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

LOUISE P. JETTE1* AND CHRISTIAN SINAVE2

Laboratoire de Santé Publique du Québec, Sainte-Anne-de-Bellevue,1 and Département de Microbiologie et Infectiologie, Faculté de Médecine, Université de Sherbrooke,2 Québec, Canada

Received 20 July 1998/Returned for modification 16 October 1998/Accepted 11 December 1998

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 detection of penicillin-resistant pneumococci and is commonly used by clinical laboratories. Although many studies have been done with disks containing ceftriaxone, cefotaxime, cefixime, cefixime, cefuroxime, and loracarbef, there is presently no method with a 1-μg oxacillin disk for the screening of cephalosporin resistance. The emergence of resistance to cephalosporins in pneumococci with this characteristic can be either sensitive, intermediate, resistant, or resistant to penicillin (30). In this situation, determination of the strain susceptibility to penicillin and ceftriaxone 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, cefixime, cefuroxime, and loracarbef, there is presently no disk diffusion test available for screening cephalosporin resistance (2, 4, 5, 14, 15, 27). The recommended interpretative zone criteria for the detection of penicillin susceptibility with the 1-μg oxacillin disk is a zone size of ≥20 mm. No interpretation is available for zone sizes of ≤19 mm, because strains of pneumococci with this characteristic can be either sensitive, intermediate, resistant, or resistant to penicillin (30). In this situation, determination of the strain susceptibility to penicillin by a quantitative method is indicated. The 1-μg oxacillin test is regarded as very sensitive for detection of penicillin resistance, but of low specificity. This study reports observations concerning the relationship between the absence of a growth inhibition zone around the 1-μg oxacillin disk and the susceptibility of S. pneumoniae to penicillin and ceftriaxone. It also reassesses the value of the oxacillin disk test, not only as a predictor of penicillin susceptibility, but also as a tool to rapidly detect decreased susceptibility to penicillin and ceftriaxone.

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 intermediately 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 inter-
mediately 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 penicil-
in (MIC, $\geq 2 \mu g/ml$) and 100% for nonsusceptibility (MIC,
$\geq 0.12 \mu g/ml$). In Quebec, the prevalence of penicillin resis-
tance 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 po-
sitive 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 microdilu-
tion. Figure 2 is a scattergram comparing oxacillin zone diam-
eters 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 $\mu g/ml$) and 22 (22.4%)
were resistant (MIC, $\geq 2.0 \mu g/ml$) to ceftriaxone. The positive
predictive value was 85%, taking into account the observed
prevalence of nonsusceptibility to ceftriaxone (MIC,
$\geq 1.0 \mu g/ml$) established at 7.1% in our surveillance program. We also
observed that among strains tested with penicillin and ceftri-
axone, 86 were found resistant (MIC, $\geq 2.0 \mu g/ml$) to penicillin,
and only 1 of them was susceptible to ceftriaxone (MIC, 0.5
$\mu g/ml$). The ceftriaxone MICs for 63 and 22 of the remaining
strains, respectively, were 1.0 (intermediate resistance) and 2.0
$\mu g/ml$ (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 pneu-
mococci nonsusceptible to penicillin (MIC, $\geq 0.12 \mu g/ml$) rose
from 1.3% to 9.8%, and the resistance rate (strains for which
MICs were $\geq 2.0 \mu g/ml$) increased from 0% to 6.9% (16, 17).
The impact of this resistance on the treatment of severe pneumo-
occal infections is serious (8, 11, 20, 21). In the microbi-
ology laboratories, the disk diffusion method with the 1-$\mu g$
oxacillin disk is largely used in the screening of penicillin non-
susceptibility. Presently, there is no disk diffusion method ac-
cepted for the detection of broad-spectrum cephalosporin re-
sistance, because minor error rates of more than 15% have
been reported (18) for cefotaxime and ceftriaxone. As pub-
lished previously (10, 18, 30) and confirmed by this study, a
zone of growth inhibition of 20 mm or more around the 1-$\mu g$
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 pen-
icillin sensitive with an oxacillin zone size of $\# 19 \mu g/ml$. The
limitations of the oxacillin diffusion test are well documented
by the NCCLS. Unfortunately, MIC determinations for strains
showing zone sizes of $\leq 19 \mu g/ml$ 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 iso-
lated from cerebrospinal fluid. The NCCLS recommends test-
ing of pneumococcal isolates from blood and the central ner-
vous 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

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
Inhibition zone diam (mm) around 1-$\mu g$ oxacillin disk & No. of isolates inhibited by penicillin & MIC (ug/ml) of: \\
\hline
& & $\leq 0.06$ & 0.12–1.0 & $\geq 2.0$ \\
\hline
6 & 0 & 62 & 280 \\
7–19 & 53 & 127 & 2 \\
$\geq 20$ & 592 & 0 & 0 \\
\hline
\end{tabular}
\caption{Distribution of 1,116 strains by MICs of penicillin and
growth inhibition zone diameter around 1-$\mu g$ oxacillin disk}
\end{table}
that the absence of a zone around the 1-μg oxacillin disk is highly predictive of penicillin and cephalosporine 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 cefoxime 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 cefoxime. 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.

We thank Martial Demers and François Robillard for technical assistance. We are grateful to Gilles Delage for helpful comments on the manuscript. Finally, we thank Lucie Carrière for secretarial assistance.

REFERENCES


