27th ECCMID Vienna, Austria 22 – 25 April 2017
The congress of ESCMID

Session: P018 Detection of resistant pathogens

Category: 4a. Diagnostic bacteriology – culture based

22 April 2017, 15:30 - 16:30
P0364

Screening culture medium for multiple aminoglycoside-resistant Gram-negative bacteria

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Background: Extended-spectrum ß-lactamases that hydrolyze extended-spectrum cephalosporins, and carbapenemases that hydrolyze in addition carbapenems are disseminating worldwide in *Enterobacteriaceae*, and therapeutic options are becoming limited. For those multidrug resistant isolates, aminoglycosides (AG) may still be considered as valuable treatment options. However, plasmid-mediated 16S rRNA methylases conferring a high level of resistance to multiple AG is increasingly reported. The 16S rRNA methylases described are ArmA, RmtB to RmtF, and NpmA, with ArmA being the most frequently identified. The amikacin plus gentamicin-containing SuperAminoglycoside medium was developed for screening multiple-aminoglycoside resistance in Gram-negative bacteria (Enterobacteriaceae, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*).

Material/methods: The optimal screening medium retained was based on the Eosine-Methylene-Blue (EMB) medium that is selective for gram negatives. This medium may also contribute to species identification by differentiating lactose fermenters (black colonies) from non-fermenters (colorless or light lavender). The optimal final concentration of amikacin and gentamicin were 30

mg/L each. Daptomycin was added as anti-Gram positive molecule at a final concentration of 10 mg/L, and amphotericin B was added as anti-fungi molecule at a final concentration of 5 mg/L.

The SuperAminoglycoside medium was evaluated by using aminoglycoside-susceptible (n=13) and aminoglycoside-resistant (n=47) Gram-negatives isolates, including 16S rRNA methylase producers (n=20). Using an inoculum with an optical density of 0.5 Mac Farland (inoculum of ~10^8 CFU/ml), serial 10-fold dilutions of the isolates were made in normal saline and 100-µl portions were plated onto the SuperAminoglycoside medium. Spiked fecal samples were also tested, being made by adding 100 µl of each strain dilution to 900 µl of fecal suspension that was obtained by suspending 5 g of freshly pooled feces from five healthy volunteers in 50 ml of distilled water.

Results: The lowest limit of detection was above the cut-off value of 10^3 CFU/ml, being ≥ 1 x 10^6 CFU/ml for all the aminoglycoside susceptible isolates and for the isolates expressing aminoglycoside-modifying enzymes. On the contrary, the 16S rRNA methylase producers grew on the SuperAminoglycoside medium in 24 h and the lowest limit of detection was below the cut-off value. The sensitivity and specificity of the SuperAminoglycoside medium for selecting amikacin- and gentamicin resistant isolates producing 16S rRNA methylases were consequently of 96% (26/27) and 100%, respectively. The spiked 16S rRNA methylase producers (except the NpmA producer) in stools grew with a lowest detection limit ranging from 10^1 to 10^2 CFU/ml

Conclusions: Overall, this medium offers the possibility to select for those multiple-aminoglycoside resistant *Enterobacteriaceae*, *P. aeruginosa* and *A. baumannii*. The use of this selective medium may contribute to rapidly identify carriers of multidrug-resistant isolates producing plasmid-mediated 16S rRNA methylases, and consequently to rapidly implement infection control measures in order to limit their spread.