Clonal dissemination of mupirocin-resistant staphylococci in Greek hospitals

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Objectives: To determine the rates of mupirocin resistance in staphylococci during a 4 year period (1999–2002) in Greece.

Materials: A total of 1200 Staphylococcus aureus and 2760 coagulase-negative staphylococci (CoNS), consecutively collected from four Greek hospitals located in different geographical areas, were tested for susceptibility to mupirocin using the Etest and a reference agar dilution method.

Results: Twenty-four S. aureus (2%) and 532 CoNS (19.2%) were found to be mupirocin-resistant during the study period. High-level mupirocin resistance was detected in 20 S. aureus (1.6%) and in 440 CoNS (15.9%), respectively. No variations in the rates of mupirocin-resistant S. aureus in relation to the year of collection were observed. In contrast, the rate of mupirocin-resistant CoNS increased dramatically from 9% in 1999, to 14% in 2000, 20% in 2001 and reached 33% in 2002. PFGE analysis revealed the presence of one main clone (A) among mupirocin-resistant S. aureus and two main clones (i and a) among Staphylococcus epidermidis isolates.

Conclusions: In Greece, the rate of mupirocin-resistant S. aureus has remained low and steady since 1999. The high rate of mupirocin-resistant CoNS (33%) in 2002 was due mainly to clonal dissemination of epidemic hospital clones.

Keywords: Staphylococcus aureus, Staphylococcus epidermidis, mupirocin resistance, epidemic clones, Greece

Introduction

The elimination of staphylococci, particularly methicillin-resistant Staphylococcus aureus (MRSA), from the nose plays a crucial role in infection control protocols. Currently, one of the most effective topical agents for eradication of nasal carriage of MRSA is mupirocin. This antimicrobial agent is also used to prevent catheter colonization by coagulase-negative staphylococci (CoNS). However, staphylococcal isolates resistant to mupirocin are found worldwide. Staphylococci expressing mupirocin resistance can be divided in two groups: low-level resistant (MuL) with MICs in the range 8–256 mg/L and high-level resistant (MuH) with MICs ≥ 512 mg/L. Low-level resistance to mupirocin is more common and is thought to arise from point mutations within the usual chromosomal staphylococcal isoleucyl-tRNA synthetase gene (ileS). High-level resistance results from acquisition of a transferable plasmid carrying a new gene, ileS-2, encoding a second novel staphylococcal isoleucyl-tRNA synthetase, which has no affinity to mupirocin. Low and high-level resistance has been detected in both S. aureus and CoNS.

In Greece, mupirocin is only used to eradicate nasal carriage of MRSA in patients and staff. The antibiotic is not used for the treatment of staphylococcal skin infections or for the prevention of bacterial colonization due to coagulase-negative staphylococci. In the present study, we investigated the rate of development of mupirocin-resistant staphylococci (S. aureus and CoNS) in Greek hospitals during 1999–2002.

Materials and methods

Bacterial isolates

A total of 3960 staphylococci—comprising 1200 S. aureus and 2760 CoNS, consecutively isolated during January 1999–December 2002,
associated with blood, skin and soft tissue infections, and recovered from clinically significant specimens—were included in the study. The samples were collected from four tertiary care Greek hospitals, located in three geographical areas (Athens, Central Greece and Southwestern Greece). Isolates recovered from different cultures (blood, catheter etc.) from the same patient with the same Staphylococcus aureus and Staphylococcus epidermidis species were included once. Identification at the species level was carried out by Gram stain, catalase and coagulase tests, and by the API Staph System (bioMérieux, SA Lyon, France).

**Results**

A total of 556 staphylococci were found to be mupirocin-resistant by both agar dilution and Etest (MIC ≥ 8 mg/L). No discrepancies were observed between the reference agar dilution and Etest MIC values. The MuL staphylococcal strains with mupirocin MICs in the range 8–256 mg/L were easily recognized by the Etest, having a faint but visible zone of inhibition around the Etest strips. The MuH staphylococcal strains with mupirocin MICs ≥ 512 mg/L all had heavy, confluent growth with no detectable zones around the Etest strips.

Among the 1200 *S. aureus* isolates, 24 (2%) expressed mupirocin resistance during the study period. These 24 isolates were collected from patients; none of them had taken mupirocin treatment for nasal carriage. The distribution of low- and high-level mupirocin resistance in relation to time of isolation is described in Table 1. The rate of mupirocin resistance among *S. aureus* isolates was low and has remained steady since 1999. MuL was detected only in four mecA-positive *S. aureus* isolates (MIC 32 mg/L), belonging to clones A (three) and B (one), which have spread in several Greek hospitals. MuH (MIC ≥ 512 mg/L) was detected in 20 *S. aureus* isolates (14 mecA-positive), sporadically isolated in two of the four participating hospitals. PFGE analysis revealed that all of the MuH strains belonged to clone A, which expressed a relatively susceptible phenotype (Table 2).

Among the 2760 CoNS isolates, 1932 were identified as *Staphylococcus epidermidis*, 400 as *Staphylococcus haemolyticus*, 380 as *Staphylococcus hominis*, 14 as *Staphylococcus saprophyticus*, 14 as *Staphylococcus simulans*, 10 as *Staphylococcus lugdunensis* and 10 as *Staphylococcus xylosus*. Mupirocin resistance was detected in 532 clinically significant isolates, comprising 528 *S. epidermidis*, one *S. haemolyticus*, one *S. hominis*, one *S. lugdunensis*, and one *S. xylosus*. The respective infections were distributed evenly over the study period and there was no evidence of outbreaks. Among mupirocin-resistant CoNS, only 10 *S. epidermidis* isolates (four expressing MuL and six expressing MuH) were collected from patients after mupirocin treatment for nasal carriage. The distribution of MuL and MuH in relation to the time of isolation is described in Table 1. MuL (MIC 8–64 mg/L) was detected in 92 *S. epidermidis* isolates, of which 86 isolates were mecA-positive. MuH (MIC ≥ 512 mg/L) was detected in 440 isolates (436 *S. epidermidis*, one *S. haemolyticus*, one

### Table 1. Distribution of low- and high-level mupirocin resistance among staphylococci in correlation to the time of isolation

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of isolates</th>
<th>MuL isolates</th>
<th>%</th>
<th>MuH isolates</th>
<th>%</th>
<th>% Mu</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>240</td>
<td>2</td>
<td>0.8</td>
<td>4</td>
<td>1.66</td>
<td>2.5</td>
</tr>
<tr>
<td>2000</td>
<td>360</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>1.66</td>
<td>1.6</td>
</tr>
<tr>
<td>2001</td>
<td>280</td>
<td>2</td>
<td>0.7</td>
<td>4</td>
<td>1.4</td>
<td>2.14</td>
</tr>
<tr>
<td>2002</td>
<td>320</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>total</td>
<td>1200</td>
<td>4</td>
<td>0.33</td>
<td>20</td>
<td>1.6</td>
<td>2</td>
</tr>
<tr>
<td>1999</td>
<td>660</td>
<td>0</td>
<td>0</td>
<td>60</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>2000</td>
<td>700</td>
<td>22</td>
<td>3.14</td>
<td>76</td>
<td>10.8</td>
<td>13.99</td>
</tr>
<tr>
<td>2001</td>
<td>680</td>
<td>34</td>
<td>5</td>
<td>102</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>2002</td>
<td>720</td>
<td>36</td>
<td>5</td>
<td>202</td>
<td>28</td>
<td>33</td>
</tr>
<tr>
<td>total</td>
<td>2760</td>
<td>92</td>
<td>3.3</td>
<td>440</td>
<td>15.94</td>
<td>19.24</td>
</tr>
</tbody>
</table>

MuL, low-level mupirocin resistance; MuH, high-level mupirocin resistance; Mu, mupirocin resistance.

### Detection of ileS-2 and mecA genes

All isolates were tested for the presence of *ileS*-2 and *mecA* genes by PCR, as described previously. The predicted size of the PCR products were 456 bp and 310 bp for the *ileS*-2 and *mecA* fragments, respectively.

### PFGE analysis

Molecular typing of the mupirocin-resistant isolates was performed by PFGE analysis. The banding patterns of the strains were compared visually following the criteria of Tenover et al. 2

**Results**

A total of 556 staphylococci were found to be mupirocin-resistant by both agar dilution and Etest (MIC ≥ 8 mg/L). No discrepancies were

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Mupirocin-resistant staphylococci

Table 2. Genotypic and phenotypic properties of mupirocin-resistant *S. aureus* and *S. epidermidis*

<table>
<thead>
<tr>
<th>Number of isolates</th>
<th>MuL</th>
<th>MuH</th>
<th>Antibiotic resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em></td>
<td>23</td>
<td>6</td>
<td>AMP</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>14</td>
<td>AMP, OXA</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1</td>
<td>AMP, OXA, ERY, CIP, FUS, SXT</td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>292</td>
<td>6</td>
<td>286 i AMP, OXA, ERY, CLI, FUS, AMK, GEN, TOB, SXT, OFX</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>64</td>
<td>– a AMP, OXA, ERY, CLI, FUS, TOB, SXT, OFX, RIF</td>
</tr>
<tr>
<td></td>
<td>62</td>
<td>6</td>
<td>56 b AMP, OXA, ERY, FUS, GEN</td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>2</td>
<td>42 b AMP, OXA, ERY, CLI, FUS, AMK, GEN, TOB, SXT, OFX</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>6</td>
<td>4 c AMP, TET, FUS</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>8</td>
<td>– e AMP, OXA, ERY, CLI, FUS, AMK, GEN, TOB, SXT, OFX, RIF</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>–</td>
<td>24 g AMP, OXA, ERY, CLI, FUS, TET, TOB</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>–</td>
<td>24 l AMP, OXA, ERY, FUS, TET, TOB</td>
</tr>
</tbody>
</table>

MuL, low-level mupirocin resistance; MuH, high-level mupirocin resistance; AMK, amikacin; AMP, ampicillin; CLI, clindamycin; OFX, ofloxacin; ERY, erythromycin; FUS, fusidic acid; GEN, gentamicin; TOB, tobramycin; OXA, oxacillin; RIF, rifampicin; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline.

*S. hominis*, one *S. lugdunensis*, and one *S. xylosus*), of which 436 were *mecA*-positive. The rate of mupirocin resistance increased dramatically from 9% in 1999, to 14% in 2000, 20% in 2001 and reached 33% in 2002. Significant differences in the rates of resistance among hospitals have not been observed, although these hospitals belong to totally different geographic (rural, urban) areas. Furthermore, no correlation was found between the site of infection and the mupirocin-resistance rate. CoNS exhibited resistance to more than four classes of antimicrobial agents (Table 2).

As expected, all MuH isolates carried the *ileS*-2 gene, which was not detected in any MuL isolate. Analysis by PFGE showed that, although MuH *S. epidermidis* strains fell into six distinct clones (i, d, b, g, l, c), the great majority of isolates, 286 out 436 (65.6%), belonged to clone i (Figure 1). Before 1999, strains belonging to this clone did not carry the *ileS*-2 gene, so the resistant mutants have emerged in the last 4 years (data not shown). The MuL *S. epidermidis* strains were grouped into six different clones (a, e, i, d, c, b), the most dominant being clone a, comprising 64 out of 92 strains (69.56%).

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**Figure 1.** PFGE of *Smal* macrorestriction fragments of mupirocin-resistant *S. epidermidis* and *S. aureus* clinical isolates. Lanes 1 and 29, molecular size standards (lambda oligomers); numbers at right show molecular sizes in kilobases; small letters in the bottom indicate PFGE types of *S. epidermidis* and capital letters PFGE types of *S. aureus*. Lanes 2–7: representatives of epidemic methicillin-resistant *S. epidermidis* clones previously characterized; lanes 8–19: mupirocin-resistant *S. epidermidis* strains; lanes 20–24: mupirocin-resistant *S. aureus* strains; lanes 25–28: representatives of epidemic MRSA clones previously characterized.
PFGE types a, b, i and l have been characterized previously as epidemic clones. PFGE types d, e, c and g emerged later, after 2000.

Discussion

During the last decade, the increasing number of methicillin-resistant S. aureus worldwide has resulted in greater use of topical application of mupirocin to prevent colonization and subsequent infection. However, the use of mupirocin, especially after prolonged duration of topical treatment and/or in areas of highly concentrated drug, such as skin infections and burns, leads to the emergence of resistance.

Mupirocin resistance is relatively unusual in S. aureus, but it is common and increasing in CoNS. It varies greatly from institution to institution regardless of geographic region monitored. According to the SENTRY antimicrobial surveillance programme 2000, mupirocin resistance rates from bloodstream infections varied by geographic area (USA, Canada, Latin America and Europe) for S. aureus from 1.9% to 5.6% and for CoNS from 12.8% to 39.9%. A previous study in 19 European hospitals in 12 countries reported high-level resistance in 1.6% of S. aureus and 5.6% of CoNS isolates, and low-level resistance in 2.3% of S. aureus and 7.2% of CoNS isolates.

The prevalence of mupirocin-resistant S. aureus in Greek hospitals in this study is lower than that reported in a previous study (1.8% in 2002 versus 4.5% in 1997). However, the rate of mupirocin-resistant CoNS has increased dramatically, ranging from 9% in 1999 to 33% in 2002. The predominance of the clones A (among MuH S. aureus, which has spread in several Greek hospitals), and i and a (among MuH and MuL S. epidermidis strains), already characterized as epidemic clones, suggests that a limited number of mupirocin-resistant clones has been disseminated in the Greek hospital environment. This is not surprising for chromosomally mediated MuL, but is less expected for plasmid-mediated MuH, where horizontal spread of the plasmid among genetically diverse strains is likely. The high prevalence of mupirocin-resistant staphylococci was due mainly to clonal dissemination and to a lesser extent to gene spread.

The resistance profiles of the isolates have shown that the overwhelming majority of these were resistant to methicillin. Linezolid, quinupristin/dalfopristin and vancomycin maintained high activity against essentially all mupirocin-resistant strains.

In the period 1999–2002 in Greece, a rising incidence of mupirocin-resistant CoNS has been observed. In contrast, mupirocin resistance in S. aureus has remained more constant. In our hospitals, the use of mupirocin is limited and it is only used for controlling the spread of MRSA. The low-rate of mupirocin-resistant S. aureus is due to the limited MRSA exposure to mupirocin and any subsequent development of resistance. On the other hand, the finding that mupirocin resistance is more common among S. epidermidis than S. aureus could be explained by the capacity of certain clones (i, a) to spread widely. Thus, the increased rate of mupirocin-resistant CoNS in Greece is related to the spread of methicillin-resistant epidemic hospital clones rather than the consumption of mupirocin. Measures to combat this spread, such as effective control of hospital clones, would appear to be prudent.

References