Cellular & Molecular Biology
Cell. Mol. Biol. 2015; 61 (4): 101-112
Published online September 26, 2015 (http://www.cellmolbiol.com)
Received on August 14, 2015, Accepted on September 7, 2015.
doi : 10.14715/cmb/2015.61.4.17

Comparative phenotypic and genotypic discrimination of methicillin resistant and susceptible *Staphylococcus aureus* in Egypt

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Abstract

The present study was designed to elucidate the phenotypic and genotypic characterization of *S. aureus* isolates in Egypt. The antibiotic susceptibility pattern of 133 identified *S. aureus* isolates revealed that over 70% of the isolates were multi-drug resistant (MDR). Fifty MDR isolates were characterized using antibiotyping and different molecular typing methods. Amplification of *mec*A gene confirmed 30 strains as methicillin-resistant *S. aureus* (MRSA) and 20 as methicillin-sensitive *S. aureus* (MSSA). Generally, 22 MRSA (73.33%) and ten MSSA (50%) harbored Panton-Valentine leukocidin (*pvl*) gene with no statistically significant difference (p=0.093). Staphylococcal cassette chromosome (SCC) *mec* typing revealed that 48% of the typeable MRSA isolates possessed SCC*mec* type IV. SCC*mec* and antimicrobial susceptibility typing signified the presence of community-acquired (CA)-MRSA in Egypt. Surprisingly, all isolates of SCC*mec* types I, II and III and 50% of both MRSA isolates of SCC*mec* types IV and V possessed *pvl* gene. It was clear that staphylococcal protein A (*spa*) and coagulase (*coa*) typing discriminated the isolates into eight different groups, whilst polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of coagulase gene yielded ten distinct RFLP banding patterns. This study presented the first baseline information on the characterization of MRSA and MSSA in Egypt.

Key words: CA-MRSA, MSSA, multi-drug resistant, SCC*mec*, Panton-Valentine leucocidin.

Introduction

Staphylococcus aureus, particularly methicillin-resistant *S. aureus* (MRSA), is an important pathogen causing wide range of infections in the hospital and community setting. Since the first discovery of the first European isolate of MRSA in 1960s, it has become a prevalent cause of nosocomial (hospital-acquired [HA]) infections associated with higher mortality than infections due to methicillin-susceptible *S. aureus* (MSSA) isolates (1). Recently, there has been an increase in the reports of community acquired MRSA (CA-MRSA) infection, which is of a great concern (2).

The success of *S. aureus* as a pathogen is influenced by both the extraordinary ability to express a large repertoire of virulence factors which cause harmful toxic effects to the host (3) as well as its aptitude to develop resistance to antimicrobial agents (4).

An important virulence factor that is predominantly associated with increased disease severity of *S. aureus* is the Panton-Valentine leucocidine (PVL), which is a bi-component pore forming cytotoxin that causes leukocyte destruction and tissue necrosis (5).

Resistance to methicillin is conferred by the expression of a variant penicillin-binding protein (PBP), PBP 2a, which is encoded by the methicillin resistance gene, *mec*A. This gene is situated on a mobile genetic element known as staphylococcal cassette chromosome *mec* (SCC*mec*) (6).

Significant geographic variations have been found in the structural organization of the SCC*mec* and these variations have been used to classify the SCC*mec* types (7). To date, up to 11 SCC*mec* types defined by combining the genetic structures of the cassette chromosome recombinase (*ccr*) gene complex and the *mec* gene complex have been described based on the criteria suggested by IWG-SCC (8), but only type I– V are globally distributed.

SCC*mec* typing can be a good marker to reveal the epidemiological changes of MRSA infection (9). The recent genomic sequence of CA-MRSA isolates indicated the presence of a novel smaller variant of methicillin resistant locus designated SCC*mec* IV besides a locus for the Panton-Valentine leucocidine (PVL) suggesting to be a stable marker of CA-MRSA cases worldwide (10). Therefore, CA-MRSA have been shown to be more virulent compared to HA-MRSA and usually have a non-multiresistant antimicrobial profile (11). The recurrent penetration of CA-MRSA into the clinical environment has blurred the boundary between HA-MRSA and CA-MRSA, making it more difficult to discriminate between them.

The increasing number of MRSA cases detected in Egypt necessitates continuous molecular epidemiological surveillance of MRSA, not only for the implementation of effective infection control measures and appropriate antibiotic treatment, but also for following up the changing epidemiology of MRSA infection to trace the evolution and spread of successful MRSA clones (12). Monitoring of these strains requires the use of rapid and precise epidemiological phenotypic antimicrobial susceptibility features and genotypic typing systems.

In light of the above and as a consequence of scarce of the epidemiological and molecular data on *S. aureus* in Egypt, the current investigation was carried out to compare phenotypic and different molecular typing methods of multi-drug resistant MRSA and MSSA strains to elect the most adequate currently available

typing techniques. The consequence of these practices among MRSA isolates may provide a fortuitous opportunity to correlate phenotypic antibiotic susceptibility patterns with the occurrence of *pvl* gene and SCC*mec* types for classifying the isolates as either CA- or HA-MRSA.

Materials and methods

Bacterial strains

This study employed a total of 133 *S. aureus* isolates of human and animal origins recovered during a two-year period from 2010 to 2012 from different provinces in Egypt. These isolates were kindly supplied by Bacteriology, Mycology and Immunology Department, Faculty of Veterinary Medicine, Zagazig University. In this regard, 55 *S. aureus* isolates representing various kinds of human infections were derived consecutively from patients attending major University Hospital and diagnostic laboratories in Zagazig and Assuit. They were isolated from several clinical specimens enclosing pus (22), sputum (17), urine (one), cerebrospinal fluid (one) and swabs from various origins (14). An additional 78 *S. aureus* isolates investigated in this study were originated from mastitic cow milk samples (61) as well as various meat products collected from local supermarkets (17) comprising minced meat (6), sausage (four) and burger (seven). All the isolates were preserved frozen in brain heart infusion broth containing glycerol 30% at −80°C prior to subsequent detailed analysis.

Microbiological analysis and identification of the strains

A subculture of *S. aureus* isolates was initially performed on mannitol salt agar. Preliminary phenotypic identification of *S. aureus* isolates was conducted by standard bacteriological procedures according to colonial and microscopical morphology based on growth and fermentation on mannitol salt agar, β hamolysis on blood agar, production of golden yellow pigments and formation of gram-positive grape-like coccoid clusters (13). These clinical isolates were then confirmed to be *S. aureus* on the basis of their biochemical reaction results comprising their ability to coagulate rabbit plasma along with their positive catalase reaction. Finally, the identities of the isolates were confirmed as *S. aureus* by PCR detection of the *nuc* gene based on the conditions described previously (14).

Antimicrobial susceptibility testing

The *in-vitro* activity of various antibiotic compounds was tested. Phenotypic antimicrobial susceptibility profiles of all confirmed *S. aureus* isolates to a range of antimicrobial agents of different groups including oxacillin (OX; 1µg), vancomycin (VA; 30 mcg), imipenem (IPM; 10 mcg), cefoxitin (FOX; 30 mcg), ceftriaxone (CRO; 30 mcg), chloramphenicol (C; 30 mcg), trimethoprim/sulfamethoxazole (SXT; 1.25/23.75 mcg), gentamicin (CN; 10 mcg), erythromycin (E; 15 mcg), clindamycin (DA; 2 mcg), ciprofloxacine (CIP; 5 mcg), rifamycin SV (RF; 30 mcg) and tetracycline (TE; 30 mcg) was determined adopting the Kirby-Bauer disk diffusion method (15) on Muller-Hinton agar (Difco, USA) using standard antibiotic disks (Oxoid, UK). The inhibition zone diameter around each disk was measured and isolates were categorized as susceptible or resistant based upon interpretative criteria developed by CLSI (Clinical and Laboratory Standards Institute) (15). Characterization based on antibiotic susceptibility testing has been regarded as a timely and inexpensive tool for *S. aureus* phenotyping. *S. aureus* isolates were considered to belong to different antibiotypes if at least one difference was observed in the antibiotic resistance profile. According to CLSI criteria, the disk testing is not reliable for evaluating vancomycin resistance, so MIC test using the broth microdilution method was performed to determine the susceptibility of *S. aureus* to such glycopeptide antimicrobial agent confirming those isolates as vancomycin resistant (16).

Determination of multiple antibiotic resistance (MAR) index

Multiple antibiotic resistance (MAR) index is a tool that reveals the spread of resistant bacteria in a given population. The MAR index values for each *S. aureus* isolate and each antibiotic were calculated (17) using the following formulas:

$$\text{MAR index for isolates} = \frac{\text{Number of antibiotics to which the isolate was resistant}}{\text{Number of antibiotics to which the isolate was exposed}}$$

$$\text{MAR index for antibiotics} = \frac{\text{Number of antibiotic resistant isolates}}{\text{Number of antibiotics x Number of isolates}}$$

Preparation of bacterial genomic DNA lysate

Total bacterial genomic DNA used as a target for all PCR assays was extracted using a simple boiling procedure as was previously described (9). Extracted DNA was used immediately or kept frozen at -20°C until used for subsequent PCR amplification.

Molecular characterization of multi-drug resistant (MDR) S. aureus isolates

Frozen crude lysates of 50 *S. aureus* isolates were assayed by PCR - based genotyping. Multiplex PCR protocol was optimized to simultaneously amplify *mec*A gene and *pvl* gene in addition to conventional staphylococcal protein A (*spa*) typing as described previously (18). Another simplified and feasible SCC*mec* multiplex PCR typing strategy was carried out for rapid and reliable characterization of the most known SCC*mec* allotypes I-V among MRSA strains using eight unique and specific pairs of primers with a gradient of melting temperatures according to the procedure reported previously (9). Optimization of multiplex PCR conditions was obtained empirically by assaying different primer and DNA concentrations and by selection of the appropriate annealing temperature which is possibly the most critical component for optimizing the specificity of a PCR reaction using the S1000 ™ thermal cycler (Bio-Rad, USA). Validation of the multiplex PCR optimizations was performed by simultaneous comparison with the results of single-target PCR assays with each individual primer pair.

Interestingly, to perform coagulase (*coa*) gene typing, the repeated units encoding hypervariable regions of the *S. aureus* coagulase gene were amplified by uniplex

Table 1. Nucleotide sequences, specificities and anticipated sizes of PCR products for *S. aureus* gene-specific oligonucleotide primers used in multiplex and single-target PCRs.

Assay type and target gene	Primer name	Specificity	Nucleotide sequence (5'-3')	Expected amplicon size (bp)	References
Multiplex PCR					
*mec*A	*mec*A P4 *mec*A P7	Methicillin resistant	TCCAGATTACAACTTCACCAGG CCACTTCATATCTTGTAACG	162	**(18)**
spa	spa-1113f spa-1514r	*S. aureus*	TAAAGACGATCCTTCGGTGAGC CAGCAGTAGTGCCGTTTGCTT	Variable	**(18)**
pvl	PVL-FP PVL-RP	PVL S/F proteins	GCTGGACAAAACTTCTTGGAATAT GATAGGACACCAATAAATTCTGGATTG	80	**(18)**
Multiplex PCR					
SCC*mec* type I	Type I-F Type I-R	Type I SCC*mec*	GCTTTAAAGAGTGTCGTTACAGG GTTCTCTCATAGTATGACGTCC	613	**(9)**
SCC*mec* type II	Type II-F Type II-R	Type II SCC*mec*	CGTTGAAGATGATGAAGCG CGAAATCAATGGTTAATGGACC	398	**(9)**
SCC*mec* type III	Type III-F Type III-R	Type III SCC*mec*	CCATATTGTGTACGATGCG CCTTAGTTGTCGTAACAGATCG	280	**(9)**
SCC*mec* type IVa	Type IVa-F Type IVa-R	Type IVa SCC*mec*	GCCTTATTCGAAGAAACCG CTACTCTTCTGAAAAGCGTCG	776	**(9)**
SCC*mec* type IVb	Type IVb-F Type IVb-R	Type IVb SCC*mec*	TCTGGAATTACTTCAGCTGC AAACAATATTGCTCTCCCTC	493	**(9)**
SCC*mec* type IVc	Type IVc-F Type IVc-R	Type IVc SCC*mec*	ACAATATTTGTATTATCGGAGAGC TTGGTATGAGGTATTGCTGG	200	**(9)**
SCC*mec* type IVd	Type IVd-F Type IVd-R	Type Ivd SCC*mec*	CTCAAAATACGGACCCCAATACA TGCTCCAGTAATTGCTAAAG	881	**(9)**
SCC*mec* type V	Type V-F Type V-R	Type V SCC*mec*	GAACATTGTTACTTAAATGAGCG TGAAAGTTGTACCCTTGACACC	325	**(9)**
Single-target PCR					
coa	Coa-F Coa-R	*S. aureus*	ATAGAGATGCTGGTACAGG GCT TCCGATTGTTCGATGC	Variable	**(19)**

Spa, staphylococcal surface protein A; *pvl*, Panton-valentine leucocidin; SCC*mec*, staphylococcal cassette chromosome methicillin; *coa*, coagulase.

PCR with the thermal profile described elsewhere (19). Furthermore, Restriction fragment length polymorphisms (RFLPs) of the amplicons were determined by digestion of amplified fragments of *coa* gene with AluI restriction endonuclease (Sigma, USA) according to the manufacturer's recommended protocol.

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, specificities and anticipated molecular sizes of PCR amplified fragments for these gene-specific oligonucleotide primer sets are outlined in Table 1.

All PCR assays were carried out in a total reaction volume of 25 µl. The reaction mixtures consisted of 12.5 µl of Dream*Taq* TM Green master mix (2X) (Fermentas, USA), 0.1 µl of 100 pmol of each primer (Sigma, USA), 2 µl of the extracted DNA template and water nuclease-free up to 25 µl. All PCR assay runs incorporated appropriate positive and negative controls.

After amplification, aliquots of the resulting amplified PCR products and restriction digest fragments of *coa* gene were subsequently visualized using an ultraviolet transilluminator (Spectroline, USA) after electrophoresis on a 1.5% agarose gel containing 0.5 µg/ml ethidium bromide (Sigma, USA) with a 100-bp standard DNA molecular weight ladder (Fermentas, USA). The interpretation criterion for identifying different strains was a single band difference.

Discriminatory power of all typing methods

To assess quantitative data of discriminatory power of the typing methods used in our study, the numerical index of discrimination (DI-value) of each typing method was assessed using Simpson's index of diversity (SID) calculating the probability that two unrelated strains sampled from the test population will be placed into different typing groups (20).

Statistical analysis

Stata Statistical Software: Release 11 (StataCorp LP, College Station, TX, USA) and MATLAB software (MathWorks, Cambridge, MA, USA) were used for data management and statistical analysis. Binary logistic regression was used to study the association between the presence of *S. aureus pvl* virulence gene and resistance to antibiotics with an odds ratios (95% CI) indicating the strength of the associations. Moreover, chi-square test was used to compare the resistance percentages of *S. aureus* isolated from human and animal origins to different antibiotics. We also applied the Fisher's exact test whenever there were not enough numbers of observations to fit logistic regression.

Results

Initial characterization of S. aureus strains

Standard conventional laboratory tests used for characterization of all the clinical isolates that were obtai-

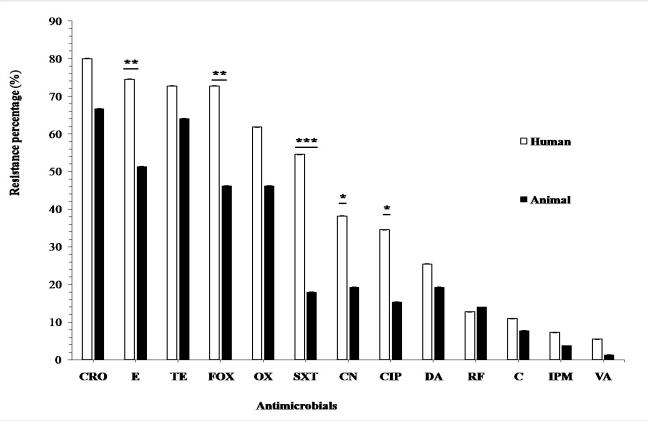

Figure 1. Distribution of antimicrobial resistance among *S. aureus* of human and animal origins. CRO, ceftriaxone; E, erythromycin; TE, tetracycline; FOX, cefoxitin; OX, oxacillin; SXT, trimethoprim-sulfamethoxazole; CN, gentamicin; CIP, ciprofloxacine; DA, clindamycin; RF, rifamycin SV; C, chloramphenicol; IPM, imipenem; VA, vancomycin. All P values were indicated by asterisks: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. All other differences in resistance were non-significant.

ned from both human and animal origins confirmed the isolates as *S. aureus*. Further confirmation of the isolates was conducted by PCR amplification of *nuc* gene.

Antimicrobial susceptibility patterns

The *in-vitro* antimicrobial susceptibility profiles of 133 *S. aureus* isolates against antibiotic agents of different classes are summarized in Table 2. Antibiogram analysis showed that *S. aureus* isolates displayed variable antimicrobial susceptibility patterns. From the therapeutic viewpoint, vancomycin and imipenem showed the maximum overall sensitivity against the isolates (96.99 and 94.74%, respectively). Another striking feature was that high levels of resistance were recorded for ceftriaxone (72.18%), followed by tetracycline (67.67%) with MAR indices of 0.056 and 0.052, respectively. Unfortunately, vancomycin resistance was observed in 3.01% of the isolates with MIC values ranged from 64-256 µg/ml.

Table 2. Antimicrobial susceptibility profiles of 133 *S. aureus* isolates.

Antibiotic	*S. aureus* (133)			
	S No. (%)	I No. (%)	R No. (%)	MAR index
OX	63 (47.37)	0 (0)	70 (52.63)	**0.040**
VA	129 (96.99)	0 (0)	4 (3.01)	**0.002**
IMP	126 (94.74)	0 (0)	7 (5.26)	**0.004**
FOX	28 (21.05)	40 (30.08)	65 (48.87)	**0.038**
CRO	18 (13.53)	19 (14.29)	96 (72.18)	**0.056**
C	111 (83.46)	10 (7.52)	12 (9.02)	**0.007**
SXT	67 (50.38)	22 (16.54)	44 (33.08)	**0.025**
CN	91 (68.42)	6 (4.51)	36 (27.07)	**0.021**
E	21 (15.79)	31 (23.31)	81 (60.90)	**0.047**
DA	77 (57.89)	27 (20.30)	29 (21.80)	**0.017**
CIP	95 (71.43)	7 (5.26)	31 (23.31)	**0.018**
RF	103 (77.44)	12 (9.02)	18 (13.53)	**0.010**
TE	31 (23.31)	12 (9.02)	90 (67.67)	**0.052**

MAR, multiple antibiotic resistance; S, susceptible; I, intermediate; R, resistant.

Antibiotic abbreviations: OX, oxacillin; VA, vancomycin; IPM, imipenem; FOX, cefoxitin; CRO, ceftriaxone; C, chloramphenicol; SXT, trimethoprim-sulfamethoxazole; CN, gentamicin; E, erythromycin; DA, clindamycin; CIP, ciprofloxacine; RF, rifamycin SV; TE, tetracycline.

As is apparent from Figure1, the resistance patterns of *S. aureus* isolates of human and animal origins were found to be highly variable. *S. aureus* strains of human origin were more resistant to most antimicrobial agents as compared to those recovered from animal origin. *S. aureus* strains of human origin showed highly significant differences in resistance percentages to erythromycin, cefoxitin and trimethoprim-sulfamethoxazole ($p<0.01$) and statistically significant differences in resistance percentages to gentamicin and ciprofloxacine ($p<0.05$) than those of animal origin.

MAR index

The frequency of MAR indices in *S. aureus* isolates of human and animal origins is given in Table 3. Analysis of the results showed that *S. aureus* isolates were resistant to at least 1 to 12 of 13 antibiotics tested generating MAR index ranged from 0.08 to 0.92. Multidrug resistance (MDR) in this study was taken as resistance to three or more different classes of antimicrobials tested. Overall, majority of the isolates (74.44%) were MDR with MAR index greater than 0.2.

Comparative study of MAR index for *S. aureus* isolates of human and animal origins revealed that the level of MDR exhibited by the isolates of human origin is alarming; such that, 58.18% of these isolates were resistant to more than four antibiotics. Conversely, 34.62% of *S. aureus* isolates of animal origin exhibited non MDR pattern, with only one isolate (1.28%) possessed resistance to more than eight antibiotics. Additionally, the maximum MAR indices of 0.92 and 0.85 were obtained for *S. aureus* isolates of human and animal origins, respectively.

Molecular characterization of MDR S. aureus isolates

With respect to the resistance pattern of *S. aureus* isolates, 50 MDR isolates obtained from pus (11), sputum (eight), swabs from various origins (seven), urine (one), CSF (one), mastitic cow milk samples (18), burger (two), minced meat (one) and sausage (one) were subsequently subjected to detailed molecular characterization by a panel of molecular methods via PCR assays. Validation of all multiplex PCR strategies described herein revealed that the results of the single-target PCR assays corresponded precisely with those from multiplex PCRs.

Genotypic validation of oxacillin and cefoxitin resistances

The PCR results for *mec*A detection were unambiguous. On the basis of the amplification of 162 base pair fragments specific for *mec*A gene, all the 30 strains (100%) which appeared oxacillin and cefoxitin resistant phenotypically by the disk diffusion technique were positive for the presence of *mec*A gene and were therefore confirmed as MRSA strains (Figure 2A). Conversely, none of the 20 isolates that showed sensitivity to oxacillin and cefoxitin in the phenotypic tests exhibited any band for the *mec*A gene in the multiplex PCR test and therefore considered as MSSA strains. Thus, it was interesting to note that there was 100% concordant between

Table 3. Frequency of multiple antibiotic resistance (MAR) indices in *S. aureus* isolates of human and animal origins against 12 antibiotics.

No. of antibiotics to which the isolates were resistant	MAR index	Frequency of MAR index (%)	
		Human origin (55)	Animal origin (78)
0	0.00	0 (0)	8 (10.26)
1	0.08	2 (3.64)	7 (8.97)
2	0.15	5 (9.09)	12 (15.38)
3	0.23	7 (12.73)	13 (16.67)
4	0.31	9 (16.36)	15 (19.23)
5	0.38	5 (9.09)	5 (6.41)
6	0.46	7 (12.73)	5 (6.41)
7	0.54	8 (14.55)	6 (7.69)
8	0.62	7 (12.73)	6 (7.69)
9	0.69	3 (5.45)	0 (0)
10	0.77	1 (1.82)	0 (0)
11	0.85	0 (0)	1 (1.28)
12	0.92	1 (1.82)	0 (0)

MAR, multiple antibiotic resistance.

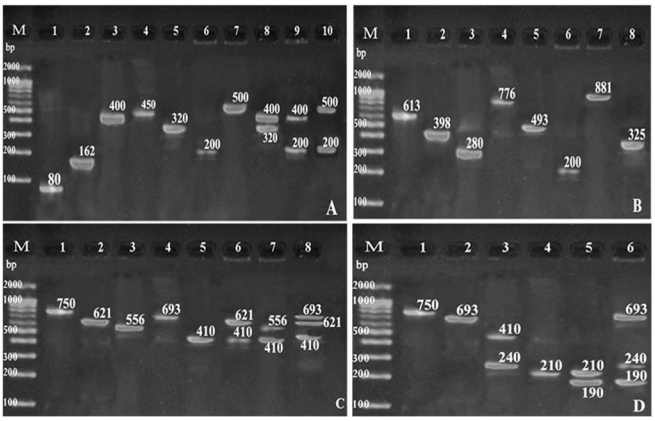

Figure 2. Agarose gel electrophoresis demonstrating predicted PCR amplicon of *pvl* (lane 1), *mecA* (lane 2) and *spa* genes (lanes 3-10) (A), PCR products produced with MRSA isolates containing SCC*mec* types I-V (B), different PCR amplified products of *coa* gene of represented isolates with C1-C8 genotypes (C) and PCR-RFLP patterns of *coa* gene of representative *S. aureus* isolates showing *coa*-RFLP patterns R1, R6, R2, R3, R4 and R10 (D). PCR product sizes of target genes are indicated.

phenotypic and PCR findings.

Frequency of pvl gene among S. aureus isolates

Analysis of *S. aureus* strains for the carriage of *pvl* gene revealed that 32 (64%) of the 50 representative isolates yielded positive PCR amplification of 80 base pair fragments specific for extracellular *pvl* toxin gene as presented in Figure 2A. Overall, 22 of 30 MRSA isolates (73.33%) and 10 of 20 MSSA isolates (50%) harbored the *pvl* gene. There was no statistically significant difference (p=0.093) in relation to the occurrence of *pvl* gene among MRSA and MSSA isolates. It is also worth noting that the *pvl* positivity rate was higher in *S. aureus* isolates from human origin (20/28, 71.43%) than that from animal origin (12/22, 54.55%). Among isolates from different types of staphylococcal infection in human, the vast majority of *pvl* positive strains were isolated from sputum specimens [7/8 (87.5%)]. Moreover, the *pvl* gene was highly recorded in *S. aureus* isolates from mastitic cow milk samples [11/18 (61.11%)].

Spa typing

Molecular diversity of *S. aureus* based on *spa* typing which is regarded as significant as *coa* for *S. aureus* were examined in the present study. The results of *spa* typing by PCR are summarized in Table 4. PCR amplification of *spa*-X region revealed that all the 50 isolates were PCR positive for *spa* gene. Most of the isolates (45; 90%) produced single PCR product in different sizes ranging from 200 to 500 bp, with a prominent amplicon size at 400 bp (68%), while the reminding five isolates (10%) had double PCR products. The prevalence of strains with two bands isolated from pus (60%) was more than in other clinical samples. Overall, the isolates were divided into eight different groups according to the *spa* polymorphisms, where five PCR products of different sizes were observed (Figure 2A) and majority of the isolates (60%) were assigned to the S1 group.

Multiplex PCR strategy for typing of SCCmec elements of MRSA isolates

Multiplex PCR for typing and subtyping of SCC*mec* elements for molecular characterization of 30 MRSA isolates produced the best results using eight pairs of primers with the refereed optimal melting temperatures. Results obtained from our one tube multiplex PCR revealed that SCC*mec* types I to V were successfully distinguished by our typing scheme with a clear and easily discriminated banding pattern for all SCC*mec* types and subtypes. The PCR amplification yielded the fragments of expected sizes as shown in Figure 2B.

SCC*mec* types could be determined for 25 (83.33%) of 30 MRSA isolates, while the remaining five MRSA

Table 4. Genotypes of *S. aureus* based on *spa* polymorphism.

Genotype code	No. of isolates (%)		Total (%)	PCR products (approximate bp)
	MRSA (30)	MSSA (20)		
S1	18 (60)	12 (60)	30 (60)	400
S2	8 (26.67)	1 (5)	9 (18)	450
S3	1 (3.33)	3 (15)	4 (8)	320
S4	1 (3.33)	0 (0)	1 (2)	200
S5	1 (3.33)	0 (0)	1 (2)	500
S6	1 (3.33)	1 (5)	2 (4)	400, 320
S7	0 (0)	2 (10)	2 (4)	400, 200
S8	0 (0)	1 (5)	1 (2)	200, 500

MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-sensitive *S. aureus*; bp, base pairs.

Table 5. Coagulase genotypes and *Coa*-RFLP patterns of methicillin-resistant and methicillin-sensitive *S. aureus* isolates.

Coagulase genotype code	PCR product (approximate bp)	*Coa*-RFLP code	*Coa*-RFLP pattern (approximate bp)	No of isolates (%)	
				MRSA (30)	MSSA (20)
C1	750*	R1	750	2 (6.67)	6 (30)
		R2	240, 410	8 (26.67)	3 (15)
C2	621	R3	210	10 (33.33)	2 (10)
C3	556*	R4	190, 210	4 (13.33)	3 (15)
		R5	210	1 (3.33)	1 (5)
C4	693	R6	693	4 (13.33)	1 (5)
C5	410	R7	210	0	1 (5)
C6	410, 621	R8	210	1 (3.33)	1 (5)
C7	410, 556	R9	190, 210	0 (0)	1 (5)
C8	410, 621, 693	R10	190, 240, 693	0 (0)	1 (5)

coa, coagulase; RFLP, restriction fragment length polymorphism; bp, base pairs; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-sensitive *S. aureus*.

* Coagulase genotype yielded two RFLP patterns.

isolates (16.67%) were non-typeable for the SCC*mec* element based on the established protocol. The vast majority (48%) of the 25 typeable MRSA isolates possessed SCC*mec* type IV, while 6 (24%), 4 (16%), 2 (8%) and 1 (4%) isolates harbored SCC*mec* types II, V, III and I, respectively. It is interesting to note that four different subtypes of the SCC*mec* type IV cassette were identified. Among the 12 diverse SCC*mec* type IV strains, 8 (66.67%), 2 (16.67%), 1 (8.33%) and 1 (8.33%) isolates were assigned to subtypes IVd, IVc, IVb and IVa, respectively. Our data demonstrated that SCC*mec* type II was recovered more frequently in MRSA isolates from human origin (33.33%), while SCC*mec* subtype IVd was mainly recovered in MRSA isolates from milk samples (36.36%).

Coa-RFLP typing

Amplification of *coa* gene revealed five and eight different coagulase genotypes in 30 MRSA and 20 MSSA isolates, respectively on the basis of their PCR amplified product patterns (Figure 2C). C1 was the predominant group and accounted for 45 and 33.33 % of MSSA and MRSA, respectively. The isolates belonging to C1-C5 groups produced single PCR product with five different band classes ranging in size from 410-750 bps, while 3.33% of MRSA and 10% of MSSA comprising C6 and C7 groups exhibited two PCR products and only one MSSA isolate belonging to C8 group (5%) yielded three PCR products (Table 5).

Digestion of all amplified *coa* gene fragments with AluI restriction endonuclease produced 1 to 3 bands in each isolate with molecular sizes ranging from 190-750 bps (Figure 2D). Two PCR products (693 and 750 bps) did not show any restriction cut with AluI. This probably indicates the absence of AluI restriction sites amongst these isolates. Overall, six band classes were produced by restriction digestion of *coa* gene fragments giving rise to seven and ten RFLP banding patterns in MRSA and MSSA isolates, respectively. A 210 bp banding pattern was found to be prevalent in 40 and 25% of MRSA and MSSA isolates, respectively (Table 5).

Comparative analysis of S. aureus isolates

This is the first time, to our knowledge, that a selection of MRSA and MSSA strains had been subjected to comparative phenotypic and genotypic perceptive analysis by a combination of antibiotyping and different molecular typing methods in Egypt. The main phenotypic and genotypic features of the representative MRSA and MSSA isolates are summarized in Tables 6 and 7 and Figure 3.

Characterization of MRSA isolates

Molecular and microbiological characterizations were conducted to determine whether MRSA isolates have traits characteristic for CA-MRSA or HA-MRSA. Despite the high rates of multiple resistance among the characterized 30 MRSA isolates, the results of the current study showed that some MRSA strains of SCC*mec* type IV were sensitive to ceftriaxone (8.33%), trimethoprim-sulfamethoxazole (58.33%) and erythromycin (8.33%) than the strains belonging to SCC*mec* I and II and to imipenem (100%) and gentamicin (66.67%) than half of the strains carrying SCC*mec* III (50%).

It is also noticed that SCC*mec* V strains possessed greater susceptibility to clindamycin (100%) than SCC*mec* II and III isolates (83.33 and 50%, respectively) and to trimethoprim-sulfamethoxazole and erythromycin (50% for each) than all SCC*mec* I and II isolates. Another observation that all SCC*mec* I, II and III isolates (100%) were resistant to cefoxitin, ceftriaxone and erythromycin; over 50% of the SCC*mec* type II isolates were resistant to trimethoprim-sulfamethoxa-

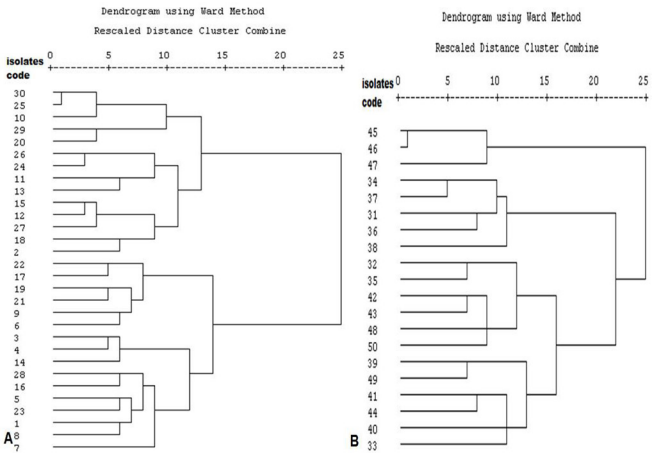

Figure 3. Dendogram deduced from the detailed data of isolates characterization by a combination of antibiotyping and four molecular typing methods depicting clustering and relatedness among 30 MRSA (A) and 20 MSSA isolates (B).

Degrees of similarity are shown in the scale at top right of the figure and strain numbers are indicated on the left side. Tested strains are indicated by numbers in ascending order from 1 to 50 correlating with those in Tables 6 and 7.

Table 6. Characteristics and typing results of 30 MRSA isolates expressing different resistance profiles.

Isolate no.	Source	Resistance pattern	MAR index	SCC*mec* type	*pvl* gene	*Spa* genotype	*Coa* genotype	*Coa*-RFLP pattern
1	Sausage	DA, RF, TE, OX, FOX, CRO, E	0.54	IVd	-	S1	C2	R3
2	Pus	DA, CIP, TE, OX, C, FOX, CRO	0.54	IVd	-	S1	C2	R3
3	Milk	DA, CIP, SXT, TE, OX, FOX, E	0.54	IVd	-	S1	C2	R3
4	CSF	DA, CIP, SXT, TE, OX, FOX, CRO, E	0.62	IVd	+	S1	C6	R8
5	Milk	CIP, RF, TE, OX, FOX, CRO, E	0.54	IVd	-	S1	C2	R3
6	Milk	CIP, TE, OX, FOX, CRO, E	0.46	IVd	-	S1	C3	R4
7	Pus	CIP, CN, TE, OX, FOX, CRO, E	0.54	IVd	+	S6	C2	R3
8	Milk	DA, TE, OX, FOX, CRO, E	0.46	IVd	-	S1	C1	R1
9	Milk	DA, CN, TE, OX, FOX, CRO, E	0.54	IVb	+	S2	C3	R4
10	Pus	CN, SXT, TE, OX, C, FOX, CRO, E	0.62	IVa	+	S2	C1	R2
11	Sputum	CN, SXT, TE, OX, C, FOX, CRO, E	0.62	V	-	S4	C1	R1
12	Pus	DA, TE, OX, FOX, CRO, E	0.46	III	+	S1	C2	R3
13	Pus	CIP, RF, SXT, OX, FOX, CRO, E	0.54	II	+	S2	C1	R2
14	Sputum	CIP, CN, SXT, TE, IPM, OX, FOX, CRO, E	0.69	III	+	S2	C1	R2
15	Swab	CIP, RF, CN, TE, OX, FOX, CRO	0.54	V	+	S1	C4	R6
16	Swab	CIP, CN, TE, IPM, OX, FOX, CRO	0.54	V	-	S1	C1	R2
17	Pus	SXT, TE, OX, FOX, CRO, E	0.46	NT	+	S1	C1	R2
18	Swab	CN, SXT, TE, OX, C, FOX, CRO, E	0.62	II	+	S1	C1	R2
19	Sputum	SXT, TE, OX, FOX, CRO, E	0.46	II	+	S1	C2	R3
20	Swab	DA, CIP, CN, SXT, TE, OX, FOX, CRO, E	0.69	II	+	S2	C3	R4
21	Swab	CIP, CN, SXT, TE, OX, FOX, CRO, E	0.62	NT	+	S5	C4	R6
22	Sputum	CIP, CN, SXT, TE, OX, FOX, CRO, E	0.62	II	+	S2	C1	R2
23	Pus	CIP, CN, SXT, OX, FOX, CRO, E	0.54	II	+	S1	C1	R2
24	Sputum	SXT, TE, OX, FOX, CRO, E	0.46	IVc	+	S2	C2	R3
25	Milk	SXT, TE, OX, FOX, CRO, E	0.46	I	+	S2	C3	R5
26	Milk	DA, CN, SXT, TE, OX, FOX, CRO, E	0.62	NT	+	S1	C2	R3
27	Milk	CN, SXT, TE, OX, FOX, CRO, E	0.54	NT	+	S1	C4	R6
28	Milk	CIP, CN, SXT, TE, OX, FOX, CRO, E	0.62	IVc	+	S1	C3	R4
29	Milk	CIP, RF, CN, TE, IPM, OX, FOX, CRO	0.62	NT	+	S1	C2	R3
30	Milk	CIP, CN, SXT, TE, OX, FOX, CRO, E	0.62	V	+	S3	C4	R6

CSF: cerebrospinal fluid; DA, clindamycin; CIP, ciprofloxacine; RF, rifamycin SV; CN, gentamicin; SXT, trimethoprim-sulfamethoxazole; TE, tetracycline; IPM, imipenem; OX, oxacillin; C, chloramphenicol; FOX, cefoxitin; CRO, ceftriaxone; E, erythromycin; MAR, multiple antibiotic resistance; SCC*mec*, staphylococcal cassette chromosome methicillin; NT: non-typeable; *pvl*, Panton-valentine leucocidin; *spa*, staphylococcal surface protein A; *coa*, coagulase; RFLP, restriction fragment length polymorphism.

zole (100%), ciprofloxacine and gentamicin (66.67% for each) and 50% of SCC*mec* type III isolates were resistant to clindamycin, ciprofloxacine, gentamicin, trimethoprim-sulfamethoxazole and imipenem was also recorded. In this context, such isolates of SCC*mec* types IV and V eventually exhibited traits typical for CA-MRSA and those of SCC*mec* types I, II and III matched the criteria of HA-MRSA on the basis of SCC*mec* complex analysis and antimicrobial susceptibility patterns.

More detailed investigations on the resistance profile revealed that 91.67 and 50%; 58.33 and 75% and 100% of both SCC*mec* IV and V isolates were resistant to erythromycin, ciprofloxacine and tetracycline, respectively. It was encouraging to imply that there was higher prevalence of *pvl* genes among the representative MRSA isolates, where all isolates of SCC*mec* types I, II and III (100%) and 50% of both MRSA isolates of SCC*mec* types IV and V harbored *pvl* gene. Within those strains, the *pvl* locus was less frequent among the isolates that harbored SCC*mec* IVd (25%) and SCC*mec* V (50%). This is unlike the SCC*mec* IVa, IVb and IVc isolates, which all harbored the *pvl* locus (100%).

It seemed clear that the antimicrobial susceptibility patterns differed among the *pvl* positive and negative MRSA isolates. Crucially, a statistically significant association was found between the presence of *pvl* gene and resistance to gentamicin (P=0.04) and trimethoprim-sulfamethoxazole (P=0.01); 68.18 and 77.27 % of *pvl* positive isolates were resistant to gentamicin and trimethoprim-sulfamethoxazole as compared to 25 % of *pvl*-negative MRSA, respectively. The odds of resistance to trimethoprim-sulfamethoxazole and gentamicin among *pvl*-positive MRSA isolates were 10.20 and 6.43 times higher than among *pvl*-negative MRSA isolates, respectively. However, by taking into account, there was no significant association between the presence of *pvl* gene and resistance to clindamycin (P=0.16); only 22.73% of the *pvl*-positive isolates were resistant to clindamycin as compared to 50% of *pvl*-negative MRSA. Moreover, the differences in resistance to imipenem, ciprofloxacine, and rifamycin were not statistically significant between the two groups (P>0.05). The odds of resistance to ciprofloxacine, imipenem, rifamycin and clindamycin among *pvl*-positive MRSA isolates were 0.72, 0.70, 0.47 and 0.29 times lower than among *pvl*-negative MRSA isolates, respectively.

Taken together, our results clearly demonstrated that all 22 *pvl* positive isolates (100%) were resistant to each of ceftriaxone and cefoxitin and a majority of them were resistant to erythromycin and tetracycline (90.91% for each), being acquired new antimicrobial resistance determinants. Therefore, the increased resistance and the fact that 50% of our SCC*mec* types IV and V isolates did not produce *pvl* suggest that the carriage of PVL as a sole marker for recognizing CA-MRSA strains may be unreliable and the criteria for classifying the MRSA

Table 7. Phenotypic and genotypic characteristics of 20 MSSA recovered from human and animal sources.

Isolate no.	Source	Resistance pattern	MAR index	*pvl* gene	*Spa* genotype	*Coa* genotype	*Coa*-RFLP pattern
31	Burger	DA, RF, TE, CRO, E	0.38	-	S7	C1	R1
32	Pus	DA, CIP, SXT, TE	0.31	+	S7	C1	R1
33	Milk	DA, RF, SXT, TE, CRO	0.38	+	S1	C7	R9
34	Urine	DA, CIP, TE, CRO, E	0.38	-	S1	C6	R8
35	Sputum	DA, CIP, SXT, TE	0.31	+	S1	C3	R4
36	Burger	DA, CIP, TE, E	0.31	+	S1	C1	R2
37	Minced meat	CIP, TE, CRO, E	0.31	-	S1	C1	R1
38	Pus	DA, RF, SXT, TE, E	0.38	-	S3	C5	R7
39	Milk	TE, CRO, E	0.23	+	S6	C8	R10
40	Milk	DA, SXT, C, E	0.31	-	S1	C1	R1
41	Pus	TE, CRO, E	0.23	-	S3	C4	R6
42	Milk	SXT, TE, CRO, E	0.31	-	S3	C1	R1
43	Milk	TE, CRO, E	0.23	+	S1	C2	R3
44	Swab	SXT, TE, E	0.23	+	S1	C2	R3
45	Sputum	SXT, TE, CRO, E	0.31	+	S1	C3	R4
46	Swab	CN, TE, CRO, E	0.31	-	S1	C1	R2
47	Sputum	CN, TE, E	0.23	+	S1	C3	R4
48	Pus	CIP, TE, E	0.23	-	S8	C1	R1
49	Milk	CN, TE, CRO, E	0.31	+	S1	C3	R5
50	Milk	SXT, TE, CRO	0.23	-	S2	C1	R2

DA, clindamycin; CIP, ciprofloxacine; RF, rifamycin SV; CN, gentamicin; SXT, trimethoprim-sulfamethoxazole; TE, tetracycline; C, chloramphenicol; CRO, ceftriaxone; E, erythromycin; MAR, multiple antibiotic resistance; *pvl*, Panton-valentine leucocidin; *spa*, staphylococcal surface protein A; *coa*, coagulase; RFLP, restriction fragment length polymorphism.

Table 8. Discrimination indices of various typing methods for methicillin-resistant and methicillin-sensitive *S. aureus* isolates.

Typing method	No. of types		No. of strains of major type (%)		Discrimination index	
	MRSA	MSSA	MRSA (30)	MSSA (20)	MRSA	MSSA
MAR index	4	2	10 (33.33)	10 (50)	0.729	0.526
Spa typing	6	6	18 (60)	12 (60)	0.584	0.632
SCC*mec* typing	9	-	8 (26.67)	-	0.860	-
Coa typing	5	8	10 (33.33)	9 (45)	0.756	0.774
Coa-RFLP	7	10	10 (33.33)	6 (30)	0.802	0.884

MAR, multiple antibiotic resistance; *spa*, staphylococcal surface protein A; SCC*mec*, staphylococcal cassette chromosome methicillin; *coa*, coagulase; RFLP, restriction fragment length polymorphism; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-sensitive *S. aureus*.

isolates as either CA- or HA-MRSA are blurring.

Characterization of MSSA isolates

There were no significant differences in resistance profiles between *pvl* positive and negative MSSA isolates against trimethoprim-sulfamethoxazole, rifamycin, ceftriaxone, erythromycin and tetracycline and in susceptibility patterns of them to trimethoprim-sulfamethoxazole, clindamycin and rifamycin (P>0.05). The odds of resistance to tetracycline and trimethoprim-sulfamethoxazole among *pvl*-positive MSSA isolates were 1.79 and 1.50 times higher than among *pvl*-negative MSSA isolates, respectively. Meanwhile, the odds of resistance to rifamycin, ceftriaxone and erythromycin among *pvl*-positive MSSA isolates were 0.44, 0.42 and 0.25 times lower than among *pvl*-negative MSSA isolates, respectively. Moreover, the odds of susceptibility to each of clindamycin and rifamycin among *pvl*-positive MSSA isolates was 2.25 times higher than among *pvl*-negative MSSA isolates, but that of susceptibility to trimethoprim-sulfamethoxazole among *pvl*-positive MSSA isolates was 0.67 times lower than among *pvl*-negative MSSA isolates.

They both had the similar resistance patterns to clindamycin (40%) and ciprofloxacine (30%) and the identical susceptibilities to ciprofloxacine (70%), ceftriaxone (20%), erythromycin (10%) and tetracycline (0%).

Finally, calculation of the discriminatory power of all typing methods revealed that SCC*mec* typing had the highest discriminatory power for typing MRSA isolates (0.860), while *coa*-RFLP typing was considered as an alternative method that provides useful information in differentiating the MRSA strains, which are otherwise non-typeable by SCC*mec* typing. Based on this observation, *coa*-RFLP typing was able to distinguish 30 MRSA isolates into seven different coagulase-RFLP genotypes. This is contrary to the routine SCC*mec* typing method, where only 83.33% (n = 25) of the isolates were typeable. Among the five non typeable isolates (16.67%), three different coagulase-RFLP genotypes were observed; R3 (two isolates), R6 (two isolates) and R2 (one isolate) (Table 6). Moreover, *coa*-RFLP was found to be the best discriminatory method for investigating the genotyping of MSSA isolates and gave a wider spectrum of types than the other techniques (DI-value = 0.884) (Table 8).

Discussion

S. aureus, in particular MRSA, has been one of the most problematic pathogens encountered by veterinary and human medicine. MRSA infection has now become

a serious and formidable public health concern that has emerged globally over the last four decades causing both nosocomial and community-acquired infections. Regular monitoring of *S. aureus* is of paramount importance for therapeutic and epidemiological purposes.

Therefore, the current large scale investigation conducted various phenotypic and molecular typing methods of *S. aureus* isolates from human and animal origins in Egypt in order to elucidate the variations in MRSA and MSSA populations from divergent sources and to choose the appropriate antibiotic therapy regimens.

In the present study, all clinical *S. aureus* isolates were characterized phenotypically by conventional tests as those from previous scientific literatures (9). Further confirmation of the isolates was conducted by PCR detection of *nuc* gene. This phenomenon was reported elsewhere (14)

Characterization based on antibiotic susceptibility testing has been regarded as a timely and inexpensive tool for *S. aureus* phenotyping.

Analyzing the antibiogram results of *S. aureus* herein revealed that imipenem showed a maximum overall sensitivity against our isolates as recorded in a previous report in India (21).

From another point of view, the relatively high activity of chloramphenicol and rifamycin observed in this study against *S. aureus* isolates is in agreement with a previous study conducted in Pakistan (22), indicating that chloramphenicol and rifamycin are excellent and effective agents for the treatment of *S. aureus* infections.

Additionally, our study revealed a high percentage of sensitivity to gentamicin (68.42%). In contrast, 96.72 % resistance to gentamicin had been reported in South Africa (23).

Notably, the continued increase in the incidence of resistant variants of *S. aureus* strains has led to widespread use of vancomycin for the treatment of staphylococcal infections. However, unfortunately, vancomycin resistance was observed in 3.01% of our isolates with MIC values ranged from 64-256 µg/ml. A similar trend in the level of vancomycin resistant *S. aureus* can be observed in an occasional report in Pakistan (4%) (24), but in contrary to our study, all *S. aureus* strains were sensitive to vancomycin in Pakistan (22). This variation in the drug resistance may be well related to the type of antimicrobial agents prescribed for treating various diseases in different geographical areas.

With regard to the considerable differences between resistance patterns of *S. aureus* isolates of human and animal origins, *S. aureus* strains of human origin were more resistant to most antimicrobial agents as compared to those recovered from animal origin. This is consistent with a previous study conducted in India (25), implying the fact that the indiscriminate use of antibiotics in Egypt has rendered the commonly used antibiotics completely ineffective in the treatment of *S. aureus* infections in human.

It is also interesting to note that the MAR indices obtained in this study is a possible indication that a very large proportion of *S. aureus* isolates have been exposed to several antibiotics. The results showed that majority of *S. aureus* isolates (74.44%) were MDR with MAR index values greater than 0.2. This suggests that such isolates originated from a high risk source of contamination, where antimicrobial agents are freely available and accessible with high potential for abuse (17,26). Conclusively, it has been revealed that the MAR index was within a closely similar range as observed previously in Nigeria (26). Moreover, the high prevalence of MDR among *S. aureus* isolates corroborated an earlier study in India (27).

Fifty multi-drug resistant (MDR) *S. aureus* isolates were subsequently subjected to our further molecular characterization to identify the potentially more virulent strains, rather than relying on epidemiological data alone.

There has been a tremendous increase in the number of MRSA cases in the past few years. Therefore, several efforts have been made for quicker and early detection of MRSA. In this study, during our screening for oxacillin and cefoxitin resistance markers in clinical *S. aureus* isolates, we observed complete consistency between the phenotypic and genotypic results for detection of those resistances as was previously demonstrated (9).

The importance of PVL as a potential virulence factor prompted us to investigate the frequency of PVL-producing *S. aureus* strains obtained from diverse origins in Egypt. Surprisingly, we identified much higher rates of *pvl*-positive *S. aureus* strains (64%) than most reports elsewhere (4.9% in UK) (28). Nevertheless, our finding of high overall prevalence of *pvl*-positive *S. aureus* isolates is consistent with another report in India (29). Although *pvl* is believed to be a stable marker of MRSA, it was also detected among MSSA isolates, but *pvl* carriage was much more frequent in MRSA strains (73.33%) than MSSA strains (50%). The obtained result concurs with data from a previously published study in India (29). This could have substantial implications on the importance of this virulence factor among resistant strains. However, this is a departure from the situation in a previously published work in the Czech Republic, where the gene coding for *pvl* was more frequent in MSSA isolates that remain an important source of infection, suggesting that MRSA has not replaced MSSA strains (30).

In the current study, we utilized *spa* genotyping as a simple, rapid and practical molecular method to monitor variations in MRSA and MSSA isolates from various sources. PCR amplification of *spa*-X region revealed that all *S. aureus* isolates were PCR positive for *spa* gene. Similar results were reported by other researchers from Turkey as well (31). It is evident that most of the isolates (90%) yielded single PCR product, while the reminding isolates (10%) had double PCR products. Likewise, in a previous study in North of Iran, it was reported that 10.6% of *S. aureus* isolates displayed double bands of *spa* gene and the reminding isolates (86.1%) had one band (32). In concordance with another study conducted in Pakistan, our isolates were divided into different groups according to the *spa* polymorphisms, where five PCR products of different sizes were observed ranging from 200 to 500 bp (33).

SCC*mec* typing is one of the most important molecular tools available for understanding the epidemiology and the genetic relatedness of MRSA isolates. The SCC*mec* element carried by 30 MRSA strains was identified by using the multiplex PCR. However,

five isolates (16.67%) could not be typed based on the established protocol. Other workers have identified strains whose SCC*mec* elements are nontypeable (28). Surprisingly, the nontypeable isolates detected in this study carried the *mec*A gene, but *ccr* genes could not be amplified. The explanation for this observation, as quoted by others, may be related to unrecognized types of *ccr* genes, the deletion of *ccr* genes from SCC, a high mutational rate in the primer-targeting regions in these ''nontypeable'' SCC*mec* isolates or *mec*A is transferred independently of *ccr* (34). The present study showed a clear overall predominance of MRSA strains carrying SCC*mec* type IV (48%). This finding confirms a tendency seen in a previous study in Basel (42.9%) (35). The previously dominance of SCC*mec* type IV in different genetic backgrounds is compatible with the suggested enhanced mobility of this *mec* element (36), perhaps because it is smaller than the other SCC*mec* types. Moreover, our data demonstrated the high prevalence of SCC*mec* type II in MRSA isolates from human origin (33.33%). In Middle Tennessee, as parallel to the above finding, the most common SCC*mec* type was found as type II (34.1%) in MRSA strains from human (37).

The coagulase gene amplification, followed by RFLP analysis of the amplicons is a rapid and reproducible method for the discrimination of *S. aureus* isolates. In the present study, 50 *S. aureus* isolates could be classified into eight different coagulase genotypes based on the sizes of their PCR amplified products; a 750 bp product was found to be prevalent in 38% of the isolates. In a previous study carried out in Turkey, 47 *S. aureus* isolates could be classified into eight groups based on the polymorphism of *coa* gene products and 42.56% of these isolates showed a 780 bp PCR product (38).

In an attempt of coagulase gene typing of *S. aureus* strains in Canada, MRSA isolates were classified into only two *coa*-RFLP patterns, while MSSA were divided into eight patterns (39). This is contrary to the results of the present study, where higher diversity in *coa*-RFLP patterns was observed; MRSA and MSSA were classified into seven and ten distinct *coa*-RFLP banding patterns, respectively. In another study conducted in Egypt, coagulase gene PCR-RFLPs of MRSA isolates exhibited 10 patterns that ranged from 1 to 8 fragments with *Alu*I digestion (40).

Finally, in order to describe the *S. aureus* population in our report, we recorded bacterial genotyping and antibiotic susceptibility results to characterize a collection of 30 MRSA and 20 MSSA isolates. MRSA is still a dominant HA- MRSA. However, there are ongoing changes in the epidemiology of MRSA. Therefore, there is an evolution of so-called CA-MRSA which grave characteristics distinct from those of the traditional HA-MRSA.

Analysis of MRSA isolates showed a higher antimicrobial susceptibility rates among SCC*mec* types IV and V strains compared with those of SCC*mec* types I, II and III in addition to a high prevalence of multidrug resistance to non β-lactam among SCC*mec* types I, II and III isolates. In light of this and consistent with a previous report in Netherlands (41), the SCC*mec* types IV and V strains examined here eventually exhibited traits typical for CA-MRSA and those of SCC*mec* types I, II and III matched the criteria of HA-MRSA concerning the SCC*mec* complex analysis and antimicrobial susceptibility patterns. The increased resistance to non β-lactam antibacterials found in the SCC*mec* types I, II and III isolates may be partly due to the fact that these cassettes contain a variety of additional drug resistance gene elements not found within SCC*mec* types IV and V cassettes (41). Nevertheless, there were high levels of resistance to other drug classes observed among the SCC*mec* type IV isolates (91.67, 58.33 and 100% were resistant to erythromycin, ciprofloxacine and tetracycline, respectively). Recently, in a large MRSA surveillance study conducted in San Francisco, almost 90% of their MRSA isolates of SCC*mec* type IV were resistant to erythromycin, over 60% were resistant to ciprofloxacin and over 24% were resistant to tetracycline (42).

Moreover, the data presented here showed that there was higher prevalence of *pvl* genes among the representative MRSA isolates, where all isolates of SCC*mec* types I, II and III (100%) and 50% of both MRSA isolates of SCC*mec* types IV and V harbored *pvl* gene. These findings complemented the results of a recent study in New York, where 75% of MRSA isolates of SCC*mec* type IV were found to contain the *pvl* genes (43).

The presence of *pvl* gene in diverse genetic backgrounds suggests that their horizontal transfer into resident *S. aureus* has occurred repeatedly. In line with this outcome, the considerable occurrence of *pvl* among *S. aureus* of different genetic background, together with the negligible differences in the antibiotic resistance profiles of MRSA strains demonstrated that the boundaries between HA and CA strains are getting blurred. This may be due to simultaneous horizontal transmission of multiple HA-MRSA strains in the community. Additionally, CA MRSA is more wide-spread and has no definite spreading vicinity as reported previously in a recent report in Egypt (44).

It also claimed that the ratio of resistance among *pvl* positive MRSA and MSSA strains was relatively high as parallel to the results obtained previously in UK (27). These findings further highlighted the clinical importance of *pvl* positive *S. aureus* strains to public health and may be crucial in international monitoring of MRSA strains with high virulence potential in Egypt as was previously recorded (45). As a result of acquired resistance by *pvl* positive strains, clinicians are likely to encounter difficulties in treating and managing resistant strains occurring in the community.

With regard to calculation of the discriminatory power of all typing methods used in the present study, *coa* typing was useful for discriminating both MRSA and MSSA isolates with DI- values of 0.756 and 0.774, respectively. Similarly, the discriminatory index of PCR-based typing method targeting the *coa* gene has been reported by a previous study in Turkey (DI- value = 0.75) (38).

This large scale surveillance study provided useful information on the antibiotic resistance and molecular diversity of MRSA and MSSA populations from human and animal origins in Egypt. This is the first attempt of using several methods, in combination, for characterization of MRSA and MSSA populations in Egypt. Considering all data of these molecular techniques, SCC*mec* and *coa*-RFLP typing proved to be useful tools for rapid

and inexpensive discriminatory typing of MRSA and MSSA isolates, respectively. Importantly, our study defined the MRSA isolates as hospital or community acquired on the basis of antimicrobial susceptibility patterns and *SCCmec* analysis. Even more alarming observed in the present study are the high proportion of *pvl* positive isolates and the emergence of multi-drug resistance in MRSA suggesting that the criteria for classifying the MRSA isolates as either CA- or HA-MRSA are blurring. In view of the high prevalence of multi-drug resistant *pvl* positive isolates, regular surveillance of antibiotic sensitivity pattern, together with implementation of appropriate infection control strategies are overwhelmingly mandatory to counter the dissemination of these strains in the community.

Authors' contributions

Marwa I. Abd El-Hamid was the principal investigator; performed data entry and analysis, laboratory work and manuscript drafting and M. Bendary participated in the planning and execution of the study and reviewed draft manuscript.

Acknowledgments

The authors are thankful to Dr. Afifi, M. (Biostatistics Assistant Lecturer), faculty of Veterinary Medicine, Zagazig University for his valuable assistance in performing the statistical analysis.

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