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Prevalence, Antimicrobial Resistance Patterns, and Risk Factors of Methicillin- Resistant Staphylococcus aureus (MRSA) Among Pets and Humans in Bangladesh

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Abstract

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a global concern due to the likelihood of bidirectional MRSA transmission between pets and humans. Nonetheless, comprehensive data regarding prevalence, antimicrobial resistance patterns, and risk factors linked to pet animals and their owners is limited. The study investigated the MRSA prevalence in pets and their owners, antimicrobial resistance (AMR) pattern and risk factors. To achieve this, 404 samples were collected from pet hospitals located in Dhaka and Mymensingh between July 2022 and June 2024. Among 404 samples, hospital visiting pets (191), pet owners (185) and veterinary personnel (28) were included for the study. *S. aureus* and MRSA were detected through PCR identification of *nuc* and *mecA*gene, respectively. AMR pattern was determined using antimicrobial sensitivity testing. To assess the risk factors, 213 responses from a structured questionnaire were recorded.

Results: Findings reveal that among 108 *S. aureus*46 isolates possess *mecA* gene. The prevalence of MRSA in pet owners is 14.05%, pets at 7.3%, and veterinary personnel at 21.43%. The antimicrobial sensitivity testing (AST) indicated high resistance to cefoxitin (97.4%), penicillin (94.8%), azithromycin (82%), and ciprofloxacin (53.9%). Conversely, vancomycin, clindamycin, and amikacin demonstrated 100% sensitivity. Risk factor analysis identified the pus (0.02) as the most significant risk factor for MRSA transmission.

Conclusions: Overall, high rate of MRSA carriage and MDR profile among veterinary personnel demands the intervention from One Health perspective. Continuous surveillance and monitoring through molecular approach is essential to track transmission events, antimicrobial stewardship and infection control in Bangladesh.

Introduction

Staphylococcus aureus, a gram-positive, spherical bacterium, is commonly found in healthy individuals; however, it can induce severe illness when it enters the bloodstream [1]. The pathogenicity was identified by Scottish surgeon Sir Alexander Ogston in 1881. Following the discovery of antibiotics by Sir Alexander Fleming in 1928, treatment became readily accessible, yet antibiotic resistance emerged as a significant concern. In 1942, the first penicillin-resistant *Staphylococcus aureus* was isolated [2]. In 1950, semisynthetic methicillin was introduced to the market, and methicillin-resistant *Staphylococcus aureus* (MRSA) was clinically isolated in 1960 [3]. Recently, World Health Organization (WHO) categorized MRSA under “high priority” due to its resistance pattern [4]. Based on the SCCmec element, clonal lineage, antibiotic resistance pattern, and virulence potential [5] MRSA can be categorised into three distinct types: healthcare-associated MRSA (HA-MRSA), community-associated MRSA (CA-MRSA), and the recently identified livestock-associated MRSA (LA-MRSA). The differentiation between HA-MRSA and CA-MRSA is becoming less pronounced, as they are predominantly found in both hospital and community settings. However, LA-MRSA strains isolated from humans account for less than 10% of the total strains [6].

The global prevalence of MRSA shows significant variation. Nonetheless, the high population density and swift urbanization increase the likelihood of MRSA development and dissemination in the Asia Pacific regions [7]. A study indicated that the prevalence of MRSA varied between 0% and 73% across 19 locations globally from the year 2000 to 2016. Furthermore, the highest prevalence rates were documented in Korea (73%), followed by the Philippines (59%), China (55.3%), Thailand (53%), and Singapore (52%) [8]. A recent investigation highlighted the prevalence rates of MRSA among healthcare professionals in India, Nepal, Pakistan, Sri Lanka, and Bangladesh, which were found to be 5.65%, 8.83%, 17.20%, 22.56%, and 4.93% respectively [9]. The consequences of MRSA infection are profoundly severe. The mortality rate associated with bacteremia due to *S. aureus* infection ranges from 15% to 60% [10]. It has also been reported that individuals over the age of 60 have a significantly higher mortality rate compared to those younger than 60 [11]. Recent trends indicate a surprising decline in the global prevalence of MRSA, suggesting effective implementation of therapeutic strategies [12].

MRSA is characterized by resistance to cefoxitin as determined by the Kirby-Bauer disc diffusion test. Typically, it exhibits resistance to β-lactam antibiotics, particularly gentamicin, azithromycin, ciprofloxacin, levofloxacin, tetracycline, doxycycline, vancomycin, clindamycin, trimethoprim/sulfamethoxazole, and chloramphenicol [13]. Molecular characterization and identification of the β-lactam resistance gene require polymerase chain reaction (PCR) [14]. The resistance gene *mecA* is present in the bacterial DNA. Additionally, the thermonuclease gene specific to *Staphylococcus aureus*, known as nuc, serves as a prevalent and highly specific target utilized in PCR for the confirmation of species identity [15]. The β-lactam antibiotic operates by interfering with bacterial cell wall synthesis, specifically by targeting penicillin-binding proteins (PBPs) that are essential for cell wall construction. MRSA produces a distinctive penicillin-binding protein (PBP2a) that exhibits low affinity for β-lactam antibiotics [16]. This particular protein enables MRSA to circumvent the effects of β-lactam antibiotics.

MRSA has mainly been linked to extended hospital stays, prolonged interaction with the healthcare system, or following antibiotic use [17]; however, LA-MRSA has heightened the risk of infection through alternative transmission routes. The recognized risk factors linked to HA-MRSA in humans include the presence of abscess, cellulitis, age, and surgical site infection [18].

Despite the increasing trend of pet ownership in Bangladesh, particularly following the COVID-19 pandemic [19], there exists a notable deficiency in the comprehension of the prevalence and antimicrobial resistance patterns of MRSA in companion animals. The current literature offers insufficient insights into the prevalence of MRSA colonization among both healthy and clinically ill pets, and evidence regarding the potential for cross-transmission between pets and their owners in the local context remains limited. Furthermore, the risk factors associated with MRSA transmission in households with pets remain insufficiently investigated. This study aims to investigate the prevalence and antimicrobial resistance profiles of MRSA in pet animals, examine the potential bidirectional transmission of MRSA between pets and their owners, and identify key risk factors associated with MRSA colonization and transmission. The findings will offer essential baseline data to support One Health-oriented AMR surveillance and guide strategies for reducing zoonotic transmission of resistant pathogens in Bangladesh.

Methods

Study population, location and sampling

The research was carried out at the Central Veterinary Hospital (CVH) in Dhaka and the Veterinary Teaching Hospital (VTH) in Mymensingh (Fig. 1). The laboratory work was conducted at the Department of Medicine, Faculty of Veterinary Science, Bangladesh Agricultural University (BAU), Mymensingh, from July 2022 to June 2024. This was a cross-sectional observational study, and protocol was not prospectively registered in a public database.

The sample size was calculated using the formula from [20] $n = Z^2[p(1-p)/d^2]$, assuming MRSA prevalence of 5% in both pets and humans [21,22]. A 95% confidence interval (CI) using a Z score of 1.96 and a precision (d) of 3% requires a sample size of 202 for both humans and pets. The sum amounted to 404.

The animals that arrived during the study period either healthy or clinically sick at the hospital with the owner, both were considered for the study. Pus swab, wound swab, and nasal swab from both the animal and the owner were collected to determine the presence of *Staphylococcus aureus*. A structured questionnaire developed by the authors tested and utilized to gather data on pet sources, pet owners, and veterinary professionals (Supplementary file 1). It assessed daily contact frequency, antibiotic usage during the study period, pets' freedom of movement within the house, sanitary conditions, extended hospital stays, recent exposure to infected/hospital areas, cohabitation with healthcare workers, types of diseases, illness duration, antibiotic treatment duration, floor cleaning frequency, recent or previous surgical procedures, and the use of gloves during pet treatment and management. Data was collected via face-to-face interviews with pet owners and veterinary professionals, along with physical examinations of the pets when possible. Participants in the study, including pet owners and veterinary professionals, were informed about the objective of research to ensure their voluntary participation during data collection and sampling. The participants were informed about the questionnaire and the estimated time required for their involvement in data collection. The research team obtained verbal and written consent from participants before conducting the interviews.

Sample and data collection

A total of 213 human samples were collected, comprising 185 pet owners and 28 veterinary personnel, including veterinary surgeons and attendants. Additionally, 191 animal samples were collected, consisting of 154 cats and 37 dogs. This study included pets visiting hospitals for illness, regular vaccination, or surgical intervention. Pets without observable illness or complaints were also included as MRSA may present in healthy individual. Nasal swabs were obtained from both healthy humans and pets. Samples including pus, wound swabs, and exudates were collected from lesions in clinical cases. Samples were collected with sterilized cotton swabs, inoculated in tryptic soy broth (Himedia, India), and transported to the laboratory in an ice box at 4°C.

Isolation and identification

Samples in tryptic soy broth were incubated for 24 hours at 37°C. *Staphylococcus aureus* was cultured on 5% sheep blood agar, exhibiting beta-hemolytic colonies, which were deemed indicative of possible *S. aureus* [23]. A colony was subsequently cultured on mannitol salt agar (Himedia), and colonies exhibiting a color change from red to yellow due to mannitol fermentation were identified as *S. aureus* [24]. Positive colonies were examined microscopically utilizing Gram's stain. Furthermore, biochemical assays, such as coagulase with 3 % hydrogen peroxide and catalase tests with lyophilized rabbit plasma, were performed to confirm the identification and isolation [25,26].

Screening with cefoxitin and antimicrobial susceptibility determination

Kirby-Bauer disc diffusion test was conducted to determine mec-A mediated resistance [27]. The protocol set forth by the Clinical and Laboratory Standards Institute (CLSI) was adhered to. Muller Hinton agar (MHA) (HiMedia, India) was prepared and incubated for 24 hours at 37°C to confirm sterility. Earlier Samples that were previously isolated and stored in glycerol stocks at -80°C were sub-cultured in MSA. Four to five colonies were gathered to prepare a 5 ml suspension in sterile saline, adjusted to achieve turbidity consistent with the 0.5 MacFarland standards. A sterile cotton swab was then immersed in the suspension and evenly spread across the MHA plate. A 30µg cefoxitin disc (Nihon Becton Dickinson) was then placed at the center. Additional antimicrobial discs, including penicillin (P), cefoxitin (FOX), ceftriaxone (CTR), cephradine (CE), gentamicin (GEN), doxycycline (DO), ciprofloxacin (CIP), tetracycline (TET), amoxicillin-clavulanic acid (AUG), amikacin (AK), azithromycin (AZM), sulfamethoxazole-trimethoprim (COT), vancomycin (VA), clindamycin (CD) and linezolid (LNZ), were positioned 30mm apart. Following an 18-hour incubation period at 37°C, the inhibition zone was assessed. If the inhibition zone for cefoxitin measures ≤ 21 mm, this suggests the presence of *mecA*-mediated resistance. An inhibition zone of 22 mm or greater was deemed indicative of a negative result for *mecA*-mediated resistance [28].

Molecular characterization

Molecular detection involved extracting DNA from the isolates via the boiling method [29,30] with samples stored at -20°C until PCR analysis. Staphylococcus spp. was identified via PCR with the *16S rRNA* primer. Primer sequences were Staph756F - AACTCT GTT ATT AGG GAA GAA CA and Staph750R - CCA CCT TCC TCC GGT TTG TCA CC, yielding an amplicon size of 756 bp [31].

The species-specific gene for *S. aureus* was confirmed using the *Nuc* gene, with sequences *Nuc*-1F-GCGATTGATGGTGATACGGTT and *Nuc*-2R-AGCCAAGCCTTGACGAACTAAAGC, resulting in an amplicon size of 279 bp [32]. Finally, MRSA was confirmed via the *mecA* gene, with an amplicon size of 147 bp and the sequences *mecA*147F- GTGAAGATATACCAAGTGATT and *mecA*147 R-ATGCGCTATAGATTGAAAGGAT, as reported by [25]. Agarose gel was utilized for gel electrophoresis. To detect the *16srRNA* gene, a 1.5% gel was employed, while a 2% gel was used for the detection of *nuc* and *mecA*.

PCR Reaction and condition

The 0.5ml microcentrifuge containing PCR master mixture (Dream teq, Thermo Scientific, EU), primers (Macrogen Co., South Korea), sample DNA, and *nuc*lease-free water (Thermo Scientific, EU) was utilized to prepare the reaction mixture. Each sample utilized 12.5 µl of master mix, 1 µl of forward & 1 µl reverse primer and, 2 µl of sample DNA, and 8.5 µl of *nuc*lease-free water. The tubes were subsequently positioned in a thermal cycler (Takara, Japan). The amplification of 16sRNA was conducted as follows: initial denaturation at 94°C for 5 minutes; followed by 30 cycles of 94°C for 1 minute, 50°C for 1 minute, and 72°C for 2 minutes; concluding with a final extension at 72°C for 10 minutes [31]. For the detection of *Nuc* and *mecA* genes, amplification involved an initial denaturation at 95°C for 5 minutes, succeeded by 30 cycles consisting of denaturation at 95°C for 1 minute, primer annealing at 55°C for 45 seconds, and extension at 72°C for 1 minute. The concluding extension occurred at 72°C for 10 minutes [31,33].

Statistical analysis

For the assessment of MRSA prevalence among animal owners, pets, and veterinary staff the statistical software R (version 4.3.3) was utilized. To identify the risk factors, we conducted logistic regression analysis using both the R statistical package and Epi Info (v.7.2.5.0). Initially, we conducted univariate logistic regression, followed by multivariate logistic regression analysis.

Results

Isolation and identification

A total of 404 samples were gathered for the investigation. A total of 185 samples were collected from animal owners, 191 from pets, and 28 from veterinary personnel, all of which were directly cultured in tryptic soy broth to facilitate bacterial growth. All samples were cultivated on blood agar and MSA to facilitate the growth of *S. aureus*. A total of 108 individuals tested positive for both cultures. Following this, all the positive isolates were examined under a microscope at 1000x magnification using Gram's stain. Similarly, the biochemical test for catalase using 3% hydrogen peroxide exhibited turbidity, while the coagulase test demonstrated the formation of a plasma clot, indicating a positive result (Fig. 2).

Fig. 3 illustrates the specific gene 16s rRNA for *Staphylococcus* spp., measuring 756bp. In the initial well, a ladder was provided, with each band indicating 100 base pairs. The 2nd and 10th samples effectively illustrate positive control (PC) and negative control (NC) respectively. Positive results in PCR were obtained from 108 samples of culture and biochemical tests (Fig. 3).

Fig. 4 illustrates the validation of *S. aureus* through the identification of the *nuc* gene. A total of 108 samples tested positive for the *nuc* gene, measuring 279 bp (Fig. 4). In summary, 58 samples from animal owners, 40 samples from pets, and 10 samples from veterinary personnel tested positive for the *nuc* gene. The data indicates the prevalence of *S. aureus* at 31.4%, 20.94%, and 35.7%, respectively. Overall, the prevalence of *S. aureus* in humans is 31.92%.

Fig. 5 illustrates the confirmation of MRSA via the identification of the *mecA* gene. Out of the 108 samples analyzed, 46 were found to be positive for the *mecA* gene (Fig. 5). The data indicates that the prevalence of MRSA among animal owners is 14.05%, whereas the prevalence in pets stands at 7.32%. Interestingly, the highest prevalence is observed among veterinary personnel, recorded at 21.4%. Nonetheless, when we consider both pet owners and veterinary staff as human subjects, the prevalence significantly decreases to 15.02% (Table 2).

Table 1: Prevalence of MRSA

Group	Total	Positive	Prevalence	95% CI_Lower	95% CI_Upper
Animal owners	185	26	14.05	9.54	20.09
Pets	191	14	7.33	4.21	12.23
Veterinary personnel	28	6	21.43	9.02	41.46
Animal owners + Veterinary personnel	213	32	15.02	10.64	20.69

Antimicrobial susceptibility pattern

The Kirby-Bauer disc diffusion test was conducted to elucidate the antibiotic resistance profile of MRSA isolates. Although the PCR results identified 46 *mecA* genes, only 39 isolates could be revived. In a sample of 39, the highest resistance was observed with cefoxitin, penicillin, and azithromycin, at rates of 97.4%, 94.8%, and 82%, respectively. Ciprofloxacin exhibited resistance exceeding 50%. Conversely, vancomycin, clindamycin, and amikacin exhibited 100% sensitivity. Cephradine, gentamicin, doxycycline, tetracycline, and sulfamethoxazole-trimethoprim exhibited sensitivities of 61.5%, 87.2%, 92.3%, 84.6%, and 92.3%, respectively (Table 2).

Table 2: Antimicrobial resistance pattern of MRSA isolates

Antimicrobials	Disc content (µg)	Disc diffusion interpretive criteria (mm)		No of MRSA isolate (%) N=39		
		S	R	R	I	S
Penicillin (P)	10 units			37(94.8)	0	2(5.1)
Cefoxitin (FOX)	30			38(97.4)	0	1(2.57)
Ceftriaxone (CTR)	30	21	13	4(10.3)	29(74.3)	6(15.4)
Cephradine(CE)	25	18		1(2.6)	14(35.9)	24(61.5)
Gentamicin (GEN)	10			0	5(12.8)	34(87.2)
Doxycycline (DO)	30			1(2.6)	2(5.1)	36(92.3)
Ciprofloxacin (CIP)	5			21(53.9)	6(15.9)	12(30.8)
Tetracycline (TET)	30			2(5.1)	4(10.3)	33(84.6)
Amoxicillin-clavulanic acid (AUG)	30		19	8(21.9)	23(59.4)	8(18.8)
Amikacin (AK)	30	15		0	0	39(100)
Azithromycin (AZM)	15			32(82)	0	7(18)
Sulfamethoxazole-trimethoprim (COT)	25			0	3(7.7)	36(92.3)
Vancomycin (VA)	30			0	0	39(100)
Clindamycin (CD)	2			0	0	39(100)
Linezolid (LNZ)	30			0	0	39(100)

*R=resistant, I= intermediate, S= Sensitive, Interpretive criteria based on the reference strain of *S. aureus* ATCC 25923 [28,34–36]

Univariate logistic regression analysis of risk factors for MRSA in Animal Owners

Univariable logistic regression analysis indicates that the prevalence of MRSA positivity is greater in the Central Veterinary Hospital (CVH), Dhaka, than in the Veterinary Teaching Hospital (VTH), Mymensingh. Additionally, it indicates that adult females predominantly test positive for MRSA. Among various factors, dining in or adjacent to animal enclosures presents the greatest risk for MRSA positivity ($p < 0.2$). Conversely, this result indicates that intimacy, such as sharing personal items, licking the owner's skin, or sleeping in the same location, recent hospital visits, or neglecting personal hygiene does not significantly elevate the likelihood of MRSA (Table 3).

Table 3: Univariable logistic regression analysis for animal owners

Variable	Co-variable	No of *S. aureus* positive	MRSA Positive (Prevalence)	95% CI		OR (95% CI)	p-Value	Likelihood ratio (p-Value)
				lower	upper			
Location	VTH,Mymensingh	133	18(13.5)	0.087	0.203	ref	0.74	0.74
	CVH,Dhaka	52	8(15.4)	0.08	0.275	1.16(0.44-2.78)		
Gender	Male	95	11(11.5)	0.065	0.195	ref	0.32	0.31
	Female	90	15(16.7)	0.103	0.256	1.527(0.6-3.6)		
Age	Adolescent	21	3(14.3)	0.049	0.346	$9.5*10^{5}$($2*10^{-123}$-NA)	0.99	0.68
	Adult	120	19(15.8)	0.103	0.234	$1.08*10^{6}$ ($1.75*10^{-123}$-NA)		
	Middle Adult	43	4(9.3)	0.036	0.216	$5.9*10^{7}$ ($1.2*10^{-123}$-NA)		
	Senior Citizen	1	0	0	0.793	Ref		
Frequent Contact	No	10	1(10)	0.017	0.404	Ref	0.70	0.69
	Yes	175	25(14.3)	0.0986	0.202	1.5(0.264-28.26)		
Eating in/near animal Shed	No	133	16(12)	0.075	0.186	Ref	0.20	0.21
	Yes	52	10(19.2)	0.107	0.318	1.74(0.71-4.09)		
Antibiotic Medication	Yes	22	2(9.1)	0.025	0.278	Ref	0.48	0.45
	No	163	24(14.7)	0.1	0.2	1.7(0.46-11.25)		
Free Movement	Yes	170	23(13.5)	0.09	0.19	Ref	0.49	0.50
	No	15	3(20)	0.07	0.45	1.59(0.35-5.5)		
Licking Owners	No	123	16(13)	0.08	0.2	Ref	0.56	0.56
	Yes	62	10(16.1)	0.09	0.27	1.3(0.5-2.99)		
Sleeping with Owner	No	30	4(13.5)	0.05	0.296	Ref	0.90	0.90
	Yes	155	22(14.2)	0.095	0.2	1.07(0.37-3.9)		
Satisfactory Sanitary condition in home	Yes	148	19(12.8)	0.08	0.19	Ref	0.34	0.35
	No	37	7(18.9)	0.09	0.34	1.58(0.57-3.9)		
Personal hygiene Satisfaction	Yes	147	19(12.9)	0.08	0.19	Ref	0.38	0.39
	No	38	7 (18.4)	0.09	0.33	1.5(0.55-3.8)		
Keeping pet	Outdoor	12	1(8.3)	0.01	0.35	Ref	0.56	0.52
	Indoor	173	25(14.5)	0.09	0.20	1.85(0.33-34.7)		
Sharing Personal Item	Yes	60	8(13.3)	0.07	0.24	Ref	0.84	0.84
	No	125	18(14.4)	0.09	0.21	1.09 (0.45-2.8)		
Presence of skin infection	Yes	16	1(6.2)	0.01	0.28	Ref	0.36	0.3
	No	169	25(14.8)	0.1	0.2	2.6 (0.49-48.1)		
Recent Surgery	Yes	8	0	0	0.32	Ref	0.99	0.11
	No	177	26(14.7)	0.1	0.2	7.3 ($3.3*10^{-48}$-NA)		

Hospitalization >7 days	Yes	3	0	0	0.56	Ref	0.99	0.33
	No	181	26(14.3)	0.09	0.2	2.6 ($9.4*10^{-73}$-NA)		
Zoonotic Vaccine	Yes	78	9(11.5)	0.06	0.2	Ref	0.40	0.39
	No	107	17(15.9)	0.1	0.24	1.4 (0.6-3.58)		
Recent contact with animal body fluid	No	66	8(12.1)	0.06	0.22	Ref	0.57	0.56
	Yes	119	18(15.1)	0.098	0.22	1.3 (0.5-3.3)		
Hand sanitization after touching sick animal	Yes	153	20(13.1)	0.08	0.19	Ref	0.4	0.41
	No	32	6(18.8)	0.08	0.35	1.53 (0.5-4.0)		
Recent Hospital visit	No	144	19(13.2)	0.08	0.19	Ref	0.53	0.53
	Yes	41	7(17.1)	0.08	0.31	1.35 (0.49-3.36)		
Living with health worker	Yes	8	1(12.5)	0.02	0.47	Ref	0.89	0.89
	No	177	25(14.1)	0.09	0.2	1.15 (0.19-21.99)		

Univariate and Multivariate logistic regression analysis of risk factors for MRSA in Pet animals

In a study of 14 *mecA* positive gene samples, univariable logistic regression analysis indicated that location, species, age, and specimens yielded the most significant results (p-value < 0.2). The pus swab from the specimen exhibited a p-value of <0.02, succeeded by values of 0.11, 0.11, 0.15, and 0.18 for the variables of species, age, and location, respectively (Table 4). The following variables are included in the multivariable logistic regression analysis. The result indicated the importance (p value < 0.02) of the pus swab, exhibiting an odds ratio of 10 in comparison to the nasal and wound swabs. In contrast, the likelihood of MRSA positivity in dogs and adult animals over 6 months of age was elevated, although these findings did not reach statistical significance (Table 5).

Table 4: Univariable logistic regression analysis for pet animals

Variable	Co-variable	No	MRSA Positive (prevalence)	95% CI		OR (95% CI)	p-Value	Likelihood ratio (p-Value)
				lower	upper			
Location	VTH,Mymensingh	139	8(5.8)	0.029	0.109	ref	0.18	0.19
	CVH,Dhaka	52	6(11.5)	0.05	0.229	2.1 (0.67-6.46)		
Species	Cat	154	9(5.8)	0.03	0.107	Ref	0.11	0.13
	Dog	37	5(13.5)	0.05	0.27	2.5 (0.73-7.8)		
Breed	Exotic	62	3(4.8)	0.02	0.133	Ref	0.36	0.34
	Indigenous	129	11(8.5)	0.04	0.15	1.8 (0.54-8.3)		
Gender	Female	76	4(5.3)	0.02	0.127	ref	0.37	0.36
	Male	115	10(8.7)	0.04	0.152	1.7(0.55-6.4)		
Age	Young (<6 months)	61	2(3.3)	0.009	0.11	Ref	0.15	0.11
	Adult (> 6 months)	130	12(9.2)	0.05	0.154	3.0 (0.76-19.68)		
Specimens	Nasal Swab	138	9(6.5)	0.03	0.11	1.04(0.29-4.87)	0.94	0.11
	Pus Swab	5	2(40)	0.11	0.76	10 (1.03-90.82)	0.02	
	Wound Swab	48	3(6.2)	0.02	0.16	Ref		
Recent infection	No	40	3(7.5)	0.02	0.19	1.03(0.22-3.5)	0.96	0.96
	Yes	151	11(7.3)	0.04	0.12	Ref		
Type of infection	Bacterial	79	6(7.6)	0.03	0.15	1.89(0.03-36.6)	0.56	0.64
	Viral	46	2(4.3)	0.01	0.14	1.04(0.09-2.31	0.97	
	Parasitic	41	5(12.2)	0.05	0.25	3.1(0.47-6.3)	0.3	
	Accidental	24	1(4.2)	0.007	0.20	Ref		
	Vaccine	1	0	0	0.79	3.9 (NA-2.47)	0.99	
Receiving Treatment	No	126	9(7.1)	0.03	0.13	Ref		0.89
	Yes	65	5(7.7)	0.03	0.16	1.08(0.32-3.2)	0.89	
Antibiotic use	Yes	60	4(6.7)	0.02	0.15	Ref		
	No	131	10(7.6)	0.04	0.13	1.15(0.3-4.3)	0.81	0.81
Treatment provider	Non-Vet	102	7(6.9)	0.03	0.13	Ref		0.79
	Vet	89	7(7.9)	0.03	0.15	1.15(0.38-3.5)	0.79	
Surgical incision or wound	Yes	24	1(14)	0.007	0.2			
	No	167	13(7.8)	0.04	0.12	1.94(0.35-36)	0.53	0.49

Incorrect Prescription	Yes	31	1(3.1)	0.005	0.16	Ref		
	No	160	13(8.1)	0.04	0.13	2.65(0.49-49)	0.35	0.29
Abundance of pets	High	105	8(7.7)	0.039	0.14	1.13(0.36-3.9)	0.82	0.96
	Medium	12	1(8.3)	0.02	0.14	Ref		
	Low	74	5(6.8)	0.01	0.35	1.25(0.06-8.8)	0.84	
Vaccinated	Yes	124	9(7.3)	0.03	0.13	Ref		0.95
	No	67	5(7.5)	0.03	0.16	1.03(0.3-3.1)	0.95	
Use of Anthelmintics	No	107	6(5.6)	0.02	0.11	Ref		
	Yes	84	8(9.5)	0.04	0.17	1.7(0.59-5.5)	0.3	0.30
Cleaning frequency	Daily	109	9(8.4)	0.04	0.14	1.5(0.47-5.7)	0.5	0.77
	Weekly	71	4(5.6)	0.01	0.37	Ref		
	Monthly	11	1(9.1)	0.02	0.13	1.67(0.08-12.8)	0.65	

Table 5: Multivariable Logistic regression analysis with outcome variable of MRSA positive in pet animals

Outcome Variable	Co-variable	OR	95% CI		p-Value
			lower	upper	
Location	VTH, Mymensingh	0.41	0.12	1.45	0.15
	CVH, Dhaka	1	Ref		
Species	Cat	1	Ref		0.13
	Dog	2.84	0.75	10.01	
Age	Young (<6 months)	1	Ref		0.10
	Adult (> 6 months)	3.42	0.80	23.93	
Specimens	Nasal Swab	1	Ref		
	Pus Swab	10.11	1.09	79.36	0.02
	Wound Swab	0.82	0.17	3.08	0.79

Univariate logistic regression analysis of risk factors for MRSA in veterinary personnel

Out of 28 veterinary personnel, 6 tested positives for the *mecA* gene. According to this result, factors including gender, age, daily contact frequency, duration of contact, current skin infection, zoonotic vaccine status, and recent exposure to sick animals' bodily fluids exhibited higher odds ratios. Nevertheless, the broad range of confidence interval values and a p-value greater than 0.05 indicate a rejection of the association with MRSA positivity (Table 6).

Table 6: Univariable logistic regression analysis for Veterinary Personnel

Variable	Co-variable	No	MRSA Positive (Prevalence)	95% CI		OR (95% CI)	p-Value	Likelihood ratio (p-Value)
				lower	upper			
Gender	Male	26	5(19.2)	0.085	0.37	ref	0.33	0.35
	Female	2	1(50)	0.094	0.90	4.2(0.15-119.6)		
Age	Adult	12	1(8.3)	0.014	0.35	Ref	0.17	0.126
	Middle Adult	16	5(31.2)	0.141	0.55	5(0.66-104.2)		
Frequent Contact	No	5	0	NA	0.43	Ref	0.99	0.1
	Yes	23	6(26.1)	0.012	0.46	4.08(00-NA)		
Duration of contact	1h	0						0.11
	2h	0						
	3h	5	0	0.0003	0.43	Ref	0.99	
	More	23	6(26.1)	0.0123	0.46	1.74(0.71-4.09)		
Antibiotic Medication	Yes	2	1(50)	0.085	0.37	4.2(0.14-119.6)	0.33	0.35
	No	26	5(19.2)	0.09	0.90	Ref		
Satisfactory hospital Sanitation	No	9	0	0	0.29	Ref		0.02
	Yes	19	6(31.6)	0.15	0.53	1.4e+8(1.2e-145-NA)	0.99	
Personal hygiene Satisfaction	No	9	0	0	0.29	Ref		0.02
	Yes	19	6(31.6)	0.15	0.53	1.4e+8(1.2e-145-NA)	0.99	
Current Skin infection	No	26	5(19.2)	0.085	0.37	ref	0.33	0.35
	Yes	2	1(50)	0.094	0.90	4.2(0.15-119.6)		
Zoonotic Vaccine	No	6	0	0	0.39	Ref		0.06
	Yes	22	6(27.3)	0.13	0.48	4.3(1.07e-82-NA)	0.99	
Recent contact with animal body fluid	No	2	1(50)	0.094	0.90	4.2(0.15-119.6)		0.35
	Yes	26	5(19.2)	0.085	0.37	ref	0.33	
Recent Hospital visit	Yes	7	4(19)	0.08	0.64	Ref	0.59	0.60
	No	21	2(28.6)	0.07	0.4	1.7(0.19-11.8)		
Using Hand gloves	Yes	24	5(20.8)	0.09	0.40	Ref		0.85
	No	4	1(25)	0.04	0.69	1.26(0.05-12.6)	0.85	

Univariate logistic regression analysis of risk factors for MRSA in animal owners and veterinary personnel

A total of 213 individuals were evaluated for this univariable logistic regression analysis. This analysis revealed that certain variables exhibited higher odds ratios; however, none of the variables demonstrated a significant association with MRSA positivity (Table 7).

Table 7: Univariable logistic regression analysis of both animal owners and veterinary personnel

Variable	Co-variable	No	MRSA Positive (Prevalence)	95% CI		OR (95% CI)	p-Value	Likelihood ratio (p-Value)
				lower	Upper			
Gender	Male	121	16(13.2)	0.1	0.26	Ref	0.4	0.4
	Female	92	16(17.4)	0.08	0.2	1.38(0.64-2.95)		
Age	Adolescent	21	3(14.3)	0.049	0.34	Ref		0.95
	Adult	132	20(15.2)	0.1	0.2	1.07 (0.32-4.86)	0.98	
	Middle Adult	59	9(15.3)	0.08	0.26	1.08(0.28-5.26)	0.91	
	Senior Citizen	1	0	0	0.79	2.8(NA-2.75)	0.91	
Sample Source	Pet owner	185	26(14.1)	0.09	0.19	Ref		0.33
	Vet personnel	28	6(21.4)	0.1	0.39	1.7(0.57-4.3)	0.31	
Frequent Contact	No	15	1(6.7)	0.01	0.29	Ref		0.30
	Yes	198	31(15.7)	0.11	0.21	2.6(0.49-47.9)	0.36	
Duration of contact (24h)	2h	4	0	0	0.48	Omitted	0.99	0.45
	3h	33	6(18.2)	0.08	0.34	1.28(0.4-3.2)	0.61	
	More	176	26(14.8)	0.10	0.2	Ref		
Antibiotic Medication	Yes	24	3(12.5)	0.1	0.2	Ref		0.70
	No	189	29(15.3)	0.04	0.3	1.26(0.4-5.6)	0.71	
Personal hygiene satisfaction	No	47	7(14.9)	0.07	0.27	Ref		0.97
	Yes	166	25(15.1)	0.104	0.21	1.01(0.42-2.6)	0.97	
Presence of skin infection	Yes	18	2(11.1)	0.1	0.21	Ref		0.61
	No	195	30(15.4)	0.03	0.32	1.45(0.38-9.4)	0.62	
Recent Surgery	Yes	8	0	0	0.32	Ref		0.10
	No	205	32(15.6)	0.11	0.21	2e+6(2e-25-NA)	0.98	
Zoonotic Vaccine	Yes	100	15(15)	0.09	0.2	Ref		0.99
	No	113	17(15)	0.09	0.23	1.0(0.47-2.1)	0.99	
Recent contact with animal body fluid	No	68	9(13.2)	0.07	0.23	Ref		
	Yes	145	23(15.9)	0.10	0.22	1.23(0.55-2.97)	0.61	0.61
Sanitization after touching sick animal	Yes	32	6(18.8)	0.08	0.35	0.72(0.28-2.10)	0.52	0.53
	No	181	26(14.4)	0.09	0.2	Ref		
Recent Hospital visit	No	165	23(13.9)	0.09	0.2	Ref		0.42

	Yes	48	9(18.8)	0.10	0.3	1.4(0.5-3.2)	0.41	

Discussion

The findings indicate that the prevalence of *S. aureus* is observed in pets, pet owners, and veterinary staff at rates of 31.4%, 20.94%, and 35.7%, respectively. The overall prevalence of *S. aureus* in humans stands at 31.92%. The elevated carriage rate of *S. aureus* emphasizes its role as a prevalent commensal while also pointing to a significant reservoir for the potential acquisition of the *mecA* gene and the subsequent emergence of MRSA in vulnerable populations [37]. Furthermore, the prevalence of MRSA among animal owners, pets, veterinary personnel, and humans was recorded at 14.05%, 7.33%, 21.43%, and 15.2%, respectively. A study carried out in Pakistan revealed that the prevalence of MRSA was 39.4% in cats, with rates of 33.9% and 25% observed in dogs and pet owners [38]. In a separate investigation conducted in the UK, MRSA was isolated from 17.9% of veterinary staff and 9% of dogs [39]. A separate investigation conducted in Trinidad indicated that the prevalence is 6% in cats and 7% among pet owners [40]. A study conducted in Tanzania found that 6.5% of dogs and 3.3% of dog owners tested positive for MRSA [41]. A separate investigation conducted in Bangladesh indicated that the prevalence of MRSA in pets was found to be 4.3% [22]. Through comparative analysis with other nations and earlier studies, the prevalence is consistently increasing in Bangladesh. This rising trend is alarming and is probably influenced by a mix of growing pet ownership and, importantly, the unregulated use of antibiotics in both veterinary and human fields, a recognized factor contributing to antimicrobial resistance [42, 43]. The occurrence of *S. aureus* and MRSA among animal owners and veterinary staff in relation to the hospital environment and occupational exposure underscores the dynamics of HA-MRSA. This rate additionally indicates the transmission occurring within the clinical environment or through regular interaction with sick or colonized animals, reinforcing the study's emphasis on veterinary staff as a population at elevated risk. The similar prevalence rates noted among pet owners (14.05%) and their pets (7.33%), although not statistically identical, highlight the possibility of a shared carriage reservoir and necessitate further exploration into cross-transmission dynamics, which are crucial to the One Health approach.

The antibiogram profile from 39 isolates of both pets and their owners indicates a significant level of resistance, with the highest rates observed against cefoxitin (97.4%), penicillin (94.8%), azithromycin (82%), ciprofloxacin (53.9%), amoxicillin-clavulanic acid (21.9%), and ceftriaxone (10.3%). In contrast, sensitivity was noted for cephradine (61.5%), gentamicin (87.2%), doxycycline (92.3%), tetracycline (84.3%), and sulfamethoxazole-trimethoprim (92.3%). The almost total resistance to cefoxitin (97.4%) aligns with the CLSI definition for *mecA* mediated resistance, affirming the selection and prevalence of MRSA clones among the isolates [44]. The notable co-resistance detected against macrolides (azithromycin, 82%) and fluoroquinolones (ciprofloxacin, 53.9%) suggests that a considerable percentage of the isolates are classified as multidrug resistant (MDR). This pattern indicates that the circulating MRSA clones are probably harbouring multiple resistance determinants, potentially through mobile genetic elements, which further complicates therapeutic options in both human and veterinary medicine [45, 46]. A study indicated that azithromycin, cefixime, imipenem, ampicillin, erythromycin, and amoxicillin exhibited complete resistance in pets, whereas cefuroxime, amikacin, ciprofloxacin, and cefotaxime demonstrated sensitivity rates of 76.9%, 69.2%, 69.2%, and 53.8%, respectively [47]. A further investigation revealed that gentamicin, amikacin, sulfamethoxazole-trimethoprim, chloramphenicol, and ciprofloxacin exhibit nearly 100% sensitivity across dogs, cats, and humans, indicating a notable similarity. However, vancomycin demonstrated resistance rates of 54.78%, 86.95%, and 57% in cats, dogs, and humans, respectively, highlighting a significant concern [38]. A separate study indicated that 40% of the isolates were Methicillin-resistant and often exhibited resistance to non-beta lactam antibiotics [48]. In comparison to other studies, our sample size was larger. This could lead to a notable effect on the precision of interpretation. The significant retention of sensitivity to last-resort or reserved antibiotics, including gentamicin, doxycycline, tetracycline, and SXT/TMP, represents an important discovery, suggesting that these medications may continue to serve as effective therapeutic options within the local context. This stands in stark contrast to the elevated resistance rates observed in certain regional studies[38], indicating differing local selection pressures.

Through univariate logistic regression analysis, consuming food near or within the animal shed has been identified as a potential risk factor for pet owners, with a p-value of less than 0.2. Furthermore, in relation to pets, the identified risk factors included location (Dhaka), species (dog), age (over 6 months), and specimens (pus swab), all of which were recognized as potential risk factors (p-value less than 0.2). Nonetheless, the analysis using multivariate logistic regression indicated that only the pus swab emerged as a significant risk factor (P value 0.02). The presence of pus in the swab indicates a significant likelihood that the risk of colonization evolves into an infection risk in cases that exhibit purulent lesions. This highlights that clinical disease, rather than mere carriage, is the main factor influencing MRSA detection in the examined pet population. In the context of veterinary personnel, age emerged as the sole potential risk factor, with a p-value of less than 0.2. This finding for veterinary personnel is consistent with existing literature indicating that age, which may be linked to years of practice, enhances exposure time and cumulative colonization risk among healthcare workers [49, 50]. By integrating information from both pet owners and veterinary staff, no potential risk factors were identified. A study also identified potential risk factors including

disease in dogs and cats, human infection on the body, prior antibiotic use, pet access to the bedroom, and kissing pets [38]. An additional study was carried out on MRSA in both rural and urban communities, examining variables such as age, gender, occupation, hospitalization history, contact with pets, antibiotic use, completion of antibiotic courses, and over-the-counter antibiotic use; however, no potential risk factors were identified [40]. In contrast, a separate investigation carried out in the UK pinpointed the variables of antimicrobial courses, duration of stay in veterinary clinics, and surgical implants as risk factors. The findings of this study indicated that the likelihood of interaction with humans who had been sick and hospitalized was greater in pets infected with MRSA [51]. The variation in the study results likely reflects changes in epidemiological trends or advancements in methodological techniques. Furthermore, the interaction patterns between pets and their owners, the design of the study, the size of the sample, and the strategy used for sampling may contribute to this phenomenon. The inability to pinpoint crucial behavioural risk factors (such as kissing pets or prior antibiotic use) in the final multivariable model, even though they have been acknowledged in other research, could stem from the study's cross-sectional design or potential recall bias among participants. This highlights the need for prospective studies to effectively delineate these interactions. Future longitudinal studies are essential to validate the possibility of matched isolates between pets and owners, which serves as the gold standard for demonstrating direct cross-transmission.

This study examines the status of MRSA in two prominent cities in Bangladesh. The current prevalence of MRSA among animal owners and veterinary personnel presents a considerable risk to public health. Maintaining proper hygiene may reduce the likelihood of MRSA transmission from pets to humans.

Even with a considerable sample size, it is important that the population is not limited exclusively to those who own animals or work in veterinary roles. A thorough population study has the potential to produce accurate results. A more thorough investigation can be conducted for the MRSA surveillance. Further molecular investigations, including whole genome sequencing, may improve the understanding of the MRSA strain and its resistance mechanisms. Finally, the variation in identifying the risk factors highlights the need for modern, context-specific studies to confirm or adjust our comprehension of these risk factors.

Conclusion

The investigation revealed the prevalence of MRSA through the detection of the *mecA* gene among various groups: animal owners at 14.05%, pets at 7.33%, veterinary personnel at 21.43%, and combined animal owners and veterinary personnel at 15.2%. AST demonstrated the greatest resistance levels with cefoxitin, penicillin, and azithromycin, showing rates of 97.4%, 94.8%, and 82%, respectively. The notable occurrence of MRSA among veterinary personnel (21.43%) and the existence of Multi-drug Resistance clones highlight a considerable public health threat arising from this occupational and animal-related source. The ongoing sensitivity to reserve medications such as gentamicin and SXT/TMP provides a limited opportunity for focused clinical intervention. Ultimately, the analysis using multivariable logistic regression revealed that the presence of pus in pet animals is a significant risk factor for MRSA transmission. The findings provide crucial baseline data for One Health surveillance in Bangladesh and strongly support the need for improved infection control protocols in veterinary hospitals, as well as targeted antimicrobial stewardship to reduce the zoonotic transmission of these resistant pathogens.

Abbreviations

MRSA - Methicillin-resistant *Staphylococcus aureus*

AMR - Antimicrobial resistance

PCR - Polymerase Chain Reaction

AST - Antimicrobial Sensitivity Testing

MDR - Multi- drug Resistance

MSA - Mannitol Salt Agar

DNA – Deoxyribonucleic Acid

CVH - Central Veterinary Hospital

VTH - Veterinary Teaching Hospital

Declarations

Human ethics

Ethical approval: All experimental protocols involving human participants were approved by the Institutional Ethical Committee of Bangladesh Agricultural University Research System (BAURES), Bangladesh Agricultural University (BAU) [Approval No.: AWEEC/BAU/2021(55)].

Accordance and ethical guidelines: All procedures involving human participants were performed in accordance with relevant institutional guidelines and regulations and in compliance with the 1964 Helsinki Declaration and its later amendments.

Informed consent: Informed consent was obtained from all participants prior to sample and data collection. For participants under the age of 18 years, informed consent was obtained from their parents or legally authorized representatives. All participants were fully informed about the objectives, procedures, potential risks, and benefits of the study and were given sufficient time to ask questions before providing written informed consent.

Animal ethics

Ethical approval: The study was approved by the Animal Welfare and Experimental Ethics Committee (AWEEC), Bangladesh Agricultural University (BAU) [Approval No.: AWEEC/BAU/2021(55)] and the Bangladesh Agricultural University Research System (BAURES).

Ethical guidelines: All animal-related procedures were conducted in accordance with relevant institutional and national guidelines and complied with internationally accepted standards for animal welfare, including the principles of the Basel Declaration.

Permissions and informed consent for collection: Animals included in this study were privately owned pets presented to the Veterinary Teaching Hospital, Bangladesh Agricultural University, and the Central Veterinary Hospital, Dhaka. Informed consent was obtained from all pet owners prior to sample and data collection. For pet owners under the age of 18 years, informed consent was obtained from their parents or legally authorized representatives. No specific field collection permits were required, as samples were collected during routine clinical visits with owner permission.

Consent for publication

Not applicable.

Availability of data and materials

The data set used in the current study is available from the Corresponding author upon reasonable request.

Competing interest

The authors declare that they have no competing interests.

Clinical Trial

Not applicable.

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Authors contributions

MMA orchestrated and oversaw the study. HS gathered the data, conducted the laboratory work, and performed the analysis. AA conducted the data analysis and drafted the initial manuscript. AKMAR evaluated and revised the manuscript. All authors sanctioned the final manuscript.

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Figures

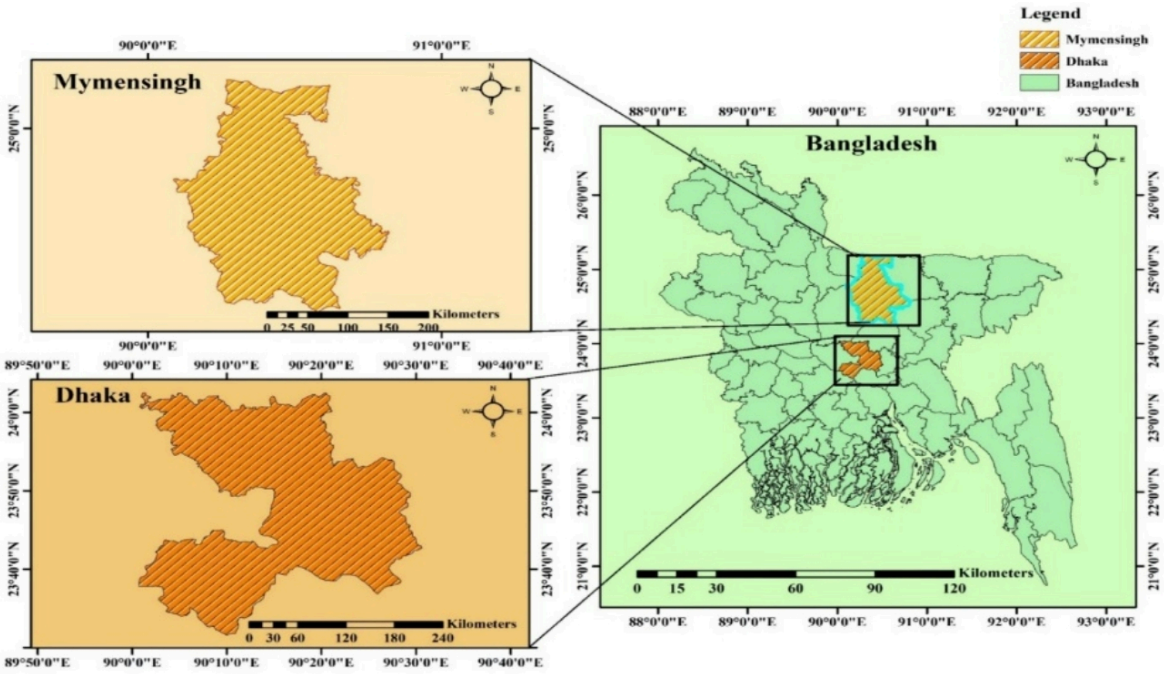


Figure 1

Geographic Locations of the study areas, Dhaka and Mymensingh is represented in the map of Bangladesh.

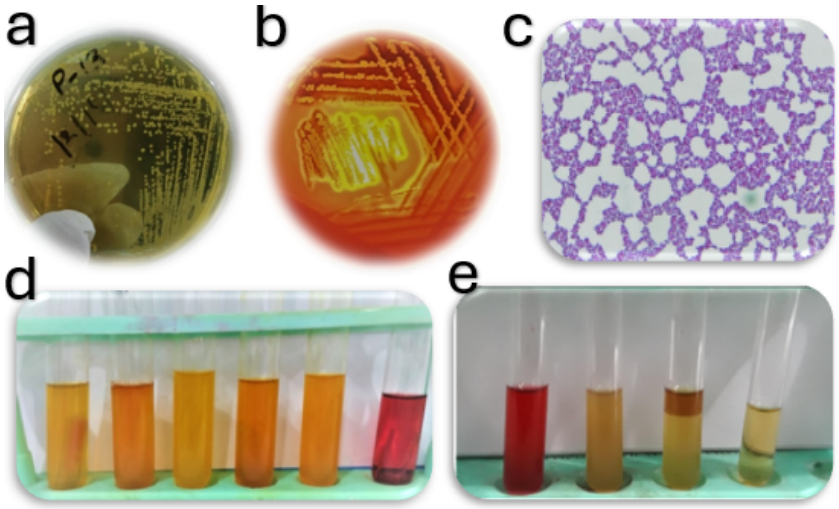


Figure 2

Phenotypic and biochemical identification of *S. aureus.* a) Golden yellow color colonies on Mannitol Salt Agar (MSA) indicating mannitol fermentation, b) Beta-hemolytic colonies on 5% Sheep Blood agar, c) Gram's staining characteristics of *S. aureus* under a light microscope (1000x magnification) showing grape like cluster indicating Gram positive cocci , d) Formation of transient oxygen bubble in catalase test, e) Positive tube coagulase test showing clot formation.

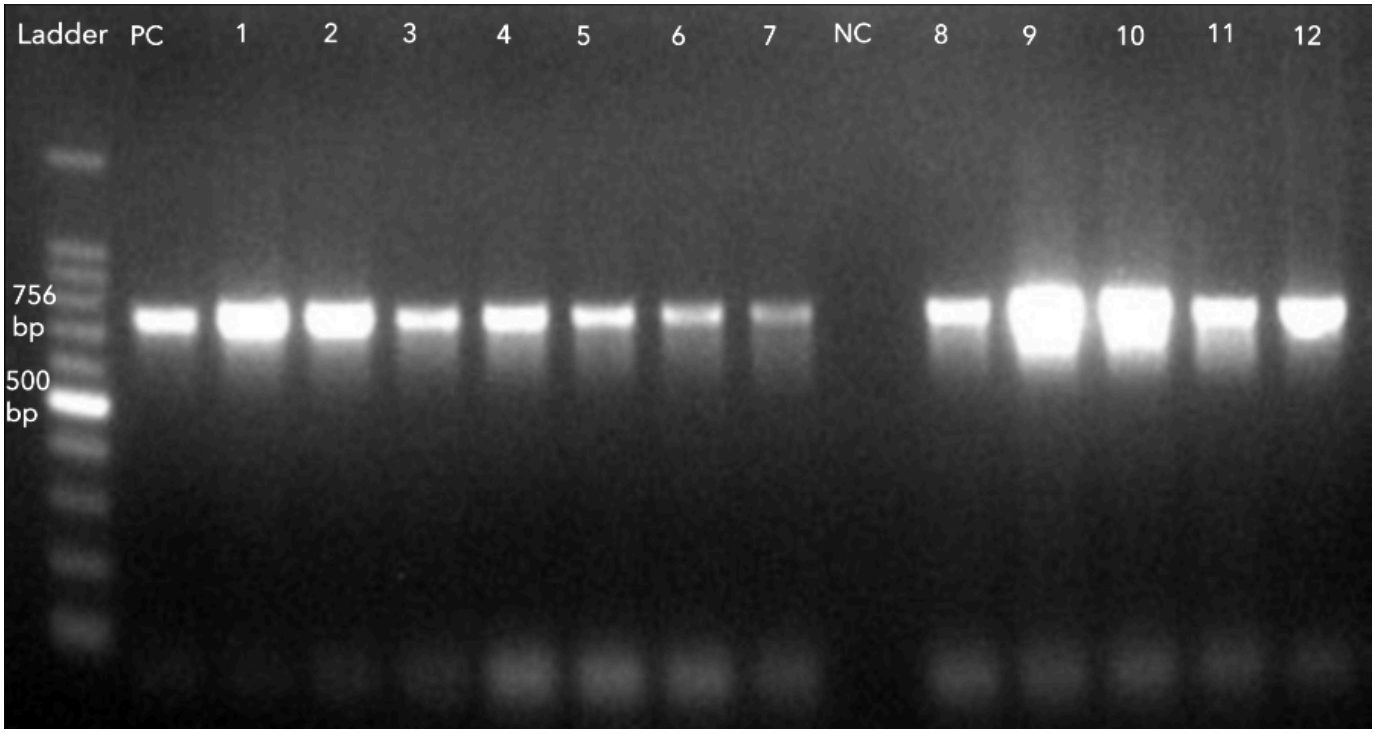


Figure 3

Molecular identification of *Staphylococcus* spp. via 16S rRNA gene PCR (756 bp). Lane M: 100 bp DNA ladder; Lanes 2–8 and 10–14: Positive isolates; Lane P: Positive control; Lane N: Negative control.

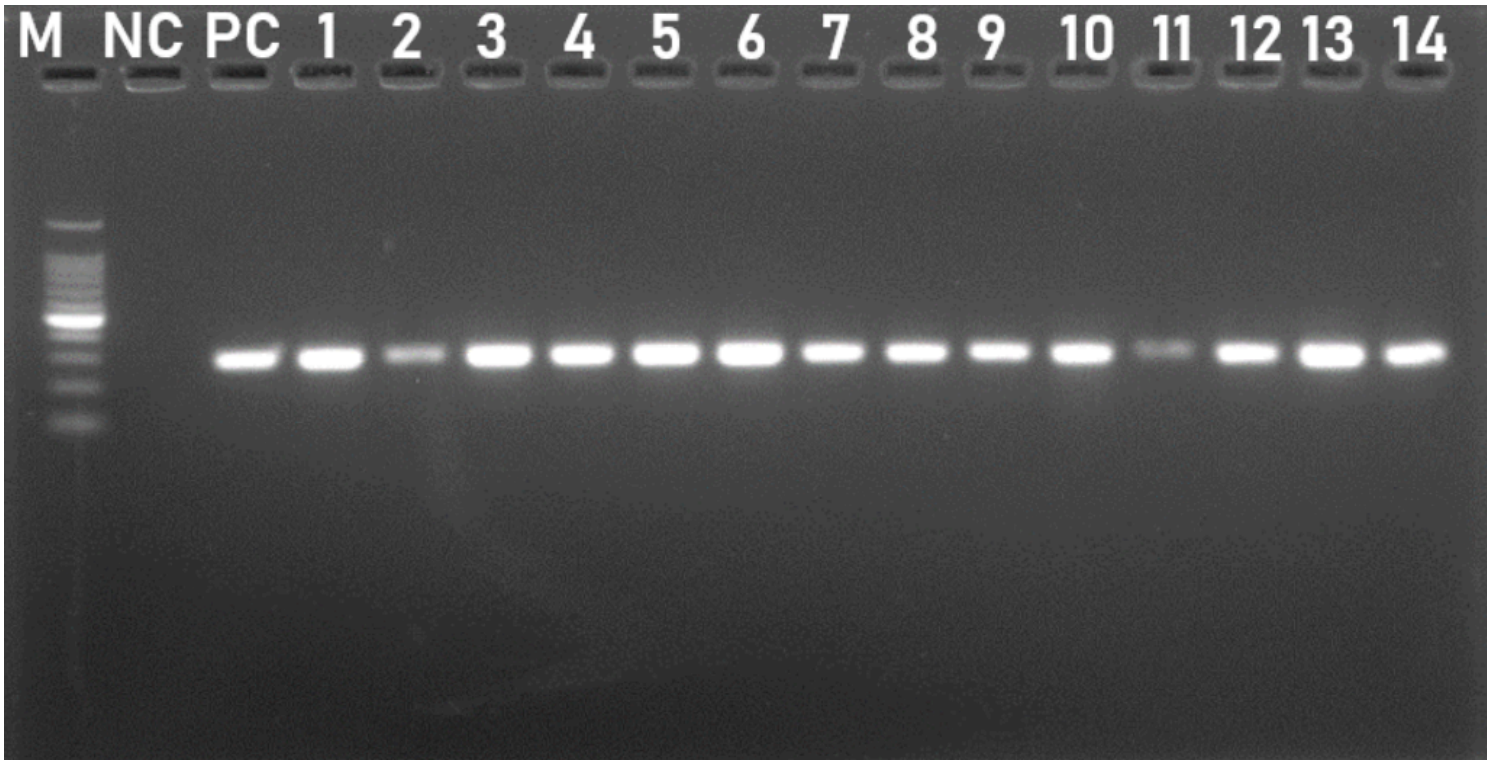


Figure 4

Confirmation of *S. aureus* via *nuc* gene PCR (279 bp). Lane M: 100 bp DNA ladder; Lanes 1–14: Positive isolates; Lane P: Positive control; Lane N: Negative control.

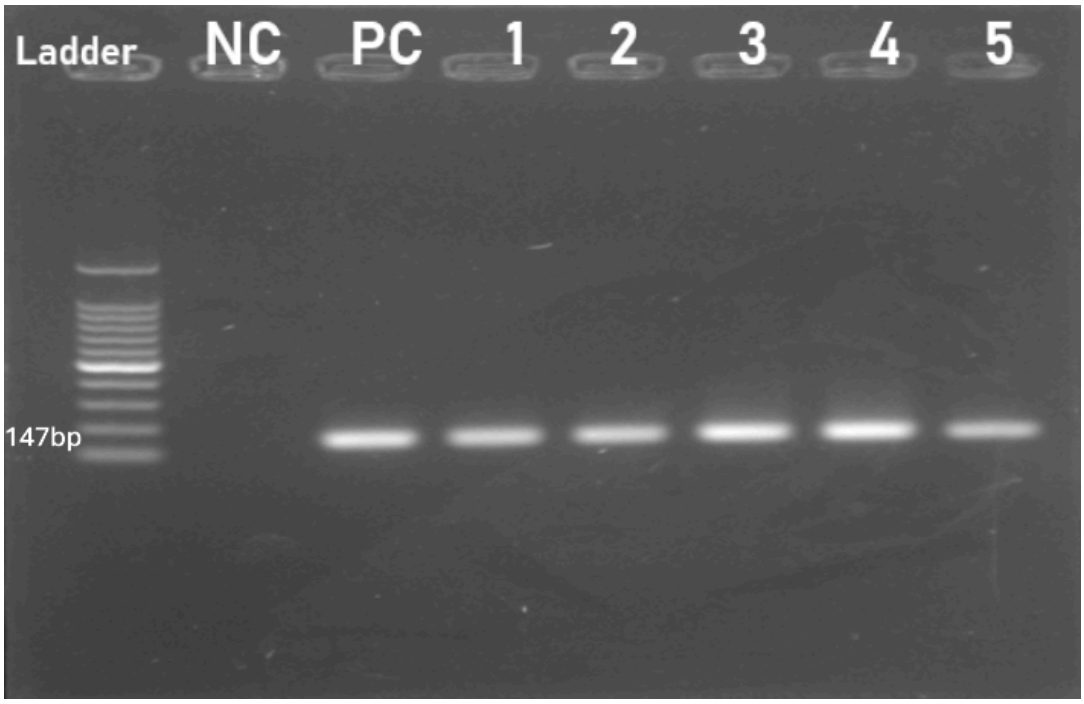


Figure 5

Detection of MRSA via *mecA* gene PCR (147 bp). Lane M: 100 bp DNA ladder; Lanes 1–5: Positive isolates; Lane P: Positive control; Lane N: Negative control.

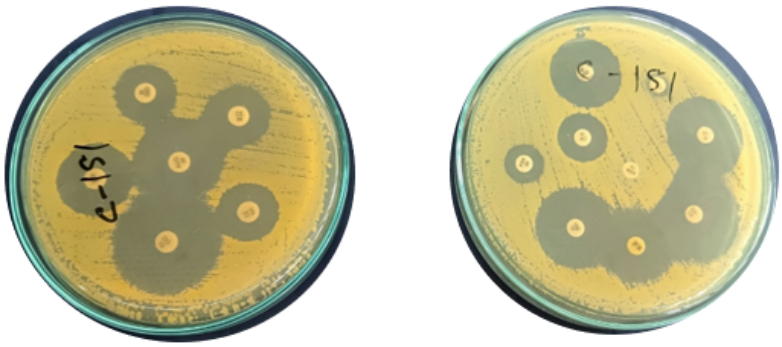


Figure 6

Antimicrobial susceptibility testing of *S. aureus*isolates. Representative image of the Kirby-Bauer disc diffusion method on Mueller-Hinton Agar showing zones of inhibition for various antibiotics.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryfile1.pdf
- SupplementaryFile2.pdf