

## ECOLOGY OF *Staphylococcus aureus* AND ITS ANTIBIOTIC RESISTANCE GENES IN DAIRY FARMS: CONTRIBUTING FACTORS AND PUBLIC HEALTH IMPLICATIONS

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**Abstract:** Dairy farms are major sources for zoonotic *Staphylococcus aureus* pathogens and their antibiotic resistance genes. This study was conducted to assess critical sources and factors related to dissemination of *S. aureus* and its resistance genes within dairy farms in Egypt. In addition, workers' knowledge, attitudes and practices (KAPs) was evaluated. A total of 102 pooled samples were collected from 3 medium-scale dairy farms in Egypt. *S. aureus* was detected in 72.5% of the examined samples: lactating cows (72.9%), workers (81.5%), barns environment (88.9%), milking equipment (40%), and bulk tank milk (100%). Cows (udder milk and nostril), workers (hand skin and nostril), barns, open-sides parlor, and lack of acid rinse were associated with *S. aureus* contamination of milking equipment ( $P= 0.004 - 0.04$ ). Methicillin resistant *S. aureus* (MRSA, *mecA+*), vancomycin-resistant *S. aureus* (VRSA, *vanA+*) and methicillin-vancomycin-resistant *S. aureus* (MVRSa, *mecA+vanA+*) represented 27.5%, 5% and 12.5% of *S. aureus* isolates, respectively. This is the first report of MVRSa in dairy farms in Egypt. For workers KAPs, 48.7% didn't know milk-borne zoonoses, while their high risk practices included consumption of raw milk (52.2%), lack of hand wash (48.7%), and willing to work with sore throat (82.6%). This study highlights the critical sources of *S. aureus* pathogens and their antibiotic resistance genes in dairy farms. This will help in reforming biosecurity plans in dairy farms; an urgent demand for consumers safety in Egypt.

**Key words:** *Staphylococcus aureus* ecology; dairy farms; antibiotic resistance genes; public health implications

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### Introduction

*Staphylococcus aureus* is the third most recorded etiology of food-borne diseases worldwide (1), and it is one of the top causes of mastitis in dairy animals (2). Dairy farms are major reservoirs of *S. aureus* pathogens. The pathogen can circulate within the farm through cow,

workers, farm environment and may occasionally pass to bulk tank milk posing a public health risk for consumers (1,3). Another major public health concern is the continuous evolving of antibiotic resistant strains of *S. aureus*. Methicillin resistant *S. aureus* (MRSA) is a global public health hazard. Reports of dairy farms associated MRSA are rising worldwide

(1, 4,5). Misuse and overuse of antimicrobials in dairy farms for therapy (e.g. clinical mastitis) or prevention (e.g. dry cow) may evolve resistance mechanisms in *S. aureus* and fasten the emergence of multi-drug resistant strains as MRSA. The ecology of MRSA in dairy farms is complex and the exchange of strains in-between cows, workers and farm environment was recorded (5). This continuous inter-sources transaction helps in persistence of MRSA infection in the farm, which may pose an animal health risk for dairy animals and a zoonotic risk for workers. Occupation exposure to MRSA may be increased by inadequate awareness and unhygienic practices as raw milk consumption, inadequate use of personal protective equipment (PPE) or hand washing (6). Dairy farm associated MRSA threat may extend to public health if these strain gain access to bulk milk or spread environmentally through contaminated air, water, or manure to population in close proximity to dairy farms (2).

In last few years, a new resistance genotype assigned as vancomycin resistant *S. aureus* (VRSA) has emerged when a MRSA isolate gained *vanA* gene and expressed resistance to vancomycin in a clinical case (7). Humans' clinical reports of VRSA infection are expanding in the Middle East, particularly in Egypt (8). However, dairy farm associated VRSA was never recorded in Egypt and also very limited data are available worldwide (9).

Identifying critical sources of *S. aureus* propagation in dairy farms will guide implementation of preventive strategies to improve both animal health and consumers' safety. Therefore, the objectives of the study were to evaluate the role of lactating cows, workers and barns environment as potential critical sources of *S. aureus* pathogens in dairy farms, to assess the factors contributing to their dissemination to milking equipment, and to determine the frequency and diversity of their antibiotic resistance genotypes. Knowledge, attitudes and practices (KAPs) of dairy workers were also recorded.

## Materials and methods

### *Dairy farms*

The study was conducted in 3 medium-scale dairy farms (A, B and C) located in Kafrelzayat district (30°49'14" N and 30°48'57" E), Gharbia Governorate, Egypt. Numbers of lactating cows were 63, 90 and 122 for A, B and C farms, respectively. Lactating cow barns in the three farms were open yards with cow sheds and soil bedding. Parlors in the three farms were pipeline milking machines. In farms A and B, the parlors were open-sides with only half length walls surrounding the parlor, while in farm C the parlor was closed with windows and gates. Teat dipping was conducted in farms B and C. Cleaning of milking equipment was conducted by hot alkaline detergent wash in farms A and B, while two-step sequential alkaline detergent wash followed by acid rinse was conducted in farm C. In farm A, milk was sold raw to consumers, while in farms B and C the milk was sold to processing plants.

### *Samples collection*

A total 102 pooled samples were collected from cows, workers, barns, milking equipment and bulk tank milk in the 3 farms during the period between October 2016 and February 2017.

Cows, workers and barn environment: all samples were collected according to Roberson et al. (10). For cows, 16 pooled samples were collected in each farm: 5 samples of composite udder milk (25 cows) and 5 samples of nasal swabs (25 cows), 3 samples (15 cows) of udder skin and 3 samples (15 cows) feces samples. For workers, 9 pooled samples were collected per farm: 3 samples (15 workers) for each of hands skin swabs, nasal swabs and stool samples. For barns environment: 3 pooled samples were collected per farm: 1 sample (500-1000 g or ml) for each of bedding, feed and water.

Milking equipment and BTM: samples were collected according to Lee et al. (3). Five pooled samples (20 teat cups) of milking equipment and one pooled sample (500 ml) of BTM were collected per each farm. All samples were transported in ice-box to the laboratory within 2 hours to be processed.

### *Detection and molecular analysis of S. aureus*

All samples were cultured on Baird Parker Agar (Oxoid, Hampshire, U.K.) supplemented with Egg Yolk Tellurite (50mL/L) (Oxoid, Hampshire, U.K.) and incubated at 37°C for 24–48 hours. Identification based on biochemical and tube coagulase tests was conducted as previously described (11).

DNA extraction from overnight broth cultures was conducted using QIAamp DNA Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Detection of *mecA* and *vanA* genes as molecular determinants of MRSA and VRSA, respectively was conducted using multiplex PCR according to Amghalia et al. (12). The primers *mecA* (F): 5'AAAATCGATGGTAAAGGTTGGC'3 and *mecA* (R): 5'AGTTCTGCAGTACCGGATTTGC'3 were used to amplify 533 bp of *mecA* gene. The primers *vanA* (F): 5'CATGAA-TAGAATAAAAGTTGCAATA'3 and *vanA* (R): 5'CCCCTTTAACGCTAATACGATCAA'3 were used to amplify 1030 bp of *vanA* gene. The reaction mixture contained 12.5 µl EmeraldAmp Max PCR Master mix (Takara Bio, Kusatsu, Japan), 1 µl (10 pmol) of each primer, 3 µl of DNA template (~ 50 ng) and water up to 25 µl reaction volume. Cycling conditions started with an initial denaturation step at 94°C for 5 min, 10 cycles of amplification (94°C for 30 sec; 64°C for 30 sec; 72°C for 45 sec), then followed by another 25 cycles of amplification (94°C for 45 sec; 50°C for 45 sec; 72°C for 1 min), and ending with a final extension at 72°C for 10 min. PCR products were electrophoresed in 2% agarose gel with ethidium bromide and photographed under UV illumination (Fig. 1).

### *Knowledge, attitudes and practices (KAPs) questionnaire*

KAPs of farm workers (23 workers) were assessed using a pre-tested semi-structured questionnaire. The questionnaire recorded knowledge of milk-borne zoonoses and practices regarding milk consumption, personal hygiene (hand washing), using PPE, and working with illness (sore throat or diarrhea).

### *Statistical analysis*

Factors association with milking equipment contamination with *S. aureus* was estimated using Fisher's Exact test and Pearson's R correlation coefficient on SPSS v19 (SPSS Inc. 2010). Significant association was recorded at  $P < 0.05$ .

### *Ethical approval*

All research details were explained to dairy farm owners and workers. Their written consents were obtained.

## **Results and discussion**

High rate of *S. aureus* dissemination in diverse sources within dairy farms will eventually reside in bulk tank milk and consequently passes to consumers as public health hazards.

### *Prevalence of S. aureus in Farm level and in bulk tank milk (BTM)*

The prevalence of *S. aureus* at farm level ranged from 67.6 to 76.5% with overall prevalence of 72.5% (Table 1). These rates were higher than other reports from dairy farms in Brazil (6.6%) and Ethiopia (19.6%) by Lee et al. (3) and Ayele et al. (6), respectively. Examined BTM samples from all farms harbored *S. aureus* (100%). Lower rate (21.7%) were reported in Brazil (3), however our finding lined with that of Haran et al. (1) who detected *S. aureus* in BTM samples from 84% of the examined dairy farms in USA.

### *Critical points for S. aureus dissemination in dairy farms*

Dairy cows, workers, barns environment, and milking equipment are critical points for dissemination of *S. aureus* within dairy farms.

### *Dairy cows*

Cows are primary reservoirs of *S. aureus* in dairy farms. The overall prevalence of *S. aureus* in Cows' samples was 72.9% (Table 1). This high rate was in line with findings of Jørgensen et al. (13), who reported *S. aureus* in 90.1% of examined cows in a small-scale dairy farm in Norway. *S. aureus* was detected in udder milk (86.7%), nostrils (80%), feces

(55.6%), and udder skin (55.6%) samples (Table 1). Prevalence of *S. aureus* in udder milk in this study was higher than other reports (5.5 – 36.4%) from Norway (13), Brazil (3), and Ethiopia (6). Nostrils carriage rate was higher than another report (3.2%) in Turkey (14). Fecal prevalence rate in this study was higher than previous reports (1.6 - 12%) in Greece (15) and USA (10). Udder skin rate was higher than that reported (8.4%) in USA (10) but lower than that

reported (90.1%) in Norway (13). There was no association between cows' samples (udder milk, nostrils, feces or udder skin). In contrast, Piccinini et al. (16) suggested a persistent association between udder skin and intra-mammary infection in dairy cows. However, our finding agreed with Zadoks et al. (17), who reported minor role of teat skin infection in intra-mammary carriage of *S. aureus*.

**Table 1:** Frequency distribution of *S. aureus* pathogens and their association with milking equipment contamination in examined dairy farms

Variables		Farm A	Farm B	Farm C	Total	<i>P</i> -value
Cows	Udder Milk	100	80	80	86.7	0.01
	Nostril	80	60	100	80	0.03
	Udder skin	66.7	0	100	55.6	0.4
	Feces	33.3	66.7	66.7	55.6	0.4
	Subtotal	75	56.3	87.5	72.9	0.02
Workers	Nostril	66.7	100	100	88.9	0.02
	Hand skin	100	100	100	100	0.004
	Stool	0	66.7	100	55.6	0.4
	Subtotal	55.6	88.9	100	81.5	0.01
Barns		100	100	66.7	88.9	0.02
Milking equipment		40	80	0	40	-
Bulk milk		100	100	100	100	-
Total		67.6	73.5	76.5	72.5	-

\*All frequency results are represented as percent

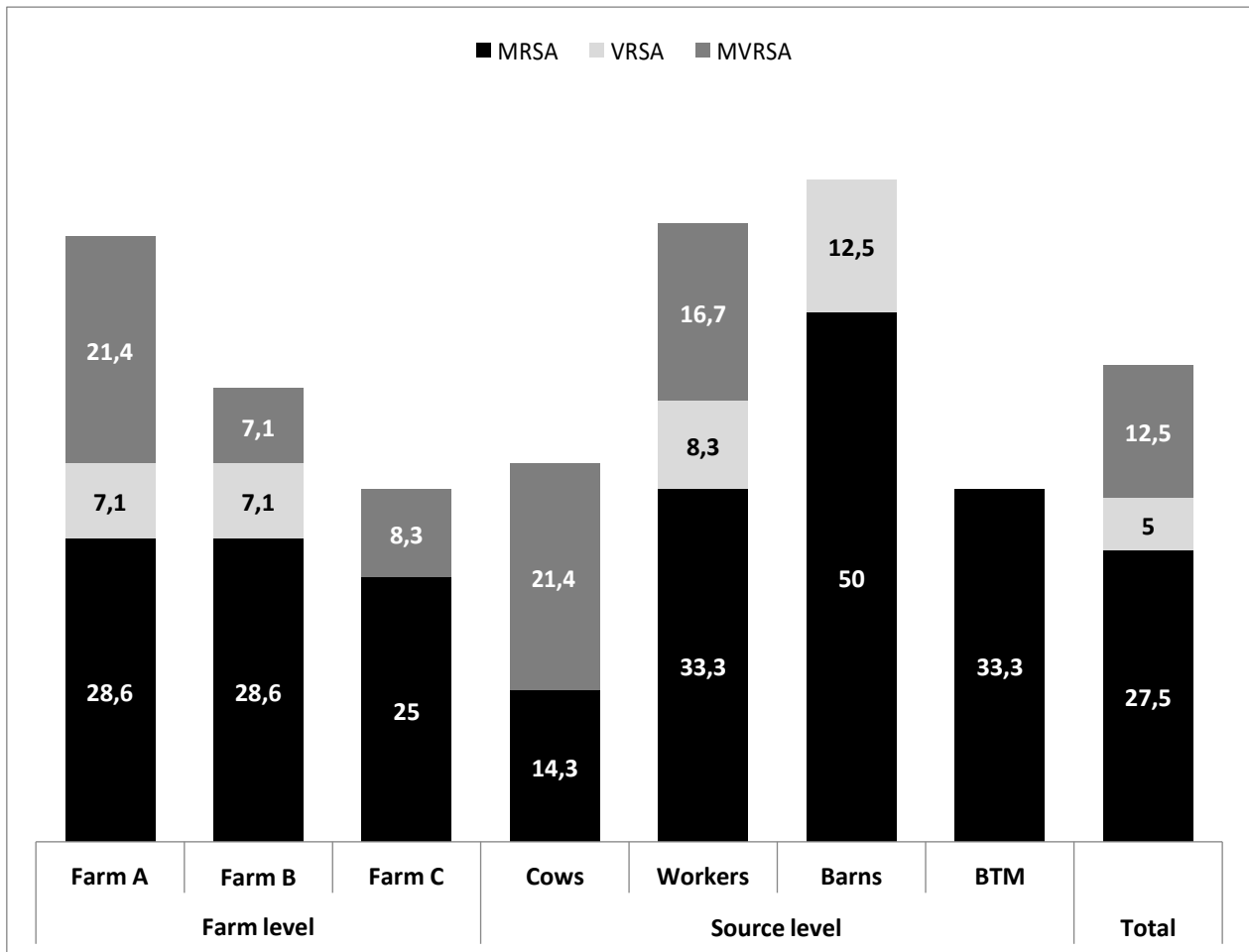
**Table 2:** Parlor design and practices associated with milking equipment contamination with *S. aureus* in this study

Variable	Categories	Farms			Percent	<i>P</i> -value	
		A	B	C		F	R
Parlor design	Open-sides	1	1	0	66.7	0.04	+0.02
	Close-sides	0	0	1	33.3		-0.02
Parlors practices	Teat dip	0	1	1	66.7	0.7	1
	No Teat dip	1	0	0	33.3		1
	>Once/day cleaning <sup>1</sup>	0	1	1	66.7	0.7	1
	Once/day cleaning	1	0	0	33.3		1
	Acid rinse	0	0	1	33.3	0.04	-0.02
	No acid rinse	1	1	0	66.7		+0.02

<sup>1</sup>: cleaning of milking equipment after milking cycle. F: Fisher's Exact test R: Pearson's R correlation coefficient



**Figure 1:** Molecular detection of antibiotic resistance genes (*mecA* and *vanA*) among *S. aureus* pathogens isolated from different samples in farm A. *mecA* gene: 533 bp, *vanA* gene: 1030 bp, Lanes 1-6: Cows' isolates, Lane 7: Milking equipment's isolates, Lanes 8-10: Barns' isolates, Lanes 11-14: Workers' isolates, M: 100 bp DNA marker, P: Positive control, and N: Negative control



**Figure 2:** Frequency distribution of antibiotic resistance genotypes among *S. aureus* pathogens isolated from examined farms. MRSA: Methicillin resistant *S. aureus* (*mecA*+), VRSA: Vancomycin-resistant *S. aureus* (*vanA*+), and MVRSA: Methicillin-vancomycin-resistant *S. aureus* (*mecA*+ -*vanA*+). BTM: Bulk tank milk. All frequency results are represented as percent

### *Farm workers*

Farm worker are at risk of occupational infection with livestock associated *S. aureus* infection. In addition, they may play a critical role in dissemination of infection to cows, equipments, farm environment, and milk (3, 10, 17). The overall prevalence of *S. aureus* in workers' samples was 81.5%, which was higher than a report (27%) in USA (10). *S. aureus* was detected in nostrils (88.9%), hand skin (100%), and stool (55.6%) samples from workers in this study (Table 1). Nostrils prevalence was higher than a report (29.3%) in Turkey (14). Lower rates for hand skin (3.3 - 32%) were reported in Brazil (3) and Ethiopia (6). Intestinal colonization and stool carriage of *S. aureus* in humans were previously reported (18). There was a significant association ( $P= 0.04$ ) between hand skin and fecal carriage of *S. aureus*. Same association was recorded in USA (18). This could be attributed to lack of adequate personnel hygiene of examined workers as improper hand washing. No other association was recorded between workers samples.

### *Barns environment*

Barns environment may act as a vehicle for *S. aureus* transmission to cows, workers, farm equipment and BTM (10, 13). *S. aureus* was detected in 88.9% of the examined barn samples (Table 1), which was higher than other reports (1-20%) in USA (10) and Norway (13).

### *Milking equipment*

Milking equipment are critical vehicles for dissemination of *S. aureus* between individual dairy cows as well as from barns, cows and worker to milk (3, 17). In the present study, *S. aureus* was isolated from 40% of milking equipment (Table 1). This was higher than other reports (2.1-11.1%) in USA (10), Brazil (3) and Ethiopia (6).

High rates of *S. aureus* detection in various sources in examined dairy farms highlight their role as critical points for *S. aureus* dissemination and may also refer to unsanitary dairy farm practices. This is an alarming threat for both

dairy animals and public health. Differences in the prevalence rates may be due to variations in the farm sanitary conditions, animal breeds, animal health status (e.g. subclinical mastitis), sampling strategies, seasons and geographical locations.

### *Factors contributing to milking equipment contamination with S. aureus in dairy farms*

Carriage of *S. aureus* via cows, workers and barns was significantly associated with milking equipment contamination ( $P= 0.01 - 0.02$ ) (Table 2). Nostrils (cows and workers), udder milk (cows) and hand skin (workers) were the sources that contributed significantly to milking equipment contamination ( $P= 0.004 - 0.03$ ) (Table 2). Our finding agreed with Zadoks et al. (17) who confirmed *S. aureus* transmission from hand skin and udder milk to the milking equipment.

Open-sides parlors design positively associated with milking equipment contamination ( $P= 0.04$ ) (Table 2). This could be linked to the significant effect of contaminated barns environment. With open-sides parlors walls, air drafts can introduce infection from contaminated barns to parlors during and in between milking cycles.

Lacking of acid rinse was significantly associated with milking equipment contamination ( $P= 0.04$ ) (Table 2). This finding agreed with Elmoslemay et al. (19) who reported the positive association between inadequate acid rinse and milk contamination within dairy farms. Acid rinse removes milk stones, which could act as niches for microbial growth within milking equipment (19). Also acid has antibacterial activity against broad range of bacteria (20). Both mechanisms may explain the significant effect of acid rinse in reducing *S. aureus* contamination of milking equipment.

Teat dip was not associated with milking equipment contamination in this study (Table 1). However it may be contributed to the elimination of teat skin role in contaminating milking equipment as recorded in this study ( $P= 0.4$ ) (Table 1).

### *Frequency distribution of S. aureus antibiotic resistance genotypes in dairy farms*

Almost half (45%) of the *S. aureus* isolates carried at least 1 antibiotic resistance gene. MRSA (*mecA*+), VRSA (*vanA*+), and MVRSA (*mecA*+, *vanA*+) resistance genotypes represented 27.5%, 5%, and 12.5% of *S. aureus* isolates respectively (Fig. 2). MRSA isolates were detected in cows, workers, barns, and BTM. VRSA isolates were from workers and barns, while MVRSA isolates were from cows and workers. Hence, workers were the only source that harbored the 3 resistance genotypes in the examined farms. In agreement with our findings, MRSA isolates were detected in cows, workers, and environment of dairy farms in Korea (4) and Italy (5). VRSA isolates were reported in cows with mastitis in China (9). However, as far as we know, this the first report of MVRSA in dairy farms in Egypt. Detection of MVRSA isolates in dairy farms is alarming and their emergence requires further investigation. MRSA acquiring *vanA* gene by plasmid transfer was previously reported in human clinical cases (21). Same mechanism of gene transfer could explain the emergence of MVRSA in this study. High rate of inter-sources transmission and mixing of *S. aureus* isolates within same farm may facilitate this gene transfer. The detection of the 3 genotypes in 2 farms (A and B) and the findings of Locatelli et al. (5), who reported MRSA genotypes exchange between cows, workers and environment within same dairy farm, support our hypothesis. Yet, further investigation is required to elucidate the ecology and molecular bases of MVRSA emergence in dairy farms.

### *Knowledge, attitudes and practices of farm workers*

Among respondents, 47.8% lacked awareness regarding milk-borne zoonoses, which was lower than that (87%) reported in Ethiopia (6). Raw milk consumption was reported by 52.2% of the workers. This was higher than another report (35%) of raw milk consumption by dairy workers in Ethiopia (6). None of workers used PPE (gloves or masks) during work. Around half (47.8%) of workers don't wash their hands,

which was lower than that reported in Ethiopia where none of the workers (100%) wash their hands (6). Finally, 82.6% and 73.9% of the worker would work with sore throat and diarrhea, respectively. Lack of awareness and unhygienic practices (raw milk consumption, lack of hand wash and PPE use) may pose an occupational zoonotic threat for the workers in these farms. In addition the unhygienic practices and willing to work with illness may contribute to the significant role of workers in disseminating *S. aureus* contamination in examined farms.

## Conclusion

This study records high dissemination rate of *S. aureus* pathogens and their antibiotic resistance genes in dairy farms in Egypt, which may impact the health of dairy products consumers in Egypt. The study also highlights the critical points and practices associated with *S. aureus* dissemination in dairy farms, which will help in improving biosecurity planning and application in dairy farms in Egypt.

## References

1. Haran KP, Godden SM, Boxrud D, Jawahir S, Bender JB, & Sreevatsan S. Prevalence and Characterization of *Staphylococcus aureus*, Including Methicillin-Resistant *Staphylococcus aureus* isolated from Bulk Tank Milk from Minnesota Dairy Farms. *Journal of Clinical Microbiology* 2012; 50: 688–95.
2. Smith TC. Livestock-Associated *Staphylococcus aureus*: The United States Experience. *PLoS Pathogens* 2015; 11: e1004564.
3. Lee SH, Camargo CH, Gonçalves JL, Cruz AG, Sartori BT, Machado MB & Oliveira CA. Characterization of *Staphylococcus aureus* isolates in milk and the milking environment from small-scale dairy farms of São Paulo, Brazil, using pulsed-field gel electrophoresis. *Journal of Dairy Science* 2012; 95: 7377–83.
4. Lim SK, Nam HM, Jang GC, Lee HS, Jung SC & Kim TS. Transmission and persistence of methicillin-resistant *Staphylococcus aureus* in milk, environment, and workers in dairy cattle farms. *Foodborne Pathogens and Diseases* 2013; 10: 731–6.
5. Locatelli C, Cremonesi P, Caprioli A, Carfora V, Ianzano A, Barberio A, Morandi S, Casula A, Castiglioni B, Bronzo V & Moroni P. Occurrence of methicillin-resistant *Staphylococcus aureus* in dairy

cattle herds, related swine farms, and humans in contact with herds. *Journal of Dairy Science* 2017; 100: 608–19.

6. Ayele Y, Gutema FD, Edao BM, Girma R, Tufa TB, Beyene TJ, Tadesse F, Geloye M & Beyi AF. Assessment of *Staphylococcus aureus* along milk value chain and its public health importance in Sebeta, central Oromia, Ethiopia. *BMC Microbiology* 2017; 17: 141.

7. Centers for Disease Control and Prevention (CDC). *Staphylococcus aureus* resistant to vancomycin—United States, 2002. *Morbidity and Mortality Weekly Report (MMWR)* 2002; 51: 565–7

8. Rahimipour F, Ghazvini K & Youssefi M. Reports of Vancomycin-Resistant *Staphylococcus aureus* from Middle East Countries, *Archives of Clinical Infectious Diseases* 2018; 13: e59522.

9. Feng Y, Qi W, Xu-rong W, Ling W, Xin-pu L, Jin-yin L, Shi-dong Z, Hong-sheng L. Genetic characterization of antimicrobial resistance in *Staphylococcus aureus* isolated from bovine mastitis cases in Northwest China. *Journal of Integrative Agriculture* 2016; 15: 2842–7.

10. Roberson JR, Fox LK, Hancock DD, Gay JM & Besser TE. Ecology of *Staphylococcus aureus* isolated from various sites on dairy farms. *Journal of Dairy Science* 1994; 77: 3354–64.

11. Elmonir W, Abo-Remela EM & Sobeih A. Public health risks of *Escherichia coli* and *Staphylococcus aureus* in raw bovine milk sold in informal markets in Egypt. *Journal of Infection in Developing Countries* 2018; 12: 533–41.

12. Amghalia E, AL-Haj N, Shamsudin M, Radu S, Rosli R, Neela V & Rahim R. Multiplex PCR assays for the detection of clinically relevant antibiotic resistance genes in *Staphylococcus aureus* isolated from Malaysian hospitals. *Research Journal of Biological Sciences* 2009; 4: 444–8.

13. Jørgensen HJ, Mørk T & Rørvik LM. The occurrence of *Staphylococcus aureus* on a farm with small-scale production of raw milk cheese. *Journal of Dairy Science* 2005; 88: 3810–7.

14. Garipcin M & Seker E. Nasal carriage of methicillin-resistant *Staphylococcus aureus* in cattle

and farm workers in Turkey. *Veterinarski Arhiv* 2015; 85: 117–29.

15. Dimitracopoulos G, Kalkani-Boussiakou H & Papavassiliou J. Animal fecal carriage and biotypes of *Staphylococcus aureus*. *Applied and Environmental Microbiology* 1977; 34: 461–4.

16. Piccinini R, Cesaris L, Daprà V, Borromeo V, Picozzi C, Secchi C & Zecconi A. The role of teat skin contamination in the epidemiology of *Staphylococcus aureus* intramammary infections. *Journal of Dairy Science* 2009; 76: 36–41.

17. Zadoks RN, van Leeuwen WB, Kreft D, Fox LK, Barkema HW, Schukken YH & van Belkum A. Comparison of *Staphylococcus aureus* isolates from bovine and human skin, milking equipment, and bovine milk by phage typing, pulsed-field gel electrophoresis, and binary typing. *Journal of Clinical Microbiology* 2002; 40: 3894–902.

18. Bhalla A, Aron DC & Donskey CJ. *Staphylococcus aureus* intestinal colonization is associated with increased frequency of *S. aureus* on skin of hospitalized patients. *BMC Infectious Diseases* 2007; 7: 105.

19. Elmoslemany AM, Keefe GP, Dohoo IR, Wichtel JJ, Stryhn H & Dingwell RT. The association between bulk tank milk analysis for raw milk quality and on-farm management practices. *Preventive Veterinary Medicine* 2010; 95: 32–40.

20. Reinemann DJ, Wolters GMVH, Billon P, Lind O, Rasmussen MD. Review of practices for cleaning and sanitation of milking machines. 2000. Available at <https://milkquality.wisc.edu/wp-content/uploads/sites/212/2011/10/review-of-practices-for-cleaning.pdf> (Accessed 10 November 2018).

21. Rossi F, Diaz L, Wollam A, Panesso D, Zhou Y, Rincon S, Narechania A, Xing G, Di Gioia TS, Doi A, Tran TT, Reyes J, Munita JM, Carvajal LP, Hernandez-Roldan A, Brandão D, van der Heijden IM, Murray BE, Planet PJ, Weinstock GM & Arias CA. Transferable vancomycin resistance in a community-associated MRSA lineage. *New England Journal of Medicine* 2014; 370: 1524–31.