Rapid detection of extended-spectrum ß-lactamase producing Enterobacteriaceae

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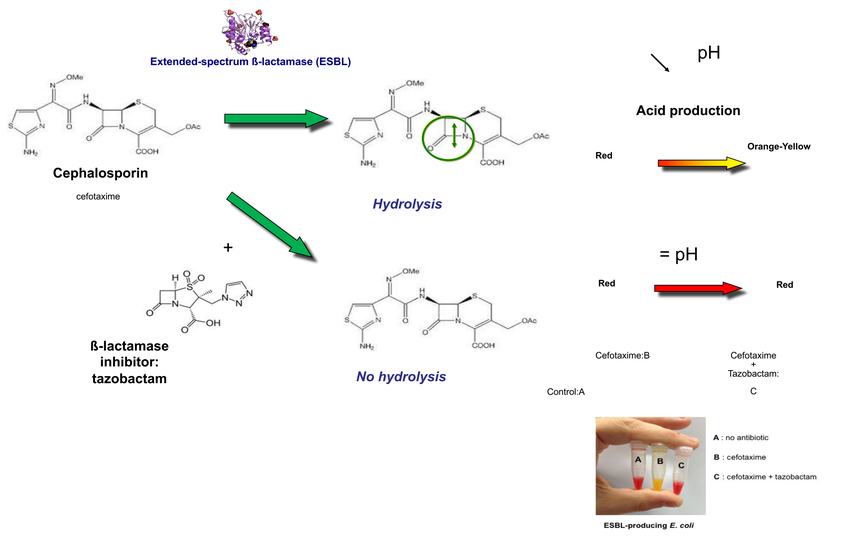
INTRODUCTION

- Multidrug resistance in Enterobacteriaceae represents a serious threat to public health, since the accumulation of resistance determinants may be the source of difficult-to-treat infections in humans.
- One of the most important resistance trait is related to resistance to broad-spectrum cephalosporins through production of extended-spectrum ß-lactamases (ESBL).
- Conventional detection of ESBL production remains time-consuming (24 to 48 h). A home-made rapid and biochemical test has been developed based on the in-vitro detection of a cephalosporin (cefotaxime) hydrolysis that is inhibited by tazobactam addition. The ESBL activity is evidenced by a color change of a pH indicator.
- Here, we have evaluated the industrial version of this test, the Rapid ESBL NP test (Liofilchem, Italy) that will be launched in 2019.

PRINCIPLE

Rapid ESBL NP test, original version (Nordmann et al., 2012)



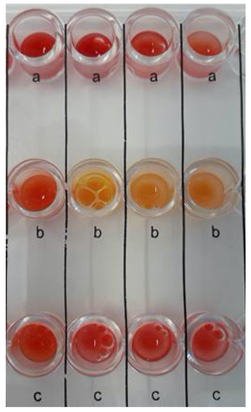
Here we evaluated an industrial version of the ESBL NP test

THE TEST

Protocol

1. Resuspend 2-4 full calibrated loops **(loop size 10 µl and not 1 µl)** of bacterial colonies in 400 µl of 20 mM Tris-HCl lysis buffer (B-PERII, Bacterial Protein Extraction Reagent, Thermo Scientific, Pierce)
2. Check that bacterial colonies have been correctly resuspended. If necessary mix up and down with a pipette
3. Dispense 100 µl of this suspension in each of the three wells in a single row
4. Alternatively,
   - Add 100 µl of 20 mM Tris-HCl lysis buffer (B-PERII, Bacterial Protein Extraction Reagent, Thermo Scientific, Pierce) in three 1.5 ml eppendorf tube
   - Resuspend a full calibrated loop **(loop size 10 µl and not 1 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 chromogenic media used for isolation of urine specimens)
   - Check that bacterial colonies have been correctly resuspended. If necessary mix up and down with a pipette
   - Transfer the contents of the three eppendorf in each of the three wells in a single row
5. Incubate at 37°C for a maximum of 20 mins
6. Follow the instructions listed below for interpreting the results

- The Rapid ESBL NP test was applied to cultures of enterobacterial strains possessing well-characterized ß-lactamase gene contents.
- Those strains were *Escherichia coli* (n=51), *Klebsiella pneumoniae* (n=30), *Enterobacter cloacae* (n=5), *Citrobacter freundii* (n=3), *Morganella morganii* (n=1), *Serratia marcescens* (n=1) and *Proteus mirabilis* (n=1). Those 92 isolates included 31 ESBL producers (CTX-M-, SHV-, TEM-, PER-, GES-, VEB-type- and 61 non-ESBL producers including carbapenemase producers (KPC-, OXA-48-, IMP-, VIM-, NDM types) and AmpC overproducers (plasmid and chromosome-encoded).



C+ C- n°1 n°2

INTERPRETATION OF THE RESULTS

Interpret the results according to the scheme below.

Column	No ESBL	ESBL	Cephalosporinase or Cephalosporinase + ESBL or Carbapenemase with or without Cephalosporinase or/and ESBL	Non interpretable
a (no antibiotic)	red	red	red	yellow
b (cefotaxime)	red	orange/yellow	orange/yellow	yellow
c (cefotaxime + tazobactam)	red	red	orange/yellow	yellow

Strains n°1 and 2 are ESBL producers

- The results of the Rapid ESBL NP test were obtained mostly within 1 h.
- Sensitivity and specificity of the test for ESBL detection were 94.6 and 86%, respectively.
- The carbapenemase producers with or without ESBL production were not identified as ESBL producers.

CONCLUSION

- This newly-developed Rapid ESBL NP test possesses high specificity and sensitivity.
- It may be used for detecting ESBL producers for infection control and antibiotic stewarship purposes.
- It clearly differentiates ESBL producers from carbapenemase producers. After further evaluation such test might be used for identification of ESBL producers from blood cultures and urines.

REFERENCE

Nordmann P, Dortet L, Poirel L. 2012. Rapid detection of extended-spectrum ß-lactamase producing Enterobacteriaceae. J Clin Microbiol.