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Molecular Characterization of *Staphylococci* Isolated from Cattle with Mastitis

Mohamed M. Ali[1], Salwa M.Helmy[2], Ibrahim E. El Desouky[2]* and **Hanaa A. Asfour[3]**

[1]*Microbiology Department, Animal Health Research Center, Kafr Elsheikh, Egypt*
[2]*Bacteriology, Mycology and Immunology Department, Faculty of Veterinary Medicine, Kafrelsheikh University 33516, Egypt*
[3]*Department of Mastitis and Neonatal Diseases, Animal Reproduction Research Institute (ARRI), Giza, Egypt*
*Corresponding author's Email: ibrahim543@yahoo.com

ABSTRACT

This study was carried out in order to investigate the occurrence of some virulence genes of *Staphylococci* isolated from cattle with mastitis. A total number of 133 milk samples (45 from clinical mastitis and 88 from subclinical mastitis) were collected from dairy cattle in Kafr El-Sheikh and EL Gharbia Governorates, Egypt. The samples were examined for the presence of *Staphylococci* by classical bacteriological methods and were further characterized geno-typically. A total of 41 *Staphylococcus* isolates were recovered from cattle with mastitis with an incidence of 30.8%. Among the isolates, 21(15.8%) of *S. aureus* [6 from clinical mastitis (13.3%) and 15 from subclinical mastitis (17%)] and 20 (15%) isolates of CNS [8 from clinical mastitis (17.7%) and 12 from subclinical mastitis (13.6%)] were identified phenotypically. All isolates were screened for the detection of binding protein A (*spa*-X), haemolysine type A (*hla)*, Haemolysine type B (*hlb)*, and toxic shock syndrome (*tsst*-1*)* by PCR. The obtained results revealed that the *spa* Xgene was detected in all *Staphylococcus* isolates recovered from subclinical mastitis while in clinical mastitis was detected with an incidence of 42.9%. Haemolysine type A was detected in clinical and subclinical mastitis with an incidence of 71.4% and 70% respectively, while haemolysine type B was detected in clinical and subclinical mastitis with an incidence of 28.5% and 40% respectively. Toxic shock syndrome was not detected in any of the isolates. The data in the study provided an overview on the distribution of some virulence genes related to *Staphylococci* isolated from cattle with mastitis in Egypt.

Key words: Cattle, Mastitis, *Staphylococci*, Virulence gene, PCR

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INTRODUCTION

A wide variety of organisms have been identified as potential mastitis pathogens including *E. coli*, *S. uberis* and *S. aureus* (Radostitis, 2008; Erskine et al., 2002 and Gitau et al., 2003). Staphylococcal mastitis is a major concern in dairy farming and a serious source of subclinical and clinical Intra-Mammary Infections (IMI) in dairy cows leading to severe economic losses to the dairy industry worldwide (Momtaz et al., 2010; Atasever, 2012; Memon et al., 2013). Several epidemiological studies have suggested that *S. aureus* is the most prevalent in intramammary infections being related to more than 80% of the cases (Song et al., 2016). Recently published work has shown that 3 % of all animals are infected with *S. aureus* (Schukken et al., 2009), however, *S. aureus* represents 10 to 12 % of all clinical mastitis infections (Tenhagen et al., 2009).

Staphylococci have a capacity to produce a large number of putative virulence factors including surface-associated adhesins, a capsular polysaccharide, exo-enzymes, and exo-toxins. Some of these factors may be of more importance than others in different diseases or at different stages of the pathogenesis of particular infections, as not all factors are

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produced by each strain (Fitzgerald et al., 2000; Kalorey et al., 2007). One of the important virulence factors is staphylococcal exo-protein A (*spa*) which is a bacterial cell wall product that binds to the FC region of immunoglobulin G and impairs opsonisation by serum complement and phagocytosis by polymorphnuclear leukocytes (Alonso and Daggett, 2000; Eman et al., 2015). Therefore, low expression of protein A on the cell on surface of *S. aureus* resulted in greater number of free receptor sites for complement C3b and in increase in phagocytosis(Gao and Stewart, 2004).Staphylococcal hemolysins are identified as important virulence factors that contribute to bacterial invasion and escape from the host immune response (Rodrigues and da Silva, 2005). Alpha-hemolysin is the most studied and characterized *S. aureus* cytotoxin and is considered as a main pathogenic factor because of its hemolytic, dermonecrotic, and neurotoxic effects (Dinges et al., 2000). Additionally, beta hemolysin is a sphingomyelinase that is highly active against bovine erythrocytes (Larsen et al., 2002). The staphylococcal enterotoxins (SEs) are recognized agents of the staphylococcal food poisoning syndrome and may be involved in other types of infections with sequelae of shock in humans and animals (Bergdoll, 1981; Marrack and Kappler, 1990). A distantly related protein, toxic shock syndrome toxin-1 (*tsst*-1), also produced by *S. aureus*, was the first toxin shown to be involved in toxic shock syndrome, in both menstrual and non-menstrual cases (Bergdoll et al., 1981 and Schlievert et al., 1981). However, no immunological identity and little amino acid homology between *tsst*-1 and the staphylococcal enterotoxins exist (Blomster-Hautamaa et al., 1986).

The importance to evaluate *Staphylococcus* pathogenic activity assessing the combination of virulence genes has been emphasized both in human and in veterinary medicine (Zecconi et al., 2006; Piccinini et al., 2009). The genotype of *Staphylococcus* affects its prevalence and the number of infected quarters within a herd (Fournier et al., 2008). As information about the genetic variability of different *Staphylococcus* populations would help in the design of efficient therapeutic approaches and improvement of control measure. Few reports exist on the prevalence of *Staphylococci* among cattle with mastitis in Egypt. Consequently, the purpose of the present study was to investigate the prevalence and molecular characterization of *Staphylococci* isolated from dairy cattle with clinical and subclinical mastitis in Kafr El-Sheikh and EL Gharbia governorates, Egypt.

MATERIALS AND METHODS

Ethics committee approval

Ethical approval handlings of animals were according to the guidelines of animal ethics committee, faculty of veterinary medicine, Kafrelsheikh University, Egypt.

Sampling and bacterial isolation

A Total of 133 milk samples were collected aseptically from lactating cow (45 from cows with clinical mastitis and 88 from cows with sub clinical mastitis) in Kafr El-Sheikh and EL Gharbia Governorates, Egypt (Table 1). The samples were collected into sterile plastic tubes and submitted to the laboratory on ice packs as soon as possible for further bacteriological examinations. Samples were processed within 24-48 hours after reception. For subclinical mastitis, apparently normal milk samples were tested by using the California Mastitis Test (CMT), and were graded as negative, trace, weak, distinct, or strongly positive (Persson et al., 2011). Isolation of *Staphylococcus* was attempted from the CMT positive milk samples.

Milk samples were centrifuged; sediment was diluted with equal amount of sterile distilled water and streaked on Mannitol Salt Agar (Oxoid) at 37˚C for 48 h. Suspected colonies were selected and picked onto nutrient agar slants, all slants were incubated aerobically at 37˚C / 24 h for further identification. The isolates were identified as *S. aureus* based on their cultural, morphological and biochemical characteristics (tube coagulase, urease, sugar fermentation, catalase tests). Haemolytic activity was evaluated by plating suspected staphylococcal strains on plates of nutrient agar supplemented with 10% sterile sheep blood according to Quinn et al. (1994). Types of haemolysins were identified according to the lysis zone of each *Staphylococcus* isolates on the blood agar plate after 24 h incubation at 37˚C aerobically.

Table 1. Number and type of samples collected from cattle with mastitis

Farms	Clinical Samples	Subclinical samples	Total samples
Kafr El-Sheikh farms farms	30	61	91
El Gharbia farms	15	27	42
Total	45	88	133

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Molecular detection of *Staphylococci* virulence genes using PCR

DNA extraction. DNA extraction from samples was performed by 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˚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.

Molecular identification was conducted for the detection of the *S. aureus*16S rRNA gene by using species-specific primers described by Wada et al. (2010) and Pradhan et al. (2011). All *Staphylococcus* isolates were analyzed for four virulent genes including, *spa*X (X-region of protein A), *hla* gene (encoding α haemolysin), *hlb* gene (gene encoding β haemolysin) and *tsst*-1 gene (encoding toxic shock syndrome toxin). Several PCR protocols were used to detect the target genes of *Staphylococcus* isolates. PCR amplification was performed with PTC-100 programmable thermal cycler (Peltier Effect cycling, MJ, Research, INC, UK). DNA amplification was performed in a final reaction volume of 25 µl consisting of: 12.5µl of Emerald Amp GT PCR master mix (2X premix), 1 µl of 20 pmol of each primer, 6 µl of the DNA template and water, nuclease-free up to 25µl. In the present study, the primer pairs used in PCR protocols were selected from published papers based on specificity, compatibility and ability to target the genes of interest. The nucleotide sequences and anticipated molecular sizes of PCR amplified fragments for these gene-specific oligonucleotide primer sets are outlined in table 2. The cycling condition of each gene has been listed in table 3. After PCR reactions, the amplified products were separated by agarose gel electrophoresis (1.5% agarose gel containing 0.5 mg/ml ethidium bromide in 0.5 X Tris EDTA electrophoresis buffer) at 5V/ cm for 1.5 hand visualized under UV trans-luminator. A 100-bp DNA ladder (Fermentas, USA) was used as molecular weight marker.

Table 2. Target genes, primers sequences, and amplicon sizes of *Staphylococci* virulence determinants

Target genes	Primers sequences	Amplified segment (bp)	References
16S rRNA	TTCGTACCAGCCAGAGGTGGA	229	Wada et al. (2010)
	TCTTCAGCGCATCACCAATGCC		Pradhan et al. (2011)
spa (X region)	CAA GCA CCA AAA GAG GAA	226	Booth et al. (2001)
	CAC CAG GTT TAA CGA CAT		
hla	GGTTTA GCC TGG CCT TC	550	Akineden et al. (2001)
	CAT CAC GAA CTC GTT CG		Pradhan et al. (2011)
hlb	GCC AAA GCC GAA TCT AAG	850	Wada et al. (2010)
	CGC ATA TAC ATC CCA TGG C		Booth et al. (2001)
tsst-1	ATGGCAGCATCAGCTTGATA	350	Booth et al. (2001)
	TTTCCAATAACCACCCGTTT		
S. aureus nuc gene	GCGATTGATGGT GATACGGTT	267	Brakstad et al. (1992)
	AGCCAAGCCTTGACGAACTAA AGC		

Table 3. Cycling conditions of *Staphylococci* virulence determinants

Target gene	Primary denaturation	Amplification (35 cycles)			Final extension
		Secondary denaturation	Annealing	Extension	
16S rRNA	95˚C, 4 min.	95˚C, 45 sec.	50˚C, 60 sec.	72˚C, 30 sec.	72˚C, 10 min.
spa (X region)	94˚C, 4 min.	94˚C, 30 sec.	55˚C, 30 sec.	72˚C, 30 sec.	72˚C, 10 min.
hla	94˚C, 4 min.	94˚C, 45 sec.	50˚C, 45 sec.	72˚C, 60 sec.	72˚C, 10 min.
hlb	94˚C, 4 min.	94˚C, 45 sec.	57˚C, 60 sec.	72˚C, 80 sec.	72˚C, 10 min.
tsst-1	94˚C, 4 min.	94˚C, 2 min.	55 ˚C, 2 min	72˚C, 60 sec.	72˚C, 10 min.

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RESULTS

Bacteriological identification of *Staphylococcus* isolates

A total of 41 *Staphylococcus* isolates were recovered from the milk of 133 mastitic cattle in a prevalence rate of 30.8 %. Among the isolates, 21 were identified as *Staphylococcus aureus* based on cultural, morphological and biochemical characteristics in a prevalence rate of 15.8%, while the rest of the isolates (20 isolates) identified as Coagulase Negative *Staphylococci* (CNS) (15%). All the *S. aureus* isolates were positive for tube coagulase test; these strains were confirmed by PCR (Figure 1). Among the examined cattle, 6 *S. aureus* isolates were recovered from 45 cattles with clinical mastitis (13.3%) and 15 isolates were recovered from 88 cattles with subclinical mastitis (17 %). On the other hand, 8 and 12 isolates of CNS were isolated from cattle with clinical and subclinical mastitis in a prevalence rate of 17.7% and 13.6% respectively (Table 4).

Molecular detection of *Staphylococcus* virulence genes

Staphylococcus genus specific primers targeting 16S rRNA were employed for the specific confirmation of the *Staphylococcus* DNA. All the examined isolates yielded a specific single DNA band of 229 bp amplicon. Furthermore, a second confirmatory PCR for confirmation of atypical *S. aureus* was used (Figure 1). All *Staphylococcus* isolates were subjected to PCR for the detection of four virulent genes (*spa*X, *hla*, *hlb*and *tsst*-1). All of the isolates were found to be positive for one or more virulence-associated genes (Table 5). Among the examined 21 isolates of *S. aureus*, *spa*X gene was the predominant one, detected in 17 isolates with an incidence of 81 % (Figure 2).

*Spa*X gene was detected in all isolates of *S. aureus* recovered from subclinical mastitis (100%) while detected in 2 out of 6 isolates from clinical mastitis (33.3%). With regard to *hla,* it was detected from *S. aureus* isolated from clinical and subclinical mastitis with an incidence of 33.3% and 60% respectively (Figure 3). On the other hand, *hlb* gene was detected in *S. aureus* recovered from clinical mastitis and subclinical mastitis with an incidence of 16.6% and 46.6% respectively (Figure 4).

Regarding to incidence of virulent genes in CNS isolates, *spa* X gene was detected in all the isolates of *Staphylococcus* recovered from subclinical mastitis (100%) while in clinical mastitis, 4 out of 8isolates were positive (50%). Haemolysine type A was detected in all isolates of CNS from clinical mastitis (100%) while in subclinical mastitis, 5 isolates gave positive amplicons out of 12 (41.6%). With regard to *hlb* gene, it was detected in CNS isolates of clinical and subclinical mastitis with an incidence of 37.5% and 8.3%, respectively. All the isolates tested, failed to amplify *tsst*-1(Table 5).

Table 4. Incidence of *Staphylococci* in cattle with mastitis

Types of samples	No. of samples	*S. aureus*		CNS		Total isolates	
		No	%	No	%	No	%
Clinical mastitis	45	6	13.3	8	17.7	14	31.1
Subclinical mastitis	88	15	17	12	13.6	27	30.7
Total	133	21	15.8	20	15	41	30.8

Table 5. Distribution of virulence determinant genes in *Staphylococcus* isolates

Items	Clinical Mastitis						Subclinical mastitis					
	S. aureus (6*)		CNS (8)		Total (14)		*S. aureus* (15)		CNS (12)		Total (27)	
	No	%	No	%	No	%	No	%	No	%	No	%
***spa* (X region)**	2	33.3	4	50	6	42.9	15	100	12	100	27	100
hla	2	33.3	8	100	10	71.4	9	60	5	41.66	14	70
hlb	1	16.6	3	37.5	4	28.5	7	46.66	1	8.3	8	40
tsst-1	0	0	0	0	0	0	0	0	0	0	0	0

*Among the isolates, 21of *S. aureus* (15.8%) [6 from clinical mastitis (13.3%) and 15 from subclinical mastitis (17%)] and 20 isolates of CNS (15%) [8 from clinical mastitis (17.7%) and 12 from subclinical mastitis (13.6%)] were identified phenotypically.

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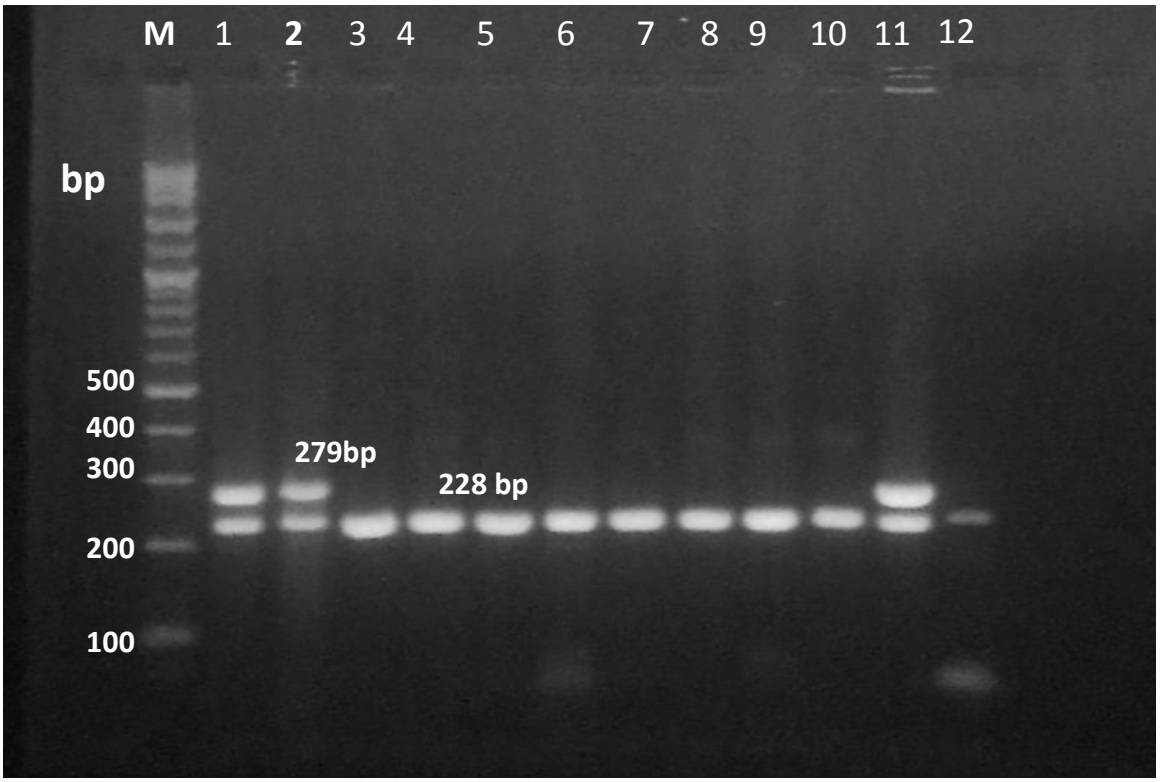

Figure 1. Agarose gel electrophoresis of duplex PCR amplification of 16S rRNA gene of *Staphylococci* (228 bp) and *S. aureus* specific *nuc* gene (267 bp). Lane M: 100 bp DNA ladder, Lane1, 2and 11: positive isolates for S. aureus. Lanes: 1-11 are positive isolates for genus Staphylococci (228 bp).

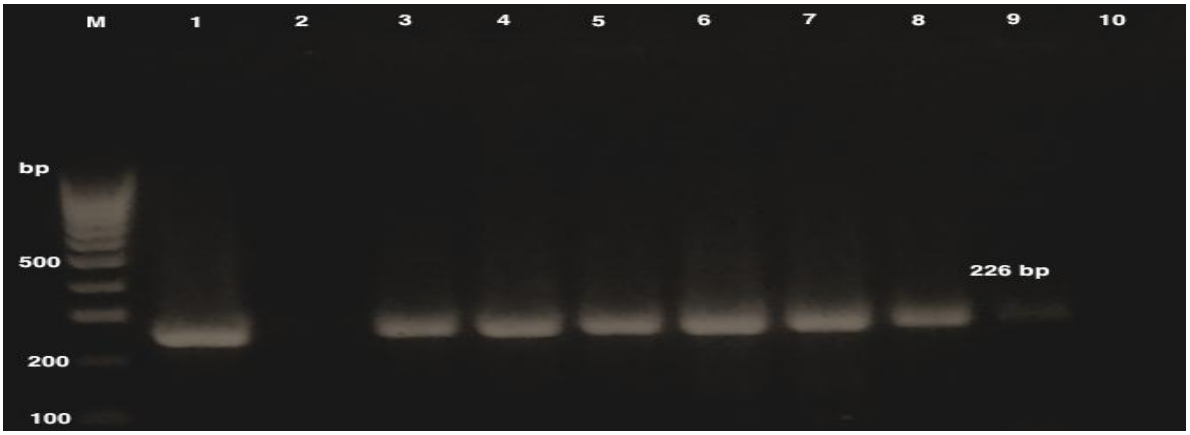

Figure 2. Agarose (1.5%) gel electrophoresis of *spa* X gene of *Staphylococcus* PCR products (226 bp). Lane M: 100 bp DNA markers, Lane 1, 3-8: positive samples, lanes 2 and 10: negative samples.

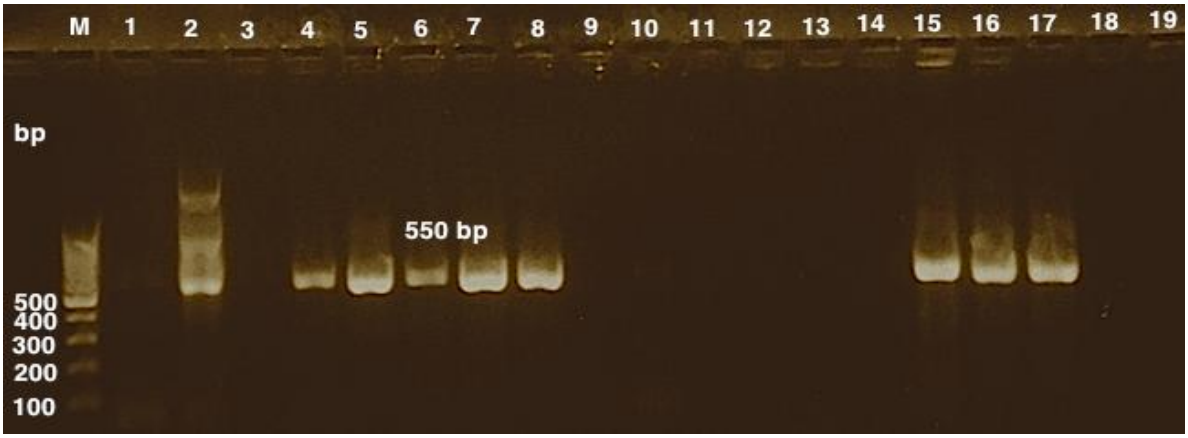

Figure 3. Agarose (1.5%) gel electrophoresis of *hla* gene of *Staphylococcus* PCR products (550 bp). Lane M: 100 bp DNA marker, Lanes: 1, 3, 9-14 and 18-19: negative isolates, lanes: 2, 4-8 and 15-17: positive isolates.

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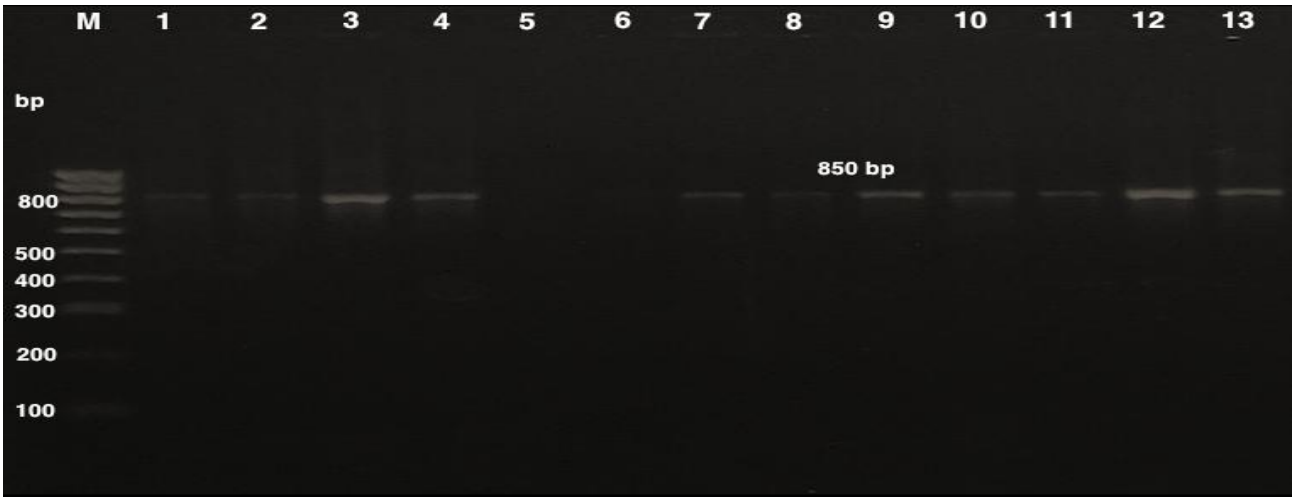

Figure 4. Agarose (1.5%) gel electrophoresis of *hlb* gene of *Staphylococcus* PCR products (850 bp). Lane M: 100 bp DNA marker, lanes 1-4 and 7-13: Positive isolates, lanes 5 and 6: negative isolates.

DISCUSSION

Cattle mastitis remains a serious and common disease in animals with significant economic losses in dairy industry worldwide (Momtaz et al., 2010; Atasever, 2012); therefore, knowing that mastitis causing bacteria and their virulent determinants using molecular methods is crucial to control the IMI (Ayman et al., 2015). A wide variety of organisms including *Staphylocooci*, *Streptococci*, *E.coli*, *Enterobacter spp.*, *Klebsiella spp.*, *Mycoplasma spp.* and *Corynebacterium* spp. are responsible for mastitis in animals. Among several bacterial pathogens that can cause mastitis, *Staphylococcus* species being the most lethal agent because it causes chronic and deep infection in the mammary glands that is extremely difficult to be cured (Momtaz et al., 2010).

The isolation of *Staphylococci* from milk alone is unequivocal in determining its role in the pathogenesis, therefore, *Staphylococci* virulent gene surveillance could be helpful in detecting genetic diversity among these major mastitis causing pathogens to develop effective control strategies against mastitis caused the pathogen (Khan et al., 2013).

In Egypt it was confirmed that *S.aureus* is considered as the predominant factor among mastitis causing pathogens followed by *S. agalactiae* (Elhaig and Selim, 2015) and *E.coli* (Hamed and Ziatoun, 2014). In the present study, out of 133 milk samples collected from cattle with mastitis, 41 *Staphylococcus* isolates were identified in a prevalence rate of 30.8%. This result was similar to the finding obtained by Barkema et al. (2009), however, a higher incidence rate was also recorded by Zecconi and Hahn (2000). Among the isolates, 21(51.2%) identified as *S. aureus* and 20 (48.78%) isolates as CNS based on biochemical tests and molecular identification.

In the current study, it was noticed that 15.7% (21/133) of *S. aureus* isolates were isolated and identified from cattle with mastitis. Similar results were reported by Botrel (2010), Nibret et al. (2011), Persson et al. (2011) and Hamid et al. (2017) who isolated *S. aureus* with an incidence of 15.8%, 16.5%, 19% and 22.5% respectively. However, higher detection rate was reported by other authors (Ahmed and Mohamed, 2009; Ashraf et al., 2016) who isolated *S. aureus* with an incidence of 77.1% and 52.5% respectively. Different PCR-based systems for the identification of *S. aureus* isolates from various origins have been used by numerous authors (Akineden et al., 2001; Nashev et al., 2004; Momtaz et al., 2010). As was found by Brakstad et al. (1992), the amplification of the gene encoding an *S. aureus*-specific part of the 16S rRNA revealed an amplicon with a size of 1,250 bp for all *S. aureus* isolates investigated.

The subclinical mastitis has special importance as it goes unnoticed and affects in a great extent the production's animal (Bhati et al., 2016). In subclinical mastitis, 15 isolates of *S. aureus* out of 88 samples were identified in a prevalence rate of 17%. Similar finding was reported by (Ak, 2000; Busato et al., 2000) who isolated *S.aureus* with a percentage of 13.3% and 16% respectively. In contrast to our data, several studies have demonstrated higher incidence of *S. aureus* in subclinical mastitis (Mokhbatly et al., 2001; Karimuribo et al., 2005; Khan and Muhammad, 2005; Ahmed and Mohamed, 2009; Alemu et al., 2014). In clinical mastitis, the incidence of *S. aureus* was 13.3% (6/45). This record agreed with the finding reported by Nevala et al. (2004), however, other studies have reported higher detection rates of *S. aureus* in clinical mastitis (Workineh et al., 2002; Elsayed et al., 2015). These variations are likely due to geographical area differences and time of sampling. In the last few years, the prevalence of CNS in mastitis was higher than those caused by *S. aureus*. In the present study, the prevalence of *S. aureus* and CNS in clinical mastitis was 13.3% and 17.7%

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respectively. Similar finding was also reported by Persson et al. (2011). In other studies, incidence of *S. aureus* was higher than that of CNS in clinical mastitis (Botrel et al., 2010; Rajeev et al., 2011; Eman et al., 2015).

Epidemiologic studies indicates that *S. aureus* strains agents of mastitis produce a group of virulence factors and it is believed that there is a relationship between the severity of mastitis and the virulence factors produced by *S. aureus* (Akineden et al., 2001). In the present study, molecular surveillance carried out in all isolates of *Staphylococci* to screen the presence of four putative virulence determinants encoding *spa* (the X-region of protein A) *hla* gene (encoding α haemolysin), *hlb* gene (gene encoding β haemolysin) and *tsst*-1 gene (encoding toxic shock syndrome toxin) by PCR. The distribution of virulent genes differed among the examined strains, some genes were present in all of the strains, but some genes were not found in any strain. The *spa*X gene typing in current study amplified (150-315bp) (Bhati et al., 2016). The genes *spa*X-region was detected in all isolates recovered from the samples of subclinical mastitis, it was consistent with the finding described by several authors (Coelho et al., 2011; Memon etal., 2013; Ashrafet al., 2016) that established the presence of *spa*X gene in nearly all of the isolates. Other reports in several countries including Italy (Dalla Pozza et al., 1999) India (Kumar et al., 2010) and Poland (Kahl et al., 2016), have previously identified this gene by 93-100% of *S. aureu s* isolated from subclinical form. The high incidence of *spa*X gene in *S. aureus* isolated from subclinical mastitis points to the potential role of this gene in this bacterium in a subclinical form, which unlike clinical mastitis, is milder and more difficult to detect.

Unlike the subclinical form, *spa*X was detected in *S. aureus* and CNS isolates in clinical mastitis with an incidence of 33.33% and 50% respectively. This is in contrast to the results described by (Stephan et al., 2001; Kalorey et al., 2007; Klein et al., 2012) who identified *spa*X in *Staphylococcus* isolates with an incidence of 76.5%, 70.3% and 85.9% respectively. In previous studies conducted by Salasia et al. (2011); Yang et al. (2012) and Wang et al. (2016), high frequency of *hla* was observed in *S. aureus* isolated from clinical mastitis (100%, 85%, and 94.3%, respectively). In our study, *hla* was observed in a percentage of 33.3%. However, *hla* was detected in all CNS isolates (100%). This finding might indicate the significant role of CNS isolates in the pathogenesis of bovine mastitis compared to *S. aureus* isolates. In subclinical mastitis, *hla* was detected in *S. aureus* and CNS with an incidence of (60%) and (41.66%) respectively, this result disagreed with Haveri et al. (2007); Salasia et al. (2011); Memon et al. (2013) they had recorded *hla*as 76%, 84% and 58% respectively. However; a higher frequency was recorded by Elsayed et al. (2015) and Ahmed et al. (2016). These different frequencies may be due to the different animal populations studied or the implemented methodologies, among other factors.

With regard to *hlb* in clinical mastitis, it was detected in *S. aureus* and CNS with an incidence of (16.66%) and (37.5%) respectively, these results correspond significantly with similar results obtained by Coelho et al. (2011). However, other investigators (Elsayed et al., 2015; Wang et al., 2016) have reported a relatively high incidence of this gene, while in subclinical mastitis, *hlb* was detected in *S. aureus* and CNS isolates with an incidence of (46.66%) and (8.3%) respectively, this percentage is higher than the one recorded by Coelho et al. (2011); Ahmed et al. (2016), however, in other studies, higher percentages of *hlb* in *S. aureus* isolates (Larsen et al., 2002, Salasia et al., 2011; Memon et al., 2013; Wang et al., 2016) were observed (97%, 84%, 71% and 79.1%, respectively). Toxic shock syndrome toxin (*tsst*-1) is one of the enterotoxogenic toxins responsible for food poisoning and is very important in the virulence of *Staphylococci*. In agreement with other studies (Nashev et al., 2004; Hassan et al., 2010; Gunaydin et al., 2011), we observed that the *tsst*-1 gene was not found in any isolate (0 %). However, these results are in disagreement with an earlier finding reported by Stephan et al. (2001) and Wang et al. (2016) that described higher *tsst*-1 gene positivity among *S. aureus* isolates (67.7% and 40% respectively).

CONCLUSION

Thus, in conclusion, the study provides a valuable insight into the virulence-associated genes of *Staphylococci*. The findings of this study indicated that all of the *Staphylococcus* isolates harbored one or more virulence-associated genes in dairy herds of cows suffering from clinical mastitis. The results also indicated that there is a direct relationship between the presence of *spa*X gene, it was the most frequent gene detected in examined isolates, and bovine mastitis especially subclinical type. Therefore, *spa* X gene could be considered as a good diagnostic method for typing of *Staphylococcus* isolates, which provided important results for the effective control of staphylococcal mastitis.

DECLARATIONS

Acknowledgments

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Author's contribution

Salwa M.Helmy and Ibrahim E. El Desouky planned and supervised the experiments and wrote the paper. Mohamed M. Ali and Hanaa A.Asfour performed the experiments and/or analyzed the data.

Competing of interest

The author declares that he has no conflict of interest with respect to the research, authorship, and/or publication of this article, the author declares that he has no competing interests.

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