Detection of mecA and class1 integron in *Staphylococcus aureus* isolated from Egyptian hospitals

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**ABSTRACT**

This study highlights the prevalence of mecA and class1 integron in multidrug resistant *Staphylococcus aureus*. A hundred clinical *Staphylococcus aureus* (SA) isolates were collected from two Egyptian hospitals (Ain-shams hospital and Abbassia fever hospital). All isolates were multidrug resistant (showing resistance to two or more antibiotic groups), antimicrobial susceptibility test showed that all isolates were resistant to methicillin, 46% were resistant to ciprofloxacin, 45% were resistant to erythromycin, 37% were resistant to vancomycin and 36% were resistant for imipenem and 11% were resistant to the seven tested antibiotic groups. Minimal inhibitory concentration showed that 58% of the isolates were resistant to imipenem. The isolates were examined for the presence of mecA, integrase gene (intI1) and class1 integron by PCR amplification. Forty two percent of the isolates were found to carry class1 integron gene cassette with variable amplicon, 36% of the isolates carried (intI1) integrase gene. Only 80% of methicillin-resistant *S. aureus* (MRSA) isolates were shown to have mecA gene.

**Keywords**: Antibiotic resistance, integron, mecA, *Staphylococcus aureus*.

**INTRODUCTION**

*Staphylococcus aureus* (SA) is opportunistic human pathogen able to cause extensive variety of diseases (Chang *et al.*, 1997; Moorem and Lindsay 2001). Due to the increasing number of infections caused by MRSA which are now most frequently multidrug resistant (MDR). MDR bacteria are defined as the bacteria resistant to more than two antibiotic groups according to Ito *et al.* (2001). MRSA harbors staphylococcal gene cassette chromosome mec (SCCmec), which mediate the methicillin resistance gene (Hotta *et al.*, 2000; Hafez *et al.*, 2009). Integron have a significant role in the dissemination of MDR via horizontal gene transfer (Mindlina and Petrovaa, 2017). It integrates exogenous open reading frames by recombination and converting them to functional genes (Mazel, 2006).

The aim of this work is to investigate the presence of mecA gene and class 1 integron between multidrug resistant MRSA.

**MATERIALS AND METHODS**

**Identification of the bacterial isolates**

A total of 100 clinical bacterial isolates were collected from two Egyptian hospitals, Ain-shams hospital and Abbassia fever hospital. The isolates were recovered from urine, pus discharge and wounds. Isolates were cultured on nutrient agar plates, purified and then subcultured on plates of blood agar, mannitol salt and Baird-Parker agar medium using the streak plate method (Mahon and Manusekis, 1995; Chapin and Lauderdale, 2003; Toidar, 2005). The plates were incubated at 37°C for 24-48h. Gram stain, catalase and coagulase production were carried out according to Koneman's color atlas (1992).
Further identification was carried out by Microscan biotyper automated system.

**Antibiotic resistance surveillance**

Muller Hinton plates were inoculated with 0.5 McFarland standard inocula. Seven different antibiotic groups were tested against the isolates as shown in Table (1). The antibiotic susceptibility test was carried out for 100 isolates according to Kirby-Bauer disk diffusion susceptibility test protocol (Bauer et al., 1966). The antibiotics inhibition zones were measured, and resistance was interpreted as recommended by (NCCLS, 1997; CLSI, 2006, 2020).

| Antibiotic group | Scientific name | Trade name | Abb. | Disc conc. (μg/disc) |
|------------------|----------------|------------|------|----------------------|
| Penicillin       | Methicillin    | Bactocil   | Ox   | 6                    |
| Quinolones       | Ciprofloxacin  | Ciprocin   | CIP  | 5                    |
| Macrolides       | Erythromycin   | Erythrocin | E    | 15                   |
| Lincosamides     | Clindamycin    | Dalacin   | DA   | 2                    |
| Tetracyclines    | Doxacycline    | Doryx     | DO   | 30                   |
| Glycopeptides    | Vancomycin     | Vancocin  | VA   | 10                   |
| Carbapenems      | Imipenem       | Tainam    | IPM  | 10                   |

**Determination of minimal inhibitory concentration (MIC):**

Multidrug resistant (MDR) *S. aureus* isolates were further investigated for their resistance to imipenem. The MIC was reported for MRSA isolates using E-test (AB BIODISK, Sweden) agar diffusion method according to (Cui et al., 2008).

**Extraction of DNA and Polymerase chain reaction (PCR)**

Genomic DNA was extracted from the bacterial isolates using Quiagen DNA extraction kit (QIAamp DNA Mini Kit) following the manufacturer instructions. PCR was carried out for amplification of meca gene which encodes the unique penicillin-binding protein (PBP2) associated with methicillin resistance in *S. aureus*. Integrase gene (*intI1*), a genetic element involved in spreading of antibiotic resistance and class1 integron gene cassette (*CSI*) (Moura et al., 2007). PCR was performed using thermocycler (Applied Biosystem, 2720). The total volume of the reaction mixture was 25μl contains 2μl DNA suspension, (0.025μmole) of each primer, 12.5 μl Dream Taq green PCR master mix (Thermo Fisher Scientific), PCR protocol was 4 min of denaturation at 94°C followed by 35 cycles of 1 min at 94°C, 30 s at annealing temperature for each primer pair (Table 2), 1 min at 72°C, final extension step of 10 min at 72°C. PCR amplicons produced distinct bands corresponding to their respective molecular sizes that were easily recognizable by electrophoresis on 0.8% TAE agarose gel stained with ethidium bromide. The gel was visualized under an ultraviolet transilluminator (UVI tec, Cambridge, UK) to investigate the presence of target gene.
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Table (2). Primers used in this study.

| Primer of target gene | Oligonucleotide Sequence | Annealing temp. | Amplicon size |
|-----------------------|--------------------------|----------------|--------------|
| mecA      | Forward 5'-GTAGAAATGACTGAACGTCCGATAA-3' | 55°C | [280 bp]   |
|           | Reverse 5'-CCAATTCCACATTGTTTCGGTCTAA-3' |     |              |
| intI      | Forward 5'-CCT CCC GCA CGA TGA TC -3' | 55°C | [430 bp]   |
|           | Reverse 5'-TCC ACG CAT CGT CAG GC-3' |     |              |
| CS1       | Forward 5'-GGAATCTGACATGGCTGACGAAG-3' | 58.5°C | variable band size |
|           | Reverse 5'-AAGCAGACTTGAAGTAGC-3' |     |              |

RESULTS

Identification of the isolates

The bacterial isolates were identified as *Staphylococcus aureus* (SA) as they produce golden yellow colonies on mannitol salt agar medium, also black, shiny and convex colonies with clear zones on Baird-Parker agar media and they were positive for catalase and coagulase. Out of 100 isolates, 47% were isolates recovered from urine, 40% from wounds and 13% from pus discharges. Moreover, 46% of the isolates were recovered from males and 54% from females.

Antibiotic susceptibility test

Antibiotic susceptibility test showed a multidrug resistance of all isolates against the seven tested antibiotics with various extents. Results of antibiotic sensitivity test are summarized in Table (3).

Table (3) Percentages of resistance to various antibiotics used in the study

| Antibiotic   | Conc. (μg) | Percentage of resistance |
|--------------|------------|-------------------------|
| Methicillin  | 6          | 100                     |
| Ciprofloxacin | 5          | 46                      |
| Erythromycin | 15         | 45                      |
| Clindamycin  | 2          | 44                      |
| Doxacycline  | 30         | 43.6                    |
| Vancomycin   | 10         | 37                      |
| Imipenem     | 10         | 36                      |

Determination of minimal inhibitory concentration (MIC):

The MIC of imipenem was determined for the hundred methicillin resistant *S. aureus* isolates against imipenem antibiotic. Results were determined by interpretation with data from Clinical and Laboratory Standards Institute (CLSI, 2020). The isolates that were resistant to >32 μg considered resistant to imipenem (CLSI, 2020; Huanga *et al.*, 2021). The isolates showed high resistance to imipenem as MIC value was 64 μg/ml for imipenem in 58% of isolates.

Detection of *mecA*, integrase gene (*intI*) and class1 integron gene cassette in *S. aureus* isolates

The result indicated that *mecA* gene was detected in 80% of MRSA isolates, unexpectedly twenty percent of the phenotypically MRSA isolates were found to be *mecA* gene negative. Furthermore, (*intI*) gene was detected in 36% of the isolates, moreover variable band sizes of class1 integron gene cassette was detected in 42% of the isolates. The pattern size of variable regions of class1 integron gene cassette in the positive isolates ranges from 100 to 1000 bp Fig (1).

The distribution of *mecA* gene among nosocomial sources was found to be predominant in urine than in pus discharge and wounds.
Fig. (1) Representative agarose gel electrophoresis of PCR product resulted from amplification of genomic DNA using the primer pair specific to:
(a) meca gene (280 bp), (b) Integrase gene (IntII) gene (430 bp), (c) a variable band size of classI integron gene cassette and (M) is 1 kbp. DNA marker

DISCUSSION
The current study investigates the presence of classI integron and meca genes in methicillin resistance *Staphylococcus aureus* isolated from 2 Egyptian hospitals. The antibiotic susceptibility test showed multidrug resistance in all tested isolates as well as high MIC values to imipenem. McClure *et al.* (2006) reported the correlation between
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phenotypic and genotypic traits that all MRSA required mecA gene but the MSSA lack the gene which means that mecA gene is the major signal for the detection of a small multidrug resistant (MRSA) isolate (Hafez et al., 2009; Curiao et al., 2011). Moreover, it was thought that the penicillin-binding protein (PBP2a) encoded by mecA is considered as the gold standard for the detection of MRSA resistance (Cui et al., 2008; Ba et al., 2014). However, the present finding gave evidence that among the MRSA isolates 20% were found to lack mecA gene. Similar findings of Curiao et al. (2011) indicated that methicillin resistant could be attributed to the presence of SMR family which confer a high level of multidrug resistance genes as β-lactamase genes. However, Lindgren et al. (2016) and Ahmed et al. (2019) indicated that mecA absence could be compensated by mecC gene, a homologue of mecA gene within newly emerging and recently recognized cassette chromosome for methicillin resistance.

The absence of mecA gene and its gene product (PBP2a) in the phenotypically methicillin resistant isolates could be attributed to several reasons such as: hyper production of β-lactamase enzyme (Olayinka et al., 2009), or specific alterations in different amino acids present in protein binding proteins cascade (PBPs 1, 2, and 3) which include three amino acid substitutions (Ba et al., 2014). Horizontal gene transfer (HGT) could be another possible factor for the dissemination of antibiotic resistance by transferring mecA gene among MRSA strains (Hanssen et al., 2004; Tolba et al., 2013; Liao et al., 2018; El-Baghdady et al., 2020).

The SCCmec chromosome contains mecA gene together with two regulatory genes mecI and mecR1 (mec complex), when mecR1 is expressed, the organism would be resistant while when mecI is expressed the isolates will be sensitive (Baig et al., 2018).

The masking of methicillin resistance of S. aureus isolates is also explained by Gallagher et al. (2017) who mentioned that accurate detection of methicillin resistance can be difficult due to (heteroresistance phenomenon) the presence of two subpopulations (one susceptible and the other resistant) that may coexist within a culture of staphylococci. All cells in a culture may carry the genetic information for resistance, but only a small number may express resistance in vitro (Wayne 2005; Figueiredo et al., 2014).

Additional genes may regulate the expression of mecA (Berger-Bachi et al., 2002; Rolo et al., 2017), although this mechanism remains unknown (Barbier et al., 2010). Resistance to several antibiotics is associated with the presence of integron (Bay and Turner 2012). In this study among the hundred MDR S. aureus isolates 42% were found to carry class1 integron gene cassette with variable amplicon size ranges from 100 to 1000 bp and 36% of isolates carry (intI) integrase gene with the amplicon size of 430 bp. The typical integron structure is known to have integrase (intI) gene and its promoter (Pint), an integration site named attl (attachment site of the integron), and a constitutive promoter (Pc) for the gene cassette integrated at the attC site. The second component is a cluster of gene cassette; a cassette is composed of an ORF flanked by two attC recombination sites (Joss et al., 2009; Yohann et al., 2017).

The In0 elements have no attC sites but having the integrase gene with its promoter and attl site. This indicates that integron lacking antibiotic resistance determinants are very common in natural populations (Mindlina and Petrovaa 2017).

The third type of integron structure is a cluster of attC site lacking integron-integrase (CALIN element) that is composed of at least two attC sites.
Integron regularly capture cassettes from CALIN elements then numerous genomes may be lacking integrase gene but carrying CALIN structure might be important reservoirs of novel cassettes (Jean et al., 2016).

In the current study 16% of the isolates were carrying a typical integron structure, 26% CALIN have a cluster of class1 integron gene cassette without integrase gene and 20% In0 isolates have integrase gene only without any gene cassette and finally 38% of the isolates have no integron element detected in them. So, the results prove a variation in integron structure genome in MRSA isolates.

The high prevalence of mecA gene and integron in multi-drug resistant isolates highlights the urgent need to employ effective means to avoid dissemination of drug-resistant bacteria.

Conclusion

Resistance to methicillin is not necessary associated with mecA gene because mecA gene was not detected in 20% of phenotypically methicillin resistance isolates. The presence of integron may lead to more extensive resistance determinants than genes alone and serve as reservoirs of antimicrobial resistance. The presence of class 1 integron in MRSA isolates could accelerates the dissemination of MRSA infections.

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