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Letters to the Editor

More about Cefuroxime Screening Test for Pneumococci

Jorgensen et al. published an extensive evaluation of disk diffusion tests for *Streptococcus pneumoniae* (3). Several antimicrobial agents were tested by a rigorous method. However, the authors did not show the results they said could be obtained using cefuroxime. They said that cefuroxime zone diameter data generated during their study were not useful for accurate recognition of cephalosporin-resistant (MICs of >2 μg/ml) strains. Similar conclusions were obtained by Tenover et al. (6). Neither of these groups has tried to use the cefuroxime disk diffusion test as an screening method to detect both cefotaxime-intermediate and cefotaxime-resistant strains (MIC, >0.25 μg/ml) (5).

One hundred and seventeen strains of *S. pneumoniae* isolated from clinically significant samples obtained from the same number of pediatric patients were selected to be tested by both a broth macrodilution method with ceftriaxone and a disk diffusion method with cefuroxime. The dilution test was performed with Mueller-Hinton broth plus 2% lysed horse blood, and the tubes were incubated for 18 to 24 h at 35°C in room air. Ceftriaxone was obtained from Productos Roche S.A.Q. e I. The diffusion method was performed by following the National Committee for Clinical Laboratory Standards (NCCLS) guidelines for pneumococci (4) using 30-μg cefuroxime disks (Laboratorios Britania S.R.L., Buenos Aires, Argentina) and 5% sheep blood Mueller-Hinton agar plates. They were incubated for 18 to 24 h at 35°C in a candle jar. *S. pneumoniae* ATCC 49619 was included as the reference strain.

No "very major" errors were obtained, and only 7.7% of the susceptible strains would be classified as resistant (or intermediate) by the screening method (Fig. 1). From this single experience, the cefuroxime disk diffusion test seemed to be a good screening method for categorizing strains of *S. pneumoniae* nonsusceptible to cefotaxime. Zone sizes of <35 mm may be indicative of either cefotaxime-resistant or cefotaxime-intermediate pneumococci by NCCLS breakpoints (5). It could be a matter of discussion which of the cutoffs is appropriate, ≤0.25 μg/ml, as the NCCLS recommended (5), or ≤0.5 μg/ml, as Jorgensen et al. proposed (3). At least one clinical failure

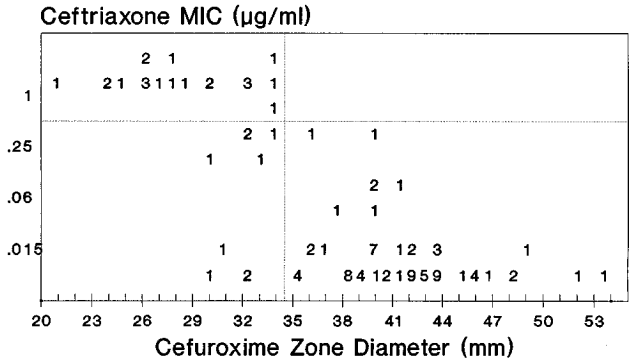

FIG. 1. Scatterplot of ceftriaxone MICs determined with 5% lysed horse blood Mueller-Hinton broth versus cefuroxime disk diffusion results determined with 5% sheep blood Mueller-Hinton agar. Breakpoints are those recommended by the NCCLS (5).

was reported when a child with meningitis caused by *S. pneumoniae* for which the MIC of cefotaxime was 0.5 μg/ml was treated with that antibiotic (1). We have no personal data to support one or the other criterion, but we preferred to be conservative in this matter and used the lower value.

Considering diameters of ≥35 mm as indicative of susceptibility by the cefuroxime screening test and adopting NCCLS breakpoints for the dilution method, we found that even in the scattergram published by Tenover et al. (6) neither resistant (MIC, ≥2 μg/ml) nor intermediate (MIC, between 1 and 0.5 μg/ml) strains of *S. pneumoniae* would be considered susceptible to cefotaxime. Twenty-seven percent of the minor errors would be introduced in this case (6). Moreover, in another study, all the strains for which MICs were ≥1 μg/ml produced inhibition zones of <35 mm when challenged with 30-μg cefuroxime disks (1).

Later, our results and some previous information tempted us to use the cefuroxime screening test for predicting not resistance but susceptibility to broad-spectrum cephalosporins, in the same way that the oxacillin test is being used for detecting penicillin susceptibility in pneumococci (4). In cases of meningitis, we believe that it will be more useful to accurately predict susceptibility, rather than resistance, to broad-spectrum cephalosporins.

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Author's Reply

In our manuscript describing the development of MIC and zone diameter interpretive criteria for pneumococci, we reported that there were numerous minor interpretive errors with the cefotaxime and ceftriaxone disk diffusion tests that precluded recommending them for routine use (2). We also stated that we did not find cefuroxime zone diameters to be useful in accurate cetegorization of the susceptibility of pneumococci to either cefotaxime or ceftriaxone, although those

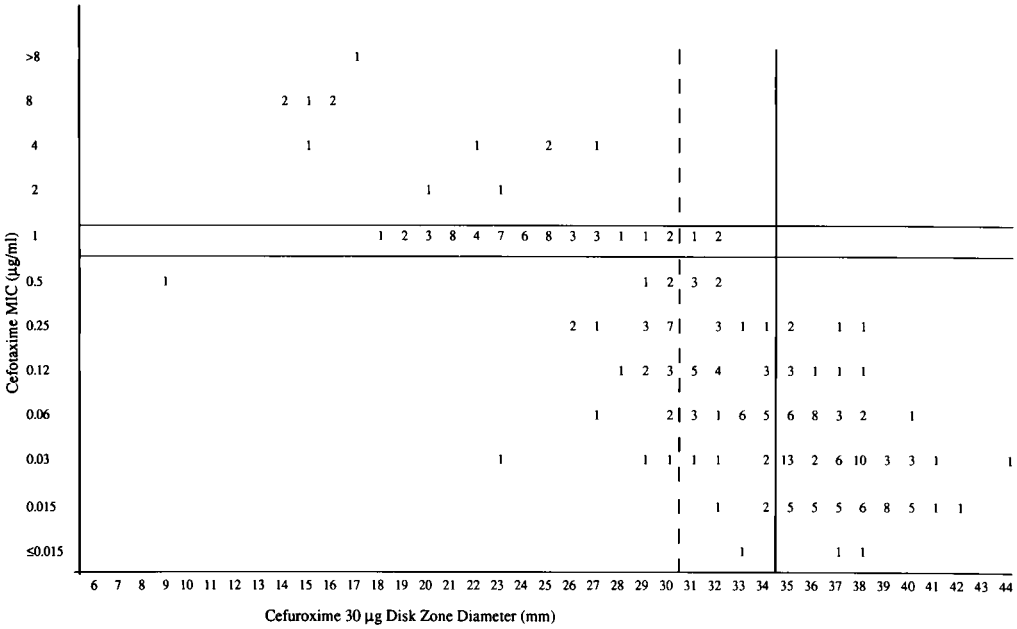

FIG. 2. Comparison of cefotaxime MICs plotted against zone diameters developed around 30-μg cefuroxime disks with 248 strains of *S. pneumoniae*. Horizontal lines represent the latest NCCLS MIC interpretive criteria from reference 3. The solid vertical line represents the zone diameter breakpoint proposed by Lopardo et al., and the dashed vertical line represents the zone diameter breakpoint proposed in reference 1.

data were not depicted in our article because of space constraints. Earlier Friedland et al. (1) suggested that cefuroxime zones of >30 mm were indicative of susceptibility to cefotaxime and ceftriaxone, whereas zones of 21 to 30 mm indicated possible cephalosporin resistance and zones of ≤20 mm should be regarded as indicating definite resistance pending MIC determinations (1). Lopardo et al. have suggested in their letter that cefuroxime zones of <35 mm are indicative of cefotaxime or ceftriaxone MICs of ≥1 μg/ml.

Figure 1 depicts a comparison of cefotaxime MICs with cefuroxime zone diameters from the 248 isolates from five medical centers included in our original organism collection. The cefotaxime MIC interpretive criteria are those from our recent article (2) and are identical to those in the latest NCCLS publication (3). Lopardo et al. appear to be correct in stating that all strains with cefotaxime or ceftriaxone (used in their graph) MICs of ≥1 μg/ml produce cefuroxime zones of <35 mm. However, the data from our study differ from those of Lopardo et al. in that the 35-mm breakpoint almost exactly bisects our susceptible-organism population (i.e., strains with MICs of ≤0.5 μg/ml), which results in 43.3% (74 of 171 susceptible isolates) major interpretive errors or false resistance with cefotaxime and 44.1% major errors with ceftriaxone (data not depicted graphically). Indeed, the earlier criteria of Friedland et al. (1) would provide fewer major interpretive errors (16.9% for cefotaxime and 19.0% for ceftriaxone) while accurately detecting all strains with MICs of ≥2 μg/ml (the current NCCLS resistance breakpoint). However, it is our opinion that >16% major errors may compromise the utility of the cefuroxime disk test, just as major interpretive errors have caused a number of laboratories to discontinue use of the oxacillin disk test to "screen" pneumococci from spinal fluids for penicillin susceptibility. Specifically, the additional day that is required to clarify the meaning of a reduced zone size in the disk screening test by determining an MIC can be problematic with life-threatening pneumococcal infections such as meningitis or bacteremia. Because extended-spectrum cephalosporins are now used empirically to treat those conditions, it is important to determine the susceptibility of pneumococci to those agents without delay. A laboratory report of false resistance could lead to a patient being treated with a less effective or more toxic alternative drug. For this reason, it is our recommendation that "all pneumococcal isolates from patients with meningitis be tested by an MIC method against penicillin and either cefotaxime or ceftriaxone as soon as isolated colonies are available" (2).

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Detection of *Salmonella* Species in Fecal Samples by Immunomagnetic Separation and PCR

A recent report by Kongmuang et al. (4) on the detection of *Salmonella* species in stool samples by immunomagnetic separation followed by PCR mentioned as one of the advantages the removal of substances which inhibit the PCR. We identified two of these inhibitory substances in our study on the use of immunomagnetic separation and PCR for the detection of *Salmonella* species in fecal samples (7). Hemoglobin degradation products were reported to be powerful inhibitors of the PCR (3), and we found that concentrations of bilirubin as low as 10 μg/ml inhibited the PCR. In the gut, bilirubin is reduced to urobilinogens by bacteria. Excretions may reach 350 mg/day for healthy people, and patients may have even higher levels (1). Bile salts also are capable of inhibiting the PCR at concentrations of 50 μg/ml. The excretions of healthy individuals reach 200 to 650 mg/day, and a 10-fold increase may be observed with patients with ileal dysfunctions (2). So the presence of bilirubin and bile acids in fecal samples may explain the inhibition of PCR when fecal samples are tested.

As a drawback Kongmuang et al. (4) mentioned the limited antibody availability. In a previous report, the generation of serogroup-specific monoclonal antibodies directed against *Salmonella* serogroups, including serogroups A, B, C1, C2, D, E, K, and Y, was described (5). These monoclonal antibodies bound live salmonellae in suspension from fecal samples and were evaluated by immunomagnetic separation followed by PCR, or magnetic immuno PCR assay (MIPA) (6). Results show that samples containing 10^5 CFU of *Salmonella* per ml of feces were positive (7). It should be noted that our samples were stored in transport medium for 2 months at 4°C before analysis, which may have resulted in a loss of viable bacteria. After enrichment, samples with fewer than 10^5 CFU/ml were also positive. MIPA was also used to detect *Clostridium difficile* in fecal samples (8). Thus, MIPA is more widely applicable for detection of *Salmonella* serogroup D as described by Kongmuang et al. (4).

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Author's Reply

Dr. Fluit's group has recently developed MIPA (10), which detects salmonellae from feces at a sensitivity of 10^5 CFU/ml. We have also applied immunomagnetic separation (IMS) procedures for isolating salmonellae and shigellae from feces, followed by enzyme immunoassay (6) or PCR (1, 2). The detection limit in either setting ranges from 10^2 to 10^3 bacteria. Dr. Fluit et al. claim the lower sensitivity of MIPA is due to prolonged transportation and storage of samples.

It is believed that inhibitory factors such as bile salts and bilirubin may affect the *Taq* polymerase enzyme activity and/or the PCR primer-DNA template annealing reaction. Immunomagnetic isolation followed by PCR (MIPA) may avoid these; but this remains rather expensive, with an estimated price tag of $10 to 15 per processing sample. Not only costly, MIPA requires specific antibodies and often relies on (i) a high affinity of antibody-antigen interaction and (ii) the nature and abundance of the antigen present on target cell surface for a satisfactory and reproducible result in fecal samples.

In general, a diagnostic microbiology laboratory processes tens to hundreds of fecal samples daily. Even though isolation of salmonellae (with a minimal 10^5 CFU/ml) can be accelerated by a couple of hours by using MIPA, it is apparently not a cost-effective approach for detecting enteric pathogens like salmonellae and shigellae that are easily and rapidly grown in culture. Therefore, it is our major objective to formulate alternative processing procedures to alleviate interference caused by host tissues, and these should be rapid and inexpensive and should accommodate the use of PCR in clinical microbiology laboratories.

Because enrichment broth cultivation of fecal samples from diarrheal patients is a routine practice, use of the suggested "enriched" PCR will not increase the cost of sample processing. Concurrently, Stone's group has also reported the enrichment broth cultivation-PCR procedure for detecting *Salmonella* serovars in bovine and equine feces (9). Both groups have observed that as few as 10^2 to 10^3 bacteria in the initial inocula were detectable following brief enrichment cultivation (2 to 4 h). In both cases, no significant inhibitory effects by the "spiked" feces on PCR sensitivity were observed. Furthermore, we have used an inexpensive StrataClean resin (Stratagene, La Jolla, Calif.) to eliminate protein substances in the DNA extracts prior to PCR analysis (3). Enriched PCR should prove a better candidate technologically and should be financially appropriate for use in publicly funded inner-city clinics or in developing countries.

Apparently, Fluit et al. concluded that our PCR assay (4) was limited by antibody availability and could detect only *Salmonella* serogroup D. Actually, we have generated serogroup A to E monoclonal antibodies (MAbs) which recognize *Salmonella* lipopolysaccharide (LPS) antigens O2, O3, O4, O5, O6,7, O8, O9, and O10 (7,8). Also, we are extending the range of our detection system by using anti-core LPS MAbs (5) which cover most or all *Salmonella* serovars.

It is possible that MIPA procedures will have great potential for isolating subpopulation of leukocytes (e.g., human immunodeficiency virus-infected lymphocytes) and microbial pathogens which are difficult to culture, like *Mycobacterium* species. For a definitive identification of *Salmonella* serovars, however, none of the present rapid tests could completely replace the standard culture technique.

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