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BACTERIOLOGICAL STUDY ON STAPHYLOCOCCAL BOVINE CLINICAL MASTITIS WITH REFERENCE TO METHICILLIN-RESISTANT *STAPH. AUREUS* (MRSA)

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ABSTRACT

This descriptive study was done on 101 milk samples obtained from clinically mastitic dairy cows in Assiut Governorate, Egypt. *Staphylococcus aureus* (*S. aureus*) was the main causative agent of clinical mastitis (34.65%) followed by *S. saprophyticus* (10.89%), *S. intermedius* and *S. epidermidis* (8.91%, for each). The other causative agents (non *Staph. Spp.*) were identified. Sensitivity test of *S. aureus* isolates was performed against 11 antimicrobial agents, where found that 21 *S. aureus* strains (60%) were methicillin resistant *S. aureus* (MRSA). Ten MRSA strains were subjected for: I- slime-producing factor on Congo Red Agar (CRA) plates phenotypically, as 6 isolates (60%) were positive for slime production. II- PCR which was optimized targeting *mecA*, *icaA* and *icaD* genes, where 5 isolates (50%) were positive for *mecA* gene. Six isolates (60%) and 8 isolates (80%) were positive for *icaA* and *icaD* genes, respectively. Five strains (50%) were positive for both *icaA* and *icaD* genes. Also 3 strains (30%) were positive for all *mecA*, *icaA* and *icaD* genes. Conclusion, it was concluded that bovine staphylococcal mastitis was the most predominant issue where *S. aureus* was the main cause. Detection of *mecA* gene in *S. aureus* isolates indicating that several cases suffering from *S. aureus* mastitis have an MRSA problem. Genotypic determination of *mecA* gene proved the most reliable method for detection of methicillin-resistant *S. aureus*. The present work paid an attention to the 3 MRSA strains (30%) were positive to all tested genes rather than slime production as the worst isolated strains all over this study (multidrug resistant, slime producing as well as carrying *mecA, icaA* and *icaD* genes). *In vitro* Enrofloxacin, Gentamicin and Doxycycline the most effective drugs for *Staph. spp.* clinical mastitis and should be recommended for treatment of such cases of bovine mastitis.

Keywords: *Cows, clinical mastitis, methicillin-resistant Staphylococcus aureus, mecA, icaA, icaD, slime factor.*

INTRODUCTION

Mastitis is one of the major challenges of the dairy industry. *Staphylococcus aureus* (*S. aureus*) is one of the most important pathogens causing mastitis in dairy cattle (Moon *et al.,* 2007; Nam *et al.,* 2011). Methicillin resistant *Staphylococcus aureus* (MRSA) has been recovered from dairy cattle in Korea (Moon *et al.,* 2007; Nam *et al.,* 2011 and Lim *et al.,* 2013); Turkey (Ciftci *et al.,* 2009); Netherland (Tavakol *et al.,* 2012); Iran (Ahmady and Kazemi, 2013) and Uganda (Kateete *et al.,* 2013). Several efforts to remove this pathogen from farms are hampered by some factors, where one of these factors is antibiotic resistance. One of the major mechanisms of resistance to β-lactam antibiotics is β-lactamase producing by staphylococci. This enzyme hydrolyzes the β- lactam ring and causes inactivation of β-lactams. In the early 1950s, it has been aware of the effectiveness of penicillin in treatment of *S. aureus* infections because of β-lactamase producing plasmids. In 1959, methicillin, synthetic, penicillinase –resistant penicillin, was introduced and solved problems in clinical practice, for a time. However, by 1960, *S. aureus* strains were found to be resistant to the new semisynthetic β-lactams (methicillin, oxacillin, flucloxacillin), and became known as methicillin-resistant *S. aureus* (MRSA). This type of resistance was termed "intrinsic resistance" because it was not due to destruction of the antibiotic by β-lactamase (Chambers, 1997). Methicillin resistance in *S. aureus* is mediated by the production of an altered penicillin-binding protein (PBP2a), a transpeptidase. *mecA* encodes this enzyme involved in cell wall peptidoglycan synthesis. Unlike conventional PBPs of *S. aureus*, PBP2a does not bind to β-lactam antibiotics with high affinity (Hiramatsu *et al.,* 2002).

It is considered that the first step in mastitis progress is adhesion of *S. aureus* to mammary epithelial cells and slime factor plays an important role for adhesion

and colonization (Vasudevan *et al.,* 2003). Production of slime factor also plays an important role in antibiotic resistance and it has been reported that slime producing strains are more resistant to antibiotics than non-slime producing strains (Amorena *et al.,* 1999). Intercellular adhesion is encoded in the *ica* locus containing *icaA, icaB, icaC, icaD* genes in *S. aureus* strains (Yazdani *et al.,* 2006). *icaA* gene encodes N-acetylglucosaminyl transferase, further, *icaD* plays an important role in expression of this enzyme. *icaA* and *icaD* were found to be in high prevalence among *S. aureus* mastitis isolates and this finding confirms that *ica* locus has a potential role as a virulence factor in the pathogenesis of mastitis in ruminants (Vasudevan *et al.,* 2003).

This study was undertaken to determine the bovine mastitis *Staph. Spp*., their resistance to antimicrobial agents approved for its control and to determine the methicillin resistance and slime factor production of *S. aureus* in bovine mastitis phenotypically and genotypically for *mecA, icaA and icaD* genes.

MATERIALS and METHODS

Milk samples:
A total of 101 milk samples were collected from 101 cows, at various private farms in Assiut, Egypt, showing clinical signs of mastitis. All samples were taken under aseptic conditions and transferred in ice box to laboratory as soon as possible.

Isolation and identification of bacterial isolates:
Amount of 0.01 ml of each milk samples was cultured on blood agar with 5% sheep blood, Mannitol salt agar (BBL), Baird-Parker medium (Oxoid) and MacConkey agar (Biomark Lab. India) which incubated at 37°c for 48 h. The suspected colonies were identified: morphologically, by Gram's stain and biochemically confirmed by using catalase activity, coagulase test as well as Novobiocin (5 µg) and polymixin-ß sulphate (300 U) sensitivity tests, according to Quinn *et al.* (2004).

Phenotypic detection of methicillin resistance:
Disc diffusion sensitivity testing was performed according to the Kirby-Bauer method, as described in the guidelines of the National Committee for Laboratory Standards (NCCLS, 2000), using discs (Bioanalyse-Turkey) containing Oxacillin (OX) 1 µg, Ampicilin (AM) 10 µg, Cefotaxime (CTX) 30 µg, Cloxacillin (CX) 1 µg, Doxycycline (DO) 30 µg, Enrofloxacin (ENR) 5 µg, Gentamicin (CN) 10 µg, Lincomycin (L) 2 µg, Oxytetracycline (T) 30 µg, Penicillin (P) 10 µ and Trimethoprim – Sulflamethaxzole (SXT) 25 µg. For Oxacillin susceptibility determinations, inhibition zones around the disc were measured after 24 and 48 h using the following breakpoints: susceptible (S) ≥ 18 mm; resistance (R) ≤ 17 mm (Quinn *et al.,* 2004).

Slime production assay:
Slime production assay was performed by cultivation of ten *S. aureus* strains, which were methicillin resistant by phenotypic test, on Congo Red Agar (CRA) plates containing 0.8 g of Congo Red dye, 21 g Mueller-Hinton broth, 15 g granulated agar and 36 g sucrose per Liter distilled water. Strains were inoculated on CRA plates and incubated for 24-72 h at 37ºC. Slime producing strains and non-slime producing strains constitutes rough black colonies and red colonies on CRA, respectively (Yazdani *et al.,* 2006).

PCR for detection of *mecA, icaA* and *icaD* genes:
Detection of *mecA, icaA* and *icaD* genes was performed on those ten *S. aureus* isolates, which were methicillin resistant by phenotypic test, as follows:

I- DNA extraction: DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56^{O}C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

II- Oligonucleotide Primers: Primers encoding for *mecA, icaA* and *icaD* genes were supplied from (Metabion, Germany) are listed in Table (1).

Table 1: Primers sequences, target genes, amplicon sizes.

Primer	Target gene	Primer sequence (5'-3')	Length of amplified product (bp)	Reference
mecA-1	*mecA*	GTA GAA ATG ACT GAA CGT CCG ATA A	310 bp	McClure *et al*. (2006)
mecA-2		CCA ATT CCA CAT TGT TTC GGT CTA A		
IcaA- AF	*IcaA*	CCT AAC TAA CGA AAG GTA G	1315 bp	Ciftci *et al.* (2009)
IcaA- AR		AAG ATA TAG CGA TAA GTG C		
IcaD- DF	*IcaD*	AAA CGT AAG AGA GGT GG	381 bp	
IcaD- DR		GGC AAT ATG ATC AAG ATA		

III- PCR amplification: Primers were utilized in a 25- µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 4.5 µl of water, and 6 µl of template. The reaction was performed in a Biometra thermal cycler. For *mecA* gene PCR, a primary denaturation step was done at 95°C for 5 min, followed by 35 cycles of 95°C for 45 sec., 50°C for 45 sec. and 72°C for 45 sec. A final extension step was done at 72°C for 10 min, according to McClure *et al.* (2006). However for the *icaA* and *icaD* genes, the cycles consisted of 95ºC for 1 min, 49ºC for 1 min and 72ºC for 1 min, according to Ciftci *et al*. (2009).

IV- Analysis of the PCR Products: The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µl of the products was loaded in each gel slot. A 100 bp DNA Ladder (Qiagen, Germany, GmbH) and 100 bp plus DNA Ladder (Fermentas, Cat.No. SM 0323) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

RESULTS

Table 2: Isolated micro-organisms from mastitic milk samples.

	Isolates	No.	%
Staph. spp	Coagulase + ve *Staph. spp.*		
	1- *S. aureus*	35	34.65
	2- *S. intermedius*	9	8.91
	Coagulase -ve *Staph. spp.*		
	1- *S. saprophyticus*	11	10.89
	2- *S. epidermidis*	9	8.91
Non *Staph. spp*	Gram –ve bacilli	32	31.68
	Corynebacterium spp.	4	3.96
	Strept. Pyogenes	1	0.99
	Total	101	100

Table 3: *In vitro* antimicrobial susceptibility test of isolated *Staph. spp*. From bovine clinical mastitis according to the agar disc diffusion method.

Antimicrobial Agents	*S. aureus* (n. = 35)		*S. intermedius* (n.= 9)		*S. saprophyticus* (n.= 11)		*S. epidermidis* (n.= 9)		Total (n.= 64)	
	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
Oxacillin	14 (40%)	21 (60%)	6 (66.67%)	3 (33.33%)	4 (36.36%)	7 (63.64%)	5 (55.56%)	4 (44.44%)	29 (45.31%)	35 (54.69%)
Enrofloxacin	35 (100%)	0 (0%)	9 (100%)	0 (0%)	11 (100%)	0 (0%)	9 (100%)	0 (0%)	64 (100%)	0 (0%)
Gentamicin	35 (100%)	0 (0%)	9 (100%)	0 (0%)	11 (100%)	0 (0%)	9 (100%)	0 (0%)	64 (100%)	0 (0%)
Doxycycline	32 (91.43%)	3 (8.57%)	9 (100%)	0 (0%)	10 (90.91%)	1 (9.09%)	9 (100%)	0 (0%)	60 (93.75%)	4 (6.25%)
Trimethoprim – Sulflamethaxzole	18 (51.43%)	17 (48.57%)	7 (77.77%)	2 (22.22%)	5 (45.45%)	6 (54.55%)	7 (77.77%)	2 (22.22%)	37 (57.81%)	27 (42.18%)
Oxytetracycline	18 (51.43%)	17 (48.57%)	6 (66.67%)	3 (33.33%)	7 (63.64%)	4 (36.36%)	6 (66.67%)	3 (33.33%)	37 (57.81%)	27 (42.18%)
Penicillin	15 (42.86%)	20 (57.14%)	5 (55.56%)	4 (44.45%)	7 (63.64%)	4 (36.36%)	5 (55.56%)	4 (44.45%)	32 (50%)	32 (50%)
Ampicilin	13 (37.43%)	22 (68.86%)	7 (77.77%)	2 (22.22%)	7 (63.64%)	4 (36.36%)	3 (33.33%)	6 (66.67%)	30 (46.88)	34 (53.12%)
Cloxacillin	14 (40%)	21 (60%)	6 (66.67%)	3 (33.33%)	4 (36.36%)	7 (63.64%)	5 (55.56%)	4 44.45%)	29 (45.31%)	35 (54.69%)
Cefotaxime	4 (11.43%)	31 (88.57%)	3 (33.33%)	6 (66.67%)	1 (9.09%)	10 (90.915%)	0 (0%)	9 (100%)	8 (12.5%)	56 (87.5%)
Lincomycin	3 (8.57%)	32 (91.43%)	3 (33.33%)	6 (66.67%)	0 (0%)	11 (100%)	0 (0%)	9 (100%)	6 (9.38%)	58 (90.63%)

Table 4: Methicillin resistant *S. aureus* strains tested for their phenotypic and genotypic characteristics and slime factor production.

MRSA strains	Congo red agar test	Genes		
		mecA	*icaA*	*icaD*
1	-ve	-ve	-ve	+ve
2	-ve	-ve	+ve	+ve
3	-ve	-ve	-ve	-ve
4	+ve	-ve	-ve	+ve
5	+ve	-ve	+ve	+ve
6	+ve	+ve	+ve	+ve
7	-ve	+ve	-ve	+ve
8	+ve	+ve	+ve	+ve
9	+ve	+ve	+ve	+ve
10	+ve	+ve	+ve	-ve

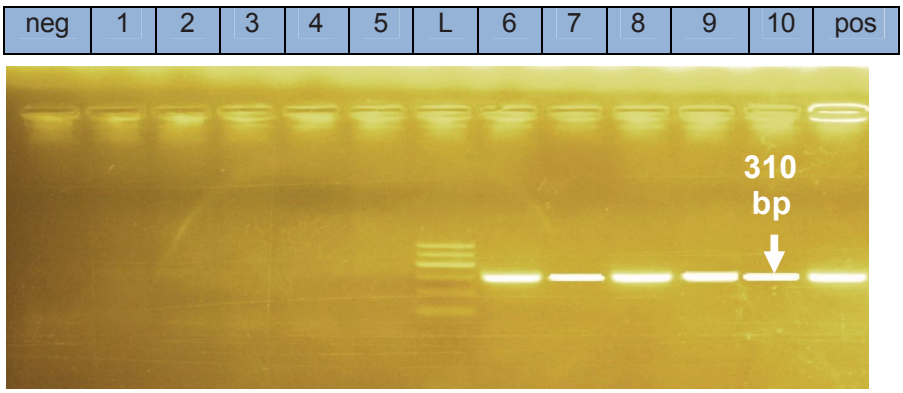

Fig. 1: PCR results for *mecA* gene showing amplification of 310 bp. (L): 100 bp ladder (QIAGEN, Gmbh) (100-600 bp), 1-10 lanes for the 10 strains respectively.

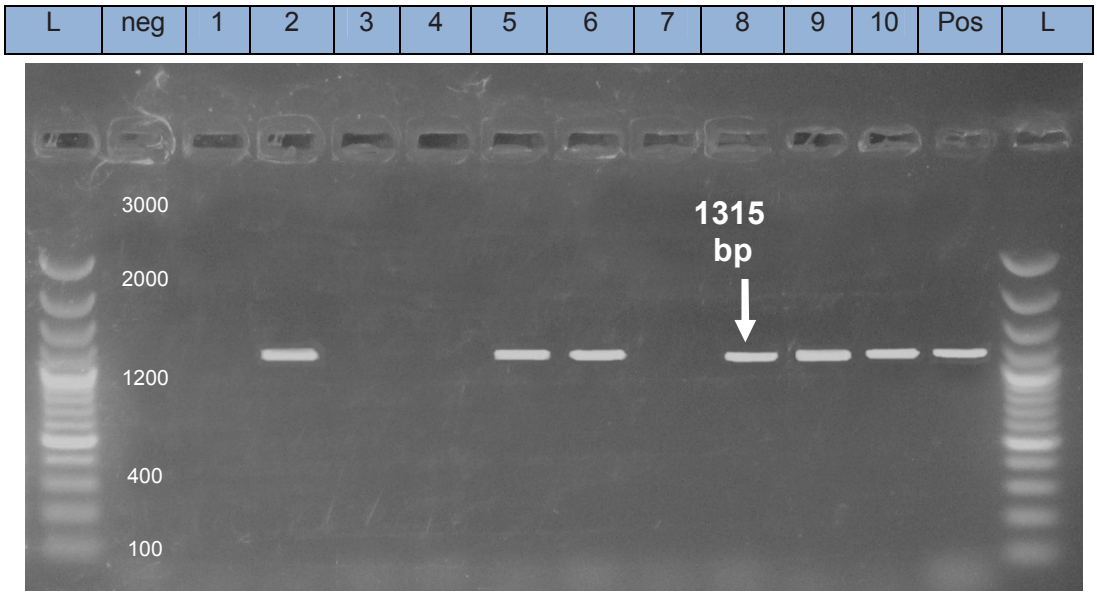

Fig. 2: PCR results for *icaA* gene showing amplification of 1315 bp. (L): 100 bp plus ladder (Fermentas, Cat. no. SM 0323) (100-3000 bp), 1-10 lanes for the 10 strains respectively.

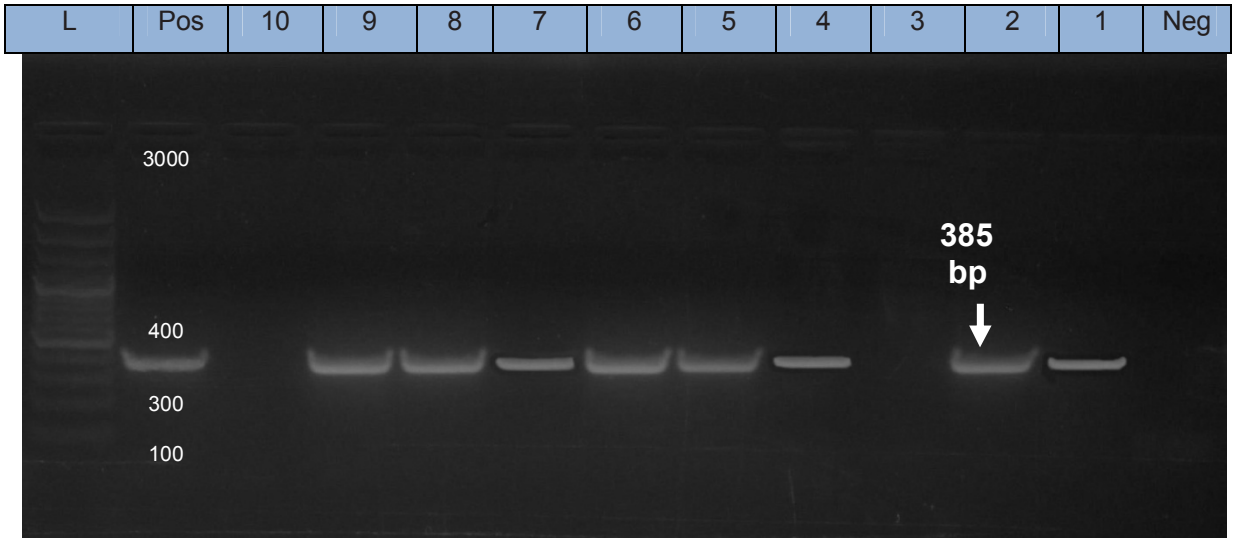

Fig. (3): PCR results for *icaD* gene showing amplification of 385 bp. (L): 100 bp plus ladder (Fermentas, Cat. no. SM 0323) (100-3000 bp), 1-10 lanes for the 10 strains respectively.

DISCUSSION

Mastitis is an important and a persistent infection producing high economies losses due to poor milk quality, reduced milk yield and increased expenditure on treatment especially staphylococcal mastitis (Abd-Elrahman, 2013) which resembled 63.37% through the current study and bacteriological examination shows that *S. aureus* was the main causative agent of clinical mastitis in cows (34.65%), followed by *S. saprophyticus* (10.89%), *S. intermedius* and *S. epidermidis* (8.91%, for each), as shown in Table (2). This result of *S. aureus* in close agreement with previous findings; 30, 30 and 36%, by Reddy *et al.* (2007); Ali *et al.* (2008) and Haftu *et al.* (2012), respectively. However, the findings (71.4%) of *S. aureus* by Abou-Zaid and El-Sawalhy (1999) are much higher than the present report. The lower prevalence reported by Eman *et al.* (2006); Moniri *et al.* (2007) and Sayed & Mohamed (2008); (21.7; 21.9 and 23.6%, respectively). High prevalence of *S. aureus* points to poor milking hygiene as this pathogen is mainly spread during milking via milkers' hands and towels (Bradley, 2002).

In vitro activities of *Staph. spp.* against 11 antimicrobial agents are summarized in Table (3). In the present work the highest rate of resistant *S. aureus* exhibited to Lincomycin followed by Cefotaxime, Ampicilin and Penicillin (91.43, 88.57, 68.86 and 57.14%, respectively) and MRSA resembled 60% of these isolates. The highest rate of sensitivity to Enrofloxacin and Gentamicin followed by Doxycycline (100, 100 and 91.43%, respectively), Table (3). Abd-Elrahman (2013) found that Enrofloxacin the most effective drugs against *S. aureus*. The highest resistance rate of *S. aureus* against Penicillin (47.6, 47.6, 56.5 and 66%) was reported by Calvinho *et al*. (2002); Gianneechini *et al*. (2002); Arshad *et al*. (2006) and Nam *et al*. (2011), respectively. Vanderhaeghen *et al*. (2010) found an unusual high prevalence of MRSA in Belgian cases of subclinical and clinical *S. aureus* mastitis in cows. *S. aureus* can adapt rapidly to the selective pressure of antibiotics and this resulted in emergence and spread of MRSA (Deurenberg *et al*., 2007).

Extracellular polysaccharides, slime factor, are considered to be significant virulence factors for some staphylococci. Slime layer surrounding the *S. aureus* strains help in adherence and colonization of these microorganisms on the mammary gland epithelium. It is reported that slime factor production in *S. aureus* isolates from mastitis cause antibiotic resistance which is due to the decreased diffusion of antibiotics through the biofilm matrix and decreased metabolic activity of bacteria (Amorena *et al.,* 1999 and Yazdani *et al.,* 2006).

In the present study, ten MRSA strains were subjected for slime production on Congo Red Agar (CRA) plates and PCR study targeting *mecA*, *icaA* and *icaD* genes. Among the tested MRSA strains only five isolates (50%) were positive for *mecA* gene genotypically, Table (4) and Fig. (1). Moon *et al*. (2007) and Ciftci *et al*. (2009) detected that 61.9 and 30.7% of MRSA were positive for *mecA* gene, respectively. MRSA resistance to methicillin and other ß-lactam antibiotics is caused by the action of *mecA* gene (Deurenberg *et al*., 2007). The discrepant results between disc diffusion methods and PCR for detection of methicillin resistance may be due to another resistance mechanism such as hyperproduction of beta-lactamase, also MRSA strains show a heterogeneous character with the level of resistance varying according to the culture conditions and β-lactam antibiotic being used. Because of this heterogeneous resistance, and time consuming the detection of MRSA by phenotypic methods becomes problematic, Ciftci *et al*. (2009). However, PCR-based methods have shown to be a rapid and reliable approach for the identification and genotypic characterization of MRSA. Detection of *mecA*- based PCR methods has accepted as "gold standard" (Sancak, 2000).

The present work found that six isolates (60%) of the tested MRSA strains were slime producing positive on CRA plates *in vitro*, Table (4). Slime-producing *S. aureus* isolates from different clinical origins such as bovine mastitis (Vasudevan *et al.,* 2003) and (Ciftci *et al*., 2009), wound infection (Yazdani *et al.,* 2006) and clinical cases (Eftekhar and Dadaei, 2011) has been detected *in vitro* by using Congo Red Agar plates in percentages of 91.4, 37.2, 52 and 53.3%, respectively. Knobloch *et al.* (2002) have reported that the phenotype on CRA was found to be an unreliable indicator of slime-forming capacity among clinical isolates of *S. aureus*. Therefore, although CRA methods may be easier to perform than a molecular analysis of the genes implicated in biofilm production and could be performed easily in a diagnostic laboratory, it may be a poor method for determining the slime producing capacity of clinical isolates in the diagnostic laboratory (Fitzpatrick *et al.,* 2005).

PCR methods provided a direct evidence of the genetic basis of slime production complementary to the CRA test. The *ica* locus consists *ica A, D, B, C* genes. Slime synthesis is controlled by the ica (intercellular adhesion) operon. Coexpression of *icaA* with *icaD* leads to a significant increase in activity and is related to phenotypic expression of the capsular polysaccharide (Arciola *et al.,* 2001). In this study, slime factor production of MRSA isolates were detected by PCR targeting *icaA* and *icaD* genes and found that 5 (50%) of the tested MRSA strains were positive for both *icaA* and *icaD* genes. Six (60%) and eight (80%) isolates were positive for *icaA* gene and *icaD* gene, respectively as shown in Table (4) and Fig. (2 & 3). Ciftci *et al.* (2009) reported that 15

(25.42%) out of 59 *S. aureus* strains were positive for both *icaA* and *icaD* genes. Sixteen (27.12%) and 38 (64.41%) out of 59 strains were positive for *icaA* and *icaD* gene, respectively. Also Eftekhar and Dadaei (2011) found that 75% of MRSA carried *ica* operon. The *icaAD* gene was detected in 32% of *Staphylococcal spp*. (Rasha *et al.,* 2012). While it was contrast to Arciola *et al.* (2001) who have reported that all strains which were positive for *icaA* gene were also positive for *icaD* gene. In addition, Vasudevan *et al.* (2003) and Yazdani *et al.* (2006) have reported that all *S. aureus* isolates possessed the *ica* locus, *icaA* and *icaD* genes. *icaA* and *icaD* genes were not be together in some isolates may due to some mutations on *icaA* gene, although coexpression on *icaA* and *icaD* is necessary for slime production it was considered that other genes in *ica* locus play role in controlling slime expression, (Ciftci *et al.*, 2009).

In present study, among four isolates which were negative for slime production on CRA plate *in vitro,* one isolate was positive for both *icaA* and *icaD* genes, two isolates were positive for *icaD* gene and the last one was negative for both *icaA* and *icaD* genes, Table (4). Ciftci *et al.* (2009) found among the 37 *S. aureus* strains which did not produce slime factor on CRA plate *in vitro,* only 7 (18.9%) strains were positive for both *icaA* and *icaD* genes. Who suggests that some environmental conditions or presence of accessory genes can influence the phenotypic behavior on the Congo red agar plate, giving colonies which did not fully express the *ica* genes.

In this work, six isolates (60%) were positive for both methicillin resistance and slime production phenotypically and three strains (30%) were positive for all *mecA*, *icaA* and *icaD* genes, Table (4). Only 2 (3.39%) of 59 *S. aureus* strains were positive for both methicillin resistance and slime producing, phenotypically, Ciftci *et al.* (2009).

In conclusion, it was showed that detection of *mecA* gene in *S. aureus* isolates indicating that several cases suffering from *S. aureus* mastitis have an MRSA problem. Genotypic determination of *mecA* gene proved the most reliable method for detection of methicillin resistance. The present work paid an attention to the 3 MRSA strains (30%) were positive to all tested genes rather than slime production as the worst isolated stains all over this study (multidrug resistant, slime producing as well as carrying *mecA, icaA* and *icaD* genes). *In vitro* Enrofloxacin, Gentamicin and Doxycycline are the most effective drugs for *Staph. spp.* clinical mastitis.

REFERENCES

Abd-Elrahman, A.H. (2013): Mastitis in housed dairy buffaloes: incidence, etiology, clinical finding, antimicrobial sensitivity and different medical treatment against *E. coli* mastitis. Life Science J. 10(1): 532 – 538.

Abou-Zaid, A. and El-Sawalhy, A. (1999): Some studies on mastitis in cattle. Alex. J. Vet., 15(1):23-34.

Ahmady, M. and Kazemi, S. (2013): Detection of the enterotoxigenic genes (sei, sej) in *Staphylococcus aureus* isolates from bovine mastitis milk in the West Azerbaijan of Iran. Comp. Clin. Path., 22(4):649-654.

Ali, L.; Muhammed, G.; Arshad, M.; Saqib, M. and Hassan, I.J. (2008): Bacteriology of mastitis buffaloes in Tehsil Samundri of district Faisalabad. Pakistan Vet. J. 28 (1); 31-33.

Amorena, B.; Gracia, E.; Monzon, M.; Leiva, J.; Oteiza, C.; Perez, M.; Alabart, J.L. and Hernandez-Yago, J. (1999): Antibiotic susceptibility assay for *Staphylococcus aureus* in biofilms developed in vitro. J. Antimicrob. Chemother., 44: 43-55.

Arciola, C.R.; Baldassarri, L. and Montanaro, L. (2001): Presence of *icaA* and *icaD* genes and slime production in a collection of staphylococcal strains from catheter-associated infections. J. Clin. Microbiol., 39 (6): 2151-2156.

Arshad, M.; Muhammad, G.; Siddique, M.; Ashraf, M. and Khan, H.A. (2006): Staphylococcal mastitis in bovine and some properties of *Staphylococcal* isolates. Pakistan. Vet. J., 26 (1); 20-22.

Bradley, A.J. (2002): Bovine mastitis an evolving disease. The Veterinary J., 164: 116-128.

Calvinho, L.F.; Toselli, F.G.; Weimann,W.R.; Canavesio, V.R.; Neder, V.E. and Iguzquiza, I. A. (2002): Antimicrobial Sensitivity of coagulase-positive staphylococcal strains isolated from bovine mastitis in the central dairy catchment area of Argentina. Rev. Argent. Microbiol., 34: 171-175.

Chambers, H.F. (1997): Methicillin resistance in staphylococci: Molecular and Biochemical Basis and Clinical Implications. Clin. Microbiol. Rev. 10: 781-791.

Ciftci, A; Findik, A; Onuk, E.E. and Savasan, S. (2009): Detection of methicillin resistance and slime factor production of *Staphylococcus aureus* in bovine mastitis. Brazilian J. of Microbiology, 40:254-261.

Deurenberg, R.H.; Vink, C.; Kalenic, S.; Friedrich, A.W.; Bruggeman, C.A. and Stobberingh, E.E. (2007): The molecular evolution of methicillin-resistant *Staphylococcus aureus.* Clin Microbiol Infect., 13: 222–235

Eftekhar, F. and Dadaei, T. (2011): Biofilm formation and detection of *icaAB* genes in clinical isolates of Methicillin resistant *Staphylococcus aureus*. Iranian Journal of Basic Medical Sciences, Vol. 14, No. 2: 132-136.

Eman, A. Ahmed; EL. Sangary, F.H. and Abou Zead, A.A. (2006): Bacterial and biochemical studies on mastitis of cattle in Sharkia Governorate. Assiut Vet. Med. J., 52 (109): 207-214.

Fitzpatrick, F.; Humphreys, H. and O'Gara, J.P. (2005): The genetics of staphylococcal biofilm formation—will a greater understanding of pathogenesis lead to better management of device-related infection? Clin. Microbiol. Infect. 11, 967-973.

Gianneechini, R.E.; Concha, C. and Franklin, A. (2002): Antimicrobial susceptibility of udder pathogens isolated from dairy herds in the West Littoral region of Uruguay. Acta Vet. Scand. 43: 31-41.

Haftu, R.; Taddele, H. and Gugsa, G. (2012): Prevalence, bacterial causes, and antimicrobial susceptibility profile of mastitis isolates from cows in large-scale dairy farms of Northern Ethiopia. Trop. Anim. Health Prod., 44: 1765 – 1771.

Hiramatsu, K.; Katayama, Y.; Yuzawa, H. and Ito, T. (2002): Molecular genetics of methicillin resistant *Staphylococcus aureus*. Int. J. Med. Microbiol., 292: 67-74.

Kateete, D.P.; Kabugo, U.; Baluku, H.; Nyakarahuka. L.; Kyobe, S.; Okee, M.; Najjuka, C. F. and Joloba, M.L. (2013): Prevalence and antimicrobial susceptibility patterns of bacteria from milkmen and cows with clinical mastitis in and around Kampala, Uganda. PLoS One, Volume 8, Issue 5, e63413.

Knobloch, J. K.M.; Horstkotte, M.A.; Rohde, H. and Mack, D. (2002): Evaluation of different detection methods of biofilm formation in *Staph. aureus*. Med. Microbiol. Immunol., 191 (2): 101- 106.

Lim, S.K.; Nam, H.M.; Jang, G.C.; Lee, H.S.; Jung, S.C. and Kim, T.S. (2013): Transmission and Persistence of Methicillin-Resistant *Staphylococcus aureus* in Milk, Environment, and Workers in Dairy Cattle Farms. Foodborne Pathogens and Diseases, Vol. 10, No., 8:731-736.

McClure, J-A.; Conly, J.M.; Lau, V.; Elsayed, S.; Louie, T.; Hutchins, W. and Zhang, K. (2006): Novel multiplex PCR assay for detection of the staphylococcal virulence marker Panton-Valentine leukocidin genes and simultaneous discrimination of methicillin-susceptible from - resistant staphylococci. J. Clin. Microbiol., 44 (3): 1141-1144.

Moniri, R.; Dastehgoli, K. and Akramian, A. (2007): Increasing resistant coagulase negative *Staphylococci* in bovine clinical mastitis. Pakistan J. of Biological Sciences, 10(15): 2465-2469.

Moon, J.S.; Lee, A.R.; Kang, H.M.; Lee, E.S.; Kim, M.N.; Paik, Y.H.; Park, Y.H.; Joo, Y.S.; Koo, H.C. (2007): Phenotypic and genetic antibiogram of methicillin-resistant staphylococci isolated from bovine mastitis in Korea. J. Dairy Sci.; 90: 1176–1185.

Nam, H.M.; Lee, A.R.; Jung, S.C.; Kim, M.N.; Jang, G.C.; Wee, S.H. and Lim, S.K. (2011): Antimicrobial susceptibility of *Staphylococcus aureus* and characterization of methicillin-resistant *Staphylococcus aureus* isolated from bovine mastitis in Korea. Foodborne Pathog. Dis.; 8: 231–238.

NCCLS (2000): Performance Standards for Antimicrobial Disk Susceptibility Test. Approved Standard M2 – A7, M100 – S10. PA: National Committee for Laboratory Standards.

Quinn, P.J.; Carter, M.E.; Markey, B. and Carter, G.R. (2004): Clinical veterinary microbiology. 6th ed., Mosby, Edinburgh, London, New York, Philadelphia, St. Louis, Sydney, Toronto.

Rasha A.Nasr; Hala M. AbuShady and Hussein, S.H. (2012): Biofilm formation and presence of *icaAD* gene in clinical isolates of staphylococci. The Egyptian J. of Med. Human Genetics 13:269–274.

Reddy, P.; Qi, C.; Zembower, T.; Noskin, G.A. and Bolon, M. (2007): Postpartum mastitis and community-acquired methicillin-resistant *Staphylococcus aureus*. Emerg. Infect. Dis., 13 (2): 298-301.

Sancak, B. (2000): S. aureus'ta metisilin direnç mekanizmalari. Mikrobiyol. Bult., 34: 381-389.

Sayed, Z.M. and Mohamed, A.E.A. (2008): Bacteriological studies on mastitis in dairy Friesian cattle in Quena Governorate. Beni-Suef, Vet. Med. J., 18 (2): 19-21.

Tavakol, M.; Richard, G.M.; Otlis, C.S.; Willem, J.B.; Alex Van B. and Theo, J.G.M. (2012): Bovine-associated MRSA ST398 in the Netherland. Acta Veterinaria Scandinavica, 54:28

Vanderhaeghen, W.; Cerpentier, T.; Adriaensen, C.; Vicca, J.; Hermans, K. and Butaye, P. (2010): Methicillin-Resistant *Staphylococcus aureus* (MRSA) ST398 Associated with Clinical and Subclinical Mastitis in Belgian Cows. Vet. Microbiology, 144:166-171.

Vasudevan, P.; Nair, M.K.M.; Annamalai, T. and Venkitanarayanan, K.S. (2003): Phenotypic and genotyping characterization of bovine mastitis isolates of *Staphylococcus aureus* for biofilm formation. Vet. Microbiol., 92: 179-185.

Yazdani, R.; Oshaghi, M.; Havayi, A.; Pishva, E.; Salehi, R.; Sadeghizadeh, M. and Foroohesh, H. (2006): Detection of icaAD gene and biofilm formation in *Staphylococcus aureus* isolates from wound infections. Iranian J. Publ. Health 35 (2), 25-28.

دراسة بكتيريولوجية علي الميكروب المكور العنقودي المسبب لالتهاب الضرع الإكلينيكي في الماشية مع إشارة إلي الميكروب العنقودي الذهبي المقاوم للميثاسيلين

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أجريت هذه الدراسة على ١٠١ عينة لبن تم الحصول عليها من الأبقار الحلوب المصابة بالتهاب الضرع الإكلينيكي في محافظة أسيوط - مصر.أسفرت النتائج أن ميكروبات المكور العنقودي كانت كالآتي [المكور العنقودي الذهبي يشكل العامل المسبب الرئيسي لالتهاب الضرع الإكلينيكي بنسبة ٣٤.٦٥٪ ويليها المكور العنقودي السابروفيتكس بنسبة ١٠.٨٩ % ثم المكور العنقودي الانترميدس والابيدرميدس بنسبة ٨.٩١ %، لكل منهما]. كما تم تصنيف باقي المسببات (غير المكورات العنقودية). تم إجراء اختبار الحساسية للميكروبات العنقودي الذهبي معمليا ضد ١١ مضاد للبكتريا، حيث وجدت ٢١ عترة منها بنسبة ٦٠ % مقاومة للميثاسيلن. تم اختيار١٠ عترات من المكور العنقودي الذهبي المقاومة للميثاسيلين حيث خضعت لاختبارات أفراز البيوفيلم معمليا باختبار نموها على أطباق الكونجو الأحمر وقد وجدت ست عترات (٦٠%) منها منتجة للبيوفيلم المسؤول عن التصاق الميكروب لخلايا أنسجة الضرع ، وبإجراء تفاعل البلمرة المتسلسل عليها للكشف عن جينات *icaA, icaD, mecA*. وجد أن خمس عترات (٥٠%) كانت ايجابية للجين *mecA*. بينما كانت ست (٦٠%) و ثماني (٨٠%) عترات ايجابية للجين *icaA* وللجين *icaD* علي الترتيب. وجد خمس عترات (٥٠%) ايجابية لوجود الجينات *icaA and icaD*. وكذلك أسفرت النتائج عن وجود ثلاث عترات (٣٠%) ايجابية لكل من الجينات *icaA* and *icaD* ، *mecA* معا، بالأضافة الى انها مفرزة للبيوفيلم ومتعددة المقاومة للمضادات الميكروبية معمليا أيضا لتكون اكثر العترات المختبرة ضراوة. ختاما أظهرت النتائج أن الكشف عن الجين *mecA* في بكتريا المكور العنقودي الذهبي يدل علي أن العديد من الحالات التي تعاني من التهاب الضرع الإكلينيكي المتسبب عن الإصابة بالمكور العنقودي الذهبي لديهم مشكلة MRSA. أن تفاعل البلمرة المتسلسل للكشف عن الجين *mecA* هو أفضل وأسرع الطرق للكشف عن المكور العنقودي الذهبي المقاوم للميثاسيلين. قدرة بعض بكتريا المكور العنقودي الذهبي المقاوم للمثاسيلين والمتعددة المقاومة للمضادات الميكروبية علي أفراز بيوفيلم وتحتوي علي الجينات *icaD* and *icaA*، *mecA* معا. معمليا وجد أن انروفلوكساسين وجنتاميسين ودوكسيسيكلين هي الأدوية الأكثر فعالية لالتهاب الضرع الإكلينيكي الناتج عن الإصابة بعترات المكور العنقودي.