A new real-time PCR for rapid detection of VIM, OXA-48, NDM and KPC carbapenemases in Gram-negative bacteria directly from rectal swabs

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Introduction

The emergence and spread of drug resistance by beta-lactamases amongst gram-negative bacteria is a serious problem, in the hospital environment as well as in the community. Since carbapenem resistance is emerging worldwide, due to the presence of various carbapenemase genes, treating infections with these multi-resistant strains has almost become impossible. Fast and accurate detection of carbapenemase genes in patient samples is extremely important:

- For immediate implementation of correct infection control safety measures to prevent further spread of carbapenemase producing bacteria
- For the use of appropriate empirical therapy

Ambler class	Most prevalent carbapenemases	More rare carbapenemases
Ambler class A	KPC	NmcA, SME, IMI-1, SFC-1 IMI-2, GES
Ambler class B	VIM, NDM	IMP, GIM-1, DIM-1, SPM-1
Ambler class D	OXA-48	OXA-162, OXA-181, OXA-204

Table 1: overview of carbapenemase genes

Materials

- 83 non-duplicate isolates were used for evaluation
  - 62 carbapenemase producers (figure 1)
  - 21 non-carbapenemase producers
- Four carbapenemase producing isolates were used for spiking in rectal swab specimens to determine the limit of detection (LoD) of the Check-Direct CPE assay and the ChromID CARBA agar.

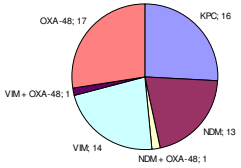

Figure 1: carbapenemase-producers used to evaluate the Check-Direct CPE

Methods

- The prototype of the Check-Direct CPE assay (Check-Points, Wageningen, The Netherlands): designed to detect all known variants of bla_{VIM} (except bla_{VIM-7}), bla_{KPC}, bla_{NDM} and bla_{OXA-48} including $bla_{OXA-162,-181,-204}$
- The well-evaluated Check-MDR CT102 assay (Check-Points, Wageningen, The Netherlands), using the same purified DNA as tested in the real-time PCR.

Limit of detection in rectal swabs

- LoD of the Check-Direct CPE was determined using seven 10-fold dilutions of the four carbapenemase producing isolates. Spiked rectal swabs were made by adding 100 µl of each dilution to a 500 µl aliquot of a pool of rectal swab specimens (gathered from rectal swab specimens collected from 25 patients for routine microbiological diagnostics).
- To determine the LoD of the ChromID CARBA, it was inoculated with 20 µl of each suspension and incubated overnight at 37°C. The number of suspected colonies (pink- or blue-coloured according to the manual) was counted the following day.

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Aim of the study

Evaluating the prototype of the Check-Direct CPE assay, a new real-time PCR for rapid and simultaneous detection of the ß-lactamase genes VIM, OXA-48, NDM and KPC in gram-negative bacteria directly from rectal swabs, as a rapid screening approach.

Screening method	Turn-around-time	Able to detect
Selective agar	>18hours	Depending on agars used
Real-time PCR	4 hours	KPC[1], NDM[2], OXA-48[3]
Micro-array	5 hours	KPC[4]

Table 2: overview of the used screeningmethods for carbapenemase genes

1: Hindiyeh et al., Rapid detection of blaKPC carbapenemase genes by mulitplex real-time PCR, JCM, 2008
2: Naas et al., Real-time PCR for detection of NDM-1 carbapenemase genes from spiked stool samples, AAC, 2011
3: Naas et al., Real-time PCR for detection of blaOXA-48 genes from stools, JAC, 2012
4: Peter et al., Direct detection and genotyping of Klebsiella pneumoniae carbapenemases from urine by use of a new DNA microarray test, JCM, 2012

Results

- All carbapenemase- and non-carbapenemase producers were detected correctly (specificity and sensitivity 100%).
- All results were available within 3 hours (including DNA extraction)
- LoD in spiked rectal swabs is shown in table 3.

Isolate	Carbapenemase-type	LoD ChromID Carba (CFU/ml)	LoD Check-Direct CPE (CFU/ml)
E. coli	VIM-2	ND	20-200
K. pneumoniae	OXA-48	200-2000	20-200
K. pneumoniae	NDM	200-2000	200-2000
K. pneumoniae	KPC	200-2000	2×10^3-2×10^4

Table 3: limit of detection determination using spiked samples

LoD, limit of detection; CFU, colony forming units; ND, not detected

Conclusion

The Check-Direct CPE assay appeared to be an accurate and rapid method to detect the most prevalent and clinically important carbapenemase genes, directly from a rectal screening swab. Therefore, this assay seems very promising as a carbapenemase screening tool and can be an important improvement in infection control programs.

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The results of this study are submitted for publishing at the Journal of Antimicrobial Chemotherapy (JAC).