Detection of antimicrobial resistance genes in beta-lactamase- and carbapenemase-producing *Klebsiella pneumoniae* by patient surveillance cultures at an intensive care unit in Rio de Janeiro, Brazil

*Detecção de genes de resistência a antimicrobianos em Klebsiella pneumoniae produtoras de betalactamases e carbapenemases por culturas de vigilância de pacientes em uma unidade de terapêutica intensiva no Rio de Janeiro, Brasil*

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**ABSTRACT**

**Introduction:** The increasing incidence of multi-resistant microorganisms has been considered a public health problem. One of the routines included in hospital practice is the screening of colonized and/or infected patients. **Objective:** The aim of this study was to evaluate the genetic variability and clonal relationships of extended-spectrum beta-lactamase (ESBL)-producing *K. pneumoniae*, from surveillance cultures, at an intensive care unit, in Rio de Janeiro, Brazil. **Material and methods:** Seventy *K. pneumoniae* isolates were obtained from rectal swabs (March 2013 to March 2014). Antimicrobial susceptibility was assessed by VITEK 2 System. Resistant genes *bla*<sub>SHV</sub>, *bla*<sub>TEM</sub>, *bla*<sub>OXA-1</sub>, *bla*<sub>CTX-M-15</sub>, *bla*<sub>VIM</sub>, *bla*<sub>IMP</sub> and *bla*<sub>NDM</sub> were investigated by polymerase chain reaction (PCR); genetic diversity, by Enterobacterial Repetitive Intergenic Consensus-PCR (ERIC-PCR). **Results:** Strains showed high resistance rates to cefepime (94%), ceftazidime (96%), ertapenem (61%), imipenem (54%) meropenem (43%) and ciprofloxacin (69%). The most prevalent genes were *bla*<sub>SHV</sub> (69%), *bla*<sub>TEM</sub> (63%), *bla*<sub>OXA-1</sub> (60%), *bla*<sub>CTX-M-15</sub> (57%), *bla*<sub>VIM</sub> (47%), *bla*<sub>IMP</sub> (16%). Genes *bla*<sub>VIM</sub>, *bla*<sub>IMP</sub> and *bla*<sub>NDM</sub> were not detected. Twenty nine profiles of resistance genes were observed, with 23% carrying at least five genes. A great genetic diversity (68 ERIC profiles) was also observed among the strains. **Conclusion:** Although no clonal relationship was observed within the isolates, this study revealed alarming data on the antimicrobial resistance deficiently monitored for preventive purposes in Brazil. Our data allow us to conclude that the inclusion of surveillance cultures in health facilities is a recommended strategy aiming particularly at preventing the spread of resistance genes in the hospital environment and, consequently, reducing morbidity and mortality.

**Key words:** infection control; epidemiological surveillance; *Klebsiella pneumoniae*; beta-lactamases; molecular typing.

**INTRODUCTION**

Healthcare-associated infections (HAIs) are a serious problem of public health and exert great impact on morbidity, mortality, length of hospital stay, and costs of diagnostic and therapeutic procedures. There are also effects on patients, their families and the community in general, such as the withdrawal from social life and work, with the resulting social, psychological and economical burden. The increased incidence of multidrug-resistant microorganisms is considered one of the main factors influencing the treatment of these infections. At intensive care units (ICU), high indices of bacterial resistance are observed, especially due to excessive use of antibiotic agents. Understanding the resistance profile of hospital microbiota against antibiotics is fundamental for prevention and control of nosocomial infections.

Bacteria can develop resistance to antibacterial drugs by means of some mechanisms already well disclosed in the literature,
such as alteration in the target site (penicillin-binding proteins [PBPs]), efflux pump, decreased expression of porins (outer-membrane proteins [OMPs]) and beta-lactamase production[4]. The production of extended-spectrum beta-lactamas (ESBLs) is the main resistance mechanism among bacteria of the *Klebsiella* genus. They are able to hydrolyze broad-spectrum beta-lactams, such as third- and fourth-generation cephalosporins, monobactams, but not cephamycins and carbapenems, such as, for example, temoneira enzyme 3 (TEM-3) and sulphhydryl variable 2 (SHV-2). TEM-2 and SHV-1 are not ESBLs, because they hydrolyze just penicillins and first- and second-generation cephalosporins[5,6].

The prevalence of ESBL in *Klebsiella pneumoniae* is increasing worldwide[7-9]. Global data show that the frequency of ESBL-producing *K. pneumoniae* was 44% in South America, 33% in Europe, 22% in Asia and 12% in the United States[10]. Nowadays, the major concerns, however, are the *K. pneumoniae* carbapenemase (KPC)-producing strains, once these enzymes are responsible for resistance to all available beta-lactam antibiotics[11].

The frequencies of the genes responsible for the production of ESBL and carbapenemases vary among themselves and among the bacterial species. These variations make each region have its own characteristics[12]. Resistance is acquired by vertical/horizontal transfer of genes such as *bla*<sub>CTX-M</sub>, *bla*<sub>KPC</sub> and *bla*<sub>NDM</sub>, frequently associated with plasmids[13,14].

One of the routines included in hospital practice is the screening of patients from several inpatient units (ICU, wards) for colonization and/or infection, by collecting materials such as nasal, oral and rectal swabs at admission and during treatment. This measure is called surveillance culture, and it is aimed at avoiding dissemination of these agents[15-17]. The screening specimens of primary surveillance that have been recommended by the Centers for Disease Control and Prevention (CDC) and by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) are stools or the rectal swab[18].

**OBJECTIVES**

The objective of the present study was to investigate antimicrobial susceptibility profiles and to research the frequencies of genes responsible for ESBL and carbapenemase-producing *K pneumoniae* based on active surveillance cultures at an ICU, besides assessing the genetic diversity of these isolates to verify the presence of cross contamination among patients and possible epidemiological implications.

**MATERIAL AND METHODS**

**Study site and population**

Samples of rectal material were collected with sterile swabs from 1,474 adult and pediatric inpatients of the ICU of a federal hospital in Rio de Janeiro, from March 2013 to March 2014 for surveillance cultures.

The study was approved by the Research Ethics Committee of Fundação Oswaldo Cruz (Fiocruz) (346.653).

**Sample processing**

The swabs were streaked on CHROMagar ESBL (Paris, France); the plates, incubated at 36ºC ± 1ºC for 24/48 hours. Bacterial isolates that presented metallic blue or pink colonies, suggestive of ESBL-producing enterobacteria, were selected and streaked on MacConkey agar.

**Phenotypic identification/antimicrobial susceptibility profile (antibiogram)**

Identification and antimicrobial susceptibility testing (AST) were performed by the VITEK® 2 system (BioMérieux). The GN Test Kit cards were used for identification, and AST-239 and AST-105 cards, for antibiogram, according to the manufacturer’s instructions.

**Molecular identification to confirm the *K. pneumoniae* species**

The genomic deoxyribonucleic acid (DNA) was extracted using the DNeasy Blood & Tissue Handbook (QIAGEN®), according to instructions by the manufacturer. The identity of *K pneumoniae* isolates was confirmed by means of amplification of 16S-23S intergenic region of the ribosomal ribonucleic acid (rRNA) genes[10]. Amplification conditions were: an initial denaturation cycle at 95ºC for 5 minutes, followed by 30 cycles of 95ºC for 1 minute, annealing temperature of 56ºC and 72ºC for 2 minutes, and a final extension cycle at 72º for 10 minutes. The fragments were analyzed by gel electrophoresis of 1.5% agarose in buffer Tris borate ethylenediaminetetraacetic acid (EDTA)-0.5× for 1 hour, at 60 V with ethidium bromide (3 mg/ml). The gel was analyzed using the ImageQuant 300 Imager (GE).
Detection of resistance genes

The following resistance genes were investigated: \textit{bla}_{KPC}, \textit{bla}_{OXA-48}, \textit{bla}_{TEM}, \textit{bla}_{SHV}, \textit{bla}_{CTX-M15}, \textit{bla}_{VIM}, and \textit{bla}_{NDM}, which despite being rarely found in enterobacteria, are reported to occur in \textit{K. pneumoniae}(20, 21).

The mixture for the polymerase chain reaction (PCR) had its final volume of 25 µl, containing 1× PCR Master Mix (Promega); 15 pmol of each primer; around 20 ng of template DNA and Gibco® water. The cycle conditions consisted of an initial step at 95ºC for 5 minutes and 35 amplification cycles at 95ºC for 1 minute, adequate annealing temperature (Table 1) during 1 minute, and 72ºC for 1 minute and a final elongation at 72ºC for 6 minutes. Amplification was carried out in an Eppendorf Mastercycler EP thermal cycler. PCR products were visualized on a 2% agarose gel (Sigma-Aldrich), in 1× Tris-acetate-EDTA buffer, stained with 0.3 ng/ml ethidium bromide. The molecular weight standard 100 bp DNA Ladder (Invitrogen) was used. Analysis was performed with the ImageQuant 300 (GE).

K. pneumoniae INCQS 00628 (ATCC BAA 1705) (\textit{bla}_{KPC} positive), INCQS 00629 (ATCC BAA 1706) (\textit{bla}_{KPC} negative), INCQS 00532 (ATCC 700603) (ESBL positive), INCQS P4475 (\textit{bla}_{NDM} positive), \textit{E. coli} INCQS 00325 (ATCC 35218) (ESBL positive) and INCQS 0033 (ATCC 25922) (ESBL negative) were used as reference.

Sequencing and identity analysis

PCR products were purified using the QIAquick® PCR Purification Kit (QIAGEN), according to the manufacturer, and subjected to sequencing with the Big Dye Terminator Kit by capillary electrophoresis in an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA) (Platform PDTIS/Fiocruz). Chromatograms were converted to FASTA format through software Sequencer 5.0 (Gene Codes Corporation, Ann Arbor, MI). Similarity between nucleotide sequences were determined using the Basic Local Alignment Search Tool (BLASTn) (http://www.ncbi.nlm.nih.gov/BLAST/), at GenBank (National Center for Biotechnology Information [NCBI]).

Clonal characterization of isolates

Enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) is a used for typing the isolates using (ERIC-PCR), using primer ERIC-2 (5'-AAGTAAGTGACTGGGGTGAGCG-3') and amplification conditions according to the previously described protocol(28). Amplification products were analyzed as described before; and the band patterns, with the BioNumerics software version 6.6 (Applied Maths, Kortrijk, Belgium). The dendrogram was built using the Dice index and the unweighted pair-group method using arithmetic averages (UP-GMA)(29).

RESULTS

Identification of isolates

Among the 1,474 samples of collected rectal material, 318 were selected that presented color suggestive of ESBL production on CHROMagar. Among these, 75 presented metallic blue color; and 48, pink color. The metallic blue isolates were phenotypically

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence (5’-3’)</th>
<th>Annealing (ºC)</th>
<th>Fragment (pb)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{bla}_{KPC-2}</td>
<td>TGTCACTGTAGCGGCTTCTCACTGCTCTCAGAAACC</td>
<td>55</td>
<td>863</td>
<td>Yigit et al. (2001) (22)</td>
</tr>
<tr>
<td>\textit{bla}_{OXA-48}</td>
<td>TGTGTTTGTTGCTGATGTAAATATGCTGTTGTCGC</td>
<td>58</td>
<td>177</td>
<td>Monteiro et al. (2012) (23)</td>
</tr>
<tr>
<td>\textit{bla}_{TEM-1}</td>
<td>ATATGTCGCTTCTTACTGCTTTAGGGTGCTCTGACC</td>
<td>55</td>
<td>1080</td>
<td>Jemina et al. (2008) (24)</td>
</tr>
<tr>
<td>\textit{bla}_{CTX-M15}</td>
<td>GTGTGGAATTGCTGACTGTCATGAATGCTGTTGTCGC</td>
<td>54</td>
<td>863</td>
<td>Barguiga et al. (2011) (25)</td>
</tr>
<tr>
<td>\textit{bla}_{SHV-1}</td>
<td>ATGGTTATTTGCTGTTGTCAGCGCGTTTGGGCGGC</td>
<td>55</td>
<td>1051</td>
<td>Barguiga et al. (2011) (25)</td>
</tr>
<tr>
<td>\textit{bla}_{IMP}</td>
<td>GAAGGCGTTTATCAGCGCGTTTGGGCGGC</td>
<td>58</td>
<td>587</td>
<td>Pitout et al. (2005) (26)</td>
</tr>
<tr>
<td>\textit{bla}_{VIM}</td>
<td>GTTGGGCTGGTCAGGGCGCGCGCGTTTGGGCGGC</td>
<td>58</td>
<td>380</td>
<td>Pitout et al. (2005) (26)</td>
</tr>
<tr>
<td>\textit{bla}_{OXA-1}</td>
<td>TGTGAGACTGCGGTCAGAGAGGCGCAGCGGCGGCGGC</td>
<td>60</td>
<td>464</td>
<td>Mulvey et al. (2011) (27)</td>
</tr>
<tr>
<td>\textit{bla}_{NDM-1}</td>
<td>TGTGAGACTGCGGTCAGAGAGGCGCAGCGGCGGCGGC</td>
<td>58</td>
<td>660</td>
<td>Mulvey et al. (2011) (27)</td>
</tr>
</tbody>
</table>

PCR: polymerase chain reaction.

TABLE 1 Detection of beta-lactamase genes by PCR
identified as *K. pneumoniae*, suggestive of ESBL production. The 48 pink isolates, suggestive of *Escherichia coli*, were not included in this work. Among the 75 isolates phenotypically identified as *K. pneumoniae*, 70 were confirmed as *K. pneumoniae* by PCR.

**Antimicrobial susceptibility**

The *K. pneumoniae* isolates presented high percentages of antimicrobial resistance. In relation to carbapenems, resistance was observed to ertapenem, 61% (43/70); to imipenem, 54% (38/70); and to meropenem, 43% (30/70) (Figure 1).

**Detection of beta-lactamase genes**

The genes *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, and *bla*<sub>CTX-M</sub> were found in 56% (39/70), 60% (42/70) and 16% (11/70) of the studied isolates, respectively. The genes *bla*<sub>TMD</sub>, *bla*<sub>TEM</sub> and *bla*<sub>CTX-M15</sub> were also detected. The genes *bla*<sub>KPC</sub>, *bla*<sub>NDM</sub> and *bla*<sub>VIM</sub> were not found (Figure 2); two isolates did not present the investigated genes.

Seventeen percent (12/70) of the isolates presented just one investigated gene; 22% (15/70), two genes; 17% (12/70), three genes; 19% (13/70), four genes; 20% (14/70), five genes; and 3% (2/70), six investigated resistance genes. In relation to the studied genes, 29 resistance profiles were observed (Table 2).

**Typing of *K. pneumoniae* strains**

Sixty-eight isolates exhibiting at least one resistance gene were subjected to ERIC-PCR. Genetic polymorphism was verified among the isolates, with 68 genotypes. However, strains with percent identity above 90% (KP15 and KP29; KP12 and KP46; KP31 and KP28; KP27 and KP45) were found (Figure 3).

### Table 2 – Resistance profile of *K. pneumoniae* isolates according to the detected genes

<table>
<thead>
<tr>
<th>Resistance profile</th>
<th>Resistance genotype</th>
<th>n°. of isolates(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>No gene</td>
<td>2 (2.9%)</td>
</tr>
<tr>
<td>II</td>
<td><em>bla</em>&lt;sub&gt;SHV&lt;/sub&gt;</td>
<td>3 (4.3%)</td>
</tr>
<tr>
<td>III</td>
<td><em>bla</em>&lt;sub&gt;SHV&lt;/sub&gt;</td>
<td>2 (2.9%)</td>
</tr>
<tr>
<td>IV</td>
<td><em>bla</em>&lt;sub&gt;TEM&lt;/sub&gt;</td>
<td>6 (8.6%)</td>
</tr>
<tr>
<td>V</td>
<td><em>bla</em>&lt;sub&gt;TEM&lt;/sub&gt;</td>
<td>1 (1.4%)</td>
</tr>
<tr>
<td>VI</td>
<td><em>bla</em>&lt;sub&gt;KPC&lt;/sub&gt; and <em>bla</em>&lt;sub&gt;SHV&lt;/sub&gt;</td>
<td>2 (2.9%)</td>
</tr>
<tr>
<td>VII</td>
<td><em>bla</em>&lt;sub&gt;TMD&lt;/sub&gt; and <em>bla</em>&lt;sub&gt;TMD&lt;/sub&gt;</td>
<td>7 (10%)</td>
</tr>
<tr>
<td>VIII</td>
<td><em>bla</em>&lt;sub&gt;KPC&lt;/sub&gt; and <em>bla</em>&lt;sub&gt;SHV&lt;/sub&gt;</td>
<td>1 (1.4%)</td>
</tr>
<tr>
<td>IX</td>
<td><em>bla</em>&lt;sub&gt;TMD&lt;/sub&gt; and <em>bla</em>&lt;sub&gt;TMD&lt;/sub&gt;</td>
<td>3 (4.3%)</td>
</tr>
<tr>
<td>X</td>
<td><em>bla</em>&lt;sub&gt;TMD&lt;/sub&gt; and <em>bla</em>&lt;sub&gt;TMD&lt;/sub&gt;</td>
<td>1 (1.4%)</td>
</tr>
<tr>
<td>XI</td>
<td><em>bla</em>&lt;sub&gt;TMD&lt;/sub&gt; and <em>bla</em>&lt;sub&gt;TMD&lt;/sub&gt;</td>
<td>1 (1.4%)</td>
</tr>
<tr>
<td>XII</td>
<td><em>bla</em>&lt;sub&gt;KPC&lt;/sub&gt;, <em>bla</em>&lt;sub&gt;TMD&lt;/sub&gt; and <em>bla</em>&lt;sub&gt;TMD&lt;/sub&gt;</td>
<td>2 (2.9%)</td>
</tr>
<tr>
<td>XIII</td>
<td><em>bla</em>&lt;sub&gt;KPC&lt;/sub&gt;, <em>bla</em>&lt;sub&gt;SHV&lt;/sub&gt; and <em>bla</em>&lt;sub&gt;TMD&lt;/sub&gt;</td>
<td>1 (1.4%)</td>
</tr>
<tr>
<td>XIV</td>
<td><em>bla</em>&lt;sub&gt;KPC&lt;/sub&gt; and <em>bla</em>&lt;sub&gt;TEM&lt;/sub&gt;</td>
<td>1 (1.4%)</td>
</tr>
<tr>
<td>XV</td>
<td><em>bla</em>&lt;sub&gt;KPC&lt;/sub&gt;, <em>bla</em>&lt;sub&gt;TMD&lt;/sub&gt; and <em>bla</em>&lt;sub&gt;TMD&lt;/sub&gt;</td>
<td>1 (1.4%)</td>
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<tr>
<td>XVI</td>
<td><em>bla</em>&lt;sub&gt;TMD&lt;/sub&gt; and <em>bla</em>&lt;sub&gt;TMD&lt;/sub&gt;</td>
<td>1 (1.4%)</td>
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<tr>
<td>XVII</td>
<td><em>bla</em>&lt;sub&gt;TEM&lt;/sub&gt; and <em>bla</em>&lt;sub&gt;SHV&lt;/sub&gt;</td>
<td>1 (1.4%)</td>
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<tr>
<td>XVIII</td>
<td><em>bla</em>&lt;sub&gt;TEM&lt;/sub&gt; and <em>bla</em>&lt;sub&gt;SHV&lt;/sub&gt;</td>
<td>1 (1.4%)</td>
</tr>
<tr>
<td>XIX</td>
<td><em>bla</em>&lt;sub&gt;TEM&lt;/sub&gt;, <em>bla</em>&lt;sub&gt;SHV&lt;/sub&gt; and <em>bla</em>&lt;sub&gt;TMD&lt;/sub&gt;</td>
<td>2 (2.9%)</td>
</tr>
<tr>
<td>XX</td>
<td><em>bla</em>&lt;sub&gt;SHV&lt;/sub&gt; and <em>bla</em>&lt;sub&gt;TMD&lt;/sub&gt;</td>
<td>2 (2.9%)</td>
</tr>
<tr>
<td>XXI</td>
<td><em>bla</em>&lt;sub&gt;KPC&lt;/sub&gt;, <em>bla</em>&lt;sub&gt;TMD&lt;/sub&gt; and <em>bla</em>&lt;sub&gt;TMD&lt;/sub&gt;</td>
<td>2 (2.9%)</td>
</tr>
<tr>
<td>XXII</td>
<td><em>bla</em>&lt;sub&gt;KPC&lt;/sub&gt;, <em>bla</em>&lt;sub&gt;TMD&lt;/sub&gt; and <em>bla</em>&lt;sub&gt;TMD&lt;/sub&gt;</td>
<td>2 (2.9%)</td>
</tr>
<tr>
<td>XXIII</td>
<td><em>bla</em>&lt;sub&gt;KPC&lt;/sub&gt;, <em>bla</em>&lt;sub&gt;SHV&lt;/sub&gt; and <em>bla</em>&lt;sub&gt;TMD&lt;/sub&gt;</td>
<td>2 (2.9%)</td>
</tr>
<tr>
<td>XXIV</td>
<td><em>bla</em>&lt;sub&gt;KPC&lt;/sub&gt;, <em>bla</em>&lt;sub&gt;TMD&lt;/sub&gt; and <em>bla</em>&lt;sub&gt;TMD&lt;/sub&gt;</td>
<td>3 (4.3%)</td>
</tr>
<tr>
<td>XXV</td>
<td><em>bla</em>&lt;sub&gt;KPC&lt;/sub&gt;, <em>bla</em>&lt;sub&gt;SHV&lt;/sub&gt; and <em>bla</em>&lt;sub&gt;TMD&lt;/sub&gt;</td>
<td>2 (2.9%)</td>
</tr>
<tr>
<td>XXVI</td>
<td><em>bla</em>&lt;sub&gt;KPC&lt;/sub&gt;, <em>bla</em>&lt;sub&gt;SHV&lt;/sub&gt; and <em>bla</em>&lt;sub&gt;TMD&lt;/sub&gt;</td>
<td>2 (2.9%)</td>
</tr>
<tr>
<td>XXVII</td>
<td><em>bla</em>&lt;sub&gt;KPC&lt;/sub&gt;, <em>bla</em>&lt;sub&gt;SHV&lt;/sub&gt; and <em>bla</em>&lt;sub&gt;TMD&lt;/sub&gt;</td>
<td>13 (18.6)</td>
</tr>
<tr>
<td>XXVIII</td>
<td><em>bla</em>&lt;sub&gt;KPC&lt;/sub&gt;, <em>bla</em>&lt;sub&gt;SHV&lt;/sub&gt; and <em>bla</em>&lt;sub&gt;TMD&lt;/sub&gt;</td>
<td>1 (1.4%)</td>
</tr>
<tr>
<td>XXIX</td>
<td><em>bla</em>&lt;sub&gt;KPC&lt;/sub&gt;, <em>bla</em>&lt;sub&gt;SHV&lt;/sub&gt; and <em>bla</em>&lt;sub&gt;TMD&lt;/sub&gt;</td>
<td>2 (2.9%)</td>
</tr>
</tbody>
</table>
Figure 3 — Molecular typing of K. pneumoniae isolated by ERIC-PCR. The dendrogram was built with the Bionumerics software 6.6 (Applied Maths) based on the Dice similarity coefficient and by means of the UP-GMA. ERIC-PCR: Enterobacterial Repetitive Intergenic Consensus-Polymerase Chain Reaction; UP-GMA: unweighted pair-group method with arithmetic averages; NI: not informed; F: female; M: male.
DISCUSSION

Monitoring ICU patients is very important because, despite comprising a small subgroup of hospitalized individuals (5%-10%), they present average risk for infection 5 to 10 times higher than inpatients of other sectors(31, 32). In Brazil, the prevalence of isolated ESBL-producing K. pneumoniae is approximately 50%, with potential to cause severe morbidity and mortality(33).

In the current study, the K. pneumonia isolates presented high resistance percentages to third- and fourth-generation cephalosporins and to carbapenems. The same was verified in a study based on surveillance cultures from ICU inpatients (69 perianal specimens), in which 37% of the K. pneumonia isolates presented resistance to carbapenems(34). Elevated indices of resistance were also observed, but in clinical isolates of the Klebsiella-Enterobacter group (58%), in Minas Gerais(35).

The production of beta-lactamase enzymes in enterobacteria has been detected in several parts of the world, as in Trinidad and Tobago, where clinical isolates of K. pneumoniae presented genes bla TEM (84.3%), bla SHV (34.5%) and bla CTX-M (58.8%; and in Iran, with bla TEM (87.5%), bla SHV (12.4%) and bla CTX-M (24.8%)34, 35). These genes were simultaneously found in E. coli (6%) and K. pneumoniae (28.5%) from clinical cultures in Iran(36). The dissemination of bla KPC genes has been described in several European countries and in the Asian region of the Pacific Ocean(37, 38). In South America, KPC strains have been reported in several countries, such as Argentina(39), Colombia(40) and Brazil(41, 42). The presence of bla KPC in K. pneumoniae in five Brazilian states – Rio de Janeiro, Espírito Santo, Minas Gerais, Goiás and Pernambuco (2006-2009) –, was revealed in studies of the surveillance network on bacterial resistance in hospital nosocomial infections(18).

It is worth emphasizing the importance of surveillance cultures in the programs of HAIs control. The comparative analysis between the number of surveillance cultures and clinical cultures demonstrated that the presence of KPC enzyme was more frequently observed in surveillance cultures. Besides, cultures grown at patients’ admission and weekly (58%) were superior to those conducted just at admission (15%)32.

In Rio de Janeiro, a study at the ICU of Instituto Nacional de Cardiologia demonstrated that the most frequent species in active surveillance were E. coli (21.95%) and K. pneumoniae (34.1%). The presence of beta-lactamases was revealed in 58% of the rectal swab isolates, encoded by genes bla TEM (54%), AmpC (50%), bla SHV (25%) and bla CTX-M (29%)44. Our results exhibited high percentages of genes bla KPC (56%) and bla TEM (60%), and 16% of bla GES-3. The genotypes bla KPC, bla SHV and bla TEM were similarly detected from surveillance cultures, in Canada(45).

Our data also reveal that 8.6% (6/70) of isolates presented resistance to carbapenems, and absence of genes bla KPC and bla GES-3. According to Poulou et al. (2013)46, this resistance may probably be associated with other mechanisms, such as loss or alteration of outer-membrane porins. An outbreak caused by a clonal lineage of K. pneumoniae CTX-M-15 was described at an ICU in Greece. This lineage presented resistance to etapenem, attributed to the interruption of the OmpK35 gene and to the presence of a porin variant, OmpK36(46).

Genes bla TEM and bla IMP, prevalent in non-glucose-fermenting Gram-negative bacteria, such as Pseudomonas aeruginosa(47), were not detected in the present study, although IMP-producing members of the Enterobacteriaceae family have been identified, mainly in China, Japan, and Australia; and VIM-producing ones in Italy and Greece(48). Recently, the coexistence of ESBL-producing K. pneumoniae lineages and other carbapenemases has been described in Colombia (KPC-2/VIM-24), Italy (KPC-2/VIM-1), and China (KPC-2/IMP), among others.

In the present investigation, strains of K. pneumoniae were detected with up to six genes responsible for beta-lactamase production. Similar results were observed in 24 strains of ESBL-producing K. pneumoniae, in Recife, Brazil, where 46% carried three resistance genes. This accumulation of genes may cause limitations to the therapeutical options available for treatment of infections caused by K. pneumoniae and E. coli(49).

In relation to molecular typing, our results demonstrated low epidemiological relationship among isolates, different antimicrobial susceptibility patterns and resistance gene profiles. A study by Cabral et al. (2012)50 also revealed great diversity among strains of K. pneumoniae, presenting similarities up to 60% in 18 isolates analyzed. Yet, six strains presented the same band pattern by ERIC-PCR, indicating clonal dissemination in the hospital where the research was conducted(50).

The high levels of genetic diversity revealed here suggests the absence of cross contamination among ICU inpatients. However, it is necessary to highlight that our study presents some limitations (absence of data on collection periodicity at admission and/or during hospitalization). In spite of the low clonal relationship, the presence of ESBL and KPC-producing K. pneumoniae may represent high potential for dissemination of resistance among patients. It is noteworthy that colonization with potential pathogens is almost always a prerequisite for the development of nosocomial infections(50).
CONCLUSION

Although no significant epidemiological relationship was demonstrated, the main contribution of this study was the disclosure of alarming data on the presence of *K. pneumoniae*, carrying genes responsible for ESBL and carbapenemase production during preventive monitoring, a still limited approach in Brazilian health services. Controls based exclusively in clinical cultures may not detect most patients who harbor resistant organisms. The inclusion of surveillance cultures is a recommended strategy, aimed principally at preventing the dissemination of resistance genes in hospital environments, and, consequently, at reducing morbidity and mortality. The data generated in this study indicate the importance of adopting measures of continuous prevention to control the spread of ESBL- and carbapenemase-producing microorganisms in hospital settings and in the community. Measures such as active surveillance, rational use of antimicrobials, isolation precautions, hand hygiene, and education for health personnel are fundamental for the success of HAI prevention and control programs.

ACKNOWLEDGEMENTS

We thank the direction, the staff of the Hospital Infection Control Committee (CCIH), and the Microbiology Laboratory of Hospital Federal da Lagoa. We also thank INQQS/Fiocruz, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) for financial support.

RESUMO

Introdução: O aumento da incidência de microrganismos multirresistentes é considerado um dos principais problemas de saúde pública. Uma das rotinas incluídas na prática hospitalar é a busca de pacientes colonizados e/ou infectados. Objetivo: Avaliar a variabilidade genética e as relações clonais de *K. pneumoniae* produtoras de betalactamases de espectro estendido (ESBL) em culturas de vigilância de unidade de terapia intensiva (UTI) no Rio de Janeiro, Brasil. Materiais e métodos: Setenta isolados obtidos a partir de swab retal, (março/2013 a março/2014). O perfil de suscetibilidade a antibióticos foi avaliado pelo sistema VITEK 2. Foram pesquisados os genes de resistência: *bla*<sub>SHV</sub>, *bla*<sub>TEM</sub>, *bla*<sub>OXA-1</sub>, *bla*<sub>KPC</sub>, *bla*<sub>OXA-48</sub>, *bla*<sub>CTX-M-15</sub>, *bla*<sub>VIM</sub>, *bla*<sub>IMP</sub>, e *bla*<sub>NDM* pela reação em cadeia da polimerase (PCR). A diversidade genética foi avaliada por Enterobacterial Repetitive Intergenic Consensus-PCR (ERIC-PCR). Resultados: Foram detectados altos percentuais de resistência a cefepime (94%), ceftazidima (96%), ertapenem (61%), imipenem (54%) meropenem (43%) e ciprofloxacina (69%). Os genes prevalentes foram: *bla*<sub>SHV</sub> (69%), *bla*<sub>TEM</sub> (63%), *bla*<sub>OXA-1</sub> (60%), *bla*<sub>KPC</sub> (57%), *bla*<sub>CTX-M-15</sub> (47%), *bla*<sub>VIM</sub> (16%). Os genes *bla*<sub>OXA-48</sub>, *bla*<sub>NDM</sub> e *bla*<sub>IMP</sub> não foram detectados. Foram observados 29 perfis em relação aos genes de resistência, com 23% apresentando pelo menos cinco genes. Uma grande diversidade genética (68 perfis) foi observada entre as cepas. Conclusão: Embora não tenha sido observada relação clonal entre os isolados, este estudo revelou dados alarmantes quanto à resistência microbiana em monitoramento preventivo, abordagem ainda pouco adotada no Brasil. Nossos dados permitem concluir que a inclusão de culturas de vigilância nas unidades de saúde é uma estratégia recomendada, visando principalmente à prevenção da disseminação dos genes de resistência no ambiente hospitalar e, consequentemente, redução da morbimortalidade.

Unitermos: infecção hospitalar; monitoramento epidemiológico; Klebsiella pneumoniae; betalactamases; tipagem molecular.

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