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ORIGINAL ARTICLE

Rapid detection of carbapenemases directly from positive blood cultures by the β-CARBA test

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Abstract

The rapid detection of blood stream infections (BSI) by carbapenemase-producing Enterobacterales (CPE) is indispensable to early optimize antibiotic treatment and to improve survival. While phenotypic tests are time-consuming and PCR is expensive and not available in many routine laboratories, colorimetric tests (e.g., Carba NP test) can provide rapid results at moderate cost. However, up to now, the detection of CPE-BSI requires a further 3-h incubation in broth supplemented with zinc sulfate and imipenem after a blood culture has become positive, thereby causing delay and additional hands-on time. The purpose of this study was to develop and evaluate a new method for the detection of CPE directly from positive blood culture without the need for incubation in broth, based on the commercially available colorimetric β-CARBA test. For the evaluation, blood cultures spiked with 140 different Enterobacterales isolates producing diverse beta-lactamases were tested with the new method. Of these, 70 were CPE (OXA-48-like, NDM, KPC, VIM, and GIM). After blood cultures turned positive, blood culture fluid was drawn, and erythrocytes were hemolyzed with SDS, washed, and equilibrated before the β-CARBA was performed on the bacterial pellet. All carbapenemases were reliably detected, including weak carbapenemases of the OXA-48 group. The sensitivity was 100% (95% CI 94.9–100) and the specificity 94.3% (95% CI 89.2–99.4). The time to result was 20 to 45 min. Carbapenemases can rapidly and reliably be detected directly from blood cultures using the new method, which could help to improve the outcome of these difficult-to-treat infections.

Keywords Carbapenemase · Carba NP · Blood stream infection · NDM · OXA-48

Introduction

Carbapenemase-producing Enterobacterales (CPE) are increasingly reported from invasive infections and are associated with higher mortality and increased cost and length of hospital stay [1]. The rapid detection of CPE from severe infections, especially blood stream infections (BSI), is of paramount importance, since the fast initiation of appropriate antibiotic therapy may help to improve patient management and clinical outcome. Currently, by standard methods, 16–20 h are required to detect carbapenem resistance after a blood culture has become positive, e.g., by disk diffusion or other susceptibility tests. If resistance to a carbapenem is observed, further confirmatory tests have to be performed for the detection of a carbapenemase, which take additional time. Several methods have been described to shorten the time for the detection of CPE from blood cultures, including nucleic acid-based techniques, magnetic resonance, MALDI-TOF analysis, immunochromatographic tests (ICT), the new rapid disk diffusion testing proposed by EUCAST, and others [2–5]. Most of these assays require further equipment, significant hands-on time or an additional incubation on agar or in broth. Additionally, several of these tests (e.g., PCR, ICT) only recognize a subset of carbapenemases.

Colorimetric assays (e.g., Carba NP test and commercial assays) indicate carbapenemase activity by the inactivation of a carbapenem (typically imipenem), resulting in a pH shift which subsequently leads to a color change of an indicator (e.g., phenol red) [6]. The advantage is the recognition of a broader spectrum of enzymes than with most available PCRs or ICTs. Colorimetric assays have

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been validated for the use from solid media, e.g., Mueller-Hinton agar. Variations have been reported for the use directly from blood cultures; however, they require an additional culture on solid or liquid media (2–4 h), followed by lysis and incubation in the presence of a carbapenem and the pH indicator, thereby leading to a long hands-on time and time to result [2].

The β-CARBA test is a new commercially available colorimetric assay, which is rapid and easy to use and has shown good performance in the detection of carbapenemases in Enterobacteriaceae grown on solid media [7]. In this study, we developed and evaluated a novel method for the rapid detection of carbapenemase activity directly from positive blood culture bottles using this assay.

Materials and methods

Bacterial isolates

A total of 140 Enterobacteriaceae isolates from the clinical microbiology laboratory of two German University hospitals (Frankfurt, Cologne) and from previous studies were included [8–13]. Of these, 70 were carbapenemase producers: OXA-48 ($n = 19$), NDM ($n = 15$), KPC ($n = 11$), VIM ($n = 14$), GIM ($n = 3$), OXA-48-like ($n = 6$), and OXA-48-like + NDM ($n = 2$) (Table 1). Additionally, 70 non-carbapenemase-producing isolates served as negative controls; of these, 68 were clinical isolates with other beta-lactamases (e.g., extended spectrum β-lactamase (ESBL), AmpC) and two were quality control strains without beta-lactamase activity (*E. coli* ATCC 25922, *E. coli* J53).

All isolates were characterized for the presence of $bla_{\text{OXA-48-like}}$, bla_{VIM}, bla_{NDM}, bla_{GIM}, and bla_{KPC} and of ESBL genes by PCR and subsequent Sanger sequencing as previously reported [8–11]. Additionally, all isolates negative for carbapenemases by PCR were tested for carbapenemase activity using the Carba NP test [6].

Inoculation and processing of blood culture bottles

A bacterial suspension equivalent to 0.5 McFarland was diluted 1:1000. Subsequently, 10 μL of this solution was mixed with 5 mL of human blood from healthy volunteers for a final inoculum of ~ 300 cfu/mL of blood and inoculated into a BD Bactec Plus Aerobic blood culture bottle (Becton Dickinson, Heidelberg, Germany). The bottles were then incubated in a Bactec FX automate (Becton Dickinson) until they flagged positive. All positive blood culture bottles were controlled for purity by inoculation of 100 μL blood culture fluid onto sheep blood agar. From the blood cultures, 2 mL of fluid was taken to perform the tests.

β-CARBA test

For the β-CARBA test, 2 mL of blood culture fluid was mixed with 200 μL of 10% SDS (AppliChem, Darmstadt, Germany) and incubated at room temperature for 5 min in order to lyse the erythrocytes. The sample was centrifuged for 1 min at 13,000×*g* in a tabletop centrifuge and the supernatant discarded. The bacterial pellet was washed first with 1 mL distilled water, followed by 1 mL phosphate buffered saline (PBS, pH 7.4) to remove remaining blood cells and balance the pH value. Subsequently, the bacterial pellet was subjected to the β-CARBA test (Bio-Rad Laboratories, Munich, Germany) as recommended by the manufacturer for bacteria grown on solid media. Briefly, the pellet was mixed with 40 μL of R1 reagent and 40 μL of R2. Following incubation for 30 min at 37 °C, the test results were read. Any color change in the first 30 min, from yellow to orange, red, or purple, was interpreted as positive.

Results

In the first part of the evaluation, a protocol for the analysis of blood cultures with the β-CARBA test was developed. To achieve a high inoculum, 2 mL of the blood culture was used and bacteria were concentrated, followed by a subsequent hemolysis of erythrocytes. We tested several hemolysing agents, some of which compromised readability or gave rise to false-positive results (e.g., saponin, data not shown). With 10% SDS, complete hemolysis was reached with no interference with the β-CARBA test. A further washing with water and PBS was done to equilibrate the bacterial suspension and to facilitate reading of a color change. Using this method, the β-CARBA test showed good results and carbapenemase-producing isolates could be easily detected by the color change from yellow to red, purple, or orange (Fig. 1). All carbapenemases were detected, resulting in a sensitivity of 100% (Table 2). There were no differences between species and carbapenemases, also, weakly hydrolyzing enzymes could be easily interpreted, including all OXA-48-like producing isolates ($n = 25$). Isolates producing more than one carbapenemase were equally well detected. The overall specificity of the test was 94.3%, and 4/70 carbapenemase-negative isolates gave rise to false-positive results. The test from blood cultures was easy to perform and results were available after 20–45 min, with 15 min hands-on-time for the preparation of the blood culture and maximum 30 min incubation time until a color change could be observed. However, in 31 out of 70 isolates, a color change could be noted within the first 5 min.

Table 1 Isolates included in the study

Carbapenemase production	No. of isolates						
	K. pneumoniae	*K. oxytoca*	*E. coli*	*E. cloacae*	*C. freundii*	Others	All species
Carbapenemase positive	25	1	18	7	14	5	70
OXA-48-like	9		12	2	2		25
OXA-48	6		9	2	2		19
OXA-162	1						1
OXA-181			2				2
OXA-204	1						1
OXA-232			1				1
OXA-244	1						1
KPC	9				1	1	11
KPC-2	8				1	1	10
KPC-3	1						1
NDM	5		4	2		4	15
NDM-1	5		3	2		4	14
NDM-7			1				1
VIM	1		1	1	11		14
VIM-1	1		1	1	9		12
VIM-2					2		2
GIM		1		2			3
GIM-1		1		2			3
Double carbapenemase producers							2
NDM-1/OXA-232	1						1
NDM-5/OXA-181			1				1
Carbapenemase negative	15	3	50	1	1		70
Total	40	4	68	8	15	5	140

Others: *Citrobacter brakii* ($n = 1$), *Proteus mirabilis* ($n = 1$), *Providencia stuartii* ($n = 1$), *Raoultella ornitholytica* ($n = 1$), *Serratia marcescens* ($n = 1$)

Discussion

This study demonstrates that with the newly developed method, the most common carbapenemases can reliably be detected directly from positive blood cultures within 20–45 min.

However, both the inoculum and hemolysis procedure were critical to obtain a sensitivity of 100%. Hemolysis was necessary because erythrocytes affected the color of the test if they had not been hemolyzed. Additionally, after hemolysis, a higher inoculum of 2 ml blood culture fluid could be used, which was important for isolates with weak carbapenemase expression, e.g., OXA-48-like. With a lower inoculum (200 μl or 500 μl), not all carbapenemases were detected (data not shown). Using the newly developed protocol, all carbapenemases could be reliably detected. Our results are in line with a previous study on the β-CARBA test from solid media, reporting a better sensitivity when a higher inoculum was used [14]. Compared to previous evaluations [7, 14, 15], in the present study, more isolates with different carbapenemases were included and the β-CARBA test was evaluated for its use directly from positive blood cultures. The sensitivity was 100%, which was similar [7] or higher than previously reported from solid media [14–16]. The specificity reached 94.3%, as four isolates gave rise to false-positive results (*K. oxytoca* ($n = 3$) and *E. coli* ($n = 1$)). Of these, three were ESBL producers (CTX-M-1, CTX-M-3, and CTX-M-9), and one *K. oxytoca* had no ESBL, but showed hyperproduction of the chromosomal K1 β-lactamase. When grown on sheep blood agar, the β-CARBA result was negative in 4/4 isolates, indicating either interference with the hemolysis procedure or culture conditions in the blood culture bottle. False-positive results with the β-CARBA test have been also reported before with isolates grown on solid agars, e.g., with *K. oxytoca* and hyperproduction of K1 β-lactamase or with *E. cloacae* and AmpC hyperproduction [19].

Other colorimetric tests have been evaluated for the use from blood cultures, e.g., the Carba NP test [2]. While this test yielded comparable results (sensitivity 97.9%, specificity 100%), it is not well suited for a routine laboratory since it requires preparation of different reagents before the test can be done. Additionally, 300 μl blood culture fluid has to be incubated with imipenem and zinc sulfate in liquid broth for

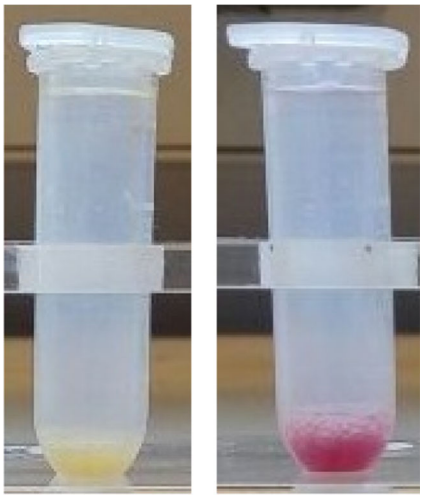

Fig. 1 Results of the β-CARBA test from blood cultures: negative (left) and positive (right)

3 h, before the Carba NP test can be performed, resulting in longer hands-on time and time to result (3 to 5 h). Recently, two other colorimetric tests were assessed for the detection of CPE from blood culture: the CNPt-direct and the Blue Carba test, both modifications of the Carba NP test [17]. In this study, sensitivity from spiked blood cultures was 87% (53/61); most false-negative results were caused by OXA-48-producing isolates (sensitivity 64% (7/11)). Compared to the two studies mentioned above, our protocol shows a comparable performance with a higher sensitivity especially for OXA-48 carbapenemases and a significantly shorter time to result (20–45 min vs. 3–5 h) and hands-on time.

Other methods have been reported for the detection of carbapenemases directly from positive blood cultures, e.g., MALDI-TOF hydrolysis assays or molecular methods, e.g., the Verigene assay [3, 4]. Each approach has specific strengths and weaknesses regarding performance, associated costs, and time to result. Compared to the MALDI-TOF hydrolysis assay, the advantage of the β-CARBA test is the easy test format and the shorter time to result. Additionally, a broth culture and further incubation are necessary after a blood culture has become positive, requiring an additional 3 to 5 h compared to 20–45 min with the β-CARBA test. With the Verigene assay, the time to result is longer (~ 2 h) and the assay is considerably more expensive than the β-CARBA test. Furthermore, for both the MALDI-TOF hydrolysis assay and the Verigene assay, additional equipment and software is necessary. The detection of carbapenemases from blood cultures using an immunochromatographic assay has recently been reported [5]. Sensitivity and specificity of 100% and a short time to result (20–45 min) have been demonstrated with this method, but currently only KPC, NDM, and OXA-48-like can be detected. In contrast, with the β-CARBA test, other carbapenemases can be detected that are not included in the panels of the molecular or immunochromatographic assays.

Table 2 Performance of the β-CARBA test from blood cultures

	β-CARBA test	
	Positive	Negative
Carbapenemase		
Ambler class D ($n = 25$)		
OXA-48 ($n = 19$)	19	0
OXA-162 ($n = 1$)	1	0
OXA-181 ($n = 2$)	2	0
OXA-204 ($n = 1$)	1	0
OXA-232 ($n = 1$)	1	0
OXA-244 ($n = 1$)	1	0
Total class D (%)	25 (100)	0 (0)
Ambler class A ($n = 11$)		
KPC-2 ($n = 10$)	10	0
KPC-3 ($n = 1$)	1	0
Total class A (%)	11 (100)	0 (0)
Ambler class B ($n = 32$)		
NDM-1 ($n = 14$)	14	0
NDM-7 ($n = 1$)	1	0
VIM-1 ($n = 12$)	12	0
VIM-2 ($n = 2$)	2	0
GIM-1 ($n = 3$)	3	0
Total class B (%)	32 (100)	0 (0)
OXA-48-like + NDM ($n = 2$)		
OXA-232 + NDM-1 ($n = 1$)	1	0
OXA-181 + NDM-5 ($n = 1$)	1	0
Total (NDM + OXA-48-like) (%)	2 (100)	0 (0)
Total (%)	70 (100)	0 (0)
No carbapenemases ($n = 70$)		
Total (%)	4 (5.7)	66 (94.3)
Sensitivity, 95% CI	100%, 94.9–100	
Specificity, 95% CI	94.3%, 89.2–99.4	

CI confidence interval

In this proof of principle study, a new protocol for the detection of CPE from blood cultures using the β-CARBA test was developed and systematically assessed, resulting in the detection of the most important carbapenemases with 100% sensitivity and 94.3% specificity. The study included a large number of molecularly characterized isolates with the most prevalent carbapenemases in Germany, OXA-48-like, KPC, NDM, VIM, and GIM making up 98.3% of all CPE sent to the National Reference laboratory for nosocomial pathogens [18]. However, the study has some limitations. Since the prevalence of BSI with CPE at our institution is too low for a prospective evaluation, we had to use spiked blood cultures to include a sufficient number of isolates with different carbapenemase variants. Additionally, only one isolate of *E. cloacae* and

C. freundii was included, which overexpressed AmpC β-lactamase. It has been shown that these strains may produce false-positive results in the β-CARBA or other colorimetric tests [19]. Furthermore, aerobic blood culture bottles from one manufacturer were evaluated (Becton Dickinson). Results might not be identical when other blood culture media are used. Therefore, our results should be verified in a routine microbiology laboratory setting with a sufficient number of patients with BSI caused by CPE. Additionally, the β-CARBA test has been shown to be suboptimal for the detection of IMI- or GES-type carbapenemases [16]. These carbapenemases are rare in Germany, but in areas where these carbapenemases are more prevalent, the low sensitivity of the β-CARBA test for these variants has to be considered.

Conclusions

The present study demonstrates that with the new method, OXA-48-like, KPC, NDM, GIM, and VIM carbapenemases from CPE can be reliably detected directly from positive blood culture bottles. With only 20–45 min time to result, the new method is more rapid than other currently available assays and can be performed in any routine microbiology laboratory, as no additional equipment is required. Especially in regions with a high CPE prevalence and/or in patients known to be colonized, our protocol may help to rapidly identify patients with CPE bloodstream infections and early optimize the management of patients with these difficult-to-treat infections. Further studies are necessary to evaluate the performance of the assay in clinical routine.

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Compliance with ethical standards

Conflict of interest AH reports grants from German Center for Infection Research and personal fees from Becton Dickinson, Bruker Daltonics, MSD/Merck, Astellas, Gilead, and Oxoid, outside the submitted work.

MM has no conflict of interest to report.

Ethical approval All procedures performed in this study were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent The study was approved by the ethics committee of the University Hospital Cologne (approval number 08-160) and written informed consent was obtained from all healthy volunteers who donated blood for this study.

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