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Editorial

Integrating manual antimicrobial susceptibility testing with automated, molecular methods

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Manual or conventional antibacterial susceptibility testing (AST) methods have evolved over several decades and stood the test of time. Disk diffusion method is the simplest, one of the most commonly used method globally and economical methods of AST.[1] Broth microdilution, agar dilution and epsilometer tests (E-Test) are also used by several laboratories. Appropriate and periodically updated guidelines for performing the tests by these methods and interpretation of the results are provided by both the Clinical Laboratory Standards Institute (CLSI)[2] and the European Committee Antimicrobial Susceptibility Testing (EUCAST).[3] In the last few decades, several laboratories have shifted to automated and molecular methods of AST for various obvious benefits including accuracy, speed, ease of testing and scalability. It is interesting to note that the conventional phenotypic AST methods are still useful in a laboratory mainly using more advanced automated and molecular AST methods.

Manual AST methods can augment the automated methods in several ways

Flexibility of testing: Automated systems use standard fixed panels, which may not include some essential antibiotics or may lack adaptability for rare bacterial strains.[4] For example, Vitek N405 card for gram negative bacterial isolates does not include nitrofurantoin, which can be easily tested separately by disk diffusion for urinary isolates. Certain CLSI-recommended adjustments for specific pathogen-antimicrobial combinations are more easily implemented manually.[2] Manual methods like E-test and disk diffusion allow customization of several testing parameters such as antimicrobial agents, culture media, incubation conditions, etc. for atypical or slow-growing pathogens, such as anaerobes or fastidious organisms.[5]

Cost-effectiveness: For laboratories with lesser sample load, manual methods are often used as more cost-effective alternatives or as an additional AST method in selected few scenarios (e.g, samples from critically ill patients in intensive care units, pathogens isolated from blood and body fluid) 2. Automated (Vitek, BD Phoenix, etc) or molecular (PCR, microarray, etc) AST methods demand higher initial procurement and regular maintenance as well as running costs, stringent technical support and training needs.[5]

Validation of AST results: Automated systems sometimes produce ambiguous or false results due to technical limitations (e.g., turbidity interference). In such situations, the manual methods can be used as effective supplemental tests for validating the doubtful results. A few such expels are - Disk diffusion enables detection of contamination or mixed cultures visually, which automated systems might miss.[4,6] Broth microdilution can be used to manually confirm MIC (minimum inhibitory concentration) values flagged as intermediate/resistant by automated systems.[7] Discordant results for oxacillin and cefoxitin for the

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same *S.aureus* isolate can be easily confirmed by supplemental manual testing.

Phenotypic relevance for AST results based on molecular methods: Though molecular techniques rapidly identify resistance genes, they cannot assess phenotypic expression or novel resistance mechanisms. MIC values obtained by manual AST can provide actionable values to guide treatment, bridging the gap between genetic prediction and clinical response.[8] Manual methods can easily detect inducible resistance (e.g., inducible clindamycin resistance in *Staphylococcus aureus*), which genotypic methods may overlook without targeted probes or automated methods are less reliable 4.

Manual AST method is sometimes the only validated AST method, for example AST methods recommended for colistin testing against Enterobacterales are agar dilution, CBDE (Colistin Broth Disk Elution) or microbroth dilution. Manual AST method can be an economical alternative. Guidelines are available for performing AST directly from the flagged blood culture bottles by disk diffusion assay. (CLSI, EUCAST)

Manual methods can be effectively used for detection of specific drug resistance: Detection of Extended spectrum beta lactamase in Enterobacterales, detection of carbapenemase production in Enterobacterales & *Pseudomonas aeruginosa* (CarbaNP Test, Modified Carbapenem Inactivation Method) can be effectively done manually. (CLSI, EUCAST)[2,3]

Synergistic laboratory workflow: Combining manual with automated and molecular AST methods can make best use of each method while providing flexibility, validity and economy in testing.[5,6]

1. Molecular assays can screen for resistance genes (e.g., *mecA* in MRSA, rifampicin resistance in *M.tuberculosis*) rapidly and accurately
2. Automated systems process high-throughput samples for common pathogens in lesser time
3. Manual methods resolve discordant results, test non-panel antibiotics, or handle fastidious organisms.

Incorporating additional manual testing for specific types of resistance in certain groups of organisms can be valuable for deciding effective treatment.

1. AST for Enterobacterales primarily by Vitek can be supplemented with manual AST for colistin (Agar dilution/ microbroth dilution/ CBDE), fosfomycin (Agar dilution), Caftazidime-Avibactam- Aztreonam synergy testing (using E-test, disk elution method, etc).
2. AST for *S. aureus* primarily done by Vitek can be supplemented with inducible clindamycin resistance detection using D-Test.

It is advisable to adapt to newer AST methods like automated systems and molecular methods for their clear advantages over the manual methods, especially in laboratories with high sample load. Manual AST methods can be incorporated appropriately for specific drug-bug combination testing and other such needs. Such integration ensures accurate, cost-effective, and clinically actionable results while considerably lowering the risks of undetected resistance.

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