Enterobacteria producing extended-spectrum β-lactamases and IMP-1 metallo-β-lactamases isolated from Brazilian hospitals

In Latin America, extended-spectrum β-lactamases (ESBLs) are commonly found in members of the Enterobacteriaceae, whereas metallo-β-lactamases (MBLs) have been found exclusively in Pseudomonas and Acinetobacter species (Paterson & Bonomo, 2005; Sader et al., 2005; Walsh et al., 2005). In this regard, following the appearance and widespread dissemination of ESBLs, carbapenems have been the therapy of choice, as they are stable with respect to these enzymes (Paterson & Bonomo, 2005). Unfortunately, ESBL- and MBL-producing members of the Enterobacteriaceae have been observed in Brazilian hospitals since 2003, as reported in this letter. Microbiological and epidemiological aspects associated with the emergence and early dissemination of these unusual strains are disclosed.

From June 2003 to June 2005, seven imipenem-resistant enterobacterial isolates (six isolates of Klebsiella pneumoniae and one isolate of Providencia rettgeri) were identified from six different hospitals (A–F) in the city of São Paulo, in southeastern Brazil (Table 1). These strains were recovered from six patients. Identification and antimicrobial susceptibility profiles were evaluated using the VITEK system (bioMérieux). The enterobacterial isolates were found to be resistant to all extended-spectrum cephalosporins, cephapirins and carbapenems and insensitive to clinically available inhibitors. Curiously, an intermediate susceptibility to aztreonam (MICs 12–16 mg l\(^{-1}\)) was detected. MICs for imipenem were subsequently determined using an agar dilution method (NCCLS, 2000) and Etest according to NCCLS guidelines and the manufacturer’s instructions (AB Biodisk), respectively. The high levels of imipenem resistance (> 32 mg l\(^{-1}\)) observed in these enterobacterial isolates suggested the presence of a carbapenemase. Hydrolysis of imipenem was confirmed by bioassays using either Staphylococcus aureus ATCC 25923 or Micrococcus luteus ATCC 9341, as described previously (Lincopan et al., 2005). A double disc diffusion test using specific β-lactam inhibitors was employed to screen for MBL and ESBL production (Arakawa et al., 2006; Thomson & Sanders, 1992). Hydrolysis of imipenem was inhibited by thiol compounds (2-mercaptopropionic acid) or EDTA, but not by clavulanic acid, suggesting that an MBL was responsible for the carbapenemase activity. The addition of EDTA to imipenem reduced the MIC from >32 mg l\(^{-1}\) to 1-0-0.5 mg l\(^{-1}\) (Table 1). On the other hand, the reduced susceptibility to aztreonam exhibited by the strains suggested an additional ESBL production, as aztreonam is not a substrate for MBL enzymes (Walsh et al., 2005). The presence of this ESBL was also confirmed by the double disc approximation test using clavulanic acid inhibition; however, a ghost zone was observed exclusively between the aztreonam- and clavulanic acid-containing discs.

DNA amplification by PCR was used to search for bla\(_{CTX-M}\), bla\(_{VIM}\), bla\(_{SHV}\) and bla\(_{PER-2}\) ESBL genes, bla\(_{IMP-1}\), bla\(_{IMP-2}\), bla\(_{VIM-1}\) and bla\(_{VIM-2}\) MBL genes and for mapping of class 1 integrons, as described previously (Lincopan et al., 2005). The presence of ESBL and MBL enzymes in the strains was confirmed by PCR (Table 1).

Nucleotide sequencing showed that the bla\(_{IMP}\) gene was identical to the bla\(_{IMP-1}\) gene first described in Serratia marcescens in Japan (GenBank accession no. S71932). Moreover, from integron mapping, the bla\(_{IMP-1}\) gene was found to be on a class 1 integron in all isolates. Epidemiological typing of K. pneumoniae isolates was

<table>
<thead>
<tr>
<th>Hospital</th>
<th>Clinical sample</th>
<th>Date (month/year)</th>
<th>Species</th>
<th>Imipenem MIC (mg l(^{-1}))</th>
<th>Identified β-lactamase</th>
<th>ERIC profile</th>
<th>Clinical outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Blood</td>
<td>6/2003</td>
<td>K. pneumoniae</td>
<td>&gt;32 + EDTA</td>
<td>IMP-1,* CTX-M</td>
<td>A</td>
<td>Death</td>
</tr>
<tr>
<td>B</td>
<td>Blood</td>
<td>10/2003</td>
<td>K. pneumoniae</td>
<td>&gt;32 + EDTA</td>
<td>IMP-1,* CTX-M</td>
<td>A</td>
<td>Death</td>
</tr>
<tr>
<td>C</td>
<td>Blood</td>
<td>1/2005</td>
<td>K. pneumoniae</td>
<td>&gt;32 + EDTA</td>
<td>IMP-1,* CTX-M, TEM-like</td>
<td>A</td>
<td>Death</td>
</tr>
<tr>
<td>D</td>
<td>Urine</td>
<td>4/2005</td>
<td>K. pneumoniae†</td>
<td>&gt;32 + EDTA</td>
<td>IMP-1,* CTX-M</td>
<td>A</td>
<td>Unknown</td>
</tr>
<tr>
<td>E</td>
<td>Peritoneal</td>
<td>4/2005</td>
<td>K. pneumoniae†</td>
<td>&gt;32 + EDTA</td>
<td>IMP-1,* CTX-M, TEM-like</td>
<td>A</td>
<td>Death†</td>
</tr>
<tr>
<td>F</td>
<td>Tracheal</td>
<td>6/2005</td>
<td>Providencia rettgeri</td>
<td>&gt;32 + EDTA</td>
<td>IMP-1,* CTX-M, TEM-like</td>
<td>–</td>
<td>Death†</td>
</tr>
</tbody>
</table>

*Inserted on a class 1 integron.
†From the same patient.
‡Cancer-related.
performed by Enterobacterial Repetitive Intergenic Consensus PCR (ERIC-PCR) using the primer ERIC-2 (5'-AAGTAAAGTACGTGGGGTGAGCG-3') (Cartelle et al., 2004). Genotyping by ERIC-PCR revealed that the six K. pneumoniae isolates were clonally related, showing identical band profiles. However, carbapenem-susceptible K. pneumoniae strains (harbouring CTX-M or SHV-18 ESBLs) showed a very different band profile by ERIC-PCR. Thus, imipenem-resistant K. pneumoniae strains could be assigned the same lineage (named ERIC profile A; Table 1).

In Latin America, MBL enzymes have been found exclusively in non-fermentative species. IMP- and VIM-like MBLs have been reported sporadically since 2001 in Pseudomonas and Acinetobacter species. SPM-1 (so far exclusive to Pseudomonas aeruginosa) is widely disseminated among unrelated Brazilian hospitals (Sader et al., 2005; Walsh et al., 2005). Recently, we described the first case report of an IMP-1-producing K. pneumoniae isolate from a patient in Brazil (Lincopan et al., 2005). In this letter, we report the emergence and early dissemination of this unusual K. pneumoniae isolate in different hospitals in São Paulo, Brazil, together with the first report of a Providencia rettgeri isolate producing an integron-borne MBL in Latin America. Surprisingly, these isolates harboured ESBL- and MBL-encoding genes (Table 1). Such an accumulation of resistance determinants in one strain imposes a tremendous limitation on the therapeutic choices available for the treatment of infections caused by Gram-negative species. In fact, the production of these two enzymes contributed to treatment failure and death of four infected patients (Table 1). This configuration of genes may be better adapted to spread among susceptible hosts in nosocomial settings. However, the fact that the blagIMP-1 gene in the isolates was found to be inserted into a class 1 integron suggests versatility in its dissemination to and from other species such as Acinetobacter species and Pseudomonas aeruginosa (Sader et al., 2005; Walsh et al., 2005).

The detection of a single genotype of K. pneumoniae among unrelated hospitals in this study indicates that this type of strain may in fact be more prevalent than previously thought, or perhaps that it has been unreported or underdiagnosed. In this respect, during the 44th Interscience Conference on Antimicrobial Agents and Chemotherapy, Grinbaum et al. (2004) performed a study using IMP-1-producing K. pneumoniae in a teaching hospital in São Paulo at the end of 2003 and beginning of 2004. It is likely that the six isolates reported here have the same genotype as those described by Grinbaum and co-workers by comparison of their characteristics disclosed in that study. Moreover, evidence of clonal spread of K. pneumoniae isolates displaying an ESBL phenotype has been well documented, revealing the existence of predominant clusters in specific geographic areas (Deshpande et al., 2004). These results strongly suggest the possibility of dissemination of ESBL- and MBL-producing isolates in nosocomial settings in Brazil.

To date, IMP-1-producing Providencia rettgeri isolates have been identified only in Japan (Shiroto et al., 2005). In the present study, imipenem-resistant Providencia rettgeri was recovered from a routine surveillance culture from an 82-year-old patient suffering from terminal cancer. In this case, although tracheal colonization by the IMP-1-producing Providencia rettgeri was documented, the patient died because of complications related to a peritoneal tumour.

In recent years, the frequency of ESBL-producing K. pneumoniae in Brazilian hospitals has been higher than that observed in many European and US hospitals, accounting for approximately 50% of K. pneumoniae strains recovered from intensive care units (Kiffer et al., 2005; Paterson & Bonomo, 2005) and related to high mortality rates (Marra et al., 2006). This widespread occurrence of ESBLs should provide a salutary lesson from which we should draw experience to aid us in combating the spread of MBLs (Paterson & Bonomo, 2005; Walsh et al., 2005).

In summary, physicians will require improved diagnostic tests from clinical microbiology laboratories to recognize the emerging threat of MBLs. Early recognition of these phenotypes will aid in the control of dissemination and in the drawing up of effective therapeutic and epidemiological measures.

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