Rapid detection of multiple aminoglycoside resistance in Enterobacteriaceae

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INTRODUCTION

• Multidrug resistant (MDR) enterobacterial isolates are disseminating worldwide and therapeutic options are becoming limited (1).
• Aminoglycosides (AG) may still be considered as valuable treatment options to treat those MDR isolates (2).
• However, plasmid-mediated 16S rRNA methyltransferases conferring a high level of resistance to multiple AG is also reported, and they are identified at a high frequency, especially among producers of NDM-like carbapenemases (3).
• In an area of paucity of novel molecules, rapidly identifying multiple resistance to AG by a simple test may be useful for implementing antibiotic stewardship and containment of those multidrug-resistant bacteria.

PURPOSE

Our aim was to develop a rapid, reliable and cost-effective test to rapidly identify multiple aminoglycoside resistance in Enterobacteriaceae.

PRINCIPLE

This test is based on the detection of the glucose metabolism related to bacterial growth in presence of a defined concentration of a mix of two aminoglycosides molecules (amikacin and gentamicin). Formation of acid mebalolites consequent to the glucose metabolism was evidenced by a color change (orange to yellow) of a pH indicator (red phenol).

PREPARATION OF THE AMINOGLYCOSIDE NP TEST

1. Reagents and solution.
• To prepare the Rapid Aminoglycoside NP solution, 6.25 g of Mueller Hinton Broth adjusted in cation (MHB-CA) powder, 0.0125 g of phenol red and 225 ml of distilled water were mixed.
• The pH of the solution was adjusted to 6.7.
• The solution was then autoclaved at 121°C for 15 min.
• After cooling the solution to room temperature, 25 ml of D(+)-glucose anhydride 10 % sterilized by filtration, was added.
• Amikacin and gentamicin were added extemporaneously to the solution.

2. Bacterial inoculum.
• A standardized enterobacterial inoculum was prepared using freshly-obtained (overnight) bacterial colonies grown on Luria-Bertani or Mueller-Hinton plates.
• The bacterial colonies were resuspended into 10 ml of sterile NaCl 0.85 % to obtain a 3 McFarland optical density (ca. 10⁹ CFU/ml).

3. Tray inoculation
• For each isolate, 2 wells are inoculated in parallel with the bacterial suspension, respectively with or without AG.
• After mixing the bacterial suspension to the reactive medium, the final concentration of bacteria was ca. 10⁹ CFU/ml and the final concentration of each AG was 30 µg/ml.

4. Tray incubation
• Incubation up to 2h at 35±2°C in ambient air, not sealed and without agitation.

5. Tray reading
• Visual inspection every hour during 2 h

EVALUATION OF THE AMINOGLYCOSIDE NP TEST

• Performances of the Rapid Aminoglycoside NP test were evaluated with a total of 48 enterobacterial isolates:
  ✓ 38 isolates resistant to AG:
    - 18 isolates harboring 16S rRNA methylases
    - 20 others producing different AG-modifying enzymes (acetyl, adeny1, and phosphoryl enzymes)
  ✓ 10 isolates susceptible to AG,
• MICs of amikacin and gentamicin were determined using the broth microdilution reference method and results were interpreted according to the EUCAST breakpoints.

RESULTS / DISCUSSION

• Good performances of the test:
  Sensitivity = 100 % and specificity = 97 %
• Rapid (less than 2h), inexpensive and reproducible.
• Two limitations:
  - Positivity of the test does not always superimpose the presence of 16S rRNA methylases because combination of aminoglycoside-modifying enzymes (nucleotidyI-, phosphoryl-, and acetyltransferases) in a given enterobacterial strain might confer resistance to both gentamicin and amikacin.
  - Test non compatible with nonfermenters, such as Pseudomonas aeruginosa and Acinetobacter baumannii, which may also produce 16S rRNA methylases.

CONCLUSIONS

• The Rapid Aminoglycoside NP test is easy to perform, rapid (2 h), sensitive, and specific.
• It detects resistance to multiple AG among Enterobacteriaceae from selective and non selective media prior to obtaining any antibiotic susceptibility testing results.
• It may guide prescription of novel broad-spectrum AG, such as plazomicin.