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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 consecutive 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 anhydre 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. 10^9 CFU/ml).

3. Tray inoculation

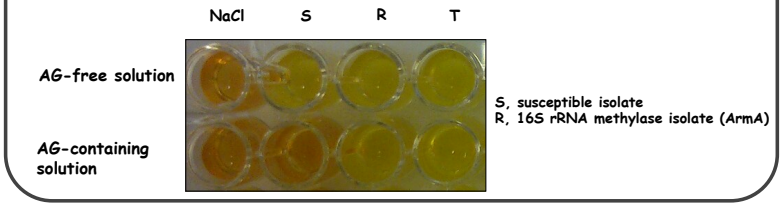
•For each isolate, 2 wells are inoculated in parallel with the bacterial suspension, respectively with or without AG.
•After mixing the bacterial suspension to the reactive medium, the final concentration of bacteria was ca. 10^8 CFU/ml and the final concentration of each AG was 30 µg/ml.

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, adenyl, 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 (nucleotidyl-, 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.

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2) Falagas ME, et al. Future Microbiol 2011. Therapeutic options for infections with *Enterobacteriaceae* producing carbapenem hydrolyzing enzymes.
3) Doi Y, et al. Infect Dis Clin North Am, 2016. Aminoglycoside resistance: the emergence of acquired 16S ribosomal RNA methyltransferases.