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Original Research

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Macrolides-resistant *Staphylococcus aureus* Associated with Clinical Mastitis in Cattle and Buffalo in Egypt

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Abstract

Staphylococcus aureus (*S. aureus*), the bacteria most frequently associated with mastitis in cattle and buffalo, has a large number of genes connected to antibiotic resistance. The objective of the current research was to determine the erythromycin and macrolide resistance of *S. aureus* isolated from mastitic milk of cattle and buffalo, particularly resistance-related genes (*ermA*, *ermB*, *ermC*, *ermT*, and *msrA*). Therefore, 150 dairy cattle and 135 dairy buffalo bred by small farmers in various governorates of Egypt (Cairo, Giza, Kalyobia, Fayoum, and Kafr El-Sheikh) provided a total of 285 milk samples. Inspection revealed that a total of 34 (22.7%) and 36 (26.7%) milk samples from cattle and buffalo, respectively, had clinical mastitis. With a total recovery of 31 (44.3%) *S. aureus* isolates. Bacterial isolation and identification of *S. aureus* verified the isolation and identification of 14 (41.2%) and 17 (47.2%) *S. aureus* isolates from cattle and buffalo, respectively. Utilizing a TaqMan probe-based real-time PCR method that targets the nuc gene, all *S. aureus* isolates were verified. In instances of bovine mastitis in India and Kenya, conventional PCR targeting the nuc gene, followed by DNA sequencing and phylogenetic analysis, revealed a high homology (100%) with that of *S. aureus* strains isolated from milk. For the tested genes, the prevalence of resistant strains was 9.6% (*ermA*), 64.5% (*ermB*), 70.9% (*ermC*), 19.3% (*ermT*), and 9.6%. (*msrA*). Therefore, effective control measures should be adopted to stop the spread of drug-resistant *S. aureus* to humans.

KEYWORDS
Clinical mastitis, Bovines, *S. aureus*, Antimicrobial resistance genes, Pathology.

INTRODUCTION

Bovine mastitis is a severe condition that has a high occurrence, causes financial loss, and is a big concern for the dairy sector globally (Enger *et al*., 2019). Mastitis was predicted to cause a $35 billion annual economic loss worldwide, including decreased milk output, milk rejection due to drug residues, veterinary expenses, the culling of chronically affected cows, and sporadic deaths (Abebe *et al*., 2016). Mastitis also constitutes a risk to human health because it may be linked to zoonoses and foodborne diseases (Bhandari *et al*., 2021).

Mastitis is a complicated condition that arises from the interaction of numerous elements related to the host, particular infections, environment, and management (Harjanti *et al*., 2018). There are around 200 distinct microbes known to cause cow mastitis (Blowey and Edmondson 2010). Studies have focused on significant mastitis bacteria like *Staphylococcus aureus* (*S. aureus*), *Streptococcus agalactiae*, and *coliforms* (Amer *et al*., 2018). According to other research, minor pathogens like coagulase-negative *Staphylococcus* and other bacilli have replaced major pathogens as the main cause of the disease (Vakkamäki *et al*., 2017).

Staphylococcus aureus has a remarkable capacity for resisting antimicrobial treatments and eluding the human immune system. Effective methods in mastitis control programs can be devised by understanding S.aureus' resistance mechanisms. It has been demonstrated that acquired resistance and intrinsic resistance both help *S. aureus* withstand particular antimicrobial stress (Baym *et al*., 2016). It can develop resistance to many other antimicrobial agents by carrying various resistance features on plasmids or transposons (Rajagopal *et al*., 2016), and it has many inherent characteristics that decrease the effectiveness of specific antimicrobial treatments (Chajecka-Wierzchowska *et al*., 2015).

A crucial component of mastitis control regimens is an antibiotic medication, however, *S. aureus* does not respond well to it (Gomes and Henriques, 2016). There is a risk that humans could contract acquired antimicrobial resistance (Ruegg *et al*., 2015). Thus, early detection and awareness of the variety of infections linked to mastitis are crucial for efficient prevention and control (Vakkamäki *et al*., 2017). Due to the significant rise in pathogens that are resistant to antibiotics, it is expected that the treatment might be more difficult shortly (Vakkamäki *et al*., 2017). Consuming unpasteurized milk has been associated with the transmission of foodborne and antibiotic-resistant mastitis pathogens to people (Beyene *et al*., 2017).

In recent years, pathogens have developed drug resistance as a result of the misuse of antibiotics, and the issue of multidrug resistance has gained significance (Rabello *et al*., 2020). Antibiotic

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resistance genes and bacterial resistance genes have a tight relationship. The discovery of methicillin-resistant *S. aureus* (MRSA) isolates in cows with mastitis raises serious concerns because these strains frequently carry genetic markers for resistance to antimicrobial drug groups other than beta-lactams (Kadlec *et al*., 2019; Liu *et al* ., 2020; Schnitt and Tenhagen, 2020; Shrestha *et al*., 2021).

The *erm(A)*, *erm(B)*, *erm(C)*, and *erm(T)* genes are frequently found in bovine mastitis MRSA, according to investigations conducted in Germany (Kadlec *et al*., 2019). The transposons Tn554 and Tn917/Tn551 are linked to the *erm(A)* and *erm(B)* genes, respectively. SCC mec elements typically include the *erm(A)* gene, while multi-resistant plasmids are connected to the *erm(B)* gene (Schwarz *et al*., 2018). The *erm(C)* gene is typically found on tiny plasmids without additional resistance genes (Lodder *et al*., 1997).

S. aureus from cases of mastitis was discovered to contain the *erm(B)* and *erm(C)* genes in China (Li *et al*., 2015). The multi-resistant plasmid can contain the *ermT* gene, which has been reported to be more prevalent in *S. aureus* than in non-aureus staphylococci associated with bovine mastitis (Qu *et al*., 2019). According to research by Youssif *et al*. (2020), *S. aureus* strains in Egypt were resistant to drugs such as tetracycline, B-lactams, macrolides, methicillin, vancomycin, and norfloxacin, which are encoded by the genes (*tetK-tetA*), (*blaZ*, bla_{TEM}), (*ermB*, *ermC*), (*mecA*, *mec1*, *mecC*), (*vanA*) and (*norA*), respectively. This study's goal was to determine the prevalence of bacteria linked to clinical mastitis in separated milk samples from dairy cattle and buffalo farmed by small farmers throughout various governorates in Egypt (Cairo, Giza, Kalyobia, Fayoum, and Kafr El-Sheikh). By using the PCR TaqMan assay and standard Polymerase Chain Reaction, *S. aureus* was discovered. All isolates underwent PCR screening for the erythromycin-resistant determinants (*ermA*, *ermB*, *ermC*, and *ermT* genes) and the macrolide determinants (*msrA* gene). After sequencing the positive PCR results for the *NUC1* gene, each animal's mammary gland underwent a histological investigation.

MATERIALS AND METHODS

Animals and milk samples processing

From small-scale farmers in various governorates of Egypt (Cairo, Giza, Kalyobia, Fayoum, and Kafr El-Sheikh), 285 milk samples were obtained from 150 dairy livestock and 135 dairy buffalo. Based on the history, clinical symptoms, and physical examination of the udder and milk as reported by Jackson and Cockroft (2002). Cases of clinical mastitis were identified. Milk samples were swiftly transported to the bacteriological examination facility of the Department of Microbiology and Immunology, Veterinary Research Institute, National Research Centre (NRC) while being tightly sealed, and kept chilled at 4°C.

Ethical approval: Medical Research Ethics Committee, NRC (No. 231712012023).

Bacteriological isolation and identification

Gram staining, catalase, coagulase (APHA, 1992 and APHA, 2003), detection of hemolytic activity, DNase agar assays (Murray *et al*., 2003), and for the biofilm activity onto Congo red medium were used to determine the presence of *Staphylococcus* spp. in milk samples from clinically and sub-clinically mastitic calves and buffalo (Arciola *et al*., 2015). Antimicrobial Susceptibility Testing (CLSI, 2017).

DNA extraction

Following the manufacturer's instructions, DNA was extracted from bacterial cultures using the QIAamp DNA Mini reagent (Qiagen, Germany, GmbH). In a nutshell, bacterial pellets were re-suspended in 200 µl of PBS and incubated for 10 min at 56 °C with 20 µl of proteinase K and 200 µl of lysis solution. 200 µl of 100% ethanol was added to the homogenate after incubation. After washing, the material was centrifuged. 50 µl of elution solution was used to elute the nucleic acid.

Molecular identification using Real-time PCR

PCR TaqMan assay was carried out targeting S.aureus, using the qTOWER 3G (AnalytikJena, Germany), which was used for thermocycling and fluorescence detection. The real-time PCR amplification was performed in a total volume of 20 µl containing 10 µl of 2X Topreal Taqman Probe quantitative PCR mixture (Cat RT600, Enzynomics) according to the manufacturer's instructions, 0.2 µl (10µm) of each primer and 0.4 µl (10µm) TaqMan probe mixture, and 2 µl of template DNA; distilled water (DW) was added for a final volume of 20µl. The specific primers (*NUC2*) and probes used for the identification of the nuc gene *S. aureus* were listed in Table 1. The cycling conditions were listed in Table 2.

Table 1. PCR primers and probes used in the study.

Gene	Sequence (5´-3´)	Amplicon size (bp)	Reference
NUC1	CTG GCA TAT GTA TGG CAA TTG TT TAT TGA CCT GAA TCA GCG TTG TCT	664bp	Graber *et al*. (2007)
NUC2	AAAGCGATTGATGGTGATACGGTT TGCTTTGTTTCAGGTGTATCAACCA FAM-Probe ATGTACAAAGGTCAACCAATGACATTYAGA	-------	Wang *et al*. (2014)
ermA	TATCTTATCGTTGAGAAGGGATT CTACACTTGGCTTAGGATGAAA	139bp	Martineau *et al*. (2000)
ermB	CTATCTGATTGTTGAAGAAGGATT GTTTACTCTTGGTTTAGGATGAAA	142bp	Martineau *et al*. (2000)
ermC	CTTGTTGATCACGATAATTTCC ATCTTTTAGCAAACCCGTATTC	190bp	Martineau *et al*. (2000)
ermT	ATTGGTTCAGGGAAAGGTCA GCTTGATAAAATTGGTTTTTGGA	536bp	Fessler *et al*. (2010)
msrA	TCCAATCATTGCACAAAATC AATTCCCTCTATTTGGTGGT	163bp	Aktas *et al*. (2007)

Molecular identification using conventional Polymerase Chain Reaction (PCR)

Using a GS-96 gradient thermocycler (hercuvan, Malaysia), PCR reactions were carried out to identify *S. aureus* (*nuc* gene). The reaction volume was 25 µl, with 12.5 µl of 2x COSMO PCR RED Master Mix (Cat. W1020300X, Willofort Co., UK), 0.5 µl (10 M) of each primer (Vivantis, Malaysia), and 1 l of target DNA. The PCR products were separated by electrophoresis on 1.5% agarose gel, and the InGenius3 gel documentation device was used to take pictures and conduct analysis (Syngene, UK). Tables 1 and 2 contain a summary of the primers (nuc 1) and cycling conditions that were used.

PCR detection of antibiotic-resistant determinants

All samples underwent PCR screening for the erythromycin-resistant determinants (*ermA*, *ermB*, *ermC*, and *ermT* genes) and the macrolide determinants (*msrA* gene). The total volume of the PCR reaction was 25 µl, which contained 1 µl of the target DNA, 0.5 µl (10 M) of each primer, and 1 µl of the 2x COSMO PCR RED Master Mix (Cat. W1020300X, Willofort Co., UK). The PCR products were separated by electrophoresis on 1.5% agarose gel, and the InGenius3 gel documentation device was used to take pictures and conduct analysis (Syngene, UK). The cycling conditions and used primers were mentioned. in Tables 1 and 2.

DNA sequencing

Thermo Fisher's GeneJETTM Gel Extraction Kit (K0691) was used to clean the positive PCR products that targeted the *NUC1* gene. MACROGEN Company (Korea) then used 3730XL sequencers to read the results (Applied Biosystems, USA). Eight PCR sequences used in this research have been deposited in the GenBank database under accession numbers OP821397-OP821400 and OP821401-OP821404 for *S. aureus* isolates from cattle and buffalo with clinical mastitis, respectively.

Histopathological examination

Each animal's mammary gland was instantly harvested for tissue samples, which were then promptly rinsed with isotonic saline and fixed in 10% buffered formalin. For histopathological analysis, tissue sections were done in paraffin (Bancroft and Stevens, 1996).

RESULTS

As shown in Table 3, an examination of a total of 150 and 135 cattle and buffalo milk samples obtained from smallholders revealed that 34 (22.7%) and 36 (26.7%) of the milk samples of cattle and buffalo, respectively, had clinical mastitis with symptoms of inflammation (hotness, redness, swelling, and pain of the udder), for a clinical mastitis prevalence of 24.6%.

With a total of 31 (44.3%), *S. aureus* isolates from bovines, the bacterial isolation and identification of *S. aureus* from milk from clinically mastitic cattle and buffalo from smallholders confirmed the isolation and identification of 14 (41.2%) and 17 (47.2%) *S. aureus* isolates out of 34 and 36 cattle and buffalo with clinical mastitis, respectively (Table 4).

Molecular identification using Real-time PCR

All 31 (100%) bacteriologically identified *S. aureus* isolates were confirmed using the Probe-Based Real-time PCR.

Table 2. Cycling conditions for the detection of genes in this study.

Gene	Init. Denat.	Denat.	Anneal.	Extention	Final Ext.	Cycles
NUC1	95°C 2min	95°C 20sec	60°C 30sec	72°C 45sec	72°C 10min	35
NUC2 (Q-PCR)	95°C 10min	95°C 10sec	56°C 20sec	60°C 40sec	----------	40
ermA	95°C 3min	95°C 20sec	59°C 30sec	72°C 45sec	72°C 10min	40
ermB	94°C 2min	94°C 30sec	55°C 30sec	72°C 30sec	72°C 7min	35
ermC	95°C 3min	95°C 20sec	55°C 30sec	72°C 45sec	72°C 7min	40
ermT	95°C 2min	95°C 30sec	57°C 30sec	72°C 30sec	72°C 7min	35
msrA	94°C 2min	94°C 20sec	54°C 30sec	72°C 45sec	72°C 7min	35

Table 3. Prevalence of clinical (CM) in dairy cattle and buffaloes at different governorates of Egypt (Smallholders).

Governorate	Cattle	Buffalo	Total
Cairo	2/12 (16.7%)	4/15 (6.7%)	6/27 (22.2%)
Giza	5/27 (18.5%)	4/25 (16%)	9/52 (17.3%)
Kalyobia	7/23 (30.4%)	9/37 (24.3%)	16/60 (26.7%)
Fayoum	8/45 (17.8%)	13/41 (30.7%)	21/86 (24.4%)
Kafr El-Sheikh	12/43 (27.9%)	6/17 (35.3%)	18/60 (30%)
Total	34/150 (22.7%)	36/135 (26.7%)	70/285 (24.6%)

Table 4. Prevalence of *S. aureus* isolates in milk samples from cattle and buffaloes with clinical mastitis (Smallholders).

Governorate	Cattle	Buffalo	Total
Cairo	2/2 (100%)	3/4 (75%)	5/6 (83.3%)
Giza	2/5 (40%)	1/4 (25%)	3/9 (33.3%)
Kalyobia	3/7 (42.9%)	4/9 (44.4%)	7/16(43.8%)
Fayoum	3/8 (37.5%)	7/13(53.8)	10/21 (47.6%)
Kafr El-Sheikh	4/12(33.3%)	2/6 (33.3%)	6/18 (33.3%)
Total	14/34 (41.2%)	17/36 (47.2%)	31/70 (44.3%)

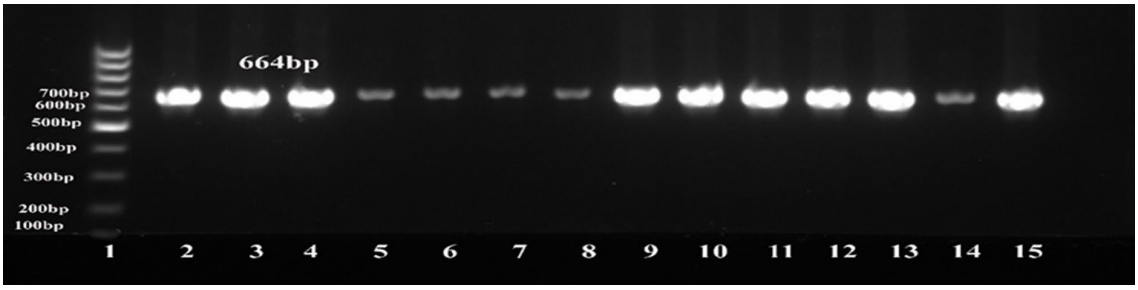

Fig. 1. Agarose gel electrophoresis of PCR product amplified from *S. aureus* nuc gene (664 bp). Lane 1 - 100 bp DNA Ladder; Lanes 2-15, representative positive samples.

Molecular identification using conventional Polymerase Chain Reaction (PCR)

For the DNA sequencing, conventional PCR reactions were performed on the positive probe-based RT-PCR samples (*S. aureus*). All samples were again positive using conventional PCR as shown in Figure 1.

Prevalence of Antimicrobial Resistance Genes

Established primers for the detection of erythromycin and macrolides genes were carried out on the genomic DNA of 31 *S. aureus* strains. Concerning erythromycin resistance, *ermA*, *ermB*, *ermC*, and *ermT* genes specific amplicons were detected in 3 (9.6%), 20 (64.5%), 22 (70.9%), and 6 (19.3%) strains, respectively as shown in Figures 2-5 and Table 3. Concerning Macrolides resistance, the *msrA* gene was harbored in 6 (7.8%) of *S. aureus* strains as shown in Figure 6 and Table 3.

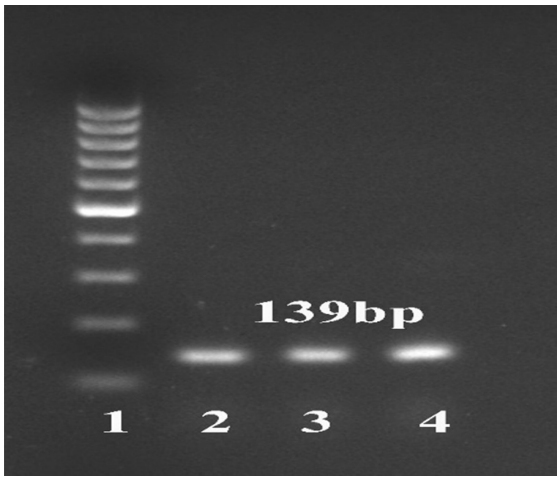

Fig. 2. PCR analysis of erythromycin-resistant determinants: *ermA* (139 bp). Lane 1, 100bp DNA ladder; Lanes, 2-4, representative positive samples.

Phylogenetic analysis

All eight PCR products had the same nucleotide sequence and were named OP821397-Op821404. The multiple sequence alignment and phylogenetic tree construction of the *S. aureus* nuc gene showed high homology (100%) with that of *S. aureus* strains isolated from milk in cases of bovine mastitis in India and Kenya (JX240349, JN247783, GU129659, MW826579) as shown in Figure 7.

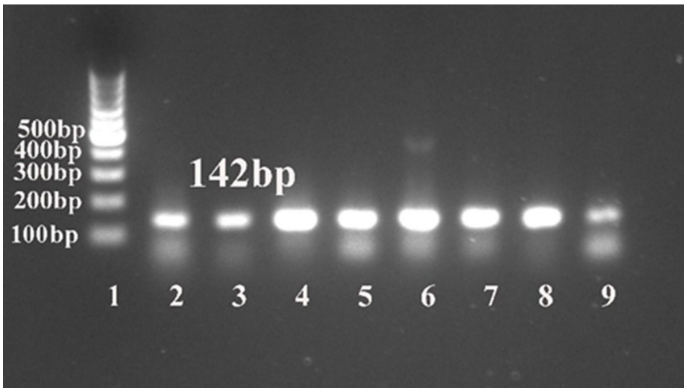

Fig. 3. PCR analysis of erythromycin-resistant determinants: *ermB* (142bp). Lane 1, 100bp DNA ladder; Lanes, 2-9, representative positive samples.

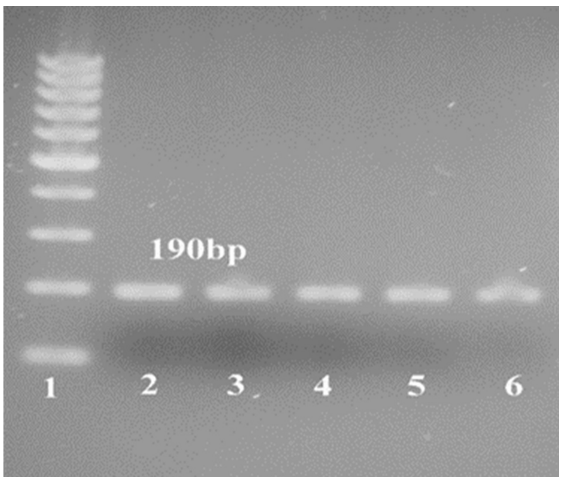

Fig. 4. PCR analysis of erythromycin-resistant determinants: *ermC* (190bp). Lane 1, 100bp DNA ladder; Lanes, 2-6, representative positive samples.

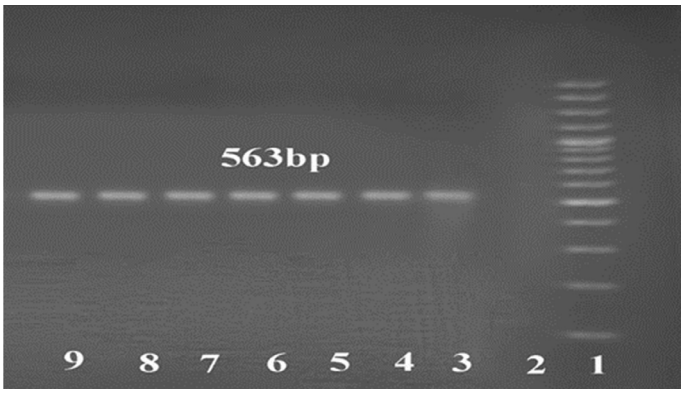

Fig. 5. PCR analysis of erythromycin-resistant determinants: *ermT* (536bp). Lane 1, 100bp DNA ladder; Lane 2, negative control; Lane 4: positive control; Lanes, 4-9, positive samples.

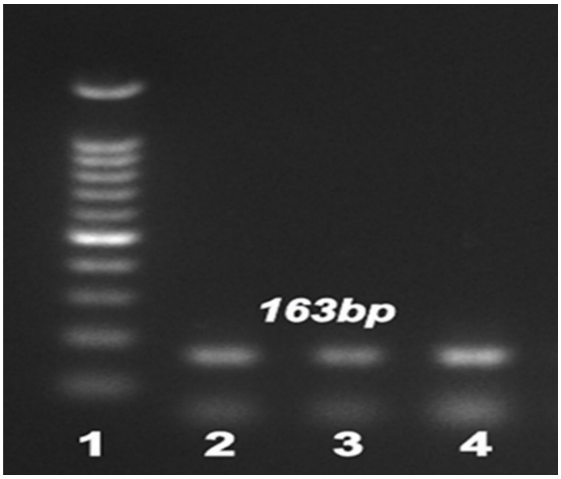

Fig. 6. PCR analysis of macrolide-resistant determinants: *msrA* (163bp). Lane 1, 100bp DNA ladder; Lanes, 2-4, positive samples.

Histopathological examination

Microscopical examination revealed lymphocytic mastitis in the mammary gland of the examined cases which is characterized by massive aggregations of mononuclear inflammatory cells mainly lymphocytes and macrophages in the interstitial connective tissue. The secretory acini showed vacuolar degeneration of the epithelial lining in some cases (Fig. 8).

Table 5. Prevalence of *S. aureus* antibiotic resistance genes (erythromycin and macrolides).

Gene	Total
NUC	31 (100%)
ermA	3 (9.6%)
ermB	20(64.5%)
ermC	22 (70.9%)
ermT	6 (19.3%)
msrA	3 (9.6%)

DISCUSSION

A significant problem for the dairy business around the world is bovine mastitis, a dangerous condition that is linked to both high incidence and financial loss. *S. aureus* is one of the most prevalent organisms that cause bovine mastitis, and antimicrobial therapy plays a crucial role in controlling mastitis brought on by *S. aureus*, although it is no longer as effective as it once was because of widespread drug resistance.

Due to the possibility of transfer of antibiotic resistance to humans as well as its impact on the efficacy of existing antibiotic therapy, antibiotic resistance has increased among several bacterial diseases (El Jakee *et al*., 2013; Algamma *et al*., 2019; El-Sayed *et al*., 2019). Moreover, MRSA strains have a high mortality rate in people and can induce nosocomial infections (Gordon and Lowy, 2008). This study's goal was to identify *S. aureus* strains with genes for virulence, drug resistance, and antibiotic resistance that could be used to control mastitis.

As shown in Table (3), a total of 34 (22.7%) and 36 (26.7%) milk samples from cattle and buffalo had clinical mastitis with a total prevalence of 24.6%. Our findings were in line with those of other studies conducted in Egypt and published by Mahmoud *et al.* (2015) who reported a prevalence rate of clinical mastitis of 22.6%. This study was carried out on 374 animals raised in El-Behera Governorate between March 2013 to January 2015. They are also in line with those of Kayesh *et al.* (2014) who reported

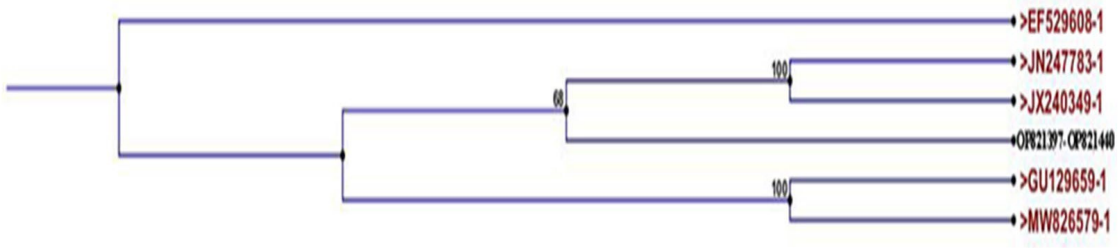

Fig. 7. Phylogenetic relationship of selected strains of *S. aureus* from different sources, based on the *nuc* gene.

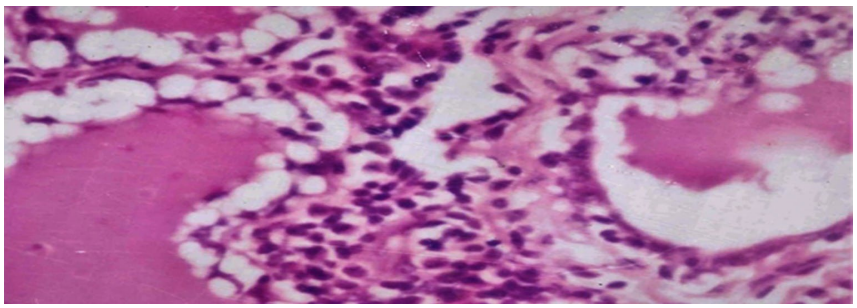

Fig. 8. Mammary gland showing lymphocytic mastitis as diffuse infiltration of lymphocytes in the intralobular interstitium associated with homogenous eosinophilic masses corpora amylacea (H&E, X100)

a 28.5% incidence rate in Bangladesh. In contrast, Mpatswenumugabo *et al*. (2017) in Rwanda reported a higher prevalence rate, showing a prevalence of 50.4%. On the other hand, in China, the incidence rate of clinical mastitis (CM) in large Chinese herds is as high as 3.3 cases per 100 cows per month (Gao *et al*., 2017). While, subclinical mastitis prevalence rate reported by Ndahetuye *et al*., 2019

S. aureus is one of the most commonly found pathogens in clinical mastitis. In our study, bacterial isolation of S.aureus from the milk of clinically mastitis cattle and buffalo confirmed 14(41.2%) and 17 (47.2%) of S.aureus isolates from 34 and 36 cattle and buffalo with clinical mastitis with a total of 31 (44.3%) S.aureus isolates from bovines with clinical mastitis as shown in Table (4).

This is consistent with Shi *et al*. (2021) investigation, which found that 276 (36.7%) of the 751 samples tested positive for *S. aureus*, with 150 (41.2%) testing positive in the spring and 126 (32.6%) testing positive in the fall. In addition, Seddek (1996) reported that *S. aureus* was the most prevalent isolated bacterium in clinically mastitis cattle (32.8%), and Waage *et al*. (1999) found that 44.3% of clinical mastitis cases in Norwegian dairy herds were caused by *S. aureus*. The prevalence rate of *S. aureus* was higher in northeastern China (45%) than in western China (33%) and southern China (31.9%), respectively. While lower incidence rates were reported by Taponen and Pyorala (2009) who found that 6.7% of mastitic cases were associated with *S. aureus*.

The nuc gene's amplification served as the basis for the identification of *S. aureus*. This was confirmed by David *et al*. (2010), which showed how amplifying the *S. aureus* nuc gene is regarded as the gold standard technique. The thermos-nucleases, or nuc gene, are encoded on the chromosome of *S. aureus*. The nuc gene is a recognized particular virulence factor in *S. aureus* that aids in the development of biofilms and immune evasion (Sultan *et al*., 2019; Andrade *et al*., 2021; Yu *et al*., 2021).

Over the years, veterinarians have recommended antibiotics as an efficient regimen for treating cow mastitis. The *msrA* gene was present in 3 (9.6%), 20 (64.5%), 22 (70.9%), 6 (19.3%), and 6 (7.8%) *S. aureus* strains in the current study, which found that the majority of *S. aureus* strains displayed MDR to several antibiotic groups, including erythromycin resistance, *ermA*, *ermB*, *ermC* and *ermT* genes, and macrolides resistance. These results are shown in Figures 2-6 and table 5. This is in line with El Faramawy *et al*. (2019), which found that 67.39% of bovine mastitis *S. aureus* isolates in Egypt were classified as MRSA strains and displayed resistance to several classes of antimicrobials.

The findings indicated a link between the usage of antibiotics and antimicrobial resistance. In a related study, Liu *et al*. (2017) discovered a positive correlation between antimicrobial resistance in isolates from mastitis samples and herd-level use of particular antimicrobials for treatment. Additionally, our findings were in line with those of Wang *et al*. (2015), who found that *S. aureus*, particularly MRSA strains, express resistance to several antimicrobial drugs, considered this to be a newly emerging etiology in bovine mastitis with a public health issue, and clarified the role of MRSA strains in challenges in treating *S. aureus* mastitis.

Erythromycin is a member of the macrolide family that functions differently in bacterial cells than penicillin and has good dispersion in the mammary gland. As a result, it is frequently employed as a substitute therapy for *S. aureus* mastitis. Erythromycin resistance genes are always thought to be present. In human infections caused by staphylococci, *ermA* and *ermC* are the most prevalent methylase genes. Erythromycin resistance in staphylococci is mostly mediated by erythromycin resistance methylase expressed by erm genes (Weisblum, 1995). Eady *et al*. (1993) reported that Type B streptogramin- and macrolide-resistant staphylococcal bacteria typically carry the gene *msrA*, which codes for an ATP-dependent efflux pump (Nicola *et al*., 1998).

The most common resistance genes found in erythromycin-resistant bacteria in this investigation were *ermB* and *ermC*, with percentages of 20 (64.5%) and 22 (70.9%), respectively. This is following Spiliopoulou *et al*. (2004) who found that the majority of their erythromycin-resistant bacteria included the *ermC* gene. On the contrary, Lim *et al*. (2012) claimed that *ermA* was the main resistance gene found in erythromycin-resistant strains.

Genotypic techniques facilitated metagenomic research of huge and diverse bacterial communities (Franco-Duarte *et al*., 2019). The phylogenetic analysis creates lineages by grouping strains with comparable spa nucleotide repeat sequences, demonstrating their genetic relatedness and the likely descendence from a common ancestor.

According to numerous studies, different herds have different *S. aureus* strains that are linked to mastitis (Cremonesi *et al*., 2015). Strains with the same genotype may have traits that give them some advantages for surviving in the environment and colonizing the udder (Mello *et al*., 2016). the results of our multilocus typing and phylogeny analysis (fig 6) of the isolates revealed that our MDR S.aureus isolates were closely related to those that have been reported from other nations and areas in the past as that of Jahan *et al*. (2015) and Xu *et al*. (2015).

In the mastitic udder, there were significantly fewer alveolar epithelial cells, fewer alveoli per plate, a decrease in alveolar luminal diameter, and an irregular shape of the alveoli. These changes appeared to be more advanced at this point, and the glandular parenchyma was losing its capacity to secrete. Reduced luminal regions, reduced secretory activity, and an abundance of connective tissue were all characteristics of involuted parenchyma. In our article, the histopathological changes include noticeably fewer alveoli, smaller alveoli, and a population of secretory alveolar cells (Fig. 8). These results suggested pathological udder tissue changes that could result from severe tissue injury brought on by various mastitis bacteria. Hussain *et al*. (2012) found that the intensity of cellular exudates changes with the different degrees of infection by the pathogen in the histomorphology of mastitis cattle udder tissue. These findings were in line with those of Fasulkov *et al*. (2015), who noted that histopathological findings indicated that epithelial cells had degenerated in vacuoles as well as interstitial alterations, edema, and mononuclear inflammatory cells that had multiplied into lymphocytes and histiocytes.

CONCLUSION

Erythromycin-resistant *S. aureus* isolates frequently exhibit co-resistance to macrolide-resistant determinants. *ErmB* and *ermC* are the two main genes identified in erythromycin-resistant bacteria. The existence of the *ermA*, *ermB*, *ermC*, *ermT*, and *msrA* genes in Egyptian MRSA strains led to erythromycin and macrolide resistance. As the association of resistance genes (*ermC* and *msrA*) with mobile genetic components may promote the dissemination of resistant features in MRSA, good infection control procedures should be used. For tracking erythromycin and macrolide resistance among MRSA strains in Egypt, this data collection may be used as a guide. Tracking staphylococci's virulence mechanisms and antibiotic resistance is essential because they can adapt to new environments; doing so enables us to completely comprehend the pathogenesis of this pathogen and may aid in the creation of new, more effective treatments in the future.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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