CARBAPENEMASES 2013: affiche 427

Comparative evaluation of Carba NP test and of ROSCO Rapid CARB Screen kit for the detection of carbapenemase-producing Enterobacteriaceae

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Introduction

- Rapid detection of carbapenemase in Enterobacteriaceae (EB) is essential for early appropriate therapeutic management and infection control purposes.[1]
- The Carba NP test has been recently proposed as a cheap and easy to perform imipenem hydrolysis-based test with high accuracy (sensitivity and specificity) for the detection of carbapenemase-producing Enterobacteriaceae (CPE).

Objectives

- However, the Carba NP test in its current format is an in-house technique requiring purchase of several reagents and home-made preparation of the test solutions (including the addition of imipenem).
- We evaluated here the performance of two imipenem hydrolysis-based rapid tests, the Carba NP test and the commercially available ROSCO Rapid CARB Screen kit for the detection of CPE.

Methods

- *Bacterial strains*: 100 well-characterized EB collection strains (44 carbapenemase producers and 56 isolates expressing other beta-lactamases) and 135 consecutive clinical EB isolates referred to the national reference center (NRC) from January to March 2013 for suspicion of CPE were tested (**Table 2**). All isolates had been verified for the presence of carbapenemase by multiplex PCR targeting bla_{VIM}, bla_{IMP}, bla_{NDM}, bla_{KPC} and bla_{OXA-48}.[2]

- *Testing methodology*: All tested isolates were subcultured twice on TSA blood agar and tested by imipenem hydrolysis using the Carba NP test (**CNP**) previously described[3] and by ROSCO Rapid CARB Screen kit (**RCS**; Rosco Diagnostica A/S, Taastrup, Denmark) according to the manufacturers instructions (**Table 1**) using the same culture grown freshly on non selective blood agar. Both tests were read at 30, 60 and 120 minutes of incubation. All isolates were screened for carbapenemase gene by the ISO15189 validated multiplex PCR described above.[2]

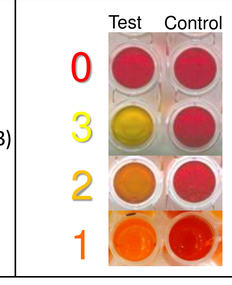
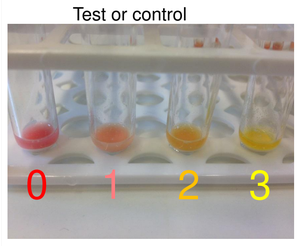
- *Reading and data recording*: For both tests, any color change observed by naked eyes from red to yellow (four shades of gradation recorded; **Table 1**) in a vial/tube was considered as a positive reaction. The interpretation of test results were as follows:

  – For CNP according to the reference publication[3]:
  - The result would be **negative** if both the test (containing imipenem) and the negative control (without imipenem) vial gave negative reaction (red; score 0)
  - The result would be **positive** if the test vial gave a positive reaction (yellow or orange; score 1, 2 or 3) and the control vial negative reaction (score 0)
  - The result would be **uninterpretable** if the control vial gave positive reaction (score ≥1)

  – For RCS according to the reading instructions provided in the product insert note (document version DBV0040C issued 15/8/2013):
  - The result would be **negative** if the test (containing imipenem) tube gave negative reaction (red; score 0)
  - The result would be **positive** if the test tube gave a strong positive reaction (yellow; score 3)
  - When the test tube gave weaker positive reaction (orange; score 1 or 2), according to the negative control (without imipenem) tube :
    - the result would be **positive** if the negative control tube gave negative reaction (score =0)
    - the result would be **uninterpretable** if the negative control tube gave positive reaction (score ≥1)

- *Performance analysis:* Sensitivity and specificity of each test was determined by comparing to the results of multiplex PCR targeting carbapenemase on all tested strains. Positive and negative predictive values (PPV and NPV) were calculated for the consecutive isolates referred to the NRC.

Table 1. Operating procedures of Carba NP test and of ROSCO Carb Screen test

Steps	Carba NP test	Rosco Carb Screen test
Bacterial inoculum	1 full 10-µl loop	Several full 10-µl loops
Bacterial lysis	30 minutes at 20°C in 100 µl B-PERII (Tris HCl)	
Centrifugation	5 minutes at 10000g	None
Inoculation	30 µl of the surnageant in 100 µl solution A ±imipenem 3 mg/ml in a vial.	50 µl of the suspension in 100 µl physio saline in a tube. Add one test (or negative control) disk.
Incubation / reading	at 37°C up to 2 hours	
Reading score (0 to 3)		

Results

Table 2. Species and carbapenemase enzyme distribution of all Enterobacteriaceae isolates tested (n=235)

Strains origin	Species	Carbapenemase OXA-48	KPC	VIM	NDM	Negative	Total
Collection	*K. pneumoniae*	7	10	2	3	15	37
	E. coli	2			2	19	23
	E. cloacae	3		4	1	4	12
	K. oxytoca	1		2		4	7
	P. mirabilis					5	5
	C. freundii	1				2	3
	S. marcescens			2		1	3
	M. morganii				2		2
	E. aerogenes					2	2
	C. braakii			1		1	2
	H. alvei					2	2
	E. kobei					1	1
	P. vermicola			1			1
Total Collection		14	10	12	8	56	100
Routine NRC	*K. pneumoniae*	42	3	1	1	24	71
	E. coli	10			1	10	21
	E. cloacae	2	1	2	2	10	17
	K. oxytoca	3				4	7
	E. aerogenes					8	8
	E. asburiae	1				2	3
	C. freundii			1	1	1	3
	E. kobei	1				2	3
	S. marcescens					1	1
	P. mirabilis					1	1
Total Routine NRC		59	4	4	5	63	135
Total		**73**	**14**	**16**	**13**	**119**	**235**

Table 3. Reading scores of CNP test vial at 30, 60 and 120 minutes of incubation and carbapenemase enzyme distribution for all Enterobacteriaceae isolates tested (n=235)

Score at 30 min	Carbapenemase OXA-48	KPC	VIM	NDM	Negative	Total
Not read		1	1	2	11	15
3		13	14	5		32
2	26		1	2		29
1	20					20
0	27			4	108	139
Total	**73**	**14**	**16**	**13**	**119**	**235**
60 min	**OXA-48**	**KPC**	**VIM**	**NDM**	**Negative**	**Total**
3	1	14	14	7		36
2	55		2	2		59
1	11			4		15
0	6				119	125
Total	**73**	**14**	**16**	**13**	**119**	**235**
120 min	**OXA-48**	**KPC**	**VIM**	**NDM**	**Negative**	**Total**
3	1	13	14	7		35
2	61	1	2	3		67
1	8			3		11
0	3				119	122
Total	**73**	**14**	**16**	**13**	**119**	**235**

Table 4. Reading scores of RCS test disk at 30, 60 and 120 minutes of incubation and carbapenemase enzyme distribution for all Enterobacteriaceae isolates tested (n=235)

Score at 30 min	Carbapenemase OXA-48	KPC	VIM	NDM	Negative	Total
3	18	14	15	7	1	55
2	37		1	2	7	47
1	14			4	19	37
0	4				92	96
Total	**73**	**14**	**16**	**13**	**119**	**235**
60 min	**OXA-48**	**KPC**	**VIM**	**NDM**	**Negative**	**Total**
3	25	14	16	7	1	63
2	31			1	8	40
1	15			5	20	40
0	2				90	92
Total	**73**	**14**	**16**	**13**	**119**	**235**
120 min	**OXA-48**	**KPC**	**VIM**	**NDM**	**Negative**	**Total**
3	21	11	16	6	1	55
2	35	3		1	10	49
1	15			6	18	39
0	2				90	92
Total	**73**	**14**	**16**	**13**	**119**	**235**

Table 5. Interpreted results and the performance of detection of CPE by CNP test and by RCS test for all Enterobacteriaceae isolates tested (n=235)

All isolates		CPE Positive	Negative	Total	Sensitivity	Specificity
Total		**116**	**119**	**235**		
CNP result	Positive	113		113	**97%**	**100%**
	Negative	3	119	122		
RCS result	Positive	114	28	142	**98%**	**76%**
	Negative	2	91	93		

Table 6. Interpreted results and the performance of detection of CPE by CNP test and by RCS test for the consecutive isolates referred to the NRC (n=135)

Referred isolates		CPE Positive	Negative	Total	PPV	NPV
Total		**72**	**63**	**135**		
CNP result	Positive	69		69	100%	95%
	Negative	3	63	66		
RCS result	Positive	70	21	91	77%	95%
	Negative	2	42	44		

Table 7. Reading scores, interpreted results and the performance of detection of CPE by RCS test for Enterobacteriaceae isolates tested with both the test disk and the negative control disk (n=166)

Isolates tested with RCS control disk			CPE		
RCS test disk score	RCS control disk score	Interpreted RCS test result	Positive	Negative	Total
3	1 or 2	Positive	7	1	8
	0	Positive	32		32
1 or 2	1 or 2	Uninterpretable	4	9	13
	0	Positive	48	18	66
0	0	Negative	2	45	47
Total tested with RCS control disk			93	73	166
Total interpreted RCS test result		**Uninterpretable**	**4**	**9**	**13**
		Positive	**87**	**19**	**106**
		Negative	**2**	**45**	**66**
Total interpretable			**89**	**64**	**153**
Corrected performance of RCS test		**Sensitivity**	98%		
		Specificity	70%		

❖ Of the total 235 strains tested (**Tables 3, 4 and 5**):
- 113/116 CPE were detected by CNP and 114/116 by RCS thus resulting in **sensitivity** of **97%** and **98%** respectively
- All of the 119 carbapenemase-negative strains yielded negative results by CNP and 91/119 by RCS (**specificity** of **100%** and **76%** respectively).

❖ Among the 135 consecutive clinical isolates referred to the NRC (**Table 6**):
- 72 were confirmed as CPE (**Table 2**) including 69 and 70 isolates detected by CNP and by RCS respectively.
- 2 and 1 OXA-48-producing *K. pneumoniae* isolates were missed by CNP and by RCS respectively; 1 OXA-48-positive *E. coli* gave negative result with both tests.
- While none of the carbapenemase-negative strain was tested positive by CNP, 21/63 (33%) yielded positive result by RCS.
- The calculated positive and negative predictive values (**PPV** and **NPV**) were therefore of **100%** and **95%** for CNP respectively, and of **77%** and **95%** for RCS.

❖ While no uninterpretable results were observed with CNP, 21/166 (13%) strains tested with RCS control disk yielded a positive reaction (**Table 7**):
- Following the reading instructions provided in the RCS insert (see Methods section), of the 79 isolates showing orange results (score 1 or 2) with RCS test disk, 13 (4 CPE and 9 non-CPE) gave positive reaction with the control disk and thus would have the test result be considered **uninterpretable**.
- No significant change in the corrected sensitivity (98%; 87/89) and specificity (70%; 45/64) was found for the RCS after excluding the uninterpretable results from the calculation.

Conclusions

➢ CNP and RCS are rapid and highly sensitive screening tests for the detection of carbapenemase in EB, although a **few OXA-48 producers may go undetected** by any of these tests. CNP performed better than RCS owing to its superior specificity and to the large number of **uninterpretable** results observed with RCS.

➢ Both CNP and RCS as CPE screening test should be used with caution in areas with high prevalence of OXA-48 producers[4] and should be evaluated in other epidemiological settings. Exclusion of suspected CPE isolates from further testing to confirm and identify carbapenemase (i.e. by molecular testing) should only be based on concordant results between CNP/RCS tests and phenotypic antimicrobial resistance patterns.

References

1. **Canton R** *et al.* Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. *Clin Microbiol Infect* 2012; 18: 413-31.
2. **Bogaerts P** *et al.* Validation of carbapenemase and extended-spectrum beta-lactamase multiplex endpoint PCR assays according to ISO 15189. *J Antimicrob Chemother* 2013; 68: 1576-82.
3. **Nordmann P** *et al.* Rapid detection of carbapenemase-producing Enterobacteriaceae. *Emerg Infect Dis* 2012; 18: 1503-7.
4. **Tijet N** *et al.* Evaluation of the Carba NP test for rapid detection of carbapenemase-producing Enterobacteriaceae and Pseudomonas aeruginosa. Antimicrob Agents Chemother. 2013 Sep;57(9):4578-80.

Funding and acknowledgements

- The study was supported in part by the research grant of Fondation Mont-Godinne. The national reference center is partially supported by the Belgian Ministry of Social Affairs through a fund within the Health Insurance System.
- We are thankful to Jose Bou Casals, Rosco Diagnostica for providing the ROSCO Rapid CARB Screen kits.