Molecular Epidemiology of Methicillin-Resistant *Staphylococcus aureus* in Israel: Dissemination of Global Clones and Unique Features

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Running title: Molecular Epidemiology of MRSA in Israel

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Abstract:

From 2006 to 2009, 315 clinical methicillin-resistant Staphylococcus aureus (MRSA) isolates were collected from 5 hospitals across Israel. Most isolates (64%) were related to the global clones spa types t001-SCCmec-I (n=99, 31%), t002-SCCmec-II (n=82, 26%) and t008-SCCmec-IV (n=21, 7%), five of which were identified as USA-300-MRSA. Seventeen Israeli-unique strains were identified. SCCmec types IV and V were common among hospital-acquired isolates.

Methicillin-resistant Staphylococcus aureus (MRSA) is an increasing problem throughout the world, both in hospitals and in the community. Several epidemic healthcare-associated (HA) MRSA clones have emerged since the 1970s. During the last decade, five major pandemic clones, designated the Iberian, Brazilian, Hungarian, New York/Japan, and Pediatric clones, have been identified, while other new or preexisting clones have emerged in certain areas (12). The recent worldwide spread of several community-associated (CA) MRSA clones, and their dissemination into hospitals, has made the understanding of this epidemiology even more complex (10).

In Israel, the proportion of MRSA among all S. aureus isolates in 2008 was 35%, similar to the proportion in Southern Europe and the UK (17) and lower compared to that in other Middle-eastern countries (4). Scattered data exist on the molecular epidemiology of MRSA in Israel. The epidemiology of CA-MRSA was studied in the pediatric population of Southern Israel, where the 5.7% of infants were found to be colonized, mostly by a unique MRSA-SCCmec-IV clonal complex (CC), 913 (1). However, the data regarding the molecular epidemiology of community-acquired MRSA among adults, and data on HA-MRSA infections in Israel, are limited to small series or case reports (6, 29, 30).
In 2008, the National Center for Infection Control initiated a national survey of MRSA in Israel. The aims of the study were: a) to describe the molecular epidemiology of hospital-associate and community-associated (CA) MRSA infections in Israel, and b) to determine whether internationally-known MRSA strains (e.g., the USA-300 strain) have spread into Israel. Five general hospitals spread around the country participated in the study: a) Tel Aviv Sourasky Medical Center (TA)-Tel Aviv area; b) Rambam Medical Center (RA)-northern Israel; c) Barzilai Medical Center (AS)-southern Israel; d) Meir Medical Center (ME)-Sharon region of central Israel and e) Bikur Cholim (BC)-Jerusalem area. Each laboratory was asked to submit prospectively collected MRSA isolates from blood or wound, isolated within 72 hours of admission (CA-MRSA), or from 72 hours on (HA-MRSA). MRSA isolates collected from 2006 to 2010 were eligible to be included in the study. Isolates were shipped and analyzed at the central study laboratory in Tel Aviv.

Identification and susceptibility testing of MRSA isolates were done by the VITEK®2 (bioMerieux, Marcy l'Etoile, France) system; DNAse testing and cefoxitin disk diffusion testing according to Clinical and Laborataory Standards Institute guidelines (7). Genetic relatedness was determined by spa typing for all strains (18) and by pulsed-field gel electrophoresis (PFGE) for t008 isolates (27). spa types were determined with Ridom StaphType software version 2.2.1 (Ridom GmbH, Würzburg, Germany) and analyzed by the BURP algorithm, with the following parameters: spa types with fewer than five repeats were considered non-group-able, and spa types belonged to the same spa-clonal complex (CC) if the cost was less than or equal to six (18). In addition, the corresponding Multi-Locus Sequence Typing (MLST)-clonal complex was assigned for each spa type based on the Ridom StaphType database (http://spa.ridom.de/spatypes.shtml) and the study by Monecke et al (9). The Panton-
Valentine leukocidin (pvl) and the arcA (t008 isolates only) genes were tested for by PCR (13, 27). SCCmec typing was assigned according to the mec and ccr complexes (36); subtyping of SCCmec IV was done for t008 isolates (23).

Three-hundred and fifteen MRSA isolates were collected from 2006 to 2009. A summary of the epidemiologic features is presented in table 1. Almost half of the isolates were collected at TASMC. Eighty six (51%) and 66 (45%) of the blood and wound cultures, respectively were collected at least 72-hours after admission.

The molecular characteristics of MRSA strains according to spa and SCCmec types are presented in table 2 and figure 1 (t008). The majority of isolates (229, 63%) belonged to 13 spa types which are related to spa-CC-002/MLST-CC 5 (table 2, figure S1). The second largest was a group of 40 isolates (11%) and 11 spa types related to spa-CC 008/MLST-CC 8 (table 2, figure S1). Except for 5 t008 isolates, all spa-CC-008 isolates were negative in pvl testing.

The 21 t008 strains were divided into 2 major types (figure 1a): a) SCCmec-IVa, pvl/arcA-positive strain, identified as USA-300 by PFGE; b) SCCmec-IVc, (1 isolate-SCCmec-IVg) pvl/arcA-negative strain, that possessed a PFGE pattern similar to that of a previously described Israeli t008 strain (30) presumably similar to the EMRSA-2/-6 clone (8). All 5 USA-300 isolates and 12 of 16 pvl-negative t008 isolates were categorized as CA based on time of isolation.

Six novel MRSA spa types were found and 9 isolates were typed with a unique combination of spa and SCCmec types (table 2). Almost all MRSA SCCmec-I-III strains (table 2) were resistant to clindamycin, whereas strains harboring SCCmec-IV/V were mostly susceptible (55/85). In contrast, most isolates were susceptible to rifampin (95%) and trimethoprim-sulphamethoxazole (98%); all strains were susceptible to vancomycin.
The present study is the first national survey of MRSA strains in Israel. The most common MRSA strains in our study were found to be related to common epidemic clones, such as the Southern Germany (t001/SCCmec-I), New-York/Japan (t002/SCCmec-II), Berlin (t065/SCCmec-IV), EMRSA-2/-6 (t008/024/SCCmec-IV/pvl negative) and Iberian (t051/052/SCCmec-I) (8, 12) clones; these clones have been previously identified in Israel (1, 6). Interestingly, these clones were absent or rarely reported in a recent study from Lebanon (35).

Three pvl-producing strains were identified. The t318-SCCmec-IV strain (n=1) has been previously reported from Israel (1) and other middle-eastern countries (3, 14). t437-SCCmec-V, a common strain in Taiwan (5), has never been reported from our region, and USA-300 MRSA infections have never been reported from other countries in our region (3, 14, 35). Although we are not able to determine whether these USA-300 MRSA infections were acquired in Israel, these cases together with the recent report of a USA-300 MRSA infection in an Israeli child (16), suggest that this strain is likely to be present at a low level in our population.

The proportions of SCCmec types IV and V were 16% and 11%, respectively. The proportion of SCCmec-V is relatively high compared with other countries, as was also reported in a recent study from Israel (2). Many of the SCCmec-IV/V strains identified in our study are uniquely reported from Israel (table 2). Four t991 SCCmec-IV isolates were cultured from wounds within 3-days of admission, from AS, TA and RA, indicating the presence of this clone in communities outside the Bedouin population in the Negev (1). t002-SCCmec-V, the most common SCCmec-IV/V-harboring strain in our study, is reported uniquely from Israel (6), and was isolated after at least 72 hours of admission in 14 out of 28 cases. Altogether, SCCmec-IV/V-harboring strains were isolated after at least 72 hours of admission in 35 of 85 cases,

5
indicating that the SCC\textit{mec}-IV/V types, can not serve as a marker for community 
acquisition in Israel (11).

Our study has several limitations concerning the extent of representation of the 
MRSA population in Israel. First, our collection lacked a significant number of 
isolates from certain parts of the country, such as Jerusalem and the Galilee. Second, 
despite the collection instructions, the proportion of wound and blood culture isolates 
was not even in all centers. Third, we are not able to determine how many of the 
isolates labeled as 'CA' represented actual community-acquired infection, as many of 
the patients from whose specimens these isolates grew may have been recently 
hospitalized.

Our study delineates molecular epidemiologic features that our MRSA isolates have 
in common with those in other parts of the world, while highlighting unique features 
of the Israeli isolates. With global transmission of MRSA (33), our local 
epidemiology may be changing due to the introduction of new strains.
REFERENCES:


Figure 1. Molecular Features of *spa*-t008 Strains in Israel. Molecular, microbiological and epidemiological features of 21 *spa*-t008 isolates in Israel. *Clin-* Resistance (R) or susceptibility (S) to clindamycin; *Onset:* CA- community associate (number of isolates cultured within 3 days of admission), HA- healthcare associate (number of isolates cultured from 3 days of admission onward); *Source:* B- blood cultures, W- wound cultures.
Table 1. Epidemiologic characteristics of MRSA isolates in the study.

<table>
<thead>
<tr>
<th>Center</th>
<th>Blood</th>
<th>Wound</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>HA&lt;sup&gt;b&lt;/sup&gt;</td>
<td>CA&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TA</td>
<td>37</td>
<td>37</td>
<td>40</td>
</tr>
<tr>
<td>RA</td>
<td>21</td>
<td>34</td>
<td>27</td>
</tr>
<tr>
<td>ME</td>
<td>13</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>AS</td>
<td>11</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>BC</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>82</td>
<td>86</td>
<td>80</td>
</tr>
</tbody>
</table>

<sup>a</sup>CA- community associate (number of isolates cultured within 3 days of admission);

<sup>b</sup>HA- healthcare associate (number of isolates cultured from 3 days of admission onward).
Table 2. Molecular and Microbiological Features of MRSA Strains (other than spa-t008) in Israel.

<table>
<thead>
<tr>
<th>spa type</th>
<th>spa CC&lt;sup&gt;a&lt;/sup&gt;</th>
<th>MLST-CC&lt;sup&gt;b&lt;/sup&gt;</th>
<th>n.</th>
<th>SCCmec type</th>
<th>Clin S&lt;sup&gt;c&lt;/sup&gt; (n)&lt;sup&gt;d&lt;/sup&gt;</th>
<th>CA (n)&lt;sup&gt;d&lt;/sup&gt;</th>
<th>W (n)&lt;sup&gt;e&lt;/sup&gt;</th>
<th>Comments</th>
</tr>
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<tr>
<td>001</td>
<td>002</td>
<td>5</td>
<td>99</td>
<td>I&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0</td>
<td>49</td>
<td>51</td>
<td>Presumed Southern Germany clone (8); previously reported from Israel (6).</td>
</tr>
<tr>
<td>002</td>
<td>002</td>
<td>5</td>
<td>82</td>
<td>II&lt;sup&gt;i&lt;/sup&gt;</td>
<td>8</td>
<td>44</td>
<td>33</td>
<td>Presumed New-York/Japan clone (8); previously reported from Israel (6).</td>
</tr>
<tr>
<td>004</td>
<td>065:004</td>
<td>045</td>
<td>8</td>
<td>II</td>
<td>0</td>
<td>5</td>
<td>3</td>
<td>Novel report&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>010</td>
<td>002</td>
<td>5</td>
<td>3</td>
<td>II (2)/I (1)</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>Novel report&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>051</td>
<td>008</td>
<td>8</td>
<td>2</td>
<td>I</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>Presumed Iberian clone (12).</td>
</tr>
<tr>
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<td>008</td>
<td>8</td>
<td>6</td>
<td>I</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>Presumed Iberian clone (12).</td>
</tr>
<tr>
<td>535</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>II</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>Novel report&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>5212</td>
<td>008</td>
<td>2</td>
<td>1</td>
<td>I</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>Novel report&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>002</td>
<td>002</td>
<td>5</td>
<td>28</td>
<td>V</td>
<td>15</td>
<td>14</td>
<td>12</td>
<td>Previously reported from hospitals in Israel (6).</td>
</tr>
<tr>
<td>024</td>
<td>008</td>
<td>8</td>
<td>2</td>
<td>IV</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>Presumed EMRSA-2/-6 (8); previously reported from Denmark (21).</td>
</tr>
<tr>
<td>032</td>
<td>S&lt;sup&gt;f&lt;/sup&gt;</td>
<td>22</td>
<td>2</td>
<td>IV</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>Presumed EMRSA-15; reported from both animals and humans in Germany (34) and the UK (25, 31).</td>
</tr>
<tr>
<td>064</td>
<td>008</td>
<td>8</td>
<td>1</td>
<td>IV</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>Previously isolated from domestic animals in Europe (20, 25, 32).</td>
</tr>
<tr>
<td>065</td>
<td>065:004</td>
<td>45</td>
<td>8</td>
<td>IV</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>Previously reported from Israel (1, 28, 29), presumed Berlin clone (12).</td>
</tr>
<tr>
<td>105</td>
<td>002</td>
<td>5</td>
<td>1</td>
<td>V</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>Novel report&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>127</td>
<td>S&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1</td>
<td>1</td>
<td>IV</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>Associated with pigs in Europe (15).</td>
</tr>
<tr>
<td>159</td>
<td>S&lt;sup&gt;f&lt;/sup&gt;</td>
<td>121</td>
<td>1</td>
<td>IV</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>Novel report&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>214</td>
<td>002</td>
<td>5</td>
<td>1</td>
<td>V</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>Novel report&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> SCCmec types I-III (n=2)  
<sup>b</sup> SCCmec types IV/V  
<sup>c</sup> Presumed EMRSA-2;  
<sup>d</sup> Presumed EMRSA-15;  
<sup>e</sup> Presumed EMRSA-15; reported from both animals and humans in Germany (34) and the UK (25, 31).  
<sup>f</sup> Presumed EMRSA-15; reported from both animals and humans in Germany (34) and the UK (25, 31).
223 223/8610 22 1 IV 1 0 1 Presumed EMRSA-15 (24).
242 002 5 1 V 0 1 0 Novel report* 
318 037 30 1 IV 0 0 1 A *pvl*-positive strain, previously reported from Israel (1) and Shanghai (19).

437 Sf 59 1 V 0 1 1 A *pvl*-positive strain, commonly reported from Taiwan (5).
509 002 5 1 IV 0 0 0 Presumed "Pediatric clone", reported from France (9).
570 002 5 1 V 1 0 1 Novel report* 
723 008 8 1 V 0 0 0 Novel report* 
796 Sf 7 1 IV 1 0 0 Previously reported from China (22).

991 E 913 4 IV 3 4 4 A common colonizing strain in Bedouin children in Israel (1).
1774 008 8 1 IV 1 0 1 Previously reported from South Africa (26).
1911 008 8 1 IV 1 1 B Novel report* 
2849 008 2 IV/V 2 1 2 Novel report* 
5160 008 1 IV 0 1 1 Novel report* 
6274 065/004 1 IV 0 1 0 Novel report* 
7544 002 1 V 0 0 1 Novel report*

Data are presented for all strains excluding 008 strains (figure 1) and single-isolate, SCCmec types I-III spa types (n=23). Additional *spa*-CC-002 strains presented in figure S1 are 008, t105, t2358, and t5712. *spa* CC- *spa* clonal complex; *MLST*-CC- Multilocus sequence typing clonal complex (presumed allocation was based on the Ridom *spa* database and Monecke et al (9), unless specified in the comment); *Clin S*- number of isolates tested susceptible to clindamycin; *CA*-community associated (number of isolates cultured within 3 days of admission); *W* wound (number of isolates cultured from wound or other non-blood sites); *S* singleton on *spa* BURP analysis; *E* excluded from *spa* BURP analysis
due to low (5-) repeat number; 3 isolates harbored SCCmec-II; 1 isolate harbored SCCmec-I. *Designate either a newly reported spa type or a new spa-SCCmec type combination.