Original Research

Journal of Advanced Veterinary Research
(2024) Volume 14, Issue 4, 621-625

Detection of multidrug-resistance *Staphylococcus aureus* from mastitic cows' milk in Dakahlia and Damietta Governorates, Egypt

Alaa Gabr, Asmaa Sadat*, Gamal Younis

Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Mansoura University, Mansoura 35516, Egypt.

ARTICLE INFO

Recieved: 11 January 2024

Accepted: 11 February 2024

***Correspondence:**

Corresponding author: Asmaa Sadat
E-mail address: asmaasadat@mans.edu.eg

Keywords:

S. aureus
Mastitis
MDR
Antimicrobials susceptibility

ABSTRACT

Staphylococcus aureus (*S. aureus*) is an important microbe which has the ability to cause a mastitis in cows and causes huge economic losses. This microorganism has a growing ability to resist antimicrobial agents which let to hinder the treatments programs. The study aimed to isolate and identify the prevalence of multi-drug resistant *S. aureus* in mastitic cows' milk in delta region (Dakahlia and Damietta governorates). A two hundred milk samples were randomly selected from clinical mastitic and sub-clinical mastitic infected cows (one hundred from each); these infected cows farms located at Dakahlia and Damietta governorates during the period of November 2020 to March 2021. The samples were diagnosed using routine culture methods to isolate *S. aureus*. All suspected colonies were subjected to biochemical analysis for the basic identification of *S. aureus* colonies. The biochemically identified *S. aureus* colonies were confirmed by using molecular marker targeting thermonuclease-nuc gene by PCR. All the confirmed *S. aureus* isolates were subjected to antimicrobial sensitivity testing against eighteen antimicrobial agents by using Kirby–Bauer disc diffusion method. Out of the 200 tested milk sample, a forty-six were identified as *S. aureus* isolate revealed a total prevalence 23%. *S. aureus* prevalence rate in clinical mastitic and sub-clinical mastitic samples was 37 (80.4%), and 9 (19.6%), respectively. *S. aureus* isolates revealed a high resistant against oxacillin, ampicillin, and ceftiofur, and moderate resistance against tetracycline, amoxicillin- clavulanic acid, cefotaxime, cefuroxime, vancomycin, and gentamycin, while a high sensitivity of *S. aureus* was displayed against ciprofloxacin, SXT and marbofloxacin. All examined *S. aureus* isolates were sensitive against imipenem. Multidrug resistance (MDR) was displayed in all the isolates. Building food tracking and farm animal surveillance systems is essential to improving the healthiness processing and guaranteeing that consumers receive safe food.

Introduction

Staphylococcus aureus (*S. aureus*) is an opportunistic infectious agent that infects humans as well as a variety of farm animals (Portillo *et al*., 2013; Liu *et al*., 2018). *S. aureus* is implicated in many human diseases, including superficial skin and soft tissue infections, pneumonia, septicemia, endocarditis, and other serious or even fatal diseases (David and Daum, 2017). It is one of the main pathogens which causes intramammary infections (IMI) in dairy cattle and represent a public health risk due to high antimicrobial resistance, food safety, and zoonotic potential (Tillotson and Zinner, 2017). Multidrug resistance of *S. aureus* and the high evasion capacity of the host immune response gives rise to persistent infections and the ability to spread fast in herds, hindering its control (World Health Organization, 2015; Semret and Haraoui, 2019). Bovine mastitis has been mentioned among the major obstacles to affect the dairy industry by incurring the dairy farms to unnecessary expenses worldwide in general and in developing countries in particular (Abebe *et al*., 2016).

Antimicrobial resistance (AMR) in *S. aureus* has resulted in the emergence of multidrug resistant (Rybak and LaPlante, 2005). Although a "national action plan for reducing the use of veterinary antimicrobials (2021-2025)" has been launched, and the use of antimicrobials is decreasing year by year, the use of antimicrobials is important for the control and treatment of bacterial disease on farms. Antimicrobials such as penicillin, ampicillin, amikacin, erythromycin, tetracycline, doxycycline, florfenicol, ciprofloxacin, enrofloxacin, and trimethoprim are frequently used by veterinarians. AMR is a public health threat that can result in increased mortality, lengthened hospital stays, and increased healthcare costs (Tillotson *et al*., 2017). AMR has a substantial effect on low and middle-income nations since they have a higher overall prevalence of infectious diseases, a low potential for AMR identification and monitoring, more restricted access to second-line antimicrobials, and fewer regulations for antimicrobial use in humans and animals (Semret and Haraoui, 2019).

The World Health Organization (WHO) has identified surveillance as a critical strategic goal for combating AMR (World Health Organization, 2015). Surveillance provides an initial assessment of the problem, enables responses to be tailored to the local context, and facilitates the evaluation of AMR-fighting strategies. In low and middle-income countries, obtaining high-quality AMR surveillance data can be difficult. Limited laboratory infrastructure, a lack of trained personnel, the absence of health information systems, and intermittent availability of consumables and reagents are all potential challenges. Surveillance provides a fundamental evaluation of the issue, allowing AMR-fighting approaches to be assessed. It can be difficult to obtain high-quality AMR surveillance data in low and middle-income countries. Limited laboratory facilities, insufficiently trained personnel, a lack of health information systems, and infrequent availability of consumables and reagents are all potential challenges (Iskandar *et al*., 2021).

The purpose of this study was to investigate the prevalence of *S. aureus* isolated from mastitic cows in Dakahlia and Damietta governorates, Egypt and to estimate the hazard of their antimicrobial susceptibility. In addition, investigate the risk of *S. aureus* transmission to between animals, or workers.

Materials and methods

Ethical approval

The study was performed following instructions of the animal re-

ISSN: 2090-6277/2090-6269/

search ethical committee of the faculty of veterinary medicine, Mansoura University (code number: M/108).

Sampling region

Our study samples were randomly collected from farms located at two large governments in delta region, Dakahlia and Damietta governorates, Egypt. Both governorates are distinguished with large farms industry as well as small farm industry due to its rich green areas.

Sampling collection

Between November 2020 and March 2021, a total of 200 milk samples were collected, 100 from each clinical and sub-clinical mastitic milk. All the milk samples were gathered from several dairy farms in Egypt's Dakahlia and Damietta governorates. Clinical samples were distinguished by appearance of observable clinical signs which included inflammation of one or all udder's quarters, fever, depression, and disturbed appetite of cow, or clot formation, discoloration, and presence of blood in milk. As well as Cow's with no observable signs were submitted to diagnosis by application of California mastitis test (CMT) to confirm subclinical infection. Milk samples were meticulously labelled, stored in an ice box, and immediately delivered to the Department of Bacteriology, Mycology, and Immunology, Faculty of Veterinary Medicine, Mansoura University for bacteriological investigation.

Isolation and identification of S. aureus

S. aureus isolates were identified using the method described by Wang *et al.* (2012), with slight modifications. All milk samples were centrifuged to remove the cream layer, and 0.1 ml of the sediment was streaked directly on the surface of Baird Parker media (Oxiod Pvt. Ltd., UK), which was supplied with 5% egg yolk and 1% potassium tellurite (Oxiod Pvt. Ltd., UK). All the cultured plates were incubated overnight at 37°C. Typical black coloured colonies surrounded by a clear halo zone were picked from the cultured plates and sub-cultured onto Tryptone Soya Agar (TSA; Oxoid Pvt. Ltd., UK). All the suspected colonies were further tested with Gram's stain and the standard biochemical tests, including catalase, oxidase, DNase, and coagulase tests (Boerlin *et al* 2003; De Freitas Guimarães *et al*., 2013). All the suspected *S. aureus* isolates were stored in 30% glycerol solution at -20°C for further investigation.

Molecular characterization of S. aureus.

DNA extraction

Whole-cell lysate was prepared from each suspected *S. aureus* isolate by suspending 3–5 colonies into 200-µL sterile nuclease free water, followed by boiling for ten minutes and centrifuging at 10000 rpm for one minute. The supernatant was then transferred to a sterile Eppendorf and was used as a DNA template for further molecular characterization. The prepared DNA samples were stored at -20°C.

Molecular characterization of *S. aureus* isolates

DNA templates of the suspected to be *S. aureus* was subjected to PCR targeting the nuc gene thermosnuclaease for species confirmation which was amplified at 270bp. The reaction mixture (25 µL) contained 12.5-µL 2x PCR master mix (WizPureTM, Gyeonggi-do, Korea), 1-µL 20-pmol of each primer (Metabion, Germany), 5-µL template DNA and completed with nuclease free water. The Primer sequence was F: GCGATTGATGGTGATACGGTT and R: AGCCAAGCCTTGACGAACTAAAG.PCRs thermocycler conditions weredone as follow: primary denaturation at 94˚C for 5 min; followed by 35 cycles of 94˚C for 30 sec, 60˚C for 1min and 72˚C for 1 min; and one cycle of 72˚C for 10 min to final extension (Oliveira *et al*., (2015). Positive control was obtained from previous studies (Sadat *et al*., 2022; Sadat *et al*., 2023).

About 5 µl of each PCR product was visualized in 1% agarose gel using agarose gel electrophoresis. Gels were stained with ethidium bromide and visualized using Gel Doc (cleaver scientific ltd UV gel documentation system, USA) and photographed using UV transilluminator.

Antimicrobial susceptibility testing

S. aureus isolates were investigated for their antimicrobial susceptibility against 18 antimicrobial agents using the disc diffusion method on Mueller-Hinton agar (Oxoid, UK) according to the guidelines of Clinical and Laboratory Standards (CLSI 2018). The following antibiotic disks (Oxoid, UK) were used: streptomycin (S, 10ug), imipenem (IPM, 10ug), ciprofloxacin (CIP, 5ug), marbofloxacin (MAR, 5ug), oxacillin (OX, 15ug), tetracycline (TE, 30ug), amoxicillin-Clavulonic Acid (AMC, 30 ug), ceftiofur (EFT, 30ug), erythromycin (E, 15ug), gentamycin (CN, 10ug), vancomycin (VA, 30ug), spiramycin (SP, 100ug), cefotaxime (CTX, 30ug), cefuroxime (CXM, 30ug), ceftriaxone (CRO, 30ug), ampicillin (AM, 10ug), penicillin (P, 10ug), sulfamethoxazole- trimethoprim (SXT, 25ug) (Table 1). Results were interpreted according to breakpoints of the clinical and laboratory standards (CLSI 2018). A multiple antibiotic resistance (MAR) strains were referred for those that demonstrated resistance to three or more antimicrobial classes (Waters *et al*., 2011). A multiple antibiotic resistance index was calculated by dividing the total number of antimicrobial resistances in each isolate by the total number of tested antimicrobials (Krumperman 1983).

Results

S. aureus prevalence in mastitic milk

Out of the 200 milk samples studied in this study, 46 (23%) isolates were identified as *S. aureus* according to their growth characters, black, shiny opaque colonies surrounded by clear zones. *S. aureus* were represented as 16 (34.8%) and 30 (65.2%) from clinical and sub-clinical samples, respectively. *S. aureus* appeared as Gram positive small round cocci and most commonly as grape-like clusters. Biochemically, *S. aureus* showed positive results with catalase, coagulase and oxidase tests and displayed a clear zone of $_{\beta}$-hemolysis around the colonies. All the biochemically identified *S. aureus* isolates were confirmed by PCR targeting the nuc gene (Figure 1).

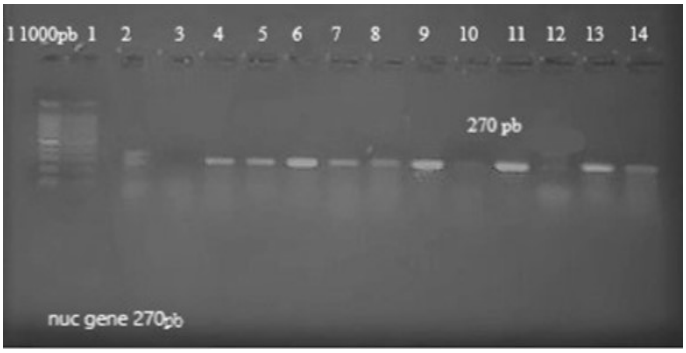

Fig. 1. Agarose gel electrophoresis showing amplification of nuc gene at 270 bp. Ladder: 1000bp, Lane 4, 5, 6, 7, 8, 10, 12, 13,14 showed positive result.

Antimicrobial susceptibility testing for S. aureus isolated strains

Antimicrobial susceptibility test was conducted on all the confirmed to be *S. aureus*, n=46 isolates, which were identified in this study.

S. aureus isolates has exhibited high resistance against oxacillin, ampicillin, and ceftiofur (100% for each), followed by penicillin and spiramycin (for each 93%), ceftriaxone (89%), streptomycin (78%), erythromycin

Table 1. Antimicrobial susceptibility testing results.

Antimicrobial Classes	Antibiotics	Disc codes	Concentrations (CPD)	*S. aureus (clinical cases)*			*S. aureus (sub-clinical cases)*		
				Resistant	Intermediate	Sensitive	Resistant	Intermediate	Sensitive
B-Lactams	Penicillin	P	10ug	34(91.89%)	-	3(8.11%)	9(100%)	-	-
	Oxacillin	OX	15ug	37(100%)	-		9(100%)	-	-
	Amoxycillin-Clavulanic Acid	AMC	30ug	27(72.97%)	-	10(27.03%)	7(77.78%)	-	2(22.22%)
Penicillins	Ampicillin	AM	10ug	37(100%)	-	-	9(100%)	-	-
Cephalosporines	Cefuroxime	CXM	30ug	27(72.97%)	4(10.81%)	6(16.22%)	8(88.89%)	1(11.1%)	-
	Cefotaxime	CTX	30ug	27(72.97%)	4(10.81%)	6(16.22%)	8(88.89%)	1(11.1%)	-
	Ceftriaxone	CRO	30ug	33(89.19%)	-	4(10.81%)	8(88.89%)		1(11.1%)
	Ceftiofur	EFT	30ug	37 (100%)	-	-	9(100%)	-	-
Macrolides	Erythromycin	E	15ug	28(75.68%)	8(21.62%)	1(2.7%)	8(88.89%)	-	1(22.22%)
Glycopeptide	Vancomycin	VA	30ug	24(64.86%)	9(24.3%)	4(10.81%)	6(66.67%)	3(33.33%)	-
Aminoglycosides	Spiramycin	SP	100ug	34(91.89%)	3(8.11%)	-	6(66.67%)	3(33.33%)	-
	Streptomycin	S	10ug	30(81.08%)		7(18.91%)	7(77.78%)	-	2(22.22%)
	Gentamycin	CN	10ug	24(64.86%)	3(8.11%)	10(27.02%)	5(55.56%)	-	4(44.44%)
Tetracyclines	Tetracycline	TE	30ug	28(75.68%)	5(13.51%)	4(10.81%)	5(55.55%)	3(33.33%)	2(22.22%)
Quinolones	Ciprofloxacin	CIP	5 ug	12(32.4%)	2(5.4%)	23(62.16%)	-	3(33.33%)	6(66.67%)
Fluroquinolones	Marbofloxacin	MAR	5ug	3(8.11%)	18(48.65%)	16(43.24%)	-	4(44.44%)	5(55.56%)
Potentiated Sulfonamides	Sulfamethoxazole/Trimethoprim	SXT	25ug	10(27.03%)	2(5.41%)	25(67.56%)	2(22.22%)	-	7(77.78%)
Carbapenems	Imipenem	IMP	10ug	-	-	37(100%)	-	-	9(100%)

(74%), tetracycline, amoxicillin- clavulanic acid, cefotaxime, and cefuroxime (for each 76%), vancomycin (67%), and gentamycin (65%) (Table 1).

Lower resistances were exposed against ciprofloxacin (26.09%), SXT (23.91%) and marbofloxacin (6.12%). All examined *S. aureus* isolates were sensitive against imipenem (Table 1).

MDR of S. aureus isolated strains

Multidrug resistance (MDR) is defined as acquired nonsusceptibility to at least one agent in three or more antimicrobial categories. Variable resistance patterns were displayed against the 18 used antimicrobial agent (Table 2). A total of 46 *S. aureus* were isolated in which all these isolates were MDR. Out of which, two isolates were resistant to three classes of antibiotics, while resistance to four classes was seen in 3 isolates, resistance to five classes was seen in 16 isolates, while resistance to six classes was seen in 15 isolates, while resistance to seven classes was seen in 8 isolates and resistance to eight classes was seen in 2 isolates. MAR index of *S. aureus* isolates was between 0.44- 0.89.

Antimicrobial Resistance Pattern of S. aureus isolated strains

S. aureus isolates displayed forty-one antimicrobial resistance patterns. The most predominant patterns were SP, TE, P, AM, OX, AMC, EFT, CXM, CRO, E; S, SP, CN, TE, P, AM, OX, AMC, EFT, CXM, CTX, CRO, E; S, SP, CN, TE, AM, OX, AMC, EFT, CTX, CRO, CIP, E, VA; S, SP, CN, TE, P, AM, OX, AMC, EFT, CXM, CTX, CRO, E, SXT.

Discussion

S. aureus is a predominant pathogen that causes one of the highest virulent forms of bovine mastitis and great challenge to dairy production in most countries (Monistero *et al*., 2018). It causes high economic losses, including a severe decline in milk production, sever reproductive complications, increased costs of veterinary medication, increases the culling rate of infected animals and replacing tainted milk (Hogeveen, 2005; Hogeveen *et al*., 2011; Deb *et al*., 2013; Botaro *et al*., 2015; Gomes and Henriques, 2016).

In the current study, *S. aureus* was found in 46 (23%) of 200 total examined milk samples. *S. aureus* was identified in milk samples from mastitic infected cows in previous research (Younis *et al*., 2018; Emeru *et al*., 2019; Girmay *et al*., 2020; Sadat *et al*., 2023). The prevalence of *S. aureus* was found to be higher than our results in Lakew *et al*. (2009) and Etifu and Tilahun (2019) findings. In dissimilarity to the current study, other finding revealed lower results (Abebe *et al*., 2016; Birhanu *et al*., 2017; Seyoum *et al*., 2018). It was also discovered that there were differences in *S. aureus* isolation rates between cows with clinical and subclinical mastitis; cows with clinical mastitis had significantly higher levels of the pathogen (37 (80.4%), and 9 (19.6%), respectively. Younis *et al*. (2018) showed similar results. In the other hand, previous studies showed unsimilar result which was the prevalence rate of *S. aureus* in subclinical was more than clinical isolates (Madut *et al*., 2009; Mekibib *et al*., 2010).

In Egypt, antibiotics treatment is of the main therapeutic choice for mastitis control. There are many factors that affect this therapeutic procedure depend on disease severity, drug choice, reasonable drug usage and measurable dosages, and prohibition of predisposing causes. However, in the recent years mastitis treatment by antibiotics became ineffectual due to persistent intracellular existence of *S. aureus* with different forms of developed defense mechanisms against antibiotics and host defense mechanism after that; they can relapse to more infectious wild-type phenotype, probably causing recurrent infection. Besides, excessive unreasonable usage of antibiotics for the long-term leads to the resistance of *S. aureus* to antibiotics (Szweda *et al*., 2014; Sadat *et al*., 2021; Alazab, *et al*., 2022).

S. aureus isolates were found resistant to the four examined cephalosporines antibiotics; cefuroxime, and cefotaxime showed resistance 76% for each; this observation agreed with Jahan *et al*. (2015); in addition, ceftiofur and ceftriaxone were found to exhibit 100% and 89% resistance, respectively. A total of 76% of the isolates were resistance to tetracycline antibiotics. This result was supported by previous findings (Jamali *et al*., 2014).

S. aureus isolates were resistant to B-Lactams antibiotics: oxacillin (100%), ampicillin (100%), penicillin (93%), and Amoxycillin-Clavulanic Acid (76%). Deyno *et al*. (2017) revealed similar result. Our result also showed resistant against aminoglycosides with a resistance rate of 93% in spiramycin, 78% in streptomycin, against macrolides antibiotic (erythromycin; 72.5%), and 65% in gentamycin. Similar higher resistance was released towards potentiated sulfonamides, sulfamethoxazole/ trimethoprim (70%).

Resistance was exhibited against glycopeptide antibiotics (vancomycin; 67%). Previous studies found resistance against vancomycin and erythromycin in accordance with our research (Lucia Ratna *et al*., 2015). Ciprofloxacin showed relative resistance of 57.5% which is supported by previous findings (Daka *et al*., 2012). As well as the *S. aureus* isolates were sensitive against fluroquinolones, marbofloxacin (47.5%). While *S. aureus* isolates showed susceptibility against carbapenems antibiotics, imipenem (100%) which agreed with Abo-Shama (2014).

The growing resistance of mastitis-causing bacteria, like *S. aureus*, to commonly used antibiotics presents another obstacle for the industry

Table 2. Antimicrobial resistance patterns and MDR in *S. aureus* isolates.

Antibiotic resistance Pattern	Antibiotics Resistance Pattern	MAR (N=46)	MAR Index	Isolate No. (%)
1	S, SP, CN, AM, OX, EFT, CTX, EFT	+	0.44	1(2.17%)
2	S, SP, TE, AM, OX, EFT, CTX, E	+	0.44	1(2.17%)
3	CN, TE, P, OX, EFT, CTX, CIP, E	+	0.44	1(2.17%)
4	S, P, AM, OX, AMC, EFT, CXM, CTX, CRO	+	0.5	1(2.17%)
5	SP, P, AM, OX, AMC, EFT, CXM, CTX, CRO	+	0.5	1(2.17%)
6	SP, CN, TE, P, AM, OX, AMC, EFT, CXM	+	0.5	1(2.17%)
7	SP, TE, P, AM, OX, AMC, EFT, CXM, E	+	0.5	1(2.17%)
8	S, SP, CN, PAM, OX, EFT, CXM, CTX, CIP, VA	+	0.56	1(2.17%)
9	S, SP, CN, AM, OX, EFT, CXM, CTX, E, VA	+	0.56	1(2.17%)
10	S, SP, TE, CN, P, AM, OX, EFT, CTX, E	+	0.56	1(2.17%)
11	SP, TE, P, AM, OX, AMC, EFT, CXM, CRO, E	+	0.56	2(4.34%)
12	S, SP, TE, P, AM, OX, EFT, CTX, E, VA	+	0.56	1(2.17%)
13	S, SP, CN, TE, AM, OX, EFT, CTX, CIP, VA	+	0.56	1(2.17%)
14	S, SP, TE, P, AM, OX, AMC, EFT, CXM, VA	+	0.56	1(2.17%)
15	SP, TE, P, AM, OX, AMC, EFT, CXM, CTX, CRO	+	0.56	1(2.17%)
16	P, AM, OX, AMC, EFT, CXM, CTX, CRO, E, VA	+	0.56	1(2.17%)
17	SP, CN, P, AM, OX, AMC, EFT, CXM, CRO, VA, SXT	+	0.61	1(2.17%)
18	SP, P, AM, OX, AMC, EFT, CXM, CTX, CRO, VA, SXT	+	0.61	1(2.17%)
19	S, CN, P, AM, OX, AMC, EFT, CXM, CTX, CIP, VA	+	0.61	1(2.17%)
20	S, SP, CN, P, AM, OX, AMC, EFT, CXM, CTX, CIP, VA	+	0.67	1(2.17%)
21	S, SP, CN, P, AM, OX, AMC, EFT, CXM, CTX, CIP, E	+	0.67	1(2.17%)
22	S, SP, CN, TE, P, AM, OX, EFT, CTX, E, VA	+	0.67	1(2.17%)
23	S, SP, TE, P, AM, OX, EFT, CTX, CRO, E, VA, SXT	+	0.67	1(2.17%)
24	S, SP, CN, TE, P, AM, OX, EFT, CXM, CTX, E, VA	+	0.67	1(2.17%)
25	S, SP, CN, TE, P, AM, OX, AMC, EFT, CXM, E, VA	+	0.67	1(2.17%)
26	S, SP, TE, P, AM, OX, AMC, EFT, CXM, E, VA, SXT	+	0.67	1(2.17%)
27	S, SP, TE, P, AM, OX, AMC, EFT, CXM, CRO, E, SXT	+	0.67	1(2.17%)
28	S, SP, TE, P, AM, OX, AMC, EFT, CXM, CTX, CRO, E	+	0.67	1(2.17%)
29	S, SP, CN, TE, AM, OX, AMC, EFT, CXM, CTX, CRO, E, VA	+	0.72	1(2.17%)
30	S, SP, CN, TE, P, AM, OX, AMC, EFT, CXM, CTX, CRO, E	+	0.72	2(4.34%)
31	S, SP, CN, TE, AM, OX, AMC, EFT, CTX, CRO, CIP, E, VA	+	0.72	2(4.34%)
32	S, SP, TE, P, AM, OX, AMC, EFT, CTX, CRO, E, VA, SXT	+	0.72	1(2.17%)
33	S, SP, CN, TE, P, AM, OX, AMC, EFT, CXM, CTX, CRO, E	+	0.72	1(2.17%)
34	S, SP, CN, TE, P, AM, OX, AMC, EFT, CXM, CTX, CRO, E, SXT	+	0.78	2(4.34%)
35	S, SP, CN, TE, P, AM, OX, EFT, CTX, CRO, CIP, E, VA, SXT	+	0.78	1(2.17%)
36	S, SP, CN, TE, P, AM, OX, AMC, EFT, CXM, CTX, CRO, E, VA	+	0.78	2(4.34%)
37	S, SP, CN, TE, P, AM, OX, AMC, EFT, CTX, CRO, E, VA, SXT	+	0.78	1(2.17%)
38	S, SP, CN, TE, P, AM, OX, AMC, EFT, CXM, CTX, CRO, E, VA	+	0.78	1(2.17%)
39	S, SP, CN, TE, P, AM, OX, AMC, EFT, CXM, CTX, CRO, E, VA, SXT	+	0.83	1(2.17%)
40	S, SP, CN, TE, P, AM, OX, AMC, EFT, CXM, CTX, CRO, E, VA, SXT	+	0.83	1(2.17%)
41	S, SP, CN, TE, P, AM, OX, AMC, EFT, CXM, CTX, CRO, CIP, E, VA, MAR	+	0.89	1(2.17%)

(Younis *et al*., 2018; Sadat *et al*., 2022). It should also be emphasized that food products have the potential to locally distribute resistant germs to other locations.

Conclusion

Based on the study's findings, we deduced that *S. aureus* continues to be the primary cause of mastitis in dairy cows. It was shown that compared to subclinical mastitis, clinical mastitis had a greater prevalence of *S. aureus*. It was also demonstrated that cow mastitis occurs often in the research location. We learned from *S. aureus*'s antibacterial susceptibility traits that they are extremely resistant to the typical antibiotics utilized in local veterinary care. Therefore, actions for controlling and preventing pathogens are necessary, and doing so will also aid in reducing the prevalence of the disease. Additionally, adding antibiotics that react better to treatment would be a smart idea. Consequently, this research has yielded significant data that may help to decrease.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Abebe, R., Hatiya, H., Abera, M., Megersa, B., Asmare, K., 2016. Bovine mastitis: prevalence, risk factors and isolation of *Staphylococcus aureus* in dairy herds at Hawassa milk shed, South Ethiopia. BMC Vet. Res. 12, 270–311.

Abo-Shama, U.H., 2014. Prevalence And Antimicrobial Susceptibility of *Staphylococcus aureus* Isolated From Cattle, Buffalo, Sheep And Goat's Raws Milk In Sohag Governorate, Egypt. Assiut Vet. Med. J. 14, 63-72.

Alazab, A., Sadat, A., Younis, G., 2022. Prevalence, antimicrobial susceptibility, and genotyping of Streptococcus agalactiae in Tilapia fish (*Oreochromis niloticus*) in Egypt. J. Adv. Vet. Anim. Res. 9, 95–103.

Birhanu, M, Leta, S., G. Mamo, G., Tesfaye, S., 2017. Prevalence of bovine subclinical mastitis and isolation of its major causes in Bishoftu Town, Ethiopia. BMC Research Notes. 10, 1 1–6.

Boerlin, P., Kuhnert, P., Hussy, D., Schaellibaum, M., 2003 . Methods for identification of *Staphylococcus aureus* isolates in cases of bovine mastitis. J. Clin. Microbiol. 41, 767-771.

Botaro, B. G., Cortinhas, C.S., Dibbern, A.G., Silva, L.F.P.E., Benites, N. R., dos Santos, M.V., 2015. *Staphylococcus aureus* intramammary infection affects milk yield and SCC of dairy cows. Trop.

Anim. Health Prod. 47, 61-66.
Clinical and Laboratory Standard Institute (CLSI), 2018. Performance Standards for Antimicrobial Susceptibility Testing, Twenty-eight Informational supplement (M100– S28) . 2018 Wayne, PA: CLSI.
Daka, D., G/silassie, S., Yihdego, D., 2012. Antibiotic-resistance *Staphylococcus aureus* isolated from cow's milk in the Hawassa area, South Ethiopia. Ann. Clin. Microbiol. Antimicrob. 26, 11-26
David, M.Z., Daum, R.S., 2017. Treatment of *Staphylococcus aureus* infections. Curr. Top. Microbiol. Immunol., 409, 325–383.
De Freitas Guimarães, F., Nóbrega, D.B., Richini-Pereira, V.B., Marson, P.M., de Figueiredo Pantoja, J.C., Langoni, H., 2013. Enterotoxin genes in coagulase-negative and coagulase-positive staphylococci isolated from bovine milk. J. Dairy Sci. 96, 2866-2872.
Deb, R., Kumar, A., Chakraborty, S., Verma, A.K., Tiwari, R., Dhama, K., Singh, U., Kumar, S., 2013. Trends in diagnosis and control of bovine mastitis: a review. Pak. J. Biol. Sci. 16, 1653-1661. doi: 10.3923/pjbs.2013.1653.1661.
Deyno, S., Toma, A., Worku, M., Bekele, M., 2017. antimicrobial resistance profile of *Staphylococcus aureus* isolates isolated from ear discharges of patients at University of Hawassa comprehensive specialized hospital. BMC Pharmacol. Toxicol. 18, 35. https://doi.org/10.1186/s40360-017-0141-x
Emeru, B.A., Messele, Y.E., Tegegne, D.T., Yalew, S.T., Bora, S.K., Babura, M.D., Beyene, M.T., Werid, G.M., 2019. Characterization of antimicrobial resistance in *Staphylococcus aureus* isolated from bovine mastitis in Central Ethiopia. Journal of Veterinary Medicine and Animal Health 11, 81-87.
Etifu, M., Tilahun, M., 2019. Prevalence of bovine mastitis, risk factors, isolation and anti-bio gram of major pathogens in Mid Rift valley, Ethiopia. International Journal of Livestock Production, 10, 14-23.
Girmay, W., Gugsa, G., Taddele, H., Tsegaye, Y., Awol, N., Ahmed, M., Feleke, A., 2020. Isolation and Identification of Methicillin-Resistant *Staphylococcus aureus* (MRSA) from Milk in Shire Dairy Farms, Tigray, Ethiopia. Veterinary Medicine International 2020, 8833973,7. https://doi.org/10.1155/2020/8833973
Gomes, F., Henriques, M., 2016. Control of bovine mastitis: old and recent therapeutic approaches. Curr Microbiol 72, 377–82.
Hogeveen, H., Huijps, K., Lam, T., 2011. Economic aspects of mastitis: new developments. N. Z. Vet. J. 59,16–23.
Hogeveen, H., 2005. Mastitis is an economic problem. Proceedings of the British Mastitis Conference; Warwickshire, UK.
Iskandar, K., Molinier, L., Hallit, S., Sartelli, M., Hardcastle, T. C., Haque, M., Lugova, H., Dhingra, S., Sharma, P., Islam, S., Mohammed, I., Naina, M.I., Hanna, P.A., Hajj, S.E., Jamaluddin, N.A. H., Salameh, P., Roques, C., 2021. Surveillance of antimicrobial resistance in low- and middle-income countries: a scattered picture. Antimicrob. Resist. Infect. Control. 10, 63. doi: 10.1186/s13756-021-00931-w.
Jahan, M., Rahman, M., Parvej, M.S., Chowdhury, S.M.Z.H., Haque, M.E., Talukder, M.A.K., Ahmed, S., 2015. Isolation and characterization of *Staphylococcus aureus* from raw cow milk in Bangladesh. J. Adv. Vet. Anim. Res. 2, 49–55. 10.5455/javar.2015.b47
Jamali, H., Radmehr, B., Ismail, S., 2014. Prevalence and antibiotic resistance of *Staphylococcus aureus* isolated from bovine clinical mastitis. J. Dairy Sci. 97, 2226–2230.
Krumperman, P.H., 1983. Multiple antibiotic resistance indexing of Escherichia coli to identify high-risk sources of fecal contamination of foods. Appl. Environ. Microbiol. 46, 165-170.
Lakew, M., Tolosa, T., Tigre, W., 2009.Prevalence and major bacterial causes of bovine mastitis in Asella, South Eastern Ethiopia. Trop. Anim. Health Prod. 417, 1525–1530.
Liu, B., Sun, H., Pan, Y., Zhai, Y., Cai, T., Yuan, X., Gao, Y., He, D., Liu, J., Yuan, L., Hu, G., 2018. Prevalence, resistance pattern, and molecular charac- terization of *Staphylococcus aureus* isolates from healthy animals and sick populations in Henan province, China. Gut Pathogens, 10, 31.
Lucia Ratna, W. M., Nainu, F., Rochmat Himawan R., 2015. Antibiotic Sensitivity Pattern of *Staphylococcus aureus* and Escherichia coli Isolated from Bovine Fresh Milk. Jurnal Veteriner December 16, 520-524.
Madut, N. A., Elamin, A., Gadir, A., Mohamed, I., Jalii, E., 2009. Host determinants of bovine mastitis in semi-intensive production system of Khartoum state, Sudan. Journal of Cell and Animal Biology 3, 71–77.
Mekibib, B, Furgasa, M., F. Abunna, F., Megersa, B., Regassa, A., 2010.Bovine mastitis: prevalence, risk factors and major pathogens in dairy farms of holeta town, central Ethiopia. Veterinary World 3, 397–403.
Monistero, V., Graber, H.U., Pollera, C., Cremonesi, P., Castiglioni, B., Bottini, E., Ceballos-Marquez, A., Lasso-Rojas, L., Kroemker, V., Wente, N., Petzer, I.M., Santisteban, C., Runyan, J., Veiga Dos Santos, M., Alves, B.G., Piccinini, R., Bronzo, V., Abbassi, M.S., Said, M.B., Moroni, P., 2018. *Staphylococcus aureus* Isolates from Bovine Mastitis in Eight Countries: Genotypes, Detection of Genes Encoding Different Toxins and Other Virulence Genes. Toxins 10, 247. doi: 10.3390/toxins10060247. PMID: 29914197; PMCID: PMC6024761.
Oliveira, C.J.B., Tiao, N., de Sousa, F.G.C., de Moura, J.F.P., Santos Filho, L., Gebreyes, W.A., 2015. Methicillin-Resistant *Staphylococcus aureus* from Brazilian Dairy Farms and Identification of Novel Sequence Types. Zoonoses and Public Health 63, 97-105.
Portillo, B.C., Moreno, J.E., Yomayusa, N., Alvarez, C.A., Cardozo, B.E., Pérez, J. A., Díaz, P.L., Ibañez, M., Mendez-Alvarez, S., Leal, A.L., Gómez, N.V., 2013. Molecular epidemiology and characterization of vir- ulence genes of community-acquired and hospital-acquired methicillin- resistant *Staphylococcus aureus* isolates in Colombia. Int. J. Infect. Dis. 17, e744–e749.
Rybak, M. J., LaPlante, K.L., 2005. Community-associated methicillin- resistant *Staphylococcus aureus*: A review. Pharmacotherapy, 25, 74–85.
Sadat, A., El-Sherbiny, H., Zakaria, A., H. Ramadan, H., Awad, A., 2021.Prevalence, antibiogram and virulence characterization of Vibrio isolates from fish and shellfish in Egypt: a possible zoonotic hazard to humans, Journal of Applied Microbiology 131, 485–498, https://doi.org/10.1111/jam.14929.
Sadat. A., Shata, R. R., Farag,A., Ramadan, H., Alkhedaide, A., Soliman, M. M., Elbadawy M., Abugomaa,A., Awad. A., 2022. Prevalence and Characterization of PVL-Positive *Staphylococcus aureus* Isolated from Raw Cow's Milk. MDPI toxins. 14, 2-16. Article ID 10.3390/toxins14020097.
Sadat, A., Farag, A.M.M., Elhanafi, D., Awad, A., Elmahallawy, E.K., Alsowayeh, N., El-khadragy, M.F., Elshopakey, G.E., 2023. Immunological and Oxidative Biomarkers in Bovine Serum from Healthy, Clinical, and Sub-Clinical Mastitis Caused by Escherichia coli and *Staphylococcus aureus* Infection.MPDI Animals 13, 892. Article ID 10.3390/ani13050892.
Semret, M., Haraoui, L.P., 2019. Antimicrobial resistance in the tropics. Infect Dis Clin. 33, 231–245.
Seyoum, B., Kefyalew, H., Abera, B., Abdela, N., 2018. Preva- lence, risk factors and antimicrobial susceptibility test of Staphylococcus aureus in bovine cross breed mastitic milk in and around asella town, oromia regional state, southern Ethiopia. Acta Tropica 177, pp. 32–36.
Szweda, P., Schielmann, M., Frankowska, A., Kot, B., Zalewska, M., 2014. Antibiotic resistance in Staphylococcus aureus strains isolated from cows with mastitis in eastern Poland and analysis of susceptibility of resistant strains to alternative nonantibiotic agents: lysostaphin, nisin and polymyxin B. J. Vet. Med. Sci. 76, 355-62. doi: 10.1292/jvms.13-0177
Tillotson, G.S., Zinner, S.H., 2017. Burden of antimicrobial resistance in an era of decreasing susceptibility. Expert Rev Anti-infect Ther. 15, 663–676.
Waters, A.E., Contente-Cuomo, T., Buchhagen, J., Liu, C.M., Watson, L., Pearce, K., Foster, J.T., Bowers, J., Driebe, E.M., Engelthaler, D.M., Keim, P.S., Price, L.B., 2011. Multidrug-Resistant Staphylococcus aureus in US Meat and Poultry. Clin Infect Dis. 52, 1227-1230. doi: 10.1093/cid/cir181
Wang, X., Meng, J., Zhang, J., Zhou, T., Zhang, Y., Yang, B., Xi, M., Xia, X., 2012. Characterization of Staphylococcus aureus isolated from powdered infant formula milk and infant rice cereal in China. Int. J. Food Microbiol. 153, 142-147.
World Health Organization, 2015. Global Action Plan on Antimicrobial Resistance. Geneva: World Health Organization; 2015.
Younis, G., Sadat, A., Maghawry, M., 2018. Characterization of Coa Gene and Antimicrobial Profiles of Staphylococcus aureus Isolated from Bovine Clinical and Subclinical mastitis. Adv.Anim. Vet. Sci. 6, 161–168.