Original article

Identification of community-acquired methicillin-resistant
*Staphylococcus aureus* (CA-MRSA) causing hospital-acquired
infections in Suez Canal University Hospitals, Egypt by
detection of its major virulence determinants

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ABSTRACT

Background: Community acquired methicillin resistant *staphylococcus aureus* (CA-MRSA) is emerging in hospitals, worldwide. Method: In Suez Canal University Hospitals (SCUH), we documented CA-MRSA causing hospital acquired- (HA-) sepsis, by polymerase chain reaction (PCR) amplification of CA-MRSA major virulence determinant genes, namely: Panton-Valentine leukocidin "pvl", accessory regulator "agr" I and III, and staphylococcal cassette chromosome mec "SCCmec" genes IV and V. Antibiograms were determined by disk diffusion and minimum inhibitory concentration (MIC). Results: Methicillin resistant *staphylococcus aureus* was detected in 90 hospital acquired infections (HAIs), including bacteremia, pneumonia, osteomyelitis, soft tissue infections, and meningitis. *Pvl* gene, characterizing CA-MRSA, was detected in 24/90 MRSA strains (26.7%). *Pvl*+ve strains were subtyped into SCCmec gene type II (8.3%), type IV (75%), type V (8.3%), and 8.3% were non-typeable. They showed only *agr* group I (62.5%), and III (37.5%). Co-trimoxazole resistance was detected in 41.6% of CA-MRSA. Only 12.5% of CA-MRSA strains were susceptible to all non-beta-lactam drugs. There was no statistical correlation between SCCmec or *agr* groups, and co-trimoxazole resistance in CA-MRSA; nor between SCCmec types and *agr* groups. Conclusion: Community aquired-MRSA is emerging in all wards of SCU hospitals, causing HAI, mostly soft tissue infections. The classical antibiogram of CA-MRSA is no longer prevailing. Diagnosis of CA-MRSA should rely upon detection of *pvl* gene, rather than classical and antibiogram-profiles. The name "CA-MRSA" is no longer satisfactory to describe such strains in hospital settings; instead, *Pvl* +ve MRSA is more accurate and reliable term to use.

Introduction

The emergence of CA-MRSA has changed the clinical and molecular epidemiology of *S. aureus* infections during the past twenty years [1]. Owing to its low rates of resistance to non-β-lactam antimicrobials, and the relatively high morbidity, CA-MRSA strains could be suspected clinically. Community acquired-MRSA infections traditionally occur in non-hospitalized, apparently healthy individuals, while HA-MRSA infections occur in individuals with risk factors for infection, such as, surgery or immune-suppression [2]. Community acquired-MRSA outbreaks have occurred among
groups of children, prisoners, members of sport teams and military personnel [3]. A third type of human MRSA infection is known as livestock-associated MRSA (LA-MRSA), first isolated in 2003, and was found to be transmitted from a reservoir of clonal complex (CC) 398 lineage isolated from pigs and cattle. All three types of MRSA infection share common characters with variable differences in genetic makeup, virulence factors, pathogenesis, epidemiologic aspects and antibiotic resistance [4].

Centers for Disease Control and Prevention (CDC) criteria stated that CA-MRSA infections typically manifest as skin and soft tissue infections, however, the pathogen can cause life-threatening infections, including osteomyelitis, necrotizing pneumonia, and fatal sepsis [5]. There is no single definition that can reliably distinguish CA-MRSA from traditional HA-MRSA. The term “CA-MRSA” has been used to describe the source of MRSA infection, the antibiogram pattern, and the genetic profile of the organism, which may not be sufficient to define the source of infection. Community acquired-MRSA clones usually cause community-onset infection and are usually non–multidrug resistant. They carry the lukSF-PV genes, which encode Panton-Valentine Leukocidin toxin which is associated with more severe virulence. Recent data showed that MRSA contains up to 11 \textit{SCCmec} gene types (I-XI), of which CA-MRSA is confined mainly to types IV, V, and VII, according to International Working Group on the Staphylococcal Cassette Chromosome elements (IWG-SCC) [6]. These types and subtypes can be discriminated by PCR-based or DNA sequencing-based protocols. \textit{SCCmec} type IV is the most isolated type among CA-MRSA, which is defined by a class B \textit{mec} complex and a \textit{ccrAB} allotype 2. \textit{SCCmec} type IV is the smallest structural \textit{SCCmec} type that is considered to be the most mobile version. Perhaps, as a consequence of its enhanced mobility, \textit{SCCmec} type IV is also more variable than other \textit{SCCmec} types, with seven recognized subtypes (IVA through IVg), differing mainly in the JI region [7]. Community acquired-MRSA strains also carry \textit{agr} gene group I and III; however, there are exceptions to these rules [8]. In Suez Canal University Hospitals (SCUH), during the past few months, it was noticed that most of nosocomial \textit{Staphylococcus aureus} strains are MRSA (unpublished data). These strains varied in antibiogram profile as well as in degree of virulence and morbidity. In order to determine the contribution of CA-MRSA in the hospital community, we decided to go further into the precise identification of CA-MRSA among the nosocomial MRSA isolates, using non-routine, molecular characterization methods.

**Material and Methods**

Isolated nosocomial \textit{Staphylococcus aureus} strains were subjected to the following procedures:

1) **Phenotypic detection of methicillin-resistance** by agar disk diffusion on Mueller-Hinton (MH) agar, using cefoxitin disk (30µg, Oxoid). \textit{S. aureus} ATCC 43300 and ATCC 25923 strains were used as positive and negative control strain, respectively [9].

2) **DNA extraction and purification** by heating the water suspension of strains at 98°C for 15 min, centrifuging at 10,000 rpm for 1 min. The supernatant was used for PCR analysis [10]. DNA concentration and purity were checked by running in 1.5% agarose gel electrophoresis and its concentration was determined by ND1.0.0.0 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

3) **Detection of mecA gene** was done by PCR to confirm the presence of methicillin-resistance gene. The primers used were: F 5’ GTG AAG ATA TAC CAA GTG ATT 3’; and R 5’ ATG CGC TAT AGA TTT AAA GGA T 3’. Using SenoQuest labcycler® thermocycler, an initial denaturation was done at 94°C (5 min), followed by 10 cycles consisted of: 94°C (45 sec), 65°C (45 sec), and 72°C (90 sec). This was followed by another 25 cycles of: 94°C (45 sec), 55°C (45 sec), and 72°C for 90 seconds. A final extension step was done at 72°C for 10 min, followed by a hold at 4°C. PCR amplicons (147 bp) were visualized using UV light, in 2% agarose gel containing 0.5 µg/ml ethidium bromide.\textit{S. aureus} NCTC8325, and ATCC 25923 were used as positive control strain, respectively [10].

4) **PCR Detection of pvl gene** in MRSA isolates was performed using following primers: **Forward**: 5’ TGC CAG ACA ATG AAT TAC CCC CAT T 3’; and **Reverse**: 5’ TCT GCC ATA TGG TCC CCA ACC A 3’. After 30 cycles of amplification, which consisted of denaturation at 94°C (30 sec), annealing at 55°C (30 sec), and extension at 72°C (60 sec). PCR products were visualized in 2% agarose gel [11]. The following 16s primer was used as internal control: **Forward** 5’ CGG TTA CCT TGT TAC GAC 3’, and **Reverse** 5’ AGA GTT TGA TYM TGG CTC 3’. \textit{S. aureus} NCTC13300 was used as pvl + control strain,

5) **DNA Extraction for multiplex PCR reaction**: Preserved MRSA isolates were subcultured with rotation in brain heart infusion broth (Oxoid). DNA was extracted using Genna Bioscience Bacterial...
Extraction kit (Genna Bioscience, Germany) according to manufacturer instructions.

6) SCCmec Typing by multiplex PCR of pvl1, MRSA strains

Four sets of primers were used to amplify specific DNA targets within the SCCmec gene. First set primer B: 5’ ATT GCC TTG ATA GCA GCC YTC T 3’; and primer a3: 5’ TAA AGG CAT CAA TGC ACA AAC ACT 3’, that produced 937 bp band. Second set primer ccrCF: 5’ CGT CTA TTA CAA GAT GTT AAG GAT AAT 3’; and primer ccrCR: 5’ CCT TTA TAG ACT GGA TTA TTC ATA AGC TGC GTA ATACCCG-3’, that produced 439 bp band; agrSa2-2: 5’ TAT TAC TTA TTG AAA AGT GCC ATA GC 3’, that produced 572 bp band; agrSa2-2: 5’ GTA ATG TAA TAG CTT GTA TAA TAC CCA G-3’, which produced 321 bp band; and and primer 1272F1: 5’ CGT CTA TTA CAA GAT GTT AAG GAT AAT 3’, that produced 937 bp band; and primer 1272R1: 0.5 µl each, and primer 5R431: 5’ CGGCTACAGTGATAACATCC 3’ that amplified 415 bp segment. PCR was performed in SenoQuest labcycler®. The reaction volume of 50µL contained: 25 µl of QIAGEN® Master Mix. Based on initial optimization experiments, primers concentration was brought to 10 pmol. Working primer concentrations were as follows: primers β and α3: 1.2 µl each; primers ccrCF and ccrCR: 1.25 µl each; primers 1272F1 and 1272R1: 0.5 µl each, and primers 1272F1 and 1272R1: 0.5 µl each. Amplification reaction included an initial denaturation at 94°C (4 min), followed by 30 cycles of: 94°C (30 sec), 55°C (30 sec), and 72°C (60 sec). Cycles were followed by a final extension step at 72°C for 10 min. Amplicons were electrophoresed in a 1.5% agarose gel with 0.5 µg/ml ethidium bromide and visualized by UV. S. aureus positive control strains NCTC10652 for agr group 1; NCTC10654, NCTC10655, and NCTC10656 strains for agr group 2; and ATCC27664 for agr group 3, were used. Automated PCR products analysis was also performed for more accurate detection of different agr groups, using QIAGEN capillary (QIAxcel ScreenGel 1.0.0.0) gel electrophoresis, according to manufacturer’s instructions [13].

8) Antimicrobial susceptibility of MRSA strains

was determined by modified Kirby-Bauer disk diffusion method, using (MH) agar medium (Oxoid), and the antibiotic disks (Oxoid): Co-Trimoxazole (1.25/23.75µg), amikacin (30µg), levofloxacin (5µg), linezolid (30µg), erythromycin (15µg), and clindamycin (15µg), while E-Test strips were used to determine MIC of vancomycin (E-test, AB BIODISK, Sweden) [9]. D-test was done to detect induced methylase-resistance to clindamycin. Statistical analysis to show correlation between genotypic and phenotypic characters was done.

Results

was detected in 90 isolates (Figure 1). Pvl gene - characterizing CA-MRSA- was detected in 24/90 MRSA strains (26.7%) (Figure 2); of which 13 strains (54%) were isolated from skin and soft tissue infections (Table 1). Identified SCCmec gene types in pvl+ strains were type II (2 strains, 8.3%), type IV (18
strains, 75%), type V (2 strains, 8.3%), and 2 strains were non-typeable (Figures 3&4). Pvl+ strains were classified into agr groups; 15 strains (62.5%) belonged to agr group I, followed by group III (9 strains, 37.5%). None of the isolates belonged to groups II or IV (Figures 5&6). All MRSA isolates were susceptible to vancomycin and linezolid (Table 2). Co-trimoxazole resistance was detected in 41.6% of pvl+ strains. None of pvl+ MRSA strains was resistance to amikacin, while 38% of pvl- isolates showed amikacin resistance. CA-MRSA (pvl+) strains showed 7 different antibiogram patterns, with the most frequent pattern was the resistance to co-trimoxazole and susceptibility to all other tested antibiotics (10 isolates, 41.6%). The classic antibiogram pattern in which CA-MRSA strains are susceptible to all non-β-lactam, anti-Gram-positive antibiotics was seen in 3 (12.5%) CA-MRSA isolates. There was no statistical correlation between SCCmec or agr groups, and co-trimoxazole resistance in CA-MRSA isolates (correlation coefficient=0.166; and 2-tailed p-value=0.538). Similarly, no statistical correlation between SCCmec types and agr groups (correlation coefficient=0.017; and 2-tailed p-value=0.95).

**Figure 1. Detection of mecA gene.** Left lane, DNA 100 bp Ladder; next lane negative control, Lane 1-9 mecA-positive; Lane 10 is mecA positive control.

![Figure 1](image)

**Figure 2. Detection of pvl gene.** First Left lane, 100 bp DNA Ladder; Lane 1-8 pvl-positive; Lane 9 and 10, pvl-negative; Last Lane (#11) is 16s primer as internal control.

![Figure 2](image)
Figure 3. Gel electrophoresis of SCCmec types. First left Lane, 100 bp DNA Ladder; Lane 1-6, SCCmec type IV; Lane 7, SCCmec type II; Lane 8, SCCmec type III (a pvl-negative strain); Lane 9, non-typable (NT); Lane 10, empty lane.

Figure 4. Capillary gel electrophoresis showing SCCmec types of pvl-positive strains. Lane A1, DNA Ladder

Figure 5. Agarose gel electrophoresis showing agr groups. Right lane, 100 bp DNA Ladder; Lanes 2,4,8,10, and 11, agr group I; Lane 5,7 and, agr group III; lanes 1,3, and 6, no amplicons.
Figure 6. *Agr* gene groups showed by automated QIGEN Capillary electrophoresis. Lane A1, DNA ladder; Lanes A2, A7, A9, and A11, group III amplicon of 321bp; Lanes A3, A4, A5, A6, A8 and A10, group I amplicon of 439 bp; Lane A12, non-specific band smearing.

Table 1. MRSA strains isolated from different infection sites.

<table>
<thead>
<tr>
<th>Site of MRSA infection</th>
<th>Pvl +ve MRSA (n=24)</th>
<th>Pvl –ve MRSA (n=66)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>No. (%)</td>
</tr>
<tr>
<td>Skin and soft tissue</td>
<td>13 (54)</td>
<td>36 (54.5)</td>
</tr>
<tr>
<td>Blood</td>
<td>3 (12.5)</td>
<td>9 (13.6)</td>
</tr>
<tr>
<td>Lower respiratory tract</td>
<td>4 (16.6)</td>
<td>5 (7.6)</td>
</tr>
<tr>
<td>Osteomyelitis</td>
<td>4 (16.6)</td>
<td>15 (22.7)</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>0 (0)</td>
<td>1 (1.5)</td>
</tr>
</tbody>
</table>

Table 2. Antibiotic resistance in 24 *pvl* + and 66 *pvl* – MRSA isolates

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Resistant <em>Pvl</em> + strains n (%)</th>
<th>Resistant <em>Pvl</em> – strains n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co-trimoxazole</td>
<td>10 (41.6)</td>
<td>25 (37.8)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>0.0</td>
<td>21 (31.8)</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>7 (29.1)</td>
<td>25 (37.8)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>6 (25)</td>
<td>30 (45.4)</td>
</tr>
<tr>
<td>Levofloxacain</td>
<td>6 (25)</td>
<td>21 (31.8)</td>
</tr>
<tr>
<td>Linezolid</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>
Discussion

The epidemiology of CA-MRSA around the world is surprisingly heterogeneous. This is due to the different reports of CA-MRSA emerging in health facilities, and rarity of current local data about these strains. We conducted this study to clarify the situation in SCUH; to document the presence of these strains, and to characterize the isolates, from both phenotypic and genotypic aspects so as to be aware of - and thus control- such infections in the future. Study design ensured selection of nosocomial MRSA isolates from different clinical wards to detect CA-MRSA strains among them. With rarity of past local published data in this aspect, this work will replenish the data in this concern.

Through ten months, 120 S. aureus strains were isolated out of 474 nosocomial samples (25.3%), from which 90 MRSA strains were identified. Recent reports in Egypt showed that 47.5%, 61%, and 100% of nosocomial S. aureus isolates were MRSA [14-16]. One of the reasons for such high rates could be the high rate of MRSA colonization in the Egyptian community. A study conducted in Minya governorate (upper Egypt) revealed 14.5% nasal colonization by MRSA [16], while higher rate of colonization (52%) was reported in Menofiya governorate, in the Nile Delta region [14]. This may be attributed to the lack of strictly applied antibiotic policy in most hospitals, and the irrational empirical use of antibiotics "over-the-counter" by the publics which favor the selection of resistant mutant strains. This led to the predominance of MRSA strains as permanent human colonizers, even in healthy people. Environmental factors help transmission of MRSA infection / colonization, such as crowdedness, working close to domestic animals, poor housing conditions, and lack of personal hygiene. As a result, CA-MRSA found its way to our hospitals through colonized patients, visitors and health-care workers. In our study, most of MRSA were isolated from skin and soft tissue infections (54.4%) (Table 1). This high prevalence compared to other sites really points to a defect in infection control measures during peri-operative care. It may also reflect high colonization rate among healthcare workers (HCW). Limited information exists regarding prevalence of CA-MRSA infections in Egypt. We tried to explore the unrevealed epidemiologic aspect of CA-MRSA infections, in SCUH. In this study we used the 48-h criterion to define nosocomial infection. Meanwhile, we used the genetic criteria to define CA-MRSA isolates, i.e., the acquisition of pvl gene, and the presence of SCCmec type IV or V, and agr group I or III. We specifically used pvl gene as a genetic marker for CA-MRSA since it is documented to be in more than 95% of all CA-MRSA isolates [17].

It is becoming more and more difficult to categorize CA-MRSA and HA-MRSA according to epidemiological criteria alone, such as duration of healthcare exposure, since typical CA-MRSA strains may cause nosocomial infections and vice-versa. The study results showed that 26.7% of all isolated nosocomial MRSA contained pvl gene. This documented CA-MRSA as a considerable contributor to nosocomial infections in SCUH, although pvl-ve MRSA are still dominating. Previous epidemiological data on prevalence of pvl-ve MRSA in SCU hospitals are essential to determine the degree of replacement of HA-MRSA by CA-MRSA. Unfortunately, such data are not available, for comparison. However, previous studies conducted in Egypt on outpatients showed different isolation rates for pvl+ve MRSA strains: these showed 19% [18], 33.3% [14] for septic lesions, and 15% [15] in healthy nasal carriers. Few studies showed that CA-MRSA circulating in Egyptian community are not uncommon. Other studies conducted in Egypt, on nosocomial infections showed data on MRSA isolates, without further identification of genetic markers that identify CA-MRSA strains.

Our CA-MRSA isolates carried the small 20.9-24.3 KD, mobile, SCCmec type IV gene which has an increased capacity to form biofilm. These genes may contribute to a higher growth rate, and capacity for persistence in host lesions [19]. The ability of tissue invasion and dissemination can be enhanced by the acquisition of additional virulence factors like PVL. This may explain the increased frequency of CA-MRSA observed in the hospital and community. The high transmissibility of CA-MRSA clones in the community, especially among people with direct contact to our patients, may well have increased the introduction of CA-MRSA into our hospitals. Likewise, we can speculate that an increase in CA-MRSA colonization in HCW subsequently lead to an increase in transmission in hospitals. Indeed, frequent introductions of a pathogen -even with low transmissibility- may still lead to patient-to-patient transmission. Global reports from different location showed further discrepancy. A study conducted in medical center in Chicago, USA, showed that 66% of nosocomial MRSA infection was due to CA-MRSA strains, especially USA300 clone. On the other hand, a study performed in a Danish Hospitals revealed that CA-MRSA accounted only for 12.8% of nosocomial MRSA infections [20]. Variation in infection rates

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between hospitals may be due to variations in infection control practices, pre-admission screening of patients for MRSA and subsequent cohort isolation of colonized subjects, with other variables that influence the transmission from non-isolated patients (such as contact rates, and consequently staffing levels, beds per room, room sizes and patient turn-around time).

Joo and co-workers [21] described clinical characteristics of nosocomial MRSA infections by a community-genotype of sequence type (ST) 72 in Korean hospitals. They concluded that as community-genotype MRSA strains spread into hospitals, the genotypes of the MRSA strains that cause hospital-acquired infections have become more diverse [21].

In our study, 75% (18 strains) of pvl+ isolates carried the SCCmec type IV, while type V, type II, and non-typeable appeared in two isolates/each. We used SCCmec typing protocol developed by Boye et al. [12]. It is a quick and easy to interpret method based on single-tube multiplex PCR reaction, using primers for specific detection of both mec class and ccr type of SCCmec type I to V. It is not useful in subtyping; moreover, this method may also misclassify SCCmec type I with type VI. However, this method can be used for confirmation of doubtful SCCmec types. In our study, two strains were non-typeable, even after repeating the SCCmec typing assay. These strains may belong to types VI through XI which are not included in the used protocol. According to figures (5&6), pvl+ strains were subtyped into agr I (15 strains, 62.5%) and III (9 strains, 37.5%). This result agreed with most studies concerning CA-MRSA agr sub-grouping [22, 23]. There was no statistically significant correlation between SCCmec types and agr sub-grouping detected in this study. Pvl+ Isolates showed different antibiogram patterns denoting heterogeneity of our isolates (16 isolates showed 7 different patterns). This may be due to the different sources of isolates, since SCUH are providing the service to patients from more than five adjacent governorates, with multiple demographic criteria. The most prominent antibiogram pattern of CA-MRSA in this study was the increased resistance to co-trimoxazole (41.6%). This phenotype is very unusual among CA-MRSA. Although it was previously found in the Egyptian pvl+ ST80 CA-MRSA [18], it was not documented in the European pvl + ST80 CA-MRSA strains. Many reports showed that CA-MRSA resistance to co-trimoxazole is rare.

Mendes and his coworkers showed that the typical antibiotic profile of USA300 is the susceptibility to co-trimoxazole, clindamycin, and tetracycline and its resistance to erythromycin and gatifloxacin [24].

Chua and co-workers mentioned that CA-MRSA strains from different regions, e.g., USA400 from USA, ST59-MRSA VT/IV from Asia, and ST93-MRSA IV, ST1-MRSA IV and ST30-MRSA IV from Australia; were almost 100% susceptible to co-trimoxazole [20]. In our study, co-trimoxazole resistance showed no statistical correlation with SCCmec types or agr groups, suggesting that it is not a clone-related phenotype. Our data showed a combined resistance to co-trimoxazole and clindamycin in 29.1% of pvl+ isolates. Another finding in this work is the susceptibility of all CA-MRSA isolates to amikacin, while 31.8% of pvl- HA-MRSA isolates showed resistance to same drug. We found two reports with high susceptibility of CA-MRSA to amikacin: 93% [25] and 88.8% [26]. Our study showed that resistance to erythromycin was 25%, whereas the clindamycin resistance rate was also 29.1%. In USA300 isolates, resistance to erythromycin was almost uniform (92.8%), whereas clindamycin resistance rate was 6.5% [5]. ST59 CA-MRSA, commonly isolated from Asian countries is typically resistant to erythromycin (89%). On the contrary, an elevated susceptibility level was reported in ST389 isolated from Europe (77%) [20]. Generally, we can conclude that the classic antibiogram pattern of CA-MRSA is no longer prevailing among our patients; a fact that further documents that pvl+ strains are exchanged between patients themselves, and between patients and HCW and visitors in our hospitals, which makes them exposed to various antibiotic therapies and acquiring such unusual antibiotic resistance. No correlation was found between different drug resistance in CA-MRSA strains and their SCCmec and agr groups. We can also conclude that diagnosis of CA-MRSA should rely upon detection of pvl gene, rather than clinical and antibiogram-dependent diagnosis. The high frequency of pathogenic CA-MRSA strains within our hospitals, and the uncommon antibiogram pattern they acquire, strongly suggest that the term "CA-MRSA" is no longer satisfactory to describe such MRSA isolates in hospital settings; instead, pvl+ MRSA is more accurate and reliable term to use. Furthermore, after exploring this aspect of CA-MRSA, it is now clear to us that this work needs more extension in many aspects. Another step of diagnosis is needed, including Pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) to define the specific isolated clones. A national surveillance program should be established to characterize isolates from carriers and infected individuals with MRSA in different Egyptian
geographic regions. Identification of isolates at the level of clonal complex will clarify the clones circulating in Egyptian community and hospitals and define their genetic and phenotypic characters.

Conflicts of interest: None.

Financial disclosure: None.

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