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RESEARCH ARTICLE

Prevalence and Antibiogram of *Staphylococcus aureus* in Clinical and Subclinical Mastitis in Holstein Dairy Cows in Egypt

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Abstract

Mastitis is a multifactorial and ubiquitous disease that results from interactions between the host, environment, and infectious agents leading to extensive economic losses. The objectives of this study were to estimate the prevalence of *S. aureus* in clinical and subclinical mastitis in Holstein dairy cows and determine the susceptibility of *S. aureus* isolatesagainst different antibiotics for screening of antibiotic resistance genes. A total of 415 Holstein dairy cows were randomly selected from three Egyptian governorates. Selected cows were examined for clinical and subclinical mastitis during the period from October 2014 to June 2018. Milk samples were examined for the presence of *S. aureus.* The *S. aureus* isolates were subjected to antibiotic sensitivity, molecular identification, and detection of the antibiotic resistance gene.The prevalence of *S. aureus* isolated from milk samples was 15.4% of which, 14.3% in clinical cases, and 15.7% in subclinical cases.The antibiogram of *S. aureus* isolates against 13 antibiotics using the disc-diffusion method revealed the highest rate of resistance to Oxacillin (OX) (96.7%), followed by Ampicillin (AM),Cefoxitin (FOX) (93.3%, each), Tetracycline (TE) (73.3%), Cefotaxime (CTX) (70%), Ampicillin/Sulbactam (SAM) (66.7%), Erythromycin (E) and Sulphamethoxazole/Trimethoprim (SXT) (56.7%, each), Gentamicin (GM) (53.3%), Ofloxacin (OFX) (40%), Chloramphenicol (C) (36.7%), Ciprofloxacin (CIP) (30%) and finally Vancomycin (VA) (0%). Molecular PCR assay revealed that all the 16 *S. aureus* isolates (100%) carried mecA gene, while 15 out of 16 isolates (93.7%) carried blaZ genebut, 8 out of 16 (50%) carried tetK gene, and only one isolate (0.06%) carried fexA gene. Uncontrolled uses of antibiotics in the treatment of mastitis should be restricted and increase awareness about the risk of antimicrobial-resistant bacteria in milk.

Keywords: Mastitis, *S. aureus*, Antibiogram, PCR, Antibiotic resistance genes.

Introduction

Mastitis is one of the most devastating diseases that results from dramatic interactions between the host, environment, and infectious agents. Mastitis is associated with huge economic losses due to reduced milk quality and quantity, and veterinary costs caused by antibiotic withdrawal time post-treatment [1, 2].

More than 140 different microorganisms have been isolated from bovine mastitis cases [3]. Contagious and environmental bacteria, moreover fungi, algae, and viruses have been incriminated as the main cause of mastitis globally [4-6]. Hygienic measures, management practices, and environmental factors have a direct effect on the distribution of mastitis and mastitis-causing microbes among countries, regions, and farms [7-10]. The most common mastitis pathogens are bacteria which can be classified into contagious pathogens include (*Staphylococcus aureus* and *Streptococcus agalactiae*, *Corynebacterium bovis*, and *Mycoplasma spp.*) and environmental pathogens including *Escherichia coli*, *Enterococcus faecalisStreptococcus dysagalactiae* and *Streptococcus uberis*, and coagulase-negative Staphylococci [4].

Staphylococcus aureus is the main causative agent of about one-third of clinical and subclinical mastitis cases in dairy cattle. Moreover, a high incidence of *S. aureus* is associated with improper farm hygiene and management system especially lacking post milking teat dipping and sanitization of the milking system as well as not applying dry cow therapy [11]. Intramammary antibiotic therapies formulated for the treatment of mastitis are generally unsuccessful in eliminating existing *S. aureus* leading to the culling of the infected animals, but the application of a combination of vaccination and extended antimicrobial treatment can reduce intramammary infectionby*S. aureus* [12]

Recently, the methicillin-resistant *S. aureus* (MRSA) isolates have become widely spread all over the world with the risk of resistance to all beta-lactams and other classes of antibiotics. Therefore, the therapeutic choices are limited significantly [13]. *S. aureus* isolates show antibiotic resistance and pathogenic characteristics via mutation, clonal evolution, and horizontal gene transfer. There are several mechanisms for expressing that resistance including hydrolysis of antibiotics by enzymes, target site modification of ribosome, and metabolic pathway alteration. Numerous antimicrobial-resistant genes have been documented including mecA which encodes for PBP2a mediated resistance to methicillin and all other beta-lactams and tetK that encodes for alteration of the ribosome or drug efflux mediated resistance to tetracycline [14-17]. Various genetic determinants such as mecA and blaZ (penicillin), tetK/M (tetracyclines) are reported to be responsible for the corresponding antimicrobial resistance mechanisms in *S. aureus*[18]. These genetic determinants enable *S. aureus* to reside for a long time inside the host or herd environment and avoid antimicrobial therapy [19]. The objectives of this study were to estimate the prevalence of *S. aureus* in clinical and subclinical mastitis in Holstein dairy cows and determine the susceptibility of *S. aureus* isolatesto different antibiotics as well as screening of antibiotic resistance genes.

Material and methods

Animals

A total number of 415 lactating Holstein dairy cows were selected from

different localities in three Egyptian governorates. From Damietta governorate, 245 cows were selected from three private dairy farms including farm A, 60 lactating cows, farm B, 65 lactating cows, and farm C, 120 lactating cows. From Sharkia governorate, 100 lactating cows were selected from one farm (farm D). From Dakahlia governorate, 70 individual cases of dairy cows were randomly selected. The selected cows were examined for clinical and subclinical mastitis during the period from October 2014 to June 2018 (Table 1). Clinical examination of the investigated lactating cows was done according to Constable*et al.*[3] to estimate the presence of any signs of inflammation for the detection of clinical mastitis cases. In addition, California Mastitis Test (CMT) was carried out as a screening test for the detection of subclinical mastitis [20]**.** The study was approved by the Ethical Committee, Faculty of Veterinary Medicine, Mansoura University.

Table1. Examined animals from Dairy farms in Damietta, Sharkia, and Dakahlia Governorates (October 2014 to June 2018)

Governorate	Farm	Examined animals	Clinical cases	Apparently healthy	Quarters No.	*S. aureus* isolates No.
Damietta	Farm A	60	12	48	64	4
Damietta	Farm B	65	0	65	48	4
Damietta	Farm C	120	3	117	60	8
Sharkia	Farm D	100	4	96	145	5
Dakahlia	Small-holders' Cases	70	31	39	150	49
	Total	**415**	**50**	**365**	**467**	**70**

Milk samples

A total of 896 quarter milk samples from clinically and apparently healthy cows were collected for bacteriological examination under aseptic conditions after cleaning and disinfection of the teat end with 70% alcohol [21].

Bacteriological examination

Milk culturing and identification were carried out at the laboratory of Animal Health Research Institute, Mansoura Provincial Lab., Egypt according to Quinn *et al.*[22]. Milk samples were initially mixed with 5 ml of pre-enrichment liquid broth (trypticase soya broth, Oxoid) and then were incubated at 37ºC overnight. The samples were streaked onto the surfaces of Baird-Parker agar and mannitol salt agar. Inoculated plates were incubated at 37ºC for 24-48 hours and then examined for bacterial growth. Identification of bacteria was performed by standard biochemical tests (catalase, coagulase, mannitol fermentation and D-Nase tests)[23, 24].

Antibiotic sensitivity test of Staphylococcus aureus

The antibiotic sensitivity test was carried out on30*S. aureus* isolates against 13 antibiotics (Oxoid) by disc-diffusion method according to Bauer *et al.*[25]. The tested antibiotics were ampicillin (AM; 10 µg), ampicillin/sulbactam (SAM; 10/10 µg), cefotaxime (CTX; 30 µg), cefoxitin (FOX; 30 µg), chloramphenicol (C; 30 µg), ciprofloxacin (CIP; 5 µg),

erythromycin (E; 15 µg), gentamicin (GM; 10 µg), ofloxacin (OFX; 5 µg), oxacillin (OX; 1 µg), sulphamethoxazole/trimethoprim (SXT; 23.75/1.25 µg), tetracycline (TE; 30 µg) and vancomycin (VA; 30 µg). Interpretation of the results was applied [26] and MAR (multiple antibiotic resistance) index of an isolate was calculated as a/b where (a) representing the number of antimicrobials to which the isolate was resistant and (b) representing the number of antimicrobials to which the isolate was subjected [27].

Molecular identification of the suspected S. aureus isolates and detection of antibiotic resistance genes

The 16 recovered *S. aureus* isolates were subjected to further molecular identification by amplification of nuc[28] and coa[29] genes and screening for antibiotic resistance genes mecA[30] and blaZ[31] for detection of β-lactam resistance, (tetK) [32] for tetracycline resistance and (fexA) [33] for chloramphenicol resistance, using primers listed in Table 2.

DNA extraction

DNA extraction was carried out using QIAamp DNA Mini Kit (Catalogue No.51304) according to manufacturer's instructions.

Polymerase chain reaction

PCR amplification was performed in T3 Thermal cycler (Biometra, Germany) in a final volume of 25µL per sample consisting of 12.5 µL of Emerald Amp GT PCR master-mix (Code No. RR310A Takara, USA) (2x premix), 1 µL (20 pmol concentration) for each forward and reverse primers, 6 µL of template DNA, and 4.5 µL PCR grade water. The following cycling conditions were conducted: primary denaturation at 94℃ for 5 min, secondary denaturation at 94℃ for 30 sec, annealing at 55 ℃ for 45 sec, extension at 72℃ for 45 sec and a final extension at 72℃ for 10 min for 35 cycles. The amplified products were separated on 1.5% agarose gel [34].

Table 2. Oligonucleotide primers sequences for detection of the suspected *S. aureus* isolates and detection of antibiotic resistance genes

Gene	Primer sequence (5'-3')	Length of amplified product	References
nuc	ATATGTATGGCAATCGTTTCAAT GTAAATGCACTTGCTTCAGGAC	395 bp	[28]
coa	ATA GAG ATG CTG GTA CAG G GCT TCC GAT TGT TCG ATG C	630 bp	[29]
blaZ	TACAACTGTAATATCGGAGGG CATTACACTCTTGGCGGTTTC	833 bp	[31]
mecA	GTA GAA ATG ACT GAA CGT CCG ATA A CCA ATT CCA CAT TGT TTC GGT CTA A	310 bp	[30]
tetK	GTAGCGACAATAGGTAATAGT GTAGTGACAATAAACCTCCTA	360 bp	[32]
fexA	GTA CTT GTA GGT GCA ATT ACG GCT GA CGC ATC TGA GTA GGA CAT AGC GTC	1272 bp	[33]

Results and Discussion

The prevalence rate of mastitis in milk samples at the quarter level was 52.1% (467/896) of which subclinical form 40.3% (361/896) was higher than clinical one 11.8% (106/896). This could be due to improper milking hygiene, lack of post milking teat dipping, and little attention given to subclinical mastitis, as the infected animal did not show any visible signs and secreted apparently normal milk [3]. The prevalence of *S. aureus* isolated from milk samples in the present study was 15.4% (70 *S. aureus* isolates/ 456 total bacterial isolates) of which, 14.3%(15 *S. aureus* from clinical mastitic cases / 105 total bacterial isolates from mastitic cases) in clinical cases, and 15.7% (55 *S. aureus* from subclinical mastitic cases / 351 total bacterial isolates from mastitic cases) in subclinical cases. These results were close to a previous report from Ethiopia in which the prevalence of *S aureus* was 13.8% in subclinical cases [35]. On the other hand, our finding is lower than other reported studies in Egypt, where *S. aureus* was isolated from 21.3% in clinical cases and 31.9% in subclinical ones [36]and 36.3% in subclinical[37]. Many factors may affect the prevalence of *S. aureus*, for instance, a high prevalence of *S. aureus* in milk could be attributed to bad hygiene, poor management including improper milking hygiene, lack of teat dipping, absence of dry therapy, and bad bedding material [38]. Moreover, *S. aureus* is one of the contagious organisms whose main reservoirs are milk of the infected gland and the udder skin that contribute to its spread with the ability to penetrate the tissue producing deep-seated foci [39].

Table (3) presented the antibiogram of 30 *S. aureus* isolates which were highly resistant to oxacillin (96.6%) followed by ampicillin and cefoxitin(93.3%, each), tetracycline(73.3%), cefotaxime (70%), ampicillin/sulbactam (66.7%), erythromycin and sulphamethoxazole /trimethoprim (56.7%, each), gentamicin (53.3%), ofloxacin (40%), chloramphenicol (36.7%), and ciprofloxacin (30%). Our results were in close alignment with the previous report that had revealed high resistance of *Staph. aureus* isolates against methicillin followed by oxytetracycline, ampicillin, and sulphamethoxazole/trimethoprim besides. About 95% of the isolates were susceptible to vancomycin[40]. In addition, they were close to the results ofDaka *et al.*[38] who reported the highest resistance of *S. aureus* to ampicillin followed by oxacillin, erythromycin, amoxicillin-clavulanic acid, trimethoprim/sulphamethoxazole, and no resistance was detected for ciprofloxacin. However, our results are inconsistent with a previous study in Egypt on the detection of high resistance levels against vancomycin (93.75%) [41]. Failure to respond to antibiotic therapy was found to be multifactorial in lactating cows, which may be attributed to the presence of micro-abscesses within the udder and inaccessibility of the drug to the causative agent and avoiding the effect of antibiotic by residing inside macrophages. Moreover, some strains of *S. aureus* can exist as latent bacteria within a capsule and can later reactivate growth when conditions normalize [42]. Other factors are related to the veterinarians such as using unsuitable drugs, reducing the dosage of the drugs, or shortening the length of the treatment protocol, less drug activity, and prescription of antibiotics without conducting antibiotic sensitivity tests on the causative organisms, which leads to increasing resistance strains of the microorganisms. The failure of these practices will result in the development of

resistant strains of microorganisms [43]. The antibiotic sensitivity of bacteria differs from one region to another where some countries showed higher resistance rates than others. In future studies, the prevalence of resistance should be used for the development of guidelines for appropriate antibiotic use in veterinary medicine[44].

Table3.Phenotypic antimicrobial sensitivity pattern of 30 *S. aureus* isolated from mastitic dairy cows at different farms in Damietta, Sharkia &Dakahlia Governorates.

Antimicrobial agent tested	Phenotypic antimicrobial sensitivity pattern					
	S		I		R	
	No	%	No	%	No	%
Ampicillin (AM)	-	-	2	6.7%	28	93.3%
Oxacillin (OX)	-	-	1	3.3%	29	96.7%
Ampicillin/sulbactam (SAM)	6	20%	4	13.3%	20	66.7%
Cefoxitin (FOX)	2	6.7%	-	-	28	93.3%
Cefotaxime (CTX)	7	23.3%	2	6.6%	21	70%
Erythromycin (E)	11	36.7%	2	6.6%	17	56.7%
Ciprofloxacin (CIP)	18	60%	3	10%	9	30%
Ofloxacin (OFX)	18	60%	-	-	12	40%
Sulphamethoxazole/trimethoprim (SXT)	12	40%	1	3.3%	17	56.7%
Tetracycline (TE)	6	20%	2	6.7%	22	73.3%
Gentamicin (GM)	12	40%	2	6.7%	16	53.3%
Vancomycin (VA)	30	100%	-	-	-	-
Chloramphenicol (C)	17	56.7%	2	6.7%	11	36.7%

Table (4) revealed that all the *S. aureus* isolates were resistant to multiple antimicrobial agents with high MAR (multiple antibiotic resistance) indexes, which was more than 0.2. These findings were in close alignment with previous reports in Egypt, where 83% of the isolated *S. aureus* exhibited multi-drug resistance to three or more antibiotics. Moreover, all MRSA strains showed resistance to nine or more antibiotics[45]. Furthermore, our results were in accordance with another study that detected resistance of all of the MRSA isolates to at least four antibiotics where 6.6% of the isolates were resistant to ≥ three antibiotics, 9% were resistant to ≥ five antibiotics, 8% were resistant to four antibiotics and 6.6% were resistant to three antibiotics[46]. In addition, Chandrasekaran *et al*.[47] reported that MDR against methicillin, amoxicillin,

penicillin, and tetracycline was commonly detected in MRSA isolates. While a previous study[48]reported a lower prevalence of multidrug resistance among *S. aureus* (9.9%). Multi-drug resistance was observed against some classes of antibiotics such as methicillin, tetracycline, and erythromycin which can limit antibiotic effectiveness [49]**.** Beta-lactamase-resistant penicillins such as methicillin and oxacillin were not used in dairy cattle except for cloxacillin, which was used in products for intramammary administration. However, MRSA had been isolated from mastitic milk samples and had the potential to complicate the treatment of bovine mastitis [50, 51]**.** It is important to highlight that oxacillin-resistant isolates were also resistant to other beta-lactams [52].Thus, the application of antibiotic sensitivity test is recommended to choose the suitable drug avoiding time waste and heavy costs to reduce the multidrug resistance phenomenon.

Table4.Antimicrobial resistance pattern of *S. aureus.*

Sample	Antibiotic resistance pattern	MAR index
1	OX, AM, TE, SAM, E, GM, SXT, CIP, CTX, FOX	0.77
2	OX, AM, TE, SAM, E, GM, SXT, C, CIP, CTX, FOX	0.85
3	OX, AM, TE, SAM, E, GM, SXT, OFX, CIP, CTX, FOX	0.85
4	OX, SXT, CIP, CTX, FOX	0.38
5	OX, AM, TE, SXT, OFX	0.38
6	OX, AM, TE, E, GM, SXT, C, OFX, CIP, FOX	0.77
7	OX, AM, TE, SAM, E, GM, SXT, C, CTX, FOX	0.77
8	OX, AM, TE, SAM, E, GM, SXT, C, OFX, FOX	0.77
9	OX, AM, TE, SAM, E, GM, SXT, C, CTX, FOX	0.77
10	OX, AM, TE, SAM, GM, SXT, CTX, FOX	0.61
11	AM, TE, SAM, GM	0.31
12	OX, TE, E, GM, SXT, OFX, CIP, CTX, FOX	0.69
13	OX, AM, TE, SAM, E, GM, SXT, FOX	0.61
14	OX, AM, E, SXT, CTX, FOX	0.46
15	OX, AM, TE, SAM, E, GM, SXT, C, CTX, FOX	0.77
16	OX, AM, TE, E, GM, SXT, OFX, CIP, CTX, FOX	0.77
17	OX, AM, TE, SAM, E, GM, SXT, OFX, CIP, CTX, FOX	0.85
18	OX, AM, TE, SAM, E, CTX, FOX	0.54
19	OX, AM, TE, SAM, E, CT, FOX	0.54
20	OX, AM, TE, SAM, E, C, OFX, CTX, FOX	0.69
21	OX, AM, SAM, FOX	0.31

22	OX, AM, SAM, OFX, CTX, FOX	0.46
23	OX, AM, GM, C, CTX, FOX	0.46
24	OX, AM, TE, SAM, C, OFX, CTX, FOX	0.61
25	OX, AM, TE, SAM, FOX	0.38
26	OX, AM, TE, FOX	0.31
27	OX, AM, OFX, CTX, FOX	0.38
28	OX, AM, SAM, C, CTX, FOX	0.46
29	OX, AM, FOX	0.23
30	OX, AM, TE, SAM, E, GM, SXT, C, OFX, CIP, CTX, FOX	0.92

* MAR: Multiple antibiotic resistance

Conventional PCR was used for the detection of nuc and coa genes in addition to antibiotic resistance genes (mecA, blaZ, tetK, and fexA) in 16 *S. aureus* isolates. All the *S. aureus* isolates harbored the amplified products of both nuc and coa genes with characteristic bands at 395 bp and 630 bp, respectively indicating a high correlation between biochemical identification and genetic detection of these isolates (Figure 1 and 2). These findings were in close alignment with the previous study of Younis *et al.*[41] who found that the nuc gene was detected in all of the *S. aureus* isolates while there was no correlation in the screening of the coa gene where 28 isolates were found positive for the coa gene despite being negative in the coagulase test. This might be explained by the unfunctionally of coa gene in these strains. The existence of coagulase enzyme could differentiate pathogenic *S. aureus* from non-pathogenic ones. Another study was conducted on 27 *S. aureus* strains recovered from both forms of mastitis, 100% were positive for the presence of coa gene alarming to the increased prevalence of pathogenic *S. aureus* isolates in the dairy animals [53]**.**

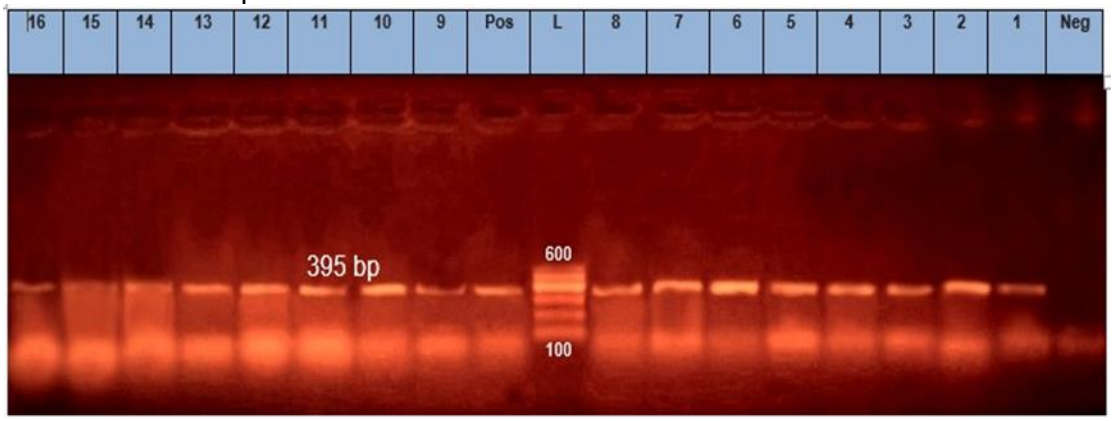

Figure 1: Agarose gel electrophoresis of amplified PCR products from *S. aureus*thermonuclease gene (nuc) isolated from cows with mastitis. Lanes 1:16 showed positive result (395 bp). Lane L:

Ladder (size range 100-600 bp), Neg.: control negative, Pos.: control positive and bp: base pair.

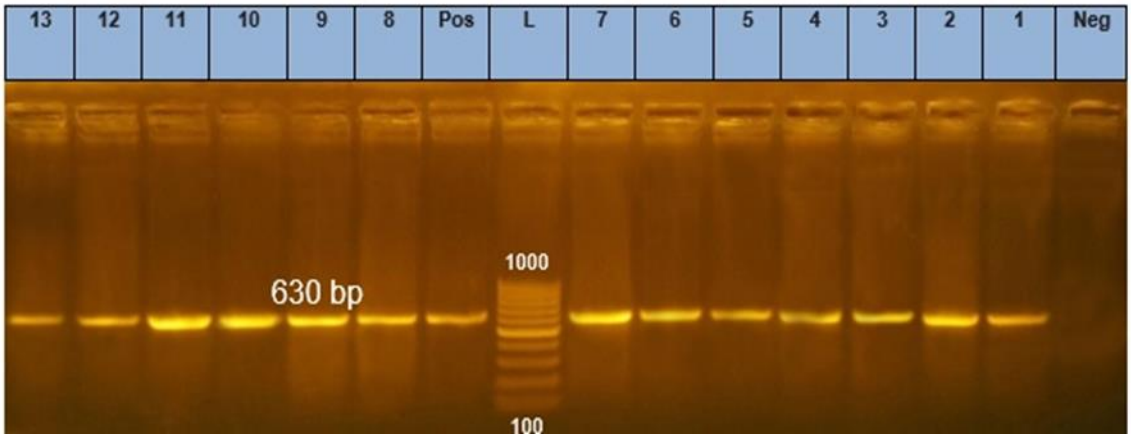

Figure 2: Agarose gel electrophoresis of amplified PCR products from *S. aureus* coagulase gene (coa) isolated from cows with mastitis. Lanes 1:13 showed positive result (630 bp). Lane L: Ladder (size range 100-1000 bp), Neg.: control negative, Pos.: control positive and bp: base pair.

Regarding the antibiotic resistance genes, all the 16 *S. aureus* isolates were positive for the presence of mecA (100%) as shown in (Figure 3), 15 out of 16 isolates were positive for the presence of the blaZ gene (93.7%) as in (Figure 4). While tetK gene was detected in 8 out of 16 isolates (50%) (Figure 5) and only one isolate was positive for the presence of fexA gene (0.06%) (Figure 6).These results were in accordance with the previous study of Jamali *et al.*[54] who found that all oxacillin-resistant *S. aureus* were positive for the mecA gene. Moreover, the blaZ gene was present in 97.4% of the penicillin-resistant *S. aureus*. The tetK gene was detected in 41.8% of the isolates resistant to tetracycline. While they detected the fexA gene with a higher prevalence of 83.3%.While Huber *et al.*[55] detected a lower prevalence of mecA with a percentage of 1.42% in Switzerland.

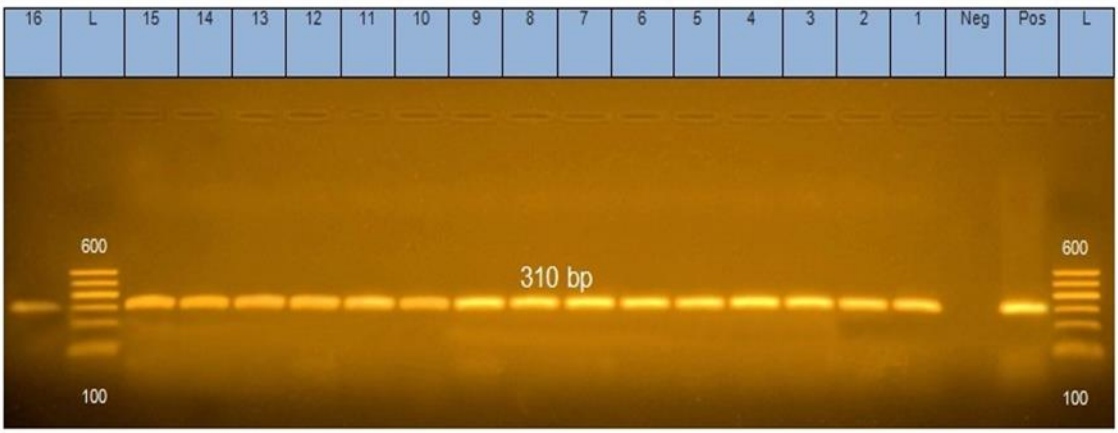

Figure 3: Agarose gel electrophoresis of amplified PCR products from *S. aureus* DNA. Lanes 1:16 showed positive results for the presence of mecA gene (310 bp). Lane L: Ladder (size range 100-600 bp), Neg.: control negative, Pos.: control positive and bp: base pair.

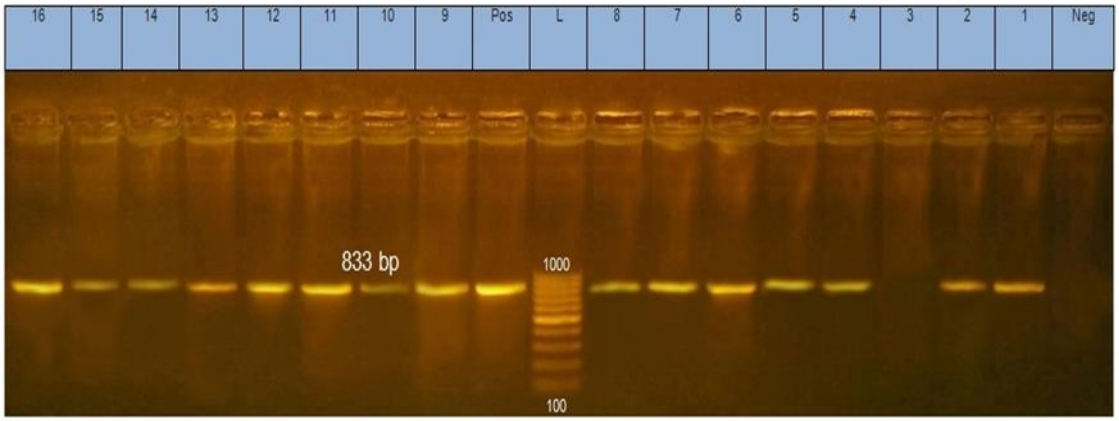

Figure 4: Agarose gel electrophoresis of amplified PCR products from *S. aureus* isolates DNA. Lanes 1:16 showed positive results for the presence of blaZ gene (833 bp) except for lane 3 showed negative result. Lane L: Ladder (size range 100-1000 bp), Neg.: control negative, Pos.: control positive and bp: base pair.

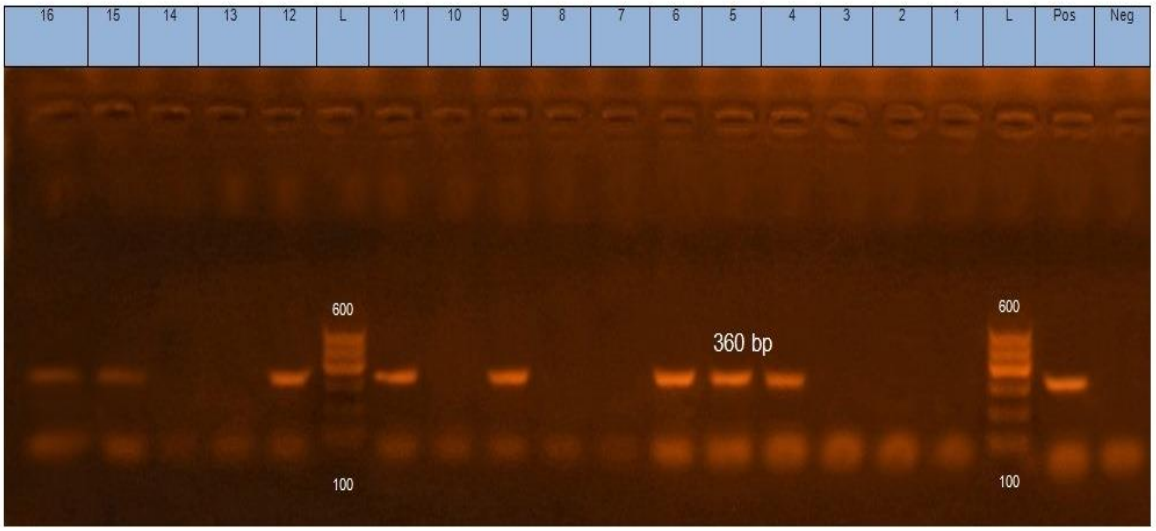

Figure 5: Agarose gel electrophoresis of amplified PCR products from *S. aureus* isolates DNA. Lanes (4, 5, 6, 9, 11, 12, 15, and 16) showed positive result for the presence of tetK gene (360 bp) while lanes (1, 2, 3, 7, 8, 10, 13, and 14) showed negative result. Lane L: Ladder (size range 100-600 bp), Neg.: control negative, Pos.: control positive and bp: base pair.

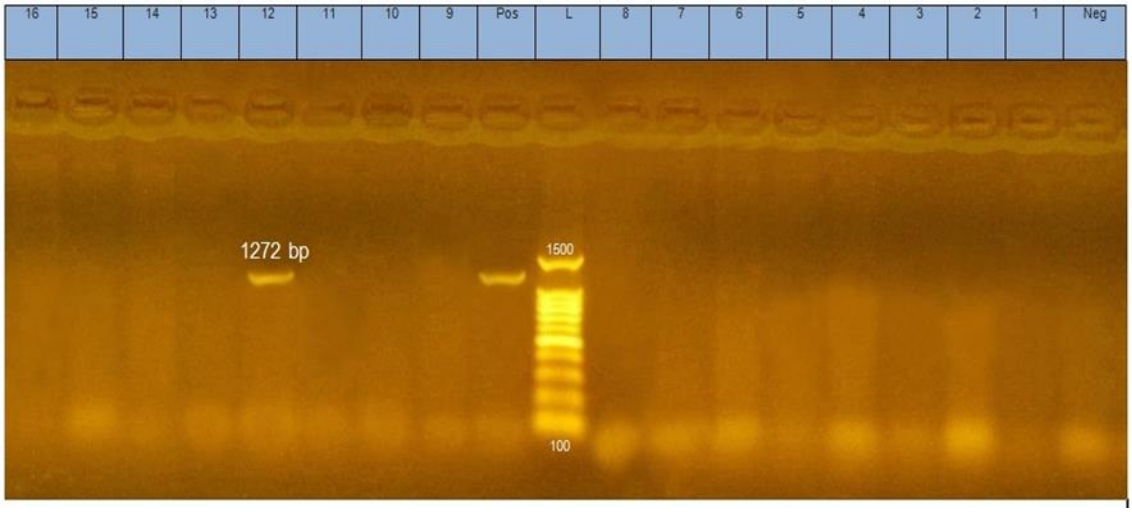

Figure 6: Agarose gel electrophoresis of amplified PCR products from *S. aureus* isolates DNA. Lanes (1: 16) showed negative result for the presence of fexA gene (1272 bp) except for lane (12) showed positive result. Lane L: Ladder (size range 100-1500 bp), Neg.: control negative, Pos.: control positive and bp: base pair.

The prevalence of the blaZ gene was 93.7% (15/16) of the *Staphylococcus* isolates. These findings were in accordance with the previous studies [45, 48] which reported a prevalence of the blaZ gene 95.45% and 95.7%, respectively. This might be due to the presence of other mechanisms of resistance to beta-lactams other than the blaZ gene [56] at which, the resistance to beta-lactams might be due to the development of β-lactamase encoded blaZ that hydrolyze penicillins[57]. Methicillin/oxacillin resistance is another β-lactam resistance mechanism, that results from the production of low-affinity penicillin-binding protein (PBP2a) encoded by the mecA gene [58].

Detection of tetK gene was 50% in (8/16) isolates. This result was in close alignment with Feng *et al.*[48], who found some difference between phenotypic and genotypic patterns of tetracycline resistance. It was noted that some tetracycline-resistant strains did not have resistance genes, while some sensitive strains carried resistance genes. In another study by Ruegg *et al.***[**59], it was reported that although 30-45% of the isolates were sensitive to tetracycline phenotypically, they carried tetK and tetM resistance genes. These results showed a clear difference between phenotypic and genotypic patterns of resistance, which recommended a wider selection of resistance genes to be tested. A study of Ho*et al.*[60] in Hong Kong demonstrated a higher prevalence of tetracycline as 97% of tetracycline-resistant isolates carried the tetK gene.

The prevalence of the fexA gene was 0.06% (1/16) of the isolates. These results were different from a previous study [60]at which, higher level of resistance to chloramphenicol (71%) was detected which was accompanied by the presence of fexA resistant gene. Florfenicol resistance genes were found in different Staphylococcal spp. and their location on mobile genetic elements might facilitate their spreading [33]

The development of antibiotic resistance among bacteria that affects

animal health is of growing concern in veterinary medicine. Antibiotic-resistant bacteria in animals have also become a potential health risk for humans, as they can cause direct or indirect transmission of the infection. The need to implement the one health concept is more urgent than ever if we consider the interconnections between humans, animals, and the environment. Therefore, establishing an antibiogram of pathogens is very important from the clinical and economic points of view [61, 62].

Conclusion:

Mastitis is considered one of the most devastating diseases responsible for huge economic losses in the dairy industry in Egypt. The current study has detected a high prevalence of multidrug resistance among *S. aureus* isolated from some Holsteinmastitic cows in Egypt against the most commonly used antibiotics. Uncontrolled use of antibiotics should be restricted with the awareness of veterinarians about the risk of antibiotic resistance. Treatment of mastitic cases should be preceded by an antibiotic sensitivity test.

Conflict of interest: The authors declare no conflict of interest.

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الملخص العربي

معدل انتشار ومقاومة الميكروب العنقودي الذهبي للمضادات الحيوية في التهاب الضرع الظاهري والخفي في الأبقار الهولشتاين الحلاب بمصر

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التهاب الضرع هو مرض متعدد العوامل وواسع الإنتشار ينتج عن تفاعلات بين العائل والبيئة والعوامل المعدية مما يؤديإلى خسائر اقتصادية واسعة النطاق. تمثلت أهداف هذه الدراسة في تقدير انتشار بكتيريا المكورات العنقودية الذهبية في التهاب الضرع السريري وتحت السريري في أبقار الألبان المصرية وفي تحديد حساسية عزلات المكورات العنقودية الذهبية ضد المضادات الحيوية المختلفة لفحص الجينات المقاومة للمضادات الحيوية. تم اختيار إجمالي 415 بقرة حلوب بشكل عشوائي من ثلاث محافظات مصرية. تم فحص هذه الأبقار المختارة للكشف عن التهاب الضرع السريري وتحت السريري خلال الفترة من أكتوبر 2014 إلى يونيو 2018. تم فحص عينات اللبن بحثًا عن وجود بكتيريا المكورات العنقودية الذهبية. تعرضت عزلات المكورات العنقودية الذهبية إلى اختبار حساسية للمضادات الحيوية والتعرف الجزيئي واكتشاف الجين المقاوم للمضادات الحيوية.بلغت نسبة انتشار بكتيريا المكورات العنقودية الذهبية المعزولة من عينات اللبن 15.4٪ منها 14.3٪ في الحالات السريرية و15.7٪ في الحالات تحت الإكلينيكية.أظهر إختبار مقاومة عزلات المكورات العنقودية الذهبية ضد 13 مضادًا حيويًا باستخدام طريقة الانتشار القرصي أعلى معدل مقاومة للأوكساسيلين (96.7٪)، يليه الأمبيسيلين و سيفوكسيتين (93.3٪, كلا منهما)، التتراسيكلين (73.3٪)، سيفوتاكسيم. (70٪)، أمبيسلين / سولباكتام (66.7٪)، إريثروميسين و سلفاميثوكسازول / تريميثوبريم (56.7٪، كلا منهما)، جنتاميسين (53.3٪)، أوفلوكساسين (40٪)، كلورامفينيكول (36.7٪)، سيبروفلوكساسين (30٪) وأخيرا فانكومايسين (0٪). أظهر فحص PCR الجزيئي أن جميع عزلاتالمكوراتالعنقوديةالذهبية (100٪) تحمل جين mecA، 15 من أصل 16 عزلة (93.7٪) تحمل جين blaZ 8 من 16 (50٪) تحمل جين tetK، بينما عزلة واحدة فقط (0.06٪) تحمل جين fexA. لذلك يجب تقييد الاستخدامات غير الخاضعة للرقابة للمضادات الحيوية في علاج التهاب الضرع وزيادة الوعي بمخاطر البكتيريا المقاومة لمضادات الميكروبات في الحليب.