One Health 18 (2024) 100680

Contents lists available at ScienceDirect

One Health

journal homepage: www.elsevier.com/locate/onehlt

Zoonotic linkage and environmental contamination of Methicillin-resistant *Staphylococcus aureus* (MRSA) in dairy farms: A one health perspective

Mithu Chandra Roy [a], Tonmoy Chowdhury [a], Muhammad Tafazzal Hossain [a], Md. Mahmudul Hasan [a], Eman Zahran [b], Md. Masudur Rahman [c], Kazi Mohammad Ali Zinnah [d], Md. Mahfujur Rahman [e], Ferdaus Mohd Altaf Hossain [a,d,*]

[a] Department of Dairy Science, Faculty of Veterinary, Animal and Biomedical Sciences, Sylhet Agricultural University, Bangladesh
[b] Department of Aquatic Animal Medicine, Faculty of Veterinary Medicine, Mansoura University, Egypt
[c] Department of Pathology, Faculty of Veterinary, Animal and Biomedical Sciences, Sylhet Agricultural University, Bangladesh
[d] Department of Animal & Fish Biotechnology, Faculty of Biotechnology & Genetic Engineering, Sylhet Agricultural University, Bangladesh
[e] Department of Medicine, Faculty of Veterinary, Animal and Biomedical Sciences, Sylhet Agricultural University, Bangladesh

ARTICLE INFO

Keywords:
MRSA
Super-antigenic
Zoonotic
Dairy cows
Environment
Human

ABSTRACT

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a ubiquitous public health challenge, with its prevalence in human, animal, and environmental interfaces posing significant concerns. This study aimed to characterize and detect the zoonotic linkages of MRSA within the cow-environment-human interfaces in dairy farms to address the One Health perspective. A comprehensive investigation, involving 636 samples (an equal number of raw milk and cow nasal swab samples, along with varying numbers of human nasal swab and environmental samples), revealed an overall MRSA prevalence of 13.4% (n = 271/636). Notably, environmental samples exhibited the highest prevalence (19.3%), emphasizing the potential role of farm surroundings in MRSA transmission, while the lowest prevalence was found in raw milk at 11.8% (n = 31/263). The prevalence in cow nasal swabs and human nasal swabs was 13.3% (n = 35/263) and 15.1% (n = 8/53), respectively. Multiplex PCR analysis revealed the presence of different Staphylococcal enterotoxin (SEa, SEb, SEc, and SEd), and exfoliative toxin-producing genes (Eta, Etb) within the MRSA isolates underlining their potential to induce public health threats. All MRSA isolates exhibited complete resistance to Oxacillin (100%) and Amoxicillin (100%), while the highest sensitivity was observed for Vancomycin (85.8%). Furthermore, these MRSA strains demonstrated varying degrees of resistance to other commonly used antimicrobial drugs, including Cefoxitin (75.3%), Ceftarolin (71.2%), Sulfamethoxazole-Trimethoprim (63.5%), Ciprofloxacin (60%), and Gentamicin (49.5%). Detection of MRSA in cow, human, and environmental samples within the same farm vicinity highlights the risk of zoonotic transmission of MRSA from cows to humans through environmental interfaces. Phylogenetic analysis of the *mecA* gene in MRSA isolates from all sources within the same farm revealed a high similarity index (>84%) among them suggesting a shared evolutionary origin. Moreover, the MRSA isolates from milk samples showed a close evolutionary relationship with isolates from Kenya and Brazil, while the isolates from humans and the environment displayed noticeable resemblance to isolates from several Asian countries. The findings emphasize the importance of collaborative efforts under the One Health framework to address this multifaceted issue and ensure the safety of our food supply and public health.

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as a significant multidrug-resistant nosocomial pathogen worldwide, posing a great public health concern due to its association with infections of high morbidity and mortality [1,2]. The isolation of MRSA strains was reported by Patricia Jevons just two years after the introduction of methicillin in clinical practice [3]. These pathogens display resistance to commonly used antimicrobial agents in both human and veterinary medicine [4]. PCR-based detection of the *mecA* gene, along with resistance to cefoxitin, is commonly employed for the clinical identification of MRSA, with the penicillin-binding protein (PBP-2 A) encoded by the

* Corresponding author at: Department of Dairy Science, Faculty of Veterinary, Animal and Biomedical Sciences, Sylhet Agricultural University, Bangladesh.
E-mail address: ferdaus.dps@sau.ac.bd (F.M.A. Hossain).

https://doi.org/10.1016/j.onehlt.2024.100680
Received 23 July 2023; Accepted 16 January 2024
Available online 17 January 2024

Table 1
Primers of *S. aureus*, MRSA and different enterotoxin producing genes by PCR.

Gene	Primer	Amplicon bp size	Primer set	Reference
nuc	F-GCGATTGATGGTGATACGGT R-AGCCAAGCCTTGACGAACTAAAGC	279	Uniplex	[21].
*mec*A	F-TGCTATCCACC CTCAAACAGG R- AACGTTGTAAC CACCCCAAGA	286	Uniplex	[22].
sea	F-GGTTATCAATGTGCGGGTGG R-CGGCACTTTTTTCTCTTCGG	102	A	
seb	F-GTATGGTGGTGTAACTGAGC R-CCAAATAGTGACGAGTTAGG	164	A	[23]
sec	F-AGATGAAGTAGTTGATGTGTATGG R-CACACTTTTAGAATCAACCG	451	A	
sed	F-CCAATAATAGGAGAAAATAAAAG R-ATTGGTATTTTTTTTCGTTC	278	A	
see	F-AGGTTTTTTCACAGGTCATCC R-CTTTTTTTTCTTCGGTCAATC	209	A	
eta	F-GCAGGTGTTGATTTAGCATT R-AGATGTCCCTATTTTTGCTG	93	B	
etb	F-ACAAGCAAAAGAATACAGCG R-GTTTTTGGCTGCTTCTCTTG	226	B	
tst	F-ACCCCTGTTCCCTTATCATC R-TTTTCAGTATTTGTAACGCC	326	B	

Table 2
Prevalence of *S. aureus* and MRSA in different types of samples by PCR.

Sources	Number of Taken sample	*S. aureus* %	MRSA %
Raw Milk	263	107 (40.7%)	31(11.8%)
Cow nasal swab	263	123(46.8%)	35(13.3%)
Human nasal swab	53	17 (32.1%)	8(15.1%)
Environment	57	24(42.1%)	11(19.3%)
Total	636	271(42.6%)	85(13.4%)

Table 3
Presence of enterotoxin and exfoliative toxin producing gene within MRSA.

Gene %	Milk (31)	Cow (35)	Human (8)	Environment (11)
sea	12.9 (4/31)	8.6 (3/35)	25 (2/8)	
seb		11.4 (4/35)		18.2 (2/11)
sec	6.5 (2/31)			18.2 (2/11)
sed	9.7 (3/31)	8.6 (3/35)	12.5 (1/8)	
see		8.6 (3/35)		9.1 (1/11)
sea- seb	3.2 (1/31)	5.7 (2/35)	12.5 (1/8)	
sea- sed	6.5 (2/31)			
sea- see		5.7 (2/35)		18.2 (2/11)
seb- sec	6.5 (2/31)		12.5 (1/8)	
sea-seb-sed	6.5 (2/31)			9.1 (1/11)
sea-seb-see		2.9 (1/35)		
eta	16.1 (5/31)	11.4 (4/35)	25 (2/8)	9.1 (1/11)
etb	9.7 (3/31)	8.6 (3/35)	12.5 (1/8)	
eta-etb	6.5 (2/31)	5.7 (2/35)		9.1 (1/11)

mecA gene playing a central role in conferring antimicrobial resistance [5,6].

MRSA strains are classified based on their epidemiological origins into healthcare-associated (HA-MRSA), community-associated (CA-MRSA), and livestock-associated (LA-MRSA) [7]. Initially, MRSA infections were primarily associated with healthcare facilities (HA-MRSA) or other healthcare settings. However, in the early 1990s, MRSA infections began to be reported in individuals without prior healthcare exposure, leading to the identification of community-associated MRSA (CA-MRSA). More recently, MRSA has been detected in livestock animals in Europe, leading to the emergence of livestock-associated MRSA (LA-MRSA) [8]. The first report of livestock-associated MRSA (LA-MRSA) in animals dates back to 1972 when it was isolated from bovine mastitis samples in Belgium [9]. LA-MRSA has become a significant public health concern due to its zoonotic potential, allowing transmission from animals to humans [10]. Individuals in close contact with animals, particularly those working on MRSA-positive farms, are considered to be at a higher risk of LA-MRSA colonization [11].

Antimicrobial drugs used to treat human infections are often similar to those used in animals [12]. Tetracyclines, macrolides, aminoglycosides, beta-lactams, fluoroquinolones, sulfonamides, and rifamycins are commonly employed for staphylococcal infection control in both humans and animals [13]. However, MRSA strains have demonstrated resistance to many antibiotics commonly used for the treatment of bacterial infections [14]. The One Health approach, emphasizing the interconnectedness of human-animal-environment health, has been repeatedly highlighted as crucial for investigating disease transmission and implementing effective control measures [15]. Concrete strategies are still needed to reduce the burden of infectious diseases and the impact of antimicrobial resistance in livestock and humans. The implementation of the One Health approach is pivotal for improving animal welfare, enhancing food safety, and promoting human health.

Of particular importance is the potential transmission of MRSA between livestock and humans, as well as host-switching events leading to the emergence of new pathogenic or resistant clones [16,17]. In Bangladesh, MRSA has been identified and characterized from various sources, including raw milk and dairy products [18,19]. However, to date, no studies have investigated the zoonotic linkage and contamination of environmental interfaces within dairy farms. As MRSA infections affect both animals and humans, this study aims to elucidate the zoonotic linkages of MRSA through molecular characterization while also assessing the contamination of environmental interfaces in dairy farms.

By exploring the transmission dynamics and sources of MRSA, this research will contribute to our understanding of the dissemination of MRSA and the potential risks associated with zoonotic transmission. Additionally, identifying and characterizing MRSA in environmental samples will provide valuable insights into the potential reservoirs and routes of contamination. Ultimately, these findings will help inform strategies for mitigating the spread of MRSA, safeguarding animal and human health, and preserving food safety.

2. Materials and methods

2.1. Ethical statement

The study was successfully approved by the Institutional Ethics Committee of Sylhet Agricultural University, Sylhet-3100; Bangladesh (#AUP2023001). All the required and applicable national, international, and institutional guidelines for the animal's care were thoroughly followed during the sample collection.

Table 4
Antibiogram profile of MRSA against different antibiotics.

Antibiotic group	Specific antibiotic disc	Concentration	Interpretive categories and zone diameter breakpoint			Resistance (%)		
			S	I	R	S	I	R
Penicillin	Oxacillin (OX)	1 μg	≥18	–	≤17	0	0	100
	Amoxicillin(AMX)	30 μg	≥17	14–16	≤13	0	0	100
Cephalosporins	Cefoxitin (CX)	30 μg	≥22	–	≤21	15.3	9.4	75.3
	Ceftarolin (CPT)	30 μg	≥25	20–24	≤19	10.6	18.8	71.2
Aminoglycosides	Gentamicin (GEN)	10 μg	≥15	13–14	≤12	23.5	27	49.5
Tetracycline	Tetracycline (TE)	30 μg	≥19	15–18	≤14	61.2	14	24.8
Sulfonamides	Sulfamethoxazole-Trimethoprim (SXT)	25 μg	≥16	11–15	≤10	20	16.5	63.5
Macrolides	Azithromycin (AZM)	15 μg	≥18	14–17	≤13	28.2	31.8	40
Fluoroquinolone	Ciprofloxacin (CIP)	5 μg	≥21	16–20	≤15	17.6	22.4	60
	Norfloxacin (NOR)	10 μg	≥17	13–16	≤12	27	24.7	48.3
Oxazolidinones	Linezolid (LZD)	30 μg	≥21	–	≤20	40	22.6	37.4
Glycopeptide	Vancomycin (VA)	30 μg	≥21	18–20	≤17	85.8	0	14.2

Here, S-Sensitive; I-Intermediate; R-Resistant.

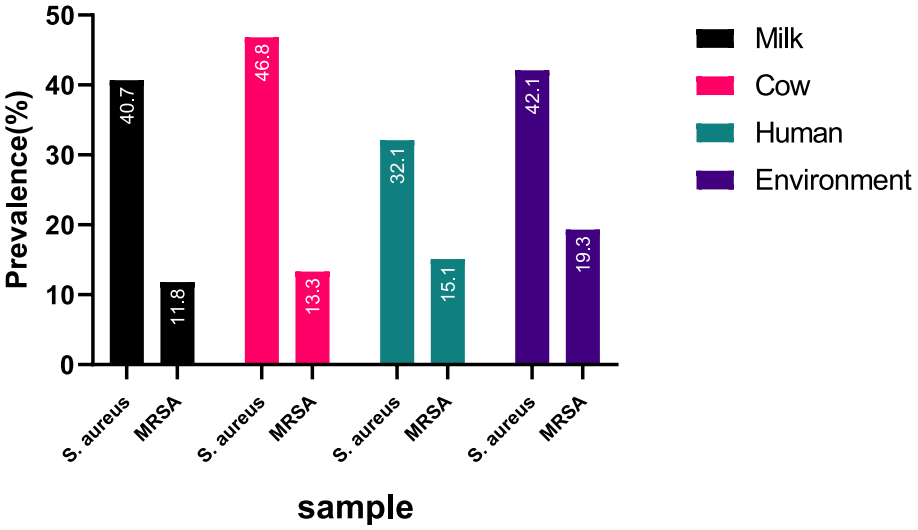

Fig. 1. Prevalence of *S. aureus* and MRSA in different type of sample those belongs to raw milk, cow nasal swab, human nasal swab and environment. Color legend in right corner refers to each type of sample. Highest prevalence of *S. aureus* has been detected in cow nasal swab while lowest in human. MRSA prevalence was highest in environment along with lowest in raw milk.

2.2. Sample collection

A comprehensive collection of 636 samples was carried out from multiple dairy farms located in Sylhet Sadar area (24.8951° N, 91.8688° E). Each dairy farm included an equal number of samples consisting of raw milk and cow nasal swabs while the sample number of human nasal swabs, and environment varied from one to another farm. Specifically, the sample distribution was as follows: cow milk (n = 263), cow nasal swab (n = 263), farm worker nasal swab (n = 53), and environmental swab (n = 57). The environmental samples were obtained from various sources such as feed troughs, gutters, and drainage systems within each farm.

For the collection of cow milk samples, approximately 5 ml of milk was aseptically collected from each cow using pre-sterilized collection tubes. Prior to sample collection, the tubes were properly and thoroughly sterilized to ensure the integrity of the samples. All the collected milk samples were then subjected to the Whiteside test (WST) to identify any cases of subclinical mastitis (Hossain et al., 2019). Samples testing positive result for subclinical mastitis were totally discarded, while only those testing negative (subclinical mastitis negative) samples were retained for further analysis.

In addition to the cow milk samples, nasal swabs were also collected from both cows and farm workers. Sterilized cotton buds were used to obtain nasal swabs, which were then placed in small containers enriched with Phosphate Buffer Solution (PBS). These containers helped to maintain the viability of the collected samples during transportation to the laboratory, which was completed within 24 h of sample collection.

The same procedure was again followed for the collection of environmental samples. Sterilized cotton buds were used to obtain swab samples from different environmental sources, including feed troughs, gutters, and drainage systems within each dairy farm. These swab samples were also placed in containers enriched with PBS to preserve their integrity during transportation to the laboratory. By employing strict aseptic techniques and utilizing appropriate containers and solutions, the collection process ensured the preservation and quality of the collected samples.

2.3. Isolation and identification of S. aureus

Samples were transferred to the Dairy Science Laboratory of Sylhet Agricultural University immediately after collection from different the dairy farms. Samples were then inoculated in the nutrient broth for their initial enrichment and incubated at 37 °C for 24 h. After enrichment, each sample was cultured on Manitol Salt Agar (HiMedia, India) and then incubated again at 37 °C for 24 h. Pure culture of the desired isolates was obtained by subculture on Manitol Salt Agar (HiMedia, India)

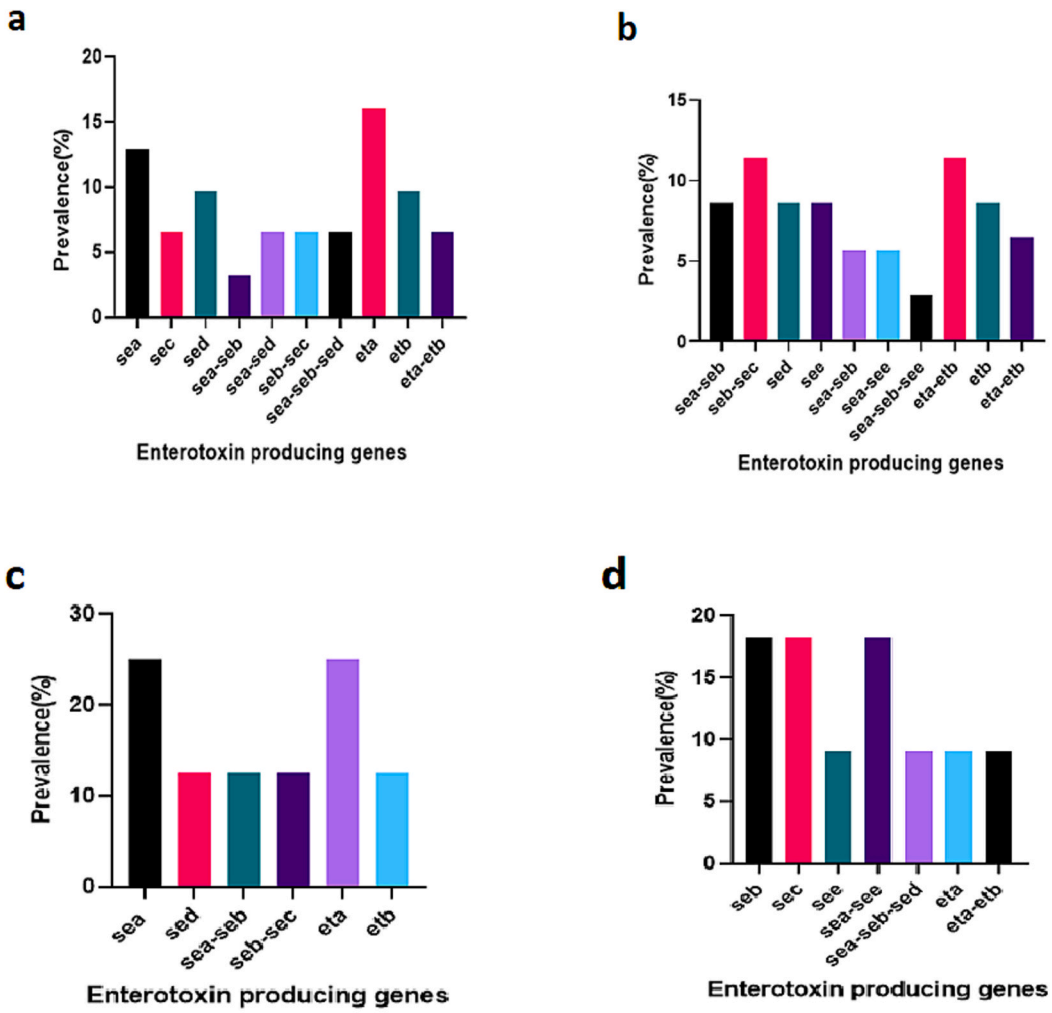

Fig. 2. Presence of different enterotoxin and exfoliate toxin-producing genes were detected within MRSA from all types of samples those belongs to milk, cow, human and environment. a. Toxin producing-genes of MRSA from milk. Presence rate of *eta* is highest and combined presence of *sea-seb* is lowest. b. Toxin producing genes of MRSA from cow nasal swab. Combined presence of *seb-sec* and *eta-etb* are highest whereas, the combined presence of *sea-seb-see* found to be lowest. c. Toxin producing genes of MRSA from human nasal swab where, *sea* and *eta* are equally highest in single pattern. d. Toxin producing-genes of MRSA from environment. The prevalence of *seb* and *sec* either in individual or combined form found to be higher, compared to other enterotoxins of MRSA.

Enterotoxin and exfoliative toxin gene profile of MRSA isolates

Each sample type was found to harbor one or more enterotoxin and exfoliative toxin-producing genes, either independently or in conjunction with other virulent genes. The prevalence of these genes ere varied across the collected samples, both individually and in combination, stressing the diverse composition of enterotoxin and exfoliative toxin profiles. The distribution (%) of these genes contrasted both individually and in grouped combinations, underscoring the dynamic nature of their presence in the study.

for several times. *Staphylococcus aureus* was identified based on their colony morphology, pigment production, Gram staining, and different biochemical test following the standard protocol.

2.4. DNA extraction

The genomic DNA from *S. aureus* was extracted using conventional boiling method that was previously described [20]. In brief, 2–3 fresh bacterial colonies were dissolved in deionized distilled water in a sterilized eppendorf tube and boiled at 95 °C for 10 mins. The tubes containing boiled colonies were then immediately transferred for instant cooling at −20 °C for 10 mins. After cooling they were centrifuged at 13500 rpm for 5 mins and the supernatant fluid was collected as DNA template. Nano Drop 2000c (Thermo Fisher Scientific Inc., Waltham, MA, USA) was used to determine the purity and concentration of the extracted DNA.

2.5. Primers

Oligonucleotides ranging from 18- to 24-mers were selected from the published DNA sequences of the *S. aureus* genes (Table 1) using Oligo software (version 3.4). Two uniplex PCR were performed to detect *nuc* gene and *mec*A gene in order to confirm *S. aureus* and MRSA respectively. For multiplex PCRs, two primers sets were prepared: set A was designed to amplify *sea, seb, sec, sed*, and see whereas set B was designed to amplify *eta, etb,* and *tst*. The primer sequences used in the multiplex PCRs were described in (Table 1).

2.6. Uniplex PCR condition to detect S. aureus

The uniplex PCR was performed to amplify the *nuc* gene of *S. aureus*. To amplify the *nuc* gene a reaction mixtures (25 µl) included 2 µl template DNA, 10× PCR buffer (Sigma Aldrich, USA), 25 mM $MgCl_2$, 200 µM of the four dNTPs, 10 pmol of each of the 2 primers (Macrogen, Korea), and 1 U *Taq* DNA polymerase (Sigma Aldrich, USA). The

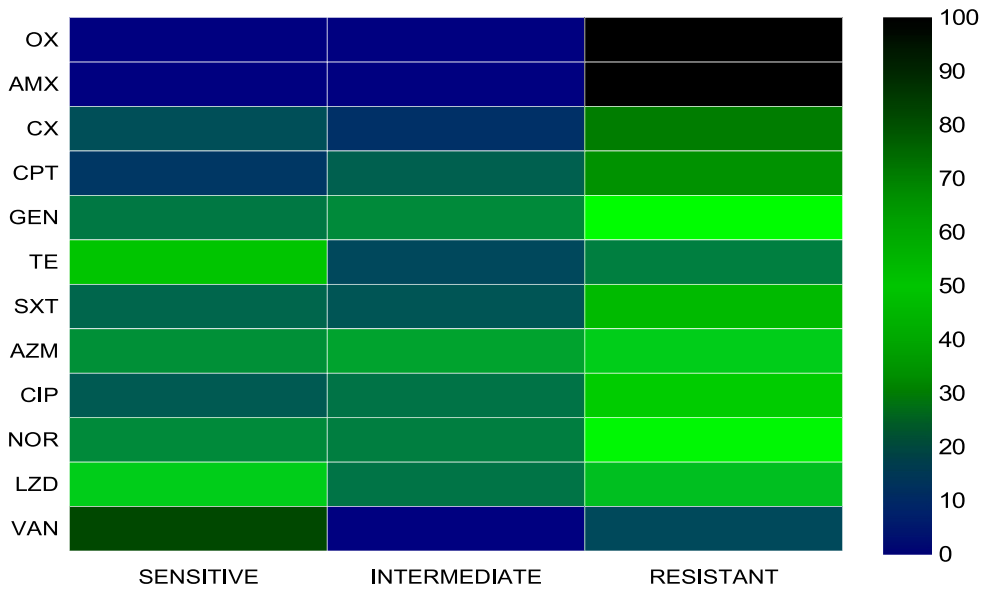

Fig. 3. Antibiotic resistance pattern of MRSA against different antibiotics. All MRSA isolates showed total resistant against Oxacillin and Amoxicillin but also demonstrated variable pattern of resistance against rest other antibiotics. Intensity of each color refers to sensitive, intermediate and resistant appearance against each antibiotics based on color intensity measuring scale on right side.

amplification of gene was carried out with thermocycler (Thermo Fisher Scientific, USA) under the following cycling conditions at initial denaturation at 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 3 min, annealing at 58 °C for 30s, and extension at 72 °C for 45 s, followed by a final extension at 72 °C for 10 min. Amplified products were then confirmed through visual observation at desired bp size by agarose gel electrophoresis (1.5% agarose containing 0.5 mg ethidium bromide in 0.5 × Tris-EDTA electrophoresis buffer) under UV illumination. A 100 bp ladder (RBC Bioscience Corp, Taiwan) was run as a molecular marker [21].

2.7. Uniplex PCR condition to detect methicillin-resistant S. aureus (MRSA)

To detect *mec*A gene, another uniplex PCR was also carried out in 25 µl reaction mixtures which contained 200 µM of dNTPs (NEB), 1× PCR buffer (NEB) and 0.5 U Taq DNA polymerase (NEB). The amplification was done by using a thermocycler (Thermo Fisher Scientific, USA) under the following cycling conditions mentioning one cycle of initial denaturation at 94 °C for 5 min, 25 cycles of denaturation at 94 °C for 30s, annealing at 54 °C for 30s, and extension at 72 °C for 30s, followed by a final extension at 72 °C for 5 mins. Amplified products were then confirmed through visual observation at desired bp size by agarose gel electrophoresis on a 1.5% low melting agarose gel (Invitrogen, USA). They were subjected for electrophoresis (0.5× TBE buffer at 150 V and 90 mA for 30 min) stained with 0.5% ethidium bromide. A 100 bp ladder (RBC Bioscience Corp, Taiwan) was run as a molecular marker [22].

2.8. Multiplex PCR conditions

Two sets of primer mixes were prepared according to the master mixes of components from the GeneAmp kit (Perkin-Elmer, USA), with slight modifications to the given instructions. Multiplex primer set A contained 200 mM deoxynucleoside triphosphates; 5 ml of 103 reaction buffer (100 mM Tris-HCl [pH 8.3], 500 mM KCl); 1.5 mM $MgCl_2$; 20 pmol of each primer for *sea, seb, sec, sed,* and *see*; 2.5 U of Taq DNA polymerase (AmpliTaq DNA polymerase; Perkin-Elmer); and 10 to 1000 ng of template DNA. Multiplex primer set B included the same constituents as in set A except for the $MgCl_2$ concentration (2.0 mM) with 20 pmol of each primer for *eta, etb,* and *tst.* DNA amplification was carried out in a Perkin-Elmer thermocycler with the following thermal cycling profile: an initial denaturation at 94 °C for 5 min was followed by 35 cycles of denaturation at 94 °C for 2 min, annealing at 57 °C for 2 min, and extension at 72 °C for 1 min ending with a final extension at 72 °C for 7 min described by [23].

2.9. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) was made by the Kirby-Basier disk diffusion method in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2020). Briefly, two-three fresh colonies were suspended in 3 ml normal saline and the turbidity of the suspension was standardized to match with 0.5 McFarland standards. This bacterial inoculum was wiped over the surface of Mueller Hinton agar plate, onto which the antimicrobial disks were placed manually within 15 min. Plates were incubated for 16–24 h at 35–37 °C prior to determination of results. The diameter of the zone of inhibition surrounding the disks was measured using millimeter (mm) scale manually and compared to the break points of CLSI. The disk diffusion was done against 12 antibiotics under 9 groups including Oxacillin (OX-1 µg); amoxicillin (AMX-25 µg); gentamicin (GEN-10 µg); cefoxitine (CX-30 µg);Ceftarolin (CPT-10 µg); tetracycline (TE-30 µg); Ciprofloxacin (CIP-5 µg); Norfloxacin (NOR-10 µg); Sulfamethoxazole-Trimethoprim (SXT-25 µg); Linezolid (LZD-30 µg); Azithromycin (AZM-15 µg); vancomycin (VAN-30 µg). All antimicrobial susceptibility testing assays were repeated at least 3 times. *Staphylococcus aureus* resistant to three or more antimicrobials were defined as MDR isolates [24]. Intermediate may be regarded as resistant since the acquisition and transition from susceptible to resistance had already begun [25]. The multiple antibiotic resistance (MAR) index was calculated and interpreted using the proven method [26].

2.10. Phylogenetic analysis of MRSA isolate

A Phylogenetic tree, based on the *mec*A gene sequence from this study, was constructed and compared with other reference MRSA strains through the maximum-likelihood method using MEGA software, version X (http://www.megasoftware.net/). The evolutionary history was inferred using the Neighbor-Joining method. The evolutionary distances were computed using the Maximum Composite Likelihood method and

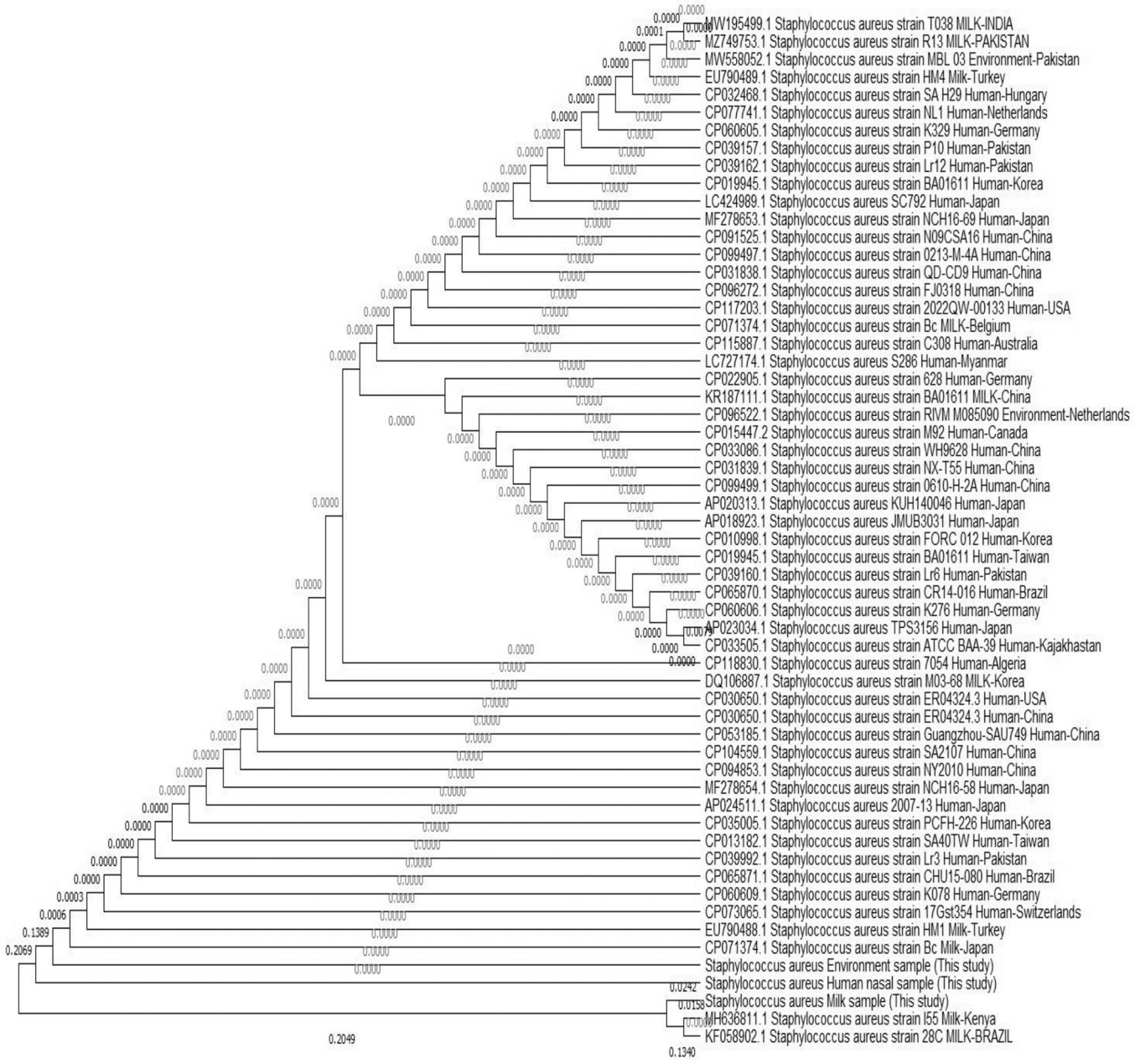

Fig. 4. Phylogenetic analysis of MRSA isolate from milk, human and environment.
Phylogenetic tree based on the *mec*A gene sequence from this study and those from other reference strains, constructed by the maximum-likelihood method using MEGA software, version X (http://www.megasoftware.net/). The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree is shown (next to the branches). The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. All ambiguous positions were removed for each sequence pair (pairwise deletion option). Accession no of milk, human and environment from this study are OR096213, OR096216 and OR096214 respectively.

are in the units of the number of base substitutions per site.

2.11. Statistical analysis and data visualization

All data was recorded on Microsoft excel 2019. Statistical analysis was performed on IBM SPSS Statistics (v.26) statistical software. The GraphPad Prism 9.3.1 statistical software was used for generating graphs. The prevalence % was calculated using the below formula:

$$\text{Percentage} = (\textit{Number of cases in a population at a pont of time})/(\textit{Total population at the same point of time}) \times 100$$

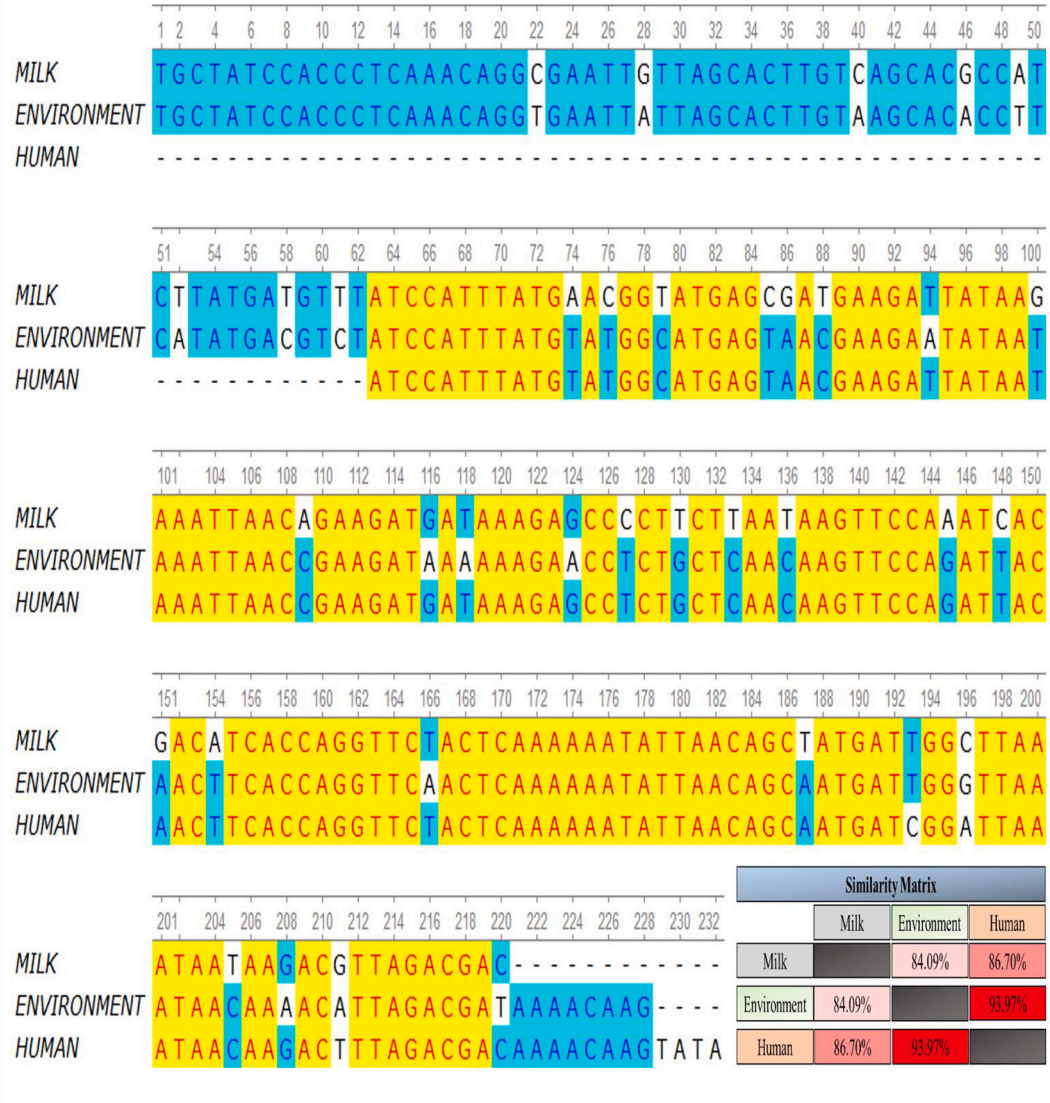

Fig. 5. Similarity matrix of *mecA* gene of MRSA from milk, human and Environment.
The similarity matrix analysis for the *mec*A gene sequences of MRSA isolates across milk, human, and environmental samples disclose the noteworthy patterns of genetic relatedness. Our finding indicates a close relationship among the sequences, with the highest similarity observed between human and environmental samples (93.97%). On the contrary, the lowest similarity was identified between milk and environmental samples (84.09%). The calculation of similarity was conducted based on nucleotide positions within the same location of each sample, providing a clear-cut understanding of the genetic relationships among the MRSA isolates in this study. Our findings accentuate the potential interconnectedness of MRSA strains across human, animal, and environmental interfaces.

3. Results

3.1. Prevalence of S. aureus and MRSA in different types of samples

Using general PCR, *S. aureus* was detected in 42.6% (271) out of 636 collected samples. Particularly, a higher prevalence was found in cow nasal swabs (46.8%), raw milk (40.7%), human nasal swabs (32.1%), and the farm environment (42.1%). This suggests a varied distribution of *S. aureus* across different sample types within the dairy farm setting. Conversely, the prevalence of Methicillin-Resistant *S. aureus* (MRSA) was present in 13.4% (85) of the samples. The majority of MRSA-positive samples were identified in the farm environment (19.3%), signifying a potential environmental reservoir. Additionally, MRSA was detected in 11.8% of raw milk samples, 13.3% of cow nasal swabs, and 15.1% of human nasal swabs. This highlights the need for a comprehensive understanding of MRSA distribution in various sample sources, emphasizing its potential impact on both animal and human health within the dairy farming context (Table 2).

3.2. Enterotoxin and exfoliative toxin gene profile of MRSA isolates from each type of sample

Most of the MRSA isolates from each type of sample were identified to carry one or more enterotoxin and exfoliative toxin producing gene. Enterotoxin and exfoliative toxin-producing gene present either alone or together with other virulent genes. The percentage of individual and group wise presence of enterotoxin and exfoliative gene vary from sample to sample (Table 3).

3.3. Antibiogram profile of MRSA

Variable degree of resistance of MRSA has been identified against different antibiotics in this study. All MRSA isolates showed total resistant against oxacillin, amoxicillin but also demonstrated variable pattern of resistance against rest other antibiotics (Table 4).

3.4. Phylogenetic analysis of MRSA from milk, human and environment

The MRSA isolates from milk samples in this study showed a close evolutionary relationship with those MRSA isolates from milk in Kenya and Brazil, while the MRSA isolates from humans and environment displayed noticeable resemblance to isolates from several Asian countries those were mentioned in the lower part of the phylogenetic tree (Fig. 4).

3.5. Similarity matrix of mecA gene of MRSA from milk, human and environment

Similarity matrix reveals close relationship among the nucleotide sequence of *mec*A gene of MRSA isolated from milk, human and environment (Fig. 5). Highest similarity was detected between human and environment mentioned as 93.97% while lowest was between milk and environment mentioned as 84.09%. Similarity was measured based on determining the nucleotide position within same location of each origin.

4. Discussion

The transmission of pathogenic bacteria from livestock to humans through milk is a critical issue with profound implications for public health, farm workers, and veterinarians. The rising incidence of *S. aureus* infections, coupled with the prevalence of MRSA in livestock, accentuates the magnitude of its impact on public health [7,29]. Livestock, including dairy cows, can serve as reservoirs for MRSA, leading to potential transmission through milk and other animal-derived products, and the transmission pathway poses an undeviating risk to consumers to be exposed to MRSA, with milk serving as a potential vehicle for infection. In this context, Methicillin-resistant *S. aureus* (MRSA) has emerged as a significant global public health concern, affecting not only humans but also animals and the environment over the past few decades [27,28]. One of the contributing factors to the intensifying concern is the indiscriminate use of antibiotics in dairying may contribute to the development and spread of antibiotic-resistant MRSA.

Our study revealed a prevalence as 40.7% for *S. aureus* in raw milk, (Fig. 1) which is notably lower than the reported prevalence of 60% in India [27] and 75% in a previous study conducted in Bangladesh [28]. In cow nasal swabs, the prevalence of *S. aureus* was found to be 46.8% (Fig. 1) which is higher than that reported in a recent study by Silva et al. [29]. For human nasal swabs, *S. aureus* was detected in 32.1% (Fig. 1) of the samples, which aligns with the findings of a prior study by Garipcin and Seker (2015) [30]. Additionally, the prevalence of *S. aureus* in the environment was identified as 42.1%, which is lower than what was reported in an Indian study by Venugopal et al. (2019) [31].

In our study, the prevalence of Methicillin-resistant *S. aureus* (MRSA) in raw milk was found to be 11.8%, (Fig. 1) slightly higher than the reported prevalence of 9.6% in raw milk from West Bengal, India [32]. In comparison to a study by Sachdev et al. [33] in India, our study found higher occurrence of MRSA in cow nasal swabs and the environment. Furthermore, the occurrence of MRSA in human nasal swabs was 15.1% (Fig. 1) in our study, which differed and was higher than the 7.5% reported in an Indian study by Singh et al. (2017) [34]. It is worth noting that our study is the first to identify the presence and prevalence of *S. aureus* and MRSA in dairy environmental samples in Bangladesh.

In our study, we have observed the presence of enterotoxin genes in samples obtained from four different sources (Fig. 2). Most of the MRSA isolates from each type of samples in our study were found to harbor at least one enterotoxin gene. The enterotoxin-producing genes, such as *sea, seb, sec, sed, and see* either alone or in combination with other SEs, are commonly reported in foods and are the primary cause of Staphylococcal food poisoning [35]. The presence of enterotoxin genes in raw milk closely aligns with the findings of a study by Khemiri et al. (2019) [36], where isolates from raw milk were also reported to be positive for one or more SEs.

The phylogenetic analysis of the *mec*A gene from milk sample in this study demonstrated a close relationship between the MRSA isolates from milk samples those originating from Kenya and Brazil (Fig. 4). Additionally, we observed a significant relationship between MRSA isolates from humans and the environment in our study with other MRSA isolates from various origins in those mentioned countries (Fig. 4). Moreover, the similarity matrix of the *mec*A gene sequences of MRSA from milk, humans, and the environment indicated a high degree of similarity among these samples. The similarity index of *mec*A gene sequences was found to be at least 84% among milk, human, and environmental samples, suggesting the possible transmission of the same MRSA strain from cows to humans, along with environmental contamination (Fig. 5). Numerous investigations have determined farmers with an elevated risk of LA-MRSA, compared to the general population [Van Cleef et al. [41]]. The prevalence of MRSA in India ranged from 31% to 39% between 2015 and 2019, spiking to 69% in 2020, compared to the national average of 37% [Patil et al. [42]]. Other Indian provinces like Jammu and Kashmir, sharing a border with Pakistan, had the highest MRSA prevalence at 55%, possibly influenced by potential illegal movement [Mohsin et al. [44]]. In contrast, Maharashtra, with more advanced hospitals, reported the lowest MRSA prevalence at 21% [Kuralayanapalya et al. [43]].

The occurrence of MRSA infections and colonization has steadily increased after the introduction of β-lactam antimicrobials, as noted in previous studies [38,39]. In our study, we observed varying degrees of resistance to several antibiotics commonly used for treating staphylococcal infections. Notably, resistance was found to be 100% for Oxacillin and Amoxicillin (Fig. 3), aligning with similar findings reported in other studies conducted in Bangladesh [18,19]. However, interestingly, we found that most of the MRSA isolates in our study demonstrated higher sensitivity to Vancomycin and tetracycline (Fig. 3). This indicates that Vancomycin and Tetracycline may be preferred antibiotics for treating *S. aureus* infections, including those caused by MRSA. The susceptibility of MRSA to Vancomycin has been previously reported in other studies as well [40]. The consequences of this scenario are twofold. Firstly, it endangers the effectiveness of antibiotics in treating *S. aureus* associated infections in both animals and humans, as the development of antimicrobial resistance (AMR) compromises the efficacy of available antibiotics. Secondly, the likely transmission of MRSA, from livestock to humans raises severe public health risks [van Cleef et al. [41]].

In our investigation, we reported the presence of MRSA in various sample types within the same dairy farm, including milk, nasal swabs from both cows and humans, and the environment. This suggests the possibility of zoonotic transmission of MRSA from cows to humans and the contamination of environmental interfaces. The widespread MRSA occurrence within the dairy farm contradicts the principles of the One Health approach, which emphasizes addressing animal-human-environmental interactions [37]. These observations underscore the importance of considering the interconnectedness of animal, human, and environmental health in addressing MRSA and other zoonotic diseases [41]. Livestock-associated MRSA (LA-MRSA) is particularly concerning from a public health perspective due to its zoonotic potential, capable of transmitting from animals to humans [11]. The environment also plays a crucial role in the interconnectedness of human and animal health, influenced by factors such as agricultural intensification, climate change, wildlife habitats, and environmental contamination. These factors have been identified as drivers for the transmission of diseases between humans and animals [41]. Addressing this overwhelming issue requires a multidisciplinary approach encompassing veterinary medicine, human health, and environmental interfaces. Strategies should include prudent use of antibiotics in livestock, surveillance programs to monitor antibiotic resistance patterns, and public awareness campaigns emphasizing safe handling and consumption of animal products [Van Cleef et al. [41]]. Besides, regulatory measures and policies promoting logical antibiotic use in dairying are essential to alleviate the mounting hazard posed by MRSA originating from livestock. Overall, a concerted

effort is needed to ensure the sustainability of agriculture, environment, and safeguard public health in the face of this emerging challenge. MRSA poses a persistent threat to human health due to its adaptability and genetic versatility. To better understand and address this challenge, future research efforts on comprehensive exploration of the complex interaction between host and pathogen, employing various techniques such as genomics, epigenetics, transcription, proteomics, and metabolomics, is decisive.

To our knowledge, this study stands as the very first comprehensive examination in Bangladesh, exploring into the detection, prevalence, molecular characterization, phylogenetic relationships, and similarity matrix of Methicillin-Resistant *S. aureus* (MRSA) across diverse sample types obtained from dairy farms. The presence of MRSA in raw milk, cow nasal swabs, human nasal swabs, and the environment, as observed in this study, raises serious concerns regarding the potential for zoonotic transmission and environmental contamination. Further complete genome sequencing of isolated MRSA is imperative to gain a more precise understanding of zoonotic interactions through environmental interfaces. Such genomic data would provide crucial insights into the dynamics of MRSA transmission and help establish effective measures to address zoonotic risks associated with dairy farming practices.

4.1. Limitations of study

This study was performed within the vicinity of dairy farms only. Other animal farm such as goat farm, buffalo farm and poultry farms weren't included to this study. So, it's too early to declare whether the same zoonotic scenario is prevailing in all types of animal farms or not. Moreover, whole genome sequencing of MRSA isolates from human, animal and environment within same farm was not performed to confirm the exact genetic linkage within human-environment-animal interfaces.

5. Conclusion

In conclusion, this study has brought attention to the concerning prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) in dairy farms in Bangladesh, posing significant risks for zoonotic transmission and antibiotic resistance. The detection of MRSA in various sample types from the same dairy farm highlights the importance of a comprehensive One Health approach, recognizing the interconnectedness of animal, human, and environmental health. The findings emphasize the urgent need for responsible antimicrobial usage to curb the development of antibiotic resistance in MRSA strains. Additionally, the study's call for genomic sequencing of MRSA isolates from different samples holds promise in identifying precise zoonotic transmission pathways through environmental interfaces, enabling targeted interventions to prevent further spread. Addressing the complex challenges posed by MRSA in dairy farms requires collaborative efforts from various stakeholders, including veterinarians, healthcare professionals, policymakers, and researchers. By embracing a One Health perspective, we can develop sustainable agricultural practices and effective disease control strategies to protect public health and ensure the well-being of both humans and animals.

Funding statement

The work was conducted through the partial funding facilitated by Ministry of Science & Technology, Bangladesh under the special research Grants-2022.

Authors' contribution statement

MCR and FMAH designed the work strategies.
MCR, TC, MTH, and MMH collected Samples.
MCR, TC, MR and KMAZ analyzed the data.
MCR, MMR, and EZ prepared the draft with the help of FMAH.

Ethics statement

The study was approved by the Institutional Ethics Committee of Sylhet Agricultural University (Approval No. AUP2023001). All applicable national, international, and institutional guidelines for the animal's care were followed during the sample collection.

Declaration of competing interest

All authors declaring that there is no conflict of interest.

Data availability

The data supporting the findings of this study are available upon request to the corresponding author. Requests for access to the data should be addressed to corresponding author. Please include a brief description of the purpose for which the data is requested, and approval will be granted in accordance with any applicable data usage restrictions.

Acknowledgements

We extend our gratitude to the National Science & Technology Ministry of Bangladesh for a partial funding for this research (Grant ID No. SRG-224575). We also thank the Sylhet Agricultural University Research System (SAURES) for administrative support and the Department of Dairy Science for providing laboratory facilities. Our appreciation goes to all participants and farm owners for their cooperation. Their contributions were vital to the success of this study.

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