EVALUATION OF A COMMERCIAL REAL-TIME PCR TO DETECT CARBAPENEM-RESISTANT ENTEROBACTERIACEAE IN RECTAL SWABS

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Background

The resistance to carbapenems in Enterobacteriaceae is mainly due to the diffusion of carbapenemase genes. To date, three main classes of carbapenemases have been identified. Ambler class A beta-lactamase are enzymes that can be chromosomally encoded (bla_{NMC}, bla_{SME}, bla_{IMI-1}, bla_{SFC-1}) or plasmid encoded (bla_{KPC} – the prevailing one, bla_{IMI-2}, bla_{GES}). Bla_{VIM} (Verona integron–encoded metallo-β-lactamase), bla_{IMP}, and the New Delhi MBL (bla_{NDM}) belong to class B metallo-β-lactamases (MBLs). Ambler class D includes the $bla_{OXA-48-like}$ carbapenemases.

Even if different bacterial genera can harbor these genes, carbapenem resistance has become more common in *Klebsiella pneumoniae* (KP) isolates, which can express different resistance mechanisms such as bla_{KPC}, bla_{VIM}, bla_{NDM}, bla_{IMP}, $bla_{OXA-48-like}$, due to the plasmid diffusion.

The treatment options for infections caused by carbapenems producing Enterobacteriacease (CPE) are currently limited and development of new classes of antibiotics is troublesome. For this reason, major efforts in managing the spread of CPE are necessary and the strict application of surveillance procedures is mandatory. Patients colonized by CPE should be promptly identified and cohorted. Surveillance cultures based on rectal swabs have been demonstrated effective, but have the disadvantage to be time consuming. Instead, the rapid detection of CPE carriers would allow the quick cohorting patients, reducing the risk of CPE spread.

This study evaluates in a clinical setting the performance of a new automated real-time PCR (RT-PCR), the Xpert® Carba-R Assay, Cepheid, USA, able to detect bla_{KPC}, bla_{VIM}, bla_{NDM}, bla_{IMP}, $bla_{OXA-48-like}$ genes from rectal swabs. The analyses were performed using the GeneXpert® Dx Systems.

Materials and methods

Clinical samples - In a 4-month period (August-November 2013), 185 paired rectal swabs belonging to 90 different patients were evaluated.

Swab #1 was analyzed using the Xpert® Carba-R Assay. Briefly, the swab was placed in a vial containing sample reagent and eluted by vortexing for 10 seconds. The sample was then transferred to the Xpert® Carba-R cartridge using a disposable pipette and evaluated using the GeneXpert® Dx Systems. The results were available for reading after one hour.

Swab #2 was placed in 10 ml of McConkey broth added with a 10 µg disk of meropenem. The day after, a drop of this broth was subcultured onto Brilliance agar (Oxoid-ThermoFisher, USA). Strains grown in culture were identified and carbapenemase production was confirmed using, as phenotypic method, the disk diffusion synergy test (meropenem disks supplemented with aminophenylboronic acid, dipicolinic acid and cloxacillin, Rosco, Denmark) and an *in-house* PCR, based on a modification of the protocol of Poirel et al.., able to detect the same genes amplified through the Xpert® Carba-R Assay.

Other analysis - To evaluate the test performances, 10 different strains known to harbor carbapenemase genes were previously analyzed using the Xpert® Carba-R cartridges. Briefly, 50 µl of a 0,5 McFarland suspension of the bacteria to be tested were inoculated in the sample reagent and eluted by vortexing for 10 seconds, then processed as above.

Finally, ten rectal swabs prepared with different CPE (random amounts for some targets) and non-CPE microorganisms were analyzed according to the previously described protocol.

Results

- The Xpert® Carba-R Assay showed very good performances. A low rate of invalid tests was initially noted, mainly due to swabs with abundant fecal material, but the problem was solved increasing the volume of the sample reagent. All the internal controls performed weekly gave the expected results, being amplified all the targets after more than 30 RT-PCR cycles (figure 1). The carbapenemase genes of the 10 known CPE analyzed were correctly detected.
- The study synopsis for the clinical samples is summarized in figure 2. 170 out of the 185 samples analyzed were both Xpert® and culture-negative.
- Thirteen samples belonging to 11 different patients were Xpert® positive (overall positivity 7.0%). One sample was positive for $bla_{OXA-48-like}$ gene, 5 for bla_{VIM}, and 7 for bla_{KPC} genes (figures 2 and 3). Agreement with cultural results was documented for 11 of 13 samples. For these 11 strains (all KP), confirmation tests (synergy and *in house* PCR) were in agreement with Xpert® results.
- The cultures of two samples, which were positive with the Xpert® Carba-R for $bla_{OXA-48-like}$ and one bla_{VIM} , resulted repeatedly negative. In both cases, the molecular target was amplified near to the end of the thermal cycles (figure 4).
- Two samples with positive cultures were Xpert®-negative. The first one was a carbapenem-resistant *Acinetobacter baumannii*; the latter an ESBL+ KP strain with negative disk diffusion synergy tests (but probably expressing outer membrane porin loss). Both isolates were negative also with the *in-house* PCR.
- All the targets of the strains inoculated in the prepared rectal swabs (2 bla_{IMP}, 2 $bla_{OXA-48-like}$, 1 bla_{NDM} and 5 negative samples) were correctly detected by Xpert®.

Discussion

- The Xpert® Carba-R Assay demonstrated exquisite specificity and sensitivity.
- The test is really easy to perform and requires only one hour to be completed (including sample preparation, run, data analysis). This short turn-around-time can be crucial for the proper decision to cohort or not patients at the admission time.
- The results were in agreement with the cultures, except for two samples, in which $bla_{OXA-48-like}$ and one bla_{VIM} genes were detected by Xpert® at the end of the thermal cycles. We postulate that in both cases a low amount of these targets, combined with a low-level MIC for meropenem (likely for these CPE) could explain the culture negativity. However, this situation could raise the question of whether these findings should be communicated to the hospital epidemiologist and if these patients should be cohorted.
- An intrinsic disadvantage of the method is the evaluation of resistance genes without the possibility to know to which microorganisms they belong (risk of cohorting of patients carrying strains that aren't alert microorganisms). Moreover, the economical impact of this molecular screening should be carefully evaluated for different settings.
- Further studies and consensus guidelines based for example on the epidemiology of the different countries and hospitals are needed to better understand the correct use of this test (first line approach instead of cultures, use for selected situations/patients etc.).

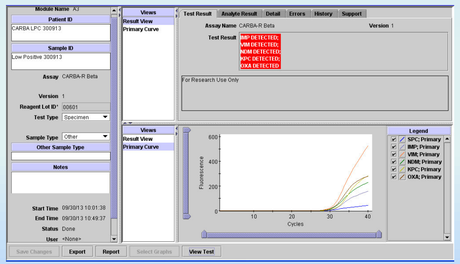

Figure 1 - Xpert® positive control

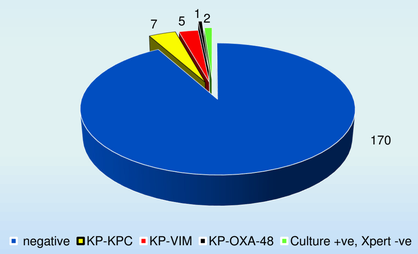

Figure 2 – Study synopsis

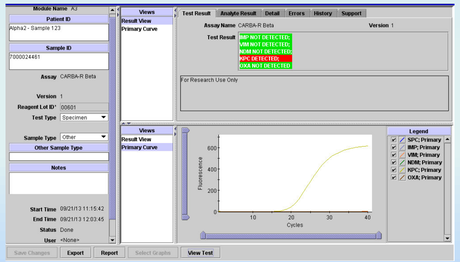

Figure 3 - Xpert®: sample positive for bla_{KPC}, culture positive for KP-KPC+

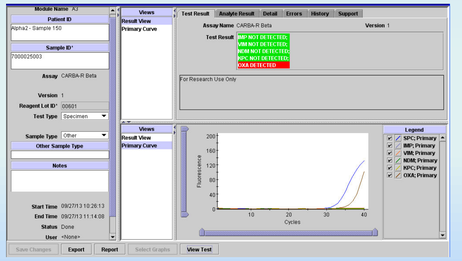

Figure 4 - Xpert®: sample positive for $bla_{OXA-48-like}$, culture negative

Acknowledgments

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