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Effects of Various Media on the Activity of NXL103 (Formerly XRP 2868), a New Oral Streptogramin, against *Haemophilus influenzae*

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The activity of NXL103 against 108 strains of *Haemophilus influenzae* was tested using *Haemophilus* test media (HTM) obtained from various sources. With the exception of those obtained with stored HTM, MICs did not differ significantly, with MIC_{50} and MIC_{90} values of 0.5 and 0.5 to 1 μg/ml, respectively, in each medium.

The major bacterial pathogens responsible for community-acquired respiratory tract infections are *Streptococcus pneumoniae*, *Haemophilus influenzae*, and to a lesser extent *Moraxella catarrhalis* (5, 12). Of existing oral compounds active against all three of these organisms, only amoxicillin-clavulanate and compounds in the broad-spectrum quinolone group are convincingly active against these three species (5). There is doubt as to the clinical activity of the macrolide-azalide-ketolide group against *Haemophilus influenzae* when MIC, pharmacokinetics/pharmacodynamics, in vitro efflux study, and (where available) double-tap otitis media study results are carefully examined and compared (1, 2, 5, 10, 11). Of the streptogramin group, quinupristin-dalfopristin has MICs against *H. influenzae* which are above achievable serum levels (9).

β-Lactamase-producing *H. influenzae* organisms are widespread throughout the world (5). Recently, more reports of increased rates of β-lactamase-negative ampicillin-resistant (BLNAR) *H. influenzae* strains have appeared in the literature, especially from France and Japan but also from other sources (3, 7). Amoxicillin-clavulanate, the oral compound most commonly used in pediatrics and for adult patients to treat infections caused by this organism (5), may not be active against BLNAR strains (3, 7). The problem is compounded by the lack of a standardized definition of this resistotype which can easily be used in the clinical laboratory. A new oral agent with a different mechanism of activity against these organisms is needed, especially for children, for whom quinolones cannot be used.

NXL103 (formerly XRP 2868), an experimental oral streptogramin which is a 70:30 combination of RPR 132552A and

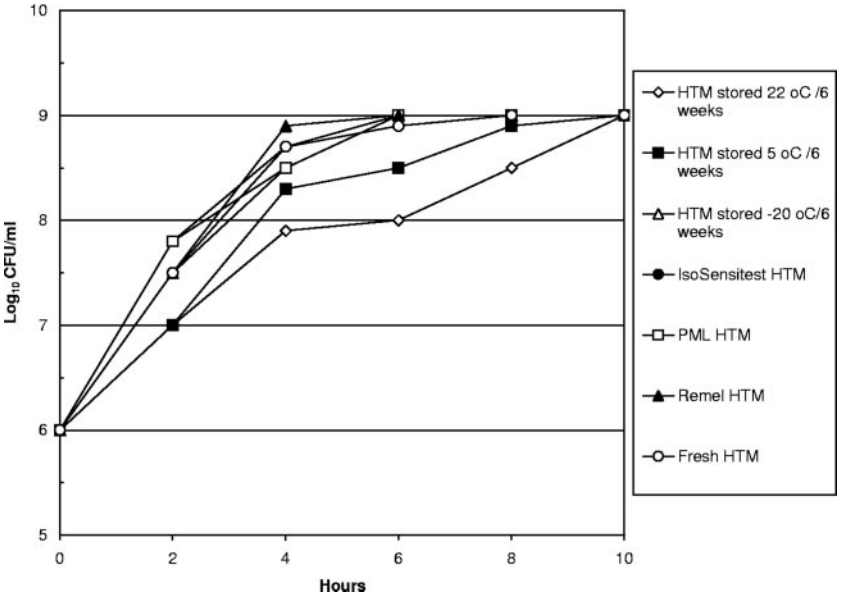

FIG. 1. Growth rates of *H. influenzae* ATCC 49247 in seven HTM broths.

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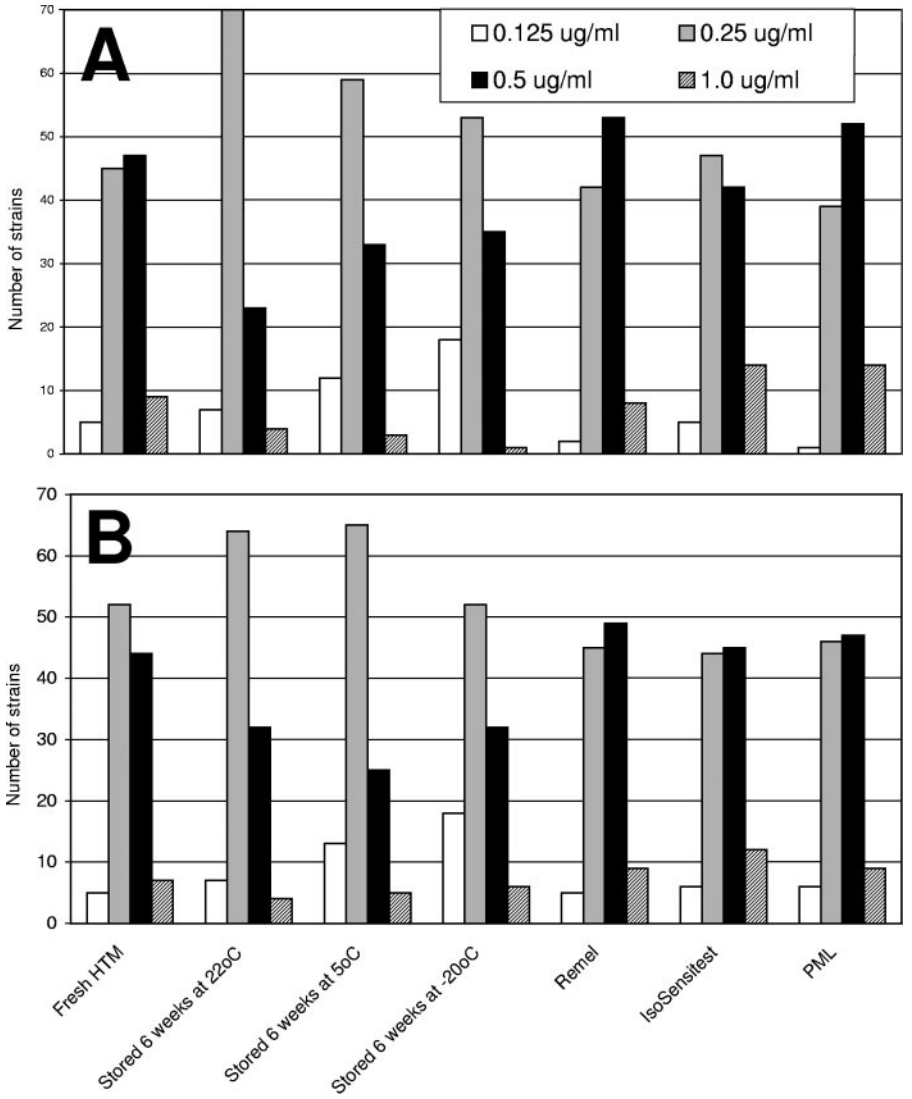

FIG. 2. MIC distributions of HTM broths incubated in air (A) and CO_2 (B).

RPR 202868, has previously been shown by our group and others to be potent against *H. influenzae*, *S. pneumoniae*, and other gram-positive organisms (4, 6, 9). Against *H. influenzae* strains of various phenotypes and genotypes, NXL103 had an overall MIC_{50} of 0.25 μg/ml and an MIC_{90} of 1.0 μg/ml, with no difference between β-lactamase positive, β-lactamase negative, and BLNAR strains. Of note was the similarly high potency of one of its components, RPR 132522A, which had MICs the same as those of the combination (9).

CLSI (formerly NCCLS) recommends the use of *Haemophilus* test medium (HTM) for routine susceptibility testing of *H. influenzae* (8). This medium has a relatively short shelf life, and all susceptibility tests need to be controlled carefully for adequate growth as well as for values for quality control strains. The present study tested the activity of NXL103 against a spectrum of *H. influenzae* strains with differing genotypes and phenotypes by use of different media.

One hundred eight *H. influenzae* strains were tested. Of these, 53 were β-lactamase positive, 35 β-lactamase negative, and 20 BLNAR. All BLNAR strains were previously genotypically characterized in our laboratory, and all had abnormalities in PBP 3 (7). Of the 108 strains, 104 were untypeable and 4 were type b. NXL103 susceptibility powder was obtained from Novexel S.A., Romainville, France.

HTM broth was purchased from Remel, Inc., Lenexa, KS, and from PML Microbiologicals, Wilsonville, OR. Iso-Sensitest broth-based HTM was prepared in-house using Iso-Sensitest broth supplemented with 0.5% yeast extract, 15 μg of NAD per ml, and 15 μg hematin per ml (Oxoid Ltd., Basingstoke, United Kingdom). Mueller-Hinton broth-based HTM was prepared in-house using BBL Mueller-Hinton II broth (BD Diagnostic Systems, Sparks, MD), 0.5% Difco yeast extract (BD Diagnostic Systems), 15 μg of NAD, and 15 μg hematin per ml (Sigma, Inc., St. Louis, MO). The latter medium was used freshly prepared and also after storage at 22°C, 4°C, and −20°C for 6 weeks.

Microtiter trays were prepared in-house using each of the above-described media, and trays were stored frozen (−70°C)

for up to 3 weeks prior to testing. MICs were determined according to CLSI criteria (8). Inoculum suspensions were prepared by suspending colonies from chocolate agar plates that were incubated overnight (20 to 24 h) into sterile saline and diluted as per CLSI recommendations. The same tube was used to inoculate each of the test broths. Inoculum checks were performed on all strains, and CLSI-recommended quality controls (*H. influenzae* ATCC 49247 and ATCC 49766) were included with each test run. *H. influenzae* ATCC 10211 was included to verify the growth-supporting properties of each HTM (8). Microdilution trays were incubated in ambient air as well as in 5 to 7% CO_2.

Growth levels of *H. influenzae* ATCC 49247 grown in each of the seven media were compared. An overnight culture diluted to obtain a concentration of 10^6 CFU/ml was used as the initial inoculum. Colony counts were obtained on chocolate agar at 0, 2, 4, 6, 8, and 10 h.

MICs from each broth were compared using the Wilcoxon rank sum test, with the use of in-house freshly prepared HTM incubated in air as the reference method, as recommended by CLSI (8). SigmaStat 3.0 (Systat Software, Inc.) was used to perform statistical analysis. The geometric means, MIC_{50}s, MIC_{90}s, and percentages of MICs within 1 and 2 dilutions of those obtained by the reference method were calculated.

Trays prepared from HTM that was stored (6 weeks at 22°C, 5°C, and −20°C) generally produced less visible growth than those prepared from the freshly prepared HTM, Remel, Iso-Sensitest, and PML broths. The lack of good visible growth in some of the trays prepared from the stored HTM made MICs more difficult to read. Four of the 108 *H. influenzae* strains failed to grow in trays prepared from HTM stored for 6 weeks at room temperature, and 1 strain failed to grow in media stored for 6 weeks either frozen or at 5°C. Growth in the trays was generally better under 5 to 7% CO_2 atmosphere than in ambient air.

Figure 1 shows the growth rates of *H. influenzae* ATCC 49247 in the seven HTM broths in ambient air. As can be seen, the growth rates were lower in the broths stored for 6 weeks at 5°C and 22°C than in the other media tested.

The MIC distributions obtained from each HTM are shown in Fig. 2. As can be seen, MIC distributions were shifted approximately 1 doubling dilution lower for the stored homemade broths incubated in air and CO_2 than those for the fresh, Remel, Iso-Sensitest, and PML HTM. The MIC difference between the fresh (air) broths and the stored HTM broths in air and CO_2 was statistically significant ($P < 0.001$).

Geometric means for the stored broths in air and CO_2 (0.28 to 0.30 μg/ml) were lower than those for the other broths (0.34 to 0.41 μg/ml). The MIC_{50}s for the stored broths were lower (0.25 μg/ml versus 0.5 μg/ml) for the reference, Remel, Iso-Sensitest, and PML broths in air. The MIC_{90}s were from 0.5 to 1.0 μg/ml for all media in air and CO_2. MICs with all media and in air or CO_2 corresponded to those found by standard CLSI methodology (microtiter methodology with freshly made *Haemophilus* test medium) in 93 to 100% of cases. We feel that in *H. influenzae* susceptibility studies, inoculum standardization is of critical importance if results of studies by different workers are to be accurately compared.

In this study the geometric means, MIC_{50}s, and MIC distributions of the stored broths were found to be 1 doubling dilution lower than those found for the other test media. Although this difference is statistically significant, MICs that are different by 1 doubling dilution are considered to be in essential agreement according to guidelines set up by the Food and Drug Administration.

The poorer growth in the stored HTM underscores the need for preparation of fresh medium before MICs are determined. However, in our hands, commercial methods proved to yield reliable and reproducible growth with MICs that did not differ significantly from one another. We therefore feel that for NXL103, the results of our study are robust enough to permit the use of trays prepared using the commercial HTM from the sources studied in the clinical laboratory.

If good pharmacodynamics are obtained and toxicity studies are satisfactory, NXL103 holds promise as an oral treatment for community-acquired respiratory tract infections. MICs are low against both *S. pneumoniae* and *H. influenzae* (9), and *Haemophilus* susceptibility testing methodology is robust with commercial media. Clinical studies will be necessary to test these hypotheses.

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