A Simple Modification of Minimum Inhibitory Concentration Determination by E-test in the Clinical Laboratory

Dear Editor,

The emergence of anti-microbial resistance among several common pathogens lays a responsibility on the clinical microbiologist to respond and report the antibiotic sensitivity pattern accurately, rapidly and at a reasonable cost.¹ The various methods include the screening techniques of disc diffusion [Stokes, Kirby Bauer, collateral disc susceptibility and the minimum inhibitory concentration (MIC) methods]. One of the popular and rapid methods for MIC evaluation, commonly used in the clinical microbiology laboratory is the antibiotic gradient diffusion (E-strip).²

According to the Center for Disease Control, USA, antibiotic resistance monitoring should be performed using MIC methods to determine emergence and evaluation of resistance in certain “key” pathogens. They emphasize the role of accuracy and timeliness in detection of resistance by the laboratory.³ The MIC methods are advantageous for this as they give a quantitative result which can be performed for fastidious, difficult to isolate and slow-growing pathogens that cannot be tested by disc diffusion. The MIC testing by micro broth, macro broth and agar dilution are time consuming, labor intensive, require technical expertise and the pure powders of the anti-microbials from reputed firms, which tend to be expensive. The macro broth dilution method is now advocated only for research facilities.⁴

The gradient diffusion method, the E test (AB Biodisk, Solna, Sweden) is a method for quantitative anti-microbial susceptibility testing wherein the antibiotic is applied in a preformed gradient across a plastic coated strip. This diffuses into the agar medium inoculated with a lawn culture of the test organism and incubated for 24 hours. The MIC is read as the point where the ellipse touches the E-strip. This test has been validated for several pathogenic bacteria including gram positive, gram negative and mycobacteria. It combines the ease of application of disk diffusion testing, rapidity and an MIC reading. However they are much more expensive and this is a huge hurdle in a resource poor setting.⁴

To overcome this difficulty, we devised a modification to this test in our laboratory - wherein a single E-strip was utilized to test, at one time, two different strains of the same genus, on the same Mueller Hinton agar plate. Lawn cultures of the two strains were separated by a space of approximately 3 mm and the E-strip was laid in the center of this gap. This is very similar to the Stokes’ method of disk diffusion testing where the test and the control strains are separated by a 3 mm gap while applying their lawn culture. The plate was incubated overnight and the reading taken for the two strains from their respective regions where the half ellipse cuts the E-strip edge (Figure).

Figure: Plate showing two strains of Salmonella typhi, one resistant to ciprofloxacin (>32 µg/mL) and the other sensitive strain (0.5 µg/mL)

This economizes on E-strip, culture media, labor and time.

This modification could not be utilized for Mycobacterium tuberculosis as these strains grew very slowly (three to seven days) and had varying growth phases. However, we have performed this test for a variety of pyogenic bacteria- Staphylococcus aureus, Group A Streptococci, Meningococci, Enterococci and Salmonella spp, with conclusive results. Hence, this simple modification of the E-strip method can halve the cost and give a rapid and accurate MIC results.

References

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