Rapid and accurate detection of carbapenemase genes in *Enterobacteriaceae* with the Cepheid Xpert Carba-R assay

Marion Smith,1 Bram Diederen,1,2 Jelle Scharringa,3 Maurine Leversteijn-van Hall,4 Ad C. Fluit3 and James Cohen Stuart1,2

1Department of Medical Microbiology, Medisch Centrum Alkmaar, Juliana Van Stolberghlaan 13, 1814 HB Alkmaar, The Netherlands
2Regional Laboratory of Public Health, Haarlem 2035 RC, The Netherlands
3Department of Microbiology, University Medical Centre Utrecht, Utrecht, The Netherlands
4Department of Medical Microbiology and Infection Prevention, Bronovo Hospital, The Hague, The Netherlands

Rapid and accurate detection of carbapenemase-producing *Enterobacteriaceae* is pivotal for adequate antibiotic therapy and infection control. The Cepheid Xpert Carba-R assay detects and identifies the most prevalent carbapenemases (KPC, VIM, IMP, NDM and OXA-48), using automated real-time PCR. The test performance of the Xpert Carba-R was evaluated using 129 well-characterized non-repeat *Enterobacteriaceae* isolates, suspected for carbapenemase production, i.e. with meropenem MICs >0.25 mg l⁻¹. The isolate collection contained 100 carbapenemase-producing isolates (36 KPC-2 or KPC-3, 20 VIM-1, 4 KPC-2 plus VIM-1, 5 NDM-1, 2 IMP-1, 1 IMP-28, 1 IMP-1 plus VIM-1 and 31 OXA-48 like) and 29 negative control isolates producing extended-spectrum β-lactamase and/or AmpC β-lactamases. PCR and sequencing of β-lactamase genes were used as reference tests. The sensitivity of the Xpert Carba-R was 100 % (100/100), with a 100 % (29/29) specificity. The time to result was approximately 55 min with a hands-on time of only 1 min per isolate. In conclusion, the Carba-R assay is a rapid and accurate instrument for the confirmation and identification of the *bla*KPC, *bla*VIM, *bla*IMP, *bla*NDM and *bla*OXA-48 genes.

INTRODUCTION

Carbapenemase-producing *Enterobacteriaceae* are an emerging problem worldwide (Doi & Paterson, 2015; Falagas et al., 2014; Tångdén & Giske, 2015). Invasive infections with these strains are associated with high morbidity and mortality rates, posing a serious threat to clinical patient care and public health. Rapid and accurate detection of carbapenemase-producing strains is therefore pivotal for adequate antibiotic therapy and infection control.

The Cepheid Xpert Carba-R assay (Cepheid) is a cartridge-based genotypic test that detects and identifies the most prevalent carbapenemases in The Netherlands and the rest of the world (KPC, VIM, IMP, NDM and OXA-48) (Vlek et al., 2016; Tångdén & Giske, 2015), using automated real-time PCR. The aim of this study was to determine the test characteristics of the Xpert Carba-R assay for the detection and identification of the *bla*KPC, *bla*VIM, *bla*IMP, *bla*NDM and *bla*OXA-48 genes in *Enterobacteriaceae* isolates suspected of carbapenemase production.

METHODS

The test performance of the Xpert Carba-R was evaluated using real-time PCR with an international collection of 129 well-characterized non-repeat *Enterobacteriaceae* isolates, suspected for carbapenemase production, i.e. with meropenem MICs >0.25 mg l⁻¹ (van Dijk et al., 2014). Of these isolates, 74 were from a β-lactamase reference centre in The Netherlands (University Medical Centre Utrecht, The Netherlands), 25 from Greece, 20 from New York, 4 from France (Nordmann et al., 2012), 5 ATCC or NCTC reference strains and 1 from the Medisch Centrum Alkmaar, The Netherlands. The collection contained 88 Klebsiella pneumoniae, 15 Escherichia coli, 2 Proteus mirabilis and 24 Enterobacter spp., of which 100 harboured one or more of the *bla*KPC, *bla*VIM, *bla*IMP, *bla*NDM and *bla*OXA-48 genes. These carbapenemases consisted of 36 KPC-2 or KPC-3, 20 VIM-1, 4 KPC-2 plus VIM-1, 5 NDM-1, 2 IMP-1, 1 IMP-28, 1 IMP-1 plus VIM-1 and 31 OXA-48 like enzymes (Table 1). The isolate collection also contained 29 negative control isolates producing extended-spectrum β-lactamase and/or AmpC β-lactamases.

PCR and sequencing of β-lactamase genes were used as the reference tests as previously described by Voets et al. (2011).
The test is enclosed in a single-use disposable cartridge that holds the PCR reagents and hosts the PCR process. All KPC variants known to date are detected, as are all VIM and NDM variants. The OXA enzymes OXA-48, OXA-162, OXA-163, OXA-181 and OXA-204 from the OXA-48 subgroup are detected, and the IMP-related enzymes IMP-1, IMP-3, IMP-6, IMP-10, IMP-25 and IMP-30 are also detected (Package insert Cepheid GeneXpert Carba-R assay, 2014).

The Carba-R assay is performed on the GeneXpert Instrument Systems, a random access device developed and manufactured by Cepheid, which performs hands-off sample processing and multiplex, real-time PCR for the detection of DNA or RNA. Sample preparation, amplification and real-time detection are all automated and integrated.

The Carba-R assay was performed according to the manufacturer’s instructions using software version 4.3. To mimic the routine clinical setting, the test characteristics were based on the first test result. For each isolate, a suspension was prepared to a density of 0.5 McFarland. The suspension was then diluted to a ratio of 1:10 in saline, and 10 µl of the dilution was transferred to a sample reagent vial. Subsequently, 1.7 ml of the sample was transferred from the reagent vial to an Xpert Carba-R cartridge, and real-time PCR was conducted using a GeneXpert Instrument System.

### RESULTS

The Xpert Carba-R assay correctly detected and identified all the carbapenemase genes and combinations thereof in all the 100 carbapenemase-producing isolates tested, corresponding to a sensitivity of 100% (100/100; 95% confidence interval, 96–100%). Although the assay is designed to detect IMP genes from the IMP-1 subgroup only, the assay also detected an IMP-28 gene, usually considered a member of the IMP-5 subgroup (Pournaras et al., 2013).

There were no false-positive results, resulting in a specificity of 100% (29/29; 95% confidence interval, 88–100%) (Table 1). The time to result was approximately 55 min with a hands-on time of only 1 min per isolate.

### DISCUSSION

This study showed 100% sensitivity and specificity of the Carba-R assay for confirmation of carbapenemase production in an international collection of well-characterized isolates.
Enterobacteriaceae isolates with a positive screen test for carbapenemase production, i.e. with a meropenem MIC >0.25 mg l\(^{-1}\). Compared to phenotypic assays for carbapenemase confirmation, the hands-on time and the time to result are short, and the results are easy to interpret. Moreover, the provided information on carbapenemase genes may be used for epidemiologic purposes and outbreak management. The easy protocol and interpretation make the test suitable for laboratories not specializing in β-lactamase detection. The assay may also be used to screen rectal swabs directly, but the performance of such application of the test remains to be evaluated.

A strength of this evaluation is that all evaluated isolates have increased carbapenem MICs (>0.25 mg l\(^{-1}\)), including the carbapenem-negative ones, reflecting the situation when confirmation for presence of carbapenemase genes is required. Two previous reports of this assay showed limited sensitivity of OXA-181, an OXA-48-like enzyme (Decousser et al., 2015; Findlay et al., 2015). However, the assay has been recently upgraded to detect OXA-181. One isolate in this study collection contained OXA-181, which was correctly detected and identified as an OXA-48-like carbapenemase. An intrinsic limitation of this assay is that newly emerging or rare carbapenemase genes cannot be detected. Therefore, isolates suspected for carbapenemase production and a negative test result should always be further evaluated for presence of carbapenemase genes in a reference laboratory.

In summary, the assay is a rapid and accurate instrument for the confirmation and identification of the bla\(_{\text{KPC}}\), bla\(_{\text{VIM}}\), bla\(_{\text{IMP}}\), bla\(_{\text{NDM}}\) and bla\(_{\text{OXA-48}}\) genes in the routine clinical setting.

REFERENCES


