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RESEARCH ARTICLE

Diversified Epidemiological Pattern and Antibiogram of *mecA* Gene in *Staphylococcus aureus* Isolates of Pets, Pet Owners and Environment

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ABSTRACT

Pets are becoming human surrogates for social life where common pathogens like *Staphylococcus aureus* may shift across to potentiate pathogenic factors. Current study was planned to investigate *mec A* gene, regressed preventive factors, and antimicrobial variants in *S. aureus* of pets, pet owners/personals, and the environment of animal treatment sites. Swab samples were put to microbiological identification of *S. aureus* and later the *mecA* gene. A dichotomous questionnaire having assumed risk factors was filled in at the time of sample collection. The representative *mecA* positive samples were subjected to antibiotic susceptibility using disk diffusion method. The descriptive statistics and logistic regression analysis were applied on collected data with 5% probability. Study found 30.43, 33.91, 25.0, and 50.0% *mecA* positive in cats, dogs, pet owners, and environment, respectively. The MRSA strains were 80, 100, 100, and 50% sensitive to chloramphenicol from cats, dogs, humans, and environment sources, respectively. On the other hands, 41.73 and 25.86% of fusidic acid sensitive MRSA from cats and the environment, respectively, while 100% fusidic acid resistant variants of MRSA were found from environment source. Diseased cases (cat OR=0.375, dog=OR=0.375, humans OR=0.333), infection on body (cat OR=0.050, dog=0.238), previous use of antibiotics (Cat OR=0.057), pet access to bed room (human OR=0.368), and often kissing to pet (human OR=0.373) were unexpected factors that did not prove to be potential risks by multiple logistic regression analysis. The present study found higher prevalence of *mecA* (MRSA), altered pattern of risk factors at animal-human-environment interface along with increased variants of antimicrobial resistance.

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INTRODUCTION

Staphylococcus aureus is a commensal bacteria as well as opportunistic pathogen and capable of colonizing at different sites in a variety of animal species and humans (Cuny *et al.*, 2010). This bacterium leads light to severe infections in humans and animals. The former infections involve skin and soft tissue infections (SSTI) with clinical presentations of impetigo contagiosa, pustules, papules, furunculosis, abscesses, and staphylococcal scalded skin syndrome (SSSS). Sever infections covers toxic shock syndrome (TSS), pneumonia, or neonatal TSS-like exanthemata's disorder in humans (Morris *et al.*, 2012). The infectivity aggravates due to increased resistance to multi antibiotics e.g. as is in case of methicillin-resistant *Staphylococcus aureus* (MRSA).

Resistance in MRSA owes to penicillin-binding protein 2a (PBP2a) encoded by *mecA* gene which is

located on one of six types of staphylococcal chromosomal cassettes (SCCs), which vary greatly in size. The smallest cassette containing a *mecA* gene, SCCmec type IV, is present in clones of community associated MRSA (CA-MRSA), which are becoming endemic in many parts of the world (Morgan, 2008; Alzomor *et al.*, 2017). Higher Prevalence of MRSA in camel, bovine, and goats (Ali *et al*., 2019; Aqib *et al*., 2019) have recently been reported. The reports of infection in humans and companion animal's colonization have exhibited the animal potential to act as a source for the spread of MRSA. Increasing interest about MRSA in the community recommends surveillance including carriage rates in healthy cats and dogs (Duquette and Nuttall, 2004). *S. aureus* has been screened from various sites on dogs, including the skin, ear, nasal cavity and anal region (Pinchbeck *et al.*, 2006). Almost 25% of humans contain *Staphylococcus aureus* in their nasal cavity which act both main source and the most significant reservoir for infection. The pathogen holds known zoonotic and humanotic transmission thus becomes potential risk in community with severe life-threatening infections (Kluytmans and Wertheim, 2005) where beta lactam activity of antibiotic is scarce to response (Lee *et al.*, 2016).

Moreover, emerging discrepancies in identification of MRSA at phenotypic and genotypic level (Aqib *et al*., 2018) is added risk toward pathogenesis of this bacteria. Studies are already fewer than needed thus allowing MRSA to unleash at animal, human and environment interface. Pakistan lacks comprehensive studies focusing MRSA colonization in dogs and cats, and their transmission to the persons taking care of them with context to antibiotic susceptibilities of these strains. Therefore, the present study was designed to check the prevalence of MRSA (*mecA*), antibiotic susceptibility profile and potential risk factors associated with spread of MRSA at animal, human and environment interface.

MATERIALS AND METHODS

Sample collection and risk factor analysis: The sampling was done from pets (dogs, cats) brought to the clinic, pet owners, and environment of veterinary clinics located in and around district Faisalabad, Punjab, Pakistan. Total of n=384 samples were collected using convenient sampling technique (Thrusfield, 2018) from pets (n=115 dog, n=115 cat), pet owners (n=96), environment of hospital (n=58). Sterile swabs dipped in phosphate buffered saline (PBS) were used for sampling from nose and ear of dogs, cats, humans and environmental sites. The collected samples were shifted to the laboratory of Institute of Microbiology, University of Agriculture Faisalabad maintaining cold chain (4°C). A dichotomous questionnaire was filled with information like species, breed, age, sex, no of pets, mode of treatment (self or veterinarian), owner's occupation (health care or not), contact with animal, animal access to bedroom, health status and previously used antibiotics.

Phenotypic identification of MRSA: Each sample (10uL) was spread on blood agar and incubated at 37°C for 24 hours, and later mannitol salt agar (MSA) following the same incubation particulars. *S. aureus* identification was done by culture characteristics, microscopic evaluation and biochemical tests following the guidelines of Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994). *S. aureus* confirmed isolates were subjected to oxacillin disk diffusion test following guidelines of Clinical Laboratory Institute (CLSI, 2016).

Molecular Confirmation of MRSA (*mecA* gene): The phenotypically confirmed MRSA were subjected to molecular confirmation by targeting *mecA* gene. DNA were extracted using the WizPrep^{TM} gDNA Mini extraction kit. The extracted DNA were processed for amplification using *mecA* forward P1: 59-TGGCATTCGTGTCACAATCG-39 and reverse primers P2: 59- CTGGAACTTGTTGAGCAGAG-3'with a product size of 310bp (Jonas *et al.*, 2002). A 20uL of reaction mixture comprising of water 3uL, forward primer 2uL, reverse primer 2uL, DNA 3uL and master mix 10uL. Initial denaturation at 95^{o}C for 5 minutes followed by 35 cycles of denaturation (95^{o}C for 30 sec), annealing (58^{o}C for 30 sec), extension (72^{o}C for 30 sec), and final extension at 72^{o}C for 10 min. The amplicons were analyzed on 2% agarose gel and visualized under UV light.

Estimation of antibiotic variants of MRSA positive bacterial isolates: *mecA* gene positive isolates were put to antibacterial susceptibility to various antibiotics such as cefoxitin (30ug), vancomycin (30ug), amikacin (30ug), gentamicin (30ug), chloramphenicol (30ug), oxytetracycline (30ug), ciprofloxacin (5ug), oxacillin (1ug) using Kirby Bauer disc diffusion test following guidelines of clinical and laboratory standard institute (CLSI, 2016). The fresh bacterial isolates adjusted at 1.5×10^{8} CFU/mL were swabbed on Mueller Hinton agar and antibiotic discs were aseptically placed. Incubation were given at 37°C for 24 hours and zone of inhibitions were measured to declare resistant, sensitive or intermediate strains (CLSI, 2016).

Ethical considerations: Current study did not involve any invasive procedure for collection of samples. Prior consent was sought for sampling. the animals presented at clinical were sampled keeping in view ethics of animals' rights. The study was approved by faculty and advanced studies board before start of work while approval of completed study notified CE/1701/M.Phil., 2019 dated11/10/2019. Post study ethical permission certificate was also sought vide FVS/379/26.02.2020.

Statistical analysis: Prevalence was determined by using formula described by (Thrusfield, 2018).

$$\text{Prevalence (\%)} = \frac{\text{No. of infected Animal (n)}}{\text{Total no. of sampled Animals (N)}} \times 100$$

The descriptive statistics was applied for estimation of antibacterial assays, while risk factor analysis was done by chi-square and regression analysis at 5% probability using SPSS version 22.

RESULTS

Prevalence of *mecA* (MRSA) in pets, pet owners and environment: The present study found overall 33.07% *mecA* from pets (dogs and cats), pet owners and environmental isolates. MRSA gene (*mecA*) was identified by amplification through PCR from isolates of different origin and *mecA* positive samples from different sources were presented in one representative picture (Fig. 1) while phenotypic identification of MRSA was done by oxacillin disk diffusion test (Fig. 2). The *mecA* gene were found higher in dogs (33.91%) as compared to cats (30.43%). The *mecA* gene were 50% present in environment isolates which were double to that of pet owners (25%). The overall 79.69% of collected samples were *S. aureus* positive while in cats 84.34%, dogs 81.73%, humans 72.91%, and environment isolates were 77.58% positive for *S. aureus*. The phenotypic identification of *S. aureus* was done by confirming mannitol sugar fermentation on mannitol salt agar and round cocci kind of colony morphology (Fig. 2). The study found non-significant difference (P<0.05) for *S. aureus* at trio i.e. pet-animal-human interface while significant difference (P<0.05) was found for *mecA* among members of this trio (Table 1).

Antibiotic variants of *mecA* positive *S. aureus* (MRSA): The present study showed 100% of *mecA* positive *S. aureus* strains to be sensitive to both the amikacin and gentamicin from cat, human, and environment sources while this percentage against aforementioned antibiotics dropped to 50 and 75%, respectively, when source of samples was dog origin. These isolates were found to be 80, 100, 100 and 50% sensitive to chloramphenicol with reference to the source of samples from cat, dog, human and environment. Similar pattern was observed in case of trimethoprim-sulphmethoxazole with little exception in that the percentage of *mecA* sensitive strains to trimethoprim-sulphmethoxazole raised to 93.93% in cats. The study also noted 100% of *mecA* positive *S. aureus* appearing as resistant strains of vancomycin and ampicillin all the way at this trio except for cat sourced samples where vancomycin sensitivity dropped to 21.74%. However, the detailed sensitivity profile of various antibiotics against *mecA S. aureus* (MRSA) of cat, dog, human and animal origin was observed during the study as shown in Table 2.

Multiple logistic regression analysis of potential risk factors

Human: Nasal samples (P=0.022), diseased animals (P=0.037), owner's occupation (P=0.037) and sleeping of pets in bedroom (P=0.020) were significantly associated with spread of MRSA but OR remained less than 1 thus invites further studies. Simple access of pets without sleeping in bedroom was non-significantly associated but with P value inclined toward significant association (P=0.073). Multiple logistic regression analysis inferred >40 year of human age to be potential risk factor (OR=1.139) for getting MRSA. Type of contact, specie of pet, and gender of human were not significantly associated (P>0.05) with spread of MRSA (Table 5).

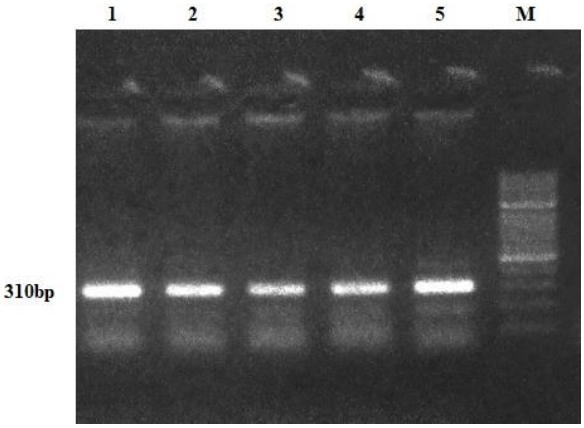

Fig. 1: PCR results of amplified 310bp DNA fragment of MRSA *mecA* gene against a known 100bp molecular weight marker. Lane M indicates 100bp molecular weight marker. Lane 5 indicates the control positive DNA fragment of MRSA *mecA* gene and Lane 1=dog, 2=cat, 3=human, and 4=environment.

Table 1: Prevalence of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* isolated from different sources

Sample sources	Prevalence of *Staphylococcus aureus*					Prevalence of MRSA				
	Total	Positive	Percentage	C.I	*P-value*	Total	Positive	Percentage	C.I	*P-value*
Cat	115	97	84.34%	0.7661-0.8987	0.19	115	35	30.43%	0.2277-0.3936	0.01
Dog	115	94	81.73%	0.7369-0.8774		115	39	33.91%	0.259-0.4296	
Human	96	70	72.91%	0.6328-0.808		96	24	25.00%	0.1741-0.3451	
Environment	58	45	77.58%	0.6534-0.8641		58	29	50.00%	0.3754-0.6246	
Total	384	306	79.69%	-	-	384	127	33.07%	-	-

P<0.05 indicate significant difference.

Table 2: Antibiotic susceptibilities of Methicillin-resistant *Staphylococcus aureus* of different origins

Antibiotic name	Potency	Cat			Dog			Human			Environment		
		R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)
Cefoxitin	30ug	40.00	0.000	60.00	33.92	0.000	66.08	25.00	0.000	75.00	50.00	0.000	50.00
Oxacillin	1ug	30.43	0.000	69.57	33.91	0.000	66.09	25.00	0.000	75.00	50.00	0.000	50.00
Vancomycin	30ug	54.78	23.48	21.74	86.95	13.05	0.000	57.39	42.61	0.000	50.00	50.00	0.000
Ampicillin	10ug	80.00	20.00	0.000	83.47	16.53	0.000	100.0	0.000	0.000	100.0	0.000	0.000
Chloramphenicol	30ug	0.000	20.00	80.00	0.000	0.000	100.0	0.000	0.000	100.0	50.00	0.000	50.00
Fusidic acid	10ug	32.19	26.08	41.73	74.00	26.00	0.000	66.66	33.34	0.000	74.14	0.000	25.86
Ciprofloxacin	5ug	0.000	13.92	86.08	0.000	8.700	91.30	0.000	15.63	84.37	0.000	0.000	100.0
Oxytetracycline	30ug	10.74	22.60	66.66	0.000	0.000	100.0	37.50	0.000	62.50	0.000	16.70	83.30
Trimethoprim-Sulphmethoxazole	25ug	6.070	0.000	93.93	0.000	0.000	100.0	0.000	0.000	100.0	50.00	0.000	50.00
Amikacin	30ug	0.000	0.000	100.0	50.00	0.000	50.00	0.000	0.000	100.0	0.000	0.000	100.0
Gentamicin	30ug	0.000	0.000	100.0	0.000	25.00	75.00	0.000	0.000	100.0	0.000	0.000	100.0

R= Resistant, I= Intermediate, S= Sensitive.

Table 3: Multiple regression analysis of the potential risk factors related with risk of MRSA infection in cats

Variable	Categories	Regression Coefficient	Standard error	Odds Ratios	C.I (95%)	P-Value
Age	<5 month			I		
	5 month- 1 year	-0.768	0.584	0.464	0.148-1.458	0.188
	>1 year	0.452	0.663	1.571	0.428-5.765	0.496
Site of sample	Nasal	-0.626	0.415	0.535	0.237-1.207	0.132
	Ear			I		
Health status	Diseased	-0.981	0.480	0.375	0.146-0.961	0.041
	Healthy			I		
Body Condition	Normal			I		
	Weak	0.617	0.416	1.853	0.819-4.189	0.139
Infection on body	Yes	-2.996	0.574	0.050	0.016-.154	0.000
	No			I		
Vaccination	Yes			I		
	No	0.732	0.413	2.078	0.926-4.666	0.076
Owners occupation	Health Care	-1.128	0.564	0.324	0.107-0.978	0.046
	Non health care			I		
Cat access to bedroom	Yes	-0.884	0.543	0.413	0.143-1.196	0.103
	No			I		
Cat sleep in bedroom	Yes	-0.767	0.483	0.464	0.180-1.197	0.112
	No			I		
Cat lies on bed	Yes	-0.455	0.411	0.634	0.283-1.420	0.268
	No			I		
Type of contact	Kissing	-0.630	0.410	0.533	0.239-1.189	0.124
	Carrying			I		
Previous use of antibiotic	Yes	-2.862	0.504	0.057	0.021-.154	0.000
	No			I		
Type of vet profession	VA	-0.940	0.452	0.391	0.161-0.947	0.038
	VD			I		

Table 4: Multiple logistic regression analysis of the potential risk factors related with risk of MRSA infection in dogs

Variable	Categories	Regression Coefficient	Standard Error	Odds Ratios	C.I (95%)	P-Value
Site of sample	Nasal	-0.626	0.415	0.535	0.237-1.207	0.132
	Ear			I		
Health Status	Diseased	-0.981	0.480	0.375	0.146-0.961	0.041
	Healthy			I		
Infection on Body	Yes	-1.437	0.436	0.238	0.101-0.559	0.001
	No			I		
No of Dogs in House	1-3			I		
	>4	0.667	0.417	1.948	0.860-4.410	0.110
Dog Access to Bedroom	Yes	-0.582	0.436	0.559	0.238-1.314	0.182
	No			I		
Dog Lies on Bed	Yes	-0.795	0.450	0.452	0.187-1.092	0.078
	No			I		
Owners Occupation	Health Care	-1.145	0.433	0.318	0.136-0.743	0.008
	Non Health Care			I		

P<0.05 indicate significant difference.

Table 5: Multiple regression analysis of the potential risk factors related with risk of MRSA infection in humans

Variable	Categories	Regression Coefficient	Standard Error	Odds Ratios	C.I (95%)	P-Value
Type of contact	Kissing	-	-	I	-	-
	Carrying	-0.762	0.495	0.467	0.177-1.232	0.124
Sex	Male	-	-	I	-	-
	Female	-0.978	0.601	0.376	0.116-1.221	0.104
Age	< 20 year	-	-	I	-	-
	20-40 year	-1.076	0.686	0.341	0.089-1.307	0.117
	>40 year	0.130	0.669	1.139	0.307-4.229	0.846
Site of sample	Nasal	-1.167	0.508	0.311	0.115-0.843	0.022
	Ear			I		
Health Status	Diseased	-1.099	0.527	0.333	0.119-.936	0.037
	Healthy			I		
Pet access to bedroom	Yes	-0.999	0.557	0.368	0.124-1.097	0.073
	No			I		
Pet lies on bed	Yes	-1.168	0.502	0.311	0.116-0.832	0.020
	No			I		
Owners occupation	Health Care	-1.099	0.527	0.333	0.119-0.936	0.037
	Non Health Care			I		
Kiss pet	Usually	-0.987	0.600	0.373	0.115-1.207	0.100
	Often	-0.987	0.600	0.373	0.115-1.207	0.100
	Never			I		

P<0.05 indicate significant difference.

Table 6: Multiple regression analysis of the potential risk factors related with risk of MRSA infection from environment

Variable	Categories	Regression Coefficient	Standard Error	Odds Ratios	C.I (95%)	P-Value
Instruments used for surgery	Sterilized			I		
	Non-Sterilized	1.361	0.617	3.900	1.163-13.078	0.027
Type of housing	Cages			I		
	Free to move	0.937	0.572	2.554	0.831-7.842	0.102

P<0.05 indicate significant difference.

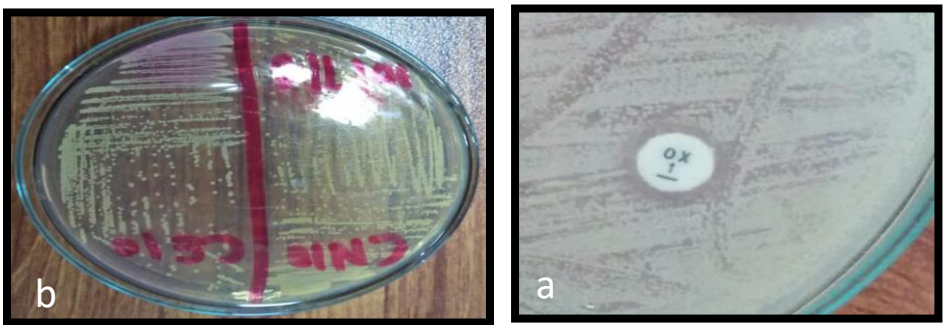

Fig. 2: a=isolated colonies on MSA showing the phenotypic identification of S. aureus; b=showing the phenotypic identification of MRS A by oxacillin disk diffusion test.

Cat: Regressed risks from multiple logistic regression gave rise to 5 month- 1 year of age having lesser odds (0.464 odds ratio) showing less susceptibility in getting MRSA infection, diseased cats (0.375 odds), secondary infection (0.05 odds), health care affiliation (0.324 odds), cats having access to bedroom (0.413 odds), cats having facility to sleep in the bedroom (0.464), kissing type of contact (0.533 odds) than to carrying type of contact, fewer than lesser use of antibiotics (0.057), and visit of professional veterinarian (0.391 odds) were the regressed factors proved to be preventive measures against cat associated MRSA infection in current study (Table 3).

Dog: The multiple logistic regression analysis found nasal source (0.535) to be safe from MRSA infection compared to that of ear proving the latter to be more susceptible in getting MRSA infection. Physical diseased health status (0.375 odds), lesser number of dogs in house (1 odds), dog access to bedroom (0.559 odds), lying of dog on bed (0.452 odds) and health care profession of owner (0.318 odds) were factor least associated with MRSA spread in dogs (Table 4).

Environment: Non-sterilized instruments and free to move type of housing did present 3.900 (CI=1.163-13.078), and 2.554 (CI=0.831-7.842) odds of MRSA spread respectively. (Table 6).

DISCUSSION

The prevalence of *S. aureus* reported in the current study was two times higher to that of reported by (Habibullah *et al.*, 2017) in dogs (42.62%) and cats (37.50%). In case of pet care personnel, in comparison to current study, nearly seven times lower prevalence (10% vs 72.91%) of *S. aureus* was noted in pet caretakers by (Tarazi *et al.*, 2015). The higher prevalence could have been because of unsanitary conditions in the study area, geographical variation, and non-protected contact with pets. The nasal carriage of *S. aureus* is reported to be positively correlated with different populations, geographical locations, influence of genetic and environmental factors (Shetty *et al.*, 2014), cell-wall lipoteichoic acid, hormonal status, and antimicrobial activity of nasal secretions (Weidenmaier *et al*., 2012). The close contact with pets was significant risk factor in current study that might be reason of higher prevalence of MRSA in pet owners. Close contact between household pets and humans offer favorable conditions for the transmission of MRSA by patting, licking, and physical injuries or through the domestic environment (contamination of food, water, and plates) and physical contact with dogs, as well as through contact with household environments contaminated by pets (floors, furniture, and carpets) (Umber and Bender, 2009).

MRSA is contagious pathogen, so once it gets horizontal transmission ability among humans and animals, the extended spread is obvious. Potential risks inclusive of which are personnel of veterinary hospitals who serve as primary source of infection, exposure to sub-inhibitory drugs, repeated exposure of same antibiotics, non-specific use of antibiotics, and exposure of contaminated sources are carrier sources for spread of MRSA. MRSA persists because of its ability to resist antibiotics, formation of pathogenic protective biofilms, and evasion of immune system through specific molecular patterns. On the other hands, transfer of typical strains associated with community (CA-MRSA), livestock associated (LA-MRSA), and hospital acquired (HA-MRSA) to non-specific hosts is another clue for extended persistent and spread of MRSA strains.

When mutant cell replicates, it passes on its resistant phenotype to its daughter cells and they to theirs. The drug kills only those cells that do not have the newly evolved drug-resistant capacity. Thus the entire bacterial population eventually become resistant to the prescribed antibiotic (Pray, 2008). In agreement to the findings of current study were the findings of Hogan *et al.* (2018) and Ng *et al.* (2017) who reported 46 and 49% of MRSA in environment of pets. The relatively higher prevalence in current study is justifiable with high contamination of hospital clinics. Higher population density at clinic may be source of shedding of pathogen in the environment.

The sensitivity of all isolates against chloramphenicol and trimethoprim-sulphamethoxazole of current study 0were in agreement with findings of (Tarazi *et al.*, 2015) in case of dog and human based isolates, while sensitivity of more than 60% of human based isolates against amikacin isolates was congruent with findings of current study of Tarazi *et al.* (2015). Contradiction to current study's results presented 20% of human based strains could show sensitivity against oxytetracycline. Bovine based MRSA were more than 90% sensitive to linezolid, trimethoprim + sulphamethoxazole, moxifloxacin, and ciprofloxcin (Aqib *et al*., 2017). Higher than the reports of current study (67.3% versus 50%) were noticed in case of trimethoprim-sulphamethoxazole resistant isolates from environment (Van Balen *et al.*, 2014).

Conclusions: The present study found more than 30% of pets, 25% humans, and 50% of surrounding of pets to harbor MRSA. Cats more than 1 year of age & having

weak body condition, while dogs more than 4 numbers in house were considered potential risks of getting MRSA. Older age of humans had higher odds of getting MRSA while from environment interface, non-Sterilized instruments and free movement type of housing proved to be potential risks. *In-vitro* antibiotic efficacy indicated higher percentage of sensitive *mecA* strains (MRSA) to gentamicin, amikacin, ciprofloxacin, oxytetracycline, trimethoprim-sulphametho-xizole, chloramphenicol while resistance was noticed against vancomycin and ampicillin from all origins of isolation. The study found variable response to antibiotic susceptibilities and variable potential risk factors in addition to higher prevalence of MRSA at animal-human-environment interface.

Authors contribution: MS did research work, compiled data, and analyzed the data. SUR conceived idea, revised manuscript. AIA did conceive idea, planned research work, analyzed data, and wrote manuscript. AN revised manuscript and worked on a part of research work. MFAK did research work, and prepared manuscript. ZAB did sampling, data collection and preparation of initial manuscript draft. MSY collected samples, screening of samples, and initial data compiling. IS did research work and final revision of manuscript. MAN did secondary data collection, and initial draft preparation. AG did Molecular analysis and data analysis.

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