Detection and prevalence of inducible clindamycin resistance in staphylococci

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INTRODUCTION

Staphylococcus aureus and coagulase-negative staphylococci (CNS) are recognized to be causing nosocomial and community-acquired infections in every region of the world. The increasing prevalence of meticillin resistance among staphylococci is an increasing problem (Fokas et al., 2005).

Once these organisms are recognized to be causing infections in a region, it is of interest to determine which of the alternatives to vancomycin are suitable for therapy. In vitro susceptibility to clindamycin (CL), trimethoprim-sulfamethoxazole, erythromycin (ER), quinolone antibiotics and tetracyclines has frequently been reported (Hussain et al., 2000; Groom et al., 2001; Naimi et al., 2001). The macrolide-lincosamide-streptogramin B (MLS\textsubscript{B}) family of antibiotics is commonly used in the treatment of staphylococcal infections (Fiebelkorn et al., 2003). However, one important issue in CL treatment is the risk of clinical failure during therapy. Therapeutic failures caused by MLS\textsubscript{B} inducible resistance are being more commonly reported.

Abbreviations: CI, confidence interval; CL, clindamycin; CNS, coagulase-negative staphylococci; ER, erythromycin; MRCS, meticillin-resistant coagulase-negative staphylococci; MRSA, meticillin-resistant S. aureus; MSCNS, meticillin-sensitive coagulase-negative staphylococci; MSSA, meticillin-sensitive S. aureus; OR, odds ratio.
The MLS family of antibiotics has three different mechanisms of resistance: target site modification, enzymatic antibiotic inactivation and macrolide efflux pumps (Goldman & Capobianco, 1990; Leclercq & Courvalin, 1991a, b). Inducible MLS\textsubscript{B} resistance cannot be determined using standard susceptibility test methods, including standard broth-based or agar dilution susceptibility tests (Fiebelkorn et al., 2003). Low levels of ER are the most effective inducer of inducible MLS\textsubscript{B} resistance (Weisblum & Demohm, 1969). Antimicrobial susceptibility data are important for the management of infections, but false susceptibility results may be obtained if isolates are not tested for inducible CL resistance (CL-R) (Fiebelkorn et al., 2003; Drinkovic et al., 2001).

This study demonstrates a simple, reliable and significant method (double-disc diffusion test) of detecting inducible resistance to CL in ER-resistance (ER-R) isolates of \textit{S. aureus} and CNS.

**METHODS**

Staphylococcal isolates were selected from clinical isolates recovered in our laboratory from 2003 to 2005; duplicate isolates were not included. A total of 214 \textit{S. aureus} and 308 CNS isolates were selected based on ER-R and CL sensitivity using standard National Committee for Clinical Laboratory Standards (NCCLS) disc diffusion testing. These isolates were tested for inducible resistance using the D-test. A 0.5 McFarland equivalent suspension of organisms was inoculated onto a Mueller–Hinton agar (MHA) plate as described in the NCCLS recommendations (National Committee for Clinical Laboratory Standards, 2003). CL (2 \mu g) and ER (15 \mu g) discs were placed 15 mm apart from the centre on the MHA. Plates were analyzed after 18 h of incubation at 35 °C. Interpretation of the diameters of zones of inhibition was as follows: ER sensitive (ER-S) \geq 23 mm, ER intermediate resistance 14–22 mm, ER-R \leq 13 mm; clindamycin sensitive (CL-S) \geq 21 mm, CL intermediate resistance 15–20 mm and CL-R \leq 14 mm. If the ER zone is \leq 13 mm and the CL zone is \geq 21 mm and both have a circular shape, the organism is negative for inducible resistance (D-test negative). If the ER zone is \leq 13 mm and the CL zone is \geq 21 mm with a D-shaped zone around the CL, the organism is positive for inducible resistance (D-test positive) (Fig. 1).

**RESULTS**

A total of 1687 staphylococcal isolates (883 \textit{S. aureus} and 804 CNS) obtained from consecutive clinical specimens were included, consisting of 27.5 % (464) meticillin-resistant \textit{S. aureus} (MRSA), 24.8 % (419) meticillin-sensitive \textit{S. aureus} (MSSA), 36.1 % (608) meticillin-resistant CNS (MRCNS) and 11.6 % (196) meticillin-sensitive CNS (MSCNS) isolates. Of the staphylococcal isolates, 49.8 % had the CL-S phenotype, 28.3 % the constitutive resistance phenotype and 21.9 % the inducible resistance phenotype (Table 1). The constitutive resistance phenotype predominated over the susceptible phenotype and inducible resistance phenotype (44.2, 31.5 and 24.4 %, respectively) among the MRSA isolates. The CL-S phenotype predominated over the inducible resistance phenotype and constitutive resistance phenotype (80.7, 14.8 and 4.5 %, respectively) among MSSA isolates. The constitutive resistance phenotype predominated over the susceptible phenotype and inducible resistance phenotype (38.3, 36.0 and 25.7 %, respectively) among the MRCNS isolates. The CL-S phenotype predominated over the inducible resistance phenotype and constitutive resistance phenotype (69.9, 19.9 and 10.2 %, respectively) among MSCNS isolates (Table 1).

While in meticillin-resistant staphylococcal isolates the constitutive CL-R phenotype level was 40.9 % and the inducible resistance phenotype level 25.1 %, in meticillin-sensitive staphylococcal isolates the constitutive CL-R phenotype level was 6.3 % and the inducible resistance phenotype level 16.4 %. When the results were statistically compared in meticillin-resistant staphylococcal isolates the

**Table 1. Susceptibility to ER and CL among all staphylococcal isolates**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>MRSA (%)</th>
<th>MSSA (%)</th>
<th>MRCNS (%)</th>
<th>MSCNS (%)</th>
<th>Total no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER-S, CL-S</td>
<td>122 (26.3)</td>
<td>323 (77.1)</td>
<td>127 (20.9)</td>
<td>116 (59.2)</td>
<td>688 (40.8)</td>
</tr>
<tr>
<td>ER-R, CL-R</td>
<td>205 (44.2)</td>
<td>19 (4.5)</td>
<td>233 (38.3)</td>
<td>20 (10.2)</td>
<td>477 (28.3)</td>
</tr>
<tr>
<td>ER-R, CL-S, D\textsuperscript{-}</td>
<td>24 (5.2)</td>
<td>15 (3.6)</td>
<td>92 (15.1)</td>
<td>21 (10.7)</td>
<td>152 (9.0)</td>
</tr>
<tr>
<td>ER-R, CL-S, D\textsuperscript{+}</td>
<td>113 (24.4)</td>
<td>62 (14.8)</td>
<td>156 (25.7)</td>
<td>39 (19.9)</td>
<td>370 (21.9)</td>
</tr>
<tr>
<td>Total</td>
<td>464 (27.5)</td>
<td>419 (24.8)</td>
<td>608 (36.1)</td>
<td>196 (11.6)</td>
<td>1687</td>
</tr>
</tbody>
</table>
constitutive CL-R phenotype was determined to be 10.2 times greater \( (P=0.000, \text{OR} = 10.2, \text{95\% CI} = 7.13-14.66) \) and the inducible resistance phenotype 1.7 times greater \( (P=0.000, \text{OR} = 1.7, \text{95\% CI} = 1.31-2.22) \) than that in meticillin-sensitive staphylococcal isolates.

There was a 21.9 % inducible MLSB resistance phenotype level among all the staphylococcal isolates (19.8 % among \( S. \) aureus and 24.3 % among CNS isolates). When the \( S. \) aureus and CNS strains among all staphylococcal isolates were statistically compared, inducible CL-R in CNS strains was determined to be 23 % more positive \( (P=0.028, \text{OR} = 0.77, 95\% \text{CI} = 0.61-0.98) \) (Table 2). MRSA and MSSA strains among \( S. \) aureus isolates were also statistically compared, and inducible CL-R in MRSA strains was determined to be 1.85 times more positive \( (P=0.000, \text{OR} = 1.85, 95\% \text{CI} = 1.30-2.65) \).

A total of 522 (30.9 %) staphylococcal isolates (214 \( S. \) aureus and 308 CNS) that showed ER-R but were CL-S were tested for inducible resistance using the D-test. Three hundred and seventy (70.9 %) staphylococcal isolates (81.8 % of \( S. \) aureus and 63.3 % of CNS) with the ER-R and CL-S phenotype were observed to have a D-shaped zone around the CL. The organism was positive for inducible CL-R. When a statistical comparison was performed among the ER-R but CL-S staphylococcal isolates, inducible CL-R in \( S. \) aureus strains was determined to be 2.6 times more positive \( (P=0.000, \text{OR} = 2.6, 95\% \text{CI} = 1.68-4.04) \).

### DISCUSSION

The increasing frequency of staphylococcal infections among patients and changing patterns in antimicrobial resistance have led to renewed interest in the use of CL therapy to treat such infections (Frank et al., 2002). CL is frequently used to treat skin and bone infections because of its tolerability, cost, oral form and excellent tissue penetration, and the fact that it accumulates in abscesses and has minimal renal toxicity. Good oral absorption makes it an important option in outpatient therapy or as follow-up after intravenous therapy. CL-R is a good alternative for the treatment of recurrent and chronic infections (Frank et al., 2003). CL-R 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 CL therapy (Drinkovic et al., 2001; Panagea et al., 1999; Siberry et al., 2003). In 1969, McGehee et al. demonstrated the development of CL-R in vitro and in vivo in ER-resistant staphylococci (McGehee et al., 1969). Other authors have also confirmed the rapid in vitro conversion of inducible to constitutive MLSB resistance in staphylococci (Panagea et al., 1999; Werckenthin et al., 1999). The 2004 NCCLS guidelines recommend use of the double-disc test and suggest that isolates with the inducible resistance phenotype should be reported as CL resistant (National Committee for Clinical Laboratory Standards, 2004). However, the clinical efficacy of CL treatment for infections caused by inducibly resistant staphylococci remains unclear. The few cases reported so far have presented conflicting results, and the elimination of a potentially useful drug such as CL is undesirable, especially for the treatment of MRSA infections (Rao, 2000).

This study demonstrates that the D shape of the CL zone adjacent to an ER disc in a conventional disc diffusion test can serve to detect \( S. \) aureus or CNS strains with inducible resistance to CL. A total of 81.8 % of \( S. \) aureus and 63.3 % of CNS isolates with the ER-R and CL-S phenotype demonstrated inducible resistance. Overall, the results indicate a high incidence (21.9 %) of the inducible MLSB resistance phenotype among all the staphylococcal isolates (19.8 % of \( S. \) aureus and 24.3 % of CNS isolates). Our results demonstrate that in the case of a staphylococcal infection inducible MLSB resistance in CNS strains is greater than that in \( S. \) aureus strains. When the clinician is faced with a staphylococcal isolate, the probability that this strain may be inducible MLSB resistant is 21.9 %. If this strain is CNS, the probability that this strain may be inducible MLSB resistant will be 20.3 % greater. Provided that this strain is metillin resistant, the possibility that the strain may be inducible MLSB resistant is 1.7 times higher compared to a metillin-sensitive strain. Provided that the clinician is informed of ER-R and CL-S phenotype staphylococcal isolates, the probability of inducible MLSB resistance is high. If it is an \( S. \) aureus strain, its inducible MLSB resistance is 2.6 times higher than that of CNS strains. In such a case, the doctor is advised to provide treatment considering inducible MLSB resistance.

In a study conducted in Brazil, 11.3 % of \( S. \) aureus and 13.7 % of CNS isolates were determined to have the inducible MLSB resistance phenotype (Van der Heijden et al., 2001). It was reported that 80 % of \( S. \) aureus and 63 % of CNS isolates with the ER-R and CL-S phenotype demonstrated inducible resistance in a New York City medical centre (Van Horn & Toth, 2003). Fiebelkorn concluded that 29 % of 114 ER-R \( S. \) aureus isolates demonstrated inducible CL-R while 30 % of CNS isolates demonstrated inducible CL-R (Fiebelkorn et al., 2003).

In our study, the inducible CL-R phenotype level was 24.4 % among MRSA isolates, 14.8 % among MSSA isolates, 25.7 %

### Table 2. Susceptibility to ER and CL in \( S. \) aureus and CNS strains

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>( S. ) aureus (%)</th>
<th>CNS (%)</th>
<th>( P )</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER-S, CL-S</td>
<td>445 (50.4)</td>
<td>243 (30.2)</td>
<td>0.000</td>
<td>2.35</td>
<td>1.91–2.88</td>
</tr>
<tr>
<td>ER-R, CL-R</td>
<td>224 (25.4)</td>
<td>253 (31.5)</td>
<td>0.005</td>
<td>0.74</td>
<td>0.60–0.98</td>
</tr>
<tr>
<td>ER-R, CL-S, D</td>
<td>39 (4.4)</td>
<td>113 (14.1)</td>
<td>0.000</td>
<td>0.28</td>
<td>0.19–0.42</td>
</tr>
<tr>
<td>ER-R, CL-S, D</td>
<td>175 (19.8)</td>
<td>195 (24.3)</td>
<td>0.028</td>
<td>0.77</td>
<td>0.61–0.98</td>
</tr>
<tr>
<td>Total</td>
<td>883</td>
<td>804</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
among MRCNS isolates and 19.9% among MSCNS isolates. In another study conducted in Turkey, 5.7% among MRSA isolates, 3.6% of MSSA isolates, 30.8% of MRCNS isolates and 15.3% of MSCNS isolates were determined to have the inducible CL-R phenotype (Azap et al., 2005). Schreckenberger et al. (2004) reported incidences of inducible CL-R of 7–12% for MRSA, 19–20% for MSSA and 14–35% for CNS in two hospitals.

In the light of the restricted range of antibiotics available for the treatment of meticillin-resistant staphylococcal infections and the known limitations of vancomycin, CL should be considered for the management of serious soft tissue infections with meticillin-resistant staphylococci that either are sensitive to CL or exhibit inducible CL-R (Rao, 2000).

The double-disc diffusion test is necessary to correctly discriminate between inducible CL-R and susceptibility to CL. However, if inducible resistance can be reliably detected on a routine basis in clinically significant isolates, CL can be safely and effectively used in those patients with true CL-S strains. ER-R staphylococci should not be assumed to have CL-R.

In this study, we describe a simple, reliable method of detecting inducible resistance to CL in ER-R isolates of S. aureus and CNS. Clinical microbiology laboratories should use the double-disc diffusion test as standard practice with all ER-R staphylococci. Furthermore, in applying the susceptibility test to staphylococcal isolates, clinical microbiology laboratories should place the ER disc 15 mm apart from the CL disc. Thanks to the early detection of inducible MLS$_B$ resistance, such a measure will enable the clinician to save time. Consequently, treatment using CL can be omitted in patients with infections caused by inducibly resistant strains, and therapeutic failures may thus be avoided.

REFERENCES


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