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Article *in* European Journal of Microbiology · September 2013

DOI: 10.1556/EuJMI.3.2013.3.5 · Source: PubMed

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European Journal of Microbiology and Immunology 3 (2013) 3, pp. 183–187
DOI: 10.1556/EuJMI.3.2013.3.5

MOLECULAR CHARACTERIZATION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATES IN THREE DIFFERENT ARAB WORLD COUNTRIES

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Received: May 11, 2013; Accepted: May 23, 2013

Molecular characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates in three different Arab world countries (West Bank of Palestine, Jordan, and Iraq) was the aim of the study presented here. This is done on the basis of *spa* sequencing and staphylococcal cassette chromosome *mec* (SCC*mec*) typing. The majority (92%) of the *spa*-tested isolates belonged to *spa* type t932 and possessed the (SCC*mec*) type III. These data suggest that MRSA clone, which harbors the *spa* type t932 and (SCC*mec*) type III, had been transferred throughout the three studied countries.

Keywords: MRSA, *spa* typing, SCC*mec* typing, epidemiology

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a multidrug-resistant pathogen associated with significant morbidity, mortality, and hospitalization costs [1–7]. Currently, sequence-based methods and protein A-encoding gene *(spa)* typing offer excellent intra- and inter-laboratory reproducibility and the opportunity to compare results internationally [8]. *Spa* typing has also been validated for long-term nationwide surveillance studies [9]. To our knowledge, no epidemiological surveillance studies have investigated the molecular nature of MRSA strains circulating in the health care settings in West Bank of Palestine, Jordan, and Iraq. The aims of the present study were to determine the resistance pattern of the current MRSA isolates circulating in these countries and to study their molecular epidemiology by *spa* sequencing and SCC*mec* typing.

Materials and methods

A total of 67 MRSA isolates were isolated between February and September 2010 from several clinical sources including the urine, wound, sputum, skin, body fluids, respiratory tract, and other sources that were randomly selected from the largest public tertiary referral hospitals in three different Arab world countries (West Bank of Palestine, 17 isolates; Jordan, 25; and Iraq 25). *Figure 1* displays the location map of study areas. All isolates were determined for *mecA*, Panton–Valentine leukocidin *(PVL)* genes, and *S. aureus*-specific fragments by multiplex polymerase chain reaction (PCR) with GenoType® MRSA-test (Hain Lifesciences, Germany) according to the manufacturer's instructions in using the reagents supplied. Briefly, the protocol consisted of DNA isolation, PCR amplification, and reverse hybridization, including chemical denaturation of the PCR products, hybridization of the biotinyl-

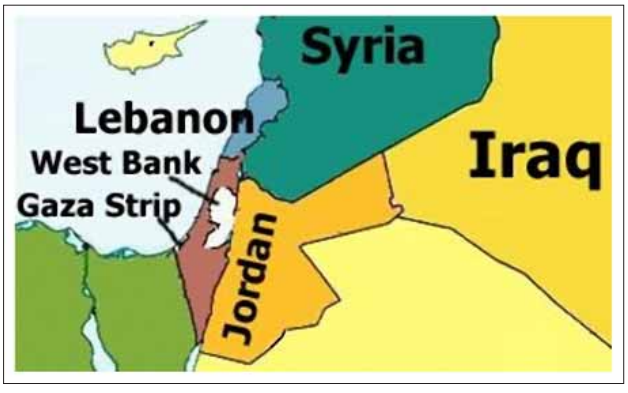

Fig. 1. Map of the study areas

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ISSN 2062-509X / $ 20.00 © 2013 Akadémiai Kiadó, Budapest

ated PCR products to membrane-bound probes, stringent washing, adding of streptavidin-alkaline phosphatase (AP) conjugate, and an AP-mediated staining reaction.

Antimicrobial susceptibility tests were performed by disc diffusion as recommended by the Clinical and Laboratory Standard Institute (CLSI) guidelines [10]. Ten antimicrobial agents were tested, these were penicillin G (1 µg), erythromycin (15 µg), clindamycin (2 µg), vancomycin (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), cefotaxime (30 µg), amikacin (30 µg), teicoplanin (30 µg), and sulfamethoxazole-trimethoprim (1.25/23.75 µg). *S. aureus* American Type Culture Collection (ATCC) 25923 was used as the control strain in this test.

Spa typing was performed on 12 isolates that included representatives of each predominant antibiogram type of the three study countries. The polymorphic X region of the *spa* gene was amplified using primers *spa*-1113f and *spa*-1514r as described previously [11, 12]. The PCR reaction conditions were as follows: 15 min at 95 °C, 30 cycles of 1 min at 94 °C, 1 min at 68 °C, 1 min at 72 °C, and 10 min at 72 °C. The PCR products were purified using the MinElute PCR purification kit (Qiagen, Germany), and the inserts were sequenced by a dideoxy chain termination method on an ABI PRISM Model 3130 Sequence Instrument at Bethlehem University, Bethlehem, Palestine. The Kreiswirth spa nomenclature obtained by the *spa* typing tool (http: //fortinbras.us/cgi-bin/spatyper/spaTyper.pl) was adopted for the present study.

The nucleotide sequences of 11 isolates reported here have been submitted to the GenBank accession numbers and were assessed as JX985621, JX985622, JX985623, JX985624, JX985625, JX985626, JX985627, JX985628, JX985629, JX985630, and JX985631.

SCC*mec* types were determined by the use of specific primers for amplification of the key genetic elements as described by Ghaznavi-Rad et al. [13]. PCR was performed with a Ready Mix PCR kit (Sigma-Aldrich). Reaction mixtures contained 2.5 µl template DNA, 12.5 µl master mix with 2.5 µl primer mix (1 µM for each primer) (Syntezza, Israel), and RNAse-free water to a final volume of 25 µl. The reaction was carried out in an Eppendorf Mastercycler gradient according to the following program: 94 °C for 4 min, 35 cycles of 94 °C for 30 s, 48 °C for 30 s and 72 °C for 2 min, and a final extension at 72 °C for 4 min. PCR products were separated by electrophoresis in agarose 2% gels and stained with ethidium bromide.

Results

All the 67 MRSA isolates were positive for *mecA* and a certain type of SCC*mec*. SCC*mec* typing identified that the majority (95.5%) of isolates carried SCC*mec* type III (n = 64), while three (4.5%) isolates harbored SCC*mec* types V. Panton–Valentin leukocidin (PVL) was detected in two (3%) of the Iraq MRSA isolates and were associated with SCC*mec* type V. Representative hybridization of MRSA isolates with the GenoType MRSA Direct is shown in *Fig. 2*.

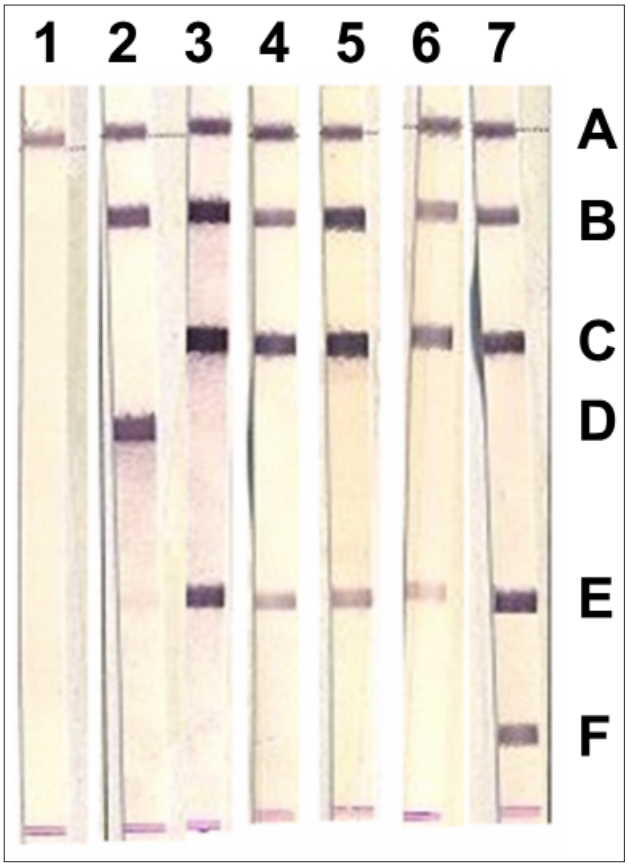

Fig. 2. Representative hybridization of MRSA isolates with the GenoType MRSA Direct. Lane 1: negative control, 2: *S. epidermidis*, 3: *S. aureus*, 4–7: MRSA. A: conjugate control, B: universal control, C: *S. aureus*, D: *S. epidermidis*, E: *mec* A gene, F: PVL gene, respectively

As can be deduced from *Table 1*, the MRSA isolates were predominantly resistant to a greater range of antibiotics. Of the ten antibiotics tested, isolates indicated extremely high rate of resistance (above 82%) to penicillin G, erythromycin, clindamycin, ciprofloxacin, gentamicin, cefotaxime, and sulfamethoxazole-trimethoprim. Resistance rates of MRSA to other antibiotics were as follows: 64.2% resistant to amikacin and 13.4% to teicoplanin. No vancomycin-resistant *S. aureus* (VRSA) nor vancomycin-intermediate *S. aureus* (VISA) isolates were identified in this study.

Table 2 shows the distribution of antibiotic resistance patterns of the MRSA isolates by country. The predominant profile was represented by 9 (53%), 10 (40%), and 9 (36%) in the isolates from Palestine, Jordan, and Iraq, respectively, with those isolates representing 28 (42%) of all isolates. It contained a combination of eight antibiotics. These antibiotics are penicillin G, erythromycin, clindamycin, ciprofloxacin, gentamicin, cefotaxime, amikacin, and sulfamethoxazole-trimethoprim.

Interestingly, all the 67 (100%) MRSA isolates were noted to be multiply resistant, that is, typically resistant to β-lactam plus three or more antibiotics of amikacin, cip-

Table 1. Resistance to various antibiotics among the MRSA isolates from the Palestine, Jordan, and Iraq countries

Source	Percent of isolates resistant to									
	AMK	CIP	CLI	CTX	ERY	GEN	SXT	TPL	VAN	PEN
Palestine	76.5	94.1	100	100	94.1	94.1	94.1	11.8	0	100
Jordan	60.0	100	100	76.0	96.0	80.0	80.0	16.0	0	100
Iraq	60.0	92.0	100	76.0	96.0	76.0	76.0	12.0	0	100
Total	64.2	95.5	100	82.1	95.5	82.1	82.1	13.4	0	100

AMK: amikacin, CIP: ciprofloxacin, CLI: clindamycin, CTX: cefotaxime, ERY: erythromycin, GEN: gentamicin, SXT: sulfamethoxazole-trimethoprim, TPL: teicoplanin, VAN: vancomycin, PEN: penicillin G

Table 2. Patterns of resistance to individual antibiotics among the MRSA isolates from the Palestine, Jordan, and Iraq

Resistance pattern	Number of isolates (%)			
	Palestine (n = 17)	Jordan (n = 25)	Iraq (n = 25)	Total
AMK, CIP, CLI, CTX, ERY, GEN, SXT, PEN	9 (53)	10 (40)	9 (36)	28 (42)
AMK, CIP, CLI, CTX, ERY, GEN, SXT, TPL, PEN	2 (12)	4 (16)	3 (12)	9 (13)
AMK, CLI, CTX, ERY, GEN, SXT, PEN	1 (06)	0 (00)	2 (08)	3 (04)
AMK, CIP, CLI, CTX, GEN, PEN	1 (06)	1 (04)	1 (04)	3 (04)
CIP, CLI, ERY, PEN	0 (00)	2 (08)	4 (16)	6 (09)
CIP, CLI, CTX, ERY, GEN, SXT, PEN	3 (18)	3 (12)	3 (12)	9 (13)
CIP, CLI, CTX, ERY, SXT, PEN	1 (06)	1 (04)	1 (04)	3 (04)
CIP, CLI, ERY, SXT, PEN	0 (00)	2 (08)	1 (04)	3 (04)
CIP, CLI, ERY, GEN, PEN	0 (00)	2 (08)	1 (04)	3 (04)

AMK: amikacin, CIP: ciprofloxacin, CLI: clindamycin, CTX: cefotaxime, ERY: erythromycin, GEN: gentamicin, SXT: sulfamethoxazole-trimethoprim, TPL: teicoplanin, VAN: vancomycin, PEN: penicillin G

Table 3. Composition of SCC*mec* and *spa* types of 12 MRSA isolates in Palestine, Jordan, and Iraq

Country	Number of	*spa* type		SCC*mec* type	
	isolates (%)	t932	t386	III	V
Palestine	4	3	1	3	1
Jordan	4	4	0	4	0
Iraq	4	4	0	4	0
Total	12	11	1	11	1

rofloxacin, clindamycin, erythromycin, gentamicin, sulfamethoxazole-trimethoprim, and teicoplanin.

The genetic association of 12 representatives MRSA isolates from the predominant antibiotic resistance pattern of the three study countries was investigated by *spa* typing. Eleven of these isolates shared a common *spa* type (t932) and were of SCC*mec* type III, indicating that 11 of the 12 isolates (92%) were clonally related *(Table 3)*.

Discussion

The current study was set up to establish the logistics for future shared studies that will continue to improve essential knowledge for clinicians and diagnostic laboratories about the cross-border dissemination of multidrug-resistant MRSA clones and their epidemic patterns in neighboring countries.

The antimicrobial susceptibility patterns of the MRSA isolates are a cause for concern as an extremely high rate (above 82%) of resistance to penicillin G, erythromycin, clindamycin, ciprofloxacin, gentamicin, cefotaxime, and sulfamethoxazole-trimethoprim was observed, a finding mirrored elsewhere [14, 15].

Of the 10 antibiotics tested, all isolates were completely sensitive to only vancomycin. Teicoplanin resistance was observed in only 13.4% of the isolates. This was not surprising since these antibiotics are relatively new, and resistance to these antibiotics is currently rare.

The high rate of resistance has major therapeutic implications, insofar as all the 67 (100%) of our population of MRSA isolates show cross-resistance to β-lactams, macrolides, ciprofloxacin, trimethoprim-sulfamethoxazole, and aminoglycosides, leaving very few therapeutic options to treat infections caused by this microorganism. This phenomenon may be related to the dissemination of transposons with insertion sequences in the 50-kb *mec* region gene [15, 16]. For example, the ability of IS431 elements through homologous recombination to trap and cluster resistance determinants with similar IS elements explains the multiple drug resistance phenotype that is characteristic of MRSA strains [15, 16].

SCC*mec* typing showed that SCC*mec* type III element was the predominant type (95.5%), followed by SCC*mec* type V (4.5%). To our knowledge, there has not been any previous similar study in the three study countries nor neighboring Arab countries to evaluate the SCC*mec* typing results. However, a high prevalence of SCC*mec* type III has been mirrored elsewhere in the world [13]. The predominance of SCC*mec* type III in the three studied countries may indicate an ancestral origin of MRSA. A selected number of isolates – 12 representative MRSA isolates from the predominant antibiotic resistance pattern of the three study countries – were also analyzed by *spa* typing since it has several advantages in terms of speed, ease of use, ease of interpretation, and database creation [17].

Eleven of these isolates shared a common *spa* type (t932) and were of SCC*mec* type III, indicating that 11 of the 12 isolates (92%) were clonally related. The regional clusters found in the three countries might be explained by cross-border patient mobility within these countries in general, between Palestine and Jordan in particular, and between Iraq and Jordan during and after the 2003 invasion of Iraq. Consequently, the cross-border transfer of patients may have an important impact on the dissemination and prevalence of MRSA in particular clone (t932/SCC*mec* III).

In our study, few MRSA isolates (4.5%) carried the PVL-toxin genes, and this could be explained by the fact that our MRSA isolates were originally hospital acquired. Only one of the PVL-positive MRSA isolates, which made up 1.5% of the overall sample, belonged to the clone (t932/SCC*mec* III). This value, when compared to the overall numbers of MRSA, is small but still deserves attention since PVL-positive community-associated MRSA (CA-MRSA) is more commonly associated with skin and soft tissue infections [18].

In conclusion, antimicrobial resistance has clearly emerged as a serious problem with MRSA in the three studied countries, with the type (t932/SCC*mec* III) being the predominant isolates. These results also indicate the geographical spread of MRSA over long distances and across cultural borders. Thus, continuous surveillance of MRSA in hospitals and communities is of great importance for understanding the epidemiology of MRSA.

Conflict of interest statement

We declare that we have no conflict of interest.

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