African J. Biol. Sci., 17 (1): 251-259 (2021)
ISSN 1687-4870 **e- ISSN 2314-5501 (online)**
www.ajbs.journals.ekb.eg **E.mail: aasdjournal@yahoo.com**

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

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Received: October 3, 2021; Accepted: October 10, 2021; Available online: October 20, 2021

ABSTRACT

This study highlights the prevalence of *mec*A 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 *mec*A, integrase gene (*intl1*) 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 (*intl1*) integrase gene. Only 80% of methicillin-resistant *S. aureus* (MRSA) isolates were shown to have *mec*A gene.

Keywords: Antibiotic resistance, integron, *mec*A, *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; Todar, 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).

Table (1). Antibiotic discs used in this study.

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
Tetracycline	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 *mec*A gene which encodes the unique penicillin-binding protein (PBP2) associated with methicillin resistance in *S. aureus*. Integrase gene (*intl1*), a genetic element involved in spreading of antibiotic resistance and class1 integron gene cassette (*CS1*) (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^{o}C followed by 35 cycles of 1 min at 94^{o}C, 30 s at annealing temperature for each primer pair (Table 2), 1 min at 72^{o}C, final extension step of 10 min at 72^{o}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.

Table (2). Primers used in this study.

Primer of target gene		Oligonucleotide Sequence	Annealing temp.	Amplicon size
*mec*A	Forward	(5'-GTAGAAATGACTGAACGTCCGATAA-3')	55°C	[280 bp]
	Reverse	(5'-CCAATTCCACATTGTTTCGGTCTAA -3')		
intl1	Forward	(5'-CCT CCC GCA CGA TGA TC -3')	55°C	[430 bp]
	Reverse	(5'-TCC ACG CAT CGT CAG GC-3')		
*CS*1	Forward	(5'-GGC ATC CAA GCA GCA AG -3')	58.5°C	variable band size
	Reverse	(5'-AAG CAG ACT TGA CCT GA -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 *mec*A, integrase gene (*intl1*) and class1 integron gene cassette in *S. aureus* isolates

The result indicated that *mec*A gene was detected in 80% of MRSA isolates, unexpectedly twenty percent of the phenotypically MRSA isolates were found to be *mec*A gene negative. Furthermore, (*intl1*) 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 *mec*A gene among nosocomial sources was found to be predominant in urine than in pus discharge and wounds.

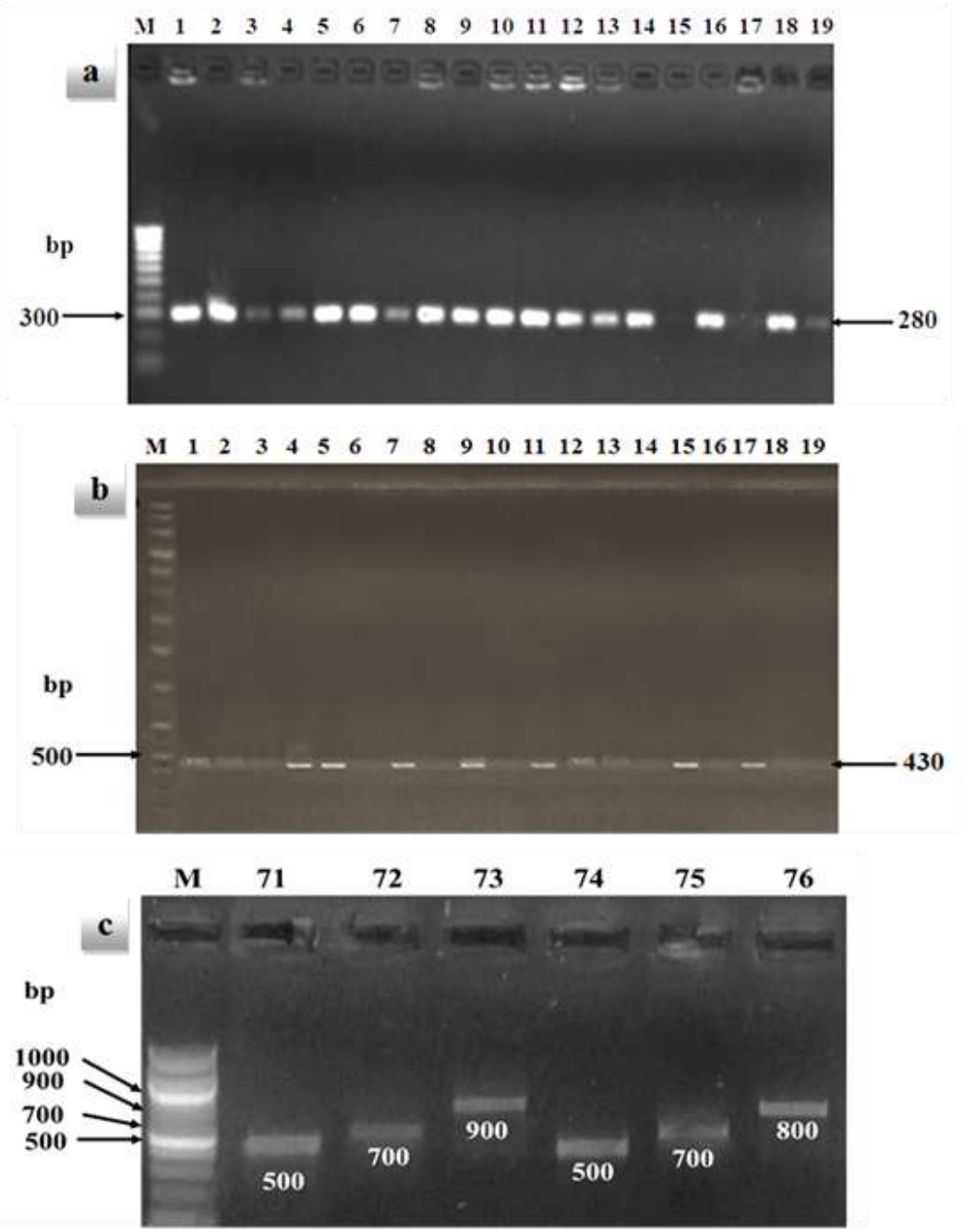

Fig. (1) Representative agarose gel electrophoresis of PCR product resulted from amplification of genomic DNA using the primer pair specific to:
(a) *mec*A gene (280 bp), (b) Integrase gene (*Intl1*) gene (430 bp), (c) a variable band size of class1 integron gene cassette and (M) is 1 kbp. DNA marker

DISCUSSION

The current study investigates the presence of class1 integron and *mec*A 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

phenotypic and genotypic traits that all MRSA required *mec*A gene but the MSSA lack the gene which means that *mec*A 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 *mec*A 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 *mec*A 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 *mec*A absence could be compensated by *mec*C gene, a homologue of *mec*A gene within newly emerging and recently recognized cassette chromosome for methicillin resistance.

The absence of *mec*A 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 *mec*A gene among MRSA strains (Hanssen *et al*., 2004; Tolba *et al*., 2013; Liao *et al.,* 2018; El-Baghdady *et al.,* 2020).

The SCC*mec* chromosome contains *mec*A gene together with two regulatory genes *mec*I and *mec*R1 (*mec* complex), when *mec*R1 is expressed, the organism would be resistant while when *mec*I 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 *mec*A (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 (*intl1*) integrase gene with the amplicon size of 430 bp. The typical integron structure is known to have integrase (*intl1*) gene and its promoter (P_{intI}), an integration site named *attI* (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 *attI* 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 *mec*A 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 *mec*A gene because *mec*A 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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الكشف عن *mec*A و class1 Integron في المكورات العنقودية الذهبية المعزولة من المستشفيات المصرية

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المستخلص

سلطت هذه الدراسة الضوء على انتشار mecA و class1 Integron في المكورات العنقودية الذهبية المقاومة للأدوية المتعددة. تم جمع مائة عزلة من المكورات العنقودية الذهبية (SA) من مستشفيين مصريين (مستشفى عين شمس ومستشفى حميات العباسية). كانت جميع العزلات مقاومة للأدوية المتعددة (أظهرت مقاومة لمجموعتين أو أكثر من المضادات الحيوية) ، أظهر اختبار الحساسية للمضادات الحيوية أن جميع العزلات كانت مقاومة للميثيسيلين ، 46٪ كانت مقاومة للسيبروفلوكساسين ، 45٪ كانت مقاومة للإريثروميسين ، 37٪ مقاومة للفانكومايسين و 36 في المائة كانت مقاومة للإيميبينيم و 11 % كانت مقاومة لمجموعات المضادات الحيوية السبع المختبرة. أظهر التركيز المثبط الأدنى أن 58٪ من العزلات كانت مقاومة للإيميبينيم. تم فحص العزلات لوجود *mec*A و class1 Integron وجين (intl1) بواسطة التفاعل المتسلسل. وجد أن 42٪ من العزلات تحمل class1 Integron لها احجام متغيرة ، وستة وثلاثون في المائة من العزلات تحمل جين (intl1) .وجد أن ثمانين في المائة فقط من عزلات MRSA كانت موجبة للجين المقاوم للميثيسيلين *mec*A.