Methicillin-resistant *Staphylococcus aureus* (MRSA) associated with mastitis among water buffaloes in the Philippines

Alona T. Badua, Sukolrat Boonyayatra, Nattakarn Awaiwanont, Paula Blanca V. Gaban, Claro N. Mingala

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**ABSTRACT**

Methicillin-resistant *Staphylococcus aureus* (MRSA) from dairy animals could pose a public health concern in the population. The study was designed to determine the prevalence of *S. aureus* and MRSA associated with mastitis among water buffaloes in the central part of Luzon island, the Philippines, and to investigate its associated factors. Three hundred and eighty-four water buffaloes were examined for mastitis using California mastitis test (CMT). Composite milk samples (*n* = 93) were collected from buffaloes showing positive reaction with CMT. *S. aureus* was identified from milk samples using biochemical tests. Cefoxitin disk diffusion assay and PCR detecting *mecA* gene were performed to identify MRSA isolates. Disk diffusion assay was used to investigate the antimicrobial resistance against 9 antibiotics. The prevalence of *S. aureus* was 41.94% (39/93). MRSA isolates resistant to cefoxitin were at 25.81% (24/93) but only 37.5% (9/24) harbored the *mecA* gene. All 24 MRSA isolates were resistant to penicillin while the majority were susceptible to clindamycin, trimethoprim-sulfamethoxazole, gentamycin, tetracycline, rifampicin, ciprofloxacin and chloramphenicol with intermediate susceptibility to erythromycin. Furthermore, 37.5% of the isolates were found resistant to two or more antibiotics. Animal-level factor associated with MRSA infection was the history of mastitis (OR = 3.18, CI = 1.03–9.79, *p* = 0.040). Herd-level factors associated with the detection of MRSA in milk included herd size (OR = 4.24, CI = 1.05–17.07, *p* = 0.042) and the presence of other animals (OR = 0.15, CI = 0.04–0.58, *p* = 0.006). High prevalence of intramammary infection with *S. aureus* and MRSA in dairy buffaloes was observed in the region. This finding raises the concern of preventing zoonotic spread of MRSA.

1. Introduction

*Staphylococcus aureus* is considered as a leading cause of mastitis in cattle and water buffaloes in Asia (Sharma and Matti, 2010). Treatment of choice for mastitis in dairy farms is the use of antibiotics (Haran et al., 2012).

Emergence of methicillin-resistant *S. aureus* (MRSA) in livestock has been implicated from the use of antimicrobials as growth promoters and for preventive and therapeutic measures (Cuny et al., 2015; Mehdiratta and Dhall, 2012). One mechanism for methicillin resistance of *S. aureus* was the acquisition of *Staphylococcal Cassette Chromosome* (SCC)*mecA* gene that alters the penicillin-binding protein (PBP2) resulting to the loss of affinity to all β-lactam antibiotics (Cuny et al., 2015; Lee, 2003).

In Asia, the prevalence of MRSA in bovine milk ranged from 1.1% in Japan (Hata et al., 2010) to 52.2% in Egypt (Elhaig and Selim, 2015). MRSA prevalence in dairy cows could be attributed to several factors. A study in Brazil showed that lacking of some hygienic measures, such as the use of pre- and post-milking teat dip, gloves and individual towels for milking cows, resulted to higher MRSA prevalence in milk (Guimarães et al., 2017). The studies of Locatelli et al. (2017) and Spohr et al. (2011) revealed a high prevalence of MRSA in dairy herds in close proximity with swine herds. In addition, the type of production system used in a dairy herd could affect MRSA prevalence. This was observed in Germany where a higher MRSA prevalence was reported in bulk tank milk of conventional dairy farms with larger herd size compared to organic dairy farms. Moreover, the higher prevalence of conventional dairy farms...
could be attributed to increased usage of antibiotics (Tenhagen et al., 2018).

There is also a great concern for treatment of mastitis caused by MRSA due to the fact that MRSA was not only resistant to β-lactams but also exhibited multi-drug resistant patterns to other commonly used antibiotics (Akindolire et al., 2015; Sharma et al., 2015; Turkylımaz et al., 2010; Wang et al., 2015). Moreover, there is an increasing concern on the public health risk of MRSA from livestock, since resistance genes can be spread from food animals to humans by direct contact or through the food chain (Marshall and Levy, 2011).

In the Philippines, dairy water buffaloes from smallholder farmers and cooperatives contribute a major part of the local milk supply (Bulatao, 2018). Thus, there is a need to assess if water buffalo milk does not pose a public health hazard from MRSA. We aimed to investigate the prevalence of intramammary infection (IMI) with MRSA together with their antimicrobial resistance among water buffaloes in the central part of Luzon island, which is the most populous area of water buffalo farms in the Luzon island of the Philippines. Moreover, the potential factors for IMI with MRSA were also determined.

2. Materials and methods

2.1. Sampling area, sample collection and data collection

The study was set to investigate the dairy water buffaloes in the province of Nueva Ecija located in the central part of Luzon island, the Philippines (Figure 1). The sampling area was composed of 153 small-holder dairy farms (averaged farm size of 2–10 dairy buffaloes) from seven dairy cooperatives and 3 large dairy farms (composed of >30 dairy buffaloes). The sample size was calculated using the formula of Charan and Biswas (2013) where a 50% prevalence rate was used since there is no baseline data for livestock MRSA prevalence in the country. The sample size was 384 dairy buffaloes. Representative animals (around 50% of the total available lactating dairy buffaloes per farm) were randomly selected during the visit.

Composite milk samples were pooled milk samples coming from four udders of each animal. Milk samples were examined for mastitis using California mastitis test (CMT). Milk samples from animals presenting positive reaction with CMT testing were collected, placed in an ice box, and transferred to the laboratory within 24 h.

A questionnaire was developed to collect animal and herd data from farmers by face-to-face interview. Animal data gathered from the questionnaire included age, parity, stage of lactation, presence of one or more lesions at the udders, history of mastitis, and the use of antibiotic treatment for mastitis. Herd data collected were herd size, type of milking (hand milking VS bucket type), hygienic practice before and after milking, milking mastitic buffaloes last, person treating mastitis, and the presence of other animals in the farm. Based on animal-level and herd-level data, potential risk factors for MRSA mastitis infection were determined.

2.2. Isolation and identification of MRSA

*S. aureus* was isolated from a milk sample by inoculating 1 mL of milk into 9 mL of Tryptone Soya Broth (HiMedia Laboratories, India) and incubating at 35 °C for 24 h. The incubated broth was streaked on Baird-Parker medium (HiMedia Laboratories, India) with Egg Yolk Tellurite Emulsion (HiMedia Laboratories, India) and incubated at 37 °C for 24–48 h. Presumptive *S. aureus* colonies were gram stained, and further confirmed using catalase test and coagulase test. Disk diffusion assay using a 30μg cefoxitin disk (HiMedia Laboratories, India) following the protocol of Hudzicki (2009) was used to phenotypically identify MRSA. A zone of inhibition ≤21mm was considered resistant for cefoxitin (CLSI, 2015).

2.3. DNA extraction

Cefoxitin resistant isolates were subjected to DNA extraction following the procedure of Zhang et al. (2004) with modifications. MRSA isolates were cultured overnight in Tryptic Soy Agar plate (HiMedia Laboratories, India) and about 3–5 bacterial colonies were picked and suspended in 50μL of DNA Rehydration Solution (Promega, USA) and heated at 95 °C for 10 min. After centrifugation at 20,000 x g for 1 min, 5μL of the supernatant was used as DNA template.

2.4. PCR detection of mecA gene

Phenotypically identified MRSA isolates were further confirmed using multiplex PCR for the detection of 16S rRNA, mecA and nuc genes following the procedure of Ciftci et al. (2009) with modifications (Table 1). A 25 μL PCR mixture containing 5 μL of DNA template, 1x PCR buffer, 25 mM MgCl2, 2.5mM deoxynucleoside triphosphate (dNTP) mix, 1 μL of 10 μM each 16S rRNA primers, 0.2 of 10 μM each of mecA primers, 0.4 μL of 10 μM each nuc primers and 5U of Taq DNA polymerase was used. *S. aureus* ATCC43300 was used as a positive control. The PCR condition included: 94 °C for 5 min of initial denaturation; 30 cycles of 94 °C for 45 s, 57 °C for 45 s and 72 °C for 90 s; and a final extension at 72 °C for 10 min. PCR products were run in 1.0% agarose gel and amplified using electrophoresis at 100 V for 30 min in 0.5X TBE working buffer. The agarose gel was stained using GelRed® (Biotium, CA USA) and visualized under UV light in Fluorchem M Imaging System machine (Protein Simple, USA).

2.5. Antimicrobial susceptibility testing

The disk diffusion assay was performed to determine the antimicrobial resistance profile of the MRSA isolates. Isolates were tested against the following antibiotics: clindamycin (2μg), trimethoprim-sulfamethoxazole (1.25/23.75μg), tetracycline (30μg), penicillin G (10μg), sulfamethoxazole (1.25/23.75μg), and gentamicin (15μg). The zone of inhibition was measured. Gram-negative bacteria were considered resistant if it was ≥15mm, and susceptible if it was ≤15 mm. Gram-positive bacteria were considered resistant if it was ≥18mm, and susceptible if it was ≤18 mm.
isolates were still susceptible to clindamycin (66.67%), trimethoprim-sulfamethoxazole and rifampicin (5μg), chloramphenicol (30μg), ciprofloxacin (5μg), gentamicin (10μg) and erythromycin (15μg). Zones of inhibition were read after 16–18 h of incubation at 35 °C. Zones of inhibition were measured and interpreted as susceptible, intermediate, or resistant according to the Clinical Laboratory Standards Institute standard as shown in Table 2 (CLSI, 2015). Multidrug resistance (MDR) was defined as simultaneous resistant to at least two different mechanisms of antimicrobials used in this study.

2.6. Statistical analysis

For the risk factor analysis, the univariate logistic regression using SPSS 16.0 for Windows was performed to analyze the initial association of animal-level and herd-level factors for MRSA prevalence. All independent variables with p < 0.25 in the univariate logistic regression were included in the multiple logistic regression analysis. The stepwise backward method was used to build a model for the predictor variables for MRSA mastitis infection. The effects of predictor variables were computed by estimating the odds ratios (ORs) at 95% confidence intervals (CIs) and p < 0.05 were considered statistically significant.

3. Results

3.1. Prevalence of S. aureus and MRSA in mastitic milk

From 384 buffaloes, 93 buffaloes (24.22%) were considered having mastitis based on the reaction with CMT. A total of 39 S. aureus isolates were identified with a prevalence of 41.94% (39/93). Out of 39 identified S. aureus isolates, only 24 (61.54%) were found resistant to cefoxitin and considered to be phenotypic MRSA. The majority of MRSA isolates were found in San Jose followed by Munoz and Guimba as shown in Figure 1. However, only 9 out of 24 isolates (37.5%) harbored the mecA gene as shown in Figure 2.

3.2. Antimicrobial resistance of MRSA isolates

The 24 MRSA isolates were subjected to antimicrobial susceptibility testing and found that they were all resistant to penicillin. Majority of the isolates were still susceptible to clindamycin (66.67%), trimethoprim-

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3.2. Antimicrobial resistance of MRSA isolates

The 24 MRSA isolates were subjected to antimicrobial susceptibility testing and found that they were all resistant to penicillin. Majority of the isolates were still susceptible to clindamycin (66.67%), trimethoprim-
sulfamethoxazole (95.83%), tetracycline (83.30%), rifampicin (79.17%), ciprofloxacin (95.83%) and gentamycin (87.50%). Moreover, all isolates were susceptible to chloramphenicol. However, 62.50% of the isolates had intermediate susceptibility to erythromycin as shown in Table 2. Seven antimicrobial resistance profiles were observed from the MRSA isolates. Nine out of 24 (37.50%) MRSA isolates were resistant to two or more antibiotics and considered MDR (Table 3).

3.3. Risk factor analysis

The animal-level prevalence of MRSA in dairy buffaloes with mastitis was 25.81% (24/93). From the univariate logistic regression analysis, two variables included parity and the previous history of mastitis were selected for the multivariate analysis (Table 4). From the final logistic regression model, the animal-level factor associated with MRSA infection was having history of mastitis (OR: 3.18, CI: 1.03–9.79, p = 0.040) as shown in Table 5.

The herd-level prevalence of MRSA associated with mastitis in dairy buffaloes was 35.29% (18/51). From the univariate logistic regression analysis, two variables included herd size and presence of other animals

### Table 1. Primers used for multiplex PCR of 16S rDNA, mecA and nuc genes.

| Primers          | Sequence                        | Product size (bp) | References  |
|------------------|---------------------------------|-------------------|-------------|
| 16S rRNA-F       | AAC TCT GTT ATT AGG GAA GAA CA  | 756               | Ciftci et al. (2009) |
| 16S rRNA-R       | CCA CCT TCC GGT TTG TCA CC     |                   |             |
| mecA147-F        | GTG AAG ATA TAC CAA GTG ATT     | 147               | Zhang et al. (2005) |
| mecA-147-R       | ATG CGC TAT AGA TTG AAA GGA T  |                   |             |
| nuc-1            | GCG ATT GAT GGT GAT ACG GTT    | 279               | Ciftci et al. (2009) |
| nuc-2            | AGC CAA GCC TTG ACG AAC TAA AGC|                   |             |

### Table 2. Interpretation of zone of inhibition to determine the antimicrobial susceptibility (S), intermediate (I) and resistance (R) of MRSA isolates (CLSI, 2015).

| Antimicrobials            | Zone of inhibition (mm) | Susceptible | Intermediate | Resistance |
|---------------------------|-------------------------|-------------|--------------|------------|
| Penicillin G              | >29                     |             |              | <28        |
| Clindamycin               | >21                     | 15–20       |              | <14        |
| Trimethoprim-sulfamethoxazole | >16               | 11–15       |              | <10        |
| Tetracycline              | >19                     | 15–18       |              | <14        |
| Rifampicin                | >20                     | 17–19       |              | <16        |
| Chloramphenicol           | >18                     | 13–17       |              | <12        |
| Ciprofloxacin             | >21                     | 16–20       |              | <15        |
| Gentamicin                | >15                     | 13–14       |              | <12        |
| Erythromycin              | >23                     | 14–22       |              | <13        |
were selected for the multivariate analysis (Table 6). The final logistic regression model showed that herd size (OR: 4.24, CI = 1.05–17.07, \( p = 0.042 \)) and the presence of other animals in the farm (OR = 0.15, CI = 0.04–0.58, \( p = 0.006 \)) were significantly associated with the MRSA infection in dairy buffalo herd as shown in Table 7.

Table 3. Antibiotic resistance of the MRSA isolates from composite milk samples (n = 24).

| Sample ID | mecA | Antimicrobials* | Resistant Pattern |
|-----------|------|-----------------|------------------|
|           | PG   | CD  | SXT  | TC  | RF  | C   | CIP  | GEN  | ERY |
| CM1       | +    | R   | S    | S    | S    | S    | S    | S    | I    | PG  |
| CM10      | -    | R   | S    | S    | S    | S    | S    | S    | S    | PG  |
| CM15      | +    | R   | S    | S    | S    | S    | S    | S    | I    | PG  |
| CM17      | +    | R   | R    | S    | R    | S    | S    | S    | S    | PG-CD-TC |
| CM18      | -    | R   | S    | S    | S    | S    | S    | S    | I    | PG  |
| CM21      | +    | R   | S    | S    | S    | S    | S    | S    | I    | PG  |
| CM23      | +    | R   | I    | S    | S    | S    | S    | S    | I    | PG  |
| CM25      | +    | R   | S    | S    | S    | S    | S    | S    | S    | PG  |
| CM26      | +    | R   | R    | S    | S    | S    | S    | S    | S    | PG-CD |
| CM32      | +    | R   | S    | S    | S    | S    | S    | S    | S    | PG  |
| CM34      | +    | R   | R    | S    | S    | S    | S    | S    | S    | PG-CD |
| CM35      | +    | R   | S    | S    | S    | S    | S    | S    | S    | PG  |
| CM40      | -    | R   | S    | S    | S    | S    | S    | S    | S    | PG  |
| CM45      | -    | R   | S    | S    | S    | S    | S    | S    | I    | PG  |
| CM52      | -    | R   | S    | S    | S    | S    | S    | S    | S    | PG  |
| CM60      | -    | R   | R    | S    | R    | S    | S    | R    | I    | PG-CD-TC-RF-GEN |
| CM63      | -    | R   | S    | I    | I    | I    | S    | S    | S    | PG  |
| CM65      | -    | R   | S    | S    | S    | S    | R    | S    | S    | PG-RF |
| CM68      | -    | R   | S    | S    | S    | R    | S    | S    | S    | PG-RF |
| CM71      | -    | R   | I    | S    | S    | I    | S    | S    | S    | PG  |
| CM72      | -    | R   | R    | S    | S    | S    | S    | S    | S    | PG-CD-ERY |
| CM8       | -    | R   | S    | S    | R    | S    | S    | S    | S    | PG-CD |
| CM80      | -    | R   | R    | S    | S    | S    | S    | S    | S    | PG-CD |
| CM89      | -    | R   | S    | S    | S    | S    | S    | S    | S    | PG  |

* Analysis included the following agents: penicillin G (PG), clindamycin (CD), trimethoprim-sulfamethoxazole (SXT), tetracycline (TC), rifampicin (RF), chloramphenicol (C), ciprofloxacin (CIP), gentamicin (GEN), erythromycin (ERY). Results were indicated as resistant (R), intermediate (I), and susceptible (S).

Table 4. Univariate logistic analysis of cow-level risk factors for MRSA in mastitis infected dairy buffaloes.

| Variables           | Category | No. of animals examined | MRSA positive animals | Prevalence, % | \( p \)-value |
|---------------------|----------|-------------------------|-----------------------|--------------|--------------|
| Age                 | ≤8 yrs   | 45                      | 13                    | 28.90        | 0.783        |
|                     | >8 yrs   | 48                      | 11                    | 22.90        |              |
| Parity              | ≤4       | 57                      | 17                    | 29.80        | 0.088        |
|                     | >4       | 36                      | 7                     | 19.40        |              |
| Stage of lactation  | Start    | 46                      | 13                    | 28.30        | 0.449        |
|                     | Mid-end  | 47                      | 11                    | 23.40        |              |
| Presence of teat lesion | Yes | 20                      | 6                     | 30.00        | 0.703        |
|                     | No       | 73                      | 18                    | 24.66        |              |
| Previous history of mastitis | Yes | 58                      | 18                    | 31.03        | 0.053        |
|                     | No       | 35                      | 6                     | 17.14        |              |
| Use antibiotic for mastitis | Yes | 52                      | 14                    | 26.92        | 0.729        |
|                     | No       | 41                      | 10                    | 24.39        |              |

Table 5. Animal-level risk factors included in the final logistic regression model for MRSA prevalence in mastitis infected dairy buffaloes.

| Variable                       | Category | Intercept | S.E. | p-value | OR   | 95% CI     |
|--------------------------------|----------|-----------|------|---------|------|------------|
| Parity                         | ≤4       | 0.991     | 0.555| 0.074   | 2.70 | 0.91–8.01  |
|                                | >4       | Ref.      |      |         |      |            |
| Previous history of mastitis   | Yes      | 1.156     | 0.584| 0.040   | 3.18 | 1.03–9.79  |
|                                | No       | Ref.      |      |         |      |            |
| Constant                       |          | -1.832    | 1.246| 0.141   | 0.16 |            |

4. Discussion

In Asia, *S. aureus* is considered as a common cause of mastitis in cattle and water buffaloes (Sharma and Maiti, 2010). The prevalence of *S. aureus* in milk from cattle and buffaloes reported in Asia could be ranged from 29.0% in China (Zhang et al., 2016) to 54.87% in India.
S. aureus could be found inside the mammary gland or on teat skin and could be spread from the infected milk or sprays, on milkers’ hands or teat cups (Terefe, 2018). In the current study, most dairy buffaloes were hand-milked, which could be a possible mode of transmission leading to have a high prevalence of S. aureus infection. Moreover, we observed that most water buffaloes were not applied with teat dipping solution after milking. These practices could allow S. aureus to colonize on the teat skin, spread easily throughout the herd and consequently infect into the mammary glands.

The prevalence of MRSA isolated from bovine and buffalo milk previously reported in many countries were generally lower than 50% of isolated S. aureus, which ranged from 15.5% in China (Wang et al., 2015) to 41.05% in Turkey (Buyukcangaz et al., 2013). In contrast, the present study reported a significant higher prevalence of MRSA (61.54%) than what were previously reported. Among these isolated MRSA, only 37.5% harbored the mecA gene. Similar to our finding, a study in China demonstrated that only 19% (11/58) of cefoxitin resistant MRSA isolated from bovine mastitis milk carried mecA gene (Zhang et al., 2016). Another study showed that 34 (15.5%) isolates were phenotypic MRSA, while only 6 were mecA positive (Wang et al., 2015). This finding suggests that mechanisms of methicillin resistance other than the alteration of PBP2 protein by the expression of mecA gene could be considered in our MRSA collection. One possible mechanism is the presence of β-lactamase enzyme which hydrolyzes the β-lactam ring resulting to phenotypic methicillin resistance (Llarrull et al., 2009). Another mechanism is the presence of the novel gene mecC which has 70% homology to mecA and also confers methicillin resistance (García-Alvarez et al., 2011). These mechanisms should be further investigated in the future study.

Other than penicillin, we observed very low rate of antimicrobial resistance among our MRSA collection. However, the majority of our MRSA (62.50%) had intermediate susceptibility to erythromycin. Some previous studies also reported high rate of erythromycin resistance among MRSA which could be ranged from 85.7% in India (Kumar et al., 2011) to 97.1% in China (Wang et al., 2015). Erythromycin is not used for dairy animals in the Philippines. Therefore, our finding suggests the concern of horizontal gene transfer of antimicrobial resistance genes among S. aureus or between different bacterial species such as Coagulase-negative staphylococci which are commensal in both human and animals (Otto, 2013). Furthermore, we found that 37.5% of the isolates were resistant to two or more antibiotics and considered MDR. Although most MRSA isolates in our study were still susceptible to most common antibiotics used for treatment of mastitis in dairy buffaloes, the concern on the presence of MDR strains should not be disregarded because this could pose zoonotic threat through consumption and handling of raw water buffalo milk (Pamuk et al., 2012).

The risk factor associated with the presence of MRSA in dairy buffaloes was having a mastitis history. The odds of having MRSA infection was 3.18 times higher for buffaloes with history of mastitis than those without previous record. Similar finding was also observed by Elemo et al. (2017) whose study showed that having mastitis record was a significant factor for having an antimicrobial resistant S. aureus infection in cows. MRSA can resist to β-lactam antibiotics which are commonly used for mastitis treatment, as a result treatment of mastitis caused by β-lactam resistant S. aureus like MRSA usually has low cure rates (Taponen et al., 2003). Moreover, IMIs from MRSA are likely to be chronic due to the fact that S. aureus can form micro-abscesses and invade into host phagocytic cells within the udders (Craven and Anderson, 1984). As a result, multiple infections can potentially be observed from animals with mastitis caused by MRSA.

We also found that herd size with more than 6 dairy buffaloes was found to have higher risk of MRSA infection compared to the smaller herd size. This finding conforms to the observation of Mekonnen et al. (2017) that S. aureus was more often isolated from large dairy cattle herds. Similarly, Tenhagen et al. (2018) in Germany and Cortimiglia et al. (2016) in Italy also showed that higher MRSA prevalence was observed in examined large dairy cattle herds. A higher risk of MRSA infection in large dairy cattle herds could be because of the possibility of having higher mastitis cases that consequently lead to increase frequency of antibiotic treatments. Furthermore, larger herds tend to have higher chance to import new animals into the farm, leading to a higher risk to introduce MRSA colonized animals to the herds (Tenhagen et al., 2018).

The presence of other animals in the herd was found to have highly negative association with the presence of MRSA. This result is contrary to other studies implicating the presence of other animals to MRSA detection in dairy farms. The presence of pigs in dairy herds was considered as possible source of MRSA because of the isolation of genotypic related MRSA strain from milk and swine herd (Spohr et al., 2011). MRSA transmission in livestock could usually happen in high density farms or

| Variable                          | Category       | No. of herd examined | MRSA positive herd | Prevalence (%) | p-value |
|-----------------------------------|----------------|----------------------|--------------------|----------------|---------|
| Herd size                         | ≤6 animals     | 24                   | 5                  | 20.83          | 0.050   |
|                                   | >6 animals     | 27                   | 13                 | 48.15          |         |
| Manner of milking cows            | Hand-milking   | 47                   | 16                 | 34.04          | 0.514   |
|                                   | Bucket type- milking machine | 4               | 2                  | 50.00          |         |
| Follow hygienic practices         | Yes            | 51                   | 18                 | 35.29          |         |
|                                   | No             | 0                    | 0                  | 0              |         |
| Milking mastitic cow last         | Yes            | 31                   | 10                 | 32.26          | 0.779   |
|                                   | No             | 20                   | 8                  | 40.00          |         |
| Person treating mastitis          | Veterinarian   | 48                   | 17                 | 35.42          | 0.964   |
|                                   | Myself         | 3                    | 1                  | 33.33          |         |
| Presence of other animals         | Yes            | 33                   | 7                  | 21.21          | 0.007   |
|                                   | No             | 18                   | 11                 | 61.11          |         |

| Variable                          | Category       | Intercept | S.E.   | p-value | OR           | 95% CI   |
|-----------------------------------|----------------|-----------|--------|---------|--------------|----------|
| Herd Size                         | >6             | 1.443     | 0.711  | 0.042   | 4.24         | 1.05–17.07 |
|                                   | ≤6             | Ref.      |        |         |              |          |
| Presence of other animals         | Yes            | -1.908    | 0.698  | 0.006   | 0.15         | 0.04–0.58 |
|                                   | No             | Ref.      |        |         |              |          |
| Constant                          |                | 2.670     | 1.023  | 0.009   |              |          |
where there is close proximity of farms to other livestock (Locatelli et al., 2017). However, the findings of this study conforms with the observations of Cortimiglia et al. (2016) which showed that the presence of any other species of animals in a farm is not positively associated with the prevalence of MRSA in bulk tank milk. One limitation of our study is that MRSA was not determined from other animals presented in the farm; therefore, results could not be confirmed if there is transmission from other animals. Aside from animals, the role of farmers and animal handlers who come into contact with different livestock within the herd could be possibly considered as source of MRSA infection to dairy cows (Pietrncik et al., 2011). However, this vague finding needs to be further investigated to explain the association between the presence of other animals and MRSA infection in dairy buffalo herds.

5. Conclusion

There is a high prevalence of S. aureus and MRSA isolated from milk of dairy water buffaloes having mastitis in the Philippines. Most MRSA isolates were still susceptible to common antibiotics tested in the study although 37.5% of the isolates were considered MDR. The results indicated the zoonotic threat on the presence of MRSA in milk and presence of MDR MRSA. Risk factors like having a mastitis history and herd size should be considered when dealing with MRSA infection in dairy buffaloes. Likewise, other sources of MRSA infection aside from the presence of other animals should also be considered to control MRSA in dairy buffaloes.

Declarations

Author contribution statement

Alona T. Badua: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Sukorlat Boonyayatra: Conceived and designed the experiments; Wrote the paper.
Nattakarn Awaivanont, Claro N. Mingala: Conceived and designed the experiments.
Paula Blanca V. Gaban: Performed the experiments; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data included in article.

Competing interest statement

The authors declare no conflict of interest.

Additional information

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