Detection of carbapenemases and other mechanisms of enzymatic resistance to β-lactams in Enterobacteria with diminished susceptibility to carbapenems in a tertiary care hospital in Zaragoza, Spain

M.M. Gómara1, A.I. López-Calleja1, B.M.P. Vela1, I Ferrer1, E. Morilla1, R. Núñez1, M. Vicente1, MJ. Capapé1, F.J. Castillo2,3,4, A. Rezusta1,2,4, MJ. Revillo1,2
1 Servicio de Microbiología-Hospital Universitario Miguel Servet, 2 IIS Aragón, 3 Servicio de Microbiología-Hospital Clínico Universitario Lozano Blesa 4 Universidad de Zaragoza, Zaragoza, Spain

mpgomara@salud.aragon.es

OBJECTIVE

Carbapenemase-producing Enterobacteriaceae (CPE) have emerged in recent years, causing important outbreaks in hospitals worldwide. In the absence of carbapenemases, carbapenem resistance can also be achieved through other mechanisms (AmpC β-lactamases, extended spectrum β-lactamases (ESBLs) or porin loss).

Our objective was to characterize the enzymatic mechanisms of carbapenems and other β-lactams resistance in clinical Enterobacteriaceae with diminished susceptibility to carbapenems in the last two years (2013-2014) at Hospital Universitario Miguel Servet.

RESULTS

- Retrospectively, 88 clinical isolates presented reduced susceptibility to carbapenems (one isolate per patient): 63 were stored and available for the study.
- The most prevalent Enterobacteriaceae were Enterobacter spp. (n=19, 30%), followed by Klebsiella spp (n=17, 27%), Escherichia coli (n=14, 22%) and others (n=13, 21%).
- Phenotypic detection of carbapenemases was positive in 15 strains (Table 1); two of these were PCR confirmed as OXA-48 producers (Klebsiella pneumoniae and Citrobacter koseri).
- ESBL detection was positive in 25 isolates (39.7%); TEM and CTX-M were the most prevalent families (Graphic 1).
- Plasmid-mediated AmpC was detected in 9 isolates (14.3%), most of them amplified with CIT primers (families LAT-1 to LAT-4)
- Derepressed AmpC β-lactamase was present in 18 isolates (28,6%) (Graphic 1).

MATERIALS AND METHODS

Antimicrobial susceptibility testing was determined by MicroScan WalkAway® (Beckman Coulter).

Isolates with reduced susceptibility to at least one carbapenem (imipenem, meropenem or ertapenem according to EUCAST breakpoints for CPE screening) were analysed for the presence of carbapenemases (KPC, OXA-48 and MBL), ESBLs and AmpC enzymes (according to Eucast criteria for ESBLs and AmpC screening respectively) by combined disk methods, followed by PCR confirmation according to Poirel et al 2011, Montseim et al 2007 and Perez Perez 2002.

CONCLUSIONS

- The decreased susceptibility to carbapenems in Enterobacteriaceae in our area is not mainly due to true carbapenemases but rather to β-lactamase activity probably combined with decreased permeability of the outer membrane.
- Most of the isolates studied (82.5%) were ESBL or AmpC producers.
- For the present only two cases of OXA-48 carbapenemase-producing Enterobacteriaceae have been detected in our hospital, unlike other Spanish or European regions.
- Given the existing situation in surrounding areas and the easy and rapid spread of these strains it is necessary to further strengthen their surveillance.

Table 1. Carbapenemase phenotypic detection compared to PCR results

<table>
<thead>
<tr>
<th>PCR</th>
<th>CARBAPENEMASE PHENOTYPIC DETECTION</th>
<th>POSITIVE</th>
<th>NEGATIVE</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>POSITIVE</td>
<td></td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>NEGATIVE</td>
<td></td>
<td>13</td>
<td>48</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>48</td>
<td>63</td>
</tr>
</tbody>
</table>

Figure 1. Examples of multiplex PCR for molecular confirmation. A) CPE Poirel et al, B) ESBL Monstein et al, C) AmpC Perez-Perez et al
M: DNA Molecular Weight Marker, OXA, CTX-M, TEM, DHA, CIT: positive controls

Graphic 1. Distribution of isolates producing ESBL, AmpC and carbapenemases

Graphic 2. Resistance mechanisms distribution