

Occurrence of KPC-2-producing *Klebsiella pneumoniae* strains in hospital wastewater

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Carbapenem-hydrolysing β -lactamase KPC-producing isolates of *Klebsiella pneumoniae* are a major problem and concern. The first KPC-producing isolate was *K. pneumoniae* from the USA, identified in 1996, with subsequent identifications in China, Europe, Israel, Central and Brazil.¹ The KPC β -lactamases, a mostly plasmid-encoded enzyme from *K. pneumoniae*, hydrolyse penicillins, cephalosporins, monobactams (aztreonam) and carbapenems, and are weakly inhibited by clavulanic acid and tazobactam.² Genetic analysis of *bla*_{KPC} genes indicates that their mobility and dissemination are related to a Tn3-based transposon, Tn4401, which is carried by large plasmids varying in size and structure.^{3,4} During the last few years, several carbapenem-hydrolysing β -lactamase KPC-producing strains have been reported in Brazil in hospitalised patients.⁵ This report describes the first detection of KPC-2-producing *K. pneumoniae* strains isolated in wastewater of a hospital sewage treatment plant, Rio de Janeiro, Brazil.

In August and December 2008, two KPC-2-producing isolates were recovered from effluents of a sewage treatment plant that services a hospital located in the metropolitan area of Rio de Janeiro, Brazil. The strains were collected from clarifier tank effluent ($N=1$) and chlorine contact tank effluent ($N=1$). The antimicrobial susceptibility profile was determined by the agar diffusion method according to CLSI guidelines.⁶ After species identification and antimicrobial susceptibility testing, isolates were screened for the ESBL and carbapenemase-producing phenotypes by the standard double-disc synergy test and a modified Hodge test. The minimum inhibitory concentrations were determined using Etest strips according to the manufacturer's recommendations. Polymerase chain reaction testing was performed, as previously described, for the presence of the *bla*_{KPC}, *bla*_{TEM}, *bla*_{SHV} and *bla*_{CTX-M} genes. Amplification products were purified and sequenced in 3730 DNA Analyser (Applied Biosystems, CA, USA), at the PDTIS-IOC DNA Sequencing Platform. Sequences were compared with those in the GenBank database.

All *K. pneumoniae* isolates showed resistance to broad-spectrum cephalosporins and carbapenems. We detected the co-resistance of piperacillin/tazobactam, ciprofloxacin and trimethoprim-sulfamethoxazole. The *K. pneumoniae* strains were of two genotypes (A, B). KPC-2-producing isolates harboured other β -lactamase enzymes detected by the presence of the genes *bla*_{KPC}, *bla*_{TEM}, *bla*_{SHV} in all strains, whereas *bla*_{CTX-M} was carried only by the genotype A. Sequencing of *bla*_{KPC} revealed KPC-2 in all strains studied (Table I).

The occurrence of KPC-2-producing *K. pneumoniae* isolates in hospital is concerning and may have a real impact on public health, principally by dissemination of these micro-organisms

and their plasmids into the environment. The rapid dissemination of KPC enzymes worldwide and the consequences for treatment and infection control measures warrant a high degree of awareness and monitoring of these enzymes. The low efficacy or lack of hospital sewage treatment may contribute to the dissemination of KPC-2-producing and other multidrug-resistant bacteria from the hospital to the environment. Thus, the hospital effluent may be considered as a potential vector of contamination and spread of these emerging resistance determinants and multidrug-resistant micro-organisms. Approaches to reducing environmental microbial contamination should be considered by hospitals. However, the vast majority of hospitals in developing countries do not have sewage treatment plants, which exacerbates the problem and may have important public health implications.

Conflict of interest statement

None declared.

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Table I

KPC-producing *K. pneumoniae* strains isolated from hospital wastewater

Strain	Date	Genotypes	MIC (mg/L)			β -Lactamases	Co-resistance ^a
			IPM	MEM	ETP		
Kp 160	August 2008	A	2	4	>32	KPC-2; CTX-M; SHV; TEM	SXT, TZP, CIP
Kp 232	December 2008	B	3	6	>32	KPC-2; SHV; TEM	TZP

MIC, minimum inhibitory concentration; IPM, imipenem; MEM, meropenem; ETP, ermapenem; SXT, trimethoprim-sulphamethoxazole; TZP, piperacillin/tazobactam; CIP, ciprofloxacin.

^a Intermediate was considered resistant in this analysis.