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Carba NP test colonies

Inserm U914 Résistances Emergentes aux Antibiotiques

Rapid detection of carbapenemase activity in *Enterobacteriaceae*

The Carba NP (Nordmann-Poirel) test using bacterial colonies

To be used with enterobacteriaceae showing any slight decreased susceptibility to any carbapenem (imipenem, meropenem, ertapenem)

"A similar protocol can be used for the detection of carbapenemase activity from *P.aeruginosa* colonies"

Protocol

1. Add 100 µl of 20 mM Tris-HCl lysis buffer (B-PERII, Bacterial Protein Extraction Reagent, Thermo Scientific, Pierce) in each of two 1.5 ml eppendorf tubes

2. Resuspend a 1/4 to 1/3 calibrated oese (10 µl) of bacterial colonies in each of those 100 µl of 20 mM Tris-HCl lysis buffer. (bacterial colonies may be recovered directly from the antibiogram around disk of carbapenem performed according to the disk diffusion techniques).

NB : Carba NP test was validated with bacterial colonies grown on Mueller-Hinton agar plates (Becton Dickinson, Le Pont de Chaix, France), blood agar plates, trypticase soy agar plates, and most of selective media used for carbapenemase producers screening. Carba NP test **CANNOT** be performed with bacterial colonies grown on Drigalski or McConkey plates.

Recommended medium : trypticase soy agar supplemented with $ZnSO_4$ at 70 µg/ml

3. Check that bacterial colonies have been correctly resuspended. If necessary mix up and down with a pipette

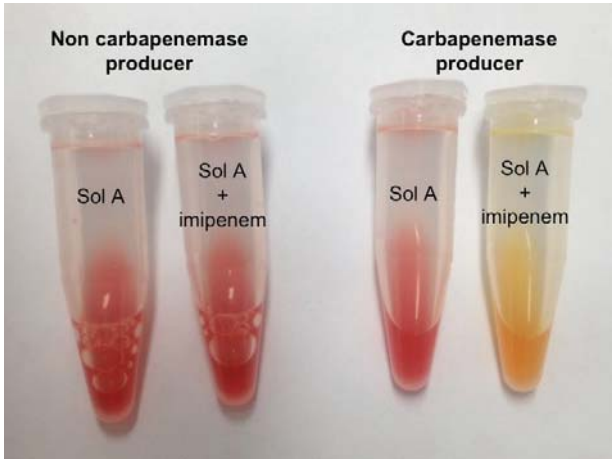
4. Add (i) 100 µl of Solution A in the first eppendorf tube and (ii) 100 µl Solution A + imipenem 6 mg/ml in the second 1.5 ml eppendorf tube.

5. Incubate at 37°C for a maximum of 2 hours

6. Optical reading of the color of each tube

Interpretation :

	No antibiotic	**Imipenem**
No carbapenemase	Red	Red
Carbapenemase producer	Red	Orange/Yellow
Not interpretable	Yellow	Yellow



Usually, the time required for obtaining positive results is as follows :
- KPC producers : 2 to 30 min
- OXA-48 like producers : 20 min to 1h
- Metallo-β-lactamases (NDM, VIM, IMP) : 15 min to 1h

Material

- 1.5 ml Eppendorf tubes
- Imipenem sodium salt (Sigma-Aldrich) or Imipenem + cilastatin (drug used for patient treatment).
- B-PERII, Bacterial Protein Extraction Reagent (Thermo Scientific, Pierce), Cat : 78260.
- $ZnSO_4$, $7H_2O$ (Sigma-Aldrich, Cat : 221376)
- Negative (wild-type *E. coli*) and positive (*K. pneumoniae* OXA-48 or *K. pneumoniae* KPC-2) controls.

Preparation and storage of Solution A

1. Prepare a concentrated solution of red phenol 0.5% w/v
2. Mix 2 ml of the concentrated red phenol solution (strongly vortex before pipetting to resuspend the solution) in 16.6 ml of distilled water
3. Adjust the pH at 7.8 by adding drops of a NaOH solution (1 N)
4. Add 180 µl of $ZnSO_4$ (Sigma-Aldrich, Cat : 221376) 10 mM to obtain a final concentration of 0.1 mM

Solution A is stable at room temperature for 1 week and may be kept at -20°C for several months.

Solution A + imipenem (6 mg/ml) has to be prepared extemporaneously.

However, batches of imipenem powders can be weighted and prepared in advance and kept at 4°C for two weeks if solution A is not added.

• 600 µl of solution A is needed for each test (solution A, solution A + imipenem, positive and negative controls).