

**Phenotypic and Genotypic Characterization of Methicillin-Resistant Coagulase-Negative *Staphylococci* as an Etiological Agent of Bovine Mastitis in Egypt**

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**ABSTRACT**

To study molecular genetic characteristics and antibiotic resistance patterns of methicillin-resistance coagulase-negative staphylococci (CoNS) from mastitic cows in Egypt. Methods: The study material consisted of 205 milk samples. Milk was bacteriologically tested. Hemolytic activity and adhesive properties of CoNS isolates were also evaluated. Fifty CoNS isolates were submitted to in vitro susceptibility testing by the disk diffusion method. From the pool of 50 CoNS isolates, 15 Methicillin resistance isolates. were screened for genes encoding staphylococcal enterotoxin B (*seb*), toxic shock syndrome toxin1 (*tss*), intracellular adhesin D (*icaD*), and Methicillin resistance (*mecA*) by PCR. Results: CoNS were serotyped into three species *S. haemolyticus*, *S. saprophyticus*, and *S. epidermidis*. *S. saprophyticus* was the predominant CoNS species isolated from cows with clinical mastitis whereas *S. epidermidis* was the most prevalent one in subclinical mastitis cases. Susceptibility screening against 10 antibiotics determined 68% CoNS isolates as multidrug-resistant. High resistance was observed against penicillin (84%) and oxacillin (50%). The methicillin resistance gene, *mecA* was found in 73.33% isolates and interestingly, 26.67% of *mecA* negative isolates were oxacillin resistance. Of all the genes examined, 33.3% of examined isolates were positive for the staphylococcal enterotoxin B (*seb*) gene. Biofilm production was confirmed in 12% of CoNS. Conclusions: CoNS as an emerging cause for 60% of clinical mastitis and 67.27% of subclinical mastitis in cows in Egypt, and harboring the virulence genes rendering them as a potential threat for veterinary and public health and are also harboring resistance against penicillin and oxacillin the core for numerous novel intramammary combinations.

**Key words:** CoNS, Mastitis, *mecA* gene, Methicillin-resistant.

**INTRODUCTION**

For a long time, coagulase-negative staphylococci (CoNS), generally spread in the common habitat and colonizing the skin and mucosa of creatures and people, have been considered non-pathogenic. On display, they are the overwhelming etiological agent of bovine mastitis in numerous countries (Honkanen-Buzalski *et al.*, 1994; Mylly *et*

*al.*, 1998; Chaffer *et al.*, 1999; Makovec and Ruegg, 2003a; Pitkälä *et al.*, 2004; Rajala-Schultz *et al.*, 2004; Taponen *et al.*, 2007; Pyorala and Taponen, 2009; Malinowski and Kłossowska, 2010). Recently, the occurrence of CoNS intramammary infections of cows, sheep and goats is particularly expanding (Deinhofer and Pernthaner, 1995; Contreras *et*

*al.*, 2007). In Egypt CoNS were isolated from the examined subclinical mastitic cattle, buffaloes, sheep and goats with percentages of 16.6%, 59.4%, 50% and 55.6%, respectively (El-Jakee *et al.*, 2013). Subclinical mastitis is the major outcome of CoNS intramammary infections (Taponen *et al.*, 2006; Persson *et al.*, 2011). CoNS intramammary infections lead to clinical mastitis, associated with elevated somatic cell count (SCC) levels and decreased milk production, resulting in economic losses (Taponen *et al.*, 2007). Control of CoNS intramammary infections is difficult because as many CoNS pathogens with heterogeneous nature are incriminated. As etiological agents of mastitis, 15 CoNS species have been recognized (Thorberg *et al.*, 2009; Persson *et al.*, 2011). Under favorable conditions, CoNS exhibit on the udder skin and in teat channels pervade the galactogenic pathway to the quarter. The CoNS pathogenic mechanism is expressed in two ways: penetration of protective barriers, adherence to host cells and formation of a biofilm (invasiveness) enzymes and toxins production, including haemolysins and proteases (toxicity) (Bartoszewicz-Potyrała M and Przondo-Mordarska A, 2002; Bochniarz and Wawron, 2012). Methicillin-resistant *staphylococci* are a major concern for public and animal health. The prevalence of methicillin resistance is higher in CoNS than in *S. aureus*. Methicillin resistant CoNS species (MR-CoNS) have the *mecA* gene, which may have horizontally transferred among staphylococci. Furthermore, *mecA*-positive CoNS may act as potential donors for the rise in new MRSA clones (Garza-Gonzalez *et al.*, 2010; Huber *et al.*, 2011). Toxin-1 that causes Toxic Shock Syndrome in humans (TSST-1) and staphylococcal enterotoxins (SEs) play an important role in staphylococcal diseases (Robert *et al.*, 2011). Biofilm-producing isolates have been reported for many CoNS species, especially in *S. epidermidis* (Tormo *et al.*, 2005; Oliveira *et al.*, 2006). Biofilm prohibits host immune defense by impairing phagocytosis and production of antimicrobial peptides by epithelial cells and neutrophils, it also protect bacteria from antimicrobial therapy (Cucarella *et al.*, 2004; Melchior *et al.*, 2006). Biofilm consists of polysaccharide intercellular adhesion [PIA] encoded by the intercellular adhesion *icaADBC* operon (Stevens *et al.*, 2008). The aim of this study

was to study molecular genetic characteristics and antibiotic resistance patterns of methicillin-resistance CoNS from mastitic cows in Menoufiya Province, Egypt.

## **MATERIALS AND METHODS**

### **Sample collection**

Individual 205 milk samples were collected according to (Mackie *et al.*, 1996) from 205 lactating cows raised in small-scale farming systems located in Menoufiya province, Egypt, comprising of 40 mastitic cows and 165 apparently healthy cows based on clinical examination of cows and macroscopic evaluation of milk (Biffa *et al.*, 2005).

### **California Mastitis Test (CMT)**

CMT was used according to (Schalm and Noorlander, 1957) for diagnosis of subclinical mastitis.

### **Isolation and biochemical characterization of CoNS**

Milk was bacteriologically tested according to (Carter and Cole, 2012). Milk samples brought to room temperature were thoroughly mixed and cultured on Mannitol salt agar media (OXOID, Hampshire, UK) and Baird parker media (OXOID, Hampshire, UK). Identification of CoNS species was performed using Tube Coagulase test (Tortora *et al.*, 2013) and standard conventional biochemical methods (Murray *et al.*, 2003).

### **Phenotypic detection of CoNS virulence characteristics**

Hemolytic activity was evaluated on sheep blood agar (5-7%) plates. Each isolate was spot-inoculated, and the plates were incubated at 37 °C for 24 h. The zone of clearance was recorded, and hemolytic activity was evaluated as previously described (Quiblier *et al.*, 2011). Bacterial adhesive properties were determined by the Congo red agar medium (CRA) (Arciola *et al.*, 2015) comprised BHI (37 g/L), sucrose (50 g/L), No. 1 agar (10 g/L) and Congo Red stain (0.8 g/L). Plates of the medium were inoculated and incubated in aerobic environment for 24 h at 37°C. Under such condition, biofilm producers form black crusty colonies on CRA, whereas non-producers form red colonies.

### **Antimicrobial sensitivity testing**

Fifty CoNS isolates were submitted to in vitro susceptibility testing by the disk diffusion method according to the guidelines of the Clinical Laboratory Standard Institute (2012). The following discs (OXOID, Hampshire, UK): Oxacillin (1 µg), Vancomycin (30 mg),

Novobiocin (30 mg), Chloramphenicol (30 mg), Erythromycin (15 mg), Gentamicin (10 mg), Penicillin G (10 IU), Ciprofloxacin (5 mg), Amoxicillin/clavulanic acid 2:1 (30 mg) and Tetracycline (30 mg) were used. The multiple antibiotic resistance (MAR) index is defined as  $a/b$  where  $a$  represents the number of antibiotics to which the strain was resistant, and  $b$  represents the number of antibiotics to which the strain was exposed (Krumperman, 1983).

#### **Molecular detection of methicillin-resistant coagulase-negative staphylococci (MR-CoNS) and virulence related genes**

From the pool of 50 CoNS isolates used for in vitro susceptibility testing, 15 Methicillin resistance isolates were selected for genomic DNA isolation using Genomic DNA bacterial QIAamp DNA Mini Kit (QIAGEN, Valencia, CA). Isolates were screened for genes encoding staphylococcal enterotoxin B (*seb*), toxic shock syndrome toxin1 (*tss*), intracellular adhesin D (*icaD*), and Methicillin resistance (*mecA*) by PCR using T3 Thermal cycler (Biometra), PCR reaction mixtures and thermal program as previously reported by (Mehrotra *et al.*, 2000; McClure *et al.*, 2006; Ciftci *et al.*, 2009). Table 1 lists the primer sets used to detect *seb*, *tst*, *icaD* and MR-CoNS (*mec-A*) genes. The amplified PCR products were analyzed on 1.5% agarose gel and were visualized using UV trans-illuminator.

#### **RESULTS**

Two hundred and five milk samples collected from lactating cows raised in small-scale farming systems located in the Menoufiya province (Egypt) were examined during this study. Clinical examination of cows and macroscopic evaluation of milk revealed forty clinical mastitis cases (19.51%) in which the udder was hot, red, painful, swollen and sometimes atrophied. Some animals suffered from systemic reaction (slightly increased temperature).

California Mastitis Test (CMT) were applied on rest of milk samples (165) where 110 cows (53.66%) were diagnosed subclinically mastitic and 55 (26.83%) were negative.

#### **Isolation and identification of CoNS**

A total of 128 *Staphylococcus* spp. isolates were obtained from cows suffered with clinical and subclinical mastitis. From these, 30 (23.44%) coagulase-positive *staphylococci* (CPS) and 98 (76.56%) coagulase-negative

*staphylococci* (CoNS). CoNS isolates were obtained from 24 out of 40 clinical mastitic cows (60.0%) and 74 out of 110 (67.27%) subclinical mastitic cows. The following CoNS species *S.epidermidis*, *S.haemolyticus* and *S. saprophyticus* were isolated from the milk of clinically and subclinically mastitic cows, (Table 2). *S. saprophyticus* (41.67%) constituted the highest percentage of CoNS species isolated from the milk of cows with clinical mastitis followed by *S.haemolyticus* (33.34%) and *S.epidermidis* (25%) whereas, *S. epidermidis* (45.94%) constituted the highest percentage of CoNS species isolated from the milk of cows with subclinical mastitis followed by *S. saprophyticus* (37.83%) and *S. haemolyticus* (16.23%).

#### **CoNS virulence characteristics**

Concerning evaluation of CoNS virulence characteristics, our study revealed 20 (20.41%) CoNS (all the *S.haemolyticus*) isolates have hemolytic activity, six (6.12%) isolates showed biofilm production on The Congo Red agar (Figure 1). Four CoNS isolates carried both characters (Hemolysis and biofilm production).

#### **CoNS antimicrobial susceptibility**

Among the randomly selected 50 CoNS isolates, 42 isolates showed resistance to penicillin G (84%), 25 isolates for oxacillin (50%), 16 isolates for erythromycin (32%), 16 isolates for tetracycline (32%), 12 isolates for Amoxicillin/clavulanic acid 2:1 (24%), 10 isolates for novobiocin (20%), 10 isolates for chloramphenicol (20%), 4 isolates for vancomycin (8%), 2 isolates for gentamicin (4%), and 2 isolates for ciprofloxacin (4%) (Table 3). The highest rate of sensitivity observed to gentamicin (96%), ciprofloxacin and vancomycin (84%, for each), followed by novobiocin (80%). Multi-resistance was detected in 34 (68 %) out of the total 50 isolates, 32% resistant one or two antibiotic class and no isolates were pan-sensitive (Table 3). The multiple antibiotic resistance (MAR) index was calculated for the recovered species: *S. epidermidis* (0.6), *S. haemolyticus* (0.5) and *S. saprophyticus* (0.4).

#### **Molecular detection of MR-CoNS and some virulence related genes**

Out of 15 phenotypically methicillin resistance strains selected at random, methicillin resistance was confirmed by amplification of the *mecA* gene for 11(73.33%) isolates (Figure

2). Concerning toxin detection, only 5 isolates (33.3 %) carried *seb* gene (Figure 3).

**Table 1: PCR primers and fragment lengths of the studied genes.**

Gene	Primer Sequence	Amplified product	Reference
tst	ACCCCTGTTCCCTTATCATC TTTTCAGTATTTGTAACGCC	326 bp	(Mehrotra et al., 2000)
seb	GTATGGTGGTGTAAGTACTGAGC CCAAATAGTGACGAGTTAGG	164 bp	
mecA	GTA GAA ATG ACT GAA CGT CCG ATA A CCA ATT CCA CAT TGT TTC GGT CTA A	310 bp	(McClure et al., 2006)
icaD	AAA CGT AAG AGA GGT GG GGC AAT ATG ATC AAG ATA	381 bp	(Ciftci et al., 2009)

**Table (2): Biochemical and Virulence Activities of Coagulase Negative Staphylococci Isolates**

Species	S. epidermidis		S. saprophyticus		S. heamolyticus	
	Clinical mastitis	Subclinical mastitis	clinical mastitis	Subclinical mastitis	clinical mastitis	Subclinical mastitis
Number (%)	6/24 (25%)	34/74 (45.94%)	10/24 (41.67%)	28/74 (37.83%)	8/24 (33.34%)	12/74 (16.23%)
Catalase	+	+	+	+	+	+
Hemolysis on blood agar	-	-	-	-	+	-
Mannitol fermentation.	-	-	-	-	-	-
Coagulase.	-	-	-	-	-	-
D-nase activity.	-	-	-	-	-	-
Biofilm formation	1	-	-	1	1	3
Novobiocin sensitivity	Sensitive		Resistant		Sensitive	
Total	40/98		38/98		20/98	

**Table (3): Phenotypic characters of Antibiotics sensitivity.**

	OX		TE		E		VA		P		C		GN		AMC		CIP		NOV	
	NO	%	NO	%	NO	%	NO	%												
R	25	50	16	32	16	32	4	8	42	84	10	20	2	4	12	24	2	4	10	20
I	5	10	5	10	14	28	4	8	-	-	8	16	-	-	-	-	6	12	-	-
S	20	40	29	58	20	40	42	84	8	16	32	64	48	96	38	76	42	84	40	80

R: Resistant I: Intermediated S: Sensitive OX: Oxacillin, TE: Tetracycline, E: Erythromycin, VA: Vancomycin, P: Penicillin G, C: Chloramphenicol, GN: Gentamicin, AMC: Amoxicillin / clavulanic acid 2:1, CIP: Ciprofloxacin, NOV: Novobiocin.



Figure (1): Biofilm on Congo red medium (positive; black color colonies).

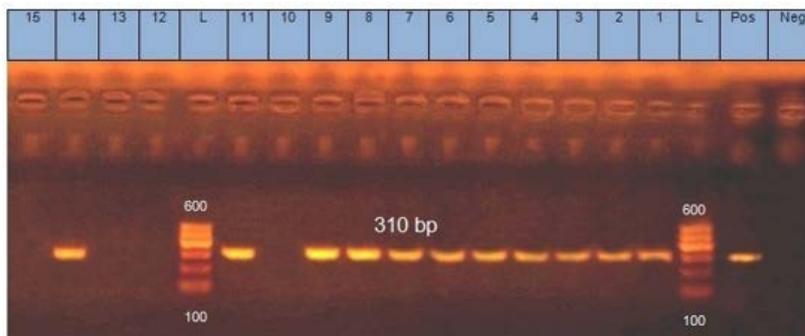


Figure (2): PCR detection of *mecA* gene  
Lane L: 100bpDNA Ladder, Post: positive control (310bp), Neg: Negative control, Lane 10,12,13,15 CNS (Negative), Lane 1-9,11,14 CNS (positive).



Figure (3): PCR detection of *seb* gene  
Lane M: 100bp DNA Ladder.  
Post: positive control (164bp), Neg: Negative control, Lane 2-8,12,13,15 CNS (Negative), Lane 1,9,10,11,14 CNS (positive).

Toxic shock syndrome toxin1 (*tss*) gene could not be detected in all tested isolates (Figure 4).

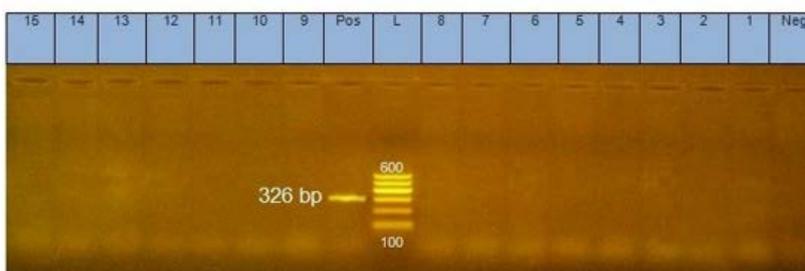


Figure (4): PCR detection of *tsst* gene  
Lane M: 100bp DNA Ladder, Neg: Negative control, Post: positive control (326bp) Lane 1:15 CNS (Negative).  
With respect to biofilm gene *icaD*, six isolates (40%) were tested positive by PCR (Figure 5).

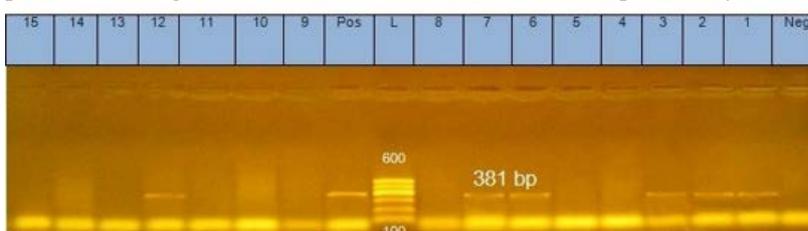


Figure (5): PCR detection of *icaD* gene  
Lane M: 100bp DNA Ladder, Post: positive control (381bp), Neg: Negative control, Lane 4,5,8,9,10,11,13,14,15 CNS (Negative), Lane 1-6,7,12 CNS (positive).

## DISCUSSION

Literature data reveal that coagulase-negative *staphylococci* considered non-pathogenic for many years and prevalent in many countries constitutes now the highest incidence of mastitis (Oliver *et al.*, 1997; Myllys *et al.*, 1998; Makovec and Ruegg, 2003b; Rajala-Schultz *et al.*, 2004; Taponen *et al.*, 2007; Malinowski and Kłossowska, 2010). In this study CoNS isolates were obtained from 24

out of 40 clinical mastitic cows (60.0%) and 74 out of 110 (67.27%) subclinical mastitic cows. Numerous studies worldwide have been investigated the prevalence of CoNS mastitis. In Egypt CoNS were isolated from the examined subclinical mastitic cattle, buffaloes, sheep and goats with percentages of 16.6%, 59.4%, 50% and 55.6%, respectively (El-Jakee *et al.*, 2013). The percentage of CoNS species isolated from mastitic cow's milk increased

from 26.6% to 53.5% during the period extended from 1988 to 1995 in Finland (Myllys *et al.*, 1998). Coagulase-negative *staphylococci* represent 49.6%, of all other isolated bovine mastitis pathogens in the same country during 2001 (Pitkala *et al.*, 2004). Moreover, several prevalence reports revealed that CoNS was the main aetiological agent of mastitis recently in various countries, Finland (Pitkala *et al.*, 2004), Norway (Østerås *et al.*, 2006), Germany (Barkema *et al.*, 1999; Tenhagen *et al.*, 2006), Belgium (Piepers *et al.*, 2007), Canada (Schukken *et al.*, 2009; Reyher *et al.*, 2011), England and Wales (Bradley *et al.*, 2007), and USA (Schukken *et al.*, 2009).

The CoNS consist of more than 50 species (www.bacterio.net, 2015). Accurate studies relying on genotypic identification, as recommended for CoNS research, revealed approximately 25 CoNS species causing bovine intramammary infection (Santos *et al.*, 2008; Sampimon *et al.*, 2009; Park *et al.*, 2011; Piessens *et al.*, 2011; Waller *et al.*, 2011). Our findings showed that *S.epidermidis*, *S.haemolyticus* and *S.saprophyticus* were isolated from the milk of clinically mastitic cows (25%, 33.33% and 41.67% respectively). While *S. epidermidis* constituted the highest percentage (45.95%) of CoNS species isolated from the subclinical mastitic cows. The results regarding other countries were slightly different. The highest percentage of CoNS species isolated from the milk of cows with mastitis in Japan by (Baba *et al.*, 1980) and in Finland by (Jarp, 1991) was *S. epidermidis*; in Sweden by (Birgersson *et al.*, 1992) was *S. simulans*; in Poland by (Malinowski *et al.*, 2006) and (Bochniarz *et al.*, 2013) was *S. xylosus*. The species differences of CoNS causing mastitis in cows may thus relate not only various countries and continents but also individual regions of a particular country, a herd- or even country-specific microbiota might exist (Piepers *et al.*, 2007; Gillespie *et al.*, 2009; Piessens *et al.*, 2011; Supre *et al.*, 2011). As well as the applied identification method and study design could influence the CoNS prevalence and distribution. The phenotypic expression of resistance can vary depending on the growth conditions (e.g., the temperature or osmolarity of the medium), making susceptibility testing of methicillin resistant *staphylococci* (MRS) by standard microbiological methods was

potentially difficult (Chambers, 1997). Bacteria in a biofilm are more resistant to antibiotics than in their planktonic form (Melchior *et al.*, 2006). The Congo Red method is rapid, sensitive, practical and reproducible for the detection of slime production in *Staphylococcus* spp. and has the advantage that colonies remain viable on the medium (Freeman *et al.*, 1989; Turkyilmaz and Eskzmrller, 2006). In the present study, slime production was examined on Congo Red Agar, six CoNS isolates (6.12%) were found to be slime production positive. These results are lower than that reported (72.1%) by (Darwish and Asfour, 2013), (47.8%) reported by (Turkyilmaz and Eskzmrller, 2006) and (48.7%) in *S. epidermidis* found by (Mohan U *et al.*, 2002). Antibiotics susceptibility or resistance of strains is very important clinically and economically. Moreover, antibiotic therapy of animal's infectious diseases may lead to introduction of resistant strains into the human food chain (Lee, 2003). In this study the in vitro susceptibility of CoNS against 10 selected antimicrobial agents were recorded. The highest resistance rate of CoNS observed to penicillin G (84%) and oxacillin (50%). Present findings are comparable with the results provided by many authors that CoNS species were penicillin resistance (56.6%) (Moniri *et al.*, 2007), and 60.2% (Moon *et al.*, 2007). More than 70% of the CoNS isolates worldwide are resistant to methicillin or oxacillin, CoNS clinical isolates were resistant to oxacillin with a percentage 62.1% (Arslan and Ozkardes, 2007). Penicillin- resistance reported in this study is higher than that previously reported (10, 5.71, 41.18%) for CoNS by (Kudinha and Simango, 2002), (Kurjogi and Kaliwal, 2011) and (EL-Berbawy *et al.*, 2015), respectively. Penicillin-resistance among CoNS can be attributed to long-term use of  $\beta$ -lactam antibiotics in human and animal healthcare settings (Moon *et al.*, 2007) and the increasing pathogenicity of the CoNS (Karabasanavar and Singh, 2013). The highest rate of sensitivity observed in this report were to gentamicin (96%, for each), ciprofloxacin and vancomycin (84%, for each), followed by novobiocin (80%). CoNS antibiotics sensitivity result were variable as reported in different studies. CoNS showed complete sensitivity to tetracycline (100%) and higher sensitivity to enrofloxacin (94.14%) (Idriss *et al.*, 2014) and gentamicin sensitive

(Giannechini *et al.*, 2002). Tetracycline more effective antibiotics against all bacteria isolated from bovine mastitis (Kurjogi and Kaliwal, 2011). CoNS isolates (97.14%) were sensitive to lincomycin (Idriss *et al.*, 2014). For identification of variable traits among the methicillin resistant CoNS from mastitic cows, from the pool of 50 CoNS isolates used for in vitro susceptibility testing, 15 Methicillin resistance isolates were screened for genes encoding staphylococcal enterotoxin B (*seb*), toxic shock syndrome toxin1 (*tss*), intracellular adhesin D (*icaD*), and Methicillin resistance (*mecA*). *S. aureus seb* and *tss* genes. . The most detected enterotoxin from food poisoning strains was *seb* showed a broader occurrence, (87.10%) from food samples and (70.97%) from milk (Carvalho *et al.*, 2013). In this study the *seb* gene can be detected in (33.3%) of examined isolates, while *tss* gene could not be detected. The *seb* gene was the most common classical staphylococcal superantigen (*Sag*) of CONS bovine intramammary infection isolates (Park *et al.*, 2011). Gene encoding the toxic shock syndrome toxin (*tss*) was not found in two separate studies considering staphylococcal toxin genes (Freitas *et al.*, 2008; Unal and Cinar, 2012). In the present work the presence of the *mecA* gene was investigated among phenotypically methicillin resistance by PCR, the incidence of methicillin resistance in the tested MRCoNS was 73.33% (11/15) by the presence of *mecA* gene. The positive detection rates of *mecA* in MR CoNS were 79% and 63.2% by (Bogado *et al.*, 2001) and (Moon *et al.*, 2007), respectively. In recent years, increased numbers of  $\beta$ - lactamase- producing CoNS and *mecA*- gene positive CoNS (MRCoNS) resistant to all groups of  $\beta$ -lactam antibiotics have been observed (Moon *et al.*, 2007). In the present study, four (26.67%) CoNS strains were positive phenotypically by disc diffusion method and negative by PCR for detection of methicillin resistance. The differences between molecular and phenotypic determinations of methicillin resistant was reported by (Bogado *et al.*, 2001), the isolates that did not carry *mecA* were phenotypically resistant to methicillin (Moon *et al.*, 2007). Combination of phenotypic and genotypic methods recommended for identifying biofilm producing strains. The intercellular adhesion (*ica*) locus, consisting of the genes *ica* ADBC, has been reported to have a potential role as a

virulence factor in the pathogenesis of mastitis in ruminants (Vasudevan *et al.*, 2003). Among the *ica* genes, *icaA* and *icaD* have been reported to play a significant role in biofilm formation in *S. aureus* and *S. epidermidis*, (Gotz, 2002). Slime factor production of MRCoNS isolates were detected by PCR targeting *icaD* genes and found that (6/15; 40%) of the tested MRCoNS strains were positive for *icaD* genes. Three (50%) isolates were positive for *icaD* gene (EL-Berbawy *et al.*, 2015). The prevalence rates of *icaD* genes was 47.1% in CoNS isolated from bovine subclinical mastitis (Darwish and Asfour, 2013). This difference in the prevalence rates can be attributed to variation in DNA sequences which may lead to failed amplification of the gene in some isolates leading to false negative results (Tormo *et al.*, 2005). In the present study, all CoNS isolates which were positive for slime production on CRA plates were also positive for detection of *icaD* gene by PCR.

## CONCLUSION

CoNS are the caustive agent of 60% of clinical mastitis and 67.27% of subclinical mastitis in cows in Egypt, CoNS are harboring the various virulence genes and are also resistance against numerous intramammary combinations specially those based on penicillin and oxacillin.

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