Detection of Carbapenemases in Enterobacteriaceae by a Commercial Multiplex PCR

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A commercial multiplex PCR (hyplex SuperBug ID) was tested with a collection of 132 clinical Enterobacteriaceae strains producing different carbapenemases. The sensitivity for the detection of KPC-, VIM-, NDM-, and OXA-48-encoding genes was 100%, whereas two IMP variants were missed.

Multidrug resistance in Enterobacteriaceae is an ever-increasing problem and might lead to dangerous limitations of treatment options. Of special importance is resistance to carbapenemases, which is caused mainly by carbapenemase production (17) or by porin loss combined with the expression of β-lactamases like an extended-spectrum beta-lactamase (ESBL) or AmpC (5). The most prevalent carbapenemases in Enterobacteriaceae are KPC, VIM, NDM, and OXA-48 (20).

Reliable detection of carbapenemases is necessary to implement contact precautions and for outbreak detection. However, carbapenemase detection in Enterobacteriaceae is challenging since carbapenemase-producing Klebsiella pneumoniae with low carbapenem MICs in the susceptible range according to CLSI or EUCAST have been described (4). On the other hand, a distinction between porin loss combined with an ESBL or by porin loss combined with the expression of metallo-β-lactamases is challenging (20).

Phenotypic tests like the modified Hodge test are useful for detecting carbapenemases but show a low sensitivity for NDM (6) and low specificity (1). For the detection of metallo-β-lactamases, phenotypic tests based on synergy with EDTA are available but can produce false-positive results with some strains and cannot differentiate between metallo-β-lactamase types (8). Class A carbapenemases like KPC can be detected by synergy with boronic acid, but false-positive synergy test results occur if AmpC β-lactamases are coproduced (16). Therefore, confirmation by molecular methods is necessary. Multiplex PCR assays for carbapenemase genes have been described (10, 15, 19) but require real-time PCR facilities or rely on amplicon detection by gel electrophoresis and might therefore not be convenient for all laboratories. In this report, we describe the use of a commercial multiplex PCR with amplicon detection by reverse hybridization.

A collection of 132 clinical Enterobacteriaceae strains from Germany was investigated. The strains were referred to the German reference laboratory for multidrug-resistant Gram-negative bacteria by 40 different laboratories because of elevated carbapenem MICs. The strain collection comprised Citrobacter freundii (n = 1), Citrobacter freundii (n = 4), Citrobacter freundii (n = 18), Enterobacter aerogenes (n = 10), Enterobacter asburiae (n = 1), Enterobacter cloacae (n = 24), Klebsiella oxytoca (n = 3), Klebsiella oxytoca (n = 65), Proteus mirabilis (n = 1), Providencia stuartii (n = 1), and Serratia marcescens (n = 4).

All of the strains were tested for the presence of carbapenemases by the modified Hodge test (3), as well as combined disk tests with boronic acid (12) for the detection of class A carbapenemases or with EDTA for the detection of metallo-β-lactamases (13). In addition, PCR, gel electrophoresis, and subsequent sequencing were performed as described before for blaKPC (22), blaOXA-48 (14), blaVIM (7, 13), and blaNDM (13). A PCR assay for blaNDM was conducted with primers NDM-1_a_fw (5'-CAATAT TATGCAACCCGTGCG-3') and NDM-1_a_rev (5'-CCTTGGCTG TCCTTGATCAGG-3'). PCR assays for rarely occurring carbapenemases like blagES (21) or blagIM (2) were performed if necessary. In order to exclude carbapenemase production, a bio-assay based on cell extracts was performed in a manner similar to that described before (9). Briefly, an imipenem disk was placed on Mueller-Hinton agar inoculated with a susceptible E. coli indicator strain. Bacterial lysates with and without potential inhibitors like EDTA, boronic acid, cloxacillin, and clavulanic acid were added to blank disks placed at edge of the expected imipenem inhibition zone. Invaginating growth into the inhibition zone indicates β-lactamase production. By PCR and subsequent sequencing, carbapenemase genes like blakPC-2 (n = 13), blakPC-3 (n = 11), blakPC-2 and blavIM-1 (n = 1), blavIM-1 (n = 17), blvIM-2 (n = 2), blavIM-4 (n = 3), blvIM-26 (n = 2), blvIMP-13 (n = 1), blvIMP-14 (n = 2), blvNDM-1 (n = 7), blvNDM-1 (n = 3), blavOXA-44 (n = 24), blavOXA-162 (n = 5), blavOXA-181 (n = 1), blavOXA-204 (n = 1), and blagES-5 (n = 1) were found (Table 1). All of the strains producing a carbapenemase, except one VIM-1-producing E. cloacae strain and one NDM-1-producing Providencia rettgeri strain, were positive by the modified Hodge test for imipenem. All KPC-producing strains except the one strain coproducing KPC-2 and VIM-1 displayed an increase of ≥4 mm for imipenem and meropenem in the combined disk test with boronic acid. All of the strains producing a metallo-β-lactamase showed an increase of ≥6 mm for meropenem in the combined disk test using EDTA, again except the one strain coproducing KPC-2 and VIM-1. Carbapenemase production could be excluded in 38 strains.

The multiplex PCR for blavIM, blavIMP, blavNDM, blavKPC, and blavOXA-48 with subsequent amplicon detection by reverse hybridization was performed with the hyplex SuperBug ID test system.

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TABLE 1 Results of the multiplex PCR

| Species | No. of isolates | No. with \textit{bla}\textsubscript{KPC} gene by: | % Agreement of \textit{bla}\textsubscript{KPC} results | No. with \textit{bla}\textsubscript{VIM} gene by: | % Agreement of \textit{bla}\textsubscript{VIM} results | No. with \textit{bla}\textsubscript{IMP} gene by: | % Agreement of \textit{bla}\textsubscript{IMP} results | No. with \textit{bla}\textsubscript{NDM} gene by: | % Agreement of \textit{bla}\textsubscript{NDM} results | No. with \textit{bla}\textsubscript{OXA-48} like gene by: | % Agreement of \textit{bla}\textsubscript{OXA-48} like results | Total no. of strains that agree/total no. of strains | % Agreement of all strains PCR hyplex |
|---------|----------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
| \textit{K. pneumoniae} | 65 | 23 | 23 | 100 | 8 | 8 | 100 | 0 | 0 | 100 | 2 | 2 | 100 | 20 | 20 | 100 | 65/65 | 100 |
| \textit{E. cloacae} | 24 | 0 | 0 | 100 | 7 | 7 | 100 | 0 | 0 | 100 | 92 | 92 | 100 | 1 | 1 | 100 | 22/24 | 92 |
| \textit{E. coli} | 18 | 2 | 2 | 100 | 2 | 2 | 100 | 0 | 0 | 100 | 2 | 2 | 100 | 1 | 1 | 100 | 4/4 | 100 |
| \textit{E. aerogenes} | 10 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0/10 | 100 |
| \textit{C. freundii} | 4 | 0 | 0 | 100 | 3 | 3 | 100 | 0 | 0 | 100 | 1 | 1 | 100 | 1 | 1 | 100 | 3/4 | 75 |
| \textit{S. marcescens} | 4 | 0 | 0 | 100 | 2 | 2 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 1 | 1 | 100 | 3/3 | 100 |
| \textit{K. oxytoca} | 3 | 0 | 0 | 100 | 2 | 2 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0/10 | 100 |
| \textit{C. freundii} | 1 | 0 | 0 | 100 | 1 | 1 | 100 | 0 | 0 | 100 | 1 | 1 | 100 | 0 | 0 | 100 | 1/1 | 100 |
| \textit{E. coli} | 1 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 1/1 | 100 |
| \textit{P. mirabilis} | 1 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 1/1 | 100 |
| \textit{P. rettgeri} | 1 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 1/1 | 100 |

| Total | 132 | 25 | 25 | 100 | 3 | 0 | 98 | 7 | 7 | 100 | 31 | 32 | 99 | 128/132 | 97 |

\( ^a \) Included among the tested isolates are 38 without any carbapenemase but with elevated carbapenem MICs (13 \textit{K. pneumoniae}, 11 \textit{E. cloacae}, 5 \textit{E. coli}, and 9 \textit{E. aerogenes} isolates) and isolates producing GES-5 (\( n = 1 \)) and GIM-1 (\( n = 3 \)).

\( ^b \) Includes isolates producing KPC-2 (\( n = 14 \)) and KPC-3 (\( n = 11 \)); one of the KPC-2-producing isolates coproduces VIM-1.

\( ^c \) Includes isolates producing VIM-1 (\( n = 18 \)), VIM-2 (\( n = 2 \)), VIM-4 (\( n = 3 \)), and VIM-26 (\( n = 2 \)); one of the VIM-1-producing isolates coproduces KPC-2.

\( ^d \) Includes isolates producing IMP-13 (\( n = 1 \)) and IMP-14 (\( n = 2 \)).

\( ^e \) All isolates produce NDM-1.

\( ^f \) Includes isolates producing OXA-48 (\( n = 24 \)), OXA-162 (\( n = 5 \)), OXA-181 (\( n = 1 \)), and OXA-204 (\( n = 1 \)).

\( ^g \) In one \textit{E. coli} strain with \textit{bla}\textsubscript{GES-5} but no other carbapenemase, a borderline result for \textit{bla}\textsubscript{OXA-48} was detected in the multiplex PCR, but this strain was negative when retested.

\( ^h \) Results were obtained with classical PCR and subsequent sequencing.

\( ^i \) Results were obtained with a commercial multiplex PCR (hyplex SuperBug ID test system).
Detection of Carbapenemases by Multiplex PCR

(AplexDiagnostics GmbH, Gars-Bahnhof, Germany) according to the manufacturer’s instructions. Briefly, bacterial DNA was prepared by suspending a single colony from MacConkey agar in lysis buffer, incubating it at 99°C, and then centrifuging it. The supernatant was used in a PCR. For hybridization, the amplification products were denatured at 95°C, added to precooled hybridization solution, and transferred to a microtiter plate containing wells coated with different specific probes (bla_VIM, bla_IMP, bla_XDR, bla_KPC, and bla_OXA-48). After incubation at 50°C, the wells were stringently washed three times. After a further washing step, a peroxidase conjugate was added to each well and the plate was incubated at room temperature and washed three times. Finally, a substrate solution was added, and after incubation at room temperature, the reaction was stopped. The test was considered positive if the extinction value at 450 nm was >0.4 and negative if it was <0.2; values in between were regarded as borderline. The test gave concordant results with all 87 strains harboring genes for KPC-2, KPC-3, VIM-1, VIM-2, VIM-4, VIM-26, NDM-1, OXA-48, OXA-162, OXA-181, and OXA-204. However, none of the three isolates producing IMP-13 or IMP-14 was detected. Out of 42 strains without any carbapenemase or with a carbapenemase gene not detectable by the multiplex PCR, 41 produced a negative result. In one E. coli strain with bla_CES-3, a borderline result for bla_OXA-48 was obtained and this strain was negative in the multiplex PCR when retested. In our study, the overall agreement between conventional PCR and the commercial multiplex PCR was 97% and the sensitivity of the multiplex PCR was 100% for bla_KPC, bla_VIM, bla_XDR, and bla_OXA-48 alike, but 0% for the bla_IMP variants tested. The specificity was 99% for bla_OXA-48 alike and 100% for the other carbapenemase genes.

The carbapenemase genes tested by this commercial system comprise the majority of the carbapenemases found in Enterobacteriaceae in different parts of the world (20). Thus, this commercial system offers convenient and accurate molecular detection of carbapenemase genes for microbiological laboratories. With an overall sensitivity of 96.7%, the results are in line with those of another commercially available molecular assay for which sensitivities of 97.6% (11) and 97% (18) have been reported. All three bla_IMP-harboring strains were missed by the commercial multiplex PCR, indicating that not all variants of the diverse IMP family can be reliably detected. In addition, it has to be emphasized that a general limitation of any molecular assay for carbapenemase detection is that only known carbapenemase genes can be detected and new variants of known carbapenemases might be missed. In certain geographic regions, carbapenemase genes not covered by the assay used might be present. Therefore, phenotypic tests like the modified Hodge test still play a role in carbapenemase detection and can additionally be used to identify strains that need molecular testing in order to reduce costs. The manufacturer claims that the test can also be used for direct detection in clinical specimens, but in this study, only carbapenemase gene detection in colonies was investigated.

In conclusion, the commercial multiplex PCR investigated in this study shows excellent performance and might supplement phenotypic tests in carbapenemase detection.

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L. Wassill is the CEO of AplexDiagnostics GmbH, Gars-Bahnhof, Germany. The rest of us have no conflict of interest.

REFERENCES


