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## Brief communication

# Evaluation of phenotypic tests to detect carbapenem-resistant Enterobacteriaceae in colonized patients hospitalized in intensive care units



Leandro Reus Rodrigues Perez<sup>a,b,c,\*</sup>, Diógenes Rodrigues<sup>c</sup>, Cícero Gomes Dias<sup>c,d</sup>

<sup>a</sup> Programa de Pós-graduação em Ciências Farmacêuticas, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

<sup>b</sup> Laboratório de Pesquisa em Resistência Bacteriana (LABRESIS), Centro de Pesquisa Experimental (CPE), Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil

<sup>c</sup> Hospital Mãe de Deus, Porto Alegre, RS, Brazil

<sup>d</sup> Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, RS, Brazil

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## ABSTRACT

In this study, we aimed to evaluate the performance of different phenotypic tests to detect carbapenem-resistant Enterobacteriaceae.

Three different phenotypic methods were evaluated: (1) combined-disk test of meropenem plus phenylboronic acid or EDTA reading after 24 h and 48 h; (2) selective/chromogenic read after 24 h and after 48 h; and (3) overnight selective enrichment broth containing 10 µg ertapenem disk followed by culture on MacConkey agar. A positive result in at least one of the methods was submitted to PCR for *bla*<sub>NDM-1</sub>, *bla*<sub>OXA-48</sub>, *bla*<sub>KPC</sub>, *bla*<sub>SPM-1</sub>, *bla*<sub>IMP</sub>, and *bla*<sub>GES</sub> detection.

Carbapenem-resistant Enterobacteriaceae was detected in 31 (30.4%) of 102 rectal swabs evaluated. All isolates showed to be KPC-2-producing organisms. Results showed excellent agreement among the evaluated tests (positive and negative) ( $\kappa = 0.88$ ).

It is important to state that combined-disk test with phenylboronic acid is not suitable for bacterial identification/isolation. Conversely, selective/chromogenic agar after 48h of incubation showed to be a useful tool, with the advantage of presumptive bacterial identification.

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\* Corresponding author at: Universidade Federal do Rio Grande do Sul, Faculdade de Farmácia, 2752 Ipiranga Avenue, 90610-000 Porto Alegre, RS, Brazil.

E-mail address: [leandro.reus@gmail.com](mailto:leandro.reus@gmail.com) (L.R.R. Perez).

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Nosocomial infections due to carbapenem-resistant Enterobacteriaceae (CRE) have been a worldwide problem in the last few decades. Falagas et al.<sup>1</sup> have recently reported that the number of deaths was significantly higher in patients with CRE infections in comparison to those with carbapenem-susceptible Enterobacteriaceae infections.

Carbapenemase production has been considered as a major challenge for microbiological laboratories due to the vast number of genes that encodes for production of carbapenemase, high ability to disseminate, and difficulties in detecting.<sup>2-5</sup>

The objective of this study was to evaluate three different types of phenotypic tests for the detection of CRE from rectal swabs collected from hospitalized patients.

As part of the routine surveillance, 102 rectal swabs for CRE screening were consecutively obtained from patients admitted to intensive care units at Hospital Mãe de Deus, Porto Alegre, from January through February 2014.

Each swab was initially plated on MacConkey agar (bioMérieux, Brazil) for growth, and combined-disk test (CDT) was applied using 10 µg meropenem (MER) disk (Oxoid, UK) alone, a MER disk plus 10 µl of 40 mg/ml phenylboronic acid (PBA) (Sigma-Aldrich, Germany) for KPC inhibition, and a MER disk plus 10 µl of 0.1 M EDTA (Sigma-Aldrich, Germany) for MBL inhibition. Bacterial growth was screened at 24 h (CDT-24h with PBA or EDTA) and 48 h (CDT-48h with PBA and EDTA) of incubation at 37 °C in ambient air. The results of inhibition were interpreted according to a previous report.<sup>6</sup> Original swabs were inoculated on selective/chromogenic ChromID agar (bioMérieux, Brazil) for reading after 24 h (ChromID-24h) and 48 h (ChromID-48h) of incubation. Suspect colonies were submitted to identification and susceptibility testing. Also, each swab was subsequently suspended in 5 ml of tryptic soy broth to which a 10 µg ertapenem (ERT) disk was added (selective enrichment broth – SEB test). The inoculated broth was incubated overnight period at 37 °C for later plating on MacConkey agar containing 10 µg ertapenem and 10 µg MER disks.<sup>7</sup> Enterobacterial colonies growing around MER and ERT disks were picked up, subcultured, identified to the species level, and subjected to susceptibility testing by the MicroScan automated system (Siemens, USA).

When a positive result was obtained from at least one of the tests, polymerase chain reaction (PCR) for the detection of *bla*<sub>NDM-1</sub>, *bla*<sub>OXA-48</sub>, *bla*<sub>KPC</sub>, *bla*<sub>SPM-1</sub>, *bla*<sub>IMP</sub>, and *bla*<sub>GES</sub> genes was applied.<sup>8</sup>

Statistical analyses were carried out using SPSS for Windows, version 13.0 (SPSS Inc., Chicago, IL). Kappa coefficient

and 95% confidence intervals (CIs) were determined for each category (positive and negative results), in order to determine agreement among the distinct phenotypic tests.<sup>9</sup>

CRE was detected in 31 (30.4%) of 102 rectal swabs evaluated. For all, *Klebsiella pneumoniae* was the sole specie that presented a positive result in the phenotypic tests evaluated while *bla*<sub>KPC</sub> was the sole carbapenemase gene detected. Positive results were more often observed in CDT-48h with PBA (31 observations), followed by Chromid-48, CDT-24 with PBA, SEB and Chromid-24 (Table 1). After 48 h of incubation, a false positive result (none gene detected by PCR) was noted for two samples in CDT with PBA. Also, one sample with no carbapenemase gene grew on ChromID-48h. For all methods, we verified the occurrence of false-negative results (negative result but presence of KPC gene detected), mainly in ChromID-24h (12 cases) and SEB test (6 cases).

Some particular characteristics of each method should be evaluated prior its application as a surveillance method. CDT with PBA shows to be an excellent and rapid method to predict the presence of KPC-producing CRE and 24 h incubation was enough to produce a reliable result. In study by Pournaras et al.<sup>6</sup> DCT with PBA was able to detect and to differentiate KPC and/or MBL production, with the advantage of obtaining results within one day. It is of note that this type of test does not favor the recovery of the isolate for later analyses, such as species identification, antimicrobial susceptibility testing, or molecular typing. Vrioni et al.<sup>3</sup> found 95.1% accuracy with ChromID-24h. However, in our study, one ESBL-producing *K. pneumoniae* grew on ChromID-48h, resulting in a false-positive detection compared with ChromID-24h. Despite some observed discrepancies, all methods tested showed an almost perfect agreement, as assessed by the kappa coefficient (kappa=0.82; 95% CI 0.75-0.88;  $p < 0.001$ ).

It should be pointed out that the SEB test protocol is recommended by the CDC<sup>10</sup> and with modification (by using ERT as inhibitor substrate) by the Brazilian Health Surveillance Agency – ANVISA.<sup>11</sup> It has been adopted as standard protocol by Brazilian laboratories in an effort to prevent CRE dissemination.

Finally, our results demonstrate that different tests have similar performance to detect CRE obtained from surveillance rectal swabs. CDT with PBA proved to be a good test, with the limitation of not allowing for bacterial isolation. Use of a selective/chromogenic medium, such as ChromID, may represent a useful tool for microbiology labs, especially after 48 h of incubation.

**Table 1 – Number of positive and negative results for CRE detection in 102 rectal swabs by using different phenotypic methods.**

Result	CDT-24h	CDT-48h	ChromID-24h	ChromID-48h	SEB test	Kappa (95% CI) <sup>a</sup>
Positive	29	31	19	30	25	0.82 (0.75-0.88)
Negative	73	71	83	72	77	0.82 (0.75-0.88)
Total	102	102	102	102	102	0.82 (0.75-0.88)

<sup>a</sup>  $p < 0.001$

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## Conflicts of interest

The authors declare no conflicts of interest.

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## REFERENCES

1. Falagas ME, Karageorgopoulos DE, Vardakas KZ. Deaths attributable to carbapenem-resistant Enterobacteriaceae infections. *Emerg Infect Dis*. 2014;20:1170-5.
2. Landman D, Salvani JK, Bratu S, Quale J. Evaluation of techniques for detection of carbapenem-resistant *Klebsiella pneumoniae* in stool surveillance cultures. *J Clin Microbiol*. 2005;43:5639-41.
3. Vrioni G, Daniil I, Voulgari E, et al. Comparative evaluation of a prototype chromogenic medium (ChromID CARBA) for detecting carbapenemase-producing Enterobacteriaceae in surveillance rectal swabs. *J Clin Microbiol*. 2012;50:1841-6.
4. Gupta N, Limbago BM, Patel JB, Kallen AJ. Carbapenem-resistant Enterobacteriaceae: epidemiology and prevention. *Clin Infect Dis*. 2011;53:60-7.
5. Carmeli Y, Akova M, Cornaglia G, et al. Controlling the spread of carbapenemase-producing Gram-negatives: therapeutic approach and infection control. *Clin Microbiol Infect*. 2010;16:102-11.
6. Pournaras S, Zarkotou O, Poulou A, et al. A Combined disk test for direct differentiation of carbapenemase-producing Enterobacteriaceae in surveillance rectal swabs. *J Clin Microbiol*. 2013;51:2986-90.
7. Lolans K, Calvert K, Won S, Clark J, Hayden MK. Direct ertapenem disk screening method for identification of KPC-producing *Klebsiella pneumoniae* and *Escherichia coli* in surveillance swab specimens. *J Clin Microbiol*. 2010;48:836-41.
8. Monteiro J, Widen RH, Pignatari AC, Kubasek C, Silbert S. Rapid detection of carbapenemase genes by multiplex real-time PCR. *J Antimicrob Chemother*. 2012;67:906-9.
9. Ludbrook J. Statistical techniques for comparing measurers and methods of measurement: a critical review. *Clin Exp Pharmacol Physiol*. 2002;29:527-36.
10. Centers for Disease Control and Prevention. CRE Toolkit – guidance for control of carbapenem-resistant Enterobacteriaceae (CRE). Atlanta, GA: CDC; 2012 <http://www.cdc.gov/hai/organisms/cre/cre-toolkit/index.html>
11. Agência Nacional de Vigilância Sanitária. Nota Técnica N° 01/2013 – Medidas de Prevenção e Controle de Infecções por Enterobactérias Multiresistentes. Brasília: ANVISA; 2013 <http://portal.anvisa.gov.br/wps/wcm/connect/ea4d4c004f4ec3b98925d9d785749fbd/Microsoft+Word+NOTA+T%C3%89CNICA+ENTEROBACTERIAS+17+04+2013%281%29.pdf?MOD=AJPERES>