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Major article

Molecular epidemiology of *Klebsiella pneumoniae* carbapenemase-producing Enterobacteriaceae in different facilities in Southern Brazil

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Background: *Klebsiella pneumoniae* carbapenemase-producing *K pneumoniae* (KPC-KP) outbreaks have been reported in many countries, including Brazil. The incidence of KPC-KP infection has increased in the first semester of 2011 in Curitiba, the capital of Parana, in Southern Brazil.The aim of this study was to characterize the infections and clonal diversity of KPC-KP isolates from several institutions in Curitiba.
Methods: KPC-KP from several clinical samples and rectal swabs taken between April 2010 and July 2012 were included. One isolate per patient was evaluated. All isolates were submitted to polymerase chain reaction (PCR) for bla_{KPC}. The genetic relatedness was evaluated using strain clustering by an automated repetitive extragenic palindromic (rep) PCR-based typing system.
Results: There were 641 samples that were positive for *K pneumoniae* carbapenemase-2 carbapenemase. There were 129 samples randomly selected for clonality evaluation. PCR and strain clustering by the automated rep PCR-based typing system identified 7 clones (A-C and E-H). Clone E was identified in only 1 hospital, and all other clones were found in >2 hospitals. Clones C and G were the most disseminated among hospitals. The infection and colonization occurred in 14 out of the 32 main hospitals in town. Similar clones were found in 2 hospitals that are administered by the same group. Another clone (H) was found in 2 hospitals receiving patients from the same municipal emergency unit.
Conclusion: The KPC-KP outbreak in Curitiba is polyclonal, and the source is unknown. Some hospitals share the same clones.

Klebsiella pneumoniae carbapenemase (KPC) was identified in KPC-producing *K pneumoniae* (KPC-KP) isolated in North Carolina in 1996. After 2001, several outbreaks of KPC-KP and other KPC-producing Enterobacteriaceae were reported in hospitals located in the New York area.[1] In 2005, KPC-KP was described in France, and some outbreaks have been reported in many countries (eg, Israel, China, Greece).[2-4] In South America, the first detection of a KPC-KP isolate occurred in Colombia in 2006.[5] In Brazil, KPC-KP has been reported in São Paulo in 2005, Recife in 2006, and in Rio de Janeiro in 2007.[6-8]

In local surveys from January 2010-December 2010, KPC-KP was related to 1%-2% of hospital-associated infections in Curitiba, the capital of Parana, in Southern Brazil. The incidence increased to 5%-8% in the first semester of 2011.[9] The clinical importance of KPC-KP infection is because of its high mortality described in some series in the city.[10,11]

Epidemiologic tools can be used to investigate outbreaks, to confirm and define the transmission behavior of ≥1 clones, to test hypotheses regarding the clonal origin or transmission modes, and to monitor and control epidemic reservoirs.

Considering the current KPC situation worldwide and the recent cases in Southern Brazil, the aim of this study was to characterize infection and colonization and clonal diversity of KPC-KP isolates from several institutions in the same city.

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MATERIAL AND METHODS

Setting

According to local surveillance recommendations, every carbapenem-resistant Enterobacteriaceae (CRE) detected in any hospital in Curitiba should be reported to the municipal health department, and the sample should be sent to the central state laboratory for molecular identification of the carbapenemase gene. Curitiba is a Southern Brazilian city with 30 major hospitals with 4,650 available beds. The local population is about 2 million people, and another 2 million people live in the metropolitan area.

All hospitals have infection control teams, and <10% of beds are in intensive care units. Less than 30% of hospitals have intensive care units. There is regular patient transferring between some hospitals because of the lack of beds in the intensive care units. One year after the first case of KPC, guidelines for continued vigilance in hospitals were developed. Vigilance rectal swab was implemented on hospital admission and intensive care unit discharge. Some hospitals have their own routine surveillance procedures.

Every patient with a positive culture for CRE is isolated according to the U.S. Centers for Disease Control and Prevention recommendations.[12] The length of isolations was determined as 6 months after 1 negative rectal swab for CRE.

Antibiotic management programs are specific for each hospital. The control is based on forms with restricted use of carbapenems and high-cost antibiotics (polymyxin, tigecycline, linezolid, daptomycin).

Clinical isolates

CRE from several clinical samples and rectal swabs between April 2010 and July 2012 were included. One isolate per patient was evaluated.

Screening of CRE samples

All CRE from clinical samples were confirmed by automated broth microdilution system or disk diffusion at the LACEN, in accordance with the Clinical Laboratory Standards Institute.[13]

Isolates showing resistance to any carbapenems were tested for the detection of carbapenemase production using the modified Hodge test.[14] All isolates were submitted to polymerase chain reaction (PCR) for bla_{KPC}. Only *K pneumoniae* was included in the study for clonality analysis.

PCR and sequencing of β-lactamase genes and genomic similarity of KPC-KP isolates

DNA extraction was performed with a MO BIO UltraClean Microbial DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA) from pure bacterial culture. The DNA is eluted with a minimum concentration of 25-50 ng/μL to the amplification quantitated on the biospectrophotometer. Performing repetitive extragenic palindromic (rep) PCR, primers bind to many specific repetitive sequences intercepted throughout the genome. Multiple fragments with several lengths are amplified, and amplicons can be separated by electrokinetics according to their mass, using microfluidic chips and a Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA).

The genetic relatedness of all KPC-KP isolates was evaluated using strain clustering by an automated rep PCR-based typing system (DiversiLab, bioMérieux, Athens, GA), and results were analyzed and interpreted with the DiversiLab Web-based software using the Pearson correlation method. A 90% similarity cutoff was chosen for molecular similarity. Samples for rep PCR were selected with random number tables using Microsoft Excel (Microsoft, Redmond, WA). The sample size was calculated considering 90% confidence and 5% sample error.

The presence of bla_{KPC} was confirmed by PCR using NucliSENS KPC (bioMérieux, Marcy-l'Étoile, France).[15] No isolates were screened for metallo-β-lactamase production.

For DNA sequencing determination, the bla_{KPC} gene was amplified as previously described[16] and acquired using an Applied Biosystems 3130 Genetic Analyzer (Life Technologies, Carlsbad, CA).

Table 1
Site of infection and colonization for 129 samples of *Klebsiella pneumoniae* carbapenemase-producing *K pneumoniae* randomly evaluated for a clonality test

Sample	n	%
Rectal swab	39	30.5
Blood	27	21.1
Urine	15	11.7
Tracheal aspiration	14	10.9
Catheter tip	12	9.4
Bronchoalveolar lavage	7	5.5
Cerebrospinal fluid	5	3.9
Surgical site	4	3.1
Abdominal fluid	3	2.3
Tissue biopsy	2	1.6
Urethral secretion	1	0.8

RESULTS

During the study period, 641 samples were positive for KPC-2 carbapenemase. There were 129 samples randomly selected for clonality evaluation. In hospitals with few samples (1-9), all isolates were included (Table 1).

PCR and strain clustering by an automated rep PCR-based typing system were performed in 129 reports of infection or colonization, and 7 clones (A-C and E-H) were identified. Clone E was identified in only 1 hospital, and all other clones were found in >2 hospitals. Clones C and G were the most disseminated among hospitals.

The infection and colonization occurred in 14 out of the 32 main hospitals in Curitiba (Fig 1). Only 7 isolates were from 2010. Initially, clones A and E were isolated. Clone A appeared in 3 hospitals, and clone E appeared in only 1 facility. Clone C was isolated in hospital 13 chronologically later than clones A and E and disseminated to 12 hospitals, becoming the predominant clone in the city. Clones A, C, F, and G, were found in hospital 11. Clone B was found in 2 hospitals that are administered by the same group. Clone G showed a pattern similar to that of clone C. Clone H was found initially in 2 hospitals receiving patients from the same municipal emergency unit.

Hospitals where KP-KPC has been detected have been between 100 and 600 beds. Two of the hospitals have >400 beds, and the rest are medium to small. All of them have an emergency department; 7 hospitals are public, and 7 are private. All hospitals had infection control services with an infectious diseases specialist. However, only 7 of the hospitals had a stewardship program.

DISCUSSION

The current study showed that KPC-KP dissemination was polyclonal in Curitiba and inside facilities, but 84% of isolates were from 2 predominant clones. The explanation for the clonal predominance was not evaluated. In Brazil, hospitals can be public or private. Large public hospitals are usually linked to universities. Even in those large hospitals, the number of beds rarely exceeds