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, cephamycins 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.*, 2000; Thomson & Sanders, 1992). Hydrolysis of imipenem was inhibited by thiol compounds (2-mercaptoacetic acid and 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*_{TEM}, *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 1. Epidemiological and microbiological data for the ESBL- and integron-mediated MBL-producing enterobacterial isolates

Hospital	Clinical sample	Date (month/year)	Species	Imipenem MIC (mg l ⁻¹)		Identified β -lactamase	ERIC profile	Clinical outcome
				Alone	+ EDTA			
A	Blood	6/2003	<i>K. pneumoniae</i>	> 32	1	IMP-1,* CTX-M	A	Death
B	Blood	10/2003	<i>K. pneumoniae</i>	> 32	1	IMP-1,* CTX-M	A	Unknown
C	Blood	1/2005	<i>K. pneumoniae</i>	> 32	0.5	IMP-1,* CTX-M, TEM-like	A	Death
D	Urine	4/2005	<i>K. pneumoniae</i> †	> 32	1	IMP-1,* SHV-like	A	Death
D	Peritoneal	4/2005	<i>K. pneumoniae</i> †	> 32	1	IMP-1,* SHV-like	A	Death
E	Blood	4/2005	<i>K. pneumoniae</i>	> 32	1	IMP-1,* TEM-like	A	Death
F	Tracheal	6/2005	<i>Providencia rettgeri</i>	> 32	0.5	IMP-1,* CTX-M, TEM-like	–	Death‡

*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'-AAGTAAGTGACTGGGGTGAGCG-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 *bla*_{IMP-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) reported an outbreak of 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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