Determination of Penicillin Resistance in *Streptococcus pneumoniae* and Use of Co-Trimoxazole in Treatment of Pneumococcal Pneumonia

We read with interest the article by Saha et al. (6) on antimicrobial resistance and serotype distribution of *Streptococcus pneumoniae*. We would like to highlight certain aspects in the article which need to be looked at. The authors state that the E test was performed to determine the MICs for all the *S. pneumoniae* strains isolated as part of the study. For penicillin, the oxacillin screen, which is an acceptable procedure (5), was used. The strains resistant to oxacillin by disc diffusion were subsequently found to be intermediately resistant or completely resistant to penicillin by the E test.

The E test is only a commercial method, not a “gold standard,” for MIC determination. The E test has the accuracy to agree within 1 doubling dilution of the reference penicillin MIC for 90% of strains and within 2 doubling dilutions for 99% of the strains (3). Even though a good correlation between the reference MIC by agar dilution methods and the E test MIC has been observed (4), a tendency for the penicillin MIC to be slightly lower by the E test than by reference agar dilution method has been observed (3). There have been reports that strains resistant to penicillin by reference MIC dilution methods were found to be intermediately resistant by E test (1). The authors do not state that internal quality control procedures such as testing of reference strains, blinded determination by independent observers, and checking for internal reproducibility of the E test results were used. A few strains resistant to penicillin by the E test could have been sent to a reference laboratory for confirmation of values.

The second issue involves co-trimoxazole resistance. The study reports an overall resistance to co-trimoxazole of 64.1%. It would have been interesting to compare the resistance to co-trimoxazole of the strains of *S. pneumoniae* isolated from invasive sites with that of strains from noninvasive sites. The authors should have looked at strains from patients with proven pneumococcal pneumonia since they have discussed the use of co-trimoxazole in treatment of pneumonia. This would have been critical to support their argument that co-trimoxazole may not be useful for the treatment of pneumonia.

At our center, a resistance level of 47.8% was found in the IBIS study (2) in cases of bacterial pneumonia confirmed by a positive blood culture across all age groups. Interestingly, the level of resistance was found to differ across the age groups studied, with levels of resistance lower, at 16%, in the below-2-year age group than in the adult age group.

In a nasopharyngeal colonization study at our immunization clinic where children below 1 year of age were enrolled, the percentage of co-trimoxazole resistance was only 5.4% (3a). However, the percentage of resistance was significantly higher, at around 64%, in children between 2 and 5 years of age (4a) in a community-based nasopharyngeal colonization study. This difference in levels of resistance with respect to invasive versus noninvasive and hospital versus community settings in children under the age of 5 is notable as this may have implications for antibiotic treatment in that age group, particularly in developing countries. This aspect may be interesting to follow up and study in detail since it may have a direct impact on the formulation of an appropriate antibiotic policy.

**REFERENCES**


M. K. Lalitha*
Anand Manoharan
Rekha Pai
Department of Clinical Microbiology
Christian Medical College & Hospital
Vellore 632004, Tamilnadu, India

*Phone: 91-416-222102 ext. 2033
Fax: 91-416-232103/232035
E-mail: mkl@micro.cmcernet.in

Kurien Thomas
Department of Medicine Unit II
Christian Medical College & Hospital
Vellore 632004, Tamilnadu, India

Authors’ Reply

We thank Dr. Lalitha and colleagues for their interest in our paper (5) and appreciate their concern about the E test; this letter will allow us to show some additional analysis of our results. We do agree that the E test is a commercial method like any of the other commercially available microdilution systems. We are aware of the tendency of the E test to show a slightly lower (a log2 dilution, 90% agreement) MIC (1–3). The E test uses a continuous-gradient scale, whereas the broth or agar dilution method uses doubling dilutions. Any strain for which the MIC is 2 μg/ml by the dilution method is anywhere within the range of 1.01 to 2.0 μg/ml as there is nothing in between. On the other hand, the E-test strip may be more precise as it contains the concentrations in between the doubling dilutions (1). Anyway, resolution of this debate needs some experimental work with fractional dilutions. In spite of this minor discrepancy between the E-test and dilution methods, the E test has possibly been the most widely used method since its introduction and all groups recommended it as a potential alternative to the conventional dilution method (1, 2). Going further in their recommendations, Lalitha et al. (3) reported this test to be simple, easy to interpret, cost-effective.
(it is rather expensive), and reliable. They have also mentioned, as a noteworthy point, that no errors either of false susceptibility or false resistance were observed. Our aim is the economization of its use on the basis of oxacillin disc screening so that routine laboratories in developing countries can use it as a quick and simple way to detect penicillin-resistant strains for the patients’ benefit.

Despite these points mentioned above, we were cautious in interpreting our results and making comments and did the fragmented analysis of MICs for our S. pneumoniae strains to understand the consequence of lower MICs (1 log₂ dilution) by the E test. The analysis showed that 12% (44 of 362) of our strains were within the MIC of 0.064 to 0.098 µg/ml; some (10%) of these strains may be classified as relatively resistant if tested by broth or agar dilution. Even if this is true, our study has identified four (1.1%) strains as sensitive which could be classified as relatively resistant by the dilution method. Similarly, we have 0.6% (2 of 362) of strains for which MICs are 1.0 to 1.5 µg/ml, which are supposed to remain the same, with possible variation in 10% of cases, by the dilution method. If we ignore the fact of the continuous gradient of the E test and consider that apparent difference of a 1-log₂-unit higher MIC as universal, the consequent changes in our results will be insignificant. Resistance to penicillin will be 13.7%, up from 12.6%, with only a change in the relative resistance rate of 1.1% and no change in the complete resistance rate.

Regarding quality control, we regularly use the ATCC strains of S. pneumoniae (ATCC 49619), Escherichia coli (ATCC 25922), Haemophilus influenzae (ATCC 49247), and Pseudomonas aeruginosa (ATCC 27853) (kindly provided by M. Steinhoff of Johns Hopkins University and G. Darmstadt of Washington University). Blinded observation and checking for internal reproducibility are routine procedures in our laboratory. Our laboratory has extensive experience in determining MICs for different organisms by broth microdilution and E tests (5–10).

Regarding co-trimoxazole resistance of strains isolated from patients with proven cases of pneumonia, it should be stated that the blood isolates were from pneumonia patients as mentioned in our previous paper (4) and, as for serotype distribution, there was no significant difference with respect to drug resistance when strains from meningitis and pneumonia patients were compared. In strains from meningitis and pneumonia patients, the rates of resistance to penicillin were 13.1% versus 10.6% (P = 0.66), respectively; similarly, resistance to co-trimoxazole was 63.9% versus 68.1% (P = 0.61), respectively. Further, as for H. influenzae type b (11), surveillance of invasive S. pneumoniae cases should focus on meningitis because the rate of isolation from patients with pneumonia is very low, in contrast to the situation for meningitis, in which diagnosis is straightforward and isolation of the organism from cerebrospinal fluid is simple. We do not have any experience with adult subjects, but the 5.4% resistance rate of the nasopharyngeal isolates from the immunization clinic versus the 64% resistance rate of community strains, as mentioned by Lalitha et al., is remarkable. Although they designated the above groups as hospital and community strains, respectively, we would be likely to consider both of them community strains because the children attending the immunization clinic were healthy and were coming from the community for a short visit (for vaccination), during which time the nasopharyngeal swabs were collected. The only difference between these two groups is the difference in age. On comparison of our cases according to age of the patient, we also have observed some difference, albeit not as striking as that observed by Lalitha et al. (3). We observed 61 and 71% co-trimoxazole resistant strains among children up to 1 year old and from 1 to 5 years old, respectively (P = 0.14).

We will soon begin the isolation of large number of nasopharyngeal strains (as mentioned in our paper) from a community near Dhaka to test for possible differences between community and hospital strains.

REFERENCES