Association of tsst-1 and pvl with mecA Genes among Clinical Staphylococcus aureus Isolates from a Tertiary Care Hospital

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Abstract

To investigate the association of both tsst-1 and pvl with mecA genes in clinical Staphylococcus aureus (S. aureus) strains obtained from a tertiary care hospital, Mansoura-Egypt. Besides, relating these genes to antibiotic resistance patterns in such isolates. A prospective study was performed over clinical samples obtained from patients showing evidence of infection at Mansoura University Children Hospital, Egypt. Staphylococcus aureus isolates were obtained to test their antibiotic sensitivity pattern by disk diffusion method, E-test strips besides oxacillin agar screen test followed by screening such isolates for mecA, pvl and tsst-1 genes by polymerase chain reaction. Out of 180 isolated S. aureus strains, 88 isolates (48.9%) were methicillin resistant. The tsst-1 gene was positive in 65.9% of methicillin resistant S. aureus (MRSA) and 55.4% of methicillin sensitive S. aureus (MSSA) strains. Carriage of mecA and tsst-1 genes was not significantly associated (P=0.69). The pvl gene was positive in 23.9% of MRSA and 9.8% of MSSA isolates showing a significant association with mecA gene (P=0.04*). The mecA gene was significantly associated with multidrug resistant isolates (p= 0.041*). There was no significant association between antibiotic resistance and the presence of tsst-1 or pvl genes. The tsst-1 gene had a high prevalence among S. aureus isolates and it was similarly distributed in both MRSA and MSSA groups. The pvl gene was significantly associated with MRSA group. Antibiotic resistance pattern of S. aureus was not significantly associated to the presence of tsst-1 or pvl genes in any way.

Keywords: Staphylococcus aureus, MRSA, mecA gene, pvl gene, tsst-1 gene, PCR.

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INTRODUCTION

Staphylococcus aureus (S. aureus) is a major bacterial human pathogen held responsible for both community-acquired as well as hospital-acquired related infections, that might cause a wide variety of clinical manifestations including sepsis, respiratory tract, surgical site and urinary tract infections. Staphylococcus aureus infection remains a tough challenge to manage due to the emergence of multi-drug resistant strains that led to drag its clinical importance under the spotlight over the past decades. Methicillin resistant S. aureus (MRSA) strains show resistance to a large group of beta-lactam antibiotics including penicillins and cephalosporins. The MRSA strains produce an altered penicillin-binding protein-2, referred to as PBP2a encoded by mecA gene, such protein has more resistance to beta-lactam antibiotics when compared to the original PBP2.

Many virulence factors are responsible for the unique pathogenicity of S. aureus such as toxins, enzymes and adhesion factors. The toxins produced by S. aureus include Panton-Valentine leukocidin (PVL), hemolysin, toxic shock syndrome toxin-1 (TSST-1), exfoliative toxins and staphylococcal enterotoxins. The PVL is a pore forming leukotoxin encoded into by pvl gene and has the ability to target and kill host leukocytes through perforation of plasma membrane together with intracellular organelle membranes. The PVL producing S. aureus strains can cause various infections ranging from as simple as infections of skin and soft tissue up to serious life endangering conditions such as severe necrotic pneumonia.

Nevertheless, another toxin known as TSST-1 encoded by tsst-1 gene is also produced by S. aureus strains, and its expression can lead to an acute life threatening toxic shock syndrome (TSS) that can present itself with rash, fever and multi-organ dysfunction. A strong relation was found between the TSS and tampon use by women due to toxin released by colonizing S. aureus strains in the vagina. However, the TSS can also occur in other different conditions such as surgical site complications, skin and respiratory tract infections. Having said that, the secretion of TSST-1 into blood also increases the rate of TSS-like neonatal exanthematous lesion, sudden infant death syndrome and Kawasaki syndrome in pediatric age group. The improper timing of prescribing the necessary antimicrobial treatment for patients with TSS may lead to fatal shock within hours after the onset of clinical presentations. Therefore, to improve the outcome of such patients it is extremely important to have sufficient data about the tsst-1 gene prevalence and its relation to antibiotic susceptibility pattern.

In literature, many studies have reported an alarming increase in the MRSA strains positive for tsst-1 and pvl genes. Similarly, previous studies conducted in Egypt detected tsst-1 and pvl genes among MRSA strains. Toxins encoded by tsst-1 and pvl genes can cause severe infections such as TSS and necrotic pneumonia. Treatment of such infections is even more challenging when caused by MRSA because of the mecA gene-associated antimicrobial resistance. Therefore, investigating the prevalence of these toxins-encoding genes among MRSA isolates in different geographical locations is necessary for proper treatment and control of these infections. Moreover, these data will enable us to set up a proper antibiotic policy to improve the outcome of these cases. In our study, we investigated the association of both tsst-1 and pvl with mecA genes among clinical S. aureus isolates obtained from a tertiary health care center, Mansoura-Egypt. In addition, the relation of these genes to antibiotic resistance patterns was evaluated.

MATERIALS and METHODS

This prospective study was undertaken at the Medical Microbiology and Immunology Department, Faculty of Medicine, Mansoura University-Egypt. The protocol was revised and proved to be fulfilling ethical criteria by our institutional review board.

Sample Collection

Various clinical samples were collected from inpatients and outpatients showing evidence of infection at Mansoura University Children Hospital, Mansoura-Egypt from January to October 2018. Suggestive signs and symptoms differed according to the site of infection such as fever, dysuria, suprapubic tenderness, dyspnea, tachypnea, purulent sputum, wound pain or tenderness, purulent wound drainage, purulent eye secretions, etc. Samples included blood, urine, endotracheal aspirates, wound drainages, sputum, eye secretions and abscesses. Enrolled...
samples were sent to the Medical Microbiology and Immunology laboratory for processing.

**Microbiological Processing**

All samples were processed by standard microbiological techniques. Resulting *S. aureus* isolates were fully identified by colonial morphology, Gram-staining and biochemical testing including catalase, coagulase and mannitol fermentation. API Staph (bioMerieux, France) was also performed in order to confirm the identification of *S. aureus* isolates.

**Antimicrobial Susceptibility Testing**

Antimicrobial susceptibility pattern of *S. aureus* isolates was tested by disk diffusion method on Mueller-Hinton agar (MHA) using the following antibiotic disks (Oxoid, UK): penicillin (10 units), cefazolin (30mg), ciprofloxacin (5mg), erythromycin (15mg), clindamycin (2mg), Trimethoprim-sulfamethoxazole (1.25/23.75mg), gentamicin (10mg), linezolid (30mg) and tetracycline (30mg). Disk diffusion procedure was carried out according to the guidelines of clinical and laboratory standard institute (CLSI)\(^{16}\). Determination of the minimum inhibitory concentration for vancomycin was achieved by E-test strips (bioMerieux, France) on MHA plates following the manufacturer’s guidelines. The breakpoints for resistance as implemented by the CLSI were used for results interpretation\(^{16}\).

**Detection of Methicillin Resistance**

Methicillin resistance in previously identified *S. aureus* isolates was phenotypically detected by cefoxitin disk diffusion and oxacillin agar screen tests following the CLSI guidelines. Cefoxitin disk diffusion test was conducted by standard disk diffusion procedure using cefoxitin (30mg) as a surrogate for oxacillin\(^{16}\). As recommended by the CLSI, oxacillin agar screen test was conducted using MHA containing 4% NaCl and 6mg/mL oxacillin. A half McFarland suspension was prepared from the *S. aureus* isolates. Then, a sterile swab was dipped in the prepared bacterial suspension. After expressing the excess liquid, an entire quadrant of the MHA plate was streaked to be incubated at 35°C for 24 hours and then examined with transmitted light. Growth of > 1 colony on this culture medium indicated methicillin resistance\(^{16}\). *Staphylococcus aureus* ATCC 29213 was used as negative control (methicillin sensitive) while *S. aureus* ATCC 33591 was used as a positive control (methicillin resistant). Isolates were then kept in tryptic soy broth supplemented by 30% glycerol at -70°C until further molecular testing. Being resistant to at least 3 or more antibiotic classes in addition to beta-lactams, define multidrug resistance\(^{17}\).

**DNA Extraction and Polymerase Chain Reaction**

Extraction of Genomic DNA was conducted by using an available commercial QiaAmpDNA Mini Kit (QIAGEN-UK) through applying the manufacturer’s guidelines. For detection of *mecA*, *pvl* and tsst-1 genes among the isolated *S. aureus* strains, polymerase chain reaction (PCR) was performed using specific primers as shown in Table 1\(^{18}\). The PCR was performed in a final 50mL volume reaction containing Taq PCR Master Mix (QIAGEN-UK), template DNA and specific primers.

PCR identification of the *mecA* gene was performed with the thermo-cycling conditions set at 5 min at 94°C for initial denaturation, followed by 40 amplification cycles: 94°C for 30s, 57°C for 45 s, 72°C for 30 s then final extension at 72°C for 5 min \(^ {18}\). Multiplex PCR for detection of *pvl* and tsst-1 genes was performed with the following cycling settings: 5 min at 94°C for initial denaturation followed by 40 cycles of amplification of successive

| Gene | Primer | Sequence (5‘→3’) | Product size (bp) |
|------|--------|------------------|------------------|
| *mecA* | F | AGAAGATGTTGATGGAAGATTAAG | 583 |
| | R | ATGTATGTCGATTGATTTGC | 583 |
| *pvl* | F | GGAACACATTATATTCTTCTTATAC | 502 |
| | R | CCGATTGAAAGTTACCTTCG | 502 |
| tsst-1 | F | TATCGTAAAGCCTTCATTTGG | 398 |
| | R | TAAAGATGTTCTATTGGAGT | 398 |
steps at 94°C for 40 s, 60°C for 40 s and 72°C for 1 min with a final extension at 72°C for 5 min. To determine the presence of the desired genes, PCR products were visualized by electrophoresis in agarose gel stained with ethidium bromide followed by UV lights examination. Reference strains of *S. aureus* ATCC 33591, *S. aureus* ATCC 49775 and *S. aureus* ATCC BAA-2094 were used as positive control for meca, pvl and tsst-1 genes respectively while distilled water was used as the negative control.

**Statistical Analysis**

Chi-square test was applied to compare variables and the results were presented as percentages (%). When P value was <0.05 the difference was statistically significant. We used SPSS 22 for Windows (SPSS Inc., Chicago, IL, USA).

**RESULTS**

A total of 180 *S. aureus* strains were isolated from the processed 375 clinical samples (48%) [88 Male (48.9%) and 92 Females (52.1%)], the mean± SD age was 6.1 ±2.8 years. Out of the *S. aureus* isolates, 75 (41.7%), 43 (23.9%) and 19 (10.6%) were recovered from blood, urine and endotracheal aspirates respectively as demonstrated in Table 2. Phenotypic evaluation of methicillin resistance by cefoxitin disk diffusion and oxacillin agar screen tests revealed that 88 out of 180 (48.9%) *S. aureus* isolates were methicillin resistant. The *meca* gene was further identified in all of these isolates that confirmed their primary identification as MRSA by phenotypic methods.

In the current study, we determined the distribution of *meca*, tsst-1 and pvl genes among the recovered *S. aureus* strains by PCR. Out of the 180 isolated *S. aureus* strains, 88 (48.9%), 109 (60.6%) and 30 (16.7%) isolates had the *meca*, *tsst-1* and *pvl* genes respectively (Table 3). Fig. 1 demonstrated a representative example of multiplex PCR for detection of *pvl* and *tsst-1* genes.

**Table 3. Distribution of meca, tsst-1 and pvl genes among Staphylococcus aureus isolates**

| Gene      | Staphylococcus aureus isolates (No=180) |
|-----------|----------------------------------------|
| meca positive | 88 (48.9%)                             |
| meca negative | 92 (51.1%)                             |
| tsst-1 positive | 109 (60.6%)                            |
| tsst-1 negative | 71 (39.4%)                             |
| pvl positive | 30 (16.7%)                              |
| pvl negative | 150 (83.3%)                             |

Values are expressed as No (%) Out of 88 isolated MRSA strains, 37 (42.0%) were positive only for the *tsst-1* gene and 21 (23.9%) were positive for both *tsst-1* and *pvl* genes. Among the 92 methicillin sensitive *S. aureus* (MSSA) isolates, 44 (47.8%) were positive only for the *tsst-1* gene, 2 (2.2%) were positive only for the *pvl* gene and 7 (7.6%) were positive for both genes as demonstrated in Table 4. Totally, the *tsst-1* gene was detected in 65.9% (58/88) of MRSA and 55.4% (51/92) of MSSA strains. No significant association was found between the carriage of *meca* and *tsst-1* genes (P=0.69). Regarding the *pvl* gene, it was found in 23.9% (21/88) of MRSA and 9.8% (9/92) of MSSA isolates. The *pvl* gene was significantly associated with the presence of *meca* gene (P=0.04).

Among the 88 *meca* gene positive *S. aureus* isolates, 39 (44.3%), 20 (22.7%) and 8 (9.1%) were recovered from blood, urine and endotracheal aspirates respectively. Of the 109 *tsst-1* gene positive *S. aureus* strains, 41 (37.6%) were isolated from blood and 29 (26.6%) from urine. As regard the 30 *pvl* positive *S. aureus* isolates, 11 (36.7%) and 7 (23.3%) were obtained from blood and urine respectively. No significant association was found between the source of clinical sample and the presence of *meca*, *tsst-1* or *pvl* genes (Table 5).
In the present study, we determined the antibiotic resistance patterns of the recovered S. aureus isolates. Of the 180 S. aureus isolates, 108 (60.0%) were resistant to penicillin, 105 (58.3%) to cefazolin, 93 (51.7%) to tetracycline, 79 (43.9%) to ciprofloxacin, 63 (35.0%) to erythromycin, 59 (32.8%) to gentamicin, 62 (34.4%) to clindamycin and 45 (25.0%) to trimethoprim-sulfamethoxazole. The highest resistance observed was to penicillin while all isolates were sensitive to vancomycin and linezolid. Antibiotic resistance patterns in relation to the existence or absence of mecA, tsst-1 and pvl genes were given in Table 6. Resistance patterns within MRSA strains was found to penicillin (100%), cefazolin (100%) followed by tetracycline (65.9%), erythromycin (48.9%), clindamycin (48.9%), ciprofloxacin (47.7%), gentamicin (45.5%) and trimethoprim-sulfamethoxazole (34.1%). Apart from ciprofloxacin, linezolid and vancomycin, resistance to evaluated antibiotics were significantly higher with the presence of mecA gene. Furthermore, the existence of mecA gene was significantly associated with multidrug resistant isolates (P=

Table 4. Distribution of tsst-1 and pvl genes among methicillin resistant and methicillin sensitive Staphylococcus aureus isolates

| Gene                        | MRSA isolates (meca positive) | MRSA isolates (meca negative) | MSSA isolates (meca negative) | Staphylococcus aureus isolates |
|-----------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Only tsst-1 positive        | 37 (42.0%)                    | 44 (47.8%)                    | 81 (45.0%)                    |                               |
| Only pvl positive           | 0                             | 2 (2.2%)                      | 2 (1.1%)                      |                               |
| Both tsst-1 and pvl positive| 21 (23.9%)                    | 7 (7.6%)                      | 28 (15.6%)                    |                               |
| Both tsst-1 and pvl negative| 30 (34.1%)                    | 39 (42.4%)                    | 69 (38.3%)                    |                               |
| Total                       | 88 (100%)                     | 92 (100%)                     | 180 (100%)                    |                               |

Values are expressed as No (%); MRSA= Methicillin resistant Staphylococcus aureus; MSSA= Methicillin sensitive Staphylococcus aureus

Fig. 1. Multiplex PCR for pvl (502 bp) and tsst-1 (398 bp) genes. M: Marker (100 bp); 1: Negative control, 2-3: Test isolates, 4: Positive control for pvl gene, 5: Positive control for tsst-1 gene
Table 5. Distribution of *mecA*, *tsst-1* and *pvl* genes among *Staphylococcus aureus* isolates recovered from different clinical samples

| Sample             | *mecA* gene |          |          |          | *tsst-1* gene |          |          |          | *pvl* gene |          |          |          |
|--------------------|-------------|----------|----------|----------|--------------|----------|----------|----------|------------|----------|----------|----------|
|                    | Positive    | Negative | *P* value | Positive | Negative    | *P* value | Positive | Negative | *P* value | Positive | Negative | *P* value |
| Blood              | 39 (44.3)   | 36 (39.1)| 0.72     | 41 (37.6)| 34 (47.9)   | 0.56     | 11 (36.7)| 64 (42.7)| 0.68       |
| Urine              | 20 (22.7)   | 23 (25.0)| 0.88     | 29 (26.6)| 14 (19.7)   | 0.68     | 7 (23.3)| 36 (24.0)| 0.89       |
| Endotracheal       | 8 (9.1)     | 11 (12.0)| 0.49     | 13 (11.9)| 6 (8.5)     | 0.72     | 4 (13.3)| 15 (10.0)| 0.69       |
| aspirates          |             |          |          |          |              |          |          |          |            |
| Wound drainages    | 6 (6.8)     | 7 (7.6)  | 0.76     | 9 (8.3)  | 4 (5.6)     | 0.64     | 3 (10.0)| 10 (6.7) | 0.43       |
| Sputum             | 5 (5.7)     | 7 (7.6)  | 0.66     | 7 (6.4)  | 5 (7.0)     | 0.65     | 2 (6.7) | 10 (6.7) | 1.0        |
| Eye secretions     | 6 (6.8)     | 5 (5.4)  | 0.59     | 6 (5.5)  | 5 (7.0)     | 0.57     | 2 (6.7) | 9 (6.0)  | 0.89       |
| Abscesses          | 4 (4.5)     | 3 (3.3)  | 0.77     | 4 (3.7)  | 3 (4.2)     | 0.69     | 1 (3.3) | 6 (4.0)  | 0.71       |
| Total              | 88          | 92       |          | 109       | 71          |          | 30       | 150      |            |

Values are expressed as No (%)

Table 6. Relation of *mecA*, *tsst-1* and *pvl* genes to antibiotic resistance patterns of *Staphylococcus aureus* isolates

| Antibiotic resistance | *mecA* gene |          |          |          | *tsst-1* gene |          |          |          | *pvl* gene |          |          |          |
|-----------------------|-------------|----------|----------|----------|--------------|----------|----------|----------|------------|----------|----------|----------|
|                       | Positive    | Negative | *P* value | Positive | Negative    | *P* value | Positive | Negative | *P* value | Positive | Negative | *P* value |
| Penicillin            | 88 (100)    | 20 (21.7)| 0.012    | 68 (62.4)| 40 (56.3)  | 0.60     | 21 (70.0)| 87 (58.0)| 0.59       |
| Cefazolin             | 88 (100)    | 17 (18.5)| 0.001    | 63 (57.8)| 42 (59.2)  | 0.88     | 21 (70.0)| 84 (56.0)| 0.48       |
| Gentamicin            | 40 (45.5)   | 19 (20.7)| 0.048    | 41 (37.6)| 18 (25.4)  | 0.08     | 9 (30.0)| 50 (33.3)| 0.66       |
| Erythromycin          | 43 (48.9)   | 20 (21.7)| 0.039    | 41 (37.6)| 22 (31.0)  | 0.49     | 12 (40.0)| 51 (34.0)| 0.58       |
| Tetracycline          | 58 (65.9)   | 35 (38.0)| 0.04     | 55 (50.5)| 38 (53.5)  | 0.51     | 14 (46.7)| 79 (52.7)| 0.7        |
| Ciprofloxacin         | 42 (47.7)   | 37 (40.2)| 0.62     | 52 (47.7)| 27 (38.0)  | 0.22     | 16 (53.3)| 63 (42.0)| 0.42       |
| Clindamycin           | 43 (48.9)   | 19 (20.7)| 0.046    | 40 (36.7)| 22 (31.0)  | 0.47     | 11 (36.7)| 51 (34.0)| 0.79       |
| Trimethoprim-sulfamethoxazole | 30 (34.1) | 15 (16.3)| 0.044    | 30 (27.5)| 15 (21.1)  | 0.37     | 8 (26.7)| 37 (24.7)| 0.65       |
| Linezolid             | 0           | 0        |          | 0         | 0           |          | 0        | 0        | -          |
| Vancomycin            | 0           | 0        |          | 0         | 0           |          | 0        | 0        | -          |
| Total                 | 88          | 92       |          | 109       | 71          |          | 30       | 150      |            |

Values are expressed as No (%); *Statistically significant
No significant association was detected between antibiotic resistance and the presence of *tsst-1* or *pvl* genes (Table 6).

**DISCUSSION**

*Staphylococcus aureus* is increasingly recognized globally as a common and serious cause of various community and healthcare associated infections with a significant increase in not only the morbidity but also in mortality. They are known producers of different types of toxins and other virulence factors that shape up their clinical significance. Molecular studies have shown that some MRSA strains that carry *tsst-1* and *pvl* genes have the ability to produce toxins encoded by these genes responsible for severe infections such as TSS and necrotic pneumonia with a mortality rate up to 75%.[19] However, treating such infections is considered a challenge to physicians in view of their antimicrobial resistant properties. Therefore, having knowledge about virulence factors of *S. aureus* and local prevalence of MRSA is beneficial for control and treatment of *S. aureus* infections.

In our study, out of the 180 *S. aureus* strains investigated, a predominance of 88 (48.9%) were found to be MRSA, similar to the results reported by Barakat and his colleagues who conducted a study in Egypt with a 45.3% of *S. aureus* isolates were MRSA.[20] Nevertheless, the reported prevalence of MRSA has varied widely dependent on location and the socioeconomic status of the region. The MRSA prevalence was found to be as low as 34% in European countries,[21,22] 18% in Russia,[23] and 41.6% in USA.[24] On the other hand, studies from developing countries have reported MRSA prevalence of as high as 72% in Lebanon,[25] 87.6% in Iran,[26] and 80% in China[27] which is considerably higher than our findings. We have reported that the source of 44.3% of MRSA isolates was the blood, going through the same stream with other studies who reported that MRSA strains were responsible for majority of bloodstream infections among hospitalized patients.[28,29]

In the current study, phenotypic detection of methicillin resistance correlated well with genotypic detection of mecA gene. However, Motamedi and his colleagues have reported that only 30% of the phenotypically identified as MRSA isolates had the mecA gene,[30] that was attributed to different mechanisms of methicillin resistance other than mecA gene. Nevertheless, our results have shown that methicillin resistance is primarily mediated by mecA gene among the studied population.

In our study, the multiplex PCR has detected *tsst-1* gene in 60.6% of the recovered *S. aureus* isolates, while Xie and his colleagues have reported that *tsst-1* gene prevalence of 48% which was lower than our findings.[19] However, other studies have reported lower prevalence of *tsst-1* gene as less as 20%.[24,32,35] This variation could be attributed to the geographical location and also to different methodology encountered in these studies as their detection of the *tsst-1* gene was carried out at the protein level only.[12,33] The expression of *tsst-1* gene does not grant the production of the toxin. Therefore, despite the presence of the gene, the toxin might not been expressed among the tested group which could explain the differences in the reported *tsst-1* gene prevalence.

We reported that the *tsst-1* gene existed in 65.9% of MRSA and 55.4% of MSSA strains. The presence of *tsst-1* gene was not significantly associated with either MRSA or MSSA groups. This was concordant with reports from Zarei Kooshka and his colleagues who detected *tsst-1* gene among 69.8% of MRSA isolates.[26] Other studies have also reported that up to 58% of MRSA strains were *tsst-1* gene positive.[33,36,37] Similar to our findings, the prevalence of *tsst-1* gene was reported up to 56% among the MSSA isolates in other studies.[24,26,38]

In our study, we found that 16.7% of isolated *S. aureus* strains were positive for the *pvl* gene, putting in consideration that in previous studies, the prevalence of *pvl* gene has shown a wide variation ranged from 2% up to 38.9%.[39,40] Moreover, we have detected the *pvl* gene in 23.9% of MRSA and 9.8% of MSSA isolates. This was similar to the findings of Goudarzi and his colleagues, who reported the prevalence of *pvl* gene of 21.4% within MRSA isolates[37] and Abiri and his colleagues who detected *pvl* gene among 21.5% of *mecA* positive MRSA strains.[1] We found that *pvl* gene was significantly prevalent in MRSA group (P=0.04). Nevertheless, these findings were contrary to what been reported by Motamedi and
his colleagues, that meCA and pvI genes did not show any association\textsuperscript{10}.

In our study, antimicrobial susceptibility patterns of \textit{S. aureus} isolates have shown higher rate of resistance to many antimicrobials that are routinely prescribed on daily bases in clinical practice for case of staphylococcal infections with the highest resistance observed toward penicillin as previously reported in other studies\textsuperscript{23,24}. Such findings highlight the urge for the availability of an accurate and geographically targeted data about local antibiotic susceptibility patterns for appropriate antibiotic selection. This measure will hinder the rapid spreading of antimicrobial resistant \textit{S. aureus} strains and it will give a solution for major challenge faced by healthcare providers in their clinical practice while treating infections caused by such isolates.

We found that 35.0\% of \textit{S. aureus} isolates were resistant to erythromycin which was similar to previous reports from Iran (51.3\%)\textsuperscript{26} and China (97.8\%)\textsuperscript{23}, others have found the resistance to erythromycin was as low as 0\%\textsuperscript{23}. On the other hand, the prevalence of tetracyclin resistant strains was 51.7\% close to the 48\% reported by Tokajian and his colleagues in Lebanon\textsuperscript{25}. Gentamicin resistance was found to be 32.8\% that is nearly similar to the reports from China (28.1\%)\textsuperscript{24} and higher than the reports from Russia (19\%)\textsuperscript{23}. In accordance with other reports, none of our isolates demonstrated resistance to vancomycin or linezolid\textsuperscript{24,25}. The reported MRSA resistance patterns in our work were in line with the findings of Goudarzi and his colleagues\textsuperscript{36}.

It has been shown that \textit{S. aureus} strains isolated from different locations had different virulence genetic profiles. Therefore, the variations between other studies and our findings in terms of prevalence of the studied genes and antimicrobial resistance patterns might be attributed to different geographical locations. Nevertheless, different study design, successful execution of infection control measures and implementation of properly designed antibiotic stewardship policy might offer another explanation as well. Moreover, the variations in antimicrobial resistance reported from different regions emphasize the importance of tailoring antibiotic policy based on local antibiotic sensitivity patterns and international guidelines. To the best of our knowledge, this is the first research conducted in our locality to find the association of both tsst-1 and pvI genes with meCA gene in \textit{S. aureus} isolates and to study their effect over the antibiotic susceptibility pattern of such isolates.

**CONCLUSION**

The pvI and tsst-1 genes positive \textit{S. aureus} strains could lead to severe infections with high morbidity and mortality. Therefore, frequent monitoring of these strains and their virulence factors are substantially important. We reported a high prevalence of tsst-1 gene among \textit{S. aureus} isolates. The tsst-1 gene was not significantly associated with either MRSA or MSSA groups. On the other hand, the pvI gene was significantly associated with MRSA group. The isolated \textit{S. aureus} strains demonstrated elevated antibiotic resistance to many of the routinely used antimicrobials and the meCA gene was significantly associated with multidrug resistant isolates. Hence, implementation of antibiotic stewardship policy based on local antibiotic resistance patterns would help the physicians in selecting the choice antibiotics for their patients.

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**CONFLICTS OF INTEREST**

The authors declare that there are no conflicts of interest.

**AUTHORS’ CONTRIBUTION**

AS developed the concept and designed the study. AS and YN did sample processing. AS collected and analysed data and did the interpretation of results. YN carried out protocol development and contributed in designing and writing of the methodological part of the article. AS wrote the manuscript. AS and YN did critical revision of the article. AS reviewed and approved the manuscript.
FUNDING
None.

DATA AVAILABILITY
All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT
All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from at least one of parents of participants included in the study. The study protocol has been approved by Faculty of Medicine - Mansoura University ethical committee.

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