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List of Abbreviations

Abbreviation	Elaboration
AMR	Antimicrobial Resistance
BHI	Brain Heart Infusion
BSAVA	British Small Animal Veterinary Association
CS	Culture Sensitivity
CLSI	Clinical and Laboratory Standards Institute
CVASU	Chattogram Veterinary and Animal Sciences University
MHB	MuellerHintonBroth
MRSP	Methicillin-resistant *Staphylococcuspseudintermedius*
PCR	Polymerase chain reaction
SAQTVH	Shahedul Alam Quadree Teaching Veterinary Hospital

Abstract

Staphylococci are the most frequent bacterial pathogen isolated from Cat. *Staphylococcus pseudintermedius* are the common colonizers in cat. Methicillin-resistant Staphylococcus pseudintermedius (MRSP) has a significant health impact on general people and pet owners. In this study we investigated the prevalence and antimicrobial resistance of *Staphylococcus pseudintermedius* in cat presented at Shahedul Alam Quadree Teaching Veterinary Hospital (SAQTVH). A total of 50 swab samples were collected from oral and perineal sites of 25 cat registered to the SAQTVH from 06 November, 2018 to 22 November, 2018. Standard bacteriological methods were followed for the isolation and identification of *S. pseudintermedius*. The identification of *S. pseudintermedius* was confirmed by the presence of pse genes by polymerase chain reactiom(PCR).The isolates were tested for susceptibility to 10 antimicrobials to determine the antimicrobial resistance phenotype. . *S.pseudintermedius* isolates were detected in 2 of 50 tested samples (4%). These two isolates were found to be resistant to multiple antimicrobials. This study gives evidence for the presence of *S. pseudintermedius* in cats in Chattogram region and their antimicrobial resistance profiles. The information can be used as a basis for further implementation of large-scale research on *S. pseudintermedius* colonization in cats in Bangladesh.

Key words : MRSP, Prevalence, PCR, Resistance

Chapter-1 Introduction

Staphylocci are Gram-positive cocci, which are nonmotile, nonspore forming and facultative anaerobes. In mammals, staphylococci are found primarily on the skin and possibly, on the anterior nares (Kloos 1990). Staphylocci have also frequently been found in the oral cavity, throat and anal mucosa (Cox *et al.* 1985; Talan *et al.* 1989).Thirty seven species have been identified (Euzeby 1997). The most common species associated with animal infections are the coagulase-positive *S. aureus* and *S. pseudintermedius*. Staphylococci do not generally appear to cause any major specific diseases in cats (Igimi *et al.* 1994) but cases of superficial dermatitis, bacterial folliculitis and superficial pyoderma caused by *Staph. Intermedius* have been reported (Austin 1978; Scott 1980).Isolation of S. pseudintermedius from cat has zoonotic potential and is an important nosocomial pathogen causing post-surgical infection in veterinary clinics.

Pathogenic organisms have long been recognized as a significant problem owing to their pathogenicity potential to animals and their zoonotic risk to humans. Among them many pathogens causing serious impact on public health (Goldberg and Rubin, 1988; Baserisalehi *et al*., 2006; Humphrey *et al*., 2007; Ethelberg*et et al*., 2004).Intimate association between potential hosts can enhance staphylococcal dispersal as they are easily spread by skin-to-skin contact, aerosols from sneezing and coughing and also through saliva.

A wide range of antimicrobials have been used to treat infections in cat by staphylococci and have led to the emergence of resistant strains. Currently, many staphylococcal species that infect humans and domestic animals exhibit some degree of antimicrobial resistance (Vandenesch *et al.,* 2003). Moreover, interest in antimicrobial resistance in companion animals has increased and a number of reports on companion animals colonized or infected with multiple-drug-resistant organisms such as methicillin resistant *S. aureus* (MRSA) and methicillin resistant *S. pseudintermedius* (MRSP) have been published (Murphy *et al*., 2009).

Antimicrobial resistance is common problem all over the world. Antibiotic resistance in clinically healthy animals is continuously increasing too (Coates *et al.,* 2002; Davis *et al.,* 2014; Henning *et al*., 2001; Manian, 2003).Various studies in recent years have shown that staphylococci isolated from cats have become resistant to at least one class of antibiotics(Love *et al.*, 1981).There are several report published on S. aureus and S. pseudintermedius isolates resistant to many antimicrobials authorized for use in veterinary medicine (Perreten *et al*., 2010; Weese *et al*., 2010). Both MRSA and MRSP in cases of refractory or recurrent human infections (Loffler and Lloyd, 2010).

Therefore, this study was carried out to determine the status of antibiotic resistance of *S. pseudintermedius* in oral and perineal region of cats.

Aims and objectives:

The overall aim of the study was to investigate the prevalence of *S. pseudintermedius* and the emergence of MRSP in cat at Chattogram metropolitan city in Bangladesh.

The specific objectives were:

i. To isolate and identify *S. pseudintermedius* from cat registered to the SAQ teaching veterinary hospital, Chattogram Veterinary and Animal Sciences University (CVASU)
ii. To determine the antimicrobial resistance pattern of *S. pseudintermedius* isolated from the cat.

Chapter-2 Materials and Methods

2.1 Study area

The study was conducted in SAQ Teaching Veterinary Hospital at Chattogram Veterinary and Animal Sciences University (CVASU), Khulshi, Chattogram, Bangladesh.

2.2 Study period

The study was conducted during the period from 06 November, 2018 through 30 December,2018.

2.3 Data collection

A pre-tested questionnaire was used to collect epidemiological as well as clinical data relevant to the (Appendix-1).

2.4 Sample collection

Cat presenting the SAQ Teaching Veterinary Hospital at CVASU for treatment, vaccination or health checkup purpose were selected for sampling. Oral and perineal samples are collected from the cats using sterile cotton swabs (Figure 1).

2.5 Isolation and identification of *S. pseudintermedius*

The swabs that were collected from oral and perineal sites were placed in 5ml Mueller Hinton Broth (MHB) (Figure 2) supplemented with 6.5% NaCl and incubated for 24 hours at 37°C.Then the enriched culture was streaked onto Mannitol salt agar (MSA) by inoculating loop and incubated for 24 hours at 37° C.

S. pseudintermedius was identified based on the colony characteristics on MSA. The *S. pseudintermedius* produced non-pigmented white colored colony(Figure 4).For further confirmation each of the colonies was streaked onto 5% bovine blood agar and incubated for 24 hours at 37° C(Figure 3).Colonies displaying the characteristic appearance of staphylococci on blood agar (pigmented or non-pigmented, raised, medium-sized and hemolytic) were selected from each plate for primary phenotypic characterization. Catalase positive and Gram-positive cocci were considered to be as Staphylococci (Figure 5). Then isolated bacterial colonies were picked up and transferred to a 10 ml test tube containing 5ml brain heart infusion broth (BHIB) and incubated at 37° C for 24 hours. All S. pseudintermedius positive isolates were stored at -80° C using 50% glycerol until further examination.

2.6 Identification of *S. pseudintermedius* by Coagulase test

2.6.1 Collection of horse plasma

Whole blood from a horse was collected into commercially available anticoagulant (citrated phosphate dextrose) treated blood collection bag. The blood was centrifuged at 3000rpm for 10 minutes using a refrigerated centrifuge machine. The resulting supernatant, the plasma, was then immediately transferred to a sterile 10 ml test tube using a sterile micropipette. The plasma was stored at -20°C for further use.

2.6.2 Tube coagulase test

The tube coagulase test was performed by adding 0.2 ml of the overnight culture grown in brain heart infusion broth to 0.5 ml of horse plasma in a glass tube (10 by 75 mm) (Figure 6). After gentle mixing, the tests were incubated at 37°C and examined after 2, 4, 6and 24 hours. The reaction was interpreted to the following criteria described by sperber and Tatini(1975).

Reaction 1+: Small disorganized coagulation

Reaction 2+: Small organized coagulation

Reaction 3+: Large organized coagulation

Reaction 4+: Coagulation of all the contents of the tubes which do not come off when inverted

The presence of *S. pseudintermedius* was confirmed when the coagulation reaction matched with Reaction 2+,3+, and 4+ reactions (Figure 7).

2.7 Identification of *S. pseudintermedius* by polymerase chain reaction

The coagulase positive isolated bacterial colonies were selected for the molecular detection of Staphylococcal species by targeting specific gene *pse* (Sasaki et al., 2010)

2.7.1 DNA extraction from bacterial culture

Bacterial DNA was extracted by boiling method (Figure 9). Firstly 200ul deionized ultrapure water transferred into Eppendorf tube and 2-3 colonies was mixed on it ,then vortexing the tubes for homogenous cell suspension. Then the tube was placed on hot water bath at 96ºC for 15min. after boiling immediately kept on -20ºC for 5 minutes. Finally the tubes were centrifuged at 13000rpm for 5 minutes. Then 100µl supernatant containing bacterial DNA from each tube was collected and preserved at -20ºC until used.

2.7.2 Polymerase chain reactions

The primer sequences used for the PCR are shown below:

Table 1: Oligonucleotide primer sequences for *Staphylococcus pseudintermedius* confirmation gene

Species	**Gene**	**Primer**	**Sequence**	**Annealing temperature**	**Aneali n size**	**Referen ces**
S. pseudintermedius	*pse*	Pse-F2	TRGGCAGTAGGATTGTTAA	56° C	926(bp)	Sasaki et al., 2010

PCR reactions were done with a 25µl reaction volume (Figure 9). Different reagents that are used for PCR for *S. pseudintermedius* are given in table. Negative and positive control were used in each reaction. Nucleus-free water was used as negative controls, and previously identified *S. pseudintermedius* were used as positive control.

Table 2: Contents of PCR reaction mixture for the detection of *pse* genes

SL. No	Contents	Volume
1	Thermo Scientific Dream Taq PCR Master mix(2x) ready to use	12.5µl
2	Forward primer	0.5 µl
3	Reverse primer	0.5 µl
4	Nuclease-free water	9.5 µl
5	DNA template	2 µl
	Total	25 µl

PCR amplification was performed in a thermo cycler (Applied Biosystem. 2720 thermal cycler. Singapore)

Table 3: Cycling conditions used for PCR detection of *S. pseudintermedius*

SL. No	Steps	Temperature and Time
1	Initial denaturation	95°C for 2 min
2	Final denaturation	95°C for 30 seconds
3	Annealing	56°C for 35 seconds
4	Initial extension	72°C for 1 minutes
5	Final extension	72°C for 2 minutes
6	Final holding	4°C for infinity

2.7.3 Visualization of amplified PCR products by agar gel electrophoresis

The PCR products were separated on 1.2% (w/v) agarose gel (Sigma-Aldrich, Germany) containing 0.5 µg/mL ethidium bromide (Sigma-Aldrich, Germany). Electrophoresis was performed in 0.5x Tris /Borate/EDTA (TBE) buffer at 100 V for one hour. The resulting PCR products were visualized under UV light using a transilluminator (BTS-20M, Japan) and the 100 bp DNA ladder plus was used as the molecular size marker. (Figure 10).

2.8 Antimicrobial susceptibility testing of *S. pseudintermedius*

The antimicrobial susceptibility testing of the obtained isolates was performed by disc diffusion method (Figure 8). Susceptibility to 10 antimicrobial agents was performed using the disk-diffusion method in accordance with the Clinical and Laboratory Standards Institute (CLSI) recommendations (Clinical and Laboratory Standards Institute 2012). Antimicrobial agents tested were (charge in lg/disk, except unit/disk for penicillin): Penicillin (10), oxacillin (1), cefoxitin (30),) tetracycline (30), trimethoprim– sulfamethoxazole (1.25/23.75), erythromycin (15), ciprofloxacin (5), vancomycin (30), nalidixic acid (10), gentamycin (10).

2.9 Statistical analysis

Data on animal signalment, antibiotic use and laboratory results were recorded. Descriptive statistics were used to summarize the data generated by the study. The data were transferred into Microsoft Excel spreadsheet for further processing and analysis for risk factor analysis. The relation of the presence of *S. pseudintermedius* with different variables was measured using Chi-squared test (X^2) from cross-tabulation of raw data in an online tool (epitools .ausvet.com.au) A P-value of less than 0.05 was considered as considered as significant association.

Figure 1: Sample Collection from oral and perineal region

Figure 2: Preparation of Mueller Hinton Broth

Figure 3 : Preparation of Agar Media

Figure 4: Isolation and identification of *Staphylococcus pseudintermedius* from swab sample

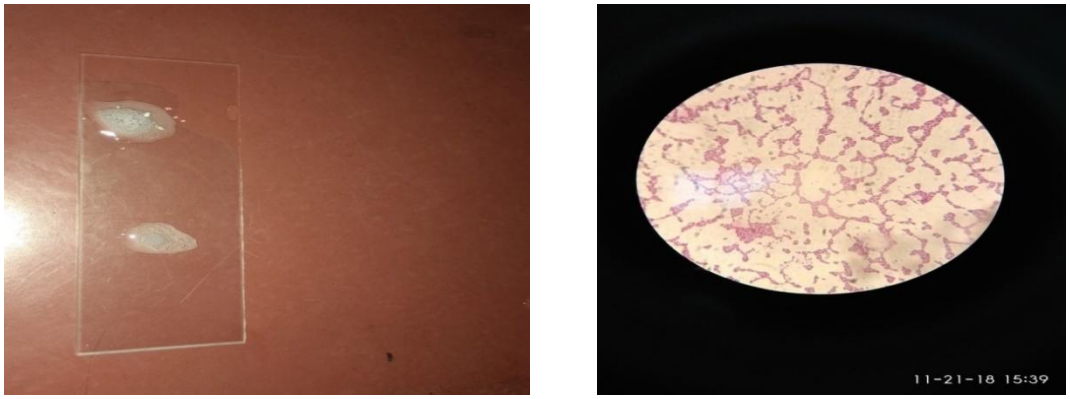


Figure 5: Identification of organism by Gram staining and catalase test

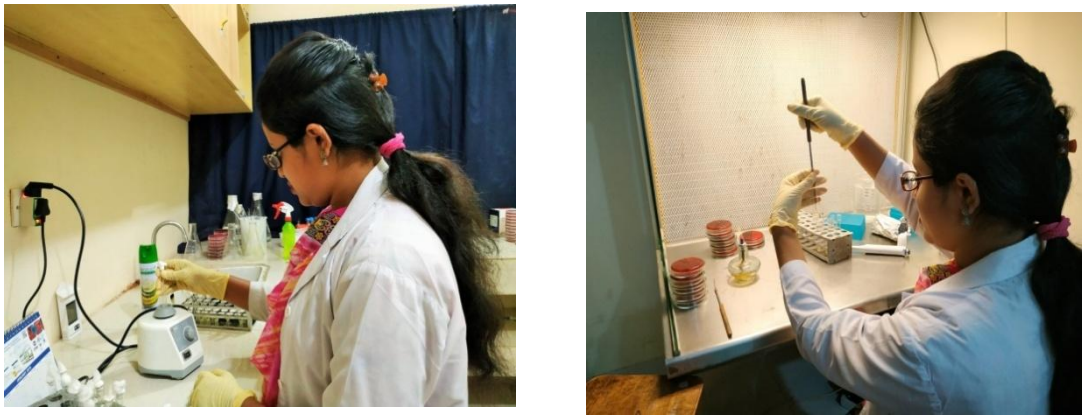


Figure 6: Performing of coagulation test

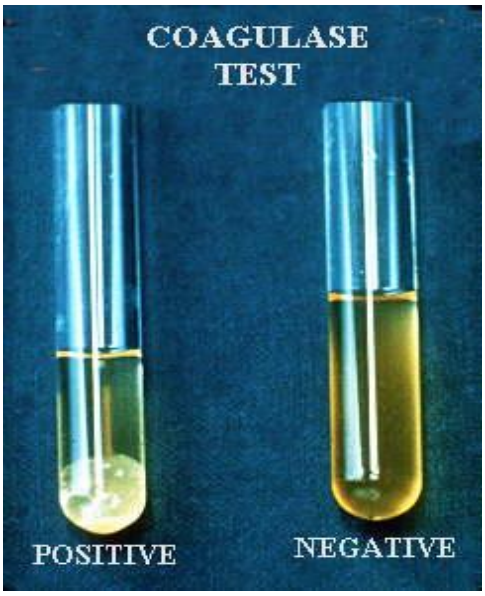
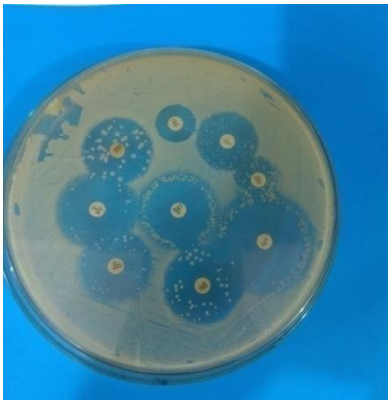


Figure 7: Identification of S. pseudintermedius by coagulase test



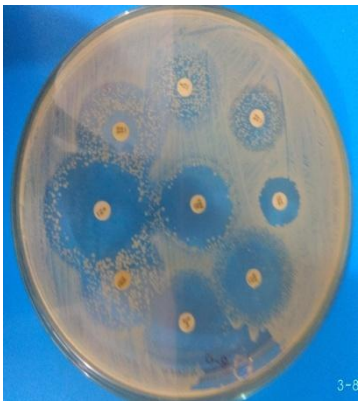


Figure-8: Culture Sensitivity test of two coagulase positive *S. Pseudintermedius*

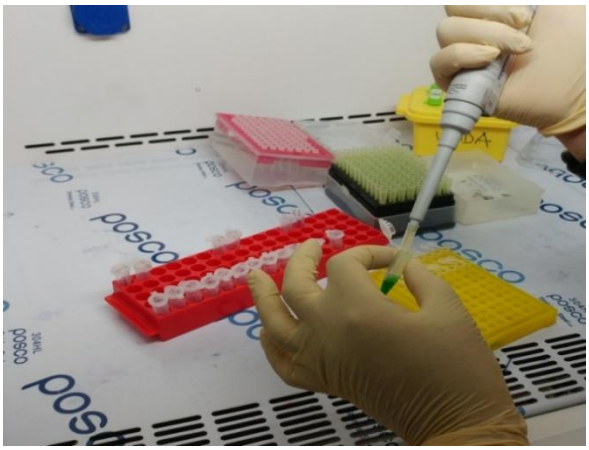
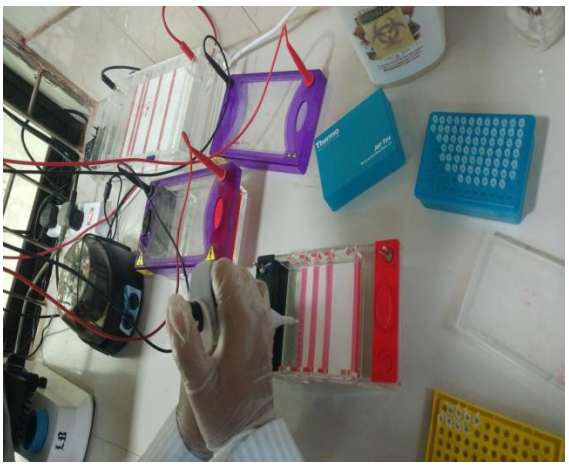


Figure 9: DNA extraction for PCR and Preparation of PCR reaction mixture



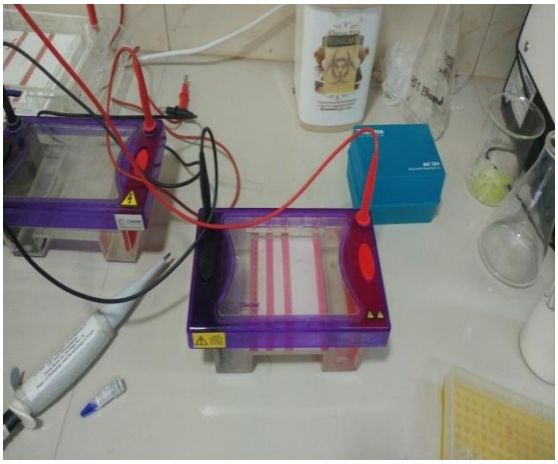


Figure 10: Gel electrophoresis of PCR product

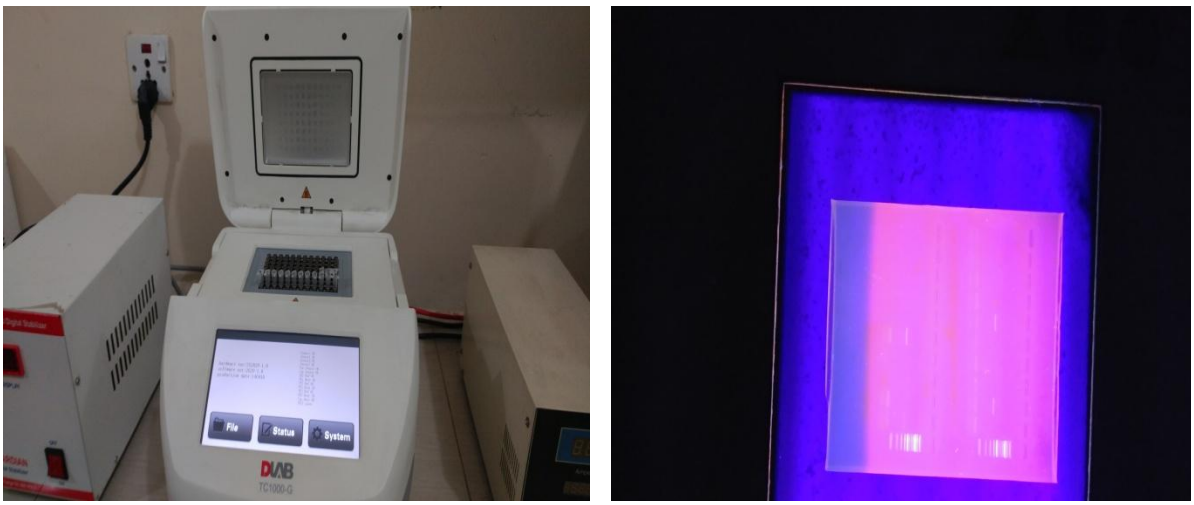


Figure 11: Performing of PCR and PCR result

Chapter-3 Results

3.1 Sample

A total of 50 samples were collected from 25 cats. Among them, 16 samples were collected from 8 healthy cat, and 34 were from 17 clinically sick cat.

3.2 Prevalence of *S. pseudintermedius*

In total 50 samples, 42 samples were found positive for *Staphylococcus* based on the results of growth properties, morphological appearance and biochemical characteristics of the bacteria. Out of 42 culturally-positive *Staphylococcus* isolates, 2 were coagulase positive. Among the two-coagulase positive *S. pseudintermedius*no one contained *pse* gene.

3.3 Antimicrobial susceptibility profiles of *S. pseudintermedius*

After performing coagulase test, there was found two-coagulase positive *S. pseudintermedius* isolates which were selected for antimicrobial susceptibility test. Antibiotic resistant of *S. pseudintermedius* identified by zone of inhibition, however 10 different antimicrobial discs were used.

Table 4: Measuring of diameter (mm) for antimicrobial

ID (Sample type)	**FOX (mm)**	**NA (mm)**	**E (mm)**	**TE (mm)**	**DO (mm)**	**VA (mm)**	**CIP (mm)**	**P (mm)**	**SXT (mm)**
7(perineal)	26	0	0	14	16	0	0	32	0
8 (Oral)	24	0	0	13	17	0	26	26	0

The highest resistance was observed in Nalidixic acid (100%), Tetracycline (100%), Erythromycin (100%), Trimethoprim-Sulfamethoxazole (100%), and Doxycycline (100%). The highest susceptibility was observed in Penicillin (50%), Ciprofloxacin (100%), Vancomycin (100%).

Here, FOX= Cefoxitiin, NA= Nalidixic acid, E= Erythromycin, TE= Tetracycline, DO= Doxycycline, VA= Vancomycin, CIP= Ciprofloxacin, P= Penicillin, SXT= Sulfamethoxazole- Trimethoprim.

ID	**FOX (mm)**	**NA (mm)**	**E (mm)**	**TE (mm)**	**DO (mm)**	**VA (mm)**	**CIP (mm)**	**P (mm)**	**SXT (mm)**
7(perineal)	S	R	R	R	R	S	R	S	R
8 (Oral)	S	R	R	R	R	S	S	R	R

Here, S=Susceptible, R= Resistant.

Table 5: Percentage of antimicrobial resistance pattern

Antimicrobials	Number	Susceptibility	Intermediate	Resistance
Penicillin	2	50%	-	50%
Cefoxitin	2	100%	-	-
Tetracycline	2	-	-	100%
Nalidixic acid	2	-	-	100%
Ciprofloxacin	2	50%	-	50%
Erythromycin	2	-	-	100%
Trimethoprim + Sulfamethoxazole	2	-	-	100%
Vancomycin	2	100%	-	-
Doxycycline	2	-	-	100%

3.5 Risk factor associated with the presence of *S. pseudintermedius* in goats Risk factorcan be detected by analysis of epidemiological data of healthy and sick animals.

In this study, I observed the highest prevalence of *S. pseudintermedius*in Local breed 2 (11.76%) rather than other breeds. I also found higher percentage of coagulase-positive in male cat 1 (11.11%) than female cat 1 (6.25%) and there was a variation in age both young 1(6.25) and adult 1(11.11). There was no variation in dermatitis and skin lesion.

Table 6: Risk factor can be detected by analysis of epidemiological data of healthy sick animals.

Variable	**Co-variable**	**No. of Cats(Sample size)**	***S. pseudintermedius*/ positive (%)**	**P-value**
Breed	Cross	2(4)	0(0)	0.39
	Local	17(34)	2(11.76)	
	Persian	6(12)	0(0)	
Age	Adult	9(18)	1(11.11)	0.001
	Young	16(32)	1(6.25)	
Sex	Female	16(32)	1(6.25)	0.36
	Male	9(18)	1(11.11)	
Health status	Yes	8(16)	1(12.5)	0.68
	No	17(34)	1(11.76)	
Dermatitis	Yes	3(6)	0(0)	0.80
	No	22(44)	0(0)	
Skin wound	Yes	2(4)	1(50)	0.87
	No	23(46)	0(0)	
Previous antibiotics	Yes	11(22)	0(0)	0.51
	No	14(28)	0(0)	
Present antibiotics	Yes	5(10)	0(0)	0.10
	No	20(40)	0(0)	

Above the table we observed.

Chapter- 4 Discussion

Staphylococcal strains are recognized as resident members of the microflora of the skin of human beings, cattle and Dogs. In cats their presence has been reported (Biberstein 1984; Medleau and Blue1988) and confirmed by the isolation rate of 4 % in the present study, lower than the 54.9% observed by Cox *et al.* (1985) as we became unable to do the coagulase test of all the sample because our facilities were minimum.

The present study was designed to know the distribution and antimicrobial susceptibility profile of *S. pseudintermedius* isolated from clinically healthy and sick cat presented at a SAQ teaching veterinary hospital in Chattogram. To the best of our knowledge, *S. pseudintermedius* has probably never been reported before and this is also the first report on the detection of MRSP in cats in Bangladesh.

S. pseudintermedius was identified in 2 (4 %) of 25 cats(50 sample) and this prevalence was lower than in comparison to previous study published by Rubin *et al.*, (2011) and Griffeth *et al.*, (2008). As our sample size was small it might be lower. If sample size would be large then it would be increase.

The body sites (oral and nasal cavity, perineum) frequently colonized with *S. pseudointermedius.* However mouth and perineum are the most common site of *S. pseudintermedius* carriage (Paul*et al.*, 2012). However, simultaneous sampling of different body sites and possible wounds could be used for screening of bacteria.

In our study we used 10 antibiotics for antimicrobial sensitivity testing of coagulase positive samples that are frequently used in small animal medicine such as penicillin, oxacillin, cefoxitin, tetracycline, gentamycine, nalidixic acid, ciprofloxacin, Vancomycin, erythromycin, and sulfamethoxazole. These two sample of *S. pseudintermedius* exhibiting resistance to penicillin(50%), Nalidixic acid(100%), Ciprofloxacin(50%), Erythromycin(100%), Trimethoprim and sulfomethoxazole (100%), Doxycycline(100%). This diversity of antimicrobial resistance was previously reported by different authors (Perreten *et al., 2011;* Garbacz *et al.,* 2013; Kern and perreten, 2013; Davis *et al.,* 2014)

Antimicrobial resistance observed might be due to the indiscriminate and irrational use of antimicrobials (Tambekar *et al*., 2007) in animals for preventive or therapeutic purposes irrespectively. Increasing antimicrobial resistance is a growing concern in both human and veterinary medicine. Because pathogens isolated from recurrent infections are more resistant and resistance is increasing over time, appropriate management of recurrent infections is critical to control antimicrobial resistance. Veterinarians should be aware of zoonotic risk and proper preventive measures should be taken to avoid MRSP transmission from animals to humans especially to pet owners.

Chapter-5 Conclusion

The prevalence of *S. pseudintermedius* was 4% regardless of the source of sample collected from oral cavity and perineal region. They showed resistance to more than three antimicrobials. Antibiotic resistance in feline staphylococci is not well studied or documented. Canine staphylococci received some attention but the techniques used in epidemiological studies have not been powerful enough to discriminate between disease causing staphylococci and nonpathogenic isolates. The information can be used as a basis for further implementation of large-scale research on *S. pseudintermedius* colonization in cats in Bangladesh.

References

1. Austin, V.H. (1978) Diagnosis and treatment of bacterial pyodermas. Part ii. *Mod Vet Pract*59, 266-271
2. Baserisalehi, M., N. Bahador and B.P. Kapadnis, 2006.*Campylobacter*: An emerging pathogen. Res. J. Microbiol., 1: 23-37.
3. Biberstein, E.L., Jang, S.S. and Hirsh, D.C. (1984) Species distribution of coagulase-positive staphylococci in animals. *Journal of Clinical Microbiology*19, 610-615.
4. Coates A, Hu Y, Bax R, Page C. The future challenges facing the development of new antimicrobial drugs. Nat Rev Drug Discov. 2002;1(11):895–910
5. Cox, H.U., Hoskins, J.D., Newman, S.S., Turnwald, G.H., Foil, C.S., Roy, A.F., Kearney, M.T., 1985. Distribution of staphylococcal species on clinically healthy cats. American Journal of Veterinary Research, 46(9): 1824-1828
6. Cox, H.U., Hoskins, J.D., Newman, S.S., Foil, C.S., Turnwald, G.H., Roy, A.F., 1988. Temporal study of staphylococcal species on healthy dogs. American Journal of Veterinary Reasearch,49(9):747-751
7. Davis J A, Jackson C R, Fedorka-Cray P J, Barrett J B, Brousse J H, Gustafson J, Kucher M. Carriage of methicillin-resistant staphylococci by healthy companion animals in the US. Letters in Applied Microbiology.2014 ISSN 0266-8254.doi: 10.1111/lam.12254. Henning S, Marianne S. Resistance to antibiotics in the normal flora of animals. INRA, EDP Sciences. Vet. Res. 2001; 32:227–241.
8. Devriese, L.A., Vancanneyt, M., Vaneechoutte, M., De Graef, E., Snauwaert, C., Cleenwerck, I., Dawyndt, P., Swings, J., Decostere, A., Haesebrouck, F., 2005. Staphylococcuss pseudintermedius sp. Nov., a coagulase-positive species from animals. International Journal of Systemic and Evolutionary Microbiology, 55 (4): 1569-1573.
9. Ethelberg, S., K.E. Olsen, P. Gerner-Smidt and K. Molbak, 2004. Household outbreaks among culture-confirmed cases of bacterial gastrointestinal disease. Am. J. Epidemiol., 159: 406-412.
10. Euzeby, J.P. (1997) List of bacterial names with standing in nomenculture: a fodder availableon the Internet. *Int ƒ Syst Bacteriol* 47, 590-592.
11. Futagawa-Saito, K., Makino, S., Sunaga, F., Kato, Y., Sakurai-Komada, N., Ba-Thein, W., Fukuyasu, T., 2009. Identification of first exfoliative toxin in Staphylococcus pseudintermedius. FEMS Microbiology Letters, 301 (2): 176-180.
12. Goldberg, M.B. and R.H. Rubin, 1988. The spectrum of *Salmonella* infection. Infect. Dis. Clin. North Am., 2: 571-598
13. Henning S, Marianne S. Resistance to antibiotics in the normal flora of animals. INRA, EDP Sciences. Vet. Res. 2001; 32:227–241.

14. Humphrey, T., S. O'Brien and M. Madsen, 2007.*Campylobacters* as zoonotic pathogens: A food production perspective. Int. J. Food Microbiol., 117: 237-257.
15. Igimi, S., Kawamura, S., Takahashi, E. and Mitsuoka, A.T. (1989) *Staphylococcusfelis*,a new species from clinical specimens from cats. *International Journalof Systemic Bacteriology* 39,373-377.
16. Kloos, W.E. (1990) Systematics and the natural history of staphylococci. *Journal ofApplied Bacteriology Symposium Supplement*, 25S-37S.
17. Loeffler, A., Lloyd, D.H., 2010. Companion animals: a reservoir for methicillin-resistant *Staphylococcus aureus* in the community? Epidemiology and Infection, 138(5): 595-605.
18. Love, D.N.,Lomas, G., Bailey, M., Jones, R.F. and Weston, I. (1981) Characterization of strains of Staphylococci from infected in dogs and cats. Journal of Small Animal Practice 22, 195-199.
19. Manian F A. Asymptomatic nasal carriage of mupirocin-resistant, methicillin-resistant *Staphylococcus aureus* (MRSA) in a pet dog associated with MRSA infection in household contacts. Clin Infect Dis. 2003; 36:26–28.
20. Martel J-L, Tardy F,Sanders P, Boisseau J: Review: New trends in regulatory rules and surveillance of antimicrobial resistance in bacteria of animal origin. Vet Res. 2001, 32: 381-392. 10.1051/vetres:2001131
21. Medleau, L. and Blue, *J*.L. (1988) Frequency and antimicrobial susceptibility of *Staphylococcus spp.* Isolated from feline skin lesions. Journal of American Veterinary Medical Association 193, 1080-1081.
22. Murphy, C., Reid-Smith, R.J., Prescott, J.F., Bonnet, B.N., Poppe, C., Boerlin; P., Weese, J.S., Janecko, N. and McEwen, S.A., 2009. Occurrence of antimicrobial resistant bacteria in healthy dogs and cats presented to private veterinary hospitals in southern Ontario: a preliminary study. The Canadian Veterinary Journal, 50(10): 1047.
23. Paul, N.C., Moodley, A., Ghibaudo, G., Guardabassi, L., 2011. Carrige or methicillin-resistant Staphylococcus pseudintermedius in small animal veterinarians: indirect evidence of zoonotic transmission. Zoonoses and Public Health 58(8): 533-539.
24. Perreten, V., Kadlec, K., Schwarz, S., Gronlund Andersson, U., Finn, m., Greko, C., Moodley, A., Kania, S.A., Frank, L.A., Bermis, D.A., Franco, A., 2010. Clonal spread of methicilin-resistant Staphylococcus pseudintermedius in Europe and North America: an international multicentre study. Journal of Antimicrobial Chemotherapy, 65 (6): 1145-1154.
25. Peton V, Le Loir Y. Staphylococcus aureus in veterinary medicine. Infect Genet Evol 2013; 21:1567–1581.

26. Sanford, M.D., Widmer, A.F., Bale, M.J., Jones, R.N., Wenzel, R.P., 1994. Efficient detection and long-term persistence of the carriage of methicillin-resistant Staphylococcus aureus. Clical Infectious Diseases, 1(6): 1123-1128.
27. Sasaki, T., Kikuchi,K., Tanaka, Y., Takahashi, N., Kamata, S. and Hiramatsu, K., 2007a. Methicillin-resistant Staphylococcus pseudintermedius in a veterinary teaching hospital. Journal of Clinical Microbiology, 45(4): 1118-1125.
28. Sasaki, T., Kikuchi,K., Tanaka, Y., Takahashi, N., Kamata, S. and Hiramatsu, K., 2007c. Methicillin-resistant Staphylococcus pseudintermedius in a veterinary teaching hospital. Journal of Clinical Microbiology, 45(4): 1118-1125.
29. Sasaki, T., Tsubakishita, S., Tanaka, Y., Sakusabe, A., Ohtsuka, M., Hirotaki, s., Kawakami, T., Fukata, T., Hiramatsu, K., 2010. Multiplex-PCR method for species identification of coagulase-positive staphylococci. Journal of Clinical Microbiology, 48(3):765-769.
30. Tambekar, D.H., D.V. Dhanorkar, S.R. Gulhane and M.N. Dudhane, 2007.Prevalence, profile and antibiotic susceptibility pattern of bacterial isolates from blood. J. Med. Sci., 7: 439-442.
31. Vandenesch, F., Naimi, T., Enright, M.C., Lina, G., Nimmo, G.R., Heffernan, H., Liassine, N., Bes, M., Greenland, T., Revery, M.E., Etienne, J., 2003. Community-acquired methicillin- resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerging Infectious Diseases,9(8): 978.
32. Weese JS. Methicillin-resistant *Staphylococcus aureus* in animals. ILARJournal 2010; 51:233–244.
33. Woods GL, Washington JA. Antibacterial susceptibility tests: dilution and disk diffusion methods. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, eds. Manual of clinical microbiology. 6th ed. Washington, DC: American Society for Microbiology, 1995:1327 – 41.

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Biography

I am Radhika Das an intern student of Chattogram Veterinary and Animal Sciences University (CVASU). I enrolled for Doctor of Veterinary Medicine (DVM) degree at Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram, Bangladesh in 2013-2014 session. At present I am doing my internship program which is compulsory for awarding my degree of DVM from CVASU. In future, I would like to work and have massive interest in microbiology sector. I feel much interest in exploring new techniques for contributing in development of veterinary field in Bangladesh.

Appendix

Title: Prevalence and antimicrobial resistance of *Staphylococcus pseudintermedius* isolated from cat presented at SAQ Teaching Veterinary Hospital at Chattrogram, Bangladesh.

Questionnaire

Sample ID:……..
Date:……..

Name of the owner:…… Cell No:……..

Breed:…… Sex: Male/ Female
Age:……..

Health Status:

1. Is the cat healthy? Yes/No
2. Any dermatitis or skin lesion: Yes/ No
3. Any wound in skin: Yes/ No
4. Presence of any oral lesions: Yes/ No
5. Any other disease:
   Tentative diagnosis:
   Confirmatory diagnosis:

History of antimicrobial therapy:

1. Previous use of any antibiotic: Yes/ No

2. If yes which group of drug use: Penicillin/ Cephalosporin(Ceftriaxone)/ Fluoroquinolone (ciprofloxacin)/Aminoglycosides(Gentamycin/neomycin)/ Macrplides (erythromycin)/ others

3. Presently use of any antibiotic: Yes/No

Management history:

1. Is showering the cat everyday? Yes/ No