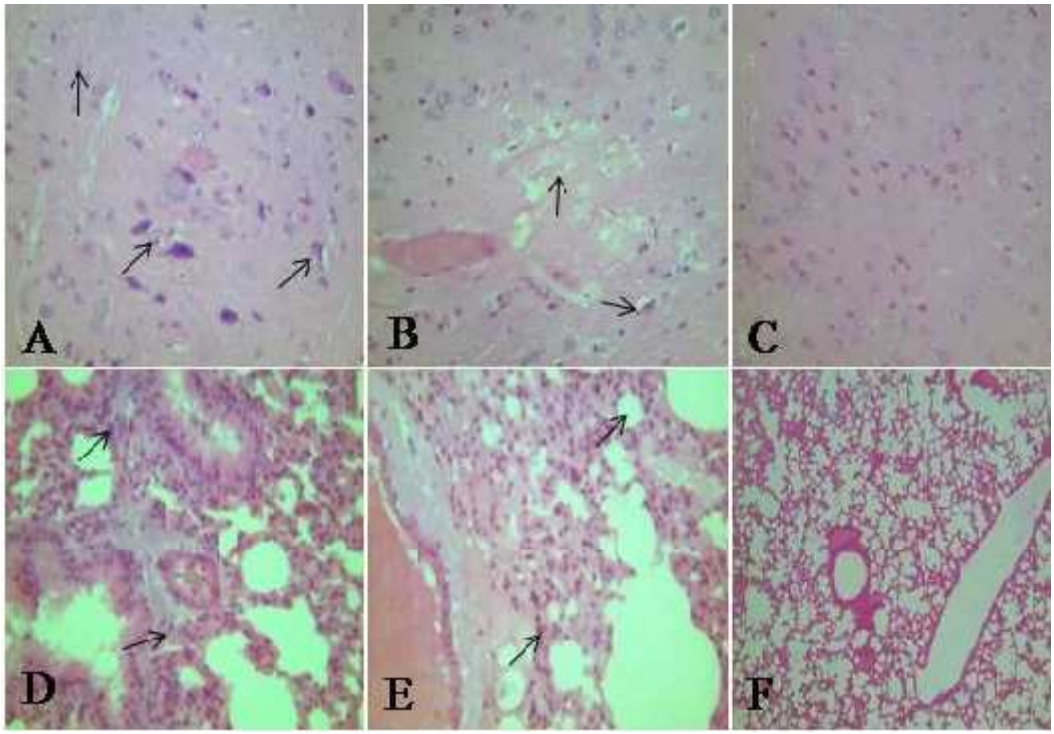


Fig 3.Histopathological changes in the lung and brain of the mice after H & E staining at 2 dpi.

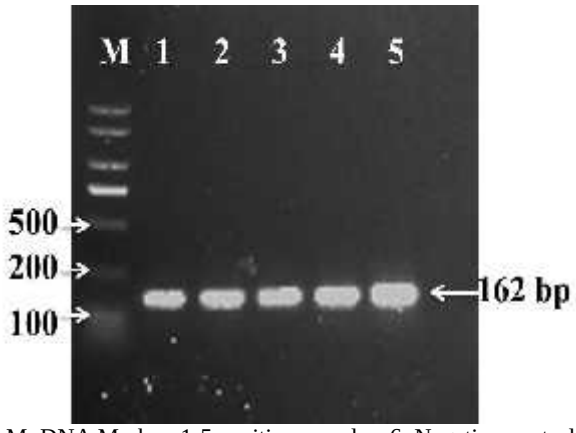
A,B. Brain of infected group (400 ×) C. Brain of control group (100 ×)

D, E. Lung of infected group (100 ×) F. Lung of control group (400 ×).

Presence of *mecA* and SCCmec typing of *S. pseudintermedius*: All oxacillin resistant isolates of *S.pseudintermedius*from dogs had *mecA* gene while oxacillin susceptible isolates did not possess the*mecA*gene. Among the *mecA*-positive isolates, 2(18.18 %) belonged to SCCmec type II–III, 2(18.18 %) belonged to SCCmec type I and 3(27.27 %) belonged to SCCmec type III. Results of *mecA* and SCCmec type are summarized in Table 2. The results for PCR amplification are shown in Fig.4-9.

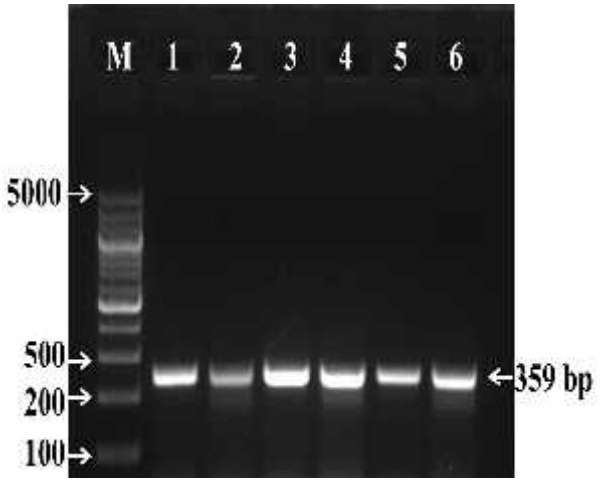
Table 2.SCCmec typing of *mecA* resistant *S.pseudintermedius*strains isolated from dogs.

Total number of samples	Number of positive strains for*mecA*(%)	SCCmec type	Number of positive strains (%)
24	11 (45.83%)	II-III	2 (18.18%)
		III	3 (27.27%)
		I	2 (18.18%)



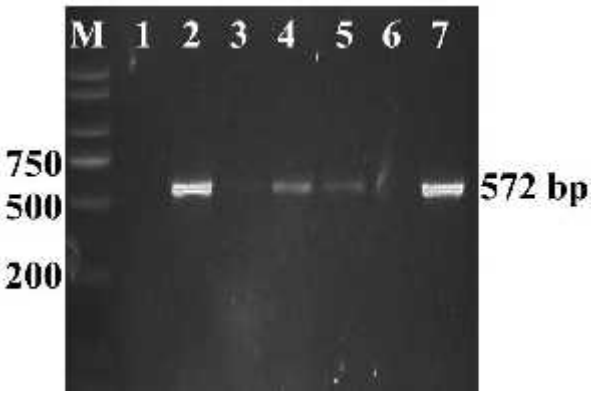
M: DNA Marker, 1-5 positive samples, 6: Negative control.

Fig. 4 PCR amplification of *mecA* gene



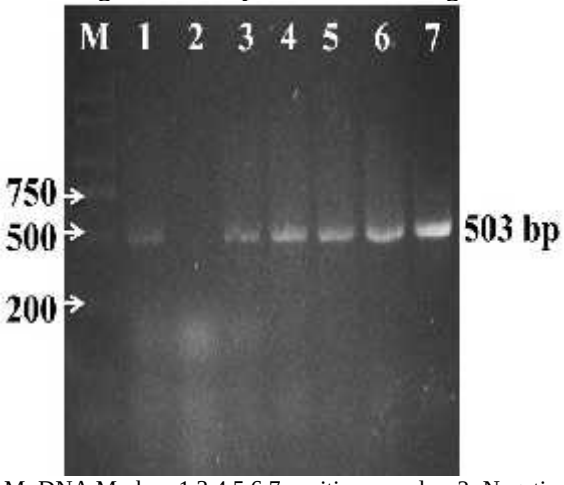
M: DNA Marker, 1-6 positive samples, 7: Negative control

Fig.5 PCR amplification of *siet*gene



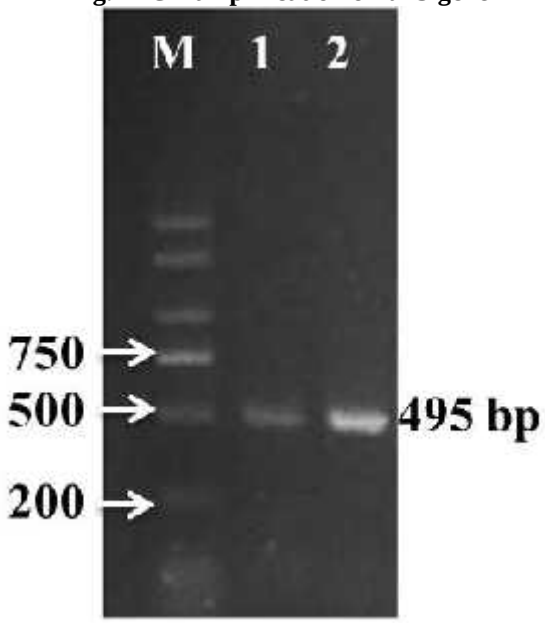
M: DNA Marker, 2,4,5,7 positive samples, 1,3,6 Negative control.

Fig. 6 PCR amplification of*lukF* gene



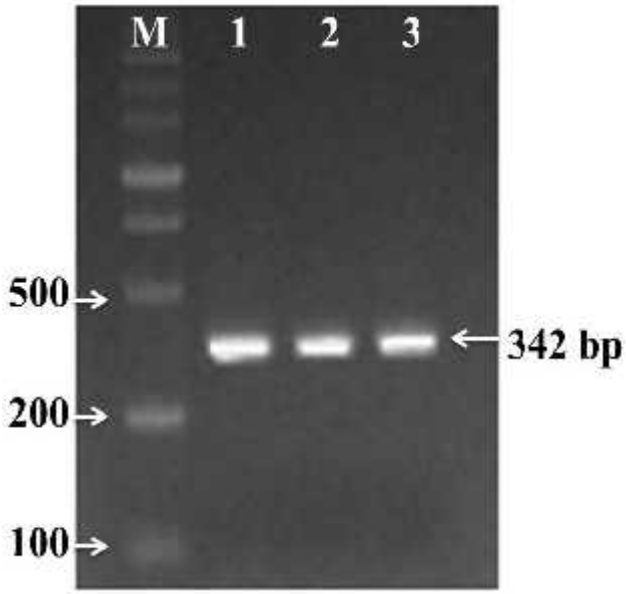
M: DNA Marker, 1,3,4,5,6,7 positive samples, 2: Negative control.

Fig.7 PCR amplification of *lukS* gene



M: DNA Marker;1-2: positive samples.

Fig. 8 PCR amplification for SCCmec type I



M: DNA Marker; 1-3: positive samples; 4: Negative control.

Fig. 9 PCR amplification for SCCmec type III

DISCUSSION

Dogs are the main reservoir of *S. pseudintermedius* having variable carriage rates subject to the different sites of the body (Bannoehr and Guardabassi, 2012). Studies have been done in past for the bacterial isolation from the upper and lower respiratory tract of both diseased and healthy dogs. In the present study twenty four *Staphylococcus pseudintermedius* spp were isolated from forty dogs having respiratory infection. Our results are in agreement with others authors who isolated *S. pseudintermedius* from indoor patient dogs (46.2%) and outdoor patient dogs (19.4%) in a Japanese veterinary teaching hospital (Sasaki *et al.*, 2007). Onuma*et al.*, (2012) reported high prevalence of *S. pseudintermedius* in dogs having pyoderma infection (76.1%during 1999-2000 and 76.4% in 2009). However, in Canada, low MRSP (2.1%) and MRSS (0.5%) isolates were detected (Hanselman *et al.*, 2008).

S. pseudintermedius is not present in the nasopharyngeal flora of the humans but can be carriers when they come in contact with infected animals (Sasaki *et al.*, 2007). A mouse model was designed to study pathogenicity of *S.pseudintermedius.* BALB/c mice were inoculated with *S.pseudintermedius*. The mice were killed at different intervals, and brains and lungs were taken and plated for bacterial count. In the present study, severe neurological signs including bending of the neck and walking in circles were observed. In previous studies, mice exhibited clinical signs of anorexia, ruffled hair, abdominal breathing, septicemic infection (Souza *et al.*, 2012) and meningitis (Gerber *et al.*, 2001). Higher level of bacterial loads in brains and lungs of the mice weredetected, leading to the severe histopathological lesions. In brain,theincreased microglial cells resulted into the formation of microglial nodules, while in lungs alveolar septa was widened with increased infiltration of the inflammatory cells including macrophages. Our findings are in agreement with other authors who observed neuronal damage, focal necrosis with shrunken eosinophilic neurons (Gerber *et al.*, 2001;Guo*et al.*, 2014), specific necrotizing encephalitis and abscesses (Vecht *et al.*, 1997). Necrosis, alveolar inflammation, interstitial edema and infiltration of the inflammatory cells have been observed after pneumococcal infection in the mice (Kalhoro *et al.*, 2015;Wang *et al.*, 2005).

Methicillin resistance of *S. pseudintermedius* is mediated by*mecA* gene. The *mecA* gene is located on a mobile element called the 'staphylococcal chromosomal cassette' (SCCmec) on the chromosome of the bacterium (Weese and Van Duijkeren, 2010). Present study showed that all oxacillin resistant strains possessed *mecA* and SCCmec type II–III in 2 isolates (18.18%), SCCmec type I in 2 isolates (18.18%) and SCCmec type III in 3 isolates (27.27%). A study by Bemis *et al.* (2006) reported 30 out of 31 staphylococci were resistant to oxacillin possessed *mecA* gene. Some studies have also shown that SCCmec II–III and SCCmec V are most prevalent in MRSP in China (Feng *et al.*, 2012; Wang *et al.*, 2012), Korea (Moon *et al.*, 2012)and Japan (Onuma *et al.*, 2012), whileSCCmec type V has been reported in USA (Moodley *et al.*, 2009). Our results are in agreement with other authors who identified SCCmec type III (Moodley *et al.*, 2009; Ruscher *et al.*, 2009; Ruscher *et al.*, 2010) as most prevalent.

Conclusion: It is concluded from present study thatmethicillin resistant *S.pseudintermedius*is potentially pathogenic in dogs. The study on pathogenicity and characterization of *S.pseudintermedius* in mouse model can be particularly important due to its ability of inducing brain damage by crossing the blood–brain barrier (BBB) and subsequent long term colonization.

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REFERENCES

Abraham, J. L., D. O. Morris, G. C. Griffeth, F. S. Shofer and S. C. Rankin (2007). Surveillance of healthy cats and cats with inflammatory skin disease for colonization of the skin by methicillin-resistant coagulase-positive staphylococci and *Staphylococcus schleiferi ssp. Schleiferi.*Vet.Dermatol. 18: 252-259.

Bannoehr, J. and L. Guardabassi (2012). *Staphylococcus pseudintermedius* in the dog: taxonomy, diagnostics, ecology, epidemiology and pathogenicity.Vet. Dermatol. 23:253-e252.

Baron, F., M.F. Cochet, J.L. Pellerin, N. B. Zakour, A. Lebon, A. Navarro, I. Proudy, Y. Le Loir and M. Gautier (2004). Development of a PCR test to differentiate between *Staphylococcus aureus* and *Staphylococcus intermedius*. J. Food Prot. 67:2302-2305.

Becker, K., R. Roth and G. Peters (1998). Rapid and specific detection of toxigenic *Staphylococcus aureus*: use of two multiplex PCR enzyme immunoassays for amplification and hybridization of staphylococcal enterotoxin genes, exfoliative toxin genes, and toxic shock syndrome toxin 1 gene.J. Clin. Microbiol. 36:2548-2553.

Bemis, D. A., R. D. Jones, L. E. Hiatt, E. D. Ofori, B. W. Rohrbach, L. A. Frank and S. A. Kania (2006). Comparison of tests to detect oxacillin resistance in *Staphylococcus intermedius*, *Staphylococcus schleiferi*, and *Staphylococcus aureus* isolates from canine hosts.J. Clin. Microbiol.44:3374-3376.

Boost, M., S. So and V. Perreten (2011). Low Rate of Methicillin-resistant Coagulase-positive Staphylococcal Colonization of Veterinary Personnel in Hong Kong.Zoonoses Public Health.58:36-40.

Bardiau, M.,K.Yamazaki, I. Ote, N. Misawa andJ.G. Mainil (2013). Characterization of methicillin-resistant *Staphylococcus pseudintermedius* isolated from dogs and cats. Microbiol.Immunol. 57:496–501.

Damborg, P., A. Moodley, B. Aalbæk, G. Ventrella, T.P. dos Santos and L. Guardabassi(2016). High genotypic diversity among methicillin-resistant *Staphylococcus pseudintermedius* isolated from canine infections in Denmark.BMC Vet. Res. 12(1), 1.

Devriese, L. A., M. Vancanneyt, M. Baele, M. Vaneechoutte, E. De Graef, C. Snauwaert, I. Cleenwerck, P. Dawyndt, J. Swings and A. Decostere (2005). *Staphylococcus pseudintermedius* sp. nov., a coagulase-positive species from animals. Int. J. Syst. Evol. Microbiol. 55:1569-1573.

Feng, Y., W. Tian, D. Lin, Q. Luo, Y. Zhou, T. Yang, Y. Deng, Y.H. Liu and J.H. Liu (2012). Prevalence and characterization of methicillin-resistant *Staphylococcus pseudintermedius* in pets from South China.Vet. Microbiol. 160:517-524.

Frank, L. A., S. A. Kania, E. M. Kirzeder, L. C. Eberlein and D. A. Bemis (2009). Risk of colonization or gene transfer to owners of dogs with meticillin-resistant *Staphylococcus pseudintermedius*. Vet. Dermatol. 20:496-501.

Futagawa-Saito, K., W. B.Thein, N. Sakurai and T. Fukuyasu (2006). Prevalence of virulence factors in *Staphylococcus intermedius* isolates from dogs and pigeons.BMC Vet. Res. 2:4.

Futagawa-Saito, K., T. Sugiyama, S. Karube, N. Sakurai, W. Ba-Thein and T. Fukuyasu (2004). Prevalence and characterization of leukotoxin-producing *Staphylococcus intermedius* in isolates from dogs and pigeons.J. Clin. Microbiol.*42*:5324-5326.

Gerber, J., G. Raivich, A. Wellmer, C. Noeske, T. Kunst, A. Werner, W. Brück and R. Nau (2001). A mouse model of *Streptococcus pneumoniae* meningitis mimicking several features of human disease.Acta Neuropathol. 101:499-508.

Griffeth, G. C., D. O. Morris, J. L. Abraham, F. S. Shofer and S. C. Rankin (2008). Screening for skincarriage of methicillin-resistant coagulase-positive staphylococci and *Staphylococcus schleiferi* in dogs with healthy and inflamed skin.Vet. Dermatol. 19:142- 149.

Guardabassi, L., M. Loeber and A. Jacobson (2004). Transmission of multiple antimicrobial- resistant *Staphylococcus intermedius* between dogs affected by deep pyoderma and their owners.Vet. Microbiol. 98:23-27.

Guo, C. M., R. R. Chen, D.H. Kalhoro, Z.F. Wang, G.J. Liu, C.P. Lu and Y.J. Liu (2014). Identification of genes preferentially expressed by highly virulent piscine *Streptococcus agalactiae* upon interaction with macrophages. PloS one 9: e87980.

Hanselman, B. A., S. Kruth and J. S. Weese (2008). Methicillin-resistant staphylococcal colonization in dogs entering a veterinary teaching hospital.Vet. Microbiol. 126: 277- 281.

Kalhoro, D. H, S. Luo, X. Xie, Y.B. Zhao, C.P. Lu and Y.J. Liu (2015). *Streptococcus pluranimalium* isolated from a canine respiratory case: identification and experimental infection in mice. Pak. Vet. J. 35: 388-390.

Lautz, S., T. Kanbar, J. Alber, C. Lämmler, R. Weiss, E. P. Berninghoff and M. Zschöck (2006). Dissemination of the gene encoding exfoliative toxin of *Staphylococcus intermedius* among strains isolated from dogs during routine microbiological diagnostics.J. Vet. Med, Series B. 53:434-438.

Moodley, A., M. Stegger, N. L. B. Zakour, J. R. Fitzgerald and L. Guardabassi (2009). Tandem repeat sequence analysis of staphylococcal protein A (spa) gene in methicillin-resistant *Staphylococcus pseudintermedius*.Vet. Microbiol. 135:320-326.

Moon, B. Y., J.H. Youn, S. Shin, S. Y. Hwang and Y. H. Park (2012). Genetic and phenotypic characterization of methicillin-resistant staphylococci isolated from veterinary hospitals in South Korea.J. Vet. Diagn. Invest. 24:489-498.

Oliveira, D. C. and H. de Lencastre (2002). Multiplex PCR strategy for rapid identification of structural types and variants of the mec element in methicillin-resistant *Staphylococcus aureus*.Antimicrob.Agents Chemother. 46:2155-2161.

Onuma, K., T. Tanabe and H. Sato (2012). Antimicrobial resistance of *Staphylococcus pseudintermedius* isolates from healthy dogs and dogs affected with pyoderma in Japan. Vet.Dermatol. 23:17-e15.

Prevost, G., T. Bouakham, Y. Piemont and H. Monteil (1995).Characterisation of a synergohymenotropic toxin produced by *Staphylococcus intermedius*.FEBS letters 376:135-140.

Ruscher, C., A. Lübke-Becker, C.G. Wleklinski, A. Şoba, L. H. Wieler and B. Walther (2009). Prevalence of methicillin-resistant *Staphylococcus pseudintermedius* isolated from clinical samples of companion animals and equidaes.Vet. Microbiol. 136:197-201.

Ruscher, C., A. Lübke-Becker, T. Semmler, C.G. Wleklinski, A. Paasch, A. Šoba, I. Stamm, P. Kopp, L. H. Wieler and B. Walther (2010). Widespread rapid emergence of a distinct methicillin-and multidrug-resistant *Staphylococcus pseudintermedius* (MRSP) genetic lineage in Europe. Vet. Microbiol. 144:340-346.

Sasaki, T., K. Kikuchi, Y. Tanaka, N. Takahashi, S. Kamata and K. Hiramatsu (2007). Reclassification of phenotypically identified *Staphylococcus intermedius* strains.J. Clin. Microbiol. 45:2770-2778.

Soedarmanto, I., T. Kanbar, H. Ulbegi-Mohyla, M. Hijazin, J. Alber, C. Lammler, O. Akineden, R. Weiss, A. Moritz and M. Zschock (2011). Genetic relatedness of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) isolated from a dog and the dog owner.Res. Vet. Sci. 91:e25-27.

Souza, C., J. Warth, J. Agottani, J. Moura, R. Maluta, M. Cardozo and F. Ávila (2012).Efficacy of seroneutralization of a systemic toxico-infection caused by *Staphylococcus pseudintermedius* on mice/utilização de modelo biológico experimental na ação soroneutralizante de toxinfecção sistêmica por cepa de *Staphylococcus pseudintermedius*. Ars Veterinaria28:022-027.

Terauchi, R., H. Sato, Y. Endo, C. Aizawa and N. Maehara (2003). Cloning of the gene coding for *Staphylococcus intermedius* exfoliative toxin and its expression in *Escherichia coli*. Vet. Microbiol. 94:31-38.

Van Duijkeren, E., D. Houwers, A. Schoormans, M. Broekhuizen-Stins, R. Ikawaty, A. Fluit and J. Wagenaar (2008). Transmission of methicillin-resistant *Staphylococcus intermedius* between humans and animals.Vet. Microbiol 128:213-215.

Van Duijkeren, E., B. Catry, C. Greko, M. A. Moreno, M. C. Pomba, S. Pyörälä, M. Ružauskas, P. Sanders, E. J. Threlfall and J. Torren-Edo (2011). Review on methicillin-resistant *Staphylococcus pseudintermedius*.J. Antimicrob.Chemother. 66:2705-2714.

Vecht, U., N. Stockhofe-Zurwieden, B. J. Tetenburg, H. J. Wisselink and H. E. Smith (1997). Virulence of *Streptococcus suis* type 2 for mice and pigs appeared host-specific.Vet. Microbiol. 58:53-60.

Wang, J., R. A. Barke, R. Charboneau and S. Roy (2005). Morphine impairs host innate immune response and increases susceptibility to *Streptococcus pneumoniae* lung infection.J. Immunol. 174:426-434.

Wang, Y., J. Yang, C. Logue, K. Liu, X. Cao, W. Zhang, J. Shen and C. Wu (2012). Methicillin-resistant *Staphylococcus pseudintermedius* isolated from canine pyoderma in North China.J.Appl. Microbiol. 112:623-630.

Weese, J. S. and E. van Duijkeren (2010). Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* in veterinary medicine.Vet. Microbiol. 140:418-429.

Weese, J. S., R. Poma, F. James, G. Buenviaje, R. Foster and D. Slavic (2009). *Staphylococcus pseudintermedius* necrotizing fasciitis in a dog. Can. Vet. J. 50:655.

Wielders, C., A. Fluit, S. Brisse, J. Verhoef and F. Schmitz (2002). mecA gene is widely disseminated in *Staphylococcus aureus* population.J.Clin. Microbiol. 40: 3970-3975.

Zakour, N. L. B., J. Bannoehr, A. H. van den Broek, K. L. Thoday and J. R. Fitzgerald (2011). Complete genome sequence of the canine pathogen *Staphylococcus pseudintermedius*. J. Bacteriol. 193:2363-2364.

Zhang, K., J. A. McClure, S. Elsayed, T. Louie and J. M. Conly (2005). Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome mec types I to V in methicillin-resistant *Staphylococcus aureus*.J. Clin. Microbiol. 43:5026-5033.