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ORIGINAL ARTICLE

Antibiotic resistance and genotyping of *mecA*-positive methicillin-resistant *Staphylococcus aureus* (MRSA) from milk and nasal carriage of dairy water buffaloes (*Bubalus bubalis*) in the Philippines

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ABSTRACT

Objective: Mastitis is considered as an economically important disease of dairy buffaloes in Asia. This study examined the mastitis milk and nasal swab samples for the detection and genotyping of methicillin-resistant *Staphylococcus aureus* (MRSA) in water buffaloes.

Materials and Methods: *Staphylococcus aureus* was identified based on biochemical tests and Polymerase Chain Reaction (PCR) detection of *nuc* gene, whereas MRSA on *mecA* gene. The disc diffusion test was used to determine the antibiotic resistance and staphylococcal cassette chromosome mec (*SCCmec*), *spa,* and multilocus sequence typing for the genotyping of isolates.

Results: *Staphylococcus aureus* was detected on 39/93 milk (41.94%) and 27/384 nasal swab (7.03%) samples. However, only nine isolates (23.08%) harbored the *mecA* gene from milk samples and three isolates (11.11%) from the nasal carriage. All MRSA isolates exhibited resistance to cefoxitin and penicillin, whereas 50% were found resistant to clindamycin. All these isolates were found susceptible to sulfa-trimethoprim and chloramphenicol, whereas the majority of the isolates were susceptible to gentamicin, ciprofloxacin, tetracycline, and rifampicin. The *SCCmec* types of the MRSA isolates were type IVc (50.00%), type II (8.33%), type I (8.33%), and non-typeable (33.33%). The *spa* types and sequence type (ST) identified were t019 (ST30), t701 (ST1649), t311 (ST5), t657 (ST1148), t015 (ST508), t1939 (ST12), t800 (ST9), t091 (ST2454), t138 (ST5991), and t1642 (ST5992).

Conclusion: Milk and nasal swab samples from dairy water buffaloes were found positive for MRSA. The MRSA isolates were still susceptible to most antibiotics tested. Moreover, the genotypes of some MRSA isolates were found similar to some human MRSA strains, suggesting a possible human to animal transmission.

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MRSA; *mecA*; water buffaloes; *SCCmec*; *spa* type; ST type

Introduction

The Gram-positive bacterium *Staphylococcus aureus* considered the anterior nares as its colonization site [1]; however, in dairy animals, the mammary gland served as its infection site, causing mastitis [2]. Besides, the anterior nares could be a possible source of contamination for udder and milk in dairy farms [3].

The emergence of methicillin-resistant *S. aureus* (MRSA) in dairy animals is implicated in the use of antibiotics, particularly β-lactams for the treatment of mastitis, which is attributed to the presence of *mecA* gene in the staphylococcal chromosome cassette (SCC) [4,5] of these bacteria. The previous studies revealed the presence of drug-resistant MRSA isolates from milk and nasal carriage [6–10]. Drug resistance could pose a zoonotic threat to humans as this could be transferred to those who have direct contact with livestock or through the consumption of animal products such as milk [11].

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Molecular typing of MRSA isolates includes staphylococcal cassette chromosome mec (*SCCmec*), *spa,* and multilocus sequence typing (MLST). *SCCmec* typing classifies the *SCC* components into types and subtypes [12]. MLST, on the other hand, uses the sequences of the seven housekeeping genes of *S. aureus* to produce an allelic profile for sequence type (ST) identification [13]. *Spa* typing involved the interpretation of succession repeats of the sequenced polymorphic region of *spa* protein A [14]. Molecular typing is essential for the epidemiological tracking of outbreaks or the establishment of a source of infection or contamination. Several reports showed similar *SCCmec*, *spa,* and ST types isolated from bovine milk and human, implicating the possible transmission of MRSA from cattle to humans or vice versa [15–19].

In the Philippines, only MRSA prevalence from hospital isolates is available [20], and there are no reports on the antimicrobial resistance status of MRSA on livestock. The increase in the population of dairy water buffaloes in the country was instrumental in boosting the local milk production. This is evident in the rise in the number of smallholder farmers involved in raising water buffaloes to meet the demand for local milk and milk products. With these, there is a need to determine the possible zoonotic threat of MRSA to consumers as well as animal handlers. Moreover, baseline data on the prevalence and genetic profiles of MRSA on livestock are lacking, and the results of this study can be used as a basis for antimicrobial resistance situation and future epidemiological studies. The objective of this study was to examine the mastitic milk and nasal swab samples for the detection and genotyping of MRSA in water buffaloes.

Materials and Methods

Ethics statement

A written or verbal permission was given to the farm owner/animal handlers/manager before the collection of milk and nasal swab samples. The collection of these samples was done by professional veterinarians. No animals were used for any kind of animal experiments in this study.

Sample collection and identification of S. aureus and MRSA isolates

A total of 93 mastitis-positive milk and nasal swab samples were collected from 384 lactating water buffaloes at the National Impact Zone (NIZ) of the Philippine Carabao Center in the Science City of Muñoz, Nueva Ecija, Philippines. Milk samples were collected aseptically using a 15-ml sterile plastic tube. For the nasal swab samples, a sterile cotton swab was inserted in the anterior nares of both the left and right nostrils of the animal. It was rotated against the anterior nasal mucosa. The swab was placed in a sterile plastic test tube containing 1 ml of sterile physiological saline solution. Milk and nasal swab samples were labeled and transported on ice for further laboratory analysis.

Milk and nasal swab samples were inoculated on Baird–Parker medium with egg yolk tellurite supplement (HiMedia, Mumbai, India) for 48–72 h at 37°C. *S. aureus* was identified using catalase and coagulase tests. Furthermore, DNA samples were extracted using the boiling method [21]. Presumptive isolates were then further confirmed as *S. aureus* and MRSA using Polymerase Chain Reaction (PCR) for the detection of *nuc* and *mecA* genes [22] (Fig. 1). PCR conditions performed were initial denaturation at 94°C for 5 min; 30 cycles of 94°C for 30 sec, 60°C for 45 sec, and 72°C for 60 sec; and a final extension of 72°C for 10 min. *S. aureus* American Type Culture Collection (ATCC) 43300 was used as a positive control for the PCR procedure. PCR products were subjected to electrophoresis using 1.0% agarose gel in 0.5× Tris-Borate-EDTA (TBE) buffer (Bio-Rad, Hercules, CA) at 100 V for 25 min.

Antibiotic susceptibility testing

All *mecA*-positive MRSA isolates were further subjected to a disc diffusion test against 10 antibiotics (HiMedia, India) to determine their antimicrobial resistance (Table 1). ATCC 25923 was used as the quality control, and the resistance was determined based on the Clinical and Laboratory Standards Institute standard [23].

Genotyping of MRSA isolates

The bacterial DNA of isolates used for the genotyping protocols was determined using a nanodrop spectrophotometer

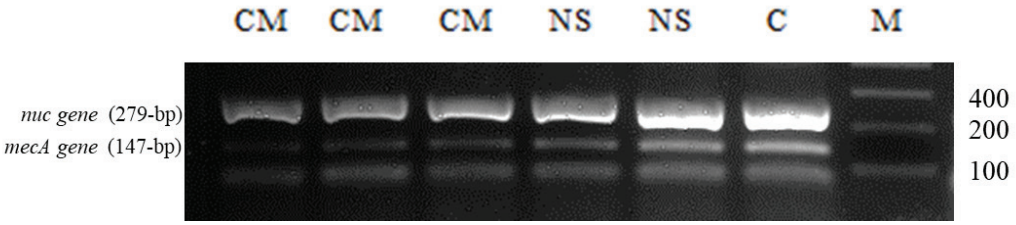

Figure 1. PCR amplification of nuc and *mecA* genes. Lanes1–3 are composite samples, lanes 4–5 are nasal swab samples, lane 6 is the control (ATCC 43300), and M is the marker (100 bp)..

Table 1. Antibiotics used for the antibiotic susceptibility test.

Antibiotic discs	Concentration
Penicillin G	10 IU
Cefoxitin	30 µg
Clindamycin	2 µg
Rifampicin	5 µg
Sulfamethoxazole/trimethoprim	23.75/1.25 µg
Tetracycline	30 µg
Erythromycin	15 µg
Chloramphenicol	30 µg
Ciprofloxacin	5 µg
Gentamycin	10 µg

(Thermo, Waltham, MA). MRSA isolates were subjected to multiplex *SCCmec* typing using specific primers [21] (Table 2). A 25 µl of volume reaction was used containing 2.5 µl of 10× PCR buffer (Promega, Madison, WI), 2.5 µl of 2mM concentration of dNTP (Promega, Madison, WI), 2.75 µl of 50 mM $MgCl_2$ (Promega, Madison, WI), 0.25 µl of Taq polymerase (Promega, Madison, WI), 0.50 µl each of the primers, and 100 ng of DNA template. The PCR procedure was performed in a thermal cycler (Applied Biosystems, Foster City, CA) following these conditions: 94°C for 5 min; 30 cycles of 94°C for 45 sec, 57°C for 45 sec, and 72°C for 1.5 min; and a final extension at 72°C for 10 min. A 2.0% agarose gel was prepared using 0.5× TBE buffer (Bio-Rad, Hercules, CA), and gel electrophoresis was done at 100 V for 35 min.

Spa typing protocol was followed using the specified primers (Table 2) for the PCR procedure [24]. The PCR products after amplification (Fig. 2) were sent for sequencing (1st Base Asia, Malaysia). *Spa* DNA sequences obtained were aligned using BioEdit 7.0, and the repeat sequences were analyzed using the DNAGear (201203012225) software to determine spa type [25].

Furthermore, MRSA isolates were subjected to MLST typing [13]. The seven housekeeping genes (*arC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi,* and *yqiL*) were amplified using specific primers (Table 2), and the PCR products were sent for sequencing (1st Base Asia, Malaysia). The sequences of the seven housekeeping genes were aligned using BioEdit 7.0 and then submitted to the pubmlst.org/saureus website to determine the allelic profile of the housekeeping genes. ST types were identified based on the allelic combination of each of the isolate.

DNA *spa* sequences of MRSA isolates were sent to the GenBank database for the assignment of accession numbers used for the phylogenetic analysis. The phylogenetic tree was constructed using Molecular Evolution Genetic Analysis version 7 software based on the neighbor-joining method using a bootstrap method of 1,000 replicates.

Results

Identification of S. aureus and MRSA

Based on biochemical tests and detection of the *nuc* gene, the prevalence of *S. aureus* on composite milk samples was 41.94% (39/93), whereas 7.03% (27/384) for the nasal swab samples. However, for the identification of MRSA based on PCR detection of the *mecA* gene, only 9/39 (23.08%) of the milk and 3/27 (11.11%) of the nasal swab isolates were found positive.

Antibiotic susceptibility

The antibiotic susceptibility test results of the 12 *mecA*-positive MRSA isolates showed a 100% resistance to cefoxitin or methicillin and penicillin. About 50% of the isolates were resistant to clindamycin, whereas 41.67% were found susceptible and 8.33% with intermediate susceptibility. For tetracycline, 8.33% of isolates were resistant, and with intermediate sensitivity, however, 83.33% of isolates were still susceptible. About 75% of MRSA isolates were susceptible to rifampicin, and the remaining 25% had intermediate susceptibility. For both ciprofloxacin and gentamicin, isolates exhibited 91.67% susceptibility and 8.33% with intermediate susceptibility. One-third or around 66.67% of isolates showed an intermediate susceptibility to erythromycin, with only 33.33% of these susceptible. All isolates were still found sensitive to sulfa-trimethoprim and chloramphenicol. Overall, the resistance was detected against cefoxitin (methicillin), penicillin, clindamycin, and tetracycline (Table 3).

Genotypes of MRSA isolates

Genotyping of the 12 MRSA isolates was based on *SCCmec, spa,* and MLST typing (Table 3). The majority of the MRSA isolates that were subjected to *SCCmec* typing belong to *SCCmec* type IVc (6/12 or 50.00%), type II (1/12 or 8.33%), type I (1/12 or 8.33%), and non-typeable (4/12 or 33.33%).

There were different *spa* and ST types identified from milk and nasal swab samples (Tables 3 and 4). The *spa* and ST types of composite milk samples were t019 (ST30), t657 (ST1148), t701 (ST1649), t311 (ST5), t1939 (ST12), and t015 (ST508), and the identification of two novel ST types were t138 (ST5991) and t1642 (ST5992). The three nasal carriage *spa* types were t800 (ST9) comprising two isolates and t091 (ST2454).

Phylogenetic tree of spa sequences

A phylogenetic tree showing the relationship of *spa* sequences of milk and nasal isolates is shown in Figure 3. The accession numbers assigned by GenBank for the 12 isolates were MT408293–MT408304. Results revealed that the *spa* sequences from buffalo nasal isolates

Table 2. Primers used in the study.

Primer	Oligonucleotide sequence (5'-3')	Amplicon size (bp)	Specificity	Reference
Multiplex PCR				
nuc-1	GCG ATT GAT GGT GAT ACG GTT	279	*nuc* gene	[22]
nuc-2	AGC CAA GCC TTG ACG AAC TAA AGC			
*Mec*A147-F	GTG AAG ATA TAC CAA GTG ATT	147	*mecA* gene	[21]
*Mec*A147-R	ATG CGC TAT AGA TTG AAA GGA T			
SCCmec typing				
Type I-F	GCT TTA AAG AGT GTC GTT ACA GG	613	*SCCmec* I	[21]
Type I-R	GTT CTC TCA TAG TAT GAC GTC C			
Type II-F	CGT TGA AGA TGA TGA AGC G	398	*SCCmec* II	[21]
Type II-R	CGA AAT CAA TGG TTA ATG GAC C			
Type III-F	CCA TAT TGT GTA CGA TGC G	280	*SCCmec* III	[21]
Type III-R	CCT TAG TTG TCG TAA CAG ATC G			
Type IVa-F	GCC TTA TTC GAA GAA ACC G	776	*SCCmec* IVa	[21]
Type IVa-R	CTA CTC TTC TGA AAA GCG TCG			
Type IVb-F	TCT GGA ATT ACT TCA GCT GC	493	*SCCmec* IVb	[21]
Type IVb-R	AAA CAA TAT TGC TCT CCC TC			
Type IVc-F2	CCT GAA TCT AAA GAG ATA CAC CG	200	*SCCmec* IVc	[21]
Type IVc-R2	GGT TAT TTT CAT AGT GAA TCG C			
Type IVd-F5	CTC AAA ATA CGG ACC CCA ATA CA	881	*SCCmec* IVd	[21]
Type IVd-R6	TGC TCC AGT AAT TGC TAA AG			
Type V-F	GAA CAT TGT TAC TTA AAT GAG CG	325	*SCCmec* V	[21]
Type V-R	TGA AAG TTG TAC CCT TGA CAC C			
Spa typing				
1095F	AGA CGA TCC TTC GGT GAG C	Varied	*spa* gene	[24]
1517R	GCT TTT GCA ATG TCA TTT ACT G			
MLST typing				
arcC-Up	TTG ATT CAC CAG CGC GTA TTG TC	456	Carbamate kinase	[13]
arcC-Dn	AGG TAT CTG CTT CAA TCA GCG			
aroE-Up	ATC GGA AAT CCT ATT TCA CAT TC	456	Shikimate dehydrogenase	[13]
aroE-Dn	GGT GTT GTA TTA ATA ACG ATA TC			
glpF-Up	CTA GGA ACT GCA ATC TTA ATC C	465	Glycerol kinase	[13]
glpF-Dn	TGG TAA AAT CGC ATG TCC AAT TC			
gmk-Up	ATC GTT TTA TCG GGA CCA TC	429	Guanylate kinase	[13]
gmk-Dn	TCA TTA ACT ACA ACG TAA TCG TA			
pta-Up	GTT AAA ATC GTA TTA CCT GAA GG	474	Phosphate acetyltransferase	[13]
pta-Dn	GAC CCT TTT GTT GAA AAG CTT AA			
tpi-Up	TCG TTCA TTC TGA ACG TCG TGA A	402	Triosephosphate isomerase	[13]
tpi-Dn	TTT GCA CCT TCT AAC AAT TGT AC			
yqiL-Up	CAG CAT ACA GGA CAC CTA TTG GC	516	Acetyl coenzyme A acetyltransferase	[13]
yqiL-Dn	CGT TGA GGA ATC GAT ACT GGA AC			

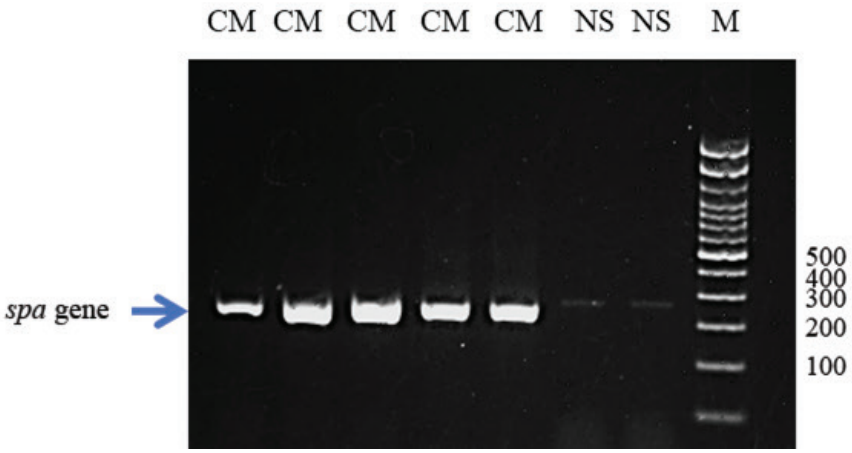

Figure 2. PCR results for the detection of *spa* gene in milk and nasal swab samples. Lanes 1–5 are composite milk samples, lanes 5–6 are nasal swab samples, and M is the marker (100 bp).

Table 3. Antimicrobial resistance and genotypes of MRSA from milk and nasal swab samples.

Sample ID	Source	Antibiotic resistance	*SCCmec* type	*spa* repeats	*Spa* type	ST type
CM1	Milk	PG-CX	Type IVc	08-16-02-16-02-25-17-24	t019	ST30
CM17	Milk	PG-CX-CD-TC	Type IVc	15-16-02-25-17-24-24	t1642	ST5992
CM21	Milk	PG-CX	Non-typeable	26-23-13-21-17-34-33-34	t657	ST1148
CM23	Milk	PG-CX	Type II	08-16-02-16-34-13-17-34-16-34	t015	ST508
CM25	Milk	PG-CX	Type IVc	26-23-17-34-20-17-12-17-16	t311	ST5
CM26	Milk	PG-CX-CD	Type IVc	08-16-02-25-17-24	t138	ST5991
CM32	Milk	PG-CX	Type IVc	11-10-21-17-34-24-34-22-25-25	t701	ST1649
CM34	Milk	PG-CX-CD	Non-typeable	07-23-02-34	t1939	ST12
CM35	Milk	PG-CX	Type IVc	11-10-21-17-34-24-34-22-25-25	t701	ST1649
NS24	Nasal Swab	PG-CX-CD	Non-typeable	07-23-21-17-34-12-23-02-12-23	t091	ST2454
NS30	Nasal Swab	PG-CX-CD	Non-typeable	07-16-12-23-02-12-23-02-34	t800	ST9
NS31	Nasal Swab	PG-CX-CD	Type I	07-16-12-23-02-12-23-02-34	t800	ST9

PG = Penicillin; CX = Cefoxitin; CD = Clindamycin; TC = Tetracycline.

Table 4. MLST allelic profile and ST of MRSA isolates.

Sample ID	Type of Sample	*arcC*	*aroE*	*glpF*	*gmk*	*pta*	*tpi*	*yqiL*	ST
CM1	Composite Milk	2	2	2	2	6	3	2	ST30
CM17	Composite Milk	18	18	6	59	13	3	2	ST5992
CM21	Composite Milk	1	61	1	8	12	4	10	ST1148
CM23	Composite Milk	10	40	8	6	10	3	2	ST508
CM25	Composite Milk	1	4	1	4	12	1	10	ST5
CM26	Composite Milk	2	2	2	2	6	287	2	ST5991
CM32	Composite Milk	12	4	1	4	12	1	67	ST1649
CM34	Composite Milk	1	3	1	8	11	5	11	ST12
CM35	Composite Milk	12	4	1	4	12	1	67	ST1649
NS24	Nasal Swab	3	3	1	1	264	1	10	ST2454
NS30	Nasal Swab	3	3	1	1	1	1	10	ST9
NS31	Nasal Swab	3	3	1	1	1	1	10	ST9

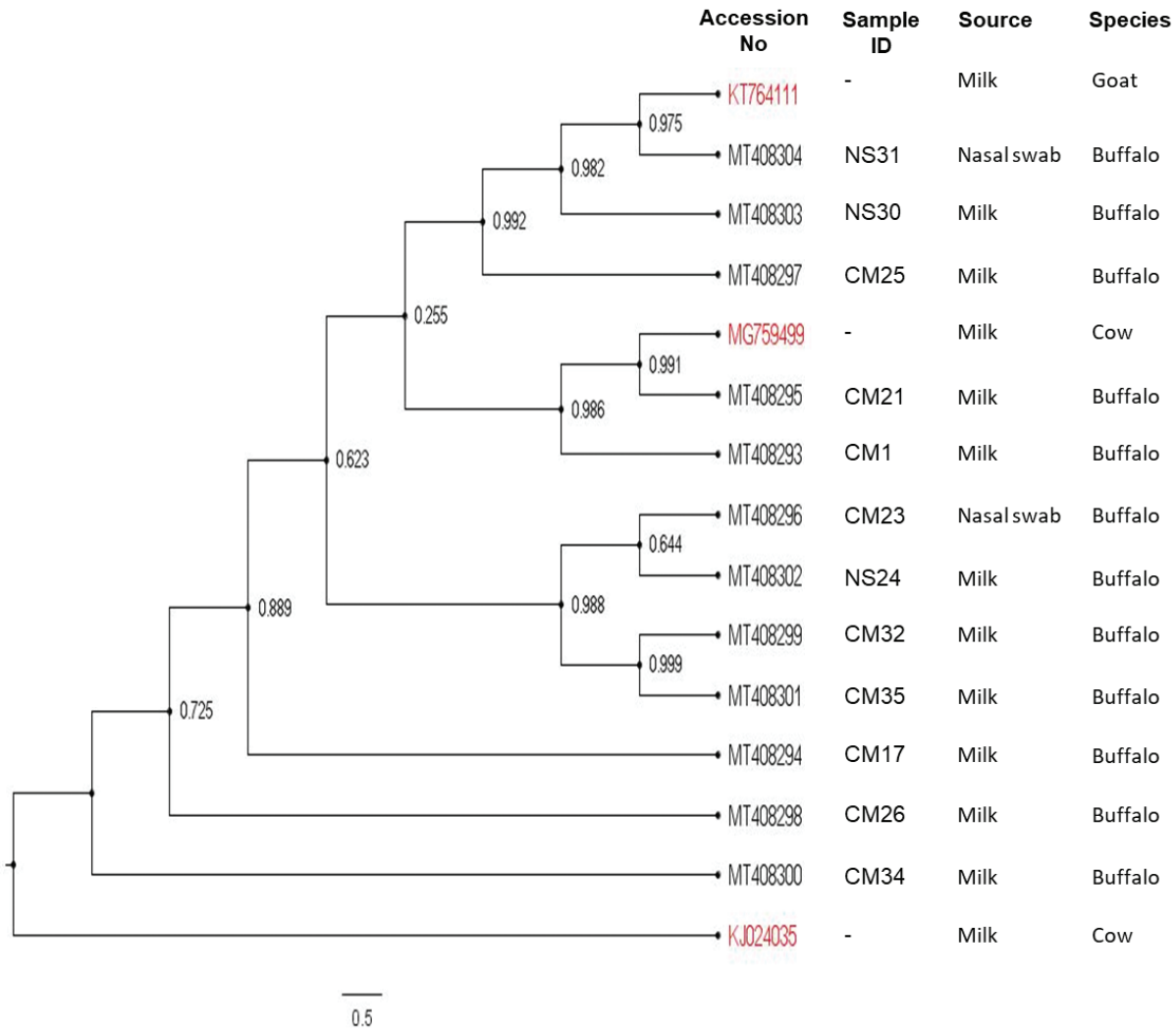

Figure 3. Phylogenetic tree of *spa* sequences of milk and nasal samples using neighbor-joining method; red-colored accession numbers were considered outgroup in the analysis.

MT408303 and MT408304 were found related to the goat milk isolate KT764111 and buffalo milk isolate MT408297. Similarly, buffalo milk isolates MT408299 and MT408301 were observed to be related to nasal isolate MT408302. Moreover, the buffalo milk *spa* sequences MT408295 and MT408293 were closely associated with the *spa* sequence of the cow isolate MG759499. The related *spa* sequences of buffalo milk (MT408299, MT408301, MT408295, and MT408293) were also observed in the study.

Discussion

Staphylococcus aureus is commonly isolated from mastitis cases in dairy animals. In Asia, water buffaloes play a major role in local milk production. The prevalence of *S. aureus* from the milk samples in the present study was found higher than the previous reports in Iran and India [26,27]. Probable reasons for a higher prevalence rate in this study is the lack of proper hygienic measures followed before and after milking of animals as well as the manner of milking used by smallholder or backyard dairy farms. The prevalence rate could vary based on geographical location, breed, management instituted, and hygienic measures followed in the farm [28].

In this study, the detection rate of the *mecA* gene in mastitis-positive milk samples was 23.08%. In Pakistan, detection of the *mecA* gene on water buffalo mastitis milk was 38%, higher than what was observed in this report [29]. A lower *mecA* gene prevalence on mastitis milk was reported from the previous studies in Asia [6,17,27,30,31].

For the nasal carriage in dairy water buffaloes, the finding was in agreement with the low nasal carriage rate on cows from Iran, which was 5.06% [2], and in Tunisia, which was 1.3% [32]. However, a higher rate of *S. aureus* nasal carriage on cattle was reported from Saudi Arabia which was 50% [33], 15% in Algeria [34], 38% in Nigeria [35], 13.9% in Norway [36], and 54.3% in Greece [37]. Although the results of the present study revealed a low nasal carriage rate, the presence of carrier animals among lactating water buffaloes that could shed bacteria in the dairy farm environment should be considered as this posed a threat to dairy farmworkers.

The presence of the *mecA* gene in the *SCC* of *S. aureus* is one resistance mechanism involved against methicillin and other β-lactam antibiotics. The PCR detection of this resistance gene in *S. aureus* is considered a gold standard for the confirmation of MRSA [38].

All *mecA*-positive isolates were found to be 100% resistant to both penicillin and cefoxitin (methicillin). The same findings were reported for bovine mastitis milk [26,39,40] and nasal carriage in cattle [9,33,35]. However, there was MRSA isolated from bovine mastitis infected milk, which was observed to be resistant to penicillin [8,41] or cefoxitin (methicillin) only [29]. A contrasting observation was seen in the previous studies, where 33.33% of MRSA isolates were resistant [30], and 100% were susceptible to cefoxitin [42]. Similarly, MRSA strains from milk and nasal carriage were reported resistant to clindamycin and tetracycline [9,35,42].

The resistance of *mecA*-positive isolates to penicillin and cefoxitin (a *β*-lactam) confirms the mechanism involved in the presence of this resistant gene in *S. aureus*. Cefoxitin is a better inducer of methicillin resistance compared to oxacillin [43] and used to determine the phenotypic MRSA. The probable reason for the presence of antibiotic resistance is the frequent use of β-lactams to treat mastitis infections in dairy water buffaloes in the area.

The usual antibiotic resistance profile of livestock-associated MRSA is their resistance to β-lactams, macrolides, lincosamides, tetracyclines, partly fluoroquinolones, and cotrimoxazole but susceptible to rifampicin [4] which was observed in this study. The high susceptibility of most isolates to the antibiotics used in the study is a good indication of the many options that can be used for the treatment of mastitis. It is also surprising to note the resistance observed against clindamycin and intermediate susceptibility to erythromycin. These two antibiotics are used in humans and not in dairy animals, suggesting the possible transmission of resistant genes from human to dairy water buffaloes in the area. The presence of drug-resistant MRSA in dairy water buffalo production poses public health concern for a possible spread through milk, in the dairy farm environment, and animal handlers from nasal carriers.

In this study, the majority of MRSA isolates from mastitis infected milk samples belonged to *SCCmec* type IVc. This result is in agreement with several studies on bovine mastitis, where the majority of MRSA isolates were typed as *SCCmec* type IV [7–8,39,41]. In Uganda, *SCCmec* type IVc was isolated in one bulk can milk samples collected from households [44]. Moreover, *SCCmec* type II isolate was identified in raw bovine milk in Iran [45]. *SCCmec* type IV was also commonly isolated as Community Acquired-Methicillin Resistant *Staphylococcus aureus* (CA-MRSA), whereas *SCCmec* type II as Hospital Acquired-Methicillin Resistant *Staphylococcus aureus* (HA-MRSA) in the Philippines and Korea [46]. Moreover, *SCCmec* type I has been reported from hospital clinical isolates in the Philippines [47] and commonly carried by HA-MRSA strains [48].

The spa type t019 (ST30)-IV from a milk isolate in this study was also a CA-MRSA strain identified in the Philippines [46,49]. ST5-IV-t311 and ST1649-IV-t701 were also reported in Argentina but as CA-MRSA clones [50]. The t015-ST508 was isolated from bovine milk with intramammary infections in Europe [51]. Similarly, this was isolated from bovine milk and human nasal swab samples in Africa [52]. Spa type t1939 was isolated from milk of dairy cows belonging to different ST types in Austria and China [53,54].

Nasal carriage isolates with *spa* type t800 (ST9) were also reported from clinical isolates in Taiwan [55]. Besides, spa type t091 was detected from persons handling raw meat and meat products in Germany [56]. Moreover, ST9 and ST2454 were reported from pigs in China [57], suggesting that these strains were not only limited to humans but also found on livestock.

This is the first documented genetic profiling of MRSA from dairy water buffaloes in the Philippines. The *spa* and ST types of MRSA reported in this study were different from the ST398 commonly identified in Europe [41,58–60] and ST9 and ST8 in Asia [7–8,39,61]. This study reported some spa types not isolated from bovine mastitis and the identification of two novel ST types (ST5991 and ST5992), implying that these results could be considered new spa and ST types associated with mastitis cases.

Similarly, the identification of CA-MRSA and HA-MRSA strains in dairy water buffaloes could suggest the presence of human strains in the dairy animal population. There were studies conducted, implicating the presence of genetically related MRSA strains from both animal handlers or milkers and dairy animals [62,63].

The bootstrap values infer the close relationship of the *spa* sequences of nasal and milk isolates (Fig. 3). This suggests that nasal carriage could be a possible transmission pathway for milk contamination [3]. The detection of related *spa* milk isolates implies that there were common *spa* types contaminating milk of dairy buffalos in different dairy farms within the province of Nueva Ecija. The

presence of closely related *spa* milk isolates from buffalo, cow, and goat indicates the presence of common *spa* types causing mastitis in ruminants.

Aside from nasal carriage, other possible modes of the transmission of MRSA to dairy buffaloes should be considered. Humans with MRSA could serve as potential risk factors for transmission to dairy animals [18,62–63]. However, this study was only limited to the detection of MRSA in dairy water buffaloes and not in animal handlers; thus, the transmission of human strains cannot be established.

Conclusion

MRSA was detected on mastitis milk and nasal carriage in dairy water buffaloes. MRSA isolates were found resistant against penicillin, cefoxitin (methicillin), and clindamycin. The susceptibility of isolates to most antibiotics used in the study offered more options for the treatment of mastitis in dairy water buffaloes. Most MRSA isolates belonged to *SCCmec* type IVc with different *spa* and ST types different from dairy animals in Asia and Europe. These results also identified two novel ST types (ST5991 and ST5992) associated with mastitis. Furthermore, the nasal carriage was considered as a possible transmission pathway for milk contamination in this study. Moreover, the detection of MRSA isolates similar to human MRSA strains suggests the need to investigate its transmission to dairy water buffaloes further.

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Conflict of interests

No competing financial interests exist.

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