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PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM CATTLE, BUFFALO, SHEEP AND GOAT`S RAWS MILK IN SOHAG GOVERNORATE, EGYPT.

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ABSTRACT

The objective of this study is to determine the prevalence rate of *S. aureus* in cow, buffalo, sheep and goat`s raw milk in Sohag Governorate, Egypt by using conventional methods and molecular technique and further characterization of their antimicrobial resistance. Isolation and identification of *S. aureus* were performed for a totally of 340 raw milk samples from cow, buffalo, sheep and goat`s milk randomly selected from dairy farms and shops. Altogether, *S. aureus was* isolated from 136 raw milk samples (40%) of the 340 milk samples 44(36.7%) from fresh cow milk samples 65(46.4%) from buffalo milk 12(30%) from sheep milk and 15(37.5%) from goat`s milk samples. Amplification of genes encoding clumping factor (*clfA*) and thermonuclease (*nuc*) gene by Polymerase Chain Reaction was used for the genotypic characterization of isolated *S. aureus* strains. All tested *S. aureus* strains yielded a single amplicon with a size of approximately 985 bp by amplification of the (*clfA)* gene, however, amplification of the (*nuc)* gene produced an amplicon of 270 bp in all examined *S. aureus* isolates. The susceptibilities of the isolates were determined for 11 antimicrobial drugs using the disk diffusion assay. Total of *S. aureus* isolates originating from cow's and buffalo's milk were more resistant than those of goat and sheep origin. The resistance pattern of *S. aureus* isolates originated from raw milk from cow's and buffalo's of this study area revealed that the most of the *S. aureus* isolates (83.7%) were resistant to one or more antimicrobial agent. Eighty isolates (13.2%) were resistant to single antibiotic and 35 isolates (25.7%) showed resistance to 2 antimicrobial agents. Multiresistance of *S. aureus* isolates were found in 44.8% of *S. aureus* isolates. Resistance in cow and buffalo raw milk to Penicillin G was the most common finding followed by Ampicillin, Oxacillin, Amoxicilin/clavulanic acid, Erythromycin and Chloramphenicol. On the other hand several isolates were found susceptible to the Ciprofloxacin, Cefoxitin, Tetracyclin, Rifampicin and Vancomycin antibiotics. In sheep and goat raw milk resistance to Erythromycin was the most common finding followed by resistance to Amoxicillin-Clavulanic Acid, Ampicillin, Oxacillin and Penicillin G. In conclusion preliminary information on prevalence of *S. aureus* as milk contaminants as well as determination of antibiotic resistant *S. aureus* isolates which may act as vehicles for the transmission of drug resistance are very important for both human and animal health care.

Keywords: *Cow, Buffalo, Sheep, Goat, Milk, S. aureus, Antimicrobial drug resistance.*

INTRODUCTION

Milk has long been referred to as the most perfect food for human from birth to senility it contains all the nutrients required for rapid growth and healthy development of the body. Milk is an excellent medium for a large number of microorganisms. When the milk is drawn from the udder of a healthy animal, it contains organisms that have entered the teat canal through its opening. They are mechanically flushed out during milking. The number is ranged during milking between several hundreds to several thousand per milliliter (Farzana *et al.,* 2004).

Staphylococcus aureus infection is estimated to be present in up to 90% of dairy farms and is responsible for 35% of the economic loss in the dairy industry. *S. aureus* is a facultatively anaerobic Gram-positive bacterium. The majority of *S. aureus* strains are catalase-positive which constitute the well known pathogenic species *S. aureus* and forms the basis of traditional identification methodology. The presence of *Staphylococcus aureus* which shows up unsanitary conditions in the dairy herd and counts above 10^3

UFC in milk increase the risk of staphylococcal toxin production more resistant to the heat processes of pasteurization (Tortora *et al.,* 2005). Normally enterotoxin is produced at temperatures of 40ºC to 45°C, although Smith *et al.* (1982) detected a production of toxins at temperatures of 10ºC to 46ºC.

Among the bacteria predominantly involved in food-borne poisoning diseases, Staphylococcus aureus is a leading cause of gastroenteritis resulting from the consumption of contaminated food *Staphylococcus aureus* is one of the most common agents in bacterial food poisoning outbreaks. It is also a major causative pathogen of clinical or subclinical mastitis of dairy domestic ruminants (Le Loir *et al.,* 2003). Staphylococcal food poisoning is due to the absorption of staphylococcal at enterotoxin performed in the food is (Le Loir *et al.,* 2003) characterized by nausea, vomiting, diarrhea, general malaise and weakness. Such symptoms appear within 1-8 hours after consumption of contaminated food. Although the illness is seldom fatal, complications including dehydration and shock, can accompany severe attacks (Balaban and Rasooly, 2001).

Antibiotics are used to treat diseases of cattle, sheep, goat, buffalo and other animals and as well as used as preservatives for milk (Devriese *et al.,* 1997). *S. aureus* has been reported to frequently show multiple antimicrobial resistance patterns (Enright, 2003). This may be due to the indiscriminate use of antibiotics has led to the development of multiple antibiotic resistances thereby rendering the antibiotic treatment ineffective. Resistant bacteria occur in soil, water, plants and animals. The resistant bacteria present in environments are in contact with human beings and animals. It has been estimated that nearly equal tonnage of antimicrobial agents are used in man (Farzana *et al.,* 2004). Antimicrobial resistance is a major public health concern in many countries due to the persistent circulation of resistant strains of bacteria in the environment and the possible contamination of water and food (Normanno *et al.,* 2007). *S. aureus* strains were once nearly uniformly susceptible to semi-synthetic penicillinase-resistant β-lactams (e.g. methicillin, oxacillin), the most commonly used class of antibiotics for skin infection. These strains were termed 'methicillin resistant *Staphylococcus aureus,* or MRSA, a term that implied cross-resistance to all β-lactams including all penicillins and cephalosporins.

Antibiotic-resistant *S. aureus* isolates pose a severe challenge to both veterinary and health professions and dairy cattle producers because they have a negative impact on therapy (Brouillette and Malouin, 2005). The usage of antibiotics correlates with the emergence and maintenance of antibiotic-resistant traits within pathogenic strains (Shitandi and Sternesjö, 2004). These traits are coded for by particular genes that may be carried on the bacterial chromosome, plasmids, transposons or on gene cassettes that are incorporated into integrons (Rychlik *et al.,* 2006), thus are easily transferred among isolates. Multiple antibiotic resistant *S. aureus* strains have been isolated from milk obtained from cattle, beef and human samples in many parts of the world (Petinaki *et al.*, 2001; Pesavento *et al.,* 2007). The prevalence of antibiotic resistance usually varies between isolates from the different sampled stations and even between isolates from different herds on the same farm (Waage *et al.,* 2002).

Determination of levels of *S. aureus* and an evaluation of the antibiotic-resistant phenotypes of the isolates could serve as a tool for determining the hygiene standards implemented during milking. Data on antibiotic resistance could also be used to characterize these opportunistic pathogens, which may further limit the risks associated with the consumption of contaminated milk and its products (Evenson *et al.,* 1988).

Therefore, the aims of this study were determine the prevalence rate of *S. aureus* in cow, buffalo, sheep and goat raw milk in Sohag Governorate, Egypt by using conventional methods and molecular technique and further characterization of their antimicrobial resistance**.**

MATERIALS and METHODS

Sample collection: A total of 340 of fresh raw milk sample (120 cow`s samples, 140 buffalo`s samples, 40 sheep`s and 40 goat`s samples) were randomly collected from various places in dairy farms and dairy shops for cow's and buffalo's milk samples and from dairy farms for sheep and goat's milk samples, all samples were collected from Sohag Governorate, Egypt. Properly packed samples were placed on ice or frozen refrigerant packs in an insulated box, and then stored in laboratory at 4°C until all the microbial examination was performed, which was within 2 h. Aseptic techniques were applied, wherein all the equipments were pre-sterilized prior to analysis. The samples collected from dairy shops were tested for heat treatment testig (Lampert; 1975).

Isolation and Identification of *Staphylococcus aureus:*

The samples were processed immediately upon arrival using aseptic techniques. To detect *S. aureus*, 1mL of each milk sample was inoculated on Baird-Parker agar (Baird-Parker, 1962) (Difco, USA) and Mannitol Salt Agar (Cruickshank *et al.,* 1973; F.D.A., 2001; Bendahou *et al.,* 2008) (Oxoid, USA). After 24 - 48 h of incubation at 37°C, suspected colonies were sub-cultured on blood agar plate (Difco, USA) and incubated for 24 h at 37°C.

Identification of bacterial cultures under microscope:
The purified cultures of bacteria isolates obtained on Mannitol salt agar media plates were further examined under the microscope for their morphological characters(convex elevation and smooth margins colonies and by gram staining the typical colonies were showed gram-positive cocci occurring in bunched, grapelike irregular clusters were taken as presumptive s*taphylococcus* species.

Biochemical Identification: Biochemical tests were performed to confirm *S. aureus* using Catalase test, Coagulase test, Oxidase test, Acetoin production, Voges-Proskauer reactions, and D-mannitol fermentation tests were conducted on suspected colonies All *S. aureus* strains were positive for all tests except for oxidase test negative. (CLSI, 2007; Huong *et al.,* 2010). For sugar fermentation *S. aureus* strains ferment glucose, lactose, sucrose, maltose and manitol and produces acid (CLSI, 2007).

Molecular biology technique (PCR): (Riffon *et al.*, 2001)
In this study PCR technique used for confirmation presence of *S. aureus* in 10 raw milk samples from cow, buffalo, sheep and goat which selected by detection of *clfA* and *nuc* genes from culture samples through the following steps:

DNA Isolation: Isolation of genomic DNA was carried out by picking three to five colonies from freshly subcultured *S. aureus.* Colonies were homogenized in 50 µl TE buffer [10 mm of Tris HCl, 1 mm of ethylenediaminetetraacetic acid (EDTA), pH 8.0], followed by the addition of 1 µl lysostaphin (1.8 U/µl; Sigma, USA). After incubation of 1 h at 37°C, 1 µl proteinase K was added and the suspension was reincubated for 2 h at 56 °C. Proteinase K was finally inactivated through boiling of the mixture for 10 min. After centrifugation at 10.000 r.p.m for 5 min the supernatant was cooled on ice before use.

PCR Amplification: For PCR amplification, a reaction mixture of (20 µl) contained 0.7 µl of primer 1 (10 pm/ µl), 0.7 µl of primer 2 (10 pm/ µl), 0.4 µl of deoxynucleoside triphosphate, 2.0 µl of 10X thermophilic 2 buffer (Promega), 1.2 µl of MgCl (25 mm; Promega), 0.1 µl of Taq DNA polymerase (5 U/ µl; Promega), and 12.9 µl of distilled water. Finally, 2.0 µl of the DNA preparation was added to each 0.2-ml reaction tube. A negative control (with no template DNA) as well as a positive control "Diagnostic PCR product of *S. aureus* DNA isolated from reference strain" were included in each PCR. The tubes were subjected to thermal cycling (Progene, Ouxford, Cambridge, U.K) with the same programme described previously by Nashev *et al.* (2004). The PCR products (10 µl) were electrophoresed on 2% agarose gels with 1X TAE buffer (40 mm Tris- HCl, 1 mm EDTA, 1.14 ml glacial acetic acid, pH 7.8) at 70–100 V. Characterization of the strains was performed by PCR amplification of virulence and regulatory genes. This included the genes encoding clumping factor (*clfA*) and thermonuclease (*nuc*). For both genes, reaction mixtures (25 µl) included 2 µl template DNA, 10 × PCR buffer (Sigma, USA), 25 mM MgCl2 , 200 µM of the four dNTPs, 10 pmol of each of the 2 primers (Integrated DNA Technologies, Inc., Coralville, Iowa), and 1U Taq DNA polymerase (Sigma, USA). Amplification parameters and primer sequences were used as described by Straub *et al.* (1999) (Table 1). Amplified products were separated by agarose gel electrophoresis (1%) agarose containing 0.5 mg ethidium bromide per ml (all reagent from Promega Corp., Madison). Visualized and photographed under UV illumination. The sizes of the amplication products were estimated by comparison with a 100 bp DNA ladder (Promega Corp., Madison).

Table 1: Primers for amplification of the Staphylococcal genes.

Gene	Sequence (5 - 3)	Size of amplified products (bp)
clfA	F: GGCTTCAGTGCTTGTAGG R: TTTTCAGGGTCAATATAAGC	**985**
nuc	F: CGATTGATGGTGATACGGTT R: ACGCAAGCCTTGACGAACTAAAGC	**270**

Antibiotic susceptibility tests: Antimicrobial susceptibility tests by disc diffusion method has been used with antibiotic discs (Oxoid, USA). Antibiotic susceptibility tests were performed on all *S. aureus* isolates to determine their antibiotic-resistance profiles (Kirby *et al.,* 1966). Fresh overnight cultures were prepared and used for antibiotic sensitivity tests. An aliquot (100µL) from each isolate suspension was spread plated on Mueller Hinton agar (Oxoid, USA). Susceptibilities of the isolates to a panel of eleven different antibiotic discs (Table, 2) were determined. Antibiotic discs were gently pressed on to the inoculated Mueller Hinton agar to ensure intimate contact with the surface and the plates were incubated aerobically at 37 °C for 18 h – 24 h. *S. aureus* isolates were then classified as resistant, intermediate resistant or susceptible according to the inhibition zone diameter for individual antimicrobial agents according to the interpretation table (supplied by the Oxoid, USA). Multiple antibiotic resistant (MAR) phenotypes were recorded for isolates showing resistance to three and more antibiotics (Rota *et al.,* 1996).

Table 2: List of antimicrobial agents tested and their symbol and volumes.

Antimicrobial agents	Symbol	Volume (µg)
Amoxicilin/clavulanic acid	AMC	30
Ampicillin	AP	10
Cefoxitin	FOX	30
Chloramphenicol	C	30
Ciprofloxacin	CIP	5
Erythromycin	E	15
Oxacillin	OX	1
Penicillin G	P	10
Rifampicin	RD	5
Tetracycline	TE	30
Vancomycin	VA	30

RESULTS

In this study the prevalence rate of *S. aureus* isolated from cow, buffalo, sheep and goat`s raw milk obtained from Sohag Governorate, Egypt were made. One hundred and thirty six of 340 samples (40%) were positive for *S.aureus*. Forty four raw cow`s milk (36.7%), 65 raw buffalo`s milk (46.4%), 12 raw sheep`s milk (30%) and 15 raw goat`s milk (37.5%) samples were contaminated with *S. aureus* (Table, 3).

Table 3: Prevalence of *Staphylococcus aureus* in raw milk isolated from cow, buffalo, sheep and goat raw milk originating from Sohag Governorate.

Source	No of samples	No of *S.aureus* positive samples	% of isolates
Cow milk	120	44	36.7
Buffalo milk	140	65	46.4
Sheep milk	40	12	30
Goat milk	40	15	37.5
Total	340	136	40

Fig. 1: Typical amplicons of the clumping factor (*clf*A) gene of some *S. aureus* strains. Lane M: 100-bp-molecular-size DNA ladder, Lane 1 – 3: strains of *S. aureus* detected from cow's raw milk, Lane 4 – 6: strains of *S. aureus* detected from buffalo's raw milk, Lane 7 - 8: strain of *S. aureus* detected from sheep raw milk, Lane 9 - 10: strain of *S. aureus* detected from goat raw milk, Lane 11: Negative control "unamplified PCR product" and Lane 12: Positive control " amplification of the 985 bp fragment of *S. aureus* from the extracted DNA of *S. aureus* reference strain".

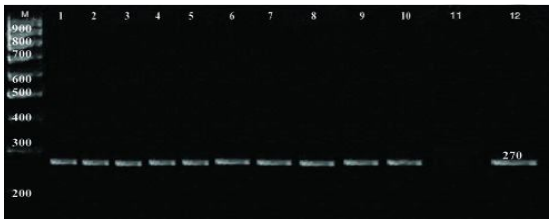


Fig. 2: Typical amplicons of the thermonuclease (*nuc*) gene of some *S. aureus* strains. Lane M: 100-bp-molecular-size DNA ladder, Lane 1 – 3: strains of *S. aureus* detected from cow's raw milk, Lane 4 – 6: strains of *S. aureus* detected from buffalo's raw milk, Lanes 7 - 8: strains of *S. aureus* detected from sheep raw milk, Lane 9 - 10: strains of *S. aureus* detected from goat raw milk, Lane 11: Negative control "unamplified PCR product"and Lane 12: Positive control "amplification of the 270 bp fragment of *S. aureus* from the extracted DNA of *S. aureus* reference strain".

The antibiotic susceptibility tests for *S. aureus* isolates were subjected to eleven antimicrobial agents, from different antibiotic classes were used. Antibiotics of veterinary and human health relevance were also considered. A summary of the percentage of *S. aureus* that were resistant to these antibiotics is provided in table (4).

Table 4: Antibiotic susceptibility profiles of *Staphylococcus aureus* isolated from cow, buffalo, sheep and goat raw milk originating from Sohag Governorate, Egypt.

Sample source of raw milk	Antibiotic sensitivity (%)										
	AMC	AP	FOX	C	CIP	E	OX	P	RD	TE	VA
Cow	70.5	54.5	100	93.3	100	79.5	63.6	45.5	100	100	100
Buffalo	64.6	56.9	100	95.4	100	90.8	60	55.4	100	100	100
Sheep	91.6	91.6	100	100	100	83.3	91.6	91.6	100	100	100
Goat	93.3	93.3	100	100	100	73.3	93.3	93.3	100	100	100

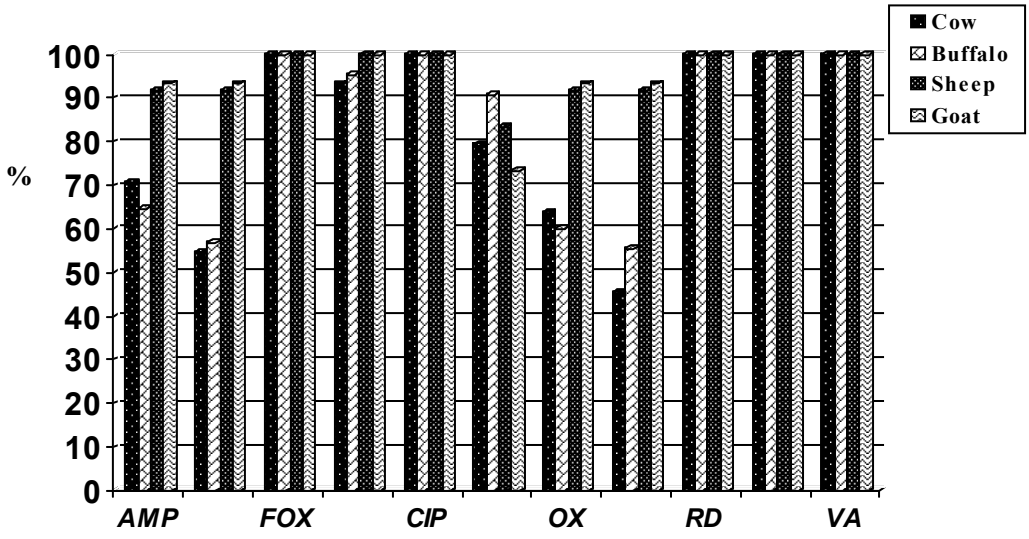
Result of antimicrobial susceptibility are shown in Figure 3. In total *S. aureus* originating from cow's and buffalo's milk were more resistant than those of goat and sheep origin. The resistance pattern of *S. aureus* isolates originated from raw milk from cow's and buffalo's of this study area revealed that most of the isolates (83.7%) were resistant to one or more antimicrobial agent. Eighty isolates (13.2%) were resistant to single antibiotic and 35 isolates (25.7%) showed resistance to 2 antimicrobial agents. Multiresistance was found in 44.8% of *S. aureus* isolates. Resistance to Penicillin G was the most common finding (54.5%, 44.6%), followed by Ampicillin (45.5%,43.1%), Oxacillin (43.1%,40%), Amoxicilin/clavulanic acid (29.5%,35.4%) Erythromycin (20.5%,19.2%) and Chloramphenicol (6.7%,4.6%) for cow and buffalo milk respectively. On the other hand several isolates were found susceptible to the Ciprofloxacin, Cefoxitin, Tetracyclin, Rifampicin and Vancomycin antibiotics.

In general observation the large percentage of Penicillin G (10µg), Ampicillin (10µg), Oxacillin (1µg) and Amoxicilin/clavulanic acid (30 µg) resistant *S. aureus* were isolated from the study area. These were also resistant to several other antibiotics. Therefore, the one can easily conclude that these are Methicillin resistant *S. aureus* (MRSA).

In sheep and goat raw milk, a resistant to Erythromycin (16.7%, 26.7%) followed by resistance to Amoxicillin-Clavulanic Acid, Ampicillin, Oxacillin and Penicillin G (8.7%, 6.7%) for sheep and goat milk respectively.

Antimicrobial susceptibility test showed similar results between cow and buffalo as well as between sheep and goat this may be due to the same rearing, breeding and handling condition to these animals and their milk production in Egypt.

Fig. 3: Comparison of sensitivity to antibiotics in *S. aureus* from raw cow, buffalo, sheep and goat milk.



DISCUSSION

In this study the prevalence rate of *S. aureus* isolated from cow, buffalo, sheep and goat`s raw milk obtained from Sohag Governorate, Egypt and further characterization of their antimicrobial resistance were made. One hundred and thirty six of 340 samples (40%) were positive for *S. aureus*. Forty four raw cow`s milk (36.7%), 65 raw buffalo`s milk (46.4%), 12 raw sheep`s milk (30%) and 15 raw goat`s milk (37.5%) samples were contaminated with *S. aureus*.

This contamination rate is similar that observed in the surveys previously conducted in other countries on several kinds of raw milk, the prevalence of *S. aureus* in the present study was 40% such as isolated by Mekonnen *et al.* (2011) (39.5%) and Zakary *et al.* (2011) (40%). On other hands, higher incidence was reported by Haggag, 2006 (56%), Ozkan *et al.* (2007) (98.7%), Ateba *et al.* (2010) (100%) and Lingathurai and Vellathurai, (2011) (61.70%). While lower prevalence was reported by Shah *et al.* (1985), Ekici *et al.* (2004), Fagundes *et al.* (2010), Kumar and Prasad, (2010), Malahat *et al.* (2010), Tambekar and Bhutda, (2010) and Santana *et al.* (2010) who found 8.3 %, 18.18%, 7.3%, 6.6%, 14% 17.39% and 18.80% prevalence respectively.

Although the prevalence of *S. aureus* has been reported to vary with the size and geographic region of the area sampled, a high proportion of these bacteria in milk relates to poor hygiene practices. Based on observations made during the collection of samples, the improper hygiene and poor farm management practices contributed to the presence of *S. aureus* in the milk. *S. aureus* is normally resident in humans; therefore the *S. aureus* present in cow's milk may have resulted from transmission from humans, which raises questions regarding the hygiene practices followed. In this study area milk was obtained from animals by washing hands of person deal with animal and/or the utensils and containers used. In certain cases, untreated groundwater was used to wash the containers that were used for milking. This may have contributed to the high level of *S. aureus* isolated. Improving the hygienic conditions of the milking environment and/or utensils may reduce the prevalence of *S. aureus* in milk and prevent its transmission to humans.

S. aureus is the most pathogenic among the mastitis causing agents in cattle, globally (Grabber *et al.,* 2009; Vyletlova *et al*., 2010; Persson *et al.,* 2011; Arga *et al.,* 2012). *S. aureus* is the most frequently isolated from the clinical cases of mastitis in small ruminants (Bergonier *et al*., 2003; Vyletlova, 2009; Arga *et al.,* 2012). Da Silva *et al*. (2004) detected that *S. aureus* represented 37% of the isolates from subclinical mastitis in goats. Contreras *et al*. (2007) identified also *Staphylococcus* spp. as the most prevalent pathogens in small ruminants and refer to necessity to eliminate this pathogen because of risk of milk contamination by thermostable toxins.

There are several disadvantages associated with microbiological culture which are as follows: it is limited by the dynamic nature of infections of the milk sample, milk culture may yield no bacteria from truly infected milks due to the presence of very low numbers of bacteria in samples and negative cultures may also be due to the presence of residual therapeutic antibiotics that may inhibit bacterial growth in vitro. The presence of leucocytes in milk samples may also inhibit growth of bacteria. Moreover, microbiological culture of milk is time consuming and species identification by standard biochemical methods requires more than 48 hours for completing (Riffon *et al.,* 2001).

Due to limitation of cultural methods, PCR has been developed to identify various bacteria in milk samples. The development of PCR- based methods provides a promising option for the rapid identification of bacteria. With this method, identification of bacterial pathogens can be made in hours, rather than days, as conventional cultural methods require. PCR can also improve the level of detection due to its high sensitivity. Theoretically, only a few cell of pathogen are necessary to yield a positive diagnosis (phueketes *et al.,* 2001). Rapid identification methods, in particular nucleic acid based tests, have the potential to be extremely specific and can also discriminate between closely related organisms such as other species of the staphylococci (Riffon *et al.,* 2001).

The ability of *S. aureus* to adhere to extracellular matrix proteins is thought to be essential for the colonization and the establishment of infections (Salasia *et al.,* 2004). *S. aureus* possesses various adhesion genes, including *clf*A, *fnb*A, and *can* (El-Sayed *et al.,* 2005). Amplification of gene sequences by PCR such as *nuc* and *clf*A is used for identification of *Staph. aureus* (Akineden *et al.,* 2001 and Nashev *et al.,* 2004).

Amplification of genes encoding clumping factor (*clfA*) and thermonuclease (*nuc*) gene by Polymerase Chain Reaction was used for the genotypic characterization of isolated *S. aureus* strains. Amplification of the clumping factor (*clfA*) gene resulted in a single amplicon with a size of approximately 985 bp for all 10 tested *S. aureus* strains isolated from raw milk samples indicating no size polymorphisms of this gene (Figure1). This agreed with (Marrack and Kappler, 1990). While, Amplification of the *nuc* gene produced an amplicon

of 270 bp in all 10 examined *S. aureus* isolated from raw milk samples (Figure 2). This result as that recorded by Rosenstraus *et al.* (1998) and Kim *et al.* (2001). Specificity of the PCR products was demonstrated with 100% of the tested isolates. This specificity of *S. aureus* was agreed to the results recorded by Ozkan *et al.* (2007) and Karahan *et al.* (2011). Lower results were detected by Karahan and Cetinkaya., 2007 (80.5%), El-Jakee *et al.,* 2010 (65.9%) and Malahat *et al.* 2010 (21%).

The accuracy of PCR in the detection of *S. aureus* in raw milk samples was more than the cultural methods. No culture positive samples were negative in PCR..` PCR amplification of the *clf*A and *nuc* genes could be used as a powerful tool for the identification of *S. aureus* in milk samples.

The antibiotic susceptibility tests for *S. aureus* isolates were subjected to eleven antimicrobial agents, from different antibiotic classes were used. Antibiotics of veterinary and human health relevance were also considered. The evolution of antibiotic resistance in *S. aureus* strains is a serious cause of concern in dairy animals (Wang *et al.* 2008). The results presented here are similar to previous studies in which Gram-positive bacteria were generally susceptible to Vancomycin, Chloramphenicol, Cefoxitin and Ciprofloxacin (Gündan *et al.,* 2006; Pereira *et al.,* 2009). These drugs are no longer used in veterinary medicine in many countries (Pace and Yang, 2006), which may account for the results reported here.

As shown in Table. 4, a large percentage of *S. aureus* isolates isolated from cow and buffalo's raw milk were Methicillin resistant *S. aureus* (MRSA). These strains in intra-mammary dissemination often produce incurable severe intra-herd infections (Moon *et al.*, 2007; Kumar *et al.*, 2010). MRSA strains have been observed to be multi-drug resistant, such as Aminoglycosides, Macrolides, Lincosamides, Streptogramins, Tetracyclines, etc., which are often used in the treatment of mastitis (Wang *et al.*, 2008; Kumar *et al.*, 2010). Lee (2003) reported that there are only a few reports on MRSA associated with mastitis although the transmission of bovine MRSA to humans is possible and may contribute to outbreaks in animal and human populations. Hence, it is necessary to know which endemic strains of *S. aureus* in dairy cattle populations are highly pathogenic and methicillin-resistant.

High levels of MRSA have been identified in patients in the United States and some European countries (Mark *et al.,* 2003). In these countries, 44.4%, 34.7%, 41.8% and 32.4% of isolates from patients in the United States, France, Italy and Spain, respectively, were resistant to methicillin. Since antibiotic-resistant isolates might be transmitted to humans by the consumption of food products containing such resistant bacteria, the use of antibiotics as growth promoters in animal husbandry, especially of those commonly used for both human and animal care should be avoided (Wise, 2007).

A further objective of the study was to characterize and compare the antibiotic-resistance profiles of *S. aureus* isolated from the study area. The motivation for this was the fact that there were no clear studies conducted in this area. Furthermore, the presence of antibiotic resistant *S. aureus* has been reported to negatively affect the treatment of its associated infections in humans and animals (Petinaki *et al.,* 2001, Brouillette and Malouin, 2005; Moneoang and Bezuidenhout, 2009). Investigation of the antibiotic-resistance profiles of these isolates may serve as a tool in assessing both the sanitary conditions employed during milking and the health risks that humans may encounter when infected by antibiotic-resistant strains.

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انتشار وحساسيه بكتيريا المكور العنقودي الذهبي المعزوله من اللبن الخام للأبقار والجاموس والاغنام والماعز في محافظه سوهاج-مصر

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الهدف من هذه الدراسة تحديد معدل انتشار بكتيريا المكور العنقودي الذهبي في الحليب الخام للبقر والجاموس والاغنام والماعز في محافظه سوهاج. اجري العزل لهذا الميكروب باستخدام الطرق التقليدية والتقنية الجزيئية وتم كذلك توصيف مقاومه عزلات هذا الميكروب للمضادات الحيوية المختلفة. تم تجميع عدد ٣٤٠ عينه عشوائية من الحليب الخام من مزارع الالبان والمحلات التجارية. تم عزل بكتيريا المكور العنقودي الذهبي في ١٣٦ عينه بنسبه (٤٠%) وكانت (٣٦.٧ %) من الحليب الخام للبقر و(٤٦.٤ %) من العزلات من البان الجاموس, (٣٠%) من ألبان الغنم و (٣٧.٥%) ألبان الأغنام. تم التوصيف الوراثي للعزلات باستخدام اختبار البلمرة المتسلسل لجين (clfA) وجين (NUC) وبفحص جميع العزلات للاختبار مقاومتها لعدد ١١ نوع من المضادات الحيوية وهى اموكسيسللين والامبيسللين والسيفوكزتين والكلورامفنيكول والسيبروفلوكساسين والاريثروميسين والاوكساسيكلين والبنيسلين جى والريفامبيسين والتتراسيكلين والفانكوميسين وذلك باستخدام مقايسة نشر القرص. وجد ان معظم العزلات (٨٣.٧ %) مقاومه لواحد او اكثر من المضادات الحيوية. ووجد ان العزلات المأخوذة من البان البقر والجاموس كانت اكثر مقاومه من تللك المأخوذة من الغنم و الماعز. ٨ عزلات فقط بنسبه (١٣.٢ %) كانت مقاومه لمضاد واحد و ٣٥ عزله (٢٥.٧ %) مقاومه لعدد ٢ من المضادات الحيوية ووجد ان عزلات المكور العنقودي الذهبي من البان البقر والجاموس كانت اكثر مقاومه للبنسيلين جي يليه الامبيسلين ثم الاوكساسيلين ثم الاريثرومايسين والكلورامفنيكول وعلى العكس وجد ان العديد من العزلات لديها حساسيه كبيره مع السيبروفلوكساسين والسيفوكزتين والتتراسيكلين والريفامبيسين واعلاهم الفانكوميسين وقد تم مناقشه الأهمية الصحية لمقاومه هذه العزلات من اللبن الخام للمضادات الحيوية حيث ان هذه الالبان تعمل كوسائل لنقل مقاومه البكتيريا للأدوية لمستهلكي هذه الالبان مما يؤثر على الناحية الصحية للإنسان.