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Detection of carbapenemase activity in Enterobacteriaceae using LC-MS/MS in comparison with the neo-rapid CARB kit using direct visual assessment and colorimetry

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ABSTRACT

It has been described that the sensitivity of the Carba NP test may be low in the case of OXA-48-like carbapenamases and mass spectrometry based methods as well as a colorimetry based method have been described as alternatives. We evaluated 84 *Enterobacteriaceae* isolates including 31 OXA-48-like producing isolates and 13 isolates that produced either an imipenemase (IMP; $n = 8$), New Delhi metallo-β-lactamase (NDM; $n = 3$), or *Klebsiella pneumoniae* carbapenemase (KPC; $n = 2$), as well as 40 carbapenemase negative *Enterobacteriaceae* isolates. We used the Neo-Rapid CARB kit, assessing the results with the unaided eye and compared it with a colorimetric approach. Furthermore, we incubated the isolates in growth media with meropenem and measured the remaining meropenem after one and 2 h of incubation, respectively, using liquid chromatography tandem mass spectrometry (LC-MS/MS). Whilst all carbapenemase producing isolates with the exception of the OXA-244 producer tested positive for both the Neo-rapid CARB test using the unaided eye or colorimetry, and the 13 isolates producing either IMP, NDM or KPC hydrolysed the meropenem in the media almost completely after 2 h of incubation, the 31 OXA-48-like producing isolates exhibited very variable hydrolytic activity when incubated in growth media with meropenem. In our study, the Neo-Rapid CARB test yielded a sensitivity of 98% for both the traditional and the colorimetric approach with a specificity of 95% and 100% respectively. Our results indicate that the Neo-Rapid CARB test may have use for the detection of OXA-48 type carbapenemases and that it may be particularly important to ensure bacterial lysis for the detection of these weaker hydrolysers.

1. Introduction

In *Enterobacteriaceae*, carbapenemases are frequently encoded by genes located on mobile elements that may transfer between bacterial strains, species or even genera (Goodman et al., 2016). Phenotypic tests such as the modified Hodge test or the Carba NP test are used to evaluate the presence of carbapenemases (Lutgring and Limbago, 2016).

The Carba NP test was first published in 2012. It utilises an acid base indicator (phenol red) to detect the hydrolysis of imipenem by a carbapenemase (Dortet et al., 2015). Many commercial tests such as the Rapidec CARBA NP test (bioMérieux, Marcy-l'Étoile, France), the Rapid CARB screen (Dortet et al., 2015) and its replacement the Neo-rapid CARB kit (Rosco Diagnostica, Taastrup, Denmark) are now available for the detection of carbapenemase activity (Casals, 2015).

It has recently been suggested that the solutions of the Carba NP test may be prepared in advance and stored at −70 °C for up to a year (Knox et al., 2016). However, the general agreement is that the test solution needs to be prepared when needed, making its preparation laborious (Gallagher et al., 2015). Furthermore, using imipenem for research purposes is not cost-effective, and it may only be economically viable if powder aliquots of pharmaceutical formulations are used. However, these formulations contain other ingredients such as cilastatin (a dehydropeptidase inhibitor blocking the renal metabolism of imipenem) and in case of the intravenous form sodium hydrogencarbonate which may interfere with the pH reaction. The intramuscular imipenem formulation lacking sodium hydrogencarbonate may be used instead, however this formulation is not available in all countries (AbdelGhani et al., 2015).

In a personal communication with Merck Switzerland we were informed that from a manufacturing perspective all ingredients in the imipenem/cilastatin preparation Tienam could be evenly distributed, but since the use as a powder for in vitro testing is not the intended

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use, the even distribution has not been tested and it cannot be guaranteed.

For these reasons it might be preferable to use a commercial kit instead of an in-house preparation of the Carba NP test.

There is variation in the literature on the reported accuracy of the Carba NP test to detect carbapenemase activity (Dortet et al., 2015; Goodman et al., 2016; Hombach et al., 2015; Lutgring and Limbago, 2016; Papagiannitsis et al., 2015). OXA-48 like β-lactamases are weak hydrolysers of carbapenems conferring only low levels of resistance in some isolates. This may impede the detection of these carbapenemases, which may have significant implications for treatment and infection control (Poirel et al., 2012).

Mass spectrometry based methods have previously been described for the detection of carbapenemases, using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) (Kulkarni et al., 2014; Peaper et al., 2013) and more commonly matrix-assisted laser desorption time of flight mass spectrometry (MALDI-TOF MS) (Hrabak et al., 2012; Lutgring and Limbago, 2016). Papagiannitsis and colleagues recently proposed that MALDI-TOF MS may be superior to the Carba NP test in the detection of OXA-48- type producers, and it has also been indicated that the use of an Elisa microplate reader may help improve the sensitivity of colour changes in order to detect OXA-48-like producers (Papagiannitsis et al., 2015).

Here we investigated 84 bacterial isolates, both carbapenemase positive and negative. Carbapenemase producing organisms included 31 OXA-48-like producing isolates and 13 isolates that produced other carbapenemases. We were using the Neo-rapid CARB kit as per package insert and comparing it with a colorimetric approach using a microplate reader. We further evaluated the hydrolysis of meropenem by these isolates using LC-MS/MS. The two latter approaches did not require subculturing for disc testing and the duration of test could therefore be cut down by one day.

2. Materials and methods

2.1. Isolates included in the study

Forty four carbapenemase producing and 40 carbapenemase negative *Enterobaceriaceae* of the following description were investigated:

The carbapenemase producing isolates included 31 OXA-48-like producing isolates, eight imipenemase (IMP) producing isolates, three New Delhi β-lactamase (NDM) producing isolates and two *Klebsiella pneumoniae* carbapenemase (KPC) producing isolates (Table 1).

The OXA-48-like producing isolates were of the following *Enterobacteriaceae* species: *Klebsiella pneumoniae* ($n = 28$), *Escherichia coli* ($n = 1$), *Klebsiella oxytoca* ($n = 1$) and *Enterobacter cloacae* ($n = 1$). The IMP producing isolates consisted of four *E. cloacae*, two *K. pneumoniae* and two *E. coli* isolates, the NDM producing isolates consisted of two *E. coli* isolates and one *K. pneumoniae* isolate, and the two KPC producing isolates were *E. coli*. Resistance testing and carbapenemase screening had been performed as previously described for the carbapenemase positive isolates as well as two carbapenemase negative *K. pneumoniae* isolates (Kim et al., 2012, Sidjabat et al., 2014, Sidjabat et al., 2015a, 2015b; Wailan et al., 2015a, 2015b; Zowawi et al., 2014). The geographic regions of origin of the carbapenemase positive isolates are indicated in Table 1.

Thirty eight *Enterobacteriaceae* isolates that were resistant to ertapenem by disc susceptibility testing as interpreted using EUCAST standard (EUCAST, 2013) were subsequently added to the study. These isolates had been tested negative for the production of carbapenemases using the modified Hodge test with ertapenem, imipenem and meropenem discs (Genc et al., 2016). These were provided by two local hospitals and included twenty two *E. coli* isolates, twelve *K. pneumoniae* isolates, three *E. cloacae* isolates and one *Enterobacter aerogenes* isolate.

2.2. Sequencing of $bla_{OXA\text{-}48\text{-}like}$ genes

The $bla_{OXA\text{-}48\text{-}like}$ genes were further investigated by amplification and sequencing with the primer set Pre_Oxa48Aplus (5′-CAGTATATTGCATTAAGCAAGGG-3′) and Pre_Oxa48B (5′-CACA CAAATACGCGCTAACC-3′). These were based on primers previously described (Poirel et al., 2011). The following PCR conditions were applied: Initial denaturation, 95 °C for 5 min followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s and extension at 70 °C for 1 min. The final extension was done for 10 min at 72 °C. Primer synthesis, PCR amplification and sequencing was performed by Macrogen, Seoul, Korea.

The sequences were aligned with the DNA Baser (v.3.5.0) and the sequences were translated making use of the EMBOSS Transeq tool (http://www.ebi.ac.uk/Tools/st/emboss_transeq/), and blasted against the NCBI Genbank (http://blast.ncbi.nlm.nih.gov/Blast.cgi) for identification.

2.3. Detection of carbapenemases in live bacterial cells using LC-MS

Two McFarland of each bacterial isolate was incubated in 5 ml of Mueller Hinton broth (Oxoid, Basingstoke, RG24 8PW, UK) with 4 μg/ml meropenem at 37 °C while shaking for one or 2 h respectively. The meropenem was subsequently extracted using methanol with 1% formic acid, and purified using a Strata X 33 μ Polymeric Reversed phase cartridge (Phenomenex, Torrance, CA 90501-1430 USA). Samples were analysed using the QTRAP 5500 System (AB Sciex, Redwood City, CA, USA) and the MicroLC 200 System (Eksigent, Redwood City, CA, USA). The 50 μm electrode was used in the TurboV source to improve the sensitivity.

The following mobile phases were used: The aqueous solvent A consisted of 5 mM ammonium formate and the organic solvent B consisted of 99.9% acetonitrile with 0.1% formic acid. A HALO C18 (AB Sciex, Redwood City, CA, U.S.A.) (2.7 μm 90 Å, 0.5 × 50 mm) column was used for the separation of compounds, and the following gradient was applied: Isocratic flow rate of 2% Solvent B for 1 min, 2–20% B for 4 min, 20–90% B for 30 s, isocratic flow at 90% B for 2.5 min, back to 2% B within 2 min. The Flow rate used was 25 uL/min.

The collision energy (CE) and declustering potential (DP) were optimised making use of the Analyst software 1.6.2, and were 25 V and 96 V respectively for both meropenem and the internal standard [$^2H_6$]-meropenem, and the following MRM settings were obtained: m/z 384.1 → 141.0 for meropenem and m/z 390.165 → 146.9 for [$^2H_6$]-meropenem.

Relative quantification was performed by peak integration making use of Analyst 1.6.2, and dividing the peak areas of 100 ng/ml of [$^2H_6$]-meropenem by the peak area of the incubated meropenem with the respective isolate for one and 2 h. The relative peak areas were subsequently compared with the relative peak area of the *E. coli* ATCC 25922 negative control.

2.4. Neo-Rapid CARB kit using colorimetry

Isolates were incubated overnight on Mueller Hinton Agar (Becton Dickinson, Sparks, USA) and one full 10 μl loop of each isolate was lysed using 150 μl of the B-PERII Bacterial Protein Extraction Reagent (Thermo Scientific, Rockford, IL, USA). The suspension was vortexed for 1 min and then kept at room temperature shaking for 30 min. Fifty microliters of each bacterial suspension was added to two tubes with 100 μl of saline, and a Neo-Rapid CARB Imipenem (×2) + Indicator Diatab (Rosco Diagnostica, Taastrup, Denmark) was added to one and a Negative Control Diatab was added to the other tube. The tubes containing the tablets were vortexed and incubated for 2 h at 37 °C shaking at 250 rpm. After incubation, the tubes were centrifuged at 17,000g for 5 min and 50 μl of the supernatant was transferred to a Nunclon™ delta surface 96 well plate (Thermo Scientific, Roskilde, Denmark).

Table 1
Results of meropenem reduction assay for $n = 44$ carbapenemase-producing *Enterobacteriaceae* clinical isolates. Isolates were cultured in Mueller Hinton broth containing 4 μg/ml meropenem, and concentrations of meropenem were measured at one hour and two hour time points using liquid chromatography tandem mass spectrometry (LC-MS/MS). Reductions in meropenem concentrations in cultured isolates were calculated relative to that observed in an *E. coli* ATCC 25922 control. The experiments were repeated for the OXA-48-like carbapenemase producers and both results are listed in the table. The isolates 1 and 14 to 44 originated from the Arabian Peninsula (Zowawi et al., 2014), the isolates 2 (Wailan et al., 2015a, 2015b), 3 and 6–13 (Sidjabat et al., 2015a) were of Australian origin and isolates 4 and 5 originated from the United States of America (Kim et al., 2012).

Isolate	Species	Geographical Origin	Name	Carbapenemase	Level of meropenem peak area reduction compared with *E. coli* ATCC 25922	
					1 h incubation	2 h incubation
1	*K. pneumoniae*	Arabian Peninsula	KPMC-10 KP	NDM	5.9	>100
2	*E. coli*	Australia	CR694	NDM-5	48	>100
3	*E. coli*	Australia	CR53	NDM-4	30	>100
4	*E. coli*	USA	YDC 419	KPC-2	71	>100
5	*E. coli*	USA	YD 648	KPC-2	57	>100
6	*E. coli*	Australia	Ec1	IMP-4	49	>100
7	*E. coli*	Australia	CR48	IMP-4	>100	>100
8	*E. cloacae*	Australia	CR19	IMP-4	>100	>100
9	*E. cloacae*	Australia	CR17	IMP-4	>100	>100
10	*E. cloacae*	Australia	CR12	IMP-4	>100	>100
11	*K. pneumoniae*	Australia	CR9	IMP-4	92	>100
12	*E. cloacae*	Australia	CR11	IMP-4	>100	>100
13	*K. pneumoniae*	Australia	CR10	IMP-4	>100	>100
14	*K. oxytoca*	Arabian Peninsula	OMN-35	OXA-48	>100; >100	>100; >100
15	*E. cloacae*	Arabian Peninsula	OMN-48	OXA-48	>100; >100	>100; >100
16	*K. pneumoniae*	Arabian Peninsula	OMN-50	OXA-48	11; 7.6	>100; >100
17	*K. pneumoniae*	Arabian Peninsula	SA-002	OXA-48	49; >100	>100; >100
18	*K. pneumoniae*	Arabian Peninsula	SA-003	OXA-48	15; 21	>100; 27.8
19	*K. pneumoniae*	Arabian Peninsula	SA-014	OXA-48	4.5; 5.3	>100; >100
20	*K. pneumoniae*	Arabian Peninsula	SA-020	OXA-48	3.7; 2.7	>100; >100
21	*K. pneumoniae*	Arabian Peninsula	SA-021	OXA-48	4.3; 3.1	>100; >100
22	*K. pneumoniae*	Arabian Peninsula	SA-027	OXA-48	17; 5.4	>100; >100
23	*K. pneumoniae*	Arabian Peninsula	Egy-6	OXA-48	17; 5.0	46; >100
24	*K. pneumoniae*	Arabian Peninsula	SA-070	OXA-48	9.0; 47	>100; >100
25	*K. pneumoniae*	Arabian Peninsula	SA-039	OXA-48	3.2; 2.1	19; 7.5
26	*K. pneumoniae*	Arabian Peninsula	SA-001	OXA-48	0.1; 0.6	1.5; 1.5
27	*K. pneumoniae*	Arabian Peninsula	SA-005	OXA-48	0.9; 0.9	3.2; 3.7
28	*K. pneumoniae*	Arabian Peninsula	SA-007	OXA-48	1.4; 0.7	2.7; 4.0
29	*K. pneumoniae*	Arabian Peninsula	SA-008	OXA-48	1.0; 0.7	3.2; 2.7
30	*K. pneumoniae*	Arabian Peninsula	SA-015	OXA-48	0.7; 0.5	1.3; 1.5
31	*K. pneumoniae*	Arabian Peninsula	SA-018	OXA-48	0.8; 0.6	1.2; 1.1
32	*K. pneumoniae*	Arabian Peninsula	SA-022	OXA-48	1.0; 0.5	1.1; 0.8
33	*K. pneumoniae*	Arabian Peninsula	SA-023	OXA-48	1.2; 0.5	2.1; 1.3
34	*K. pneumoniae*	Arabian Peninsula	SA-025	OXA-48	1.2; 0.4	1.9; 1.9
35	*K. pneumoniae*	Arabian Peninsula	SA-028	OXA-48	2.5; 1.8	6.5; 5.0
36	*K. pneumoniae*	Arabian Peninsula	SA-029	OXA-48	1.1; 1.3	2.6; 2.5
37	*K. pneumoniae*	Arabian Peninsula	SA-031	OXA-48	1.4; 1.4	4.0; 5.5
38	*K. pneumoniae*	Arabian Peninsula	SA-032	OXA-48	1.4; 1.3	2.9; 4.0
39	*K. pneumoniae*	Arabian Peninsula	SA-034	OXA-48	1.3; 1.0	1.1; 1.2
40	*K. pneumoniae*	Arabian Peninsula	SA-036	OXA-48	1.3; 1.1	1.8; 1.9
41	*K. pneumoniae*	Arabian Peninsula	SA-038	OXA-48	1.1; 1.4	1.6; 2.1
42	*K. pneumoniae*	Arabian Peninsula	QTR-46	OXA-181	0.2; 1.3	3.0; 2.5
43	*K. pneumoniae*	Arabian Peninsula	QTR-35	OXA-232	0.2; 1.0	2.4; 1.9
44	*E. coli*	Arabian Peninsula	QTR-23	OXA-244	0.2; 1.8	6.9; 14

Absorbance was measured at 560 nm making use of a Spark 10 M multimode microplate reader (Tecan Group Ltd. Maennedorf, Switzerland).

2.5. Neo-Rapid CARB kit using the unaided eye

The test was performed as per insert, using Ertapenem Antimicrobial Susceptibility Test Discs, BBL™ Sensi-Disc™, 10 μg (Becton Dickinson, Sparks, USA). Stored isolates were cultured overnight using Mueller Hinton agar plates (Becton Dickinson, Sparks, USA) and the isolates were subcultured by lawning 0.5 McFarland onto new plates and an ertapenem disc was added. One full 10 μl loop was suspended in 200 μl B-PER II buffer diluted 1:1 with 0.9% NaCl and the suspension was vortexed for 1 min and maintained at room temperature for 30 min. Fifty microliters of the suspension were added to two tubes with 150 μl of 0.9% NaCl, and one Imipenem(×2) + Indicator (CARB) diatab was added to one tube and the CARB Negative Control Diatab was added to the other tube. The tubes were vortexed for 1–2 s in order to disintegrate the tablet. The samples were incubated at 37 °C and results were read by blinded lab personnel after 15 min, 30 min, one and 2 h respectively.

3. Results

3.1. Sequencing of $bla_{OXA\text{-}48\text{-}like}$ genes

Full sequencing of the $bla_{OXA\text{-}48\text{-}like}$ genes revealed that twenty eight out of the thirty one isolates carried $bla_{OXA\text{-}48}$, whereas one isolate each carried $bla_{OXA\text{-}181}$, $bla_{OXA\text{-}232}$ and $bla_{OXA\text{-}244}$, respectively (Table 1).

3.2. Detection of carbapenemases in live bacterial cells using LC-MS/MS

All the 40 carbapenemase negative isolates had their relative peak area reduced by a factor that ranged between 0.7 and 2.3 compared with the *E. coli* ATCC 25922 negative control.

The isolates that produced either NDM, IMP or KPC demonstrated strong hydrolytic activity. After 1 h of incubation, one NDM positive *K. pneumoniae* isolate had meropenem reduced by a factor of six, and the

other twelve isolates had the meropenem reduced by a factor of thirty or more. All thirteen isolates had meropenem decreased by a factor of above one hundred after 2 h of incubation (Table 1).

The 31 OXA-48-like producing isolates showed variable degradation of meropenem in the MHB media culture assay as measured by LC-MS/MS. This was also the case amongst the 28 isolates that were producing OXA-48 and not a variant. The experiments for these 31 Oxa-48-like producing isolates were repeated with results shown in Table 1.

The $bla_{OXA-244}$ carrying *E. coli* isolate showed no detectable hydrolysis after 1 h of incubation compared with the carbapenemase negative isolates. However, the amount of meropenem was reduced by a factor of seven and fourteen after two hours of incubation in two separate experiments.

The amount of meropenem was not reduced compared with the *E. coli* ATCC 25922 negative control in the OXA-181 producing *K. pneumoniae* isolate after one hour of incubation. After two hours of incubation, meropenem was reduced by a factor of two or three respectively, showing very little hydrolysis. For the $bla_{OXA-232}$ positive *K. pneumoniae* isolate, no reduction of meropenem could be detected after one or two hours of incubation compared with the carbapenemase negative isolates investigated in this study.

One bla_{OXA-48} positive *K. oxytoca*, one *E. cloacae* and one *K. pneumoniae* isolate each hydrolysed meropenem almost completely after one hour already.

In the case of eight bla_{OXA-48} positive *K. pneumoniae* isolates, meropenem was reduced by a factor of three or more compared with the *E. coli* ATCC 25922 negative control after one hour of incubation. After two hours of incubation with these eight isolates, meropenem was hydrolysed almost completely.

The remaining twenty bla_{OXA-48} positive *K. pneumoniae* isolates showed little or no hydrolysis (Table 1).

3.3. Neo-Rapid CARB kit using colorimetry

Using colorimetry, the Neo-Rapid CARB test yielded a sensitivity of 98% and a specificity of 100%.

Whilst the absorbance at 560 nm was the same or higher for the imipenem containing test than the control for all carbapenemase negative isolates, the absorbance was lower for the imipenem containing test than the control for all but one carbapenemase positive isolate.

When dividing the absorbance of the Neo-Rapid CARB negative control by the absorbance of the test containing imipenem, all 40 carbapenemase negative isolates had a relative absorbance of one or less.

Forty-three out of the 44 carbapenemase positive isolates (98%) had an absorbance which was lower in the sample with imipenem than the control and had therefore a relative absorbance above one. The exception was the OXA-244 positive isolate. One OXA-48 positive, one IMP positive and the OXA-232 positive *K. pneumoniae* isolate had a relative absorbance that ranged between 1.1 and 1.5. All other isolates had a relative absorbance of 1.6 or higher (Fig. 1).

3.4. Neo-Rapid CARB kit using the unaided eye

Using the unaided eye and the Neo-Rapid CARB kit as per package insert, a sensitivity of 98% was obtained, with a specificity of 95%. The only carbapenemase producing isolate that gave false negative results was the OXA-244 producer included in the study. Two carbapenemase negative isolates were identified as positive, one of which was an *E. coli* isolate which was positive after 15 min and then turned uninterpretable due to colour change to yellow of the control. The other was a *K. pneumoniae* isolate which turned positive after 2 h of incubation.

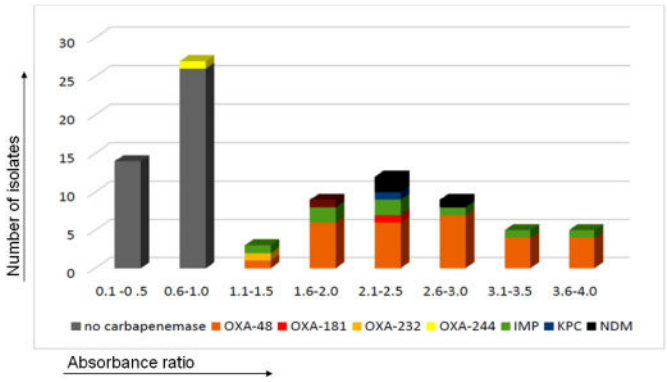

Fig. 1. Absorbance ratio at 560 nm of the Neo Rapid CARB negative control versus the test containing imipenem for the 84 isolates investigated in this study (x-axis). The y axis indicates the number of isolates with the respective absorbance ratios. All carbapenemase negative isolates had a relative absorbance of either 1.0 or less. The OXA-244 isolate had a relative absorbance of 0.6 and was therefore considered negative. The OXA-232 positive isolate had a relative absorbance of 1.1. All other isolates had a relative absorbance of 1.3 or more.

4. Discussion

We tested 84 *Enterobacteriaceae* isolates including 40 carbapenemase negative isolates, 31 OXA-48 like producers (OXA-48; $n = 28$, OXA-181; $n = 1$ and OXA-244; n = 1), as well as 13 isolates producing either IMP ($n = 8$), NDM ($n = 3$) and KPC (n = 1) using the Neo-Rapid CARB test. We compared the Neo-Rapid CARB test using the unaided eye versus a colorimetric approach using a multiplate reader to interpret colour change. We also assessed the reduction in meropenem by incubation of the isolates in MHB growth media for one or 2 h respectively, using a LC-MS/MS instrument for quantitation.

We found a specificity 95% for the Neo-Rapid CARB test using the unaided eye and 100% for the colorimetric approach. When incubating the 40 carbapenemase negative isolates in growth media with meropenem, the amount of remaining meropenem varied between 0.43 and 1.43 times the *E. coli* ATCC 25922 control after 2 h of incubation.

Previous studies suggested that the Carba NP test may have low sensitivity for OXA-48-like producing isolates (Goodman et al., 2016; Lutgring and Limbago, 2016; Papagiannitsis et al., 2015). However in our isolate collection we identified 30 out of the 31 OXA-48-like producing isolates by both the Neo-Rapid CARB test interpreted using the unaided eye as well as the colorimetric approach. The only exception was the OXA-244 producing isolate, an OXA-48-type carbapenemase that had previously been described not to be reliably detected with the Carba NP test (Dortet et al., 2015, 2016).

Whilst the sensitivity was 98% for the Neo-Rapid CARB test using both the unaided eye and the colorimetric approach, a very variable hydrolytic activity was detected when incubating the isolates in growth media with meropenem. All 13 isolates that produced either NDM, IMP or KPC had a remaining amount of meropenem that was <1% after 2 h of incubation compared with the remaining meropenem of the *E. coli* ATCC 25922 negative control. However, this was only the case for 11 out of the 31 OXA-48-like producers, some of which showed no increased hydrolytic activity compared with the 40 carbapenemase negative isolates.

The fact that there was no major issue with detecting OXA-type carbapenemases with the Carba NP test, where a lysis step is involved, may indicate that the differences in meropenem degradation in the presence of live bacterial cells is not due to variability in expression of the enzyme, but due to additional resistance mechanisms such as decreased permeability (Poirel et al., 2012). In Gram-negative organisms β-lactamases are located in the bacterial periplasm, and carbapenemases generally enter Gram-negative bacteria through

porins (Papp-Wallace et al., 2011). Weaker carbapenemases such as the OXA-48 type may need additional resistance mechanisms such as porin loss in order to confer high levels of carbapenem resistance (Poirel et al., 2012). Therefore, it may be particularly important to ensure lysis for the detection of OXA-48-type carbapenemase activity. In our study we were adhering to the package insert of the Neo-rapid CARB tests which states that the bacterial samples need to be vortexed in lysis buffer for 1 min and leave them at room temperature for an additional 30 min.

It has been suggested that an increased bacterial inoculum may be needed for the Carba NP test, in order to appropriately detect IMP, NDM and OXA-48 producers (Gallagher et al., 2015; Hombach et al., 2015). Here we used a full 10 μl loop and both the colorimetry based Neo-Rapid CARB test and the traditional test using the unaided eye had high specificity and sensitivity.

Whilst the OXA-244 producer included in our study was the only isolate that tested falsly negative, meropenem degradation was detected when incubating the isolate in growth media. It has been suggested that whilst OXA-244 has similar hydrolytic activity as OXA-48 for ertapenem and meropenem, it hydrolyses imipenem much less efficiently (Potron et al., 2016), and the Neo-Rapid carb test may therefore not be suitable to detect OXA-244.

5. Conclusions

The traditional and the colorimetric approach of the Neo-Rapid carb test showed high levels of sensitivity (98% in both cases) and specificity (95% for the traditional approach and 100% for the colorimetric approach). Using colorimetry, the test was performed without disc testing and consequently results would be achieved one day earlier.

OXA-244 producers may not reliably be detected by the Neo-Rapid CARB test (Dortet et al., 2015, 2016; Potron et al., 2016), and sequencing of the $bla_{\text{OXA-48-like}}$ genes may help detecting this carbapenemase.

Our results using the LC-MS/MS indicate that bacterial lysis may be particularly important for isolates producing OXA-48-like carbapenemases, as these are relatively weak carbapenem hydrolysers and other resistance mechanisms such as porin loss are likely to play a role (Poirel et al., 2012).

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