Evaluation of a rapid method to detect KPC carbapenemase based on Maldi-TOF spectra analysis

Anna Rita Centonze¹, Anna Oliani² Giuseppe Cornaglia¹-², Annarita Mazzariol¹-²

¹ Department of Diagnostic and Public Health, Verona University, Italy
² UOC Microbiology and Virology, Azienda Ospedaliera Universitaria Integrata, Verona, Italy

Introduction

The resistance to β-lactams in Enterobacteriaceae, with particular reference to carbapenems is actually one of the main international concern in the last two decades. In hospitals outbreaks occur with high rates of mortality by clonally related strains of bacteria. Clinical microbiology lab needs to quickly identify patients who carry bacterial strains positive for carbapenemases production, so as, to contain their spreading and to address proper pharmacological therapy.

The objective of this study is to standardize the use of Maldi-TOF for quickly identify KPC producers by characteristic peaks related to this carbapenemase activity.

Materials and methods

The study included strains isolated from clinical samples of the Microbiology laboratory of the Azienda Ospedaliera Universitaria di Verona, namely:

- 218 Klebsiella pneumoniae strains: 163 carbapenem resistant and 55 cephalosporins resistant by means of ESBL production or by other resistance mechanisms.
- 30 K. pneumoniae, used as control group, susceptible to all β-lactams
- 139 Escherichia coli strains: 122 ESBL producers, 4 with decreased susceptibility to carbapenems, and 13 carbapenemases producers.
- Other strains included in the study were:
  - 17 carbapenem-resistant Enterobacteriaceae strains, carrying either an NDM or VIM enzyme.

Susceptibility testing was performed for all strains by microdilution test following the EUCAST interpretative guideline (Eucast website). Carbapenemase and ESBL production were confirmed phenotypically by CarbaNP test and ESBL NDP test, respectively. (Nordmann, 2012).

Carbapenemases genes (blaKPC, blaVIM, blaNDM and blaOXA132) on K. pneumoniae and E. coli with decreased susceptibility to carbapenems were detected by multiplex and single PCRs. (Dallenne et al. 2010)

Maldi-TOF Vitek MS (BiomerieuxTM) was used to analyze spectra and identify a peak of 11109 Da (Fig. 1) correlated with KPC enzyme (AF Lau et al. 2014). All carbapenem-resistant strains showed carbapenemase production by a positive CarbaNP test and carried a blaKPC gene. One strain co-carried also a blaVIM gene.

All cephalosporin-resistant strains, showed ESBL production by a positive ESBL NDP test. All showed a negative CarbaNP test, and a negative PCR for all carbapenemases genes.

All K. pneumoniae strains (163/163) harboring blaKPC gene, only 0,61% co-carried also a blaVIM gene (1/163)

Discussion and conclusion

One hundred sixty one out of 163 of K. pneumoniae and 12 out 13 E. coli carbapenem resistant by Maldi-TOF spectra analysis present the 11109 Da peak (Tab. 1) correlated with the plasmid harboring the blaKPC gene.

Table 1. Results of spectra analysis

<table>
<thead>
<tr>
<th>Strain</th>
<th>Present</th>
<th>Absent</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>K. pneumoniae KPC</td>
<td>161</td>
<td>2</td>
<td>163</td>
</tr>
<tr>
<td>K. pneumoniae ESBL or Ertapenem R</td>
<td>0</td>
<td>55</td>
<td>55</td>
</tr>
<tr>
<td>E.coli KPC</td>
<td>12</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>E.coli ESBL or Ertapenem R</td>
<td>0</td>
<td>126</td>
<td>126</td>
</tr>
<tr>
<td>K. pneumoniae susceptible</td>
<td>0</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Enterobacteriaceae carrying NDM or VIM</td>
<td>0</td>
<td>17</td>
<td>17</td>
</tr>
</tbody>
</table>

Neither the cephalosporin-resistant (ESBL bearing) or the beta-lactams susceptible strains showed the 11109 Da peak in the Maldi-TOF spectra analysis. Enterobacteriaceae strains harboring carbapenemase other than blaKPC, namely blaKPC and blaVIM did not show the 11109 Da peak in the Maldi-TOF spectra analysis.

One hundred seventy three out of 176 Enterobacteriaceae strains (K. pneumoniae and E. coli) carbapenem resistant and KPC producers assayed by Maldi-TOF presented the 11109 Da peak correlated with the presence of a KPC enzyme.

The 11109 Da peak was absent in all ESBL producers, susceptible strains and Enterobacteriaceae strains harboring different enzyme.

Maldi-TOF is a good and rapid method for identify KPC producers. Our results confirm that the presence of 11109 Da peak, is a good indicator for the Enterobacteriaceae harboring the pKpQIL plasmid coding for KPC enzyme.