resistance. However, the present study highlights the importance of continuous monitoring of susceptibility patterns in order to observe the development of resistance over a period of time. Such laboratory-based data will be of immense value in countries, such as India, where there are no controls on the prescription and use of antibiotics in the community.

REFERENCES


RESEARCH NOTE

Prevalence of inducible clindamycin resistance in macrolide-resistant Staphylococcus spp.

S. Fokas, S. Fokas, M. Tsironi, M. Kalkani and M. Dionysopoulou

Sparta General Hospital, Microbiology, Sparta, Laconia, Greece

ABSTRACT

Between January 2002 and December 2003, macrolide-resistant isolates of Staphylococcus aureus (n = 45) and coagulase-negative staphylococci (CoNS; n = 7) from a Greek hospital were examined phenotypically for inducible clindamycin resistance. The constitutive macrolide resistance phenotype predominated (60%) in S. aureus, followed by the inducible (35%) and the clindamycin-susceptible (5%) phenotypes. In CoNS, the inducible phenotype was more common than the constitutive phenotype (50% vs. 41%). There was...
a significant incidence of inducible clindamycin resistance, and screening of all staphylococci is necessary in order to differentiate inducibly resistant isolates from those that are truly sensitive.

**Keywords** Clindamycin resistance, coagulase-negative staphylococci, macrolide resistance, resistance, *Staphylococcus aureus*, susceptibility testing

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*Staphylococcus aureus* and coagulase-negative staphylococci (CoNS) have been recognised worldwide as important causes of nosocomial and community-acquired infections. The increasing prevalence of methicillin resistance among staphylococci is an increasing problem, and clindamycin is considered to be one of the alternative agents available to address this issue. In staphylococci, macrolide resistance is usually caused either by ribosomal modification mediated by 23S rRNA methylases encoded by the *erm* genes [1], or by active efflux of the antimicrobial agent by an ATP-dependent pump encoded by the *msrA* gene [2]. Methyases confer inducible (iMLS_B) or constitutive (cMLS_B) resistance to macrolides, lincosamides and type B streptogramin agents (MLS_B resistance), while the efflux mechanism affects macrolides and type B streptogramins only. Other more rare macrolide resistance mechanisms include ribosomal mutations and antibiotic inactivation by specific hydrolases or phosphotransferases [3,4].

Three macrolide resistance phenotypes have been described in staphylococci, based on their susceptibility to clindamycin, namely constitutive resistance, inducible resistance and susceptibility. In vitro tests show that strains with constitutive resistance are resistant to all macrolides (14- and 16-membered rings), lincosamides and streptogramin B, while inducibly-resistant strains are resistant only to 14- and 15-membered-ring macrolides. Broth dilution susceptibility tests fail to detect inducible resistance, but this phenotype can be detected by the double-disk diffusion test, which is an induction test using closely positioned erythromycin and clindamycin disks [5].

The objective of the present study was to investigate MLS_B resistance phenotypically and to record the current trend regarding the incidence and distribution of inducible clindamycin resistance in clinical isolates of *S. aureus* and CoNS from Greece.

In 2003, the prevalence of community-acquired methicillin-resistant *S. aureus* (MRSA) in the Laconia area was 18.7%, with a higher and increasing frequency (36.2%) of hospital-acquired MRSA. The frequency of erythromycin resistance in hospital-acquired MRSA was significantly higher than in community-acquired MRSA (25% vs. 12%). In total, 120 consecutive, erythromycin-resistant, clinical isolates of staphylococci (45 *S. aureus* and 75 CoNS) were recovered from wounds, pus, catheters, urine and blood cultures at the General Hospital of Sparta (Laconia, Greece) during the period 2002–2003. Erythromycin-resistant community-acquired MRSA isolates were obtained from four patients with skin abscesses, while the other 41 (91%) *S. aureus* isolates were hospital-acquired MRSA strains. Identification to the species level was achieved by catalase test, Gram’s stain, mannitol fermentation, growth on NaCl 6.5% w/v, latex slide agglutination assay for detection of clumping factor/protein A/capsular polysaccharides (Staphytec Plus; Oxoid, Basingstoke, UK), coagulase tube test and, for CoNS, the API Staph system (bioMérieux, Marcy-L’Étoile, France). Antibiotic susceptibility testing was performed by the NCCLS disk diffusion method with Mueller–Hinton agar, an inoculum of 0.5x McFarland standard, and incubation for 18 h at 35°C (24 h for oxacillin) in ambient air [6]. Methicillin resistance was detected with an oxacillin 1-µg disk (Bio-Rad, Marnes La Coquette, France), while PBP2’ was detected by a latex slide agglutination assay (Slidex MRSA detection; bioMérieux).

All erythromycin-resistant, clindamycin-susceptible isolates were also tested by the double-disk test to determine the macrolide resistance phenotype. Disks of erythromycin (15 µg) and clindamycin (2 µg) were placed 16 mm apart (edge to edge) [7,8]. Isolates with the clindamycin-susceptible phenotype demonstrated erythromycin resistance with clindamycin susceptibility, while isolates with the inducible resistance phenotype were erythromycin-resistant with a flattening or blunting of the clindamycin zone in the area between the two disks (D-shaped zone).
Statistical analysis was by the chi-square test with Yates’s correction, with the level of statistical significance defined as $p < 0.05$. Most (60%) of the *S. aureus* isolates had the constitutive phenotype, but 35% had the inducible phenotype, and only 5% had the clindamycin-susceptible phenotype. The constitutive phenotype predominated over the inducible phenotype (75% vs. 25%) among the MRSA isolates, and the clindamycin-susceptible phenotype was found only among methicillin-susceptible *S. aureus* isolates. Without the double-disk test, all *S. aureus* isolates with inducible clindamycin resistance would have been misclassified as clindamycin-susceptible, resulting in an underestimated clindamycin resistance rate of 60% instead of 95%.

The distribution of macrolide resistance phenotypes among CoNS isolates revealed a higher incidence of the inducible phenotype than the constitutive phenotype (50% vs. 41%). In particular, for *Staphylococcus epidermidis*, the species cultured most frequently from clinical samples, 55% of the isolates had the inducible phenotype and no isolate with the clindamycin-susceptible phenotype was observed. A statistically significant association between methicillin resistance and the constitutive phenotype was observed for *S. aureus* ($p < 0.05$) and *S. epidermidis* ($p < 0.001$) isolates. The macrolide resistance phenotypes found in the *S. aureus* and CoNS isolates following the double-disk induction test are summarised in Table 1.

Overall, the results indicated that there was a high incidence of the inducible MLSB resistance phenotype among *S. aureus* and CoNS isolates. The double-disk test was necessary to discriminate inducible clindamycin resistance from susceptibility to clindamycin correctly. The clindamycin-susceptible phenotype was less common among both *S. aureus* and CoNS isolates, but erythromycin-resistant staphylococci should not be assumed to be clindamycin-resistant. The incidence of MLSB resistance varies significantly according to geographical region. In Europe, there is a high incidence (93%) of the constitutive phenotype in MRSA, while the inducible phenotype is predominant in methicillin-susceptible *S. aureus* [9–11]. In contrast, a study from the USA reported a high incidence of the clindamycin-susceptible phenotype in *S. aureus* (50%) and in CoNS isolates (33%) [8].

Clindamycin resistance can develop in staphylococcal isolates with the inducible phenotype, and spontaneous constitutively resistant mutants have been selected from such isolates both *in vitro* and *in vivo* during clindamycin therapy [12–14]. The 2004 NCCLS guidelines recommend use of the double-disk test and suggest that isolates with the inducible resistance phenotype should be reported as clindamycin-resistant (with an optional comment). However, the clinical efficacy of clindamycin treatment for infections caused by inducibly resistant staphylococci remains unclear. The few cases reported so far have presented conflicting results, and elimination of a potentially useful drug such as clindamycin is not desirable, especially for the treatment of MRSA infections [15]. Additional clinical investigations are needed to determine whether or not staphylococci with the inducible phenotype should be reported as clindamycin-resistant. Clinical microbiology laboratories should use the double-disk test as standard practice with all erythromycin-resistant staphylococci, in order to identify the precise macrolide resistance phenotype and enable clinicians to be informed about the possibility of clindamycin treatment failure in patients with infections caused by inducibly resistant strains.

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>Constitutive (%)</th>
<th>Inducible (%)</th>
<th>Clindamycin-susceptible (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MR</td>
<td>MS</td>
<td>MR</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>45</td>
<td>21 (47)</td>
<td>6 (13)</td>
<td>7 (15)</td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>53</td>
<td>22 (41)</td>
<td>2 (4)</td>
<td>11 (21)</td>
</tr>
<tr>
<td><em>S. haemolyticus</em></td>
<td>11</td>
<td>6 (55)</td>
<td>0</td>
<td>2 (18)</td>
</tr>
<tr>
<td><em>S. saprophyticus</em></td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>3 (60)</td>
</tr>
<tr>
<td><em>S. lugdunensis</em></td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>1 (25)</td>
</tr>
<tr>
<td><em>S. hominis</em></td>
<td>2</td>
<td>3 (50)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

MR, methicillin-resistant; MS, methicillin-susceptible.
REFERENCES


