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Evaluation of the Role Of *bla* Genes in Beta Lactam and Methicillin Resistant *Staphylococcus aureus*

Sarah H. Ahmed[(1)#], Sahar T.M. Tolba[(1)], Yehia A. El-Zawahry[(2)]

[(1)]Department of Microbiology, Faculty of Science -Ain-Shams University, Cairo, Egypt; [(2)]Department of Botany, Zagazig University, Zagazig, Egypt.

ONE HUNDRED and nineteen clinical samples were isolated from patients admitted in different hospitals in El-Sharkia Governorate. Sixty six isolates were confirmed to be *S. aureus*. Susceptibility to different antimicrobial agents and Minimum inhibitory concentration tests showed that all the isolates were resistant to β-lactam antibiotics, 77.2% (n= 51) isolates were methicillin resistant *S. aureus* MRSA, while almost all the isolates were sensitive to vancomycin and tigecycline. Polymerase chain reaction (PCR) of *mec*A, encoding methicillin resistance, and *bla*Z, β-lactamase biosynthetic gene, revealed the coexistence of both genes in 56.8% (n= 29/51) of the isolates. Meanwhile, 11.7% (n= 6/51) of MRSA isolates phenotypically resistant to oxacillin were found to be *mec*A$^-$. This data support the fact that the expression of *bla* genes enhanced the phenotypic expression of oxacillin resistance as a result of β-lactamase hyperproduction. On the other hand, 33% of MRSA (n=17/51) were *blaZ*$^-$ suggesting a mutation event in *blaZ* or the existence of an alternative mechanisms for β-lactam resistance that may compete with *mec*A gene.

Key words: *Staphylococcus aureus*, *mec*A, *bla*Z, MRSA, MIC- β-lactamase resistant MRSA.

Introduction

Staphylococcus aureus is an extraordinarily versatile pathogen that can survive under hostile external environmental conditions, colonize mucous membrane and skin and cause severe toxin-mediated diseases or severe invasive purulent infections in humans (Archer, 1998 and Lowy, 1998). It represents a progressive problem in hospitals for decades, in addition, the occurrence of community-acquired infections have been increasing (Tokajjan, 2014).

Many studies reported the prevalence of MRSA isolates in Egypt (Tolba et al., 2013; Abdel-Maksoud et al., 2016 and Abdelsalam et al., 2017). Data from the European Antimicrobial Resistance Surveillance System (EARSS) showed that in more than one-third of countries the proportion remained >25% (Johnson, 2011). In Ireland, MRSA has been endemic in hospitals since the 1980s and different strains have predominated at different time periods (Shore et al., 2005). The pandemic sequence type (ST)8 USA300 of MRSA is the dominant CA-MRSA clone in the United States (Uhlemann et al., 2014).

The severity of staphylococcal infections combined with feeble treatment is due to the specific suite of virulence and antibiotic resistance-associated genes (Paul et al., 2015). Most methicillin-resistant *Staphylococcus aureus* (MRSA) infections occur in people who have been in hospitals or other healthcare settings, such as nurseries and dialysis centers (David & Daum, 2010 and Fukunaga et al., 2016). Staphylococcal resistance to beta-lactam antibiotics is mediated by either of two mechanisms: (i) Production of beta-lactamase and (ii) Production of an altered target penicillin-binding protein (PBP2a) (Pantosi et al., 2007).

Methicillin resistant gene *mec*A is embedded in a large heterologous chromosomal cassette, the SCCmec element (Ito et al., 1999). MRSA strains carry upstream to the *mec*A gene *mec*I-*mec*R1 encoding for a repressor and a sensor/inducer of the *mec*A expression, respectively. The *mec*A gene is present in a *mec* gene complex in *SCCmec* elements. Four classes of the *mec* gene complex (A-D) have been described which were differ in their genetic organization (Katayama et al., 2001 and Ito et al., 2004). The *SCCmec* types are listed on the *SCCmec* website by the International

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Working Group on the Classification of SCC Elements (IWG-SCC) (http://www.sccmec.org/Pages/SCC_TypesEN.html). This genetic organization is similar to the beta-lactamase locus that encodes for penicillin-resistance only, and contains the structural gene (*bla*Z), a repressor (*bla*I) and a sensor/inducer (BlaR1) (Shore et al., 2011). There is a cross-talk between both regulatory systems, as each one alone is able to control the transcription of *mec*A and *bla*Z (Hackbarth & Chambers, 1993). Based on the aforementioned, it has been postulated that full resistance to beta-lactamase of many contemporary MRSA clinical strains, implies a non-functional *mec*I-*mec*R1 regulatory system (Hiramatsu et al., 1992). The cross link between *mec* genes and *bla* genes are not yet resolved. PCR detection for both genes has been tested. This study aims to assess the role of *bla*Z in β lactam resistant methicillin resistant *S.aureus* isolates.

Materials and Methods

Bacterial isolates

A total of 119 samples were recovered from patients with age ranged from 22-86 years old, suffering from severe infections in different body sites. Those patients were submitted to different hospitals in the period from September 2014 to September 2015. Samples were collected from wounds, blood, sputum, urine (Table 1). Swabs were streaked on the surface of blood agar, Baired-Parker and mannitol salt agar media for selective isolation of *Staphylococcus aureus*. The purified colonies were then checked microscopically using Gram-stain and biochemically using catalase and coagulase tests (Cheesbrough, 2000 and Ryan & Ray, 2004).

Antibiotic susceptibility test

Antimicrobial resistance to twelve antibiotics was carried out by Kirby-Bauer disk diffusion method according to (Bauer, 1966). Muller Hinton plates were inoculated with bacterial suspensions of approximately 0.5 McFarland. The antibiotic disks were placed on the agar surface and incubated at 37˚C for 18-24h. Inhibition zone diameter (mm) was measured as recommended by CLSI (2016). The antibiotics discs used in µg were azithromycin 15, ampicillin 10, ceftazidime 30, cefotaxime 30, vancomycin 30, oxacillin 1, erythromycin 15, Doxycycline 30, imipenem 10, ciprofloxacin 5, ceftriaxone 30.

Determination of minimum inhibitory concentration

Minimum inhibitory concentration (MIC) for isolates that were phenotypically oxacillin resistant was evaluated using Vitek 2 card AST-GP67 (biomerieux, France) according to Kumar et al. (2013) and Bobenchik et al. (2014). Isolates were considered methicillin resistant *Staphylococcus aureus* if their MIC for oxacillin were ≥4 µg/ml (CLSI, 2016).

Polymerase chain reaction

DNA was extracted from the bacterial isolates according to Wilkening et al. (2013). PCR was carried out using the primer pair *mec*A1(5'-GTAGAAATGACTGAACGTCCGATAA-3') corresponded to nucleotides 318 to 342 and *mec*A2 (5'-CCAATTCCACATTGTTTCGGTAA-3') complementary to nucleotides 603 to 627 for amplification of 310 bp from the highly conserved region of the 2,456-bp *mec*A gene which encodes for the unique penicillin-binding protein associated with oxacillin resistance (Zhang et al., 2004). A second pair of primers was used for amplification of 533 bp of *bla*Z gene blaZ-1(5'-GAT AAG AGA TTT GCC TAT GC-3') corresponding to nucleotide position 165 to 186 and blaZ-2 (5'-GCA TAT GTT ATT GCT TGA CC-3') complementary to nucleotides 678 to 699 (Oliveira & de Lencastre, 2011). A single colony was picked and boiled in 50 µl of ultra pure water and then 1µl of the supernatant was used as DNA template. PCR was performed in a total volume of 25µl containing 20 ρmol of each primer, 12.5µl Bioline mastermix (Sigma) using AB applied Biosystem thermocycler 2720. The thermocycler protocol was 5min at 95˚C and then 30 cycles of denaturation at 95˚C for 40sec, annealing at 55˚C for 1min and extension at 72˚C for 1min, post extension step at 72˚C for 7min. The PCR products of *mec*A and *bla*Z genes were run in 1% agarose gel stained with 0.05% ethidium bromide.

Results

Isolation of clinical isolates

Clinical samples were collected from hospitalized patients with age range from 22 to 86 years old. Samples were collected from different sources, a total of 119 samples, 54% (n= 65) of which collected from wounds, 22% (n= 27) from blood, 12% (n= 15) from sputum (Table 1). The rest of the samples were collected from cerebrospinal fluid and urine.

TABLE 1. Sources and numbers of *S. aureus* isolates.

Source	Total number	Age range	No. of females	No. of males	No. of *S.aureus* isolates	Percentage (%)
Wound infections	65	22-86	18	18	36	54.54
blood	27	40-59	10	11	21	31.82
Sputum	15	70-82	2	3	5	7.57
Cerebrospinal fluid	3	55-63	1	2	3	4.55
Urine	9	29-50	0	1	1	1.52
Total No.	119	119	31	35	66	100

Out of the 119 samples, 66 isolates were recovered and identified by conventional and biochemical methods as *S. aureus*. They produced black colonies surrounded by a clear zone on Baired-Parker agar media, yellow colonies on mannitol salt agar. They were catalase and coagulase positive. Most of the isolates were recovered from wound 54.5% (n= 36) and blood 31.8% (n= 21) (Table 1).

Antibiotic susceptibility and determination of MIC

Antibiotic susceptibility test showed various degrees of resistance to the tested antibiotics. Out of 66 *S. aureus* isolates, 77.2% (n= 51) isolates were oxacillin resistant *S. aureus* (ORSA) and 2.7 % (n= 15) were oxacillin sensitive *S. aureus* (OSSA). All the isolates were *β*-lactam resistant, they exhibited 100% resistance to ampicillin and ceftazidime, while 77.2% were resistant to cefotaxime. The isolates were also carbapenem resistant 75.7% were resistant to meropenem while, 22% were resistant to imipenem, 68% were resistant to ceftriaxone, 44% were resistant to erythromycin. Only 13.6 % were resistant to vancomycin as shown in Fig.1.

Determination of minimum inhibitory concentrations

The 51 oxacillin resistant isolates were selected for the study of MIC values against different antibiotics, using AST-GP67 card in Vitek 2 system. Isolates that were resistant to ≥ 4µg/ml of oxacillin were regarded as oxacillin resistant *S. aureus* (ORSA) according to CLSI (2016). The results demonstrated that all the isolates were resistant to benzyl-penicillin ≥0.5µg/ml, 86.2% (n= 44) of isolates were resistant to ≥4µg/ml oxacillin. In addition, 90% (n= 46) of the isolates were resistant to tetracycline as they showed resistant to ≥16µg/ml. Most of the isolates were sensitive to flouroquinones, only 15.7% (n= 8) were resistant to ciprofloxacin, 31.4 % (n= 16) were resistant to levofloxacin, those isolates were able to grow at concentration ≥8µg/ml. Only 37% of the isolates (n= 19) were resistant to macrolides, they exhibited resistant to ≥8µg/ml to erythromycin and clindamycin. Most of the isolates were sensitive to vancomycin, only 11% (n= 6) were resistant to ≥16µg/ml. Almost all the isolates were sensitive to tigecycline (Table 2).

PCR detection of mecA and blaZ gene

PCR detection of *mec*A and *bla*Z gene revealed single band of the right size (Fig. 2 a, b). The PCR data showed that most of ORSA isolates 88% (n= 45) were found to harbor *mec*A gene. However, 11.7% (n= 6) of the phenotypically oxacillin-resistant isolates were *mec*A^{-}. On the other hand, *bla*Z gene was detected in 66.6% (n= 34) of the isolates (Table 2). Coexistence of both *mec*A and *bla*Z genes were recorded in 56.8% (n= 29) of isolates (Table 2).

Discussion

Many bacteria have become resistant to most used antimicrobial agents. The high resistance rate was linked to the extensive antibiotic use and selective pressure (Davies & Davies, 2010). Many studies reported the effect of sex, gender and the extent of exposure to microorganisms on the immune response (Oertelt-Prigione, 2012 and Fink & Klein, 2015). In the present study, It was noticed that males could be more vulnerable to infections as the rate of infection in males (53%) was higher than in females (46%).

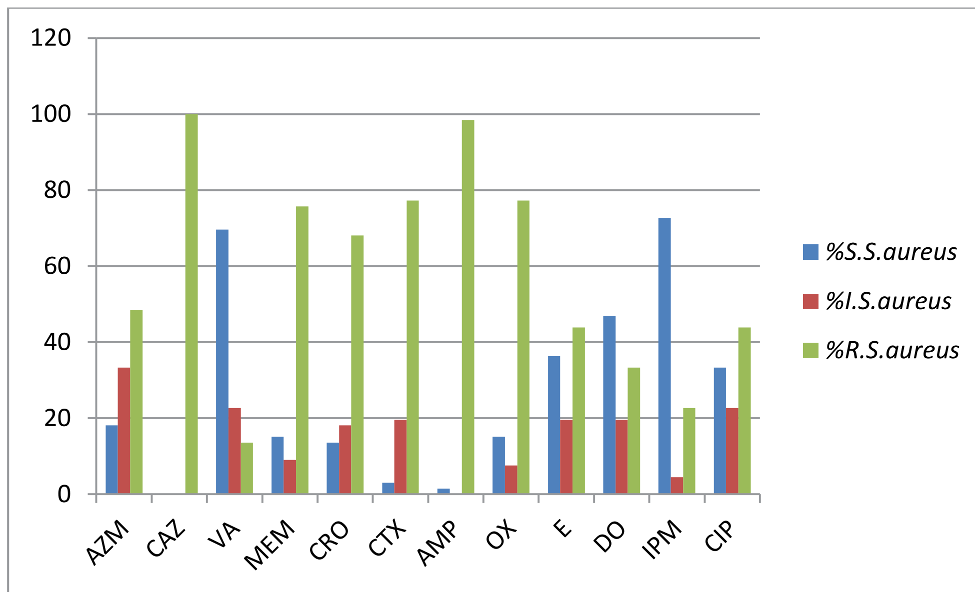

Fig. 1. Antibiotic resistance pattern of *S. aureus* isolates (AZM: Azithromycin, CAZ: Ceftazidime,VA: Vancomycin, MEM: Meropenem, CRO: Ceftriaxone CTX: Cefotaxime, AMP: Ampicillin, OX: Oxacillin, E: Erythromycin, DO: Doxacillin, IPM: Imipenem and CIP: Ciprofloxacin).

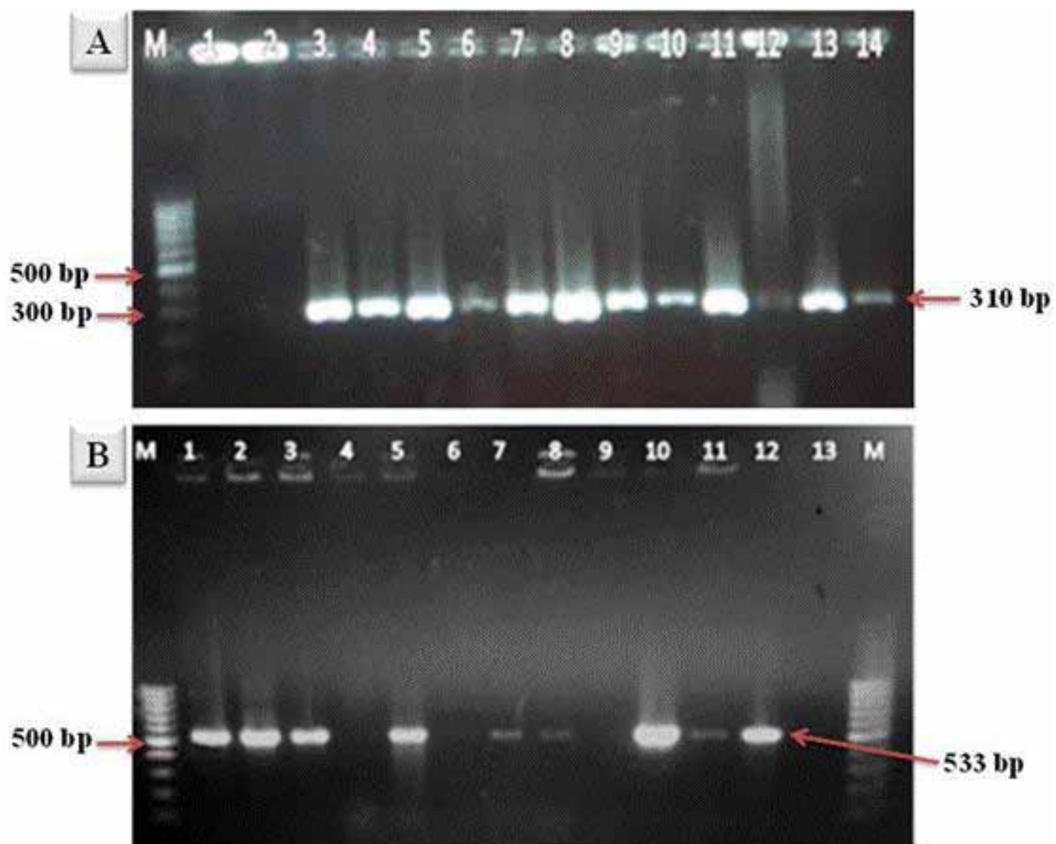

Fig. 2. Representative agarose gel photo of PCR amplicons of A- amplification of *mec*A gene, M is 1kb ladder, lane 1 is negative control, lanes 2-14 are amplicons of *mec*A gene from *S. aureus* isolates. B-amplification of blaZ gene. M is 1kb ladder, lanes 1-12 are amplicons of *bla*Z and lane 13 is negative control.

TABLE 2. Minimum inhibitory concentrations of tested antibiotics and genotypic characterization of the clinical isolates.

Isolates	BP	Ox	Gen	CIP	Lev	Mox	E	DA	Q.	Van	Tet	Tig	Rif	Tri	*mecA*	*blaZ*
SW1	≥ 0.5	≥ 4	≥16	≥ 8	≤ 0.12	≤0.25	≤0.25	≤0.25	≤0.25	≤0.5	≥16	0.5	≤ 0.5	≤10	+	+
SW2	0.12	≥ 4	8	≤0.5	≤ 0.12	≤0.25	≤0.25	≤0.25	≤0.25	≤0.5	≤ 1	≤0.1	≤ 0.5	≤10	+	+
SB3	≥ 0.5	≥ 4	≥16	≥8	≥ 8	4	≥ 8	≥ 8	0.5	1	≥16	0.5	4	≤10	+	-
SW5	≥ 0.5	≥ 4	8	≤ 0.5	0.25	2	≥ 8	≥ 8	≤0.25	2	≥16	≤0.1	2	≤10	+	+
SB6	≥ 0.5	≥ 4	≥16	≤ 0.5	≥ 8	4	≥ 8	≥ 8	0.5	≤0.5	≥16	≤0.1	4	≤10	+	-
SU7	≥ 0.5	≥ 4	≤ 0.5	≤ 0.5	≤ 0.12	≤0.25	≤0.25	≤0.25	≤0.25	≤0.5	≥16	≤0.1	≤ 0.5	≤10	+	-
SA8	≥ 0.5	≥ 4	≥16	≤ 0.5	≥ 8	4	≥ 8	≥ 85	0.5	≤0.5	≥16	≤0.1	16	≤10	+	+
SW9	≥ 0.5	≥ 4	≤0.5	≤ 0.5	0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.5	≥16	≤0.1	≤ 0.5	≤10	+	+
SB10	≥ 0.5	≥ 4	≥16	≤ 0.5	≥ 8	4	≥ 8	≥ 8	0.5	1	≥16	≤0.1	4	≤10	+	+
SW11	≥ 0.5	1	≤0.5	≤ 0.5	≤ 0.12	≤0.25	≤0.25	≤0.25	≤0.25	≤0.5	≥16	≤0.1	≤ 0.5	≤10	+	+
SB12	≥ 0.5	1	≤0.5	≤ 0.5	0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.5	≥16	≤0.1	≤ 0.5	≤10	+	+
SW13	≥ 0.5	≥ 4	≤0.5	≤ 0.5	0.25	≤0.25	≤0.25	1	≤0.25	1	≥16	≤0.1	≤ 0.5	≤10	+	+
SW14	≥ 0.5	≥ 4	8	≤ 0.5	≤ 0.12	≤0.25	≤0.25	≤0.25	≤0.25	1	≥16	≤0.1	≤ 0.5	≤10	+	+
SB16	≥ 0.5	1	≤0.5	≤ 0.5	≤ 0.12	≤0.25	≤0.25	≤0.25	≤0.25	≤0.5	≥16	≤0.1	≤ 0.5	≤10	-	+
SB17	≥ 0.5	≥ 4	≥16	≤ 0.5	≥ 8	4	≥ 8	≥ 8	≤0.25	≤0.5	≥16	≤0.1	2	≤10	+	+
SB18	≥ 0.5	≥ 4	≥16	≤ 0.5	4	2	≤0.25	≤0.25	≤0.25	≤0.5	≥16	≤0.1	≥32	≤10	+	+
SW19	≥ 0.5	≥ 4	≥16	≤ 0.5	≥ 8	4	≥ 8	≥ 8	≤0.25	≤0.5	≥16	≤0.1	2	≤10	+	-
SW20	≥ 0.5	≥ 4	8	≤ 0.5	0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.5	≤ 1	≤0.1	≤ 0.5	≤10	+	-
SB21	≥ 0.5	≥ 4	≤0.5	≤ 0.5	0.25	≤0.25	≤0.25	≤0.25	≤0.25	1	≥16	≤0.1	≤ 0.5	≤10	+	-
SU23	≥ 0.5	≥ 4	≤0.5	≤ 0.5	≤ 0.12	≤0.25	≤0.25	≤0.25	≤0.25	≤0.5	≥16	≤0.1	≤ 0.5	≤10	+	+
SU24	≥ 0.5	≥ 4	≥16	≤ 0.5	≥ 8	4	≥ 8	≥ 8	0.5	1	≥16	≤0.1	16	≤10	+	+
SW 25	≥ 0.5	≥ 4	≤0.5	≤ 0.5	0.25	≤0.25	≤0.25	≤0.25	≤0.25	≥ 32	≥16	≤0.1	≤ 0.5	≤10	+	+
SW 26	≥ 0.5	≥ 4	≥16	≤ 0.5	≥ 8	4	≥ 8	≥ 8	0.5	1	≥16	≤0.1	2	≤10	+	-
SB 27	≥ 0.5	≥ 4	≥16	≤ 0.5	≥ 8	4	≥ 8	≥ 8	0.5	≤0.5	≥16	≤0.1	16	≤10	-	+
SW 30	≥ 0.5	≥ 4	≤0.5	≤ 0.5	≤ 0.12	≤0.25	≤0.25	≤0.25	≤0.25	1	≥16	≤0.1	≤ 0.5	≤10	+	+
SU 31	≥ 0.5	≥ 4	≥16	≤ 0.5	≥ 8	4	≥ 8	≥ 8	0.5	≤0.5	≥16	≤0.1	2	≤10	+	+
SB 32	≥ 0.5	1	≤0.5	≤ 0.5	≤ 0.12	≤0.25	≤0.25	≤0.25	≤0.25	≤0.5	≥16	≤0.1	≤ 0.5	≤10	+	+
SW 33	≥ 0.5	≥ 4	≤0.5	≤ 0.5	≤ 0.12	≤0.25	≤0.25	≤0.25	≤0.25	≤0.5	≥16	≤0.1	≤ 0.5	80	-	+

TABLE 2 Cont.

Isolates	BP	Ox	Gen	CIP	Lev	Mox	E	DA	Q.	Van	Tet	Tig	Rif	Tri	*mecA*	*blaZ*
SB 34	≥ 0.5	≥ 4	≤ 0.5	≤ 0.5	0.25	≤0.25	≤0.25	≤0.25	≤0.25	1	≥16	≤0.1	≤ 0.5	80	+	+
SW 35	≥ 0.5	≥ 4	4	≤ 0.5	≤ 0.12	≤0.25	≤0.25	≤0.25	≤0.25	≤0.5	≤ 1	≤0.1	≤ 0.5	≤10	+	+
SU 36	≥ 0.5	2	4	≤ 0.5	≤ 0.12	≤0.25	≤0.25	≤0.25	≤0.25	≤0.5	≥16	≤0.1	≤ 0.5	≤10	+	-
SB 38	≥ 0.5	≥ 4	≥16	≤ 0.5	≥ 8	4	≥ 8	≥ 8	0.5	≤0.5	≥16	0.2	4	≤10	+	+
SW 40	≥ 0.5	1	4	≤ 0.5	≤ 0.12	≤0.25	≤0.25	≤0.25	≤0.25	1	0.25	≤16	≤ 0.5	≤10	+	+
SW 41	≥ 0.5	≥ 4	≥16	≤ 0.5	0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.5	≥16	≤0.1	≤ 0.5	≤10	+	+
SW 43	≥ 0.5	≥ 4	≤0.5	≤ 0.5	≤ 0.12	≤0.25	≤0.25	≤0.25	≤0.25	≤0.5	≥16	≤0.1	≤ 0.5	≤10	+	-
SW 44	≥ 0.5	≥ 4	≥16	≥ 8	≥ 8	≥ 8	≥ 8	≥ 8	4	≥ 32	≥16	1	≥32	20	+	+
SB 45	≥ 0.5	≥ 4	≥16	≥ 8	≥ 8	4	≤0.25	≥ 8	≤0.25	≤0.5	≥16	0.25	4	≤10	+	+
SW 46	≥ 0.5	1	8	≤ 0.12	≤ 0.12	≤0.25	≤0.25	≤0.25	≤0.25	≥ 32	≥16	≤0.12	≤ 0.5	≤10	+	-
SU 49	≥ 0.5	≥ 4	≥16	≥ 8	≥ 8	4	≥ 8	≥ 8	0.5	≤0.5	≥16	0.5	2	≤10	-	+
SB 53	≥ 0.5	≥ 4	≤0.5	≤ 0.5	≤ 0.12	≤0.25	≤0.25	≤0.25	≤0.25	1	≥16	≤0.12	≤ 0.5	160	+	+
SW54	≥ 0.5	≥ 4	≤0.5	≤ 0.12	0.25	≤0.25	≤0.25	≤0.25	≤0.25	1	≥16	≤0.12	≤ 0.5	≤10	-	+
SW55	≥ 0.5	≥ 4	≤0.5	0.25	0.25	≤0.25	≤0.25	≤0.25	≤0.25	1	≥16	≤0.12	≤ 0.5	≤10	-	-
SU56	≥ 0.5	1	≤0.5	≤ 0.12	≤ 0.12	≤0.25	≥ 8	≤0.25	≤0.25	≤0.5	≥16	≤0.12	≤ 0.5	20	+	-
SB57	≥ 0.5	≥ 4	≤0.5	0.25	0.25	≤0.25	≤0.25	≤0.25	≤0.25	2	≥16	≤0.12	16	≤10	+	-
SW58	≥ 0.5	≥ 4	≤0.5	0.25	0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.5	≥16	≤0.12	≤ 0.5	≤10	+	-
SW59	≥ 0.5	2	≤ 0.5	0.25	0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.5	≥16	0.25	≤ 0.5	≤10	+	+
SW61	≥ 0.5	≥ 4	≤ 0.5	1	1	0.5	≥ 8	≥ 8	0.5	16	≥16	0.25	16	≥320	+	+
SB62	≥ 0.5	≥ 4	≥16	≥ 8	≥ 8	≥ 8	≥ 8	≥ 8	0.5	≤0.5	≥16	0.25	4	≤10	+	-
SU63	≥ 0.5	≥4	≥4	≥ 8	≥ 8	≥ 8	≥ 8	≥ 8	≤0.25	≤0.5	≥16	1	2	≤10	+	-
SW64	≥ 0.5	≥ 4	4	≤ 0.5	0.25	≤0.25	≤0.25	≤0.25	≤0.25	≥32	≤ 1	≤0.12	≤ 0.5	≤10	+	-
SW65	≥ 0.5	≥ 4	≥16	≥ 8	≥ 8	≥ 8	≥ 8	≥ 8	≥ 16	≥32	≥16	≥2	≥32	≥320	+	+

(BP) Benzylpenicillin, (Ox) Oxacillin, (G) Gentamicin, (CIP) Ciprofloxacin, (Lev) Levofloxacin, (Mox) Moxifloxacin, (E) Erythromycin, (DA) Clindamycin, (Q) Quinupristin/Dalfopristin, (Van) Vancomycin, (Tet) Tetracyclin, (Tig) Tigecycline, (N) Nitrofurantoin, (Rif) Rifampicin, (Tri) Trimethoprim/Sulfamethoxazole. SW: *S. aureus* isolated from wound infections, SB: *S. aureus* isolated from blood, SU: *S. aureus* isolated from urine

Most *Staphylococcus aureus* isolates associated with nosocomial infections have developed multidrug resistance and had relatively high MIC values to oxacillin and tetracycline, erythromycin and ciprofloxacin (Duin & Paterson, 2016). Our results showed that high levels of resistance was detected against oxacillin, *β*-lactam and tetracycline however low level of resistance was recorded against macrolides and flouroquinones. In addition, most of the isolates were sensitive to vancomycin and tigecycline.

Marlowe & Bankowski (2011) suggested that the use of molecular techniques in the detection of MRSA is essential for rapid diagnosis. Ostojić & Hukić (2015) demonstrated a correlation between phenotypic and genotypic characteristics. Their results revealed that all MRSA isolates were *mec*A$^+$, while Panda et al. (2016) stated that using conventional methods for detection of MRSA is more significant than detection with PCR. In the present study, 15.6% (n= 8) isolates showed low level of resistance to oxacillin (1 or 2µg/ml) were found to harbor *mec*A gene, while four isolates that were resistant to ≥4µg/ml oxacillin lacked *mec*A gene. This finding is in agreement with a study by Elhassan et al. (2015) who suggested low burden of the *mec*A gene in MRSA isolates. In addition, Wielders et al. (2002) found that 5% of the phenotypically methicillin-resistant isolates did not carry *mec*A. This finding opens the door to search for other intrinsic factors that may compete with *mec*A gene in producing resistance phenomenon in regions with high prevalence of MRSA.

In most clinically hospitalized cases, *β*-lactam is the drug of choice. MRSA strains resistant to *β*-lactam antibiotics frequently harbor *bla*Z in addition to *mec*A (Munita et al., 2015). In our study, the coexistence of both resistance genes *mec*A and *bla*Z were recorded in only 56.8% of the tested *β*-lactam resistant MRSA isolates, while 17(33%) of the isolates were *bla*Z negative. It was also found that *bla*Z gene was present in 5 isolates that were *mec*A negative. Ba et al. (2014) identified four MRSA isolates that lacked *mec*A, but still resistant to penicillin-resistant *β*-lactam antibiotics.

Arêde et al. (2013) suggested that there is a cross-talk between the two regulatory systems, and it has been demonstrated that *bla* regulators stabilize the *mec*A acquisition. The preservation of *bla*Z gene in MRSA isolates, even in the absence of *mec*A, could be implicated as "a first-line defense" against *β*-lactams. Recent studies analyzed *bla* genes among *S. aureus* clonal types and found that a selective pressure has long played a role to keep the bacteria sustain the *bla* locus fully functional and improve its activity. However, most existing MRSA strains that still carry the *β*-lactamase locus along with the SCC*mec* element, begin to lose the *bla* genes. This could occur as a result of the poor fitness cost associated with the *bla* genes and the recent acquisition of *mec*A gene (Foster, 2017). It is also hypothetically possible that there is a link between *bla* genes to other positively selected genes (e. g., the cadmium resistance genes present in some *β*-lactamase plasmids). The *bla* locus enhances the stabilization and *in vitro* acquisition of *mec*A, explicating the retention of a functional *bla* regulatory system. Moreover, regulation of *mec*A, by *bla* regulatory system, mimics that of *bla*Z gene in an unknown manner Arêde et al (2013).

ELhassan et al. (2015) reported the complete absence of *mec*A gene as well as penicillin-binding protein in isolates which were phenotypically classified as MRSA suggesting that *β*-lactamase hyperproduction is the cause of this resistance pattern. In our study the absence of *mec*A and *bla*Z genes was detected in 2% of the isolates. Hence, the hypothesis 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 may be accepted (Paterson et al., 2014 and Lindgren et al., 2016).

Conclusion

The presence of β lactamase resistant MRSA strains lacking *mec*A gene suggests that phenotypically resistant MRSA could be misdiagnosed using molecular methods alone and provides evidence of alternative mechanisms for β-lactam resistance in MRSA. Our results suggest the possibility of the existence of unidentified mechanism of regulation involved in the transcriptional control of *mec*A gene in MRSA strains which is contradictory to the idea that in most clinical MRSA strains *mec*A gene is under the control of the *bla* regulatory genes. Mutagenesis and gene expression studies of the *bla* genes are still needed to resolve the mechanism of β-lactam resistance in MRSA strains.

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تقييم لدور جينات ال *bla* فى بكتيريا المكورات العنقودية الذهبية المقاومة للبيتالاكتام والميثيسيللن

سارة حسين أحمد[1] ، سحر طلبه محمد[1]، يحيى أحمد الظواهرى[2]
[1]قسم الميكروبيولوجى - كلية العلوم - جامعة عين شمس - القاهره - مصر و [2]قسم النبات - كلية العلوم - جامعة الزقازيق - الزقازيق - مصر.

تم عزل مائة تسعة عشر عينة سريرية من المرضى في مستشفيات مختلفة في محافظة الشرقية. تم تعريف ستة وستون عزلة انها بكتريا *S. aureus*. أظهر اختبار قابلية العزلات للمضادات الميكروبية المختلفة وتحديد أقل تركيز مثبط للبكتريا أن جميع العزلات كانت مقاومة للمضادات الحيوية β-lactam، و أن 77.2% (n= 51) عزلة مقاومة للميثيسيلين MRSA، في حين كانت جميع العزلات تقريبا حساسة لفانكوميسين وتيغاسيكلين. أظهر تفاعل البلمرة المتسلسل (PCR) لـجين *mec*A، الخاص بمقاومة المثيثلين و جين *bla*Z ، الخاص بتكوين انزيم b-lactamase، تلازم كلا الجينين في 56.8% (n= 29/51) من العزلات. في الوقت نفسه، 11.7% (n= 6/51) من عزلات MRSA والتي أظهرت مقاومة ظاهرية للأوكساسلين وجد أنها عديمة جين *mec*A، هذه النتائج تدعم حقيقة أن التعبير عن الجينات *bla* عزز التعبير المظهري لمقاومة oxacillin نتيجة لغزارة انتاج انزيم β Lactamase. ومن ناحية أخرى ، كان 33% من MRSA (n= 17/51) عديمة جين *bla*Z مما يشير إلى حدوث طفرة في *bla*Z أو وجود آليات بديلة لمقاومة β-lactam التي قد تتنافس مع الجين *mec*A.