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Educational Workshop

Antibiotic resistance determination; dilution methods versus disc diffusion. The old story comes back

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Antimicrobial susceptibility testing (AST) of anaerobic bacteria using dilution methods such as broth microdilution and agar dilution (described in the CLSI guideline M11-A7) has been and still is the gold standard. For many years these methods have been used primarily for research and surveillance of national and local susceptibility patterns in order to choose appropriate antimicrobial agents for empirical therapy. However, as increasing resistance has been observed for many of the frequently encountered anaerobic bacteria, there is a need for more simple methods in the routine clinical microbiology laboratory. Gradient strips are used as a simple but expensive method for AST. Disk diffusion on Wilkins-Chalgren agar (former CLSI recommended agar) for anaerobic bacteria has also been evaluated several years ago, and although results were promising at that time, the method has not been generally accepted. With the introduction of the EUCAST disk diffusion method the intention was to develop a standardised method for anaerobic bacteria. The following test conditions were suggested: the Brucella Blood Agar medium supplemented with hemin and vitamin K (BBA, currently recommended by the CLSI) with strictly controlled test conditions (temperature, atmosphere and time of incubation). The use of the disk diffusion method was limited to rapidly growing (<24 hours) anaerobic bacteria such as members of the Bacteroides fragilis group, Clostridium spp. and Fusobacterium necrophorum. Studies on BBA are in progress and preliminary studies with Bacteroides fragilis group and Fusobacterium necrophorum reference strains have been promising. Furthermore, studies with clinical isolates of Clostridium difficile shows that isolates with reduced susceptibility to metronidazole and vancomycin can be separated from wild-type isolates with disk diffusion. Clostridium perfringens or other Clostridium spp. may well be the next candidates to be evaluated for AST with disk diffusion. Although the reference methods are still the AST methods of choice for a large number of slow growing anaerobic species, disk diffusion seems to be a potential alternative for certain rapidly growing anaerobic bacteria.