A culture medium for screening pandrug- resistance to aminoglycosides in Gram-negative bacteria

Multidrug resistance in Gram negatives is now recognized as an issue of worldwide interest. Those bacteria possess various resistance mechanisms compromising the efficacy of several class of antibiotics such as beta-lactams and aminoglycosides. Therefore, it is essential to develop effective tools such a screening culture medium to detect quickly the potential spread of aminoglycoside-resistant bacteria in human and animal isolates. This medium allows to perform prospective screening and epidemiological surveys to ensure the use of aminoglycosides.

The main mechanism of resistance to aminoglycosides relies on the production of enzymes that modify either the antibiotic itself or the target of the antibiotic. Nucleotidyltransferase (ANT), phosphotransferases (APH) and acetyltransferases (AAC) alter the structures of the only specific aminogylcosides but not all. They are usually plasmid-encoded and may be co-associated conferring pandrug resistance to aminoglycosides but this is an exceptional phenomena. However, the 16S rRNA methylases that modify the target of the aminoglycosides by methylating the 16s rRNA and therefore conferring pandrug resistance to aminoglycoside are of increasing prevalence being associated with many carbapenemase producers such as NDM producers. This methylation confers resistance to the widely used aminoglycosides such as gentamicin, tobramycin, amikacin, isepamicin, kanamycin, and netilmicin except neomycin and avoparcin (Fig. 1).Therefore, a medium was developed for screening pandrug resistance to aminoglycoside.

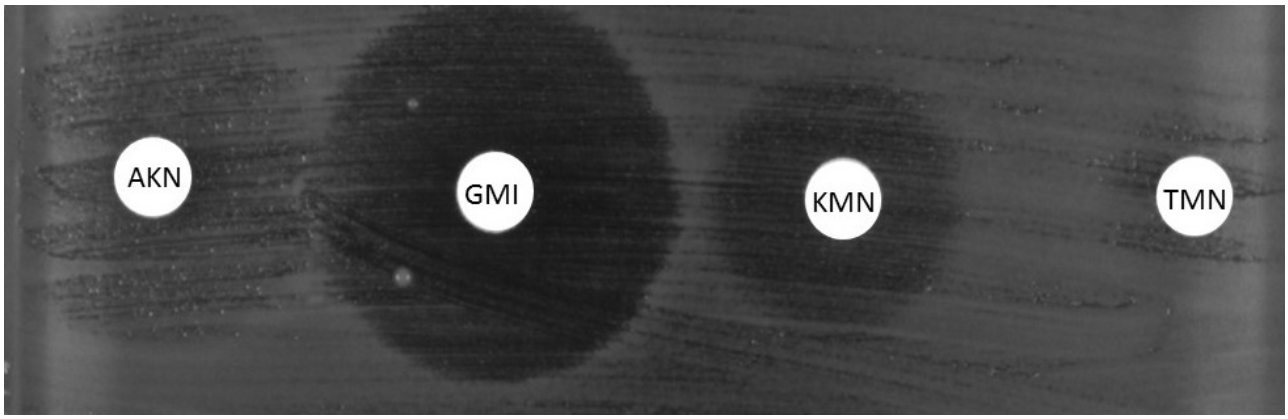

Fig. 1. Antibiogram of a Klebsiella pneumoniae strain producing 16S rRNA methylases (AKN – Amikacin , GMI - Gentamicin, KMN - Kanamycin , TMN - Tobramycin). An indicator of the 16S rRNA methylases is the phantom zone around the antibiotic discs of aminoglycosides.

The base of the medium is the Eosine-Methylene-Blue (EMB) agar, which allows the differentiation of lactose fermenters (black colonies) from most of the nonfermenters (colorless or light lavender)

(Fig. 2). Vancomycin and amphotericin B were included to prevent the growth of Gram-positives bacteria and fungi, respectively. Given that 16S rRNA methylase producers are simultaneously resistant to amikacin and gentamicin, both antibiotics were incorporated in the medium to select specifically bacteria that are pandrug resistant to aminoglycoside.

Concerning the inoculum, a cutoff value was set at 10^3 CFU/ml. Indeed, a higher inoculum of a susceptible strain or of an isolate producing aminoglycoside-modifying enzymes could lead to the growth of few colonies. So, 100 µl of a bacterial suspension in normal saline are plated onto the SuperAminoglycoside medium. Then, the plate is incubated during 18 hours at 37°C. If no growth was observed, the incubation was extended up to 48 hours to confirm that no growth actually occurred.

Fig. 2. 16S rRNA methylase-producing K. pneumoniae growing onto the SuperAminoglycoside medium.

To evaluate the performance of the SuperAminoglycoside medium, 69 isolates of various species (Enterobacteriaceae, *P. aeruginosa*, and *A. baumannii)* were tested in this study. Twelve isolates were susceptible to aminoglycosides, and 57 isolates were resistant to aminoglycosides. Twenty isolates produced 16S rRNA methylases, and 37 isolates produced aminoglycoside-modifying enzymes. The isolates were classified as resistant or susceptible according to their MIC to amikacin and gentamicin (obtained by broth microdilution method in cation-adjusted Mueller-Hinton broth).

The sensitivity and specificity of the SuperAminoglycoside medium for selecting amikacin- and gentamicin resistant isolates producing 16S rRNA methylases were 95% (19/20) and 96% (47/49), respectively. When considering detection of amikacin- and gentamicin-resistant isolates only (regardless of production of 16S rRNA methylases), the specificity of the medium was 100%.

The SuperAminoglycoside medium contributes to species identification according to the color of the colonies growing on the medium (Fig. 2). It allows to rapidly identify isolates producing plasmid-mediated 16S rRNA methylases which enables to implement quickly infection control measures to restrict the spread of resistance-plasmid.

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