Detection of \textit{bla}_{GES-5} in carbapenem-resistant \textit{Kluyvera intermedia} isolates recovered from the hospital environment

Vanessa B. Ribeiro$^{1}$; Alexandre P. Zavascki$^{1,2}$; Franciéli P. Rozales$^{3}$; Mariana Pagano$^{3}$; Cibele M. Magagnin$^{4}$; Carolina S. Nodari$^{5}$; Renato Cassol Ferreira da Silva$^{6}$; Micheline G. Dalarosa$^{6}$; Diego R. Falcê$^{6}$; Afonso L. Barth$^{1,3,5}$

$^{1}$ Centro de Pesquisa Experimental, Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre, Brasil; $^{2}$ Infectious Diseases Service, Hospital de Clínicas de Porto Alegre, Porto Alegre, Brasil; $^{3}$ Programa de Pós-Graduação em Ciências Farmacêuticas; Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brasil; $^{4}$ Programa de Pós-Graduação em Ciências Médicas, UFRGS, Porto Alegre, Brasil; $^{5}$ Faculdade de Farmácia, UFRGS, Porto Alegre, Brasil; $^{6}$ Hospital Nossa Senhora da Conceição, Porto Alegre, Brazil

Running title: Detection of \textit{bla}_{GES-5} in \textit{K. intermedia}

Keywords: carbapenem; resistance; \textit{Kluyvera}; GES-5 carbapenemase

* Corresponding Author: Vanessa B. Ribeiro. Centro de Pesquisa Experimental, Hospital de Clínicas de Porto Alegre, 2350 Ramiro Barcelos St, Porto Alegre, 90.035-903, Brasil. Phone/fax: +55 (51) 33598607, e-mail: vanebley@hotmail.com

† Both authors contributed equally to the article
Kluyvera spp. are uncommon human pathogens. Although a \textit{bla}_{KPC-2} gene has been recently reported in a \textit{K. georgiana} isolate [1], multidrug-resistance among the species of this genus remains rare. In this report, we described the presence of an Ambler Class A carbapenemase, a GES-5, in a carbapenem-resistant Kluyvera spp. recovered from the hospital environment.

Three isolates were recovered from one sink and two distinct taps, used for hand washing of health professionals in an intensive care unit (ICU) from a tertiary-care hospital from Porto Alegre, Brazil. Swabs were collected during May 2013 during an environment surveillance after the detection of NDM-1-producing isolates.

The isolates were identified as \textit{K. intermedia} by the VITEK2 system (bioMérieux, France) and were resistant to carbapenems by the disk-diffusion method. The presence of \textit{bla}_{GES} was detected in all isolates by a multiplex real-time PCR [2]. Sequencing was performed [3] and revealed the presence of \textit{bla}_{GES-5}. Plasmidial DNA, obtained from alkaline lysis, was electroporated into an \textit{Escherichia coli} Top10. Transformants were selected on Luria-Bertani agar containing 0.5 µg/ml of ceftazidime and all of them confirmed the presence of \textit{bla}_{GES} by multiplex real-time PCR. Minimum inhibitory concentrations (MIC) for the isolates and for the transformants, assessed by Etest®, phenotypic tests for carbapenemase detection and molecular typing of the isolates are shown in the table.

GES-type carbapenemases (GES-2, GES-4, GES-5, GES-8, GES-11, GES-14; GES-18, GES-20) have been described in several Gram-negative bacteria from distinct parts of the world [4-6], but are relatively rare in Enterobacteriaceae whether compared to other carbapenemases, such as KPC and metallo-\(\beta\)-lactamases. To our knowledge, this is the first description of \textit{bla}_{GES-5} in isolates of \textit{K. intermedia}. Although this organism is usually non-pathogenic, it has already been demonstrated that Kluyvera spp. may act as
a reservoir of resistance genes to species of clinical relevance [7]. Continued

surveillance in Enterobacteriaceae with reduced susceptibility to carbapenems from the
institution where K. intermedia was obtained did not reveal the presence of \textit{blaGES-5} in
any other isolate (clinical or environmental), until October 2013.

The three carbapenem-resistant \textit{K. intermedia} presented the same clonal profile by
PFGE, but a distinct susceptibility profile to ceftriaxone, cefepime and aztreonam was
observed. Indeed, two isolates presented susceptibility to these drugs, as expected for a
GES-5 enzyme [6,8] and one showed resistance to these antibiotics. Additionally, the
low MICs to carbapenems observed in the transformants indicated that the presence of
GES-5 was not the only determinant for the high level resistance to carbapenems.

Noteworthy, the Modified Hodge Test failed to detect the presence of
carbapenemase activity and the combined-disks assay with boronic acid detected two of
the three isolates.

In summary, this report presents the first description of a GES-5 enzyme in \textit{K.}

intermedia. This finding highlights the need for a continuous monitoring of the presence
of carbapenemases even in non-pathogenic bacteria recovered from the nosocomial
environment.

Acknowledgments

This work was supported by Fundo de Incentivo à Pesquisa e Eventos do Hospital de
Clinicas de Porto Alegre, Coordenação de Aperfeiçoamento de Pessoal de Nível
Superior and Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul
(10/0026-1), Brazil. We thank to Fabiane Jamono Vieira, Laura Czekster Antochevis
and Lisiane Pancotto for their useful assistance with the experiments.
References


Table. Minimum inhibitory concentration, phenotypic and molecular characterization of blaGES-5-producing isolates.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Site of isolation</th>
<th>Minimum Inhibitory Concentration (µg/ml)</th>
<th>MHT</th>
<th>APB</th>
<th>PFGE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IPM MEM ERT DOR SAM TZP CRO FEP ATM GEN AMK PMB CST TGC</td>
<td>IPM MEM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>148F</td>
<td>Sink swab from tap A(^a)</td>
<td>&gt;32 &gt;32 &gt;32 8 &gt;256 32 1 0.5 0.19 6 2 0.75 0.38 0.25 N N N A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T 148Fa</td>
<td></td>
<td>1 0.19 0.047 0.125 48 24 0.5 0.064 0.094 4 2 0.5 0.19 0.19 N N N</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>157F</td>
<td>Tap swab B(^b)</td>
<td>&gt;32 &gt;32 12 8 &gt;256 48 0.75 0.5 0.125 4 1.5 0.19 0.19 0.25 N N P A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T 157Fa</td>
<td></td>
<td>0.75 0.19 0.064 0.125 48 16 0.38 0.064 0.094 2 2 0.125 0.125 0.125 N N N</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>185F</td>
<td>Tap swab A(^b)</td>
<td>&gt;32 &gt;32 &gt;32 &gt;32 &gt;256 &gt;256 &gt;256 &gt;256 &gt;256 &gt;256 &gt;256 48 8 4 0.75 0.125 0.125 0.125 0.125 N N P A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T 185Fa</td>
<td></td>
<td>0.38 0.047 0.023 0.032 12 8 0.19 0.5 0.5 0.75 3 0.19 0.19 0.38 N N N</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Transformants;  
\(^b\) Used for hand washing of health professionals in an intensive care unit;  
\(^c\) Assessed by Etest;  
IPM, imipenem; MEM, meropenem; ERT, ertapenem; DOR, doripenem; SAM, ampicillin-sulbactam; TZP, piperacillin-tazobactam; CRO, ceftiraxone; FEP, cefepime; ATM, aztreonam; GEN, gentamicin; AMK, amikacin; PMB, polymyxin B; CST, colistin; TGC, tigecyclin; MHT, Modified Hodge Test; APB, combined-disk with phenyl boronic acid; N, negative; P, positive.