



## Standardization of Antimicrobial Sensitivity testing in Clinical Microbiology Laboratory: Bangladesh Perspective

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The performance of antimicrobial susceptibility testing in the clinical microbiology laboratory is very crucial to confirm the susceptibility of the drugs to chosen empirical antimicrobial agents, even to detect resistance in individual bacterial isolates. It is worth noting that empirical therapy continues to be effective for some bacterial pathogens because the resistance mechanisms have not been observed like continued penicillin susceptibility of *Streptococcus pyogenes*. The susceptibility testing of individual isolates is important with species which possess the acquired resistance mechanisms like the members of the Enterobacteriaceae, *Pseudomonas* species, *Staphylococcus* species, *Enterococcus* species and *Streptococcus pneumoniae*.

The individual microbiology laboratory must test and report the antimicrobial agents that are most appropriate for the organism isolated, for the site of the infection, and the institution's formulary. The CLSI provides the zone of diameter; thus the list of these antimicrobial agents can be used for appropriately to the members of the Enterobacteriaceae, *Pseudomonas*, and other gram-negative lactose non-fermenters, staphylococci, enterococci, streptococci, *Haemophilus* species and others bacteria as well. The listings include recommendations for agents that are important to test routinely and those that may be tested or reported selectively based on the institution's formulary.

The availability of antimicrobial agents for testing by the laboratory's routine testing methodology must next be determined. The disk diffusion and gradient diffusion procedures offer the greatest flexibility including testing of newly available drugs. Most broth microdilution or automated test

panels contain  $\leq 96$  wells, effectively limiting the number of agents tested or the range of dilutions of each drug that can be included. Manufacturers of commercially prepared panels have attempted to deal with this problem by offering a number of different standard panel configurations, or by including fewer dilutions of each drug in a single panel. Another solution to this problem is testing antimicrobial agents that have activities that are essentially the same as the desired formulary drugs. The CLSI susceptibility testing document lists groups of some antimicrobial agents with nearly identical activities that can provide practical alternatives for testing.

When assessing the accuracy of various susceptibility testing methods as compared to standard reference methods, the terms very major and major errors have been used to describe false-susceptible or false-resistant results, respectively. In evaluations of new susceptibility testing methods it is important to examine a representative number of strains that are resistant to various drugs to verify the ability of the new test to detect resistance and to test a number of susceptible strains to determine the rate of major errors that might be expected in a typical clinical laboratory setting. To be cleared for marketing in the United States, the FDA requires that very major errors attributable to a test device should be  $< 1.5\%$  for individual species/drug comparisons, major errors should not exceed  $3\%$ , and an overall essential MIC agreement of  $> 90\%$  of device MICs within one doubling dilution of a CLSI reference MIC. A recent, international standard on susceptibility test device evaluation proposes similar but not identical criteria for acceptable accuracy. The emergence of new antimicrobial resistance mechanisms, including some that may be difficult to detect (eg,

vancomycin intermediate susceptibility in *S. aureus* and carbapenemase production in some gram-negative organisms) requires that the performance of susceptibility devices be constantly reassessed and updated when needed. In some cases, it has been necessary to employ special ancillary testing methods like single concentration screening agars, modified Hodge test for carbapenemase production) to supplement routine testing by a commercial instrument system.

The antimicrobial susceptibility testing methods provides reliable results when used according to the procedures defined by the CLSI or by the manufacturers of the commercial products. However, there is considerable opportunity for improvement in the area of rapid and accurate recognition of bacterial resistance to antibiotics. There is a need for development of new automated instruments that could provide faster results and also save money by virtue of lower reagent costs and reduced labor requirements. To accomplish

this, it will likely be necessary to explore different methodological approaches for detection of bacterial growth. The direct detection of resistance genes by polymerase chain reaction or similar techniques has limited utility, because only a few resistance genes are firmly associated with phenotypic resistance like *mecA*, *vanA*, and *vanB*. There are hundreds of  $\beta$ -lactamases, and numerous mutations, acquisitions, and expression mechanisms that result in fluoroquinolone, aminoglycoside, and macrolide resistance; too many to be easily detected by current molecular techniques.

Therefore, it seems likely that phenotypic measures of the level of susceptibility of bacterial isolates to antimicrobial agents will continue to be clinically relevant for years to come.

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