Multiplex PCR for rapid detection of genes encoding oxacillinases and metallo-β-lactamases in carbapenem-resistant Acinetobacter spp.

Acinetobacter spp. resistant to carbapenems have become common in hospitals worldwide (Dijkshoorn et al., 2007). Carbapenem resistance mechanisms described in Acinetobacter spp. include hydrolysis by β-lactamases, alterations in outer-membrane proteins and penicillin-binding proteins, and increased activity of efflux pumps (Peleg et al., 2008). However, carbapenemases, such as metallo-β-lactamase (MBL) or oxacillinases, cause the most concern due to the chance of rapid dissemination (Lee et al., 2005; Poirel & Nordmann, 2006; Brown & Amyes, 2006). Four families of oxacillinases have been described in Acinetobacter baumannii so far: OXA-23-like, OXA-24-like and OXA-58-like enzymes, and OXA-51-like, a chromosomal oxacillinase enzyme present in A. baumannii that when overexpressed can be associated with carbapenem resistance (Turton et al., 2006; Poirel & Nordmann, 2006).

Acinetobacter spp. is one of the most frequent agents of health-care associated infection in Brazil (Mendes et al., 2005; Girão et al., 2008); however, data regarding the prevalence of carbapenemases in Brazilian hospitals is scant (Tognim et al., 2006; Carvalho et al., 2009).

The aim of the present study was therefore to develop a multiplex PCR assay for detecting alleles encoding oxacillinases and MBL to evaluate the presence of carbapenemase genes among nosocomial carbapenem-resistant Acinetobacter spp. isolated from four hospitals in the state of São Paulo, Brazil, during a 7 year period (2002 to 2008).

The study included a total of 68 isolates of A. baumannii, 64 carbapenem-resistant and 4 carbapenem-susceptible strains; 50 (78%) of the resistant strains were isolated from bloodstream infections. The following reference strains were used in this study: P. aeruginosa producing IMP-1 and VIM-1, Acinetobacter spp. producing SIM-1, OXA-23 and OXA-24 enzymes as positive controls, and A. baumannii ATCC 19606 was used as a negative control for all carbapenemase except OXA-51. The isolates were identified by API (NE) test (bioMérieux). MICs of carbapenemases were determined by broth microdilution and interpreted using Clinical and Laboratory Standards Institute breakpoints (CLSI, 2005).

Both multiplex PCRs were performed at the same time with seven pairs of specific primers, one pair for each of the seven gene families (Table 1). The DNA template was obtained at the concentration of 20 ng μl⁻¹ using a genomicPrep cell and tissue DNA isolation kit (Amersham Pharmacia Biotech). The PCR mixture used was as follows: 1 μl DNA template in a 49 μl mixture containing 10 mM Tris/HCl (pH 8.8), 4 mM MgCl₂, 50 mM KCl, 0.1 % Triton X-100, 200 μM each dNTP, 30 nM oxacillinase primers, 200 nM IMP primers, 100 nM VIM primers, 50 nM SIM primers and 1 U Taq DNA polymerase. The PCR conditions were as follows: initial denaturation at 94 °C for 5 min, 33 cycles of 94 °C for 25 s, 53 °C for 40 s and 72 °C for 50 s, followed by a single, final, elongation step at 72 °C for 6 min. The PCR products were then purified using GFX PCR DNA gel band purification kit (GE Healthcare) and subjected to automated DNA sequencing. The aligned sequences were then analysed with the BioEdit sequence program and similarity searches for the nucleotide sequences were performed with the BLAST program (http://www.ncbi.nlm.nih.gov).

An MBL Etest (AB Biodisk) was carried out following the manufacturer’s recommendations to confirm the phenotypic expression of MBL in the strains. Amplification by PCR with oligonucleotide primers specific for ISAba1 region was performed as described elsewhere (Stoeva et al., 2008). The MIC₅₀ values were 64 and 128 μg ml⁻¹, and the MIC₉₀ values were 128 and >128 μg ml⁻¹ for imipenem and meropenem, respectively. All clinical isolates were positive for blaOXA-51-like genes. Twenty-two (34%) of these isolates had

Table 1. Sequences of primers used for multiplex PCR for detection of genes encoding MBLs and oxacillinases in isolates of Acinetobacter spp.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Length (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imp-F</td>
<td>5’-GAATAGAATGGTTAATCTCCT-3’</td>
<td>188</td>
<td>Mendes et al. (2007)</td>
</tr>
<tr>
<td>Imp-R</td>
<td>5’-CCTATCATCATCAACTTTCC-3’</td>
<td>382</td>
<td>Mendes et al. (2007)</td>
</tr>
<tr>
<td>Vim-F</td>
<td>5’-TTTCCATACCGGATTGC-3’</td>
<td>569</td>
<td>Mendes et al. (2007)</td>
</tr>
<tr>
<td>Vim-R</td>
<td>5’-GCATGCTTCATTCATTCGAT-3’</td>
<td>353</td>
<td>Woodford et al. (2006)</td>
</tr>
<tr>
<td>Sim-F</td>
<td>5’-TTTCCATACCGGATTGC-3’</td>
<td>569</td>
<td>Mendes et al. (2007)</td>
</tr>
<tr>
<td>Sim-R</td>
<td>5’-GAATAGAATGGTTAATCTCCT-3’</td>
<td>188</td>
<td>Mendes et al. (2007)</td>
</tr>
<tr>
<td>Oxa-51-like-F</td>
<td>5’-TAATATTTGATGGAGAACCAGA-3’</td>
<td>501</td>
<td>Woodford et al. (2006)</td>
</tr>
<tr>
<td>Oxa-51-like-R</td>
<td>5’-GAATAGAATGGTTAATCTCCT-3’</td>
<td>426</td>
<td>Woodford et al. (2006)</td>
</tr>
<tr>
<td>Oxa-23-like-F</td>
<td>5’-TATTCCTGAGGAGAGGAGGAG-3’</td>
<td>599</td>
<td>Woodford et al. (2006)</td>
</tr>
</tbody>
</table>
blaOXA-23-like genes and four (6%) had the gene blaIMP (Fig. 1). The imipenem MIC among blaOXA-23-like positive strains ranged from 16 to 128 µg ml⁻¹ and was lower compared with IMP-1 positive strains (MIC >128 µg ml⁻¹). Insertion element ISAba1 was detected in all carbapenem-resistant strains except one. Phenotypic expression of MBL was confirmed by MBL Etest in all four IMP-1 positive strains.

OXA-23 carbapenemase has been reported in Brazil during an outbreak of multiresistant Acinetobacter spp. that occurred in two hospitals in the south of the country (Dalla-Costa et al., 2003), and more recently in a study of 110 carbapenem-resistant A. baumannii isolates from eight hospitals in Rio de Janeiro that showed that 87% of strains had the gene encoding carbapenemase OXA-23 (Carvalho et al., 2009). The present study showed that all strains had blaOXA-23-like genes and 34% had blaOXA-23-like genes. However, IMP-1 has been identified in several Brazilian hospitals since 2003 (Gales et al., 2003; Sader et al., 2005; Tognim et al., 2006). A SENTRY Antimicrobial Surveillance Program study of the dissemination and diversity of MBL in Latin America showed that the greatest concentration of carbapenemase-containing strains was in Brazil, and that only IMP-like was identified among isolates of carbapenem-resistant Acinetobacter spp. (Sader et al., 2005). IMP-1 was less frequent in the present study, being identified only in four strains of A. baumannii.

In conclusion, the present study showed that blaOXA-23-like was the most frequent carbapenemase identified among carbapenem-resistant A. baumannii strains isolated from four Brazilian hospitals, and that ISAba1 was present in all carbapenem-resistant strains except one; IMP-1 was present only in four strains. The multiplex PCR assay results were consistent with previous single PCR assays, and the multiplex assay could be a useful tool for the rapid detection of genes encoding both oxacillinas and MBLs, and could help in the implementation of measures for the control of the dissemination of carbapenem resistance in the hospital setting.

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