Comparison of E test and agar dilution for testing activity of ceftriaxone against *Neisseria gonorrhoeae*

The Centers for Disease Control and Prevention’s sexually transmitted disease (STD) treatment guidelines, 2015, recommend combination therapy comprising ceftriaxone (250 mg) and azithromycin (1 g) for management of gonorrhoea (CDC, 2015). The recent data from WHO SEAR (South-East Asia region) countries attest to the effectiveness of in-use therapies. Further, no immediate region) countries attest to the effectiveness of in-use therapies. Further, no immediate threat to the efficacy of the extended-spectrum cephalosporins (ESCs) is suggested (Bala et al., 2013). However, in view of the reports of creeping MICs to ESCs world over (Martin et al., 2006; Unemo et al., 2010; Ison et al., 2011; Ohnishi et al., 2011; Sood et al., 2013; Lahra et al., 2014), we can now envisage that gonococcus is slowly walking towards resistance. Therefore, there is a need to keep a close watch on changing susceptibility to ESCs. We are well aware that the disc-diffusion method does not detect small changes in antimicrobial susceptibilities and that these are reflected only by methods producing quantitative MIC results. E test is a very convenient method for MIC determination in a clinical bacteriology laboratory and is widely used. We therefore sought to determine how closely E test results correlate to those of the agar dilution method, the reference ‘gold standard’ for MIC determination of ceftriaxone in *Neisseria gonorrhoeae*.

A total of 60 *N. gonorrhoeae* clinical isolates collected during a 2-year period (July 2010–June 2012) from patients attending the Dermatology Out-patient Department of the All-India Institute of Medical Sciences (AIIMS) and Dr. R.M.L. Hospital & PGIMER, New Delhi, were revived for the present study on chocolate agar plates at 36 °C in an atmosphere of 5 % CO₂. In addition, eight WHO reference strains (F, G, K–P) were included in the present investigation. The agar dilution test was conducted as per the standard protocol (CLSI, 2012), and the E test as per the manufacturer’s instructions. The strains were defined as susceptible (S; MIC <0.03 µg ml⁻¹) and those exhibiting decreased susceptibility (DS; MIC 0.032–0.25 µg ml⁻¹) using breakpoint criteria of the calibrated dichotomous sensitivity technique (Bell et al., 2011). For both the agar dilution and E test, control strains passed quality control if the MICs were within ±1 log₂ of the predetermined MIC.

The MICs of ceftriaxone by agar dilution and E test ranged from <0.002 to 0.25 µg ml⁻¹ and <0.002 to 0.125 µg ml⁻¹, respectively. The E test values were rounded up to the nearest log₂ agar dilution MIC value to facilitate comparison of the two methods (Table 1). The overall agreement of MICs (±1 log₂ dilution) between the two methods was 95 %. Twenty-four (40 %) of 60 strains gave the same MIC in both tests, 33 (55 %) of the strains agreed within 1 log₂ dilution step, while 3 (5 %) strains agreed by two dilution steps. Overall, 36 (60 %) read lower in the E test method. The MIC values of the WHO reference strains were well within the published range by both techniques (Unemo et al., 2009).

All isolates categorized as S (n=55) by the agar dilution method were correctly categorized as S by the E test. On the other hand, out of five categorized as DS by agar dilution, four were DS while one was S by E test. The MIC of the isolate with conflicting susceptibility category was 0.032 µg ml⁻¹ by agar dilution and 0.023 µg ml⁻¹ by E test. The minor error in categorization (U.S. Department of Health and Human Services, Food and Drug Administration, and Center for Devices and Radiological Health, 2009) can be explained by the lower read in the E test method during our study and close proximity of the MICs to the category breakpoints.

MIC determination by agar dilution is technically demanding and beyond the ability of most laboratories in developing countries. Further, testing by agar dilution technique is often batched. For a laboratory like ours that gets few gonococcal isolates per year, E test is a simple and practical alternative which provides quantitative measure of susceptibility and can be performed on isolates on an emerging basis. Although the correlation between the E test and agar dilution method was very good, we note that small differences between the results obtained by the two methods did lead to discordance in the categorization of one clinical isolate. However, this does not have any clinical implications in the present scenario as the treatment modality remains unchanged.

Further, it is pertinent to mention that presently we do not have any reference resistant strains of *N. gonorrhoeae*. Availability of the same in future could add value to the study.

| Table 1. Comparison of *N. gonorrhoeae* MICs obtained by E test and agar dilution method |
|-----------------------------------------------|-------------------------------------------------|-----------------|-----------------|-----------------|
| **Conc. (log₂)** | **No. of isolates with E test MIC within indicated Conc. (log₂) of agar dilution** | **Agreement within 1 log₂ concn (%)** | **Categorical discrepancy** |
| (+3) | (+2) | (+1) | (0) | (−1) | (−2) | (−3) | Major | Very major | Minor |
| Ceftriaxone | 0 | 0 | 0 | 24 | 33 | 3 | 0 | 95 | 0 | 0 | 1 |
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