Screening rectal swabs for carbapenemase genes

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Abstract

In an outbreak setting we screened 16296 samples from 3644 patients by PCR for the presence of blaOXA-48, blaVIM, blaIMP, blaNDM, blaKPC. The gene for blaOXA-48 was found in 43 patients in 9 different species of Enterobacteriaceae. Five patients had Pseudomonas aeruginosa containing blaVIM. The negative predictive value of screening was 100%, the positive predictive value 86%.

We describe here the results of screening for the most prevalent carbapenemase genes VIM, IMP, NDM, KPC and OXA-48 by a real-time multiplex PCR, in the aftermath of a K.pneumoniae containing blaOXA-48 outbreak (5). The mean number of samples per patient was 4.5 (range 1-20). Swabs were collected from discharged contact patients (n=3200), from weekly ICU surveillance (n=351), and from patients (re)admitted and/or considered otherwise at risk (n=361). Rectal swabs were overnight incubated in Brain Heart broth containing 0.25 mg/L ertapenem and, if positive or inhibited in the PCR screen, plated on MacConkey No. 3 agar (Oxoid) supplemented with ertapenem (0.25mg/L). PCR protocols, primer/probes sequences, and their validation have been described previously (13). Positive controls in the PCR screen consisted of P. aeruginosa containing blaVIM2 or blaIMP18 and K. pneumoniae containing blaOXA-48, blaNDM1 or blaKPC1. Antibiotic susceptibility testing was performed using the Vitek 2 system, and MICs for ertapenem and meropenem were determined by E-test (Vitek 2, Etest; bioMérieux, Marcy L’Etoile, France). ESBL confirmatory tests were performed by disk diffusion (3).

In total, 186 rectal swabs from 87 patients were positive by PCR screen, 17 showed inhibition, and 16093 samples were negative. Screen positive and inhibited samples were cultured on MacConkey agar plates supplemented with ertapenem, in addition to 167 screen negatives as a control group, totalling 370. Of these, 318 cultures became positive, growing respectively Escherichia coli (n=170), K. pneumoniae (n=81), Morganella morganii (n=16), P. aeruginosa (n=15), Proteus mirabilis (n=14), Shewanella spp. (n=5), Enterobacter.
cloacae (n=5), Citrobacter spp. (n=4), Klebsiella oxytoca (n=4), Serratia marcescens (n=2), and 1 each of Providencia stuartii and Kluuyvera cryocrescens. Fifty one cultures remained negative, including 17 inhibited samples, and 34 screen positives.

PCR identified blaOXA-48 in 63 of 81 K. pneumoniae isolates, in 60 of 170 E. coli isolates, in 4 K. oxytoca isolates, and in one each of C. braakii, C. farmerii, E. cloaca, K. cryocrescens, M. morganii, and S. marcescens. The blavIM gene was identified in 10 of 15 P. aeruginosa isolates. No carbapenemase genes were detected in screen negative specimens. The negative predictive value of screening was thus 100% and the positive predictive value 86%.

Forty nine patients carried carbapenemase producers: 43 an isolate containing blaOXA-48, 4 a P. aeruginosa containing blavIM, 1 a P. aeruginosa containing blavIM as well as an E. coli containing blaOXA-48. Gene sequencing confirmed blaOXA-48 and blavIM-2. The blavIM gene was found only in P. aeruginosa. Fourteen patients carried an E. coli containing blaOXA-48 only, 10 patients a K. pneumoniae containing blaOXA-48 only, and 9 patients carried both. Two patients carried blaOXA-48 positive K. oxytoca and 1 patient C. braakii. 2 patients had E. coli containing blaOXA-48 in addition to either a blaOXA-48 positive E. cloaca or K. oxytoca, and 2 patients carried K. pneumoniae containing blaOXA-48 with either a blaOXA-48 positive M. morganii or K. cryocrescens. Two patients had 3 blaOXA-48 positive Enterobacteriaceae isolates, including either C. farmerii or S. marcescens in addition to E. coli and K. pneumoniae.

For 158/167 blaOXA-48 negative isolates, antibiotic susceptibilities ranged from ≤0.25-≥16 mg/L for imipenem, and ≤0.25-1 mg/L for meropenem, while 93/133 blaOXA-48 positive isolates, including all different species, showed ranges of 0.5-≥16 mg/L for imipenem, and ≤0.25-4 mg/L for meropenem.

Using the Vitek2 and applying CLSI recommended clinical breakpoints for resistance (3), 27 E. coli and 5 K. pneumoniae isolates would have escaped phenotypic detection (Figure 1). Using the EUCAST ECOFF (7), all blaOXA-48 positive E. coli and K. pneumonia isolates would have been detected. However, this applies also to a large number of blaOXA-48 negatives. The observed phenotypic heterogeneity does not allow for a discrimination between OXA-48 positives and negatives. For E. coli imipenem appears to be the best indicator antibiotic, whereas meropenem appears best for K. pneumoniae.

E-testing was performed with 22 K. pneumoniae, and 29 E. coli blaOXA-48 positive isolates, and included every first isolate of each patient (Figure 2). The ranges of the E-test MIC values for blaOXA-48 positive E.coli and K.pneumoniae are concordant with susceptibility testing using Vitek2.
Most blaOXA-48 positives were from patients considered at risk in the outbreak setting. Two 
blaOXA-48 positive isolates were obtained at the first admission and were therefore considered 
non-outbreak related. Timely knowledge of carbapenemase carriage provides the information 
needed to implement appropriate infection control measures, and to guide adequate treatment 
of these patients. Since the observed negative predictive value of our screening assay was 
100%, new admissions may be considered safe when screening is negative. However, carriage 
may remain undetected until selection for resistant microorganisms after broad-spectrum 
antibiotic therapy.

The positive predictive value of screening was 86%, with false-positives mainly due to 
negative cultures on selective media, or growth failure of carbapenemase producing isolates. 
These patients can be asked to submit a follow-up sample to ensure their status. 
The blaOXA-48 gene is plasmid encoded (4, 9). As a consequence, blaOXA-48 can easily be 
transferred horizontally to other members of the Enterobacteriaceae family, as was observed 
in our study, yielding 9 different species positive for blaOXA-48, excluding Shewanella spp. 
because it can harbour OXA-like enzymes.

The blaVIM gene is integron-encoded and most often part of the chromosomal DNA (2). 
Hence, a much lower frequency of horizontal transfer of the gene is expected, and only P. 
aeruginosa isolates were found to be blaVIM positive. K. pneumoniae containing blaNDM and 
P. aeruginosa containing blaIMP have previously been found, but no clinical isolate containing 
blaKPC has been detected in this study.

In contrast to other carbapenemase genes, blaOXA-48 can be difficult to detect by phenotypical 
methods. We have shown that antibiotic susceptibility testing using Vitek2 or Etests is often 
unable to detect blaOXA-48, corroborating the findings described by Woodford et al. (11).

Our assay can reliably detect the most prevalent carbapenemase genes, but it cannot detect 
genomes that have not (yet) been specifically included in the PCR reaction. Whenever needed, 
other laboratory methods can supplement our assay, as described elsewhere (1, 6, 8, 10, 12).

Acknowledgement 
We thank Matthew McCall MD, PhD for critical reading of the manuscript.
References


Figure 1. Comparison of MIC values for imipenem and meropenem of different $bla_{OXA-48}$ positive and negative isolates

<table>
<thead>
<tr>
<th>$bla_{OXA-48}$</th>
<th>Organism</th>
<th>Imipenem MICs (mg/L)</th>
<th>Meropenem MICs (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤0.25 0.5 1 2 4 8 16</td>
<td>≤0.25 0.5 1 2 4</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=158/157</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C. freundii</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>E. cloacae</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>E. coli</td>
<td>3 4 54</td>
<td>104 1</td>
</tr>
<tr>
<td></td>
<td>K. pneumoniae</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>M. morganii</td>
<td>1 7 6 1</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>P. mirabilis</td>
<td>11 1 2</td>
<td>12 1 1</td>
</tr>
<tr>
<td></td>
<td>P. stuartii</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>S. marscescens</td>
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<td>1</td>
</tr>
<tr>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=93</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C. braakii</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>C. farmerii</td>
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<td>1</td>
</tr>
<tr>
<td></td>
<td>E. cloacae</td>
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<td>1</td>
</tr>
<tr>
<td></td>
<td>E. coli</td>
<td>1 26 14 3 1</td>
<td>32 12 1</td>
</tr>
<tr>
<td></td>
<td>K. cryocrescens</td>
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<td>1</td>
</tr>
<tr>
<td></td>
<td>K. oxytoca</td>
<td>1 1 1</td>
<td>3</td>
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<td>34 4 1</td>
</tr>
<tr>
<td></td>
<td>M. morganii</td>
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</tr>
<tr>
<td></td>
<td>S. marscescens</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

1. no meropenem MIC was available for C. freundii.
2. isolates were negative for other carbapenemase genes.

CLSI recommended breakpoints for resistance (≥4 mg/L) are indicated by darker shading. EUCAST ECOFFs are indicated by lighter shading only for E. coli (≥0.5 mg/L) and K. pneumoniae (≥1 mg/L) for imipenem, and ≥0.25 for meropenem.
Figure 2. Comparison of antibiotic susceptibility testing for meropenem by Vitek2 and E-test (left panels) for bla\textsubscript{OXA-48} positive \textit{E. coli} and \textit{K. pneumoniae}. MIC values represented in mg/L are shown on the y-axes. The lines connect the values of Vitek (left) and E-test (right) of each isolate. The right panels show the MICs for imipenem and ertapenem of bla\textsubscript{OXA-48} positive \textit{E. coli} and \textit{K. pneumoniae}. The x-axe represents each patient isolate. On the y-axes MICs represented in mg/L.