Outbreak of carbapenem-resistant *Klebsiella pneumoniae*: two-year epidemiologic follow-up in a tertiary hospital

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This study describes a carbapenem-resistant Klebsiella pneumoniae *(CRKP) outbreak that occurred from October 2008-December 2010. Polymerase chain reaction assays were performed to detect the* bla_{KPC} *gene and molecular typing was performed using pulsed-field gel electrophoresis (PFGE). There were 33 CRKP infections; PFGE revealed five genotypes: genotype A in five (15%), B in 18 (55%), C in eight (24%) and two unique profiles. Genotype B was disseminated in all hospital units and belonged to the same clone identified in 11 different hospitals in the state of São Paulo. Sixteen (48%) patients died. Seven isolates (21%) were resistant to polymyxin B and 45% were resistant to tigecycline and amikacin.*

Key words: carbapenem-resistant *K. pneumoniae* - nosocomial outbreak - polymyxin resistance

The emergence of strains of multidrug-resistant *Klebsiella pneumoniae* has been reported with increasing frequency in several countries worldwide. Carbapenem-resistant *K. pneumoniae* (CRKP) can cause nosocomial infections and outbreaks with high mortality rates (Maltezou 2009). Such infections occur mainly in patients admitted to Intensive Care Units (ICU) with several underlying diseases and histories of having received prolonged courses of antibiotics (Maltezou 2009). Several resistance mechanisms have been described. Carbapenemase production (KPC), metallo-β-lactamase (MBL) and porin loss, combined with the production of extended-spectrum beta-lactamase (ESBL), are described as the most common mechanisms of carbapenem resistance (Souli et al. 2008, Peirano et al. 2009).

Control measures, including contact precautions for infected or colonised patients and active surveillance in high-risk units, have been recommended as strategies to control CRKP outbreaks (Ben-David et al. 2010). In our hospital, CRKP had never been detected until October 2008. The objectives of this study were to describe the epidemiological findings in a CRKP outbreak and to verify the existence of clones that are present in other hospitals in the state of São Paulo (SP).

Hospital Brigadeiro is a public, tertiary-care teaching hospital that is part of the Brazilian National Health Care System. It has the following specialised clinics: Oncology, Haematology, Bone Marrow Transplantation, Nephrology, Renal and Liver Transplant Units, ICU in addition to other clinical and surgical units. Renal and Liver Transplant Units were recently opened for patient admission in December 2009 and May 2010, respectively. The hospital has 130 beds and 4,300 admissions per year and offers medical and surgery training. During the period from October 2008-December 2010, there were 33 cases of infections caused by CRKP.

The isolates evaluated in the hospital's laboratory were sent to the Adolfo Lutz Institute, a public health laboratory, to confirm our initial identification of CRKP, to assay for the production of carbapenemases and to determine the level of genetic relatedness between samples.

K. pneumoniae was confirmed by classical phenotypic methods. Disk-diffusion tests were performed to determine susceptibility to antimicrobials, according to the Clinical and Laboratory Standards Institute (CLSI 2010). Isolates were screened for the ESBL phenotype by the standard double-disk synergy test and KPC was screened both by evaluating breakpoints and using modified Hodge test, again according to CLSI guidelines (2010).

Minimal inhibitory concentrations (MICs) were determined by E-test (AB Biodisk, Solna, Sweden) for the following antimicrobial drugs: ceftazidime, cefotaxime, cefotaxime plus clavulanic acid, cefepime, aztreonam, ertapenem, imipenem, meropenem, piperacillin/tazobactam, gentamicin, amikacin, tigecycline tobramycin and polymyxin (CLSI 2010). Imipenem plus ethylenediamine tetraacetic acid (EDTA) strips were used to detect the presence of metallo-beta-lactamases. Tigecycline and polymyxin B were evaluated using breakpoints for Enterobacteriaceae recommended by European Committee on Antibiotic Susceptibility Testing (≤ 2 and ≥ 4 μg/L for susceptible and resistant to polymyxin B, respectively, and ≤ 1, 2 and ≥ 4μg/mL for susceptible, intermediate and resistant to tigecycline, respectively).

Mueller Hinton agar (Oxoid Ltd, Basingstoke, Hampshire, England) plates were freshly made for determining the susceptibilities of the samples to antimicrobial agents.

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Pseudomonas aeruginosa ATCC 27853, *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218, *K. pneumoniae* ATCC 700603 and *Enterococcus faecalis* ATCC 29212 were used for quality control of susceptibility tests.

Polymerase chain reaction (PCR) analysis was performed to detect the bla_{KPC} gene using primers described by Rasheed et al. (2008). Molecular typing was performed using pulsed-field gel electrophoresis (PFGE) in which genomic DNA was digested by the restriction enzyme *Xba*I, according to the protocol of the PulseNet Program and standardised by the Centers for Disease Control and Prevention (CDC) (USA) (cdc.gov/pulsenet) based on the methodology described by Gautom (1997). Electrophoresis was performed using the Chef-III DR system (BioRad, Hercules, CA). Relatedness of the isolates was determined according to criteria by Tenover et al. (1995) and the Bionumerics Program, version 5.0, 2007 (Applied Maths, Kortrig, Belgium).

PFGE profiles were also compared with those obtained from CRKP isolates received by the Adolfo Lutz Institute from several different hospitals in SP.

Standard infection control measures were implemented plus the following specific measures: (i) Care was reinforced with urinary catheters and the disposal of urine. The quality and specifications of urinary catheters were reviewed; (ii) Urinary cultures were taken for all patients who were submitted to renal transplantation two, five and seven days after the procedure, starting in January 2010; (iii) Surveillance cultures were performed according to the CDC protocol (cdc.gov/ncidod/dhqp/pdf/ar/Klebsiella_or_Ecoli.pdf). Briefly, rectal swabs were cultured on tryptic soy broth (TSB) containing a meropenem disk (10 µg) and were incubated at 35ºC overnight. Growth on TSB was streaked out on MacConkey agar plates and imipenem (10 µg) and meropenem (10 µg) disks were added. Colonies around the disks were selected for further analysis. Surveillance cultures were started in June 2010 with rectal swabs taken weekly for patients in ICU, quarterly in Haematology and Transplant Units and monthly for all other units. Additionally, surveillance cultures were performed for all patients who shared rooms with CRKP-infected or colonised patients. Isolates obtained from surveillance cultures were neither genotyped nor submitted to PCR for bla_{KPC}.

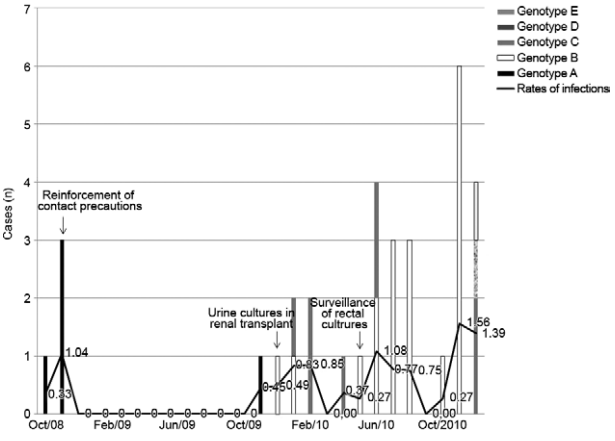
During the period from October 2008-December 2010, 33 patients presented with infections caused by CRKP. The median age of patients was 54 years (range 3-94 years) and 20 (61%) patients were male. Fourteen (42%) patients had urinary tract infections, 11 (33%) had bloodstream infections, seven (21%) had pneumonia and one had a skin infection. Six patients had either been transferred from or had undergone procedures before CRKP infection in five different hospitals of the city of São Paulo.

PFGE revealed five genotypes, which are named genotypes A through E: genotype A in five (15%) patients, genotype B in 18 patients (55%), genotype C in eight patients (24%) and unique profiles in two remaining patients (6%). The monthly distribution of genotypes, intervention measures and rates of infection per 1,000 patient-days are described in Figure.

Genotype A was the first genotype identified and few patients in the ICU were affected. Genotype C was limited to patients with renal diseases in the nephrology, urology and renal transplant units. Neither of these genotypes disseminated to other locations in the hospital. After its introduction to the hospital, genotype B spread to all units and became the predominant clone. This genotype affected mainly haematological and intensive care patients and was the same as that identified in 11 hospitals in SP. This clone seems to have caused a statewide spread and has persisted in our hospital despite control measures. CRKP has been reported previously in Brazil (Pereira et al. 2011, Seki et al. 2011); we believe that, in our hospital, the introduction of new clones on different occasions led to patient-to-patient spread and caused additional control measures to be necessary. The control measures, such as reinforcement of contact precautions, urinary culture surveillance and rectal swabs, were adopted to avoid an outbreak, but several introductions of new strains coming from other hospitals may have made control difficult. One limitation of this study was that it did not measure compliance to control measures. It is also possible that after the initial introduction of genotype B to the hospital, the gene may have jumped to other *K. pneumoniae* clones.

All of the strains were ESBL producers and none of the isolates produced MBL. PFGE genotype A was not a KPC producer, but it presented porin loss combined with ESBL (data not shown). One interpretation of these data is that genotype A was not part of the outbreak and may be a clone that had already been circulating in the hospital and that had lost a porin over time. All isolates of genotypes B and six (75%) isolates of genotype C were KPC producers. Two isolates from genotype C were different from the others of that same genotype in that they did not produce KPC. It is possible that intrinsic changes led to the loss of the KPC gene.

All *K. pneumoniae* isolates were resistant to all cephalosporins, aztreonam, ertapenem and piperacillin/tazobactam. Seven isolates (21%) were resistant to poly-



Monthly distribution of genotypes and rates of infection per 1,000 patients-days of carbapenem-resistant *Klebsiella pneumoniae* and interventions measures, from October 2008-December 2010 in Hospital Brigadeiro, state of São Paulo, Brazil.

myxin B and resistance or intermediate susceptibility to tigecycline and amikacin occurred in 15 patients (45%), to gentamicin in eight patients (24%), to tobramycin in 18 patients (55%), to meropenem in 26 patients (79%) and to imipenem in 31 patients (94%). The resistance to polymyxin in our study is higher than in other studies. A recent study reported 6.8% colistin-resistant in isolates from 221 patients with bacteraemia (Suh et al. 2010). Polymyxin-resistant *K. pneumoniae* has been described during polymyxin treatment or as emerging in an ICU after long periods of colistin administration (Antoniadou et al. 2007, Lee et al. 2009), but in our study only one of seven patients had received polymyxin previously, mostly of genotype A and isolated in ICU (data not shown).

Sixteen of the 33 patients (48%) died during hospitalisation: nine of 11 (82%) with bloodstream infections, three of seeven (57%) with pneumonia and three of 21 (21%) with urinary tract infections. Patients infected with KPC producers, non-KPC producers and polymyxin-resistant strains presented mortality rates of 40%, 75% and 71%, respectively.

Few therapeutic options are available against CRKP*:* imipenem or meropenem (if MIC $\leq$ 1 µg/mL), polymyxin, tigecycline or a combination of antibiotics associated with the removal of invasive devices (Souli et al. 2010). For polymyxin-resistant infections, the options may be tigecycline (although experience in treating severely ill patients is limited), aminoglycosides, high doses of polymyxin or a combination of antibiotics. However, more studies are necessary.

Surveillance rectal cultures were performed in 313 patients and 38 (12%) were positive during the period from June-December 2010. These isolates were not genotyped. Our carriage rates were higher than those described in other studies, which varied from 5.4-9% (Ben-David et al. 2010, Wiener-Well et al. 2010). Our strategy of limiting surveillance to high-risk patients, predominantly in the ICU, haematology unit, may explain this.

In conclusion, CRKP infections were caused by five different genotypes, three of which presented a clonal distribution. One genotype was identical to isolates from 11 other hospitals in SP.

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