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Evaluation of the automated BD Phoenix CPO Detect panel in combination with the β-CARBA assay for detection and classification of carbapenemase-producing Enterobacterales

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ABSTRACT

Recently, the CPO Detect panel for the detection of carbapenemase-producing, Gram-negative bacteria was introduced for the Phoenix semi-automated antimicrobial susceptibility testing system. The CPO Detect assay aims to detect carbapenemase activity (P/N test) and to type carbapenemase producers according to the Ambler classification (Ambler test). The P/N test-based detection of carbapenemase producers was 100% sensitive and 55.3% specific in the assessment of 57 carbapenemase-producing and 38 non-carbapenemase-producing Enterobacterales. False-positive test isolates in the P/N test arose from carbapenemase-non-producing, but carbapenem-non-susceptible isolates. In contrast, using the Ambler test-based approach for carbapenemase detection resulted in a specificity of 100% and a sensitivity of 79%. In order to improve the overall performance, we established an algorithm that additionally included the colorimetric β-CARBA assay as downstream test for P/N test-positive isolates, which remained un-typed in the Ambler test. This algorithm displayed an overall sensitivity and specificity of 98.3% and 100%, respectively. Our data demonstrate that the combination of the CPO Detect assay with the β-CARBA test allows for rapid detection and classification of carbapenemase-producing Enterobacterales.

1. Introduction

Currently, carbapenemase-producing Gram-negative bacteria are spreading worldwide and constitute a major challenge for global health (Bonomo et al., 2018). The reliable detection and further characterization of carbapenemase types is a prerequisite for the surveillance. On that basis it is possible to develop containment strategies for carbapenemase-producing Gram-negative bacteria and to optimize diagnostic methods. Furthermore, determining the Ambler class of β-lactamases (Ambler, 1980) provides useful information for the treatment with novel β-lactam/β-lactamase inhibitor combinations. For example, ceftazidime/avibactam is effective against KPC-type carbapenemase producers (class A); however, avibactam cannot inhibit the activity of metallo-β-lactamases (class B) (Wright et al., 2017). Therefore, there is a great interest in the development of reliable methods for fast detection and characterization of carbapenemase-producing Gram-negative bacteria.

Automated test formats offer the advantage of highly standardized test execution and operator-independent interpretation. Thereby, these tests increase reproducibility and may provide time savings. Recently, Becton Dickinson introduced the first automated carbapenemase detection test (BD Phoenix CPO Detect assay) as part of antimicrobial susceptibility testing (AST) panels. The CPO Detect assay aims to detect carbapenemase activity (P/N test) and to classify carbapenemase producers according to the Ambler classification (Ambler test). Thomson et al. (2017) published a study examining the CPO Detect assay with Enterobacterales, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Although the study revealed a high overall sensitivity to detect carbapenemase activity with the P/N test (97.1%) the specificity was only moderate (68.6%).

Here, the performance of the CPO Detect assay was investigated with a collection of 57 carbapenemase-producing and 38 non-

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carbapenemase-producing Enterobacterales. Moreover, a diagnostic algorithm including the automated carbapenemase test and the colorimetric β-CARBA assay was established in order to optimize the overall performance.

2. Material and methods

2.1. Bacterial strains

The strain collection for this study included 57 carbapenemase-producing and 38 non-carbapenemase-producing Enterobacterales. Most isolates were obtained from routine clinical samples processed at the Institute of Clinical Microbiology and Hygiene, Regensburg. 8 isolates were kindly provided by the Robert Koch Institute, Wernigerode, and 13 isolates by the German National Reference Laboratory, Ruhr-University Bochum. One isolate was the type strain *Escherichia coli* ATCC 35218. Carbapenemase-producing test strains were identified by demonstrating the presence of a carbapenemase gene as described previously (Simon et al., 2018). The carbapenem susceptibility of the isolates was determined for ertapenem, imipenem and meropenem using the BD Phoenix NMIC-502 AST panel. All isolates were stored at −80 °C. In order to obtain fresh, overnight cultures for further testing the isolates were sub-cultured at a temperature of 35 ± 1 °C in ambient air on Columbia agar plates +5% sheep blood (Oxoid, Cambridge, UK) and McConkey agar plates (Merck, Darmstadt, Germany).

2.2. BD Phoenix CPO detect panel

The BD Phoenix NMIC-502 AST panel (Becton Dickinson, Heidelberg, Germany) includes 27 antimicrobial agents and nine test wells containing a β-lactam agent with or without various β-lactamase inhibitors for the detection of carbapenemases. The assay is suitable for Enterobacterales, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* (Thomson et al., 2017). As the carbapenemase test is incorporated into the AST test panel, every isolate that will undergo AST, will be evaluated regarding its carbapenemase content. Overnight grown bacterial colonies were taken from Columbia agar plates +5% sheep blood or McConkey agar plates, inoculated into the NMIC-502 panels using the Phoenix AP instrument and measured with the Phoenix M50 semi-automated AST system according to the manufacturer's recommendation (Becton Dickinson, Heidelberg, Germany). The interpretation of the measured values is algorithm-based and was performed by the EpiCenter software, version V7.00A/ V6.35A (Becton Dickinson, Heidelberg, Germany).

The EpiCenter delivers results on (1) carbapenemase activity as positive or negative (which is further referred to as P/N test) and provides (2) the carbapenemase type according to the Ambler classes A, B and D (which is further referred to as Ambler test). The isolates determined positive by the P/N test may or may not be assigned to an Ambler class by the Ambler test and hence some carbapenemase producers may remain un-typed (Thomson et al., 2017). P/N test-negative results with Ambler classification are not possible.

All results deviating from the reference result were repeated at least once. If the second test run led to a conflicting result compared to the first test run, the test was repeated additionally for two or three times. The final result corresponds to the result of the majority of all independent test runs and is included in Table 1.

2.3. β-CARBA test

The assay was performed according to the manufacturer's recommendations (Bio-Rad, Marnes-la-Coquette, France) except for the incubation period, which was extended from 30 to 60 min (Compain et al., 2016; Simon et al., 2018). Briefly, overnight grown colonies of the test strains were picked from Columbia agar plates +5% sheep blood. One 1 µl loop full of bacterial mass was thoroughly homogenized in 80 µl of reaction mixture by rigorous rotation of the loop in the liquid. After incubation at 35 ± 1 °C for 60 min, the colour change was estimated as positive or negative. In the presence of carbapenemases a chromogenic substrate is hydrolyzed, which leads to a colour change from yellow to orange, red or violet. Determination of Ambler classes is not possible.

2.4. Carbapenemase inactivation method

Where indicated, carbapenemase-production was re-assessed using the carbapenemase inactivation method (CIM) (van der Zwaluw et al., 2015). Briefly, a 10 µl loop was filled with overnight grown bacterial colonies from Columbia agar plates +5% sheep blood. The bacterial mass was homogenized in 400 µl water by rigorous rotation of the loop in the liquid. A 10 µg meropenem disk (Becton Dickinson, Heidelberg, Germany) was added into the suspension and incubated for 2 h at 35 ± 1 °C. During this incubation, carbapenemase-producing test strains are able to hydrolyze and inactivate the carbapenem. For the next step, the meropenem-susceptible quality control strain *Escherichia coli* ATCC 25922 was suspended in saline to a density of a McFarland 0.5 turbidity standard using the Densimat photometer (bioMérieux, Marcy-l'Étoile, France). The suspension was streaked in three directions with a sterile cotton swab on a Mueller Hinton 2 agar plate (bioMérieux, Marcy-l'Étoile, France) and the meropenem disk was placed onto the inoculated agar plate. After overnight incubation at 35 ± 1 °C, the agar plate was evaluated regarding the formation of an inhibition zone. The development of an inhibition zone indicates a non-carbapenemase-producing test strain and no inhibition zone indicates a carbapenemase-producing test strain.

2.5. Quality control

Quality controls were performed with *Klebsiella pneumoniae* ATCC 700603 harboring SHV-18 extended spectrum β-lactamase as negative control and a laboratory *Escherichia coli* isolate with a genetically determined OXA-48-like carbapenemase as positive control (Dortet et al., 2016).

2.6. Statistical analysis

Sensitivity (true positives/ [true positives + false negatives]) and specificity (true negatives/ [true negatives + false positives]) are stated as percentages. 95% confidence intervals for sensitivities and specificities were calculated using the MedCal statistical software (MedCalc, 2018). The presence of differences between the three matched sets were assessed using Cochran's Q test (MedCalc, 2018). If Cochran's Q test is positive (P-values < .05), a post-hoc test according to Sheskin (2004) was used for pairwise comparison of variables.

3. Results

3.1. Evaluation of the P/N test for detection of carbapenemase activity and the Ambler test for typing of carbapenemases

Our investigation aimed to evaluate, if the CPO Detect assay allowed for (1) detection of carbapenemase activity and (2) the Ambler classification of carbapenemases. First, the ability of the CPO Detect assay to identify carbapenemase activity with the P/N test was examined. The P/N test identified all 57 carbapenemase-producing isolates. However, 17 of 38 non-carbapenemase-producing isolates were falsely judged to be carbapenemase producers. Thus, the P/N test yielded an overall sensitivity of 100% (95% confidence interval [95% CI], 93.7% to 100%) and specificity of 55.3% (95% CI, 38.3% to 71.4%).

Of note, 17 of 20 non-carbapenemase-producing isolates with carbapenem non-susceptibility were falsely classified as carbapenemase-

positive. This resulted in a specificity of 15% (95% CI, 3.2% to 37.9%) for non-carbapenemase-producing test strains with carbapenem MIC values in the intermediate or resistant range (Table 1B).

In contrast, 14 carbapenem-susceptible non-carbapenemase-producing test strains were correctly categorized as negative for carbapenemase production; they included 10 test strains with phenotypic resistance to oxyimino-cephalosporins, one wild type *Klebsiella oxytoca* isolate, one TEM-1 β-lactamase-positive *Escherichia coli* isolate (ATCC 35218) and two isolates (one *Enterobacter cloacae* complex, one *Citrobacter freundii* complex isolate) harboring wild type chromosomal AmpC β-lactamases. Four carbapenem-susceptible test strains with meropenem MICs above the EUCAST screening breakpoint of 0.125 mg/l were properly determined as negative for carbapenemase production. Taken together, the specificity for 18 carbapenem-susceptible isolates was 100% (95% CI, 81.5% to 100%).

Second, the ability of the CPO Detect assay to correctly classify carbapenemase producers to their respective Ambler class was examined. The Ambler test, aims to attribute the Ambler class to the P/N test-positive isolates. Five of 13 test strains with Ambler class A carbapenemases were determined properly. 19 of 21 tested Ambler class B-positive isolates and 21 of 22 Ambler class D carbapenemases harboring isolates were specified correctly. Three isolates provided conflicting results within multiple test runs; however, the majority of the results corresponded with the reference value, therefore, the results were regarded as correctly identified. Of note, all false-positive test results of the P/N test remained un-typed in the Ambler test. Therefore, the P/N test compromises the overall specificity of the CPO Detect assay since the P/N test category ultimately defines the overall performance of the CPO Detect assay for detection of carbapenemase-producing organisms (Thomson et al., 2017).

3.2. Evaluation of the Ambler test for detection of carbapenemase activity

Alternatively, carbapenemase production could be assessed by using the Ambler test. For that purpose the attribution of the organisms either to the category of carbapenemase producing organisms or to the group of non-carbapenemase producing organisms was based on their Ambler class typing results. All isolates were regarded as carbapenemase producers if the Ambler test attributed any Ambler class to the tested organism (i. e. if an organism was typed with Ambler class A, B or D). In contrast, all isolates, including P/N test-positive isolates, were categorized as non-carbapenemase-producing organisms if no information on the Ambler class was provided by the Ambler test (i.e. if the respective isolate remained un-typed by the Ambler test). Following these Ambler test-based criteria for carbapenemase detection, the test panel correctly detected 45 of 57 carbapenemase-positive isolates. This approach yielded an overall sensitivity of 79% (95% CI, 66.1% to 88.6%) (Table 1A). There were no false-positive results (specificity: 100% [95% CI, 90.8% to 100%]) (Table 1B).

Taken together, these findings demonstrate that the identification of carbapenemase activity based on the Ambler test allows for detection of carbapenemases in Enterobacterales with high specificity of 100%, but with moderate sensitivity of 79%. In contrast, identification of carbapenemase activity based on the P/N test results in a sensitivity of 100%, but comes with a specificity of 55.3%. However, for diagnostic purposes it would be desirable to combine the sensitivity provided by the P/N test with the specificity provided by the Ambler test-based carbapenemase detection.

3.3. Evaluation of a new algorithm including the CPO Detect assay and the β-CARBA test for carbapenemase detection

In contrast to the Ambler test-based carbapenemase detection, the P/N test produced false-positive results. Therefore, further testing of P/N test-positive isolates, which were un-typed in the Ambler test, might increase the overall specificity. Based on this reasoning, a new algorithm for carbapenemase detection was evaluated that included the CPO Detect assay (i.e. the P/N test and the Ambler test) and the colorimetric β-CARBA test as a downstream test (Fig. 1). According to this algorithm, all isolates with negative results in the P/N test were considered as carbapenemase-negative without further testing due to the high sensitivity of the P/N test (Table 1A). Due to the high specificity of the Ambler test-based carbapenemase detection (Table 1B) every isolate was regarded as a carbapenemase producer, if the Ambler test revealed the presence of a carbapenemase. All P/N test-positive but Ambler class un-typed isolates were subjected to the β-CARBA test. If the β-CARBA test confirmed the carbapenemase activity, the isolates were considered as carbapenemase producers. If the carbapenemase activity was not confirmed by the β-CARBA test, the isolates were estimated as carbapenemase-negative. 30.5% of all tested Enterobacterales in our strain collection required examination with the β-CARBA test in addition to the CPO Detect assay (Fig. 1).

The combination of CPO Detect assay and β-CARBA test yielded a single isolate with a false final result. It was a false-negative GES-type carbapenemase harboring *Enterobacter cloacae* complex isolate, which was detected in the P/N test without Ambler classification, but remained non-reactive in the β-CARBA assay. The carbapenemase activity of this isolate was confirmed with the CIM. The new algorithm resulted in a sensitivity of 98.3% (95% CI, 90.6% to 100%) and a specificity of 100% (95% CI, 90.8% to 100%) (Table 1A, B).

3.4. Comparison of P/N test, Ambler test and the novel algorithm for carbapenemase detection

Cochranes's Q test and post hoc testing revealed that the sensitivity of the new algorithm-based approach (98.3%) exceeded significantly the moderate sensitivity of the Ambler test (79%) while there was no difference regarding specificity (both 100%). Moreover, the novel

Table 1A
Sensitivities of the P/N test, the Ambler test and the combination of the CPO Detect assay with the β-CARBA test for the detection of carbapenemases. Results are given as number of positive test results/ number of tested isolates.

		Class A			Class B				Class D	Class A + B	All CPE	Sensitivity % (95% CI)
		KPC	GES	IMI	NDM	VIM	GIM	IMP	OXA-48-like	KPC + VIM		
CPE[a] (n = 57)	P/N test	6/6	6/6	1/1	14/14	5/5	1/1	1/1	22/22	1/1[c]	57/57	100 (93.7-100)
	Ambler test[b]	2/6	2/6	1/1	12/14	5/5	1/1	1/1	21/22	0/1[c]	45/57	79 (66.1-88.6)
	CPO Detect assay with β-CARBA test	6/6	5/6	1/1	14/14	5/5	1/1	1/1	22/22	1/1[c]	56/57	98.3 (90.6–100)

[a] CPE: carbapenemase-producing Enterobacterales.
[b] Positive test result: detection of any Ambler class; negative test result: detection of no Ambler class.
[c] No Ambler class detected.

Table 1B
Specificities of the P/N test, the Ambler test and the combination of the CPO Detect assay with the β-CARBA test for the detection of carbapenemases. Results are given as number of positive test results/ number of tested isolates.

		Phenotypic carbapenem susceptibility[c]			All non-CPE	Specificity % (95% CI)
		Non-susceptible	Susceptible and MIC_{MEM} > 0.125 mg/l[d]	Susceptible		
Non-CPE[a] (*n* = 38)	P/N test	17/20	0/4	0/14	17/38	55.3 (38.3–71.4)
	Ambler test[b]	0/20	0/4	0/14	0/38	100 (90.8–100)
	CPO Detect assay with β-CARBA test	0/20	0/4	0/14	0/38	100 (90.8–100)

[a] Non-CPE: carbapenemase non-producing Enterobacterales.
[b] Positive test result: detection of any Ambler class; negative test result: detection of no Ambler class.
[c] Antimicrobial susceptibility testing of ertapenem, meropenem and imipenem was performed using the CPO Detect panel.
[d] Meropenem MICs > 0.125 mg/l and ≤ 2 mg/l are used as screening values, which may be indicative of carbapenemase production.

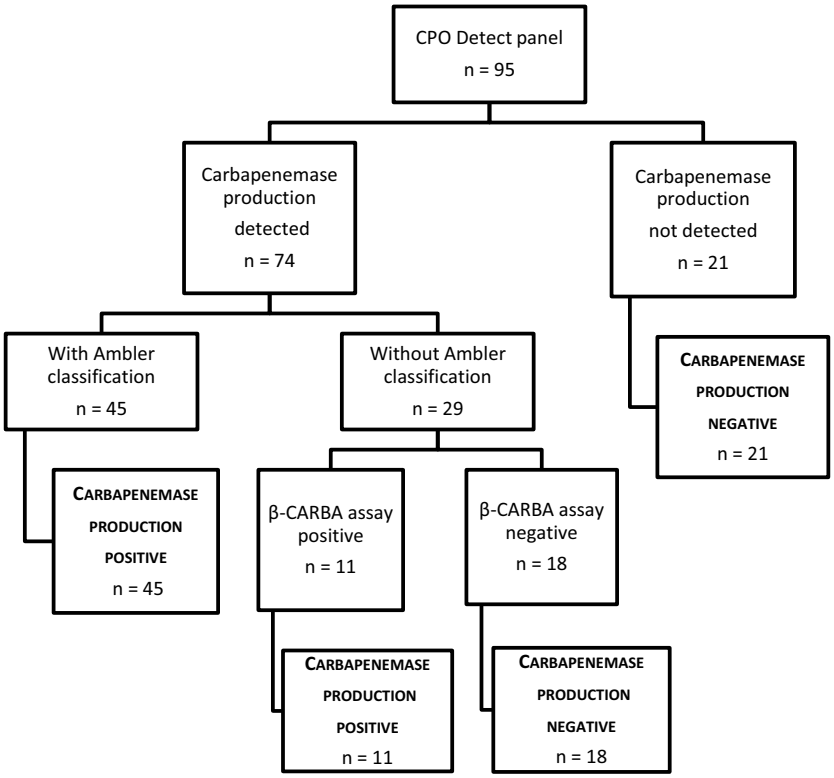

Fig. 1. Exemplary diagnostic algorithm using the CPO Detect assay in conjunction with the β-CARBA test. Final results are given in bold, small capitals.

algorithm-based approach resulted in a significant increase in specificity compared to the P/N test (100% vs. 55.3%) while the sensitivity remained unaltered (98.3% vs. 100%). Overall, these data demonstrate that the new algorithm-based approach significantly improves the test performance.

4. Discussion

The BD Phoenix CPO Detect panel is an automated platform that offers several advantages in comparison with manual test formats for the detection of carbapenemase producers: It reduces hands-on-time, is operator-independent and provides additional information about the carbapenem resistance mechanism at the same time point as the results of the initial phenotypic AST are available (Thomson et al., 2017).

Our findings comply partially with those of Thomson and co-workers (Thomson et al., 2017) and with a recent study published by Ong and co-workers (Ong et al., 2018). Our investigations and the study published by Thomson et al. (2017) revealed a high sensitivity of the P/N test reaching 100% and 97.1%, respectively. Ong et al. (2018) reported a moderate sensitivity of 89.4%. Detection of carbapenemase production based on the Ambler test was comparable in all three studies: Our study revealed a sensitivity of 79%. We re-analyzed the data given by Thomson et al. (2017) and Ong et al. (2018) according to our Ambler test-based approach for carbapenemase detection. In Thomson's (2017) study 197 isolates were typed with an Ambler class, which resulted in a sensitivity of 81.1% (95% CI, 75.6% to 85.8%). Ong et al. (2018) reported that 133 isolates were typed with an Ambler class, which resulted in a sensitivity of 83.1% (95% CI, 76.4% to 88.6%).

The capability to correctly type Ambler class D-carbapenemases was similar for our study and for Thomson et al. (2017) and Ong et al. (2018) (sensitivity 95.5%, 88.6% and 92.3%, respectively). However, the investigations varied in their sensitivities to classify isolates harboring carbapenemases of the Ambler classes A and B. In line with Ong et al. (2018) our study revealed that typing of Ambler class A carbapenemases was error prone (sensitivity of 43.3% and 38.5%, respectively), while Thomson et al. (2017) reported a sensitivity of 82.7%. In reverse, Ong's (2018) and our investigation obtained a better sensitivity of Ambler class B classification compared to Thomson's (2017) study

(90.2% and 90.5% vs. 69.2%).

Compared to the studies by Thomson et al. (2017) and Ong et al. (2018), our results provided a substantial lower specificity (68.6% and 66.7% vs. 55.3%) of the P/N test. Detection of carbapenemase production based on the Ambler test revealed an excellent specificity of 100% in our investigation. Re-analysis of data published in the online supplemental material by Thomson, et al. resulted in a specificity of 82.4% (95% CI, 69.1% to 91.6%) due to 9 false-positive non-carbapenemase-producing isolates, which were typed as Ambler class B- or D-positive isolates (Thomson et al., 2017). Of note, re-analysis of the data published by Ong revealed a specificity of 96.7% (95% CI, 82.8% to 99.9%) due to a single non-carbapenemase-producer typed with an Ambler class (class B), which conforms to our findings.

These differences in sensitivities and specificities between the studies might be traced back to the different sample sizes and the fact, that Thomson's (2017) strain collection was the only one that included *Pseudomonas aeruginosa* and *Acinetobacter baumannii* complex isolates. Moreover, the compositions of the carbapenemase types and subtypes in the three strain collection varied which might underlie reported differences. For instance, Ong and co-workers (2018) report that the CPO detect assay does not reliably detect IMI-type carbapenemases which are abundant in their strain collection ($n = 23$) but largely missing in Thomson's (n = 2) and our collection ($n = 1$) of isolates.

In order to increase the overall specificity of the CPO Detect assay and render positive P/N test results without Ambler classification more reliable, a two-part algorithm was established (Fig. 1), thereby the β-CARBA assay served as a confirmatory test for P/N test-positive, Ambler test-negative results. The colorimetric assay is easy to use and provides same day results (Noël et al., 2017). Thus, the time saving advantage of the CPO Detect panel was maintained. The overall specificity was increased from 55.3% to 100% and the overall sensitivity was not impaired significantly (100% vs. 98.3%). One GES-type harboring isolate was not detected using the algorithm since it was missed by the β-CARBA test. GES-type carbapenemases are known to be difficult to detect using colorimetric methods (Bernabeu et al., 2017; Simon et al., 2018). Our data suggest that also the Ambler test failed to reliably detect GES-type carbapenemases: four of six GES-type carbapenemase harboring isolates were false-negative. GES-type harboring strains were not evaluated by Thomson et al. (2017) and by Ong et al. (2018). Moreover, the detection of the more prevalent KPC-type carbapenemases (Pfennigwerth, 2018) by the Ambler test was also difficult in our study (Table 1A). However, this was not reported by Thomson et al. (2017) and Ong et al. (2018). The algorithm in our study identified correctly one IMI-type carbapenemase by the Ambler test and colorimetric confirmation was not necessary. It is conceivable, that it would have been missed by the β-CARBA test alone, because IMI-type carbapenemases are probably undetectable in the β-CARBA test (Mancini et al., 2017). In this situation the CPO Detect assay was capable to cover weaknesses of the colorimetric assay.

Of note, the excellent specificity of our proposed algorithm for carbapenemase detection relies on the absence of false-positive Ambler test results in our test strain collection. However, misclassifications of non-carbapenemase-producing isolates by the Ambler test are possible (Ong et al., 2018; Thomson et al., 2017), which will impact on the performance of our proposed algorithm. If a significant number of non-carbapenemase producing, Ambler test-positive isolates would appear, this would ultimately compromise the overall specificity of the algorithm approach. In order to estimate the impact of such isolates in routine diagnostic processing, the assessment of their prevalence is required. However, Thomson et al. (2017) underline, that the investigated strain collection was not composed of routine clinical isolates, but rather of isolates that are difficult to diagnose. Therefore, further studies are needed that especially focus on the analysis of routine clinical isolates in order to clarify this issue.

5. Conclusions

Taken together, in our investigation of 95 carbapenemase-producing and non-carbapenemase-producing Enterobacterales the CPO Detect assay revealed that the Ambler test was well suited to rapidly classify carbapenemase-producing organisms. However, the P/N test displayed a low specificity due to the misclassification of carbapenem-non-susceptible, non-carbapenemase-producing test strains. The combination of the CPO Detect assay with the β-CARBA test provided an excellent specificity without significantly impairing the sensitivity. Finally, this testing strategy includes the identification of the Ambler classes which provides useful information e. g. for the treatment with β-lactam/β-lactamase inhibitor combinations, such as ceftazidime/avibactam.

Declaration of interest

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