Use of an Oxacillin Disk Screening Test for Detection of Penicillin- and Ceftriaxone-Resistant Pneumococci

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In a context of worldwide emergence of resistance among Streptococcus pneumoniae strains, early detection of strains with decreased susceptibility to β-lactam antibiotics is important for clinicians. If the 1-µg oxacillin disk diffusion test is used as described by the National Committee for Clinical Laboratory Standards, no interpretation is available for strains showing zone sizes of ≤19 mm, and there is presently no disk diffusion test available for screening cephalosporin resistance. The zones obtained by the diffusion method by using the 1-µg oxacillin disk were compared with penicillin MICs for 1,116 clinical strains and with ceftriaxone MICs for 695 of these strains. Among the 342 strains with growth up to the 1-µg oxacillin disk margin, none were susceptible (MIC, ≤0.06 µg/ml), 62 had intermediate resistance (MIC, 0.12 to 1.0 µg/ml), and 280 were resistant (MIC, ≥2.0 µg/ml) to penicillin. For ceftriaxone, among 98 strains with no zone of inhibition in response to oxacillin, 68 had intermediate resistance (MIC, 1.0 µg/ml), and 22 were resistant (MIC, ≥2.0 µg/ml). To optimize the use of the disk diffusion method, we propose that the absence of a zone of inhibition around the 1-µg oxacillin disk be regarded as an indicator of nonsusceptibility to penicillin and ceftriaxone and recommend that such strains be reported as nonsusceptible to these antimicrobial agents, pending the results of a MIC quantitation method.

The emergence of resistance to β-lactam antibiotics in clinical isolates of Streptococcus pneumoniae has been reported throughout the world with increasing frequency (1, 8, 11, 12, 21, 25, 31). Because of the consequences of β-lactam resistance to the clinical response to antimicrobial therapy and the possible need to modify such a therapy based on susceptibility results, early detection of strains with decreased susceptibility to these antibiotics is important (20, 24). The diffusion method with a 1-µg oxacillin disk is currently recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (22, 23) as an effective screening method for the detection of penicillin-resistant pneumococci and is commonly used by clinical laboratories. Although many studies have been done with disks containing ceftriaxone, cefotaxime, cefixime, cefuroxime, and loracarbef, there is presently no method with a 1-µg oxacillin disk margin, none were susceptible (MIC, ≤0.06 µg/ml), 62 had intermediate resistance (MIC, 0.12 to 1.0 µg/ml), and 280 were resistant (MIC, ≥2.0 µg/ml) to penicillin. For ceftriaxone, among 98 strains with no zone of inhibition in response to oxacillin, 68 had intermediate resistance (MIC, 1.0 µg/ml), and 22 were resistant (MIC, ≥2.0 µg/ml). To optimize the use of the disk diffusion method, we propose that the absence of a zone of inhibition around the 1-µg oxacillin disk be regarded as an indicator of nonsusceptibility to penicillin and ceftriaxone and recommend that such strains be reported as nonsusceptible to these antimicrobial agents, pending the results of a MIC quantitation method.

Isolates were sent to our laboratory for susceptibility testing or as part of a multicenter surveillance study of invasive S. pneumoniae infections in the province of Quebec (17). Between January 1995 and December 1996, strains (n = 1,116) were isolated from normally sterile body fluids (63%) and respiratory tract sources (37%).

Susceptibility methods were performed as outlined by the NCCLS (22, 23). Disk diffusion tests were performed on Mueller-Hinton agar supplemented with 5% defibrinated sheep blood, and test samples were incubated for 20 to 24 h at 35°C in a 5 to 7% CO2 atmosphere. Oxacillin disks were purchased from Oxoid (Unipath, Ontario, Canada). Broth microdilution tests were carried out with an inoculum prepared from an overnight growth on blood agar plates and adjusted to achieve approximately 5 × 105 CFU/ml. MICs were recorded after 20 to 24 h of incubation at 35°C in ambient air. Twofold dilutions of benzylpenicillin (32 to 0.03 µg/ml) of ceftriaxone (32 to 0.03 µg/ml) were performed with cation-adjusted Mueller-Hinton broth supplemented with 2 to 5% lysed horse blood. Antimicrobial powders were purchased from Sigma Chemical Co. (St. Louis, Mo.). S. pneumoniae ATCC 49619 was used as a control throughout the investigation and included with each batch of diffusion and microdilution tests. Results were interpreted according to NCCLS recommendations.

Figure 1 is a scattergram comparing oxacillin zone diameters to penicillin MICs for 1,116 strains. As expected, all strains (n = 592) showing a zone size of ≥20 mm were found susceptible (MIC, ≤0.06 µg/ml) to penicillin by the microdilution method. Among a total of 645 strains found susceptible by microdilution, 53 (8.2%) yielded oxacillin zone sizes of ≤19 mm and would be misclassified as nonsusceptible on the basis of the disk test. For the non-penicillin-susceptible strains (MIC, ≥0.12 µg/ml), all 189 intermediate resistant (MIC, 0.1 to 1.0 µg/ml) and all 282 resistant (MIC, ≥2.0 µg/ml) strains had oxacillin zone diameters of ≤19 mm. However, more interestingly, among the 342 strains with growth up to the disk margin (zone diameter = disk diameter = 6 mm), none were
susceptible to penicillin: 62 and 280, respectively, were immediately resistant and resistant to penicillin (Table 1). The absence of a zone of growth inhibition around the oxacillin disk had positive predictive values of 82% for resistance to penicillin (MIC, ≥2 µg/ml) and 100% for nonsusceptibility (MIC, ≥0.12 µg/ml). In Quebec, the prevalence of penicillin resistance found in our prospective surveillance program in 1996 was 6.9%, while the prevalence of nonsusceptibility was 9.8% (17). Taking into account these prevalence rates, the new positive predictive values for resistance as well as nonsusceptibility to penicillin were 87.6 and 100%, respectively.

Among the 1,116 strains tested by disk diffusion, 695 were also tested for ceftriaxone susceptibility by broth microdilution. Figure 2 is a scattergram comparing oxacillin zone diameters with ceftriaxone MICs. Among the 98 strains with no zone diameter of inhibition in response to oxacillin, 68 (69.4%) were intermediately resistant (MIC, 1 µg/ml) and 22 (22.4%) were resistant (MIC, ≥2.0 µg/ml) to ceftriaxone. The positive predictive value was 85%, taking into account the observed prevalence of nonsusceptibility to ceftriaxone (MIC, ≥1.0 µg/ml) established at 7.1% in our surveillance program. We also observed that among strains tested with penicillin and ceftriaxone, 86 were found resistant (MIC, ≥2.0 µg/ml) to penicillin, and only 1 of them was susceptible to ceftriaxone (MIC, 0.5 µg/ml). The ceftriaxone MICs for 63 and 22 of the remaining strains, respectively, were 1.0 (intermediate resistance) and 2.0 µg/ml (resistant).

All strains were tested along with the quality control strain, S. pneumoniae ATCC 49619 (23). Results obtained with this strain were always within the recommended MIC limits of penicillin (n = 147) and ceftriaxone (n = 104). For the disk diffusion method, concordance was 98%; in two occasions among 96 tests, the zone diameters were out of the expected range. Clinical strains belonging to these two batches were retested.

In Quebec, during the last 10 years, the prevalence of pneumococci nonsusceptible to penicillin (MIC, ≥0.12 µg/ml) rose from 1.3% to 9.8%, and the resistance rate (strains for which MICs were ≥2.0 µg/ml) increased from 0% to 6.9% (16, 17). The impact of this resistance on the treatment of severe pneumococcal infections is serious (8, 11, 20, 21). In the microbiology laboratories, the disk diffusion method with the 1-µg oxacillin disk is largely used in the screening of penicillin nonsusceptibility. Presently, there is no disk diffusion method accepted for the detection of broad-spectrum cephalosporin resistance, because minor error rates of more than 15% have been reported (18) for cefotaxime and ceftriaxone. As published previously (10, 18, 30) and confirmed by this study, a zone of growth inhibition of 20 mm or more around the 1-µg oxacillin disk is always predictive of susceptibility to penicillin. Similarly to what was previously reported by Doern et al. (10) and Jorgensen et al. (18), we observed some strains fully penicillin sensitive with an oxacillin zone size of ≤19 mm. The limitations of the oxacillin diffusion test are well documented by the NCCLS. Unfortunately, MIC determinations for strains showing zone sizes of ≤19 mm introduce a delay of at least 1 day in reporting the final result. For laboratories with sufficient technical resources, Doern et al. (10) recently recommended that MIC tests be performed directly, at least for strains isolated from cerebrospinal fluid. The NCCLS recommends testing of pneumococcal isolates from blood and the central nervous system (CNS) by using a MIC method, since reliable disk diffusion tests with agents such as ceftriaxone and cefotaxime do not yet exist (23). Unfortunately, the necessary technical resources are not always available on-site. Our study shows

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**TABLE 1.** Distribution of 1,116 strains by MICs of penicillin and growth inhibition zone diameter around 1-µg oxacillin disk

<table>
<thead>
<tr>
<th>Inhibition zone diam (mm) around 1-µg oxacillin disk</th>
<th>No. of isolates inhibited by penicillin MIC (µg/ml) of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤0.06</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>7–19</td>
<td>53</td>
</tr>
<tr>
<td>≥20</td>
<td>592</td>
</tr>
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</table>
that the absence of a zone around the 1-µg oxacillin disk is highly predictive of penicillin and ceftriaxone nonsusceptibility. The results obtained by Doern et al. (10) and Barry et al. (4) were similar to ours. These studies presented scattergrams comparing MICs of penicillin or cefotaxime to zone inhibitions around a 1-µg oxacillin disk and showed that 99 and 98% of pneumococci with no zone of inhibition in response to oxacillin were nonsusceptible to penicillin, and 99% of them were also nonsusceptible to cefotaxime. The oxacillin disk test was also useful in predicting nonsusceptibility to cephalosporins when strains grew up to the margin of the disk. Unfortunately, discrimination between intermediate susceptibility and resistance cannot be achieved with enough confidence to be of any use, even with other β-lactam disks.

Penicillin remains the antibiotic of choice for treatment of infections caused by susceptible strains of S. pneumoniae. A high dosage is recommended for treatment of meningitis and other infections of the CNS. Penicillin at a higher dosage is recommended for pneumococci with intermediate susceptibility to penicillin in non-CNS infections, whereas for meningitis, other antimicrobial agents should be used, because many reports of penicillin failure with even moderately resistant strains have been published. Obviously, penicillin is not an option for any CNS infection with pneumococci showing resistance to penicillin. For the treatment of severe non-CNS infections with resistant strains, many experts recommend another antimicrobial agent (6, 7, 9, 13, 19, 25, 26).

Studies have shown that an increase in MICs of penicillin is usually accompanied by increases in MICs of other β-lactams (3, 28, 29). The extremely high rate of decreased ceftriaxone susceptibility among our penicillin-resistant strains is disturbing, because this antimicrobial agent or cefotaxime is part of the antimicrobial regimen for the treatment of meningitis caused by these strains.

As recommended by the NCCLS, MIC determinations are indicated for strains with a zone inhibition of ≤19 mm with oxacillin. For pneumococcal meningitis, immediate MIC testing is certainly a legitimate option. However, for the majority of other infections caused by S. pneumoniae, the 1-µg oxacillin disk screening test, due mainly to its simplicity and cost-effectiveness, remains an attractive method. We propose that strains with growth up to the margin of the oxacillin disk be reported as nonsusceptible to penicillin and ceftriaxone pending MIC results. This would be of particular interest to small institutions which have to rely on reference laboratories for MIC testing, further delaying the presentation of final results. Performance of MIC testing of such strains should be dictated by the severity of patient infection. In hospitals with a very high prevalence of penicillin resistance, immediate MIC testing of penicillin and broad-spectrum cephalosporins, by the dilution or E-test method, for all or the majority of pneumococci is an option to be considered.

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