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Occurrence of *Staphylococcus aureus* in dairy farms and humans with reference to antimicrobial profile in Qalyobia governorate

Manar, G.A. Sallam [1], Nashwa O. Khalifa [1], Ashraf M.A Barakat [2] , Lobna M.A. Salem [1]

[1] *Department of Zoonosis, Faculty of Veterinary Medicine, Benha University, Egypt*

[2] *Department of Zoonosis, National Research Center, Dokki, 12622, Egypt*

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ABSTRACT

Staphylococcus aureus is considered one of the Zooanthroponotic diseases that normally colonize skin and mucus membrane of human and can also spread from human to animals by direct or indirect contact during milking and causes disease to animal and human. The occurrence of *S. aureus* was determined by using conventional cultural techniques and biochemical identification. A total of 304 samples were collected from different dairy farms, 237 animal samples collected from (66 cows and 13 buffaloes) including 3 samples (milk sample, teat swab and nasal swab) from each animal.In addition to 15 samples were collected from dairy utensils (6 Hand and 9 machine milking). As well as 52 samples from dairy workers (26 nasal swabs and 26 hand swabs).The results of nasal swabs were 3/66(4.55%) and 2/13(15.38%)from cattle and buffaloes respectively, and teat swabs were 4/66(6.06%) and2/13(15.38%)from cattle and buffaloes respectively.Also, 5/66(7.58%) of cow's milk were positive for *S.aureus*. Moreover, the results of machine and hand milking dairy utensils were 11.11% and 50%respectively. The results of dairy worker's hand and nasal swabs were 23.08% and 15.38%.*S. aureus* isolates (n=30) showed resistance againstamoxicillin/clavulanic acid (96.7%), ampicillin (90%), and cefotaxime (53.3%).and greater sensitivity to levofloxacin (100%), ciprofloxacin (70%), erythromycin and azithromycin (both 56.7%), ceftriaxone (26.7%). The risk of contamination of the milk increased by contaminated hand workers, dirty utensils with poor hygiene of the farm.

1. INTRODUCTION

Staphylococcus aureus is a major zoonotic pathogen that not only produce large economic losses in dairy animal production but also poses a risk to public health (Zhou *et al*., 2017).*Staphylococcus aureus* is a well-known opportunistic bacterium that can infect dairy animals as well as people. It is the third most prevalent causes of foodborne illness globally (Banu *et al*., 2022).The bacterium is a frequent colonizer and is thought to be a part of the natural commensal flora of people and animals, colonizing about 30–50% of the human population(Dweba *et al.*, 2018). In humans, *S. aureus* colonizes the skin and nasal passages. Additionally, it is an opportunistic pathogen that can cause potentially fatal invasive diseases such bacteremia, pneumonia, endocarditis, and osteomyelitis in addition to superficial skin and soft tissue infections(Crosby *et al*., 2016). The pathogenicity of *Staphylococcus aureus*, which causes numerous human illnesses is primarily regulated by a number of virulence factors (Rasmi *et al*., 2022). Human staphylococcus occurs in three significant forms: food poisoning, suppurative disease or as a carrier state (subclinical commensal infection).*Staphylococcus aureus* may be the principal food-borne pathogen that affects people and animals with health issues. Additionally, it is one of the main contributors of bovine mastitis, which has a significant negative impact on milk quality and production, causing financial losses in the dairy industry (Merz *et al*., 2016).

Staphylococcus aureus is a major cause of intramammary infections in dairy cows; the various prevalence's of mastitis reported may be due to a combination of *S. aureus* virulence factors other than host factors. *S. aureus* produces haemolysins, leukocidins, enterotoxins, and superantigens that cause intramammary infection and aid the pathogen in evading the immunological response of the host (Magro *et al*., 2017). Staphylococcal food poisoning is produced by ingesting the toxins that *S. aureus* produces. Food handlers have enterotoxin-producing *S. aureus* on their hands, in their noses, and are the primary direct or indirect sources of food contamination (Argudín *et al*., 2010)Additionally, it is crucial to take consideration that this organism has the capacity to develop resistance to nearly all antimicrobial drugs (Zhang *et al*., 2018). *Staphylococcus aureus'* development of antibiotic resistance presented a significant veterinary and public concern on a global scale. As a very adaptable pathogen, *S. aureus* can rapidly acquire resistance genes. Bacterial resistance has existed before the widespread use of antibiotics. Yet, since the discovery of penicillin-resistant *S. aureus* in the early 1940, reports of *S. aureus* resistance developments have been made. Since then, *S. aureus* has gained widespread recognition as the most frequent reason for nosocomial, public, and livestock-related infection (Bitrus *et al*., 2018). The aim of this study was to detect the presence of *S. aureus* in milk; nasal, teat swabs of dairy animals; utensils and also, hand swabs and nasal swabs

Correspondence to: manar.sallam@fvtm.bu.edu.eg

from dairy workers from different dairy farms, also detect its antimicrobial resistance and risk factors.

2. MATERIAL AND METHODS

2.1. Sampling

A total of 304 samples include (237 animal samples, 15 utensils and 52 human samples) were collected from four different dairy farms in Qalyobia governorate

2.1.1. Animal samples

A total of 237 samples were collected from 79 Animals (66 cows and 13 buffaloes). From each animal, we collected 3 samples including milk sample, teat swab and nasal swab.in addition to 15 samples were collected from dairy utensils (Hand and machine milking). Milk sample were collected from the udder of the examined dairy animals according to Hogan *et al*., (1999). Teat skin swabs were collected according to Da Costa *et al*., (2014). The swabs were immediately put into sterile tubes with 5 ml of buffered peptone water after collection and carried in ice box to the laboratory to be examined. Nasal swabs were collected according to VandenBergh *et al*., (1999).Sample from dairy utensils used in hand and machine milking(Automated milking suction machine with teat cups) according to Silva et al., (2000) by using sterile swabs previously soaked in buffered peptone water.

2.1.2. Human samples

A special consent was obtained from dairy workers at each dairy farm for collection of hand and nasal swabs. Ethical approval number (BUFVTM-18-06-23). A total of 52 human samples included nasal swabs from the anterior nares and hand swabs from the palm of the hand and area between fingers, and also finger tips and nails. The collected samples were labeled and transport in ice box to the laboratory for examination (table 1).

Table (1): The number and type of collected samples from each farm

Farm	Animals	Milk samples	Teat swabs	Nasal swabs	Dairy utensils	Human nasal swabs	Human hand swabs	Total human samples
Farm(1)	16	16	16	16	3(Hand milking)	3	3	6
Farm(2)	3	3	3	3	2(Hand milking)	3	3	6
Farm(3)	8	8	8	8	1(Hand milking)	5	5	10
Farm(4)	52	52	52	52	9(Machine milking)	15	15	30
Total	79	79	79 (69 healthy animals and 10 with skin lesion)	79 (75 healthy animals and 4 with nasal catarrh)	15	26 (25 healthy and 1 with nasal catarrh)	26 (19 healthy and 7 with skin lesion)	52

2.2. Isolation of S. aureus

Pre-enrichment was carried out in Buffered peptone water (BPW) (Oxoid, CM509, Adelaide, Australia) (Addis *et al*., 2011).5 ml of milk sample were homogenized with 45 ml of BPW for pre-enrichment and then incubated at 37°C for 24 hours. Hand, nasal, teat swabs and milking utensils samples were inserted into sterile tubes with 5ml BPW, and then incubated at 37°C for 24 hours. Isolation of *S. aureus* was attempted according to Singh *et al*., (2008)with slight modification. The selective medium used for isolation of *S. aureus* was Baird Parker Agar (BPA) (HIMedia PvtLtd). A loopful from the pre enriched samples in BPW were streaked on BPA and incubated for 48 hours at 37°C. Suspected colonies (black, shiny, convex colonies surrounded by clear halo zone) were streaked on Mannitol salt agar plates (HIMedia™ PvtLtd) and then incubated at 37°C for 24-28 hours for further identification.

2.3. Biochemical identification

2.3.1. Staining technique (Habib et al., 2015)

Smears of the isolated colonies of pure culture were prepared and stained with Gram's Method and the characteristics of the organisms were recorded as its being Gram-negative or Gram-positive.

2.3.2. Coagulase test: according to Gillies and Dodds, (1984) using slide test and tube test

2.3.3. Catalase Test(Harrigen and McCance, 1966)

Add 2-3 drops of 3% hydrogen peroxide to 24 hours agar slant culture, bubbling will instantly occur and is regarded as positive and indicate presence of catalase.

2.4. Determination of Antimicrobial resistance of S. aureus isolates

Thirty *S. aureus* isolates were tested using the disk diffusion method on Mueller- Hinton agar plates (MHA) (Oxoid) for 8 commonly used antibiotics (Oxoid). The antibiotic discs used in this study included ampicillin (AM 10), amoxicillin/ clavulanic acid (AMC30), ceftriaxone (CRO30), ciprofloxacin (CIP5), erythromycin (E15), cefotaxime (CTX30), levofloxacin (LEV5), azithromycin (AZM15) were applied onto MHA plates .the plates were aerobically incubated at 37°c for 24 h, and the diameter of inhibition zone was measured (in mm)(CLSI, 2011).

2.5. Risk factors associated with S. aureus infection within the examined dairy farms

Investigation of risk factors for infection with *S. aureus* in dairy farms using history data which covered those points (type of milking, disinfection of milking equipment, signs of mastitis and hygienic condition of the farm).

3. RESULTS

3.1. Occurrence of S. aureus from the samples

Out of 304 samples from tested dairy animal ,human and dairy utensils that were cultured on Baired parker Agar(BPA) and Mannitol salt agar we found that 136 strains were identified on BPA as typical colony(black, shiny, convex colonies 1-4 mm in diameter) appeared after 24 h of incubation due to tellurite reduction , lightening clear halo develops around colonies from coagulase positive Staphylococcus aureus, and upon further incubation after 48 h produce an opaque zone due to an egg yolk – lecithinase reaction (lypolytic activity) (fig1). On Mannitol salt agar appear yellow colony surround by yellow halo (Mannitol fermentation).*S. aureus* isolates showed positive results for gram staining with grape like clusters gram positive cocci by oil immersion lens(fig1). *Staphylococcus aureus* isolates were positive for coagulase, catalase. Out of 136 strains, 30

strains were purified as coagulase positive *S. aureus as* showed in table (2) and table (3).

Table (2): Percentage of *S. aureus* isolated from the examined animal samples

Species		Cow	buffaloes	Total
Nasal swabs from	A	63	12	75
	B	3	1	4
	Total	66	13	79
Teat swabs from	A	58	11	69
	C	8	2	10
	Total	66	13	79
Positive No and % from nasal swabs	A	2(3.17)	1(8.33)	3(4)
	B	1(33.33)	1(100)	2(50)
	Total	3(4.55)	2(15.38)	5(6.33)
Positive No and % from teat swabs	A	2(3.45)	0(0)	2(2.89)
	C	2(25)	2(100)	4(40)
	Total	4(6.06)	2(15.38)	6(7.59)
Milk sample	No	66	13	79
	Positive No and %	5(7.58)	0	5(6.33)
Dairy utensils	No	9 Machine milking + 6 Hand milking		15
	Positive No and%	1(11.11) + 3 (50)		4(26.67)

A: apparently healthy animal, B: animal with nasal cattarhal, C: animal with skin lesion

Table (3): Percentage of *S. aureus* isolated from Dairy worker samples

Samples	Examined. No.	Positive	%
Nasal swabs from			
A	25	3	12
B	1	1	100
Total	26	4	15.38
Hand swabs from			
A	19	2	10.53
C	7	4	57.14
Total	26	6	23.08
Total hand and nasal swabs	52	10	19.23

A: apparently healthy human, B: Human with nasal catarrh, C: Human with skin lesion

3.2. Antimicrobial resistance of S. aureus isolates

We performed the antimicrobial resistance analysis of the purified *S. aureus* isolates (n=30) obtained from animal, human and utensil samples using disc diffusion technique (fig 2). A total of 8 antibiotics were used in this analysis. Diameter of inhibition zone was measured (in mm) and compared to CLSI (2011).The antibiotic sensitivity test results were listed in table (4)

Table (4): Antimicrobial resistance of *S. aureus* isolates (n=30)

		Resistance		Intermediate		Sensitive	
Antibiotic discs	Antimicrobial classes	No	%	No	%	No	%
Ampicillin (AM10)	Penicillins	27	90	0	0	3	10
Amoxicillin/ clavulanicacid (AMC30)	B-lactams	29	96.7	0	0	1	3.3
Ceftriaxone (CRO30)	Cephalosporins	1	3.3	21	70	8	26.7
Cefotaxime (CTX30)	Cephalosporins	16	53.3	10	33.3	4	13.3
Levofloxacin (LEV5)	Fluoroquinolones	0	0	0	0	30	100
Ciprofloxacin (CIP5)	Fluoroquinolones	4	13.3	5	16.7	21	70
Erythromycin (E15)	Macrolides	10	33.3	3	10	17	56.7
Azithromycin (AZM15)	Macrolides	7	23.3	6	20	17	56.7

% according to total number of S. aureus isolates (n=30)

3.2. Risk factors associated with S. aureus infection

The results of the questionnaire listed in table (5)

Table (5): Risk factor associated with contamination of milk according to the collected history data

Variable	Examined. No.	Positive	%
Milk collection			
Automatic	52	1	1.92
Manual	27	4	14.81
Total	79	5	6.33
Disinfection of milking utensils			
Yes	9	1	11.11
No	6	3	50
Total	15	4	26.67
Hygienic condition of the farm			
Good	197	9	4.57
poor	107	21	19.63
Total	304	30	9.87
Signs of mastitis			
Yes	9	2	22.22
No	70	3	4.29
Total	79	5	6.33

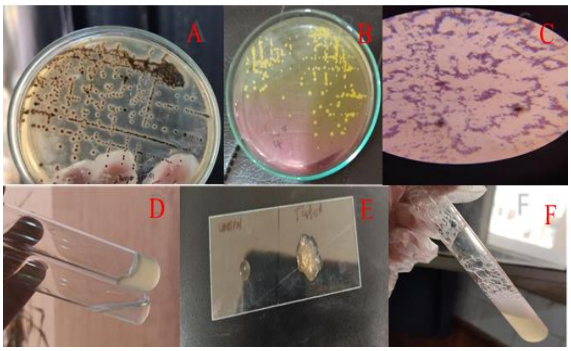

Fig.1. Isolation and identification of *staph. aureus*

A**. S. aureus colonies on Baird parker Agar (Black, shiny, convex colonies 1-4 mm in diameter surrounded by outer clear halo and opaque zone).*, ***B**. S. aureus on Mannitol salt agar.*,C**. Gram staining of S. aureus showing grape like clusters.*,***D**. Positive tube coagulase test (The entire contents of the tube were coagulated and were not displaced when the tube was inverted),* ***E**. Positive slide coagulase test (Staph aureus clumped within 15 seconds).*,***F**. Positive catalase test (Bubbling will instantly occur)*

Fig. 2. Antimicrobial susceptibility test for *S. aureus*

4. DISCUSSION

The results recorded in table (2) revealed that the occurrence of *S. aureus* in the examined 66 cows was 4.55% from nasal swabs (33.33% with nasal catarrh and 3.17% apparently healthy animals), 6.06 % from teat swabs (25% with skin lesion and 3.45%apparently healthy animals) and 7.58% from milk. These results were nearly similar to (Dastmalchi *et al*., 2020)(5.1%)for nasal swabs,(Tibebu *et al*., 2021)(10%) for teat swabs and(Sotohy *et al*., 2022)(11.6%) for milk, higher than (Leigue *et al*., 2017)(2%) for milk and lower than , (Rasol *et al*, 2023)(62.7%)for nasal swabs and (Badawy *et al.*, 2022)(25%) for teat swabs .

Concerning the occurrence of *S. aureus* in the examined 13 buffaloes, it was 15.38% from nasal swabs (100% with nasal catarrh and 8.33% apparently healthy animals), 15.38% from teat swabs (100% with skin lesion and 0% apparently healthy animals) .These results were nearly similar to (Badawy *et al*., 2022) (16.7%) for teat swabs, higher than (Badua *et al*., 2020) (7.03%)and lower than , (Hassan *et al*.,

2017) (40%)for nasal swabs. In this study, *S. aureus* not isolated in buffaloes' milk, this may be attributed to *S. aureus* was the most common pathogens found (76.2% of the pathogens found), and the prevalence of mastitis was reported to be 10% per cow on a private farm. On the other hand, mastitis frequency reached as high as >60% on the examined government farm that raised Holstein-Friesian cattle. Contrary to popular belief, buffaloes appear to have higher mastitis resistance than cows(Vink, 1995),It indicates that buffaloes have a stronger innate immunity to fight against infection(Chanu *et al*., 2018). The discrepancy between results of animals in this study and other authors may be due to the level of hygienic measures adopted in animal housing and environment.

Regarding to the occurrence of *S. aureus* in examined dairy utensils, it was higher in hand milking (50%) than machine milking (11.11%).This may be attributed to inadequate cleaned and sanitized hands of milkers may be a source of infectious diseases spreading during milking procedures . Assessment of the effects of manual and automated milking methods on udder health depends on the production of high-quality and sanitary milk. Therefore, hand milking is typically seen as carrying a larger risk of contamination than mechanical milking, Milker's hands can be a key factor in spreading of udder diseases; however, if machine milking is done properly, this problem may be avoided(Singh *et al*., 2019).

Staphylococcus aureus is the cause of a wide range of different kinds of major and minor pyogenic infections, and also occurs harmlessly as a commensal in the anterior nares and on moist areas of skin in 20 to 30 % of healthy persons. Examined dairy workers reveled that out of 26 nasal swabs, 4 isolates (15.38%) (100% with nasal catarrh and 12% apparently healthy humans) were obtained and 6 isolates (23.08%) (57.14% with skin lesion and 10.53% apparently healthy humans) were obtained from hand swabs. These results were nearly similar to (Pala *et al*., 2010)(15.2%) for nasal swabs, higher than (Banu *et al*., 2022)(22.73%)and lower than , (Diab *et al*., 2021)(44.2%)for hand swabs.

Results in Table (4) showed high resistance to ampicillin(90%),nearly similar to(Neelam *et al*., 2022) (89.9%) and (Gebremedhin *et al*., 2022),who detected high resistance against ampicillin (95%). Resistance rate of isolates to amoxicillin/clavulanic acid in the current study was 96.7%, nearly similar to(Rasmi *et al*., 2022),who found that 91.5% of isolates were resistant to amoxicillin/ clavulanic acid. On the other hand,21.4% of isolates were resistant to amoxicillin/clavulanic acid according to (Algammal *et al*., 2020). These results could be explained by the frequent use of beta-lactam antibiotics like ampicillin on dairy cattle farms in the study area may be the cause of the high ampicillin resistance profiles of *S. aureus* found in this investigation. The most effective antibiotics for treating mastitis are still β-lactams, which are thought to put selective pressure on *S. aureus* and increase the resistance due to their widespread use (Guimarães *et al*., 2017).

The current study also revealed resistance to cefotaxime (53.3%), nearly similar to(Elias *et al*., 2020) (47.06%) . On the other hand, (Banu *et al*., 2022)found the highest numbers of isolates were susceptible to Cefotaxime (70.7%).The current study revealed that 100% of all *S. aureus* isolates were sensitive to levofloxacin, nearly similar to(Akanbi *et al*., 2017)(86.7%) . Lower percentage was recorded by(Neelam *et al*., 2022) (16.36%).The results showed sensitivity of *S. aureus* against erythromycin and azithromycin were 56.7% for both. These were nearly similar to (Hassani *et al*., 2022) who reported that 44.68% of isolates were sensitive to azithromycin.(Algammal *et al.*, 2020) reported that 63.1 % of isolates were sensitive to erythromycin. On the other hand,(Geletu *et al*., 2022) reported that 19.4% of *S. aureus* isolates were sensitive to erythromycin while higher resistance to erythromycin, 70% was recorded by(Akanbi *et al*., 2017) .on the other hand, (Wang *et al*., 2016)who revealed that 68.6% of all *S. aureus* isolates were resistant to erythromycin and azithromycin. The table showed higher sensitivity to ciprofloxacin (70%).nearly similar to (Banu *et al*., 2022) (82.9%) .but higher than that obtained by (Tiwari *et al*., 2022) (15.8%).The sensitivity for ceftriaxone was (26.7%),and this is nearly similar to (Neelam *et al*., 2022) (29.09%).but higher than(Geletu *et al*., 2022)(0%)and lower than (Ullah *et al.*, 2022)(85%).

One of the primary and last processes that affect a dairy farm's profitability is milking. Farmers, however, deal with a number of difficulties, such as low productivity, poor hygiene, and times for daily milking. The frequency of intra mammary infections might vary according on the method of milking, whether it is by machine or by hand. Hand milking dairy cows exposes them to risks of injury, disease transmission, and incomplete udder emptying, which affects both the health and subsequent milk production of the cow (Ombuna, 2018). In this study as illustrated in table (5), the isolation of *S. aureus* in milk that collected either automated or hand was 1.92% and 14.8% respectively and the isolation rate is higher in mastitic animals than apparently healthy animals. The hygienic condition of the farm revealed 3 farms were poor hygiene and not disinfects the udder or teats or equipment and 1 farm was good. Carrier states in animals and humans are dangerous because devitalized skin or mucous surfaces generally serve as portal of entry to *S. aureus* in nasal and skin carriers, in addition environmental stresses or other diseases are also predisposing factors for changing carrier states to staphylococcal infections(Acha and Szyfres, 2003).

5. CONCLUSION

It could be concluded that *S. aureus* is ubiquitous in nature and isolated from animals' milk, teat swabs and nasal swabs with high prevalence rate occurs due to contamination by hand of dairy workers and milking utensils through hand milking indicating that there is a relationship between isolation from animals and dairy workers. The isolates have shown resistance against antibiotics duo to uncontrolled use of antibiotics in treatment of animals in dairy farms which causes problems to animal and human. Additionally, bad hygienic measure during milking procedure and poor housing of animals help spread *S. aureus* among animals.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest for current data

6. REFERENCES

1. Acha, P.N., Szyfres, B., 2003. Zoonoses and communicable diseases common to man and animals. Pan Am. Heal. Organ. Washingt. 1: 1–395.
2. Addis, M., Pal, M., N.Kyule, M., 2011. Isolation and Identification of Staphylococcus Species from Raw Bovine Milk in Debre Zeit ,Ethiopia.Vet .Res.,4:45-49.
3. Akanbi, O.E., Njom, H.A., Fri, J., Otigbu, A.C., Clarke, A.M., 2017. Antimicrobial susceptibility of Staphylococcus aureus isolated from recreational waters and beach sand in Eastern Cape Province of South Africa. Int. J. Environ. Res. Public Health. 14: 1–15.
4. Algammal, A.M., Enany, M.E., El-Tarabili, R.M., Ghobashy, M.O.I., Helmy, Y.A., 2020. Prevalence, Antimicrobial Resistance Profiles, Virulence and Enterotoxins-Determinant

Genes of MRSA Isolated from Subclinical Bovine Mastitis in Egypt. Pathogens .9: 362–372.
5. Argudín, M.Á., Mendoza, M.C., Rodicio, M.R., 2010. Food Poisoning and Staphylococcus aureus Enterotoxins. Toxins. 2: 1751–1773.
6. Badawy, B., Elafify, M., Farag, A.M.M., Moustafa, S.M., Sayed-Ahmed, M.Z., Moawad, A.A., Algammal, A.M., Ramadan, H., Eltholth, M., 2022. Ecological Distribution of Virulent Multidrug-Resistant Staphylococcus aureus in Livestock, Environment, and Dairy Products. Antibiotics .11: 1651–1662.
7. Badua, A., Boonyayatra, S., Awaiwanont, N., Gaban, P., Mingala, C., 2020. 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. J. Adv. Vet. Anim. Res., 7: 397–406.
8. Banu, M.G., Zewdu Geberemedhin, E., 2022. Occurrence and antimicrobial susceptibility of Staphylococcus aureus in dairy farms and personnel in selected towns of West Shewa Zone, Oromia, Ethiopia. PLoS One. 17: 1–19.
9. Bitrus, A.A., Peter, O.M., Abbas, M.A., Goni, M.D., 2018. Staphylococcus aureus: A Review of Antimicrobial Resistance Mechanisms. Vet. Sci. Res. Rev., 4: 43–54.
10. Chanu, K.V., Thakuria, D., Kumar, S., 2018. Antimicrobial peptides of buffalo and their role in host defenses. Vet. World. 11: 192–200.
11. CLSI, 2011. Performance Standards for Antimicrobial Susceptibility Testing, Twenty-First Informational Supplement M100-S21,Wayne,PA,USACLSI.
12. Crosby, H.A., Kwiecinski, J., Horswill, A.R., 2016. Staphylococcus aureus Aggregation and Coagulation Mechanisms, and Their Function in Host–Pathogen Interactions, in: Physiology & Behavior. pp. 1–41.
13. Da Costa, L.B., Rajala-Schultz, P.J., Hoet, A., Seo, K.S., Fogt, K., Moon, B.S., 2014. Genetic relatedness and virulence factors of bovine Staphylococcus aureus isolated from teat skin and milk. J. Dairy Sci., 97: 6907–6916.
14. Dastmalchi Saei, H., Panahi, M., 2020. Genotyping and antimicrobial resistance of Staphylococcus aureus isolates from dairy ruminants: differences in the distribution of clonal types between cattle and small ruminants. Arch. Microbiol. 202: 115–125.
15. Diab, M.S., Ibrahim, N.A., Elnaker, Y.F., Zidan, S.A., Saad, M.A., 2021. Molecular detection of Staphylococcus aureus enterotoxin genes isolated from mastitic milk and humans in El-Behira, Egypt. Int. J. One Heal., 7: 70–77.
16. Dweba, C.C., Zishiri, O., El Zowalaty, M., 2018. Methicillin-resistant Staphylococcus aureus: livestock-associated, antimicrobial, and heavy metal resistance. Infect. Drug Resist., 11: 2497–2509.
17. Elias, L., Balasubramanyam, A.S., Ayshpur, O.Y., Mushtuk, I.U., Sheremet, N.O., Gumeniuk, V. V., Musser, J.M.B., Rogovskyy, A.S., 2020. Antimicrobial susceptibility of staphylococcus aureus, streptococcus agalactiae, and escherichia coli isolated from mastitic dairy cattle in Ukraine. Antibiotics. 9: 1–7.
18. Gebremedhin, E.Z., Ararso, A.B., Borana, B.M., Kelbesa, K.A., Tadese, N.D., Marami, L.M., Sarba, E.J., 2022. Isolation and Identification of Staphylococcus aureus from Milk and Milk Products, Associated Factors for Contamination, and Their Antibiogram in Holeta, Central Ethiopia. Vet. Med. Int., 2022: 1–13.
19. Geletu, U.S., Usmael, M.A., Ibrahim, A.M., 2022. Isolation, Identification, and Susceptibility Profile of E. coli, Salmonella, and *S. aureus* in Dairy Farm and Their Public Health Implication in Central Ethiopia. Vet. Med. Int., 2022:1–13.
20. Gillies, R.R., Dodds, T.C., 1984. Bacteriology Illustrated. Churchill Livingstone.pp. 1–224.
21. Guimarães, F.F., Manzi, M.P., Joaquim, S.F., Richini-Pereira, V.B., Langoni, H., 2017. Short communication: Outbreak of methicillin-resistant Staphylococcus aureus (MRSA)-associated mastitis in a closed dairy herd. J. Dairy Sci., 100: 726–730.
22. Habib, F., Rind, R., Naeemullah, D., Bhutto, A.L., Buriro, R.S., Tunio, A., Aijaz, N., Lakho, S.A., Bugti, A.G., Shoaib, M., 2015. Morphological and Cultural Characterization of Staphylococcus Aureus Isolated from Different Animal Species. J. Applied, Environ. Biol. Sci., 5: 15–26.
23. Harrigen, W.F., McCance, M.E., 1966. Laboratory Methods in Microbiology, in: Academic Press. Elsevier, pp. 1–4.
24. Hassan, A.A., Howayda, M.El-Shafei and Hanan, K.M., 2017. Antimicrobial Potential of Ozone on Fungal and Bacterial Contamination of Animal Feed That Caused Diseases in Some Buffalo Farms. 1st Int. Conf. Anim. Heal. Res. Inst., 9–13.
25. Hassani, S., Moosavy, M.-H., Gharajalar, S.N., Khatibi, S.A., Hajibemani, A., Barabadi, Z., 2022. High prevalence of antibiotic resistance in pathogenic foodborne bacteria isolated from bovine milk. Sci. Rep., 12: 3878–3887.
26. Hogan JS, Gonzalez RN, Harmon RJ, Nickerson SC, Oliver SP, P.J. and S.K., 1999. Laboratory Handbook on Bovine Mastitis. Natl. Mastit. Counc. Madison. 78: 1–222.
27. Leigue, L., Hilgert, A.R., Fiorini, A., Santos, M.F. dos, Vendruscolo, E.C.G., 2017. Occurrence and genetic characterization of Staphylococcus aureus in milk samples of cattle with mastitis, and in the Veterinary Hospital personnel and dairy workers. Brazilian J. Vet. Res. Anim. Sci., 54: 117–128.
28. Magro, G., Biffani, S., Minozzi, G., Ehricht, R., Monecke, S., Luini, M., Piccinini, R., 2017. Virulence Genes of *S. aureus* from Dairy Cow Mastitis and Contagiousness Risk. Toxins. 9: 195–206.
29. Merz, A., Stephan, R., Johler, S., 2016. Staphylococcus aureus Isolates from Goat and Sheep Milk Seem to Be Closely Related and Differ from Isolates Detected from Bovine Milk. Front. Microbiol. 7: 1–7.
30. Neelam, Jain, V.K., Singh, M., Joshi, V.G., Chhabra, R., Singh, K., Rana, Y.S., 2022. Virulence and antimicrobial resistance gene profiles of Staphylococcus aureus associated with clinical mastitis in cattle. PLoS One. 17:1–11.
31. Ombuna, C., 2018. Trends in Hand Milking and Machine Milking in Kenya.Journal of Engineering and Applied Sciences. 13:5655-5660.
32. Pala, K., Ozakin, C., Akis, N., Sinirtas, M., Gedikoglu, S., Aytekin, H., 2010. Asymptomatic carriage of bacteria in food workers in Nilüfer district, Bursa, Turkey*. Turkish J. Med. Sci., 40: 133–139.
33. Rasmi, A.H., Ahmed, E.F., Darwish, A.M.A., Gad, G.F.M., 2022. Virulence genes distributed among Staphylococcus aureus causing wound infections and their correlation to antibiotic resistance. BMC Infect. Dis., 22:652–662.
34. Rasol, V.A., Abdulrahman, R.F., 2023. Detection and Molecular Characterization of Staphylococcus aureus and Methicillin-Resistant Staphylococcus aureus (MRSA) Nasal Carriage Isolates from Healthy Domestic Animal in Duhok Province. Egypt. J. Vet. Sci., 2:263–273.
35. Silva, W.P. da, Destro, M.T., Landgraf, M., Franco, B.D.G.M., 2000. Biochemical characteristics of typical and atypical Staphylococcus aureus in mastitic milk and environmental samples of Brazilian dairy farms. Brazilian J. Microbiol. 31: 103–106.
36. Singh, M., Rathi, B., Mukherjee, R., Shakya, M., 2019. Comparative Analysis of Hand v/s Machine Milking on Bovine Intramammary Infection. Int. J. Curr. Microbiol. Appl. Sci., 8: 1940–1949.
37. Singh P and Prakash A, 2008. Isolation OF Escherichia coli, Staphylococcus aureus and Listeria monocytogenes from milk products sold under market conditions at agra region. Acta Agric. Slov., 92: 83–88.
38. Sotohy, S., Emam, S., Ewida, R., 2022. Incidence of Staphylococcus aureus and enterotoxin A gene in marketable milk and some milk products sold in New Valley governorate, Egypt. New Val. Vet. J., 2: 9–15.
39. Tibebu, L., Belete, Y., Tigabu, E., Tsegaye, W., 2021. Prevalence of Staphylococcus aureus, Methicillin-Resistant Staphylococcus aureus and Potential Risk Factors in Selected Dairy Farms at the Interface of Animal and Human in Bishoftu, Ethiopia. Vet. Med. Res. Reports . 12: 241–251.
40. Tiwari, B.B., Subedi, D., Bhandari, S., Shrestha, P., Pathak, C.R., Chandran, D., Pandey, G., Mohankumar, P., Dhama, K., 2022. Prevalence and Risk Factors of Staphylococcal Subclinical Mastitis in Dairy Animals of Chitwan, Nepal. J. Pure Appl. Microbiol. 16: 1392–1403.
41. Ullah, H., Bashir, K., Idrees, M., Ullah, A., Hassan, N., Khan,

S., Nasir, B., Nadeem, T., Ahsan, H., Khan, M.I., Ali, Q., Muhammad, S., Afzal, M., 2022. Phylogenetic analysis and antimicrobial susceptibility profile of uropathogens. PLoS One .17: 1–12.

42. VandenBergh, M.F.Q., Yzerman, E.P.F., van Belkum, A., Boelens, H.A.M., Sijmons, M., Verbrugh, H.A., 1999. Follow-Up of Staphylococcus aureus Nasal Carriage after 8 Years: Redefining the Persistent Carrier State. J. Clin. Microbiol. 37: 3133–3140.
43. Vink, D., 1995. Subclinical mastitis in the Nile Delta—A cross-section- al study. PhD thesis. Faculty of Veterinary Medicine, University of Utrecht, the Netherlands.
44. Wang, D., Zhang, L., Zhou, X., He, Y., Yong, C., Shen, M., Szenci, O., Han, B., 2016. Antimicrobial susceptibility, virulence genes, and randomly amplified polymorphic DNA analysis of Staphylococcus aureus recovered from bovine mastitis in Ningxia, China. J. Dairy Sci., 99:9560–9569.
45. Zhang, L., Gao, J., Barkema, H.W., Ali, T., Liu, G., Deng, Y., Naushad, S., Kastelic, J.P., Han, B., 2018. Virulence gene profiles: alpha-hemolysin and clonal diversity in Staphylococcus aureus isolates from bovine clinical mastitis in China. BMC Vet. Res., 14: 63–74.
46. Zhou, Z., Zhang, M., Li, H., Yang, H., Li, X., Song, X., Wang, Z., 2017. Prevalence and molecular characterization of Staphylococcus aureus isolated from goats in Chongqing, China. BMC Vet. Res., 13: 352–359.