Background

The main carbapenemases identified in Enterobacteriaceae belong to Ambler class A (KPC), class B (VIM, IMP and NDM), and class D (OXA-48). Currently identification of CPE carriers is a difficult task for laboratories.

Methods

Screening rectal swabs

Two clinical rectal swabs from 81 patients were collected in semi-solid Stuart’s transport medium during an OXA-48 outbreak situation at one hospital. The first swab of each patient was tested within the 4 hours in the collection hospital. The second swab was kept at -20°C and sent to the reference laboratory for enrichment and molecular testing and was cultured within the 48 hours after collection.

Bacterial screening and enriched bacterial identification

The swabs were analyzed by direct plating on Brilliance chromogenic agar (CRE, Oxoid) at the local laboratory. Following transfer of the specimens to the reference laboratory, the swabs were incubated at 37°C for 4 hours in 2.5 mL of BHI broth supplemented with 0.25 µg/mL of ertapenem. Twenty µL of the enriched broth were spread on CRE selective chromogenic agar.

Real-time PCR assays

Eighty rectal swabs were analyzed by direct plating on Brilliance chromogenic agar (CRE, Oxoid) at the local laboratory. Following transfer of the specimens to the reference laboratory, the swabs were incubated at 37°C for 4 hours in 2.5 mL of BHI broth supplemented with 0.25 µg/mL of ertapenem. Twenty µL of the enriched broth were spread on CRE selective chromogenic agar.

16 patients culture-positive for OXA-48-producing Enterobacteriaceae by direct plating

8 additional patients culture-positive for OXA-48-producing Enterobacteriaceae after enrichment

81 clinical rectal swabs

Conclusions

Four-hour enrichment in presence of 0.25 µg/mL of ertapenem permits the detection of 30% additional carriers (8/24 positive by culture).

Low Ct values (strong signal) observed with RT-PCR 2 and 3 suggests to use this method on clinical samples avoiding the preliminary enrichment step and reducing the time to result.

Several positive samples by RT-PCR could not be confirmed by culture, underlining the need of cut-off determination for the risk of interpatients transmission.

Real-time PCR 2 targets the most relevant carbapenemase (KPC, OXA-48, VIM and NDM) but does not permit to distinguish between NDM and VIM carriers (same fluorophore for both NDM and VIM).

Real-time PCR 3 targets only OXA-48 and VIM but a new product version includes now KPC.

RT-PCR 1 is not available commercially.

Results

Table 1. Cycle threshold (Ct) obtained with the 3 RT-PCR for the detection of OXA-48 coding gene

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Patient 32 as OXA-48 carriers (Table 1 B).

Compared to culture, false positive results were observed for several patients with any RT-PCR (Table 1 C). Each RT-PCR includes an internal control permitting to avoid false negative results because of inhibitors.

Expected Ct for RT-PCR 1 presents no false negative results whereas RT-PCR 2 and 3 do not detect Patient 32 as OXA-48 carriers (Table 1 B).

Table 1. Cycle threshold (Ct) obtained with the 3 RT-PCR for the detection of OXA-48 coding gene

A) Patients positive by direct culture . B) Patients positive by culture only after enrichment. C) Patients only positive by RT-PCR

References


Mailing address:
Pierre Bogerts
Laboratory of Microbiology
CHU Mont-Godinne (UCL)
1 Avenue Dr. G. Thérasse, 5530 Yvoir, Belgium
E-mail: pierre.bogaerts@uclouvain.be