SCCmec and spa types of methicillin-resistant *Staphylococcus aureus* strains in Israel. Detection of SCCmec type V

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Abstract Molecular characterization of methicillin-resistant *Staphylococcus aureus* isolates from three hospitals in Israel was the aim of the study presented here. We identified 11 distinct genetic clones by pulsed-field gel electrophoresis. Molecular typing identified four different SCCmec types—I, II, IV, and V—and nine spa types. Spa type t002 was the most common.

Introduction

*Staphylococcus aureus* is an important cause of community- and hospital-acquired infections. Since the 1980s, methicillin-resistant strains (MRSA) have spread in hospitals and become a common nosocomial pathogen. In the last decade, community-acquired strains of MRSA (CA-MRSA) have spread in many communities, causing mostly skin and skin structure infections. These strains are often, but not always, susceptible to non-beta-lactam antibiotics [1]. CA-MRSA strains were reported to penetrate hospitals and cause nosocomial outbreaks [2].

The proportion of nosocomial MRSA differs greatly among countries. The European Antimicrobial Resistance Surveillance System (EARSS) reports that 44.1% of hospital-acquired (HA) *S. aureus* bloodstream isolates in Israel are methicillin-resistant [3]. The proportion of CA-MRSA among all MRSA infections is also highly variable, and figures as high as 70% have been reported [4]. In Israel, surveillance of children and their guardians attending pediatricians’ outpatient clinics found a CA-MRSA carriage rate of 6/10,000 population [5]. We recently observed a growing number of CA-MRSA skin and soft-tissue infections caused by nonmultidrug-resistant (non-MDR) strains among patients with no recent contact with the health-care system, although these are still relatively uncommon (unpublished data) and the evolution of these strains is unclear.

Molecular-typing techniques may shed light on the epidemiology and the evolutionary relationships of MRSA. Characterization of MRSA lineages requires identification of both the mobile genetic element responsible for methicillin resistance, i.e., the staphylococcal cassette chromosome *mec* (SCCmec), and the genetic background of the strains.

To date, five major types of SCCmec elements (I–V) have been defined based on *mec* gene complex and *ccr* gene allotypes [6–8]. SCCmec types I, II, and III are mostly found in hospital-acquired strains, whereas type IV is mostly found among strains that disseminate primarily in the community [5, 9, 10]. However, certain strains asso-
ated predominantly with health-care-related infections, for example strains USA500, USA 600 and USA800, also carry SCCmec type IV [11]. At present, reports regarding strains carrying SCCmec type V are still rare. Several strains harboring this SCCmec type were identified in community MRSA isolates from Australia [12, 13] and Taiwan [14].

SCCmec includes the mec gene complex, sorted into four classes A–D, and the ccr gene complex encoding for site-specific recombinases. Other regions of the SCCmec have been referred to as the J (junkyard) regions and contain a variety of genes, including those coding for resistance to other classes of antibiotics (non-beta-lactams) [6–8, 15]. SCCmec types can be further sub-typed (i.e., into variants) based on differences in J regions [10].

Spa typing is a highly discriminating genotyping technique for evolutionary and epidemiologic investigation. There is a good correlation between clonal relatedness as determined by Smal macrorestriction analysis, MLST and spa typing (http://www.spaserver.ridom.de/). The analysis of the resolution of different sequence-based methods performed by Robinson and Enright showed that diversity resolved by the spa typing was even greater than that of the multi-locus sequence typing (MLST) [16]. Spa typing of certain clone groupings using the based upon repeat pattern (BURP) algorithm reflects their sequence type (ST). As previously described [17], spa type 001 was found for isolates of ST228 collected in Germany, Poland, and Slovenia, while spa type t002 and, clustered with it, type t045 have been reported for MRSA isolates of lineage ST5 from Central Europe, the USA, Japan and South Korea. Different spa types grouped in spa-CC065 were reported for MRSA isolates ST45.

Molecular data on MRSA in Israel are lacking. Here we attempted to characterize MRSA isolates from three Israeli hospitals and describe their PFGE pattern, spa types, and SCCmec types.

Materials and methods

Study strains

A total of 25 MRSA isolates collected from three Israeli hospitals were examined. These strains are a sample of MRSA strains isolated during 2002–2003 in three teaching hospitals in central Israel, representing 2,000 beds. Isolates were selected in each hospital for this study based on their antibiotic susceptibility pattern in an attempt to represent a wide spectrum of phenotypes. This sample should not be viewed as a representative sample of all MRSA strains in Israel. For isolates that had similar phenotype and similar PFGE pattern, only one representative was further studied. Isolates were considered health-care-associated when they were isolated, after more than 72 h in the hospital or in other health-care settings; the isolates were considered community-acquired if the patient had been hospitalized for less than 72 h when the culture was drawn, and the patient did not have contact with other health-care settings such as dialysis, ambulatory hospital visits, residence in long-term care facility, home intravenous care, or other frequent contact with hospitals. We report the detailed analysis of 17 MRSA isolates that were selected to represent molecular diversity and/or various antibiotic susceptibility phenotypes.

Antimicrobial susceptibility

Identification and susceptibility testing were performed using BactiStaph reagent (Remel), DNase test agar with toluidine blue (Novamed) and VITEK–2 system (bioMerieux, Hazelwood, MO, USA). Oxacillin resistance was verified on MRSA screening agar (with 6 μg ml⁻¹ oxacillin, 0.68M NaCl, MH, HY–PD MRSA plates, Hy–Labs, Israel).

SCCmec typing and detection of PVL

SCCmec classification was performed using both the simplex polymerase chain reaction (PCR) typing with primers designed by Okuma et al. [1] and the multiplex PCR developed by Oliveira and de Lencastre [18]. Primers ccr F 5′-GTCGGAGCATGGGTACTCAATC and ccr R 5′-CTTCTTCGCTGAACAGCCCAAT were used for amplification of ccrC gene. The presence of mecA gene and the pvl gene was examined by PCR using primers described previously [18, 19]. Bacterial cell lysates, prepared from overnight cultures and boiled in 100 μl of sterile water, were used as a DNA template. Amplifications were performed using Hot-StarTaq DNA polymerase (Qiagen, Hilden, Germany). The PCR reaction conditions for SCCmec determination by both methods were as follows: 15 min at 95°C, 30 cycles of 1 min at 94°C, 2 min at 62°C, 3 min at 72°C, and 10 min at 72°C.

Pulsed-field gel electrophoresis (PFGE) and spa typing

Genetic relatedness was analyzed using two approaches: PFGE [20, 21] and spa typing. The polymorphic X region of spa gene (protein A) was amplified using the primers spa-1113f and spa-1514r [17]. Spa types as well as BURP spa clonal complexes (spa-CCs) were determined using the Ridom StaphType software version 1.4.11 (Ridom, Würzburg, Germany) as described by Harmsen et al. [22]. 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.
Results and discussion

Seventeen MRSA isolates were studied, representing a variety of isolation sites including blood, wounds, ears, sputum, or phlegm. Patients affected represented all age groups, including newborn babies in neonatal intensive care units and old debilitated patients with multiple comorbidities. All MRSA isolates harbored the mecA gene. Eleven different PFGE clones and nine different spa types were detected (Table 1). There were eight different PFGE clones and eight spa types in hospital A, and five different PFGE clones belonging to two spa types (one of these PFGE clones was also detected in hospital A and another was found in all three hospitals) in hospital C. One PFGE clone was an outbreak strain, belonging to the t002 spa type, which was the most frequent type among the examined isolates. Five different PFGE clones (B, D, H, J and K) belonged to this spa type (Table 1).

Using the BURP algorithm, spa types were clustered into three different groups: (1) types t002, t001, and t045 classified by BURP into spa-CC002/001, (2) types t008, t052, and t064 classified into spa-CC008, and (3) types t004 and t065 classified into spa-CC065. Spa type t362 was excluded from grouping because of BURP clustering parameters (only types with more than five repeats were included). However, t362 type contains two repeats, r09 and r34, that are present in both t004 and t065 types. Moreover, r09 is considered as a repeat specific to spa-CC065 [17], thus allowing us to classify t362 within this

Table 1 Pulsed-field gel electrophoresis (PFGE) pattern, and SCCmec and spa typing

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Hospital</th>
<th>PFGE</th>
<th>SCCmec type</th>
<th>Spa type</th>
<th>Clustering by BURP</th>
<th>Antibiotic resistance profile</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Multiplex</td>
<td>Simplex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>A</td>
<td>A</td>
<td>I</td>
<td>I</td>
<td>t001</td>
<td>Spa-CC002/001 β-lactams, CLI, ERY, GEN, OFX, TOB</td>
</tr>
<tr>
<td>2</td>
<td>A</td>
<td>B</td>
<td>II</td>
<td>II</td>
<td>t002</td>
<td>Spa-CC002/001 β-lactams, CLI, ERY, OFX, TOB</td>
</tr>
<tr>
<td>3</td>
<td>A</td>
<td>B</td>
<td>II</td>
<td>II</td>
<td>t045</td>
<td>Spa-CC002/001 β-lactams, CLI, ERY, OFX, TOB</td>
</tr>
<tr>
<td>4</td>
<td>A</td>
<td>C</td>
<td>II</td>
<td>II</td>
<td>t004</td>
<td>Spa-CC065</td>
</tr>
<tr>
<td>5</td>
<td>A</td>
<td>D</td>
<td>NT</td>
<td>V</td>
<td>t002</td>
<td>Spa-CC002/001 β-lactams, GEN, OFX, TOB</td>
</tr>
<tr>
<td>6</td>
<td>A</td>
<td>F</td>
<td>IV</td>
<td>IVa</td>
<td>t065</td>
<td>Spa-CC065</td>
</tr>
<tr>
<td>7</td>
<td>A</td>
<td>F</td>
<td>IV</td>
<td>IVa</td>
<td>t362</td>
<td>Excluded β-lactams</td>
</tr>
<tr>
<td>8</td>
<td>A</td>
<td>E</td>
<td>IVA</td>
<td>IV</td>
<td>t008</td>
<td>Spa-CC008 β-lactams, OFX, TOB</td>
</tr>
<tr>
<td>9</td>
<td>A</td>
<td>E</td>
<td>IVA</td>
<td>IV</td>
<td>t008</td>
<td>Spa-CC008 β-lactams, GEN, OFX, TOB</td>
</tr>
<tr>
<td>10</td>
<td>A</td>
<td>G</td>
<td>IV</td>
<td>IVc</td>
<td>t064</td>
<td>Spa-CC008 β-lactams, GEN, OFX, TOB, TET, SXT</td>
</tr>
<tr>
<td>11</td>
<td>A</td>
<td>H</td>
<td>NT</td>
<td>V</td>
<td>t002</td>
<td>Spa-CC002/001 β-lactams, GEN, OFX, TOB</td>
</tr>
<tr>
<td>12</td>
<td>B</td>
<td>H</td>
<td>NT</td>
<td>V</td>
<td>t002</td>
<td>Spa-CC002/001 β-lactams, GEN, OFX, TOB</td>
</tr>
<tr>
<td>13</td>
<td>C</td>
<td>I</td>
<td>NT</td>
<td>V</td>
<td>t052</td>
<td>Spa-CC008 β-lactams, ERY, GEN, OFX, TOB</td>
</tr>
<tr>
<td>14</td>
<td>C</td>
<td>J</td>
<td>I</td>
<td>I</td>
<td>t002</td>
<td>Spa-CC002/001 β-lactams, CLI, ERY, GEN OFX, RIF, TET, TOB</td>
</tr>
<tr>
<td>15</td>
<td>C</td>
<td>K</td>
<td>II</td>
<td>II</td>
<td>t002</td>
<td>Spa-CC002/001 β-lactams, CLI, ERY, OFX, TOB</td>
</tr>
<tr>
<td>16</td>
<td>C</td>
<td>H</td>
<td>NT</td>
<td>V</td>
<td>t002</td>
<td>Spa-CC002/001 β-lactams, GEN, OFX, TOB</td>
</tr>
<tr>
<td>17</td>
<td>C</td>
<td>B</td>
<td>II</td>
<td>II</td>
<td>t002</td>
<td>Spa-CC002/001 β-lactams, CLI, ERY, OFX, TOB</td>
</tr>
</tbody>
</table>

BURP Based upon repeat pattern, NT nontypable
group. Since no other published studies have examined the molecular epidemiology of MRSA in Israel, we do not know the representation of these clones in other settings.

All examined PFGE clones except D, H, and I showed known multiplex PCR patterns and were classified as SCCmec types I, II, IV, and IVA (Table 1). PFGE clone D from hospital A and PFGE clone I from hospital C (one isolate each) had an unusual SCCmec multiplex PCR pattern: positive bands for loci E and F, absence of the typical locus C band, and an additional band of locus A (the 495-bp amplicon of the pls gene downstream region that is usually part of SCCmec type I) (Fig. 1, lanes 6, 7). These clones belonged to different spa types (t002 and t052, respectively) clustered in two different groups. PFGE clone H, which was present in all three hospitals, belonged to spa type t002 and showed a multiplex PCR pattern of SCCmec type IIIA variant that was described in Taiwan [9] as exhibiting amplicons of loci E and F.

PCR analysis of mec and ccr complexes was performed for further SCCmec classification. PCR amplification using primers IS2 and mA6 [1] yielded a 800-bp amplicon in isolates of PFGE clones D, I, and H, corresponding to the predicted size of this part of the SCCmecV element (relative to accession numbers AB121219 or AY894415) [8, 14]. Sequencing of the amplicons revealed high homology with the class C2 mec complex typical of SCCmec type V. PCR performed with ccrC specific primers and sequencing revealed the presence of the ccrC gene in these clones. Thus, these isolates should be classified as SCCmec type V (Table 1). SCCmec type V has been described as being related to CA-MRSA, but, in our case, PFGE clones D and I, which were isolated from hospitals A and C as carrying SCCmec type V, were health-care-associated. PFGE clone H, which caused a MRSA outbreak in the neonatal intensive care unit of hospital B, had been introduced into the unit by a staff member [23], and therefore it is difficult to determine whether this was community- or health-care-associated. Clone H isolates from hospitals A and C were health-care-associated.

The results of simplex PCR analysis of the remaining isolates included in this study are presented in Table 1. PCR amplifications using primers specific to the J1 region [10] of clone F, which were classified as type IV by multiplex PCR, revealed that they harbored type IVA SCCmec. One unique isolate (PFGE clone G) harbored type IVc SCCmec. Two isolates of PFGE clone E that exhibited an IV A multiplex PCR pattern had no amplicons with primers for a, b, or c types, indicating differences in the J1 region. The pvl gene was not detected in any of the tested strains, while SCCmec type V was detected in three clones.

Antibiotic susceptibility

PFGE clone A, with SCCmec type I, was resistant to CLI, ERY, GEN, OFX, and TOB. PFGE clone J, with SCCmec type I, also showed resistance to RIF and TET. Isolates of PFGE clones B, C, and K, with SCCmec type II, were resistant to CLI, ERY, OFX, and TOB. PFGE clones D and H were resistant to GEN, OFX, and TOB, and PFGE clone I was also resistant to ERY (Table 1). Two SCCmec type IVA isolates belonging to PFGE clone F were susceptible to all examined non-beta-lactam antibiotics, while...
one belonging to a PFGE clone G isolate with SCC\textit{mec} type IVc was resistant to OFX, TET, GEN, TOB, and SXT. One of the two SCC\textit{mec} type IVA strains and all the SCC\textit{mec} type II isolates displayed unusual resistance phenotypes, with susceptibility to gentamicin (GS-MRSA) and resistance to tobramycin, a phenotype that was described previously from France [24].

In the present study, we determined clonal relatedness, SCC\textit{mec}, \textit{spa} typing and antibiotic susceptibilities of selected MRSA isolates from three Israeli hospitals, and found high diversity. Four different SCC\textit{mec} types were found (I, II, IV, and V) in the same PFGE clone as well as across clones (Table 1). PFGE clone F, which was isolated in hospital A, was identical to a previously described CA-MRSA clone in Israel [2]. Two isolates of this PFGE clone with SCC\textit{mec} type IVa had different \textit{spa} types of t065 and t362 belonging to the \textit{spa}-CC0065 group. SCC\textit{mec} type II PFGE clone C had \textit{spa} type t004, also belonging to the \textit{spa}-CC0065 group. On the other hand, two strains of PFGE clone B that also had SCC\textit{mec} type II and showed the same antibiotic susceptibility phenotypes as PFGE clone C had \textit{spa} types t002 and t045, belonging to \textit{spa}-CC002/001 group. Molecularly identical isolates of PFGE clone E had different antibiotic susceptibility phenotypes. \textit{Spa} type t002 was the most frequent \textit{spa} type and was found in five different PFGE clones, including clone D, with SCC\textit{mec} types II or V. On the other hand, PFGE clone I, possessing SCC\textit{mec} type V, had \textit{spa}-type t052, belonging to \textit{spa}-CC008 group. To the same \textit{spa} group belonged isolates of PFGE clone G, possessing SCC\textit{mec} type IVc and \textit{spa}-type t064, and PFGE clone E, possessing SCC\textit{mec} type IV and \textit{spa} type t008. Thus, two isolates carrying SCC\textit{mec} type V with the same nontypical multiplex PCR pattern belonged to different PFGE and \textit{spa} groups. All these data imply that multiple and independent insertions of different SCC\textit{mec} elements to various genetic backgrounds had occurred.

The commonly held belief is that SCC\textit{mec} types I, II, and III are present in HA-MRSA strains and that types IV and the infrequently reported type V [8, 14] are usually associated with community-acquired strains, although some reports described CA-MRSA strains harboring SCC\textit{mec} types I, II, or III [25, 26], while another described HA-MRSA strains with SCC\textit{mec} type IV [11, 27]. Although rare in the world and to our knowledge previously only found in CA-MRSA strains, SCC\textit{mec} type V was found in five HA-MRSA strains. One of these strains, PFGE clone H, an outbreak strain from hospital B, introduced by a staff member, was difficult to classify. These findings reveal that any distinction between CA-MRSA and HA-MRSA based on the SCC\textit{mec} type is not absolute. The antibiotic susceptibility profiles of the studied strains differed among SCC\textit{mec} types and PFGE clones. In general, isolates belonging to SCC\textit{mec} types IV and V were resistant to fewer classes of antimicrobials.

In conclusion, this study reveals high diversity of HA-MRSA strains in Israel. Various PFGE clones carrying different SCC\textit{mec} types and \textit{spa} types were found. Although rarely reported in the world, SCC\textit{mec} type V existed in three different PFGE clones from three hospitals.

References


