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Using of (SPA) protein of *Staphylococcus aureus* as a genetic marker for characterization of methicillin resistant *S. aureus* (MRSA) recent isolates from bovine mastitis

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A total number of 140 milk samples were collected from four dairy farms in Cairo, Giza, Qalioubia, Sharkia governorates. The lactating cows were clinically examined for the manifestations of clinical signs. Milk samples from apparently healthy animals were subjected to CMT. Quantifying of SCC by using of Bentley soma count 150 (SCC). A threshold of 200,000 cells/ml is often used to distinguish subclinical mastitis from healthy udders. The prevalence of examined dairy cows at the cow level were 12.8% for clinical mastitis and 32.8%, 42.6% for subclinical mastitis based on (CMT) and (SCC) results respectively. Ten locally field isolates were detected from clinical and subclinical mastitis to be *S. aureus* (MRSA) in prevalence of 14.2%. The prevalence of MRSA isolates was 38.8% in clinical mastitis and was 5.8% in subclinical mastitis. The prevalence of MRSA from ten *S. aureus* isolates was 100% (10 isolates). Molecular identification of *spa* gene of *S. aureus* isolates revealed that products with approximate sizes of (280, 150 and 250 bp). The PCR amplification with *mecA* gene and *23SrRNA* gene specific primers resulted in products with approximate sizes of 310 bp and 1250 bp, respectively. *Spa, mecA* and *23SrRNA* were detected in all ten (100 %) *S. aureus* isolates. Phylogenetic tree, nucleotide sequence and deduced amino acids alignment for *S. aureus spa, mecA* and *23SrRNA* genes partial sequences showed clear clustering of the Egyptian isolated strain (*S. aureus* ASM strain) and different *S. aureus* strains uploaded from GenBank with (94% to 99.2%), (99.7% to 100%) and (99.9% to 100%), homology respectively. The X-region of the *spa* gene can be used as a molecular marker typing in studying the genetic diversity among the strains of *S. aureus* MRSA for epidemiological investigation of origin and source of infection and comparing the differences in virulent phenotypes amongst various strains.

Keywords: *mecA*, MRSA, *Staphylococcus aureus*, SCC, sequencing, *spa, 23SrRNA*.

INTRODUCTION

Staphylococcus aureus regarded as one of the most important and frequent etiological agents of contagious bovine mastitis global and has emerged as a large public health problem as it is frequently accountable for intramammary infection in bovine. *Staphylococcus aureus* considered as one of the most well-known etiologic agents that has an important role in clinical and subclinical mastitis, characterized by persistent and recurrent infections with low cure rate in response to antimicrobial remedy (Barkema et al., 2006, Gao

et al., 2017).

Staphylococcus aureus has many virulence factors that include the surface IgG binding protein A (*spa*) whose characteristic and function is to capture the Fc region of immunoglobulin of most mammalian species therefore prevent phagocytosis of the bacterial cells with the aid of the host immune system (Foster, 2005). The gene harboring and encoding protein A (*spa*) is consists of some functionally clear and distinct regions: Fc binding, X-region and a C-terminus regions, a sequence required for cell wall attachment. The X-region of the *spa* gene contains a different number of 24-bp repeats (Frenay et al., 1994; Kuzma et al., 2005) and so can be used as a molecular marker typing in studying the genetic diversity among the strains of *Staphylococcus aureus* for epidemiological investigation of origin and source of infection and comparing the differences in virulent phenotypes amongest various strains (Choudhary et al., 2018).

S. aureus strains showed a single or multiple antibiotic resistance and regarded as one of the most important threat to public health (Pereira et al., 2009). Methicillin- resistant *Staphylococcus aureus* (MRSA) considered as one of the most important pathogens worldwide. There are many studies reported and isolated MRSA from cattle mastitis (Fessler et al., 2010; Moon et al., 2007).

MRSA regarded as one of the most common known carrier of the *mecA* gene. The *mecA* gene plays a distinct and important role in characterization of MRSA. The *mecA* gene containing the penicillin binding protein 2a (*PBP2a*), which has a reduced affinity for βlactams (Haenni et al., 2010). So, the *mecA* gene is regarded as a very useful molecular typing marker and method for methicillin-resistance in all staphylococci. The *23SrRNA* intergenic spacer of the ribosomal RNA operon (rrn) has proven useful for identification of strains and species.

The main objective of this study is quantification of the prevalence of MRSA isolated from clinical and subclinical mastitic cattle dairies in Egypt by studying the polymorphism of *spa* gene (X-region) and using staphylococcal chromosome cassette mec *(mecA*) in typing methicillin- resistant *S. aureus* (MRSA) recent isolates from bovine mastitis and conducting phylogenetics of *spa, mecA* and *23SrRNA* genes sequence analysis of the recently isolated MRSA cattle clinical and subclinical mastitis.

MATERIALS AND METHODS

Milk samples:

No. of 140 compsitve milk samples were collected from 140 cows and were screened from four dairy farms in Cairo, Giza, Qalioubia, Sharkia. The lactating cows were clinically examined for the manifestations of general clinical signs associated with udder and presence of any gross lesions and physical appearance of milk was done according to Quinn et al., (2004). The milk samples were collected by a customary milk sampling methods (NMC, 1999). Milk samples from apparently healthy animals were subjected to California Mastitis test (CMT) (American Public Health Association (APHA) 1992; IDF 1975; NMC 1987; Quinn et al., 2004; Schalm et al., 1971). Quantifying and measuring Somatic Cell Count (SCC) by means of Bently soma count 150 (SCC) according to Zecconi et al., (2002). A threshold of 200,000 cells/ml has been proposed and is often used to distinguish subclinical mastitis (SCM) from healthy udders (Djabari et al., 2002; Dohoo and Leslie 1991; Pantoja et al., 2009; Schepers et al., 1997).

Isolation of Methicillin-resistant *Staphylococcus aureus* (MRSA):

Milk samples were pre-incubated for 18-24 hours at 37°C, then centrifuged at 3000 rpm for 20 minutes the cream and supernatant fluid were discarded. A loopful from sediment was streaked on the surface of nutrient agar, blood agar, Mannitol salt agar and Baird Parker agar. All plates were incubated aerobically at 37°C for 24-72 hr. The suspected colonies were picked up and subcultured for purification. Microscopical examination was done according to Quinn et al., (2002).

Biochemical identification:

Biochemical identification was carried out by using Analytical Profile Index-Staph (API-Staph Kit, bioMerieux, France) was used according to the manufacturer's instructions in accordance to methods described by Taponen et al.,(2006).

Phenotypic characterization of some virulence factors:

Coagulase activity of each isolate was tested by tube coagulase test using sterile human plasma (Qunin et al., 2002). Haemolysin production assay (Koneman et al., 1997). All gram positive purified collected samples identified by hemolysis test, hemolysis characters were

recorded on sheep blood agar 7% for alpha (α), beta (β) haemolysis and incubated at 37°C for 24h.

Molecular identification of *spa* (x-region),*mecA* and *23SrRNA* genes of *S. aureus*isolates:

DNA extraction

DNA extraction from samples was carried out by using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) according to the manufacturer's recommendations. Oligonucleotide primers used were supplied from Metabion (Germany) are listed in (Table 1). Specific primers as previously described (Bhati et al., 2016, McClure et al., 2006 and Momtaz et al., 2013) were uesd for typing of *spa* (x-region), *mecA* and *23SrRNA* genes respectively.

Table (1): Primers sequences, target genes, amplicon sizes and cycling conditions

Target gene	Primers sequences 5'--3'	Amplified segment (base pair)	Primary Denaturation	Amplification (35 cycles)			Final extension	Reference
				2ry denaturation	Annealing	Extension		
S. aureus 23S rRNA	AC GGAGTTACAAAGGACGAC AGCTCAGCCTTAACGAGTAC	1250	94°C 5 min.	94°C 30 sec.	55°C 40 sec.	72°C 1.2 min.	72°C 12 min.	Bhati et al., 2016
mecA	GTA GAA ATG ACT GAA CGT CCG ATA A CCA ATT CCA CAT TGT TTC GGT CTA A	310	94°C 5 min.	94°C 30 sec.	50°C 30 sec.	72°C 30 sec.	72°C 7 min.	McClure et al., 2006
Spa (x-region)	CAA GCA CCA AAA GAG GAA CAC CAG GTT TAA CGA CAT	Variable	94°C 5 min.	94°C 30 sec.	60°C 40 sec.	72°C 40 sec.	72°C 7 min.	Momtaz et al., 2013

PCR amplification

Primers were utilized in a 25-µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of every primer of 20 pmol concentration, 6 µl of DNA template and 4.5 µl of water. The reaction was performed in an Applied biosystem 2720 thermal cycler.

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the products was loaded in each gel slot. Gelpilot 100 bp DNA Ladder (Qiagen, Germany, GmbH) and generuler 100 bp ladder (Fermentas, Thermo, Germany) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and therfore the data was analyzed through computer software.

Phylogenetic and gene sequence analysis of *spa* (x-region), *mecA* and *23SrRNA* genes of *Staphylococcus aureus*

PCR products were purified using QIAquick PCR Product extraction kit. (Qiagen, Valencia). Big dye Terminator V3.1 cycle sequencing kit (Perkin- Elmer) was used for the sequence reaction so it had been purified using Centrisep spin column. DNA sequences were obtained by Applied Biosystems 3130 genetic analyzer (HITACHI, Japan), a BLAST® analysis (Basic Local Alignment Search Tool) (Altschul et al., 1990) was initially performed to as certain sequence identity to GenBank accessions. The phylogenetic tree was created by the Mega Align module of Laser gene DNA Star version 12.1 (Thompson et al., 1994), and Phylogenetic analyses was done using neighbor joining in MEGA6 (Tamura et al., 2013).

RESULTS

The prevalence of clinical and subclinical mastitis:

The prevalence of clinical and subclinical mastitis at the cow level in examined dairy cows were 12.8% (18/140) for clinical mastitis these results agree with Ahmed (2006), Petrosski et al., (2009) and Rafik et al., (2014) and disagree with Lakew et al., (2009). The prevalence of subclinical mastitis based on the results of CMT was 32.8% (40/122), These results agree with Lakew et al., (2009) and disagree with Karimuribo et al., (2008), Kivaria and Noordhuizen (2007). Somatic cell count was determined in all samples and bacterial

examination of the milk samples with a SCC exceeding 200,000 SCC/ ml was performed. Examination of the 122 compositve milk samples from the apperentelly healthy cows using SCC revealed that 70 cases at range < 200,000 SCC/ ml and 52 subclinically mastitc cows (42.6%) at range 200,000 SCC / ml – 500,000 SCC / ml. Table (2) these results nearly agree with Mdegela et al., (2009) (51.6%), Sayed et al., (2014) (56.3%) and disagree with El-haig and Selim (2015) (71.6 %), Karimuribo et al., (2008) (75.9%).

Isolation of MRSA *Staphylococcus aureus* field isolates.

Out of a total number of 140 milk samples were collected from cattle showing clinical and subclinical mastitis, ten locally field isolates were detected from clinical and subclinical mastitis and confirmed phenotypically by culturing, gram staining, biochemical and molecular identification to be *Staphylococcus aureus* in prevalence of 14.2% (10/70) these results agree with Botrel et al., (2010), Nibret et al., (2011), Persson et al., (2011) and disagree with Ahmed and Mohamed (2009), Ashraf et al., (2016), Hamid et al., (2017). In clinical mastitis the percentage of *S. aureus* isolates was 38.8% (7/18), these results agree with Elsayed et al., (2015), Workineh et al., (2002) and disagree with Nevala et al., (2004). In subclinical mastitis the percentage of *Staphylococcus aureus* isolates was 5.8% (3/52), these finding disagree with Ahmed and Mohamed (2009),Alemu et al., (2014), Khan and Muhammad (2005). On the other hand the prevalence of methicillin- resistant *Staphylococcus aureus* (MRSA) from ten *Staphylococcus aureus* isolates was 100% (all ten isolates) these findings agree with Ashraf et al., (2016 a) and disagree with Enany et al., (2013). Bacteriological examination revealed that the colonies morphology of *Staphylococcus aureus* were small to medium sized and golden yellow on BHI agar, while the colonies appeared surrounded by a double zone of beta haemolysis on blood agar plates, on mannitol salt agar, they were yellow color surrounded by yellow halo with yellow colored medium, the colonies were typically black smooth with entire margin on Baird Parker agar media. Phenotypic characterization of some virulence factors as haemolysin production assay and coagulase production revealed that all ten *S. aureus* strains were positive for coagulase test, these in agreement with Brown and Ngeno (2007).

Table (2): Results of CMT and SCC tests

Animals	CMT		SCC/ ml	
	NO.	%	NO.	Range
Healthy	82	67.2	70	< 200.000
Subclinical Mastitis	40	32.8	52	>200.000
Total	122	-	122	-

Staphylococcus aureus produce a large number of potential virulence factors which have an important role in the pathogenesis of mastitis Kalorey et al., (2007), these include, Coagulase which is considered the most important virulence factor that clot plasma and coats the bacterial cell, so prevent the phagocytosis Panizzi et al., (2004). All ten MRSA *S. aureus* strains produce *Beta* hemolysins. *S. aureus* produces a wide array of virulence elements including enzymes and toxins, which are responsible for the invasion of host cells such as hemolysins (Da Silva *et al.*, 2005). Hemolysins are considered the most important virulent elements in development of the disease (Ariyanti et al., 2011; Dinges et al., 2000). Beta-hemolysin (sphingomyelinase), it also has a high activity against erythrocytes of sheep and bovine (Larsen et al., 2002).

Molecular identification of *spa* (x-region),*mecA* and *23SrRNA* genes of *Staphylococcus aureus* isolates:

The PCR amplification with *mecA* gene specific primers was conducted with genomic DNA, which revealed in a product of approximate size 310 bp (Figure 1). Also *mecA* gene was found in all ten (100%) *S. aureus* isolates these results agree with Mehrotra et al., (2000), Omar et al., (2014), Saidi et al., (2015). Typing of the staphylococcal chromosome cassette *mec* (SCC *mec*) elements allows to categorize the methicillin-resistant *Staphylococcus aureus* (MRSA). The *mecA* gene is harbored by chromosomal cassette encoding penicillin-binding protein2a *(PBP2a)*

(Becker et al., 2014), and play an important and distinct role for resistance to methicillin and to a majority of beta- lactam antibiotics. The PCR amplification with *spa* gene specific primers were conducted with genomic DNA, which revealed in a products of approximate sizes 280 bp, 150 bp and 250 bp. *SPA* gene was detected in all ten (100%) *S. aureus* isolates. These results agree with the findings described by several studies (Ashraf et al., 2016b, Coelho et al., 2011, Dalla Pozza et al., 1999, Kahl et al., 2016, Kumar et al., 2010 and Memon et al., 2013) that established the presence of *spa* X-region gene in nearly all of the isolates and disagree with (Kalorey et al., 2007, Klein et al., 2012 and Stephan et al., 2001) who identified *spa* (X-region) gene in *Staphylococcus aureus* isolates with incidences of 76.5%, 70.3% and 85.9%, respectively.

Spa (X-region) gene considered as one of the most often and important used methods primarly based on single locus sequencing (Mitra et al., 2013, Sabat et al., 2013a). It is a very common and popular technique used for genotyping staphylococci from mastitis (Budd et al., 2015, Bar-Gal et al., 2015, Lundberg et al., 2016). *spa* gene typing considered as a for most method or a supplementary tool for typing of *Staphylococcus aureus* isolates which provided a vital role for controlling of staphylococcal mastitis .

On the other hand, The PCR amplification with *23SrRNA* gene specific primers revealed in a product of approximate size 1250 bp. *23SrRNA* gene was detected in all ten *Staphylococcus aureus* isolates (100%). Molecular identification and characterization were performed by these results agree with Kuzma et al., (2003). PCR amplification of genes encoding *23SrRNA* have been commonly used for molecular typing of *S. aureus* Annemüller et al., (1999). The target sequence of *23SrRNA* was chosen because it has been found as important molecular marker for phylogenetic relatedness Ludwig et al.,(1992).

Concerning *Staphylococcus aureus spa* gene nucleotide sequence analysis revealed great homology and identity between the Egyptian isolated strain (*S. aureus* ASM strain) and the different *Staphylococcus aureus* strains uploaded from GenBank. Phylogenetic tree for *S. aureus spa* gene partial sequences showed clear clustering of the Egyptian isolated strain (*S. aureus* ASM strain) and different *S. aureus* strains uploaded from GenBank. Sequence distance of *S. aureus spa* virulence gene showed 94% to 99.2% homology between the Egyptian isolated strain (*S. aureus* ASMstrain) and GenBank *S. aureus* strain.

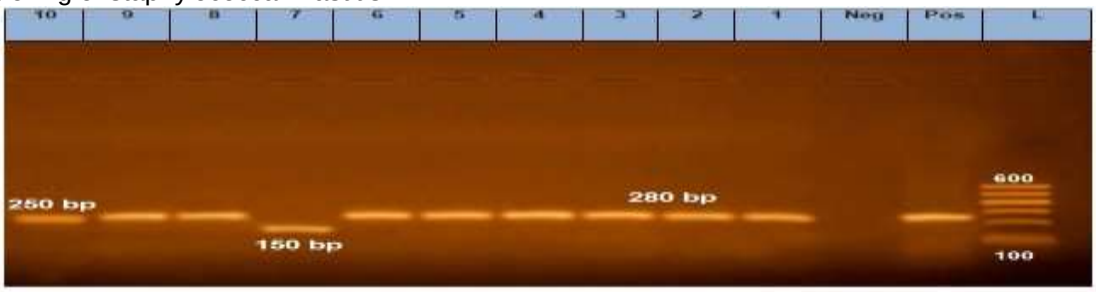

Figure 1: Agarose gel showing polymerase chain reaction (PCR) amplified product of 280, 150 and 250 bp of methicillin resistant *spa* gene for *Staphylococcus aureus* MRSA, lanes (1) to (10): samples positive for *spa* gene, lane (pos.): positive control, lane (Neg.): Negative control, Lane (L): MW 100bp ladder (DNA marker) (Qiagen, Germany).

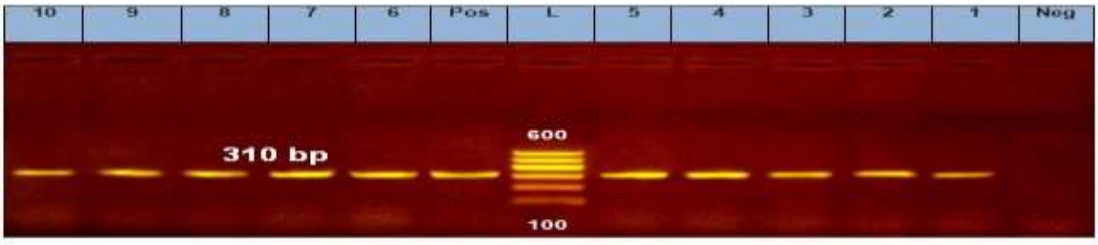

Figure 2: Agarose gel showing polymerase chain reaction (PCR) amplified product of 310 bp of

methicillin resistant gene *mecA* (*mecA)* virulence gene for *Staphylococcus aureus* MRSA, lanes (1) to (10): samples positive for gene*mecA*, Lane (pos.): positive control, Lane (Neg.): Negative control, Lane (L): MW 100bp ladder (DNA marker) (Qiagen, Germany).

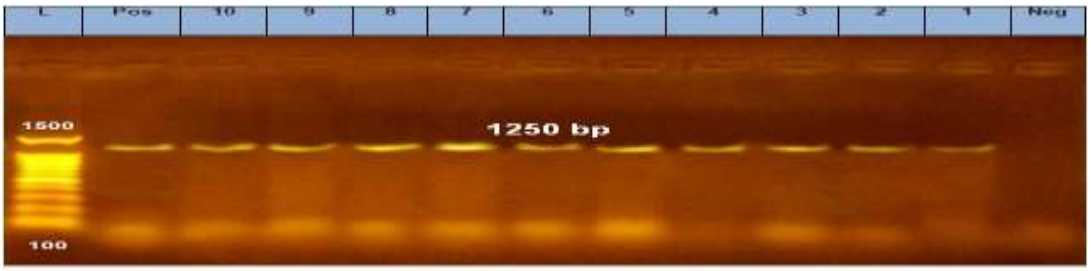

Figure 3: Agarose gel showing polymerase chain reaction (PCR) amplified product of 1250 bp of methicillin resistant *23SrRNA* gene for *Staphylococcus aureus* MRSA, lanes (1) to (10): samples positive for *23SrRNA* gene, lane (pos.): positive control, lane (Neg.): Negative control, Lane (L): MW 100bp ladder (DNA marker) (Qiagen, Germany).

Nucleotide sequence of *spa* gene of the Egyptian isolated strain (*S. aureus* ASM strain) showed 99.2% and 98% identity with the Indian *S. aureus* strains (t345-27 and t1234-94), (accession No. KY110975 and accession No. KY110983), respectively by Singh et al., (2016). 96.8% identity with the Iraquian *S. aureus* strain 7-t2802 by Idabies and Khudor (2018) (accession No. KY 130836), 95.6% with Czech Republic *S. aureus* strain SAH 756 by Ruzckova and Pantucek (2007) (accession No. EF450766), and *S. aureus* strain by Cai et al., (2007) (accession No. EF094521).

Deduced amino acids alignment report of the sequenced 250 amino acid of *Staphylococcus aureus spa* showed great homolgy between the Egyptian strain *(S. aureus* ASM strain) and the different *S.aureus* strains uploaded from GenBank

Phylogenetic tree for the nucleotide partial coding sequance of *Staphylococcus aureus mecA* gene showed clear clustering of the Egyptian isolated strain (*S. aureus* ASM strain) and different *S. aureus* strains uploaded from gene bank.

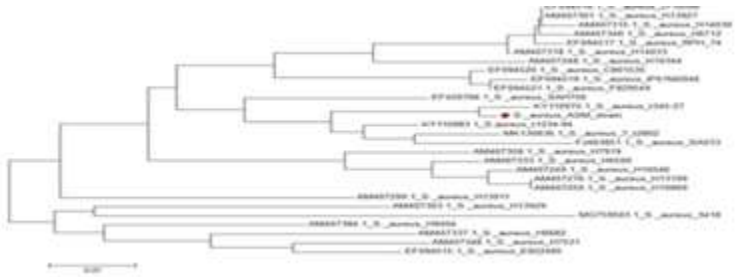

Figure 4: Phylogenetic tree for *Staphylococcus aureus* MRSA (*spa*) virulence gene partial sequences that was generated using neighbor joining in MEGA6. It showed clear clustering of the Egyptian isolated strain (*S. aureus* ASM strain) and different *Staphylococcus aureus* strains uploaded from gene bank.

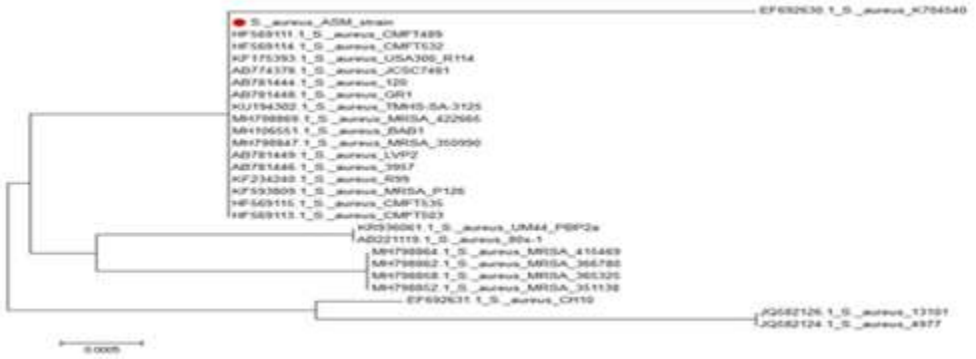

Figure 5: Phylogenetic tree for *Staphylococcus aureus* MRSA (*mecA*) virulence gene partial sequences that was generated using neighbor joining in MEGA6. It showed clear clustering of the Egyptian isolated strain (*S. aureus* ASM strain) and different *Staphylococcus aureus* strains uploaded from gene bank.

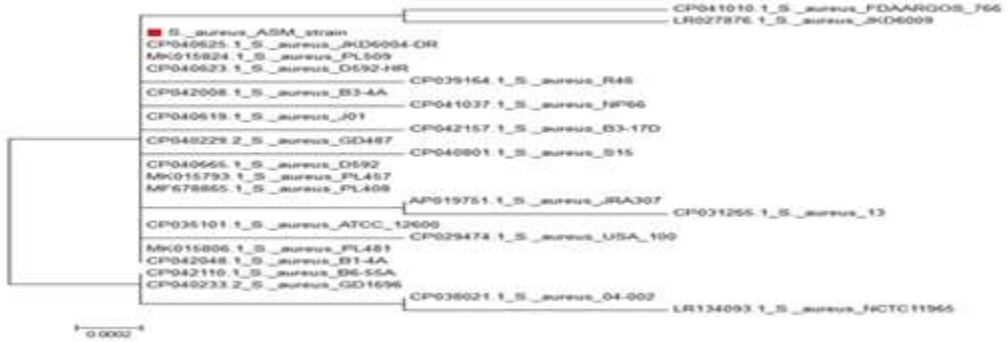

Figure 6: Phylogenetic tree for *Staphylococcus aureus* MRSA (*23SrRNA*) virulence gene partial sequences that was generated using neighbor joining in MEGA6. It showed clear clustering of the Egyptian isolated strain (*S. aureus* ASM strain) and different *Staphylococcus aureus* strains uploaded from gene bank.

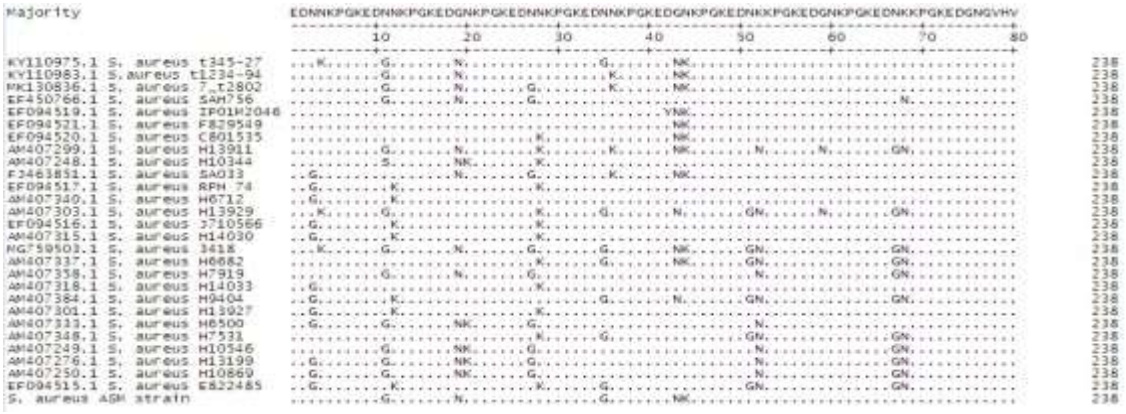

Figure 7: Amino acids alignment of *mecA* gene of *Staphylococcus aureus* of Egyptian isolated strain (*S. aureus* ASM strain) using CLUSTALW multiple sequence alignment program version 1.83 of Mega Align module of laser gene DNA star and different *Staphylococcus aureus* strains uploaded from gene bank

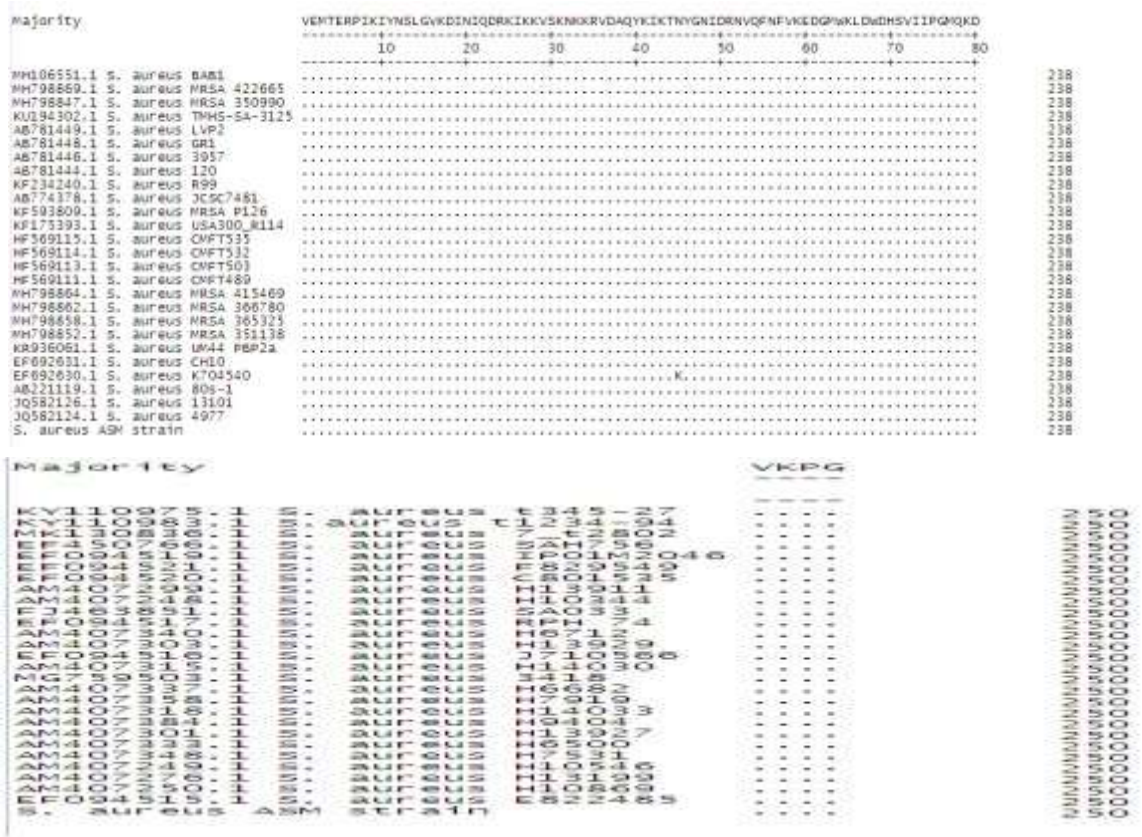

Figure 8: Amino acids alignment of *spa* gene of *Staphylococcus aureus* of Egyptian isolated strain (*S. aureus* ASM strain) using CLUSTALW multiple sequence alignment program version 1.83 of Mega Align module of laser gene DNA star and different *Staphylococcus aureus* strains uploaded from gene bank.

Sequence distance of *Staphylococcus aureus mecA* virulence gene showed 99.7% to 100% homology between the isolated Egyptian strain (*S. aureus* ASM strain) and different *S. aureus* strains uploaded from gene bank. Sequence distance of *Staphylococcus aureus mecA* virulence gene showed 99.7% to 100% homology between the isolated Egyptian strain (*S. aureus* ASM strain) and different *S. aureus* strains uploaded from gene bank. When analyzing nucleotide sequence of *mecA* gene of the Egyptian isolated strain (*S. aureus* ASM strain) in the current study, it showed 100% identity with the Pakistanian*S. aureus* BAB1 Penicillin binding protein 2A (*mecA*) gene strain recovered by Mushtaq et al., (2018) (accession No. MH106551), the Italian *S. aureus* MRSA (422665, 350990) penicillin binding protein PBP2A (*mecA*) gene (accession No. MH798869, MH798847) by Morroni et al., (2018), the American *S. aureus* strain TMHS-SA-3125 ceftaroline-resistant penicillin-binding protein PBP2a (*mecA*) gene, *mecA*- Y446N/E447K by Long et al., (2015) (accession No. KU194302), the Indian *S. aureus* strain LVP2 by Balakuntla et al., (2014) and Nadig et al., (2012) (accession No. AB781449), the Indian *S. aureus* strain subsp. aurues GR1 by Balakuntla et al., (2014) and khedkar et al., (2012) (accession No. AB 781448), the the Indian *S. aureus* strain by Balakuntla et al., (2014) and Shambat et al., (2012) (accession N0. AB781446), the Indian *S. aureus* strain subsp. *Aurues* 120 by Balakuntla et al., (2014) and Shambat et al., (2012) (accession No. AB781444), the Netherlands *S. aureus* strain R99 by Sabat et al., (2013b) (accession No. KF234240), the japanise *S. aureus* strain JCSC7841 by Zhang et al., (2013) (accession No. AB774378), the Belgium *S. aureus* strain MRSA P126 by Vandendriessche et al., (2014) (accession No. KF593809) and 99.7% identity with the Italian *S. aureus* MRSA strains 415469, 366780, 365325 by Morroni et al., (2018) (accession No. MH798864, MH798662 and MH798858 respectively), the Germany *S.aureus* strain UM44PBP2A by Schaumburg et al., (2015) (accession No. KR936061) and the Australian *S. aureus* strains CH10 and K704540 by Stephenes et al., (2007) (accession No. EF692631 and EF692630 respectively). Deduced amino acids alignment report of the sequenced 307 amino acid of *Staphylococcus aureus mecA* showed great homolgy between the Egyptian strain *(S. aureus* ASM strain) and the different *Staphylococcus aureus* strains uploaded from gene bank.

On the other hand, Phylogenetic tree of the nucleotide partial coding sequance for *Staphylococcus aureus 23SrRNA* gene showed clear clustering of the Egyptian isolated strain (*S. aureus* ASM strain) and different *Staphylococcus aureus* strains uploaded from gene bank. Sequence distance of *Staphylococcus aureus 23SrRNA* gene showed 99.9% to 100% homology between the Egyptian isolated strain (*S. aureus* ASM strain) and different *S. aureus* strains uploaded from gene bank. Nucleotide sequence of *23SrRNA* geneof the Egyptian isolated strain (*S. aureus* ASM strain) showed 100% identity with the American *S. aureus* strain B3-4A by Neyaz et al., (2019) (accession No. CP042008), the Netherlands *S. aureus* strain PL509, *S. aureus* strain PL481, *S. aureus* strain PL457 and *S. aureus* strain PL408 by Kosecka-strojek et al., (2019) (accession No. MK015824, MK01586, MK015793 and CPMF678865), the Chinese *S. aureus* strain ATCC 12600 by Chen (2019) (accession No. CP035101), the Canadian *S. aureus* strains GD487 and GD1696 by McClure et al., (2019) (accession No. CP040229 and CP040233), the American *S. aureus* strains D592, JKD6004, J01and D592HR by Miller et al., (2019) (accession No.CP040665, CP040625, CP040619 and CP040623), and showed 99.9% identity with the Japanse *S. aureus* strain JRA307 by Sekizuka et al., (2019) (accession No. CP040665 and AP019751), the American *S. aureus* strains B3-17D, B1-4A and B6-55 by Neyaz et al., (2019) (accession No. CP042157, CP042048 and CP042110), the South African *S. aureus* strain NP66 by Allam (2019) (accession No. CP041037), the Pakistanian *S. aureus* strain R46 by Ullah and Alli (2019) (accession No. CP039164), and the American *S. aureus* strain USA100 by Chilpala et al., (2018) (accession No. CP029474).

CONCLUSION

The X-region of the *spa* gene can be used as a molecular marker typing in studying the genetic diversity among the strains of *Staphylococcus aureus MRSA* for epidemiological investigation of origin and source of infection and comparing the differences in virulent phenotypes amongst various strains, and the *mecA* gene typing considered as an important and useful tool for the study of MRSA molecular epidemiology.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

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AUTHOR CONTRIBUTIONS

ASM designed the concept of the review article, ASM and SAE designed and performed the experiments of isolation and identification of milk samples, MIA designed the molecular identification of the samples, all authors shared in writing, reviewed and approved the manuscript.

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