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DETECTION OF ENTEROTOXIN GENES IN *STAPHYLOCOCCUS AUREUS* ISOLATED FROM COW MILK

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ABSTRACT

Staph. aureus is considered one of the main causes of food poisoning and clinical infections in different hosts. So this work intended to investigate the occurrence of enterotoxin genes and antibiotics susceptibility of *Staph aureus* isolated from raw and local pasteurized milk. To realize this, 125 samples of raw milk and 75 samples of local pasteurized milk were obtained from supermarkets, dairy shops and farms in Sohag Province, Egypt. These samples were subjected to bacteriological examination. The result showed that 68 out of 125 raw milk samples and 37 out of 75 local pasteurized milk samples were positive for *Staphylococcus* spp. on mannitol salt agar. Biochemical scheme supposed that 18 isolates of raw milk samples and 8 isolates of local pasteurized milk samples were positive for *staph aureus*. PCR by using *nuc* gene specific primer, discriminated the existence of *Staph aureus* DNA in 15 isolates out of 18 isolates of raw milk samples, while 7 isolates out of 8 isolates of local pasteurized milk samples showed a postive bands at 267bp. *Staphylococcal* enterotoxin genes were detected in 53.33% and 42.86% of *Staph aureus* isolates from raw and local pasteurized milk, respectively. *sea* and *sed* genes were detected in most isolates of *Staph aureus* while none of these isolates harbored *seb* and *see* genes. *sec* gene could be detected only in the raw milk isolates. The antibiotics sensitivity profile of enterotoxigenic *Staph aureus* was showed a high percentage of resistance to penicillin and tetracycline while all isolates were fully susceptible to vancomycin.

Key words: Milk, *Staph aureus*, *nuc gene*, enterotoxins, antimicrobials.

INTRODUCTION

Milk is an imperative food because it is rich by various essential components including proteins, vitamins, and minerals also it considers a nutritive media for many microorganisms such as *Staph. aureus* which mainly criminalized in food poisoning cases (Pandey *et al.,* 2014), because it can adapt to grow in various types of foods and secreting enterotoxins (Balaban and Rasooly,2000).

The contamination of milk by *Staph. aureus* was occurred through the infection of the mammary gland or by bad sanitary conditions, during or after milking, and these happened by human activity who responsible for the contamination (Rehman *et al.*, 2014)

Staph. aureus is a gram-positive microorganism, grows in a different temperatures, ranged from 7 ″C to 48.5 ″C. It produce a broad extracellular toxins, the antigenic-base classification of *Staphylococcal* enterotoxins (SEs) includes five classical types of toxins (SEA- SEE) (Riva *et al*.,2015). The most imperative SEs are SEA and SEB which usually more common in milk (Chiang *et al*., 2006).

Staphylococcal enterotoxins resists the majority of proteolytic enzymes and thus remains their action in gastrointestinal tract. They are highly heat resistant toxin (Sutejo *et al*., 2017), they keep their activities even after pasteurization (Ralln *et al.,* 2008).The SEs toxins resulting in nausea, brutal vomiting, abdominal pain and occasionally diarrhea (Rosengren *et al.,* 2013).

Staph. aureus became more complicated problem for it's ability to resist different types of antibiotics. The spreading of the MRSA strains worldwide lead to high costs in terms of treatment and lead to life threatening infections (Yamamoto *et al*., 2013).

Routine identification of *Staph. aureus* usually carried out by traditional methods but these methods

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were discomfit and time consuming. Furthermore, these methods lead to indistinct results. Rapid and accurate methods for identification of food borne pathogens are important for microbiological safety. In the last 10 years, unrestricted detection methods using molecular techniques, suth as polymerase chain reaction (PCR) method particularly multiplex PCR was proven as one of the most suitable way for sensitive and fast detection of pathogenic bacteria in food (Shawish and Al-Humam, 2016 and Kim *et al*., 2017).

Considering to these facts, the existing work intended to study: The presence of SEs coding genes (*sea, seb, sec, sed and see*) in *Staph aureus* strains isolated from raw and local pasteurized milk by using multiplex-PCR and evaluation the sensitivity of these isolates to different types of antimicrobials.

MATERIALS AND METHODS

I-Milk samples:
A total of 200 cow milk samples (125 raw cow milk and 75 local pasteurized cow milk) were collected randomly from local markets, street vendors and farms in Sohag Governorate, Egypt, during the period from July to September 2017. The samples were collected in sterile plastic bags. All the samples were taken to the laboratory under refrigerate conditions where they were prepared for bacteriological examination.

II- Isolation of *Staph. aureus*:
One milliliter of each sample was added aseptically in a sterile test tube contain 9 ml of the nutrient broth. Inoculated test tubes were incubated at 37°C for 18 hours. A loopful from each incubated broth tube was plated on mannitol salt agar by using Streaking plate method. Plates were incubated at 37ºC for 24-48 hours according to (Arora, 2003). The suspected colonies were purified and then transferred to nutrient agar slopes for preservation and further identification.

III-Biochemical Identification of *Staph. aureus* isolates:
The identification of suspected colonies were identified according to Holt *et al*. (1993) this identification based on colony morphology, staining reaction and biochemical tests such as catalase, coagulase test and thermonuclease test. The isolates which pretended a positive results in pervious tests were submitted to the Voges-Proskauer to discriminate *Staph. aureus* (positive) from other coagulase and thermonuclease positive (negative).

IV-Genotypic identification:

1-DNAextraction:
DNA was extracted from 5 ml of a coagulae-positive *Staphylococcal* culture grown at 35ºC (±2ºC) for 16-24h in nutrient broth (oxoid). DNA was extracted by QIAamp DNA Mini kit (Qiagen, Germany, GmbH) according to the manufacture instructions.

2-Molecular confirmation of *Staph aureus* by using conventional PCR:
Amplification reaction were performed according to Mansour *et al*. (2017) with slight modification, in a final volume of 50 µl containing: 25µl PCR Mastermix (Emerald Amp GT), 1.5µl for each primer for *nuc* gene (Table. 1), 5µl of DNA Template and 17µl of PCR grade water. Reactions were carried out in thermal cycler (MJ Research, Inc. Watertown, MA) with the following program: initial denaturation at 94ºC for 5 min followed by 35 cycles of 94ºC for 2 min, 52ºC for 1min and 72ºC for 1min with a final extension at 72ºC for 10 min. PCR product was alienated by electrophoresis in 1.5% agarose gel (Bioshop^{R}, Candainc.) stained with ethidium bromide, then visualized in a UV transilluminator.

3-Detection of classical enterotoxin genes in *Staph. aureus* isolates by using multiplex PCR:
Multiplex PCR was performed by using the following primers for enterotoxin genes (*sea, seb, sec, sed* and *see*) (Table 1), the PCR mixture was prepared as following: 12.5 µl PCR Taq green Mastermix (Thermo), 1 µl of each primer and 1µl DNA. The final volume was adjusted to 25 µl by adding sterile PCR grade water. Amplification profile was standardized in 94ºC for 5 min followed by 35 cycles of 94ºC for 2 min, 52ºC for 2 min and 72ºC for 3 min with a final extension at 72ºC for 7 min. PCR products were separated by gel electrophoresis (1.5% agarose gel stained with ethidium bromide) then were visualized in a UV transilluminator (Mansour *et al.*, 2017).

V-Antibiotic sensitivity Test:
The disk diffusion technique was used according to (Mohanty and Cock, 2010), to perform the antimicrobial susceptibility test for enterotoxigenic *Staph. aureus* strains, 0.1ml of bacterial suspension ($1x10^8$ CFU/mL) equivalent to (0.5 McFarland) was plated on surface Mueller-Hinton agar. The plates was left for 2-5minutes for dry then antimicrobial disks were placed on surface of agar and incubated at 35ºC±2 for 24-48hrs. Different antimicrobials were used like: PencillinG, amoxicillin, amikacin, erythromycin, clindamycin, tetracycline, vancomycin and sulfamthaxazole-trimethoprime. The diameter of inhibition zone was measured for each antimicrobial agents used and the interpretation was compared with the measurements of CLSI (2018) for each antimicrobial was used.

RESULTS

The current results illustrated in (Fig.1) showed that *Staphylococcal* isolates were recovered from 54.40% (68/125) of raw milk samples and 49.33% (37/75) of local pasteurized milk samples on mannitol salt agar.

The biochemical profile revealed that the coagulase production was clear in 38 isolates out of 68 isolates of raw milk samples and 18 isolates out of 37 isolates of local pasteurized milk samples. Most of coagulase positive *staphylococcal* isolates of raw and local pasteurized milk declared a thermonuclease activity with percentages 78.9% (30/38) and in 72.2% (13/18), respectively. The positive coagulase and thermonuclease isolates were submitted to Voges-Proskauer test to discriminate *Staph aureus*, 18 out of 30 raw milk isolates and 8 out of 13 local pasteurized milk isolates were positive for Voges-Proskauer test (Table2).

PCR by using specific primer for (*nuc* gene), confirmed the presence of *Staph aureus* DNA in 15 out of 18 isolates of raw milk identified positive biochemically and in 7 out of 8 isolates from local pasteurized milk (Table 2&Fig.2).

The data postulated in Table (3) and Fig (3&4) revealed that enterotoxin genes were detected in 8 out of 15 *Staph. aureus* isolates (53.33%) from raw milk samples and 3 out of 7 *Staph. aureus* isolates (42.86%) from local pasteurized milk samples, *sea* and *sed* genes were detected in most *Staph. aureus* isolates, *sec* gene could be detected only in the raw milk isolates while *seb* and *see* genes were not detected in any *Staph. aureus* isolates from both raw and local pasteurized milk.

Enterotoxigenic *Staph aureus* (8 from raw milk and 3 from local pasteurized milk) were tested for their susceptibilities to eight antimicrobial agents. The data postulated in Table (4) demonstrated that a large section of enterotoxigenic *Staph. aureus* isolates showed resistance to penicillinG (72.73%) and tetracycline (72.73%). Intermediate resistance were showed against clindamycin (27.27%) and erythromycin (18.18%). All isolates showed a high sensitivity to vancomycin (100%) followed by amikacin (90.91%), sulfamethoxazole- trimethoprim (81.82%) and amoxicillin (81.82%).

Tables:

Table1: Primers sequence.

Gene	Primers sequence 5′ ′3	Size	Reference
nuc	**5′ GGTTATCAATGTGCGGGTGG ′3** **5′ CGGCACTTTTTTCTCTTCGG ′3**	**267**	**Brakstad *et al.* (1992)**
sea	**F- 5′ GGTTATCAATGTGCGGGTGG ′3** **R-5′ CGGCACTTTTTTCTCTTCGG ′3**	**102**	**Mehrotra *et al.* (2000)**
seb	**F-5′ GTATGGTGGTGTAACTGAGC ′ 3** **R-5′ CCAAATAGTGACGAGTTAGG ′3**	**164**	
sec	**F-5′AGATGAAGTAGTTGATGTGTATGG′3** **R- 5′ CACACTTTTAGAATCAACCG ′3**	**451**	
sed	**5′CCAATAATAGGAGAAAATAAAAG′3** **5′ATTGGTATTTTTTTTCGTTC′3**	**278**	
see	**5′AGGTTTTTTCACAGGTCATCC′3** **5′CTTTTTTTTCTTCGGTCAATC′3**	**209**	

Table 2: Specificity of PCR method for *Staph. aureus* DNA in raw and local pasteurized milk isolates.

Samples	Biochemical identification	PCR			
	No. of *Staph aureus* isolates	No. of positive isolates		No. of negative isolates	
		No.	%*	No.	%
Raw milk	**18**	**15**	**83.33%**	**3**	**16.67%**
Pasteurized milk	**8**	**7**	**87.50%**	**1**	**12.50%**

Table 3: Enterotoxigenic genes in *Staph. aureus* isolated from raw and local pasteurized milk samples.

Samples	Total No. of *Staph. aureus* isolates	No. of Enterotoxogenic *Staph. aureus* isolates	Enterotoxin genotyping pattern
Raw milk	**15**	**1**	**Sec**
		3	**Sed**
		3	**Sea+Sed**
		1	**Sec+Sed**
Local pasteurized milk	**7**	**2**	**Sea**
		1	**Sea+Sed**

Table 4: Sensitivity of enterotoxigenic *Staph. aureus* isolated from raw and local pasteurized milk to different antimicrobials.

Antibiotics	No. of Enterotoxigenic *Staph. aureus* isolates (no =11)		
	Sensitive	Intermediate	Resist
PenicillinG	3(27.27 %)	0(0%)	8(72.73%)
Amoxicillin	9(81.82%)	0(0%)	2(18.18%)
Amikacin	10(90.91%)	0(0%)	1(9.1%)
Vancomycin	11(100%)	0(0%)	0(0%)
Sulfamethoxazole- Trimethoprim	9(81.82%)	1(9.1%)	1(9.1%)
Clindamycin	7(63.64%)	3(27.27%)	1(9.1%)
Tetracycline	3(27.27%)	0(0%)	8(72.73%)
Erythromycin	7(63.64%)	2(18.18%)	2(18.18%)

Figures:

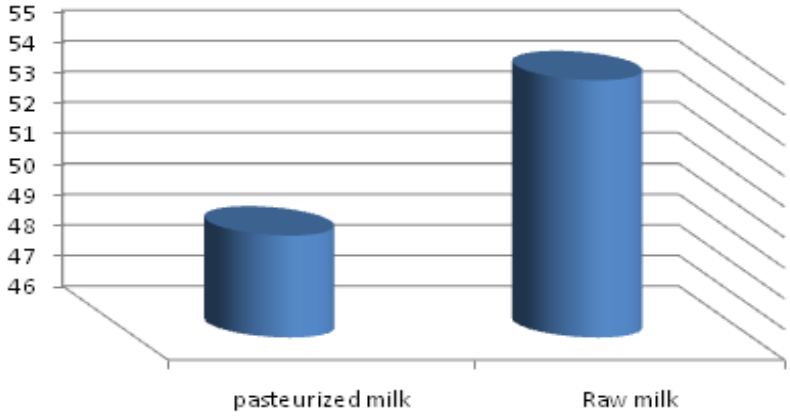

Fig. (1): Incidence of *Staphylococcus* spp. in raw and local pasteurized milk samples on mannitol salt agar

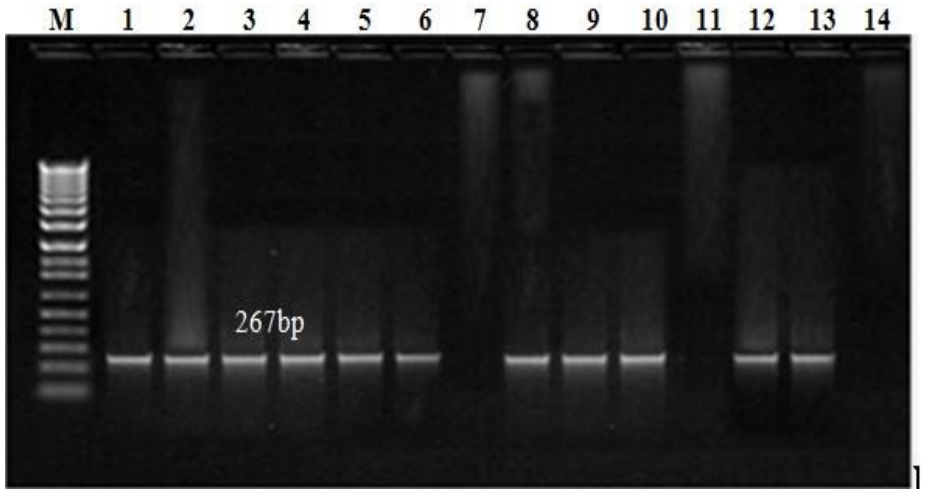

Fig. (2): Amplified profile of *Staph. aureus* DNA positive for *nuc* gene at 267bp. M: Gel Pilot 100 bp ladder (QIAGEN, no. 239035), Lane1: positive control, Lane: 2,3,4,5,6,8,9,10,12,13positive isolates, Lane 14: negative control.

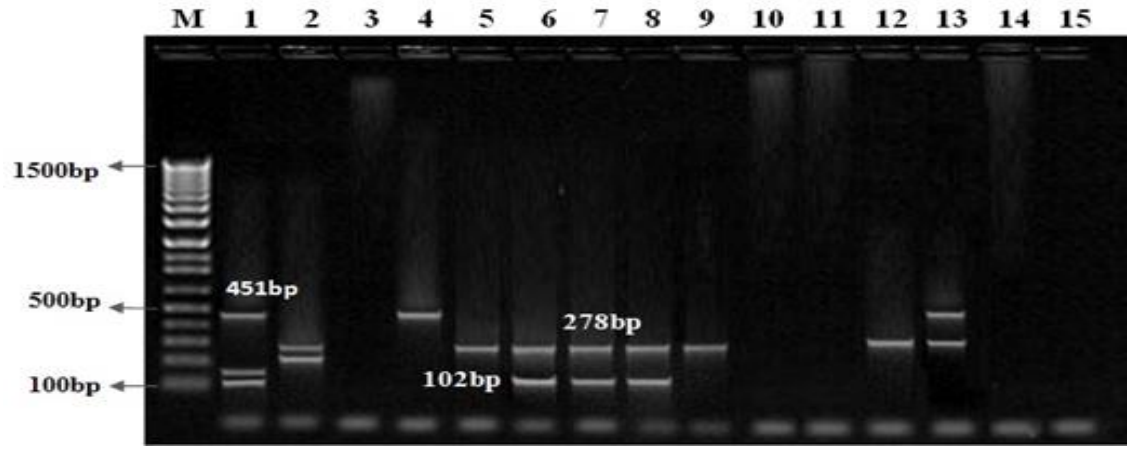

Fig. (3): Agrose gel electrophoresis of multiplex PCR products amplified from enterotoxigenic genes extracted from *Staph. aureus* isolated from raw milk. Lane1: positive control for *sea* (102 bp), *seb* (164 bp) &*sec* (451 bp), Lane 2:positive control for *see* (209 bp) & *sed* (278 bp) genes. Lane 4 positive isolate for *sec* gene, lane 5,9,12 positive isolates for *sed* gene, lanes 6,7,8 positive isolate for *sea* and *sed* genes, lane 13 positive isolate for *sed* and *sec* genes and lane 15 negative control.

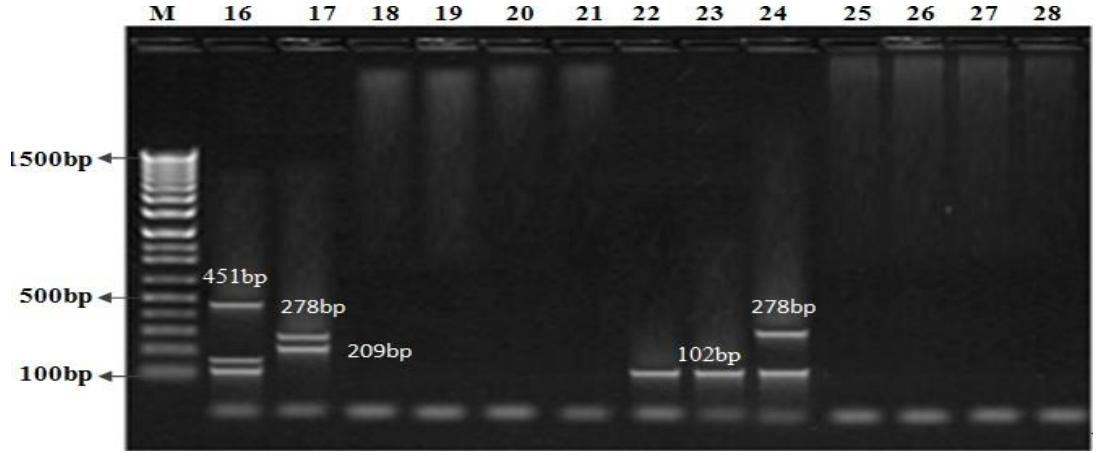

Fig. (4): Agrose gel electrophoresis of multiplex PCR products amplified from enterotoxigenic genes extracted from *Staph aureus* isolated from local pasteurized milk. Lane1: positive control for *sea* (102 bp), *seb* (164 bp) &*sec* (451 bp), lane 2: positive control for *see* (209 bp) & *Sed* (278 bp) genes. Lanes 22, 23 positive isolate for *sea*, lane24 positive isolate for *sea* and *sed* genes and lane 28 negative control.

DISCUSSION

Staph. aureus is the principle cause of food poisoning and clinical infections in humans and animals (Chiang *et al.*, 2006). Enterotoxigenic *Staph. aureus* in milk possess a public health problem to consumers. So the identification of such strain should be used as a part of a risk in analysis of milk (Zouharova and Rysanek, 2008).

The conventional identification of *Staphylococcus* spp. based on many diagnostic methods such as growing on selective media containing 8-10% NaCl, production of coagulase, thermostable nucleases and acetoin production (Kateete *et al*., 2010) also Quinn *et al*. (2002) reported that mannitol salt agar is one from the important media used specifically in food microbiology for identification of *Staphylococcus* spp.

Staphylococcus isolates were recovered from 54.40% of raw milk samples on mannitol salt agar (Fig.1). Nearly similar incidences of *Staphylococcus* spp. were showed by Ghaleb *et al.* (2005) 57.5% and Uddin *et al.* (2011) 50%. A higher results were recorded by Daka *et al*. (2012) and Duguma (2018) who found that the incidences of *Staphylococci* were100%, and 76.2% in raw cow milk samples, respectively. On opposing with, Donkor *et al.* (2007) and Tessema and Tasegaye (2017) who recorded that 14.6% and 28.2% of raw milk samples were contaminated by *Staphylococcus* spp.

On the other hand 49.33% (37/75) of local pasteurized milk samples were contaminated by *Staphylococcus* spp. (Fig.1). Lower percentage was conceded by EI-Jakee *et al*. (2013) who detected *Staphylococci* species with percentage of 16% in the pasteurized milk samples. pasteurization always done at 60–65ºC for 30 minutes and this can't kill *Staph.*

aureus but reduce the level of contamination in food products, especially milk and milk products (Yaniarti *et al.,* 2017), beside different sanitation levels during the packaging of the products (Soomro, 2003).

The variety in percentage of *Staphylococcus* spp. in milk may be due to different reasons include level and sources of contamination (human and animal sources), geographical regions, samples numbers and method of isolation (Fagundes and Oliveira, 2004).

The biochemical profile of our results showed that 55.88% of raw milk isolates were coagulase positive. Our result in accordance with Wani and Bhatt (2003), Abd EL-Tawab *et al*. (2015) and Asiimwe *et al.* (2017) who identified coagulase postive *Staphylococci* spp. from milk samples with percentage 51.1 %, 53% and 53.57%. While Kumar *et al.* (2011), Jahan *et al.* (2015) and Duguma (2018) recorded a higher percentages of coagulase positive isolates as 89.7%, 100% and 66.67% respectively. Lower results were recorded by Ghosh *et al.* (2003) who found that 35.4% of isolates were coagulase positive *Staphylococci* spp., respectively.

Additionally, 48.6% of *Staphylococcus* spp. recovered from the local pasteurized milk samples were coagulase positive. Oliveira *et al.* (2011) attained a lower percentage of coagulase positive isolates (30%).

Coagulase positive *staphylococci* are frequently involved in suppurative infections (Ryan and Ray, 2004), so the presence of these microorganisms in milk occurs especially with neglected sanitary precautions during milking.

Coagulase test was considered a primary test in identification of *Staphylococcal spp.* but diagnosis of these species needs many biochemical tests to guarantee a consistent results. (Quinn *et al.,* 2002) also coagulase positive *Staphylococci* have the same phenotypic characters so coagulase test not considered a single species-specific biochemical test (Sasaki *et al*., 2010).

In the current study, most of coagulase positive *Staphylococcal* isolates of raw and local pasteurized milk declared a thermonuclease activity. This result was supported by many authors who found a correlation between a results of these tests (Ratner and Stratton ,1985; Kaplan, 2003 and Suarez *et al*., 2008). Thermo nuclease test excluded 8 and 5 of coagulase positive isolates from raw and local pasteurized milk isolates, respectively, this may be due to false-positive results of coagulase test because there are other microbial extracellular products are produced during the production of coagulase enzyme which leading to unreliable results (Buchanan and Gibbons,1974). On other hand, Bello and Qahtani (2006) noticed that the performance of coagulase test was differed according to settings for test and experience of laboratory technicians.

Coagulase and thermonuclease tests are the criteria used by many laboratories for the identification of *Staph. aureus* (Kaplan 2003), but there were other coagulase and thermonuclease positive *Staphylococci* like (*Staph. hyicus* and *Staph. Intermedius*) (Downes and Ito, 2001 and Viçosa *et al.,* 2010), these strains also caused intramammary infections in dairy cows and reached to milk (Roberson *et al*., 1996).

Voges-Proskauer is an important test in determination of *Staph aureus* from other coagulase and thermonuclease positive isolates (Quinn *et al.,* 2002; Arora, 2003 and Vos *et al.,* 2009). These findings were supported our following results, the positivity of Voges-Proskauer were recorded in 18 isolates from raw milk samples and 8 isolates from local pasteurized milk samples (Table2). Higher results were illustrated by Rusenova and Rusenov (2017) who found that 73% of coagulase positive *Staphylococcal* strains isolated from different animals were Voges-Proskauer positive. This variation may back to level of contamination, number of samples and method of isolation (Fagundes and Oliveira, 2004).

Most of traditional methods for the identification of *Staph. aureus* not reached to accurate identification for important veterinary pathogens. PCR assay can be used as a rapid and sensitive diagnostic method for diagnosis of *Staph aureus* in raw milk samples and it can be used in conniving accurate pasteurization methods of milk as a main food source (Brakstad *et al.*,1992). The PCR amplification of the *nuc* gene has a potential for the rapid and accurate diagnosis of *Staph aureus* infections (Kilic *et al.,*2010).

In our study, we used *nuc* gene primers in confirmation the presence of *Staph. aureus* DNA. This gene is a specific genetic marker for detection and confirmation of *Staph. aureus* (Hedge *et al*., 2013 and Hu *et al.,* 2013). Furthermore, some previous studies suggested that there was a relationship between enterotoxin production and presence of *nuc* gene which considered as an indicator of food contamination with enterotoxigenic *Staph. aureus* (Tamarapu *et al*., 2001 and Cremonesi *et al.,* 2005).

The data shown in table (2) and fig (2) cleared that PCR confirmed the presence of *staph aureus* DNA in 15 isolates and denied its existence in 3 isolates out of 18 isolates of raw milk also one isolate only of local pasteurized milk samples not confirmed as *staph aureus* by using specific primer (*nuc* gene), these results in accordance with Karahan and Cetinkaya (2007) in addition Bennett and Lancette (1998) reported that most international standards specially FDA found that PCR is the most essentially

equivalent method in detection of *Staph aureus* also Speers *et al.* (1998) found that the sensitivity of biochemical tests was low in compared to PCR, furthermore genetic method not necessarily correspond to the same results of conservative phenotypic tests (Bosshard *et al.*, 2004).

Staph aureus microorganisms are able to produce enterotoxins which pose a risk factor on public health (Wu *et al.*, 2016) and most of the food industry contaminated by *Staph aureus* containing SEs genes, especially moist foods containing starch and protein, such as meat, poultry products and milk (Tamarapu *et al*., 2001).

The results postulated in Table (3) and Fig. (3) showed that SEs genes were detected in 53.33% of *Staph aureus* isolates from raw milk samples. This result is in agreement with this reported by Jorgensen *et al.* (2005), Zouharova and Rysanek (2008) and Murphy *et al.* (2010). Lower recovery of enterotoxigenic *Staph aureus* was reported by EI-Jakee *et al*. (2013) and Mansour *et al.* (2017) 35.7% and 26.1% respectively. While Rahimi and Alian (2013) recorded a higher percentage of enterotoxigenic *Staph aureus* isolates (75%).

On the other hand, 42.86% of *Staph aureus* isolates from local pasteurized milk samples were enterotoxigenic isolates (Table3 & Fig. 4). Higher percentage was recorded by Breurec *et al.* (2010) 90% while EI-Jakee *et al*. (2013) found that no isolate showed a positive result for enterotoxin genes.

In our study, it was noticed that *sea* and *sed* genes were the most detectable genes in most *Staph aureus* isolates, *sec* gene could be detected only in two isolate of raw milk while *seb* or *see* genes were not detected in any isolate. These results supported Rall *et al*. (2008) and Carfora *et al*. (2015).

Pasteurized milk may also be disposed to toxin production because in various situations, the shop owners turn off the chillers at night to save electricity, leaving the product exposed to different degree of temperature as cited by Chapaval *et al.* (2010) also the highest risk of SEs production is associated with storage the pasteurized milk at room temperature, incubation (Janštova *et al.*, 2012).

The classical *Sea* and *Sed* genes were a common concern in cases of *Staphylococcal* food poisoning (Tamarapu *et al.*, 2001). Normanno *et al.* (2005) found that the *sea* gene is the most frequent SEs genes observed among enterotoxigenic strains of *Staph aureus* and the more common SEs in milk (Chiang *et al*., 2006), the presence of *Staph aureus* strains have *sea* gene isolated from milk may be due to the bad handling during milking and packaging the products, because *sea* gene is more common in human isolates than animal origin (El-Baradie, 1993).

In this study, the absence of *Staph aureus* isolates harbored *seb* or *see* genes were recorded in previous studies (Marija *et al.*, 2016; Karahan *et al.*, 2009; Neder *et al.*, 2011; EI-Jakee *et al*., 2013 and Rahimi and Alian, 2013).

The development of Antibiotic resistance among the bacteria poses a problem of concern. Many original studies have exposed an increasing concern towards the existence of several antibiotic resistant of *Staph aureus* isolates in worldwide. So, our study was concerned by determining the susceptibilities of enterotoxigenic *Staph aureus* isolates against different families of antimicrobials. Table (4) demonstrated that a high resistance of these isolates were recorded against penicillin (72.73%) and tetracycline (72.73%) followed by clindamycin (27.27%) and erythromycin (18.18%), while all isolates were fully susceptible to vancomycin. Our results were consolidated by Gündgan *et al*. (2006) Pereira *et al*. (2009) and Waters *et al.* (2011), who noticed that multidrug resistance were shown against tetracycline, Penicillin and ampicillin. Abera *et al*. (2010) and Thaker *et al*. (2013) reported a high resistance to Penicillin-G 94.4% and 100% respectively, while Asiimwe *et al.* (2017) reported a high resistance to tetracycline (73.2%).

The high resistance against penicillin was explained by Lee (2003) who returned the causes to the presence of resistance genes that coded for an alteration of penicillin-binding protein 2a which reduced the affinity for β-lactam antibiotics, another causes were recorded by Yamamoto *et al.* (2013) such as *Staph aureus* harbor a several antibiotic resistant plasmids that may lead to the phenotypes resistance.

Additionally, the mistreatment of infection by different antibiotics in dairy farms is known to be one of the major factors responsible for the multiple drug resistant of bacteria worldwide especially ampicillin and tetracycline were the mostly used on dairy cattle (Chee-Sanford *et al.*, 2009).

CONCLUSION

Phenotypic methods not reach to a high grade in specificity of *Staph aureus* identification while, PCR play a confirmative role in detection of *Staphylococcus aureus* and focus a light on presence of enterotoxin types of these isolates in raw and local pasteurized milk which consider a public health problem. The existence of *Staph aureus* resistance to some antibiotic specially penicillin in milk reflect our need to management practices and appropriate sanitary procedures to be during milking operations also we suggest the occurrence of *Staph aureus* in local pasteurized milk may not only back to expire

date but attributed to improper handling and storage temperature.

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الكشف عن جينات الانتيروتوكسين في المكورالعنقودي الذهبي المعزول من البان الأبقار

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يعتبرالمكورالعنقودي الذهبي من اهم مصادرالتسمم الغذائي والعدوي في العوائل المختلفة ولذلك هدف هذا العمل للكشف عن جينات الانتيروتوكسين والحساسيه لمختلف انواع المضادات الحيويه في ميكروبات المكورالعنقودي التي تم عزلها من اللبن الخام والمبستر ولتحقيق هذا تم تجميع ١٢٥عينه من اللبن الخام و٧٥ عينه من اللبن المبسترتم تجميعها من المحلات والمزارع بمحافظة سوهاج، هذه العينات خضعت للفحص البكتريولوجي وقد اظهرت النتائج أن ٦٨ من ١٢٥ من عينات اللبن الخام و٣٧ من٧٥ عينة من اللبن المبستر اثبتت ايجابيتها لانواع الميكروبات العنقودية على المانيتول اجار كما افترضت الاختبارات البيوكيميائية ايجابية ١٨ عزله من اللبن الخام و٨ عزلات من اللبن المبستر للمكورالعنقودي الذهبي ولكن اختبار البلمره المتسلسل باستخدام بادئ معين (*nuc* gene) اثبت وجود الماده الوراثيه الخاصه بالمكورالعنقودي في ١٥ عزلة من ١٨ معزولة من اللبن الخام التي افترضت ايجابيتها بالاختبارات البيوكيميائيه بينما ٧ عزلات من ٨ عزلات من اللبن المبستر اظهروا نتائج ايجابية عند (bp ٢٦٧). وقد تم الكشف عن جينات الأنتيروتوكسين في٥٣,٣٣ و ٤٢,٨٦ من عزلات المكورالعنقودي الذهبي المعزوله من اللبن الخام والمبسترعلى الترتيب. جينات الانتيروتوكسين من النوع (a,d) من اكثرالجينات التي تم الكشف عنهافي معزولات المكورالعنقودي والجين (c) تم الكشف عنه في معزولات اللبن الخام فقط بينما جينات الانتيروتوكسين (e,b) لم يتم الكشف عنه في اي معزوله، كما اظهر اختبار الحساسية الذي اجري علي عزلات المكور العنقودي الذهبي المحتويه علي جينات الانتيروتوكسين مدى مقاومة هذة العزلات لانواع المضادات الحيوية المختلفة مثل البنسلين والتتراسيكلين بينما اظهرت هذه المعزولات حساسيتها للفانكومايسين.