Slov Vet Res 2019; 56 (Suppl 22): 755–63
DOI 10.26873/SVR-816-2019

Original Research Article

ANTIMICROBIAL PHENOTYPES OF GEOGRAPHICALLY MATCHED *Staphylococcus aureus* ISOLATED FROM BUFFALO'S MILK AND CLINICAL HUMAN CASES IN EGYPT: POTENTIAL ZOONOTIC RISKS

Walid Elmonir[1*], Hader Aglan[1], Ehab K.Elmahallawy[2], Wael F. El-Tras[3]

[1]Hygiene and Preventive Medicine (Zoonoses) Department, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh 33516, Egypt, [2]ZoonosesDepartment, Faculty of Veterinary Medicine, Sohag University, Sohag82511 Egypt, [3]Faculty of Aquatic and Fisheries Sciences (Zoonoses), Kafrelsheikh University, Kafrelsheikh33516, Egypt

*Corresponding author: walid.elmonir@gmail.com

Abstract: Global emergence of antibiotic-resistant food-borne pathogens is a major public health problem. This study aimed to determine the potential zoonotic risk of *Staphylococcus aureus* in buffalo's milk in Egypt. A total of 102 raw buffalo's milk samples and 51 human nasal swabs were collected at Kafrelsheikh city, Egypt. All samples were examined for occurrence of *S. aureus*. Detected isolates were characterised based on *DNase* activity, *mecA* gene acquisition, and antibiotic resistance patterns. *S. aureus* was detected in 33.3% of buffalo's milk samples and29.4% of human nasal swabs. Multiple drug-resistant *S.aureus* (MDRSA) represented 88.2% and 90% of buffalo and human *S. aureus* isolates, respectively. Buffalo and human *S. aureus* isolates showed highest resistance rate for erythromycin (100%), and lowest resistance rate for gentamicin (22.2%). Interestingly, there was no significant difference in resistance patterns between methicillin-resistant *S. aureus* (MRSA) and methicillin-sensitive *S. aureus* (MSSA) (P<0.46 - 0.97). One-third of the detected *S. aureus* phenotypes (5/15, 33.3%) were identical between buffalo and human isolates. Moreover, there was no significant difference in antibiotic resistance patterns between buffalo and human isolates (P<0.1 - 0.97).This study highlights the potential public health risk of MDRSA transmission via buffalo's milk.

Key words: buffalo's milk, *S. aureus*, *DNase*, *mecA*, antibiogram, zoonotic risk

Introduction

The last few years have witnessed an alarming increase in the reports of antibiotic-resistant pathogens in human and veterinary practices. Special concern is given to the possibility of food contamination with multiple drug-resistant (MDR) bacteria or bacteria carrying antibiotic-resistant genes (1,2). Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are β-lactam-resistant *S. aureus* that can easily acquire resistance to a wide spectrum of other antibiotics (3). Thus, the high rate of dissemination of these pathogens worldwide has created an additional infection control problem in both human and veterinary medicine (4). Multiple drug-resistant *S. aureus* (MDRSA) strains, including MRSA strains, have been isolated from bovine milk and different dairy products worldwide (5-7). Bovine MRSA strains have

Received: January 2019
Accepted for publication: February 2019

emerged as zoonotic organisms based on several lines of evidence showing the possibility of direct transmission of MRSA between bovines and humans (4, 8).

In Egypt, buffalo's milk and its derived dairy products are more popular than those derived from cows owing to the higher fat content, whiter colour and creamier flavour of buffalo's milk than of cow's milk (9). Buffalo's milk is the second most produced milk in Egypt, with over2 million tons produced annually (10), making Egypt the fourth largest buffalo's milk producer worldwide (10). Despite this important aspect, there is not sufficient data on the role of Buffalo's milk in maintaining the epidemiological foci of MDRSA at the national level. Therefore, the aim of this study was to elucidate the potential public health risk of *S. aureus* in buffalo's milk by reporting the prevalence and antibiotic resistance patterns of *S. aureus* isolates from buffalo's milk used for consumption by residents of Kafrelsheikh, Egypt. Furthermore, the phenotypes of buffalo *S. aureus* isolates were compared with those isolated from geographically matched human clinical cases to evaluate the potential zoonotic risk of buffalo's milk-borne *S. aureus* isolates.

Materials and methods

Sampling

Buffalo's milk and human nasal samples were collected from Kafrelsheikh city, the capital of Kafrelsheikh Governorate, which is located in the northern region of the Nile Delta, Lower Egypt (31°06′42″N 30°56′45″E). Buffalo milk used for consumption in the study region was bought from either markets or livestock smallholders. A total of 102 milk samples (100 ml per sample) were collected from Kafrelsheikh between September and December 2016. The buffalo's milk samples were divided as follows: half of the samples (51 samples) were bought from various markets at different localities in the city, while the other half were collected directly from buffaloes owned by smallholders. Potential heat treatment of market milk samples was evaluated by the *peroxidase* (Storch) test (11), and only raw samples were included in this study. Milk samples from buffaloes were collected under aseptic conditions as composite milk samples from the 4 quarters. All sampled buffaloes were apparently healthy, and the collected milk samples showed no physical or organoleptic abnormalities. During the same study period, a total of 51 nasal swabs were collected from outpatients at Kafrelsheikh Chest Hospital. All human samples were collected by the medical staff of the hospital.

Isolation and identification of S. aureus

Isolation of *S. aureus* were conducted according to the guidelines of the Food and Drug Administration (12). Suspected *S. aureus* colonies were identified based on the following criteria: grape-like clusters of gram-positive cocci by Gram staining and yellow colonies on mannitol salt agar (Oxoid, Hampshire, U.K.), beta haemolysis on sheep blood agar (Oxoid, Hampshire, U.K.), and firm coagulation in the tube coagulase test using rabbit plasma (12).

Phenotyping of S. aureus isolates

From the 49 detected *S. aureus* isolates, 27 (17 buffalo and 10 human) isolates were chosen for phenotyping based on biochemical profiles, *mecA* gene acquisition, and antibiotic resistance patterns.

For biochemical profiles, the following tests were used (13): *DNase*, *catalase*, *oxidase*, Voges-Proskauer, growth in 10% NaCl, arginine *decarboxylase*, esculin hydrolysis, and sugar fermentation (lactose, maltose, ribose, arabinose, sorbitol, and raffinose). All *S. aureus* isolates showed the same biochemical profile except for the *DNase* test results; *DNase* activity was used as a marker for the biochemical profiles. For molecular detection of the *mecA* gene, *S. aureus* cultures incubated in broth overnight were used for DNA extraction by the QIAamp DNA Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Amplification of the *mecA* gene was conducted according to Murakami *et al*. (14) using the following primers: forward *mecA1*-5' AAAATCGATGGTAAAGGTTGG 3' reverse *mecA2*-5'AGTTCTGCAGTACCGGATTTG

3'. Five μL of DNA extracted from *S. aureus*-culture was added to a PCR mixture that contained 1 μL (20 pmol) of each primer, 12.5 μL of DreamTaq PCR Master Mix (Thermo Fisher Scientific, Waltham, USA) and distilled water up to the 25 μL reaction volume. PCR was performed in a Mastercycler (Eppendorf, Hamburg, Germany) using the following conditions: 95°C for 3 min followed by 35 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 1 min and a final extension at 72°C for 10 min. Positive control (*mecA*+ *S. aureus* isolate) was kindly provided by Prof. Mohamed Hassan, Prof. of Food Hygiene, Faculty of Veterinary Medicine, Benha University, Egypt. PCR products were examined by gel electrophoresis and UV illumination (Fig. 1).

For antibiotic resistance patterns, a standard disk diffusion assay was used for antibiotic sensitivity testing according to the guidelines of the Clinical and Laboratory Standards Institute (15). The antibiotic disks (Oxoid, Hampshire, U.K.) used in this study are listed in table 2. *S. aureus* isolates resistant to cefoxitin were considered resistant to all β-lactams and were designated as MRSA. *S. aureus* isolates sensitive to cefoxitin were designated as methicillin-sensitive *S. aureus* (MSSA). MSSA isolates were further tested using ampicillin (10 μg) disks. *S. aureus* isolates that showed resistance to ampicillin were tested by nitrocefin discs (Thermo Scientific, Lenexa, USA) for β-lactamase activity.

Statistical analysis

The potential difference in antibiotic resistance patterns among the chosen 27 *S. aureus* isolates was assessed using a univariate logistic regression model to compare MRSA versus MSSA isolates or buffalo versus human isolates as the response variables. The model was built for all tested antibiotics with the exception of erythromycin and ampicillin. These antibiotics were removed because all 27 isolates were resistant to erythromycin and because ampicillin was used only for MSSA isolates. The statistical analyses were carried out using SAS 9.2 (SAS Institute Inc., 2008). Statistical significance was considered at $P<0.05$.

Ethical approval

The research details and methods were approved by the Ethics Committee of the Hygiene and Preventive Medicine Department, Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt. For human samples, all nasal samples were collected by the medical staff of Kafrelsheikh Chest Hospital. The research details and risks of participation were explained to the participating outpatients, and written consent was obtained.

Results and discussion

In the present study, *S. aureus* was isolated from 33.3% of the examined buffalo's milk samples (table 1), which is similar to the results of a previous study (33.3%) in Turkey (5). However, our result was higher than another report (17.5%) in Iran (16). *S. aureus* was detected in 29.4% of nasal swabs from outpatients at Kafrelsheikh Chest Hospital (table 1). Likewise, Ungureanu *et al*. (17) isolated *S. aureus* from 35.38% of nasal exudates collected from hospitalized patients and outpatients in Romania.

Bovine milk could act as a vehicle for MRSA transmission to humans (4, 8). Acquisition of the *mecA* gene defines MRSA isolates (4).A total of 35.5% of *S. aureus* isolates from buffalo's milk were MRSA (table 1). This value was higher than a report (9.2%) in Turkey (5). However, a higher rate of MRSA (56.1%) was reported in Uganda (6). The relatively high rates of MRSA reported in this study may be attributed to unhygienic milking and rearing of buffaloes in Egypt. The majority of buffalo populations in Egypt are household reared, and their milk is sold in informal markets (7). Manual milking and unhygienic milking equipment, milk storage conditions, and milk transportation are common practices in this production system. These practices may contribute to the high contamination rate observed in this study. MRSA represented 40% of human *S. aureus* isolates, which was higher than that in other studies (18.5–30%) reported elsewhere (17,18).

MDR to more than 3 classes of antibiotics was observed in 88.2% and 90% of buffalo and

human *S. aureus* isolates, respectively (table 1). In agreement with our findings, high rate (95.5%) of MDRSA were previously reported in bovine milk in Egypt (7). In contrast, other studies reported high sensitivity of bovine milk *S. aureus* isolates to several antibiotics in Brazil (19) and in Pakistan (20). The majority (90%) of the human *S. aureus* isolates were MDRSA (table 1). This value was higher than that in a study in Romania (18.75 - 45.45%) by Ungureanu et al. (17) but comparable to that in another report in Iran (61 - 93%) (18).

All MRSA isolates in this study were cefoxitin-resistant (table 2). This result implies a broad-spectrum resistance to all ß-lactams, with the exception of anti-MRSA cephalosporins (15). Interestingly, 47.1% of the MSSA isolates were resistant to ampicillin (table 2). Ampicillin-resistant MSSA is also resistant to penicillinase-labile ß-lactams (15). Both buffalo and human MSSA isolates showed ampicillin resistance at rates of 45.5% and 50%, respectively (table 2). In line with our findings, several pre vious studies reported resistance of bovine MSSA (2, 5), and human MSSA (17, 18) to penicillinase-labile ß-lactams at variable rates. MRSA isolates harboured the *mecA* gene, which mediates resistance to ß-lactams by encoding penicillin-binding protein 2a (PBP2a). On the other hand, MSSA isolates in this study lacked the *mecA* gene but produced the *ß-lactamase* enzyme (100%); the *ßlactamase* enzyme is encoded by the *blaZ* gene (21). In agreement with this finding, Yokoyama *et al*. (21) attributed ß-lactam resistance in MSSA to the production of the *ß-lactamase* enzyme. They also reported a higher prevalence of the *blaZ* gene among MSSA isolates than among MRSA isolates (21).

All buffalo and human *S. aureus* isolates were resistant to erythromycin (100%) (table 2). High rates of erythromycin resistance among bovine milk *S. aureus* isolates (50.5 – 77.3%) were also reported by Pamuk *et al*. (5) and Elmonir *et al*.(7). In contrast, AiresdeSousa *et al*. (19) and Asiimwe *et al*.(6) reported much lower erythromycin resistance rates (3.3 – 6.5%). Similarly, high rates of erythromycin resistance (61.3 - 93%) were shown in human *S. aureus* isolates in other studies (17, 18).

In addition, buffalo and human *S. aureus* isolates showed high resistance rates (70.4 - 88.9%) for kanamycin, sulphamethoxazole/trimethoprim, and tetracycline (table 2). Previous reports recorded comparable resistances of bovine and human *S. aureus* isolates for kanamycin, sulphamethoxazole/trimethoprim, and tetracycline (6, 7, 17, 18). However, other reports showed high efficacy of sulphamethoxazole/trimethoprim and tetracyclines on bovine *S. aureus* isolates (6, 19, 20). All *S. aureus* isolates in this study showed low resistance for ciprofloxacin (33.3%) and gentamicin (22.2%) (Table 2). High efficacy of ciprofloxacin was also reported elsewhere for bovine isolates (6, 20) and for human isolates (17). In contrast, Rahimi *et al*. (18)reported high resistance (95%) of human MRSA isolates for ciprofloxacin. Gentamicin is used in combination with vancomycin or cephalosporins as an alternative therapeutic choice for severe MRSA infections that show reduced susceptibility to vancomycin or daptomycin (22, 23). Hence, resistance to gentamicin limits treatment options and raises public health concerns. A total of 11.8% and 40% of buffalo and human isolates, respectively, showed resistance to gentamicin (table 2). In line with our findings, several studies reported resistance of bovine *S. aureus* isolates to gentamicin at rates ranging from 10% to 64.3% (5, 7, 20, 24).However, the findings of this study disagree with those of other studies that showed high rates of gentamicin efficacy (6, 19). High rates (18.5 – 59%) of gentamicin resistance among human *S. aureus* isolates were previously reported (17, 18), which agrees with our findings. The discrepancies in the present results compared with the results from various previous studies could be attributed to multiple factors, including differences in national policies for antibiotic administration to animals, animal production systems, sanitary measures of animal rearing and animal byproduct marketing, personal hygiene, and sampling methods as well as demographic and regional differences.

Table 1: Frequency distribution of *S.* aureus isolated from buffalo's milk and human nasal swabs in this study

Isolates	Buffaloes			Humans	Total
	Market Milk	Household Milk	Total Milk	Nasal swabs	
S. aureus	16/51 (31.4)	18/51 (35.3)	34/102 (33.3)	15/51 (29.4)	49/153 (32.03)
MRSA	3/8 (37.5)	3/9 (33.3)	6/17 (35.3)	4/10 (40)	10/27 (37.04)
MSSA	5/8 (62.5)	6/9 (66.7)	11/17 (64.7)	6/10 (60)	17/27 (62.96)
MDRSA	7/8 (87.5)	8/9 (88.9)	15/17 (88.2)	9/10 (90)	24/27 (88.9)
MDR-MRSA	3/3 (100)	3/3 (100)	6/6 (100)	3/4 (75)	9/10 (90)
MDR-MSSA	4/5 (80)	5/6 (83.3)	9/11 (81.8)	6/6 (100)	15/17 (88.2)

Brackets: Percent; MRSA: Methicillin-resistant *S. aureus*; MSSA: Methicillin-sensitive *S. aureus*; MDRSA: multiple drugs resistant *S. aureus*

Table 2: Antibiotic resistance diversity of *S. aureus* isolates detected in this study

Antimicrobial agent		Species		Methicillin resistance		Total No. (%)
		Buffalo No. (%)	Human No. (%)	MRSA No. (%)	MSSA No. (%)	
Cephems	FOX (30µg)	6 (35.3)	4 (40)	10 (100)	0 (0)	10 (37.04)
Penicillins	AMP (10µg)	11 (64.7)	7 (70)	10 (100)	8 (47.1)	18 (66.7)
Aminoglycosides	CN (10µg)	2 (11.8)	4 (40)	3 (30)	3 (17.6)	6 (22.2)
	K (30µg)	15 (88.2)	9 (90)	9 (90)	15 (88.2)	24 (88.9)
Macrolides	E (15µg)	17 (100)	10 (100)	10 (100)	17 (100)	27 (100)
Fluoroquinolones	CIP (5µg)	4 (23.5)	5 (50)	4 (40)	5 (29.4)	9 (33.3)
Nitrofurantoins	F (300µg)	5 (29.4)	5 (50)	4 (40)	6 (35.3)	10 (37.04)
Tetracyclines	TE (30µg)	12 (70.5)	7 (70)	7 (70)	12 (70.6)	19 (70.4)
Phenicols	C (30µg)	9 (52.9)	6 (60)	6 (60)	9 (52.9)	15 (55.6)
Sulfonamides	SXT (25µg)	15 (88.2)	9 (90)	9 (90)	15 (88.2)	24 (88.9)

FOX: cefoxitin; AMP: ampicillin; CN: gentamicin; K: kanamycin; E: erythromycin; CIP: ciprofloxacin; F: nitrofurantoin; TE: tetracycline; C: chloramphenicol; SXT: sulfamethoxazole/trimethoprim

The main difference between MRSA and MSSA is the spectrum of antibiotic resistance. Previous studies showed that the majority of MRSA strains are multi-resistant to β-lactams and to a wide spectrum of other antibiotics, while MSSA strains show much lower rates of resistance to multiple antibiotics in both human and bovine infections (3, 17, 24). Unlike these studies, our study showed no significant difference (P<0.46 - 0.97) in resistance patterns between MRSA and MSSA isolates for 7 classes of antibiotics (table 3). This study recorded unprecedentedly high rate (81.8%) of MDR among MSSA isolates from buffaloes (Table 1). Reports of MDR-MSSA in bovine milk are mostly from developing countries (2, 7). This fact could be attributed to unhygienic milk production and misuse of antibiotic therapy in veterinary practices in these countries. This evidence also highlights the role of buffaloes in the carriage and dissemination of MDR-MSSA,

Table 3: Univariate regression model for the association of antibiotic resistance among MRSA vs. MSSA and Buffalo vs. Human *S. aureus* isolates

Variable	MRSA vs. MSSA				Buffalo vs. Humans			
	C.	OR	*P*<	95% CI	C.	OR	*P*<	95% CI
K	MRSA MSSA	- 0.83	- 0.89	- 0.1 - 10.6	Buffalo Human	- 1.2	- 0.88	- 0.1 - 15.20
SXT	MRSA MSSA	- 0.83	- 0.88	- 0.1 - 10.6	Buffalo Human	- 1.2	- 0.88	- 0.1 - 15.20
TE	MRSA MSSA	- 1.03	- 0.97	- 0.19 - 5.68	Buffalo Human	- 0.97	- 0.97	- 0.18 - 5.37
C	MRSA MSSA	- 0.75	- 0.72	- 0.15 - 3.65	Buffalo Human	- 1.33	- 0.72	- 0.27 - 6.0
F	MRSA MSSA	- 0.82	- 0.80	- 0.16 - 4.10	Buffalo Human	- 2.4	- 0.3	- 0.48 - 12.13
CIP	MRSA MSSA	- 0.63	- 0.57	- 0.12 - 3.22	Buffalo Human	- 3.25	- 0.17	- 0.61 - 17.28
CN	MRSA MSSA	- 0.5	- 0.46	- 0.08 - 3.15	Buffalo Human	- 5.0	- 0.10	- 0.72 - 34.92

C.: Categories; OR: Odd ratio; CI: Confidence interval

which raises concerns regarding potential hazards to the animal industry and safety of milk in these countries, including Egypt. MSSA is characterized by higher fitness and shorter generation time than MRSA; hence, MSSA causes a higher number of cases than MRSA does (3). If such strains attain MDR, which hinders the treatment and eases the dissemination of these pathogens, a serious public health hazard is predictable. Klein *et al*. (25) reported an increase in hospitalization costs associated with MSSA-related infections relative to MRSA-related infections between 2010 and 2014 in the USA. They hypothesized that ineffective treatment approaches for MSSA may be one of the reasons for this shift (25). We may expect a further increase in the burden of MSSA infections at the human-animal interface with the potential emergence of MDR-MSSA.

Zoonotic transmission of *S. aureus* between bovines and humans is well documented (4, 8). Fifteen phenotypes of *S. aureus* were determined in this study (table 4). Approximately one-third of these phenotypes (5/15, 33.3%) were shared by 8/10 (80%) and 6/17 (35.3%) of the human and buffalo isolates, respectively. Additionally, none of the tested antibiotics showed a significant difference between buffalo and human isolates by univariate analysis ($P<0.1 - 0.97$), (table 3). In contrast, Jayaweera and Kumbukgolla (2) reported significantly higher odds of resistance to gentamicin and ciprofloxacin for animal *S. aureus* isolates than for human isolates; however, they included isolates from other species (e.g., poultry and pigs) in their analysis, which may explain the contradiction with our findings. The high phenotypic similarity of human isolates with buffalo isolates, especially in terms of antibiotic resistance patterns, highlights the potentially high rate of zoonotic interspecies transmission between humans and buffaloes and emphasizes the role of buffalo's milk as a vehicle of MDRSA for humans in the study region.

Table 4: Phenotyping of isolated *S. aureus* isolates from buffalo's milk and human nasal swabs samples

P*	*Dnase*	*mecA*	Antibiogram profile	Source	No.
P1	+	+	FOX, E, K, SXT, TE, C, F, CIP, CN	Human	2
				Buffalo	1
P2	-		FOX, E, K, SXT, TE, C, F, CIP	Human	1
P3	+		FOX, E, K, SXT, TE, C	Buffalo	2
P4	+		FOX, E, K, SXT, TE	Buffalo	1
P5	+		FOX, E, K, SXT	Buffalo	2
P6	+		FOX, E	Human	1
Total MRSA					10
P7	+	-	AMP, E, K, SXT, TE, C, F, CIP, CN	Human	2
				Buffalo	1
P8	+		AMP, E, K, SXT, TE, C, F, CIP	Buffalo	2
P9	+		AMP, E, K, SXT, TE, C, F	Buffalo	1
P10	+		AMP, E, K, SXT, TE, C	Human	1
				Buffalo	1
P11	+		E, K, SXT, TE, C	Buffalo	1
P12	+		E, K, SXT, TE	Human	1
				Buffalo	2
P13	+		E, K, SXT	Human	2
				Buffalo	1
P14	+		E	Buffalo	1
P15	-		E	Buffalo	1
Total MSSA					17

P: Phenotype

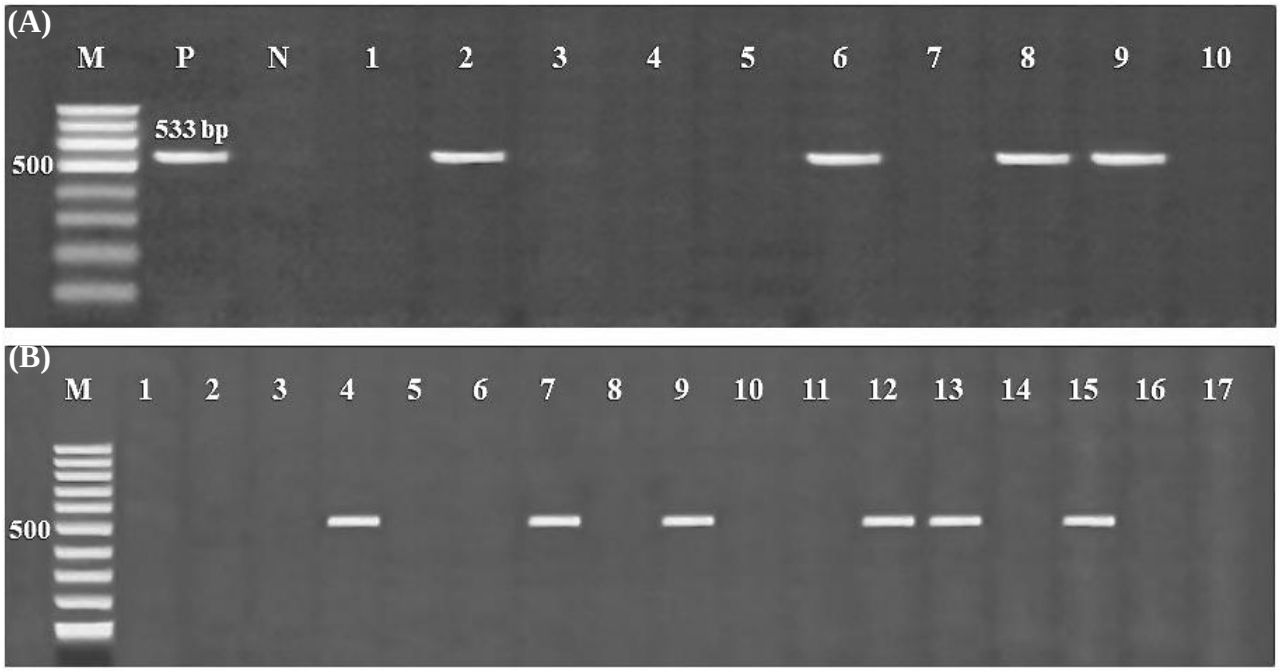

Figure 1: Molecular detection of *Methicillin* resistance gene (*mecA*) among *S. aureus* pathogens isolated from Buffalo's milk and human nasal swabs. (A) Human nasal swabs isolates. (B) Buffalo's milk isolates. M: 100 bp DNA marker, P: Positive control, and N: Negative control.

Conclusion

Our study highlights the possibly high risk of MDRSA dissemination to humans via buffalo's milk in the study region. The high rates of buffalo's milk-borne MDR-MRSA and MDR-MSSA need further investigation to explore resistance mechanisms and risk factors that contribute to the emergence of these pathogens in the study region. This study also highlights the genuine need for hygienic production and marketing of buffalo's milk, restriction and supervision of antibiotic therapy in veterinary practices, and public awareness about the potential risks of raw buffalo's milk consumption and processing.

Acknowledgements

We thank Yamen Hegazy for assistance with statistics.

Conflict of interest

None declared.

References

1. Laxminarayan, R., Matsoso, P., Pant, S., Brower, C., Rottingen, J.A., Klugman, K. and Davies, S. Access to effective antimicrobials: a worldwide challenge. Lancet 2016;387: 168–75.

2. Jayaweera, J. and Kumbukgolla, W.W. Antibiotic resistance patterns of methicillin-resistant Staphylococcus aureus (MRSA) isolated from livestock and associated farmers in Anuradhapura, Sri Lanka. Germs2017;7: 132–9.

3. Rozgonyi, F., Kocsis, E., Kristof, K. and Nagy, K. Is MRSA more virulent than MSSA? Clin Microbiol Infect 2007;13: 843–5.

4. Becker, K., Ballhausen, B., Kock, R. and Kriegeskorte, A. Methicillin resistance in Staphylococcus isolates: the "mec alphabet" with specific consideration of mecC, a mec homolog associated with zoonotic S. aureus lineages. Int J Med Microbiol 2014;304: 794–804.

5. Pamuk, S., Yildirim, Y., ŞEKER, E., Gürler, Z., Kara, R. A survey of the occurrence and properties of methicillinresistant Staphylococcus aureus and methicillin-resistant Staphylococcus intermedius in water buffalo milk and dairy products in Turkey. International Journal of Dairy Technology2012; 65: 416–22.

6. Asiimwe, B.B., Baldan, R., Trovato, A. and Cirillo, D.M. Prevalence and molecular characteristics of Staphylococcus aureus, including methicillin resistant strains, isolated from bulk can milk and raw milk products in pastoral communities of South-West Uganda. BMC Infect Dis 2017;17: 422.

7. Elmonir, W., Abo-Remela, E.M. and Sobeih, A. Public health risks of Escherichia coli and Staphylococcus aureus in raw bovine milk sold in informal markets in Egypt. . Infect Dev Ctries 2018;12: 533–41.

8. Juhasz-Kaszanyitzky, E., Janosi, S., Somogyi, P., Dan, A., van der Graaf-van Bloois, L., van Duijkeren, E. and Wagenaar, J.A. MRSA transmission between cows and humans. Emerg Infect Dis 2007;13: 630–2.

9. Borghese, A. Recent developments of buffaloes in Europe and Near East. Proc of the Seventh World Buffalo Congress, Manila, Philippines 20 to 23 Oct 2004: 10–6.

10. FAOSTAT Food and agriculture organization statistics.2016available at: http://www.fao.org/faostat/en/#data/QL. (accessed in 10-10-2018).

11. Roberts, D.G., M. Practical food microbiology, 3rd edition. Oxford: Blackwell publishing Ltd. 2003, 206 p.

12. FDA, Bacteriological Analytical Manual Online. Chapter 12: Staphylococcus aureus (January 2001 update) Available: https://wwwfdagov/Food/FoodScienceResearch/LaboratoryMethods/ucm071429htm (Accessed: 10 October 2018).

13. MacFaddin, J.F. Biochemical tests for identification medical bacteria. . Warery Press Inc, Baltimore, Md 21202,2000, USA.

14. Murakami, K., Minamide, W., Wada, K., Nakamura, E., Teraoka, H. and Watanabe, S. Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction. J Clin Microbiol 1991;29: 2240–4.

15. Clinical and Laboratory Standards Institute [CLSI] Performance Standards for Antimicrobial Susceptibility Testing; twenty second Informational Supplement. CLSI document M100-S22, 2012,ISBN 1-56238-785–5.

16. Rahimi, E. and Alian, F. Presence of enterotoxigenic Staphylococcus aureus in cow, camel, sheep, goat, and buffalo bulk tank milk. Veterinarski Arhiv2013; 83: 23–30.

17. Ungureanu, A., Zlatian, O., Mitroi, G., Drocas, A., Tirca, T., Calina, D., Dehelean, C., Docea, A.O., Izotov, B.N., Rakitskii, V.N., Cioboata, R., Spandidos, D.A., Tsatsakis, A.M. and Gaman, A. Staphylococcus aureus colonisation in patients from a primary regional hospital. Mol Med Rep 2017;16: 8771–80.

18. Rahimi, F., Bouzari, M., Katouli, M. and Pourshafie, M. Antibiotic Resistance Pattern of Methicillin Resistant and Methicillin Sensitive Staphylococcus aureus Isolates in Tehran, Iran. Jundishapur J Microbiol 2012;6: 144–9.

19. Aires-de-Sousa, M., Parente, C.E., Vieira-da-Motta, O., Bonna, I.C., Silva, D.A. and de Lencastre, H. Characterization of Staphylococcus aureus isolates from buffalo, bovine, ovine, and caprine milk samples collected in Rio de Janeiro State, Brazil. Appl Environ Microbiol2007; 73: 3845–9.

20. Aqib, A.I., Ijaz, M., Anjum, A.A., Malik, M.A.R., Mehmood, K., Farooqi, S.H. and Hussain, K. Antibiotic susceptibilities and prevalence of Methicillin resistant Staphylococcus aureus (MRSA) isolated from bovine milk in Pakistan. Acta Trop 2017;176: 168–72.

21. Yokoyama, T., Honda, J., Kawayama, T., Kajimura, K. and Oizumi, K. Increased incidence of beta-lactamase-plasmid negative, high level methicillin-resistant Staphylococcus aureus (MRSA). Kurume Med J 1996;43: 199–206.

22. Nimmo, G.R. and Coombs, G.W. Community-associated methicillin-resistant Staphylococcus aureus (MRSA) in Australia. Int J Antimicrob Agents2008; 31: 401–10.

23. Unger, N.R., Osiyemi, O.O., Richardson, R.M., Jose Menajovsky, J. and Jennifer Kuretski, J. Ceftaroline and gentamicin for the treatment of daptomycin-non-susceptible meticillin-resistant Staphylococcus aureus bacteraemia and endocarditis in a pregnant patient. JMM Case Reports 2014; DOI 10.1099/jmmcr.0.002394.

24. Li, T., Lu, H., Wang, X., Gao, Q., Dai, Y., Shang, J. and Li, M. Molecular Characteristics of Staphylococcus aureus Causing Bovine Mastitis between 2014 and 2015. Front Cell Infect Microbiol 2017;7:127.

25. Klein, E.Y., Jiang, W., Mojica, N., Tseng, K.K., McNeill, R., Cosgrove, S.E. and Perl, T.M. National Costs Associated with Methicillin-susceptible and Methicillin-resistant S. aureus Hospitalizations in the United States, 2010–4. Clin Infect Dis2018; doi: 10.1093/cid/ciy399.