D-Zone test for detection of inducible clindamycin resistance using SirScan paper disks and Rosco Neo-Sensitabs at 25 and 15 mm distances

Erlangga Yusuf, Annelies de Bel, Jamel Bouasse and Denis Piérard

Department of Medical Microbiology and Infection Control, Universitair Ziekenhuis Brussel, Brussels, Belgium

Clindamycin is a possible antibiotic treatment of infections by Gram-positive cocci. However, its use can be limited by inducible clindamycin resistance. To screen for the presence of this type of resistance, the D-zone test is used. The aim of this study was to compare the performance of SirScan paper disks with that of Rosco Neo-Sensitabs for the D-zone test at distances according to dispensers provided by the manufacturers (25 mm) and when the disks are placed at a distance of 15 mm. We studied 364 Gram-stain-positive cocci representing clinical isolates that were resistant to erythromycin and susceptible to clindamycin. Out of these isolates, 207 (57 %) gave a positive D-zone test result at 25 mm distance using SirScan paper disks. When the test was repeated with the same disks placed 15 mm from the 157 (43 %) isolates that had previously given a negative result, 58 (36.9 %) gave a positive D-zone test result. The same isolates were also found to test positive when Rosco Neo-Sensitabs were used. Placing the disks at a distance of 15 mm instead of 25 mm led to an 84.3 % increase in positive D-tests among Staphylococcus aureus, 43.8 % among group B streptococci (GBS) and 6.4 % among coagulase-negative staphylococci (CNS). In conclusion, the SirScan paper disks are equivalent to Rosco Neo-Sensitabs in screening for inducible resistance to clindamycin. The D-test needs to be performed at a shorter distance (15 mm) to prevent false-negative reporting.

INTRODUCTION

Gram-positive cocci are an important cause of nosocomial and community-acquired infections (Jones et al., 1999). Treating infections caused by these micro-organisms can be challenging because of the increasing number of Gram-positive cocci that are resistant to β-lactam antibiotics and because some patients may experience allergic reactions to this class of antibiotics. To address these issues, an attractive alternative is clindamycin, an antibiotic belonging to the lincosamide group (Lewis & Jorgensen, 2005). However, one of the major concerns of using clindamycin is the possible presence of inducible resistance. It is caused by ribosomal target modification that affects the activities of both macrolides and clindamycin, resulting in so-called macrolide–lincosamide–streptogramin B (iMLSB) resistance, which is encoded by the erm(A) or erm(C) gene (Hamilton-Miller & Shah, 2000). It is important to differentiate this iMLSB type of resistance from constitutive macrolide resistance that affects only macrolides and not clindamycin (Siberry et al., 2003).

Inducible clindamycin resistance might not be detected by some automated susceptibility-testing platforms, the standard disk diffusion test or the Etest (Jorgensen et al., 2004). In routine antimicrobial susceptibility tests, strains with inducible clindamycin resistance appear as erythromycin resistant and clindamycin susceptible. Strains expressing iMLSB resistance show a flattened inhibition zone around the clindamycin disk (positive ‘D-zone test’) when erythromycin and clindamycin disks are placed close to each other (Swenson & Patel, 2011).

It has not been investigated previously whether the size and type of the disks have any influence on the D-zone test. In our laboratory, we recently made a switch to SirScan paper disks (2A) that have a 6 mm diameter from Neo-Sensitabs (Rosco A/S) that have a 9 mm diameter. Automated disk dispensers generally place disks up to 26 mm apart (Swenson & Patel, 2011). According to the European Committee on Antimicrobial Susceptibility Testing (EUCAST), the range of distances between the disks is wide. To detect inducible clindamycin resistance in staphylococci, the committee advises placing the erythromycin and clindamycin disks...
edge to edge at a distance of 12–20 mm, and for streptococci at a distance of 12–16 mm (EUCAST, 2013).

The aim of this study was to compare the performance of SirScan paper disks with Rosco Neo-Sensitabs for the D-zone test at an edge-to-edge distance determined by the dispensers provided by the manufacturers (25 mm) and when the disks are placed at a distance of 15 mm.

**METHODS**

**Isolates.** This study was performed in a medical microbiology laboratory of a university hospital with approximately 700 beds. We identified and included 364 staphylococci and streptococci clinical isolates collected between November 2013 and January 2014. These clinical isolates appeared to be resistant to erythromycin and susceptible to clindamycin when inhibition zones were measured without taking into account eventual flattening of the clindamycin zone under the influence of an adjacent erythromycin disk. The erythromycin breakpoint for β-haemolytic streptococci was used for viridans-group streptococci since there is no EUCAST breakpoint for this type of micro-organism.

All inhibition-zone diameters were measured, reviewed and adjusted on screen with the SirScan instrument (i2A) by a laboratory technician as recommended by the manufacturer (Hombach et al., 2013).

**D-Zone test.** The primary D-zone test was performed by using an automatic disk dispenser (i2A) to place 15 μg erythromycin and 2 μg clindamycin SirScan paper disks at 25 mm edge-to-edge distance on isolates inoculated on Mueller–Hinton (bioMérieux) or on Mueller–Hinton–F agar (bioMérieux) in the case of streptococci. The isolates were then incubated at 35 ± 1 °C for 18 ± 2 h according to the EUCAST guideline.

The isolates that were read as negative for the primary D-zone test were re-isolated under the same conditions, and the D-zone tests were repeated at a shorter distance. The isolates were inoculated on Mueller–Hinton–F agar (bioMérieux) or on Mueller–Hinton–F agar (bioMérieux) in the case of streptococci. The isolates were then incubated at 35 ± 1 °C for 18 ± 2 h according to the EUCAST guideline.

The D-Zone tests were read independently by two observers, one who read the plate directly and another who read the image of the plate on a computer screen. Tests showing flattening of the clindamycin inhibition zone adjacent to the erythromycin disk were classified as D-zone test positive, and those with a circular inhibition zone were classified as D-zone test negative.

**Statistical analysis.** Analyses were performed using IBM SPSS Statistics for Windows, Version 20.0. (IBM). We calculated the number of positive D-tests for every distance and every type of disk.

### RESULTS

**Isolates**

Of 364 included isolates, 207 (57 %) gave positive D-zone test results with SirScan paper disks placed at a 25 mm distance. Therefore, we repeated the D-zone test in 157 (43 %) isolates, including 51 *S. aureus*, 78 coagulase-negative staphylococci (CNS) and 28 streptococci [16 of which were group B streptococci (GBS)] that gave negative D-zone test results.

**Comparison of performance of SirScan paper disks with Rosco Neo-Sensitabs for D-zone test**

The readers agreed in all D-zone test readings. Among the 157 isolates that gave a negative D-test result at a distance of 25 mm, 58 (36.9 %) tested positive in a D-zone test when the SirScan paper disks were placed at a 15 mm distance. The same isolates tested positive in the D-zone test using Rosco Neo-Sensitabs at a distance of 15 mm.

When the results were stratified according to the type of bacterium, placing the disks at a shorter distance led to 84.3 % more positive D-test results among *S. aureus*, 43.8 % among GBS and 25 % among streptococci other than GBS (Table 1). Only for CNS was the additional yield minimal (i.e. 6.4 %).

**DISCUSSION**

To the best of our knowledge, no earlier report has compared SirScan paper disks with Rosco Neo-Sensitabs in the D-zone test. For isolates with negative test results when the SirScan paper disks were placed at a distance of 25 mm, we found no differences in the D-zone test results between the SirScan paper disks and Rosco Neo-Sensitabs when the disks were placed at a distance of 15 mm.

The convenience of using automated disk dispensers that place the disks at up to 26 mm distance comes at the cost of false-negative D-tests. We showed that placing the disks

<table>
<thead>
<tr>
<th>Type of bacterium</th>
<th>No. of isolates testing D-test negative at 25 mm distance</th>
<th>No. of isolates at 15 mm distance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D-test positive (%)</td>
<td>D-test negative (%)</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>51</td>
<td>43 (84.3)</td>
</tr>
<tr>
<td>CNS</td>
<td>78</td>
<td>5 (6.4)</td>
</tr>
<tr>
<td>GBS</td>
<td>16</td>
<td>7 (43.8)</td>
</tr>
<tr>
<td>Other streptococci</td>
<td>12</td>
<td>3 (25.0)</td>
</tr>
</tbody>
</table>
at a shorter (i.e. 15 mm) distance will lead to more positive D-test results, especially for \textit{S. aureus} and GBS. Therefore, one cannot report that inducible resistance is absent in cocci resistant to erythromycin and susceptible to clindamycin on the basis of a negative D-test result with disks placed at a distance of 25 mm.

The results of our study support those from O’Sullivan and colleagues that showed a 12.3 % lower sensitivity of the D-test at a distance of 22 mm versus 15 mm (87.7 % versus 100 %) for \textit{Staphylococcus} sp. (O’Sullivan et al., 2006). Yet, our results differ from those of the study by Fiebelkorn and colleagues (Fiebelkorn et al., 2003) that showed sensitivities of disk-induction testing of 100 % at 15 mm and 97 % at 26–28 mm for \textit{S. aureus}. They also showed that the sensitivity was 100 % at both 20 and 26 mm for CNS. We can only speculate that the difference between our results and those of Fiebelkorn and colleagues is caused by the difference in the genes that encode the inducible resistance. It has been shown that isolates with \textit{erm}(A) show more false-negative D-test results than isolates with \textit{erm}(C) (O’Sullivan et al., 2006). The difference in the genes encoding inducible resistance to clindamycin might also explain why there are more false-negative D-tests for \textit{S. aureus} isolates in tests where disks are placed at a distance of 25 mm, while for CNS isolates the number of false-negative isolates at this distance is low. It has been reported that constitutive resistance is more predominant than inducible resistance (60 vs 35 %) among \textit{S. aureus} isolates, but the inverse is true among CNS isolates, where the incidence of the inducible phenotype is higher than the constitutive phenotype (50 vs 41 %) (Fokas et al., 2005). Since the number of false-susceptible isolates (i.e. representing very major error) appears to be low in CNS isolates, it may be reasonable to report isolates with negative D-test results at distance of 25 mm as susceptible to clindamycin.

The variation in reported distances in the above-mentioned studies is caused by the use of automated disk dispensers from different manufacturers. It is also reasonable to compare the distances at both ends of the range given by the EUCAST (e.g. 20 and 12 mm for staphylococci, and 16 and 12 mm for streptococci). A limitation of this study should be mentioned: no PCR on the \textit{erm} gene was performed. Therefore, we cannot exclude the possibility that some of the isolates with negative D-test results may harbour the gene, and, thus, both disk tests could lack sensitivity.

**REFERENCES**


