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Molecular typing of a large nosocomial outbreak of KPC-producing bacteria in the biggest tertiary-care hospital of Quito, Ecuador

M. Belen Prado-Vivar[a,b], Lizeth Ortiz[b], Jorge Reyes[a,c], Eduardo Villacis[c], Marco Fornasini[b,d], Manuel E. Baldeon[b,d], Paul A. Cardenas[a,b,*]

[a] Instituto de Microbiología, COCIBA, Universidad San Francisco de Quito, Diego de Robles s/n y Vía Interoceánica, Quito, Pichincha, Ecuador
[b] Centro de Investigación Traslacional (CIT), Universidad de las Américas, Quito, Pichincha, Ecuador
[c] Instituto Nacional de Investigación en Salud Pública (INSPI), Quito, Pichincha, Ecuador
[d] Center for Biomedical Research (CENBIO), Eugenio Espejo College of Health Science, Universidad Tecnológica Equinoccial, Quito, Pichincha, Ecuador

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ABSTRACT

Objectives: *Klebsiella pneumoniae* is an opportunistic pathogen associated with nosocomial infections worldwide. Isolates with a *K. pneumoniae* carbapenemase (KPC)-producing phenotype show reduced susceptibility to first-choice antibiotics. Between 2012–2013, the largest public tertiary-care hospital in Quito (Ecuador) reported an outbreak of KPC-producing bacteria with more than 800 cases. We developed a molecular epidemiological approach to analyse the clonality of *K. pneumoniae* isolates recovered from selected hospital services and patient samples.

Methods: A retrospective cohort study was performed based on microbial isolates and their corresponding records from the hospital and referred to Instituto Nacional de Investigación en Salud Pública (INSPI). From 800 isolates that were collected between 2012–2013, a total of 100 isolates were randomly selected for this study. Antimicrobial susceptibility testing was performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Genotypic detection and phylogenetic relationship analysis were performed by multilocus sequence typing (MLST). The bla_{KPC} carbapenemase gene was also amplified by PCR and was sequenced using Sanger sequencing.

Results: Molecular analysis showed that the outbreak had a polyclonal origin with two predominant genotypes, comprising sequence types ST25 and ST258, present in 38 and 36 cases, respectively. These genotypes were found in all studied hospital services including general surgery, intensive care unit and emergency. Thebla_{KPC-5} gene was the most prevalent bla_{KPC} variant in this study.

Conclusion: These data indicate that KPC-producing polyclonal *K. pneumoniae* are frequent causes of nosocomial hospital outbreaks in South America. Similar genotypes have been reported in Colombia, Argentina, Brazil, North America and Asia.

1. Introduction

Nosocomial infections are infections caused by fungi, bacteria or viruses that affect hospitalised patients within 48 h after admission. The causative micro-organisms usually produce serious infections, including pneumonia, urinary tract infection and sepsis [1,2]. Hospitals are important reservoirs for antimicrobial-resistant bacteria [3,4]. In Latin America, approximately 11.6% of hospitalised patients develop a nosocomial infection, usually related to surgery, injury and invasive procedures including catheterisation or mechanical ventilation [5,6]. There are no reports on general mortality rates due to nosocomial infections in Ecuador, however patients with nosocomial pneumonia have a mortality rate of approximately 50% [7].

Klebsiella pneumoniae is a major cause of nosocomial outbreaks worldwide [8]. *Klebsiella pneumoniae* can acquire resistance to carbapenems by production of carbapenemases, e.g. *K. pneumoniae* carbapenemase (KPC) [9]. Carbapenemases have a broad spectrum of hydrolysis against β-lactams, including carbapenems. These enzymes may be encoded on the bacterial chromosome or on plasmids, embedded within mobile genetics elements, e.g. transposons [10]. Dissemination of resistance to carbapenems in Enterobacteriaceae is currently a major public-health problem owing to the wide distribution of resistance genes and the capacity for horizontal transfer of these genes to other bacterial species [11].

* Corresponding author at: Centro de Investigación Traslacional (CIT), Universidad de las Américas, Quito, Pichincha, Ecuador
E-mail address: pacardenas@usfq.edu.ec (P.A. Cardenas).

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KPC-producing *K. pneumoniae* has been reported in several countries of South America. It was detected for first time in Colombia in 2005 [12], followed by Brazil [13], Argentina [14] and Chile [15]. Complete surveillance of KPC-producing *K. pneumoniae* in Ecuador has not been reported previously, where only particular cases have been described (23 cases reported in 2016) [16]. However, to completely characterise strains in molecular epidemiological studies it is necessary to categorise bacterial strains at the clonal level [17].

The aim of this study was to characterise *K. pneumoniae* isolates producing carbapenemases in a nosocomial outbreak that occurred in a public hospital in Quito, Ecuador, between 2012–2013.

2. Materials and methods

2.1. Patients and study design

A retrospective cohort study was conducted of samples positive for KPC-producing *K. pneumoniae* stored at the Department of Bacterial Resistance of the Instituto Nacional de Investigación en Salud Pública (INSPI) in Quito, Ecuador. Studied isolates were obtained from a nosocomial outbreak that occurred between January 2012 and December 2013 in a public tertiary-care hospital in Quito, with 414 beds and 180 000 annual inpatients. Original samples were obtained within 72 h after hospitalisation from female and male patients aged 15–95 years, except those patients who were transferred to the hospital and were attended at the emergency service with a diagnosis of nosocomial infection, in which case samples were collected before 72 h of hospitalisation.

Beginning in 2012, an epidemiological outbreak of nosocomial infections emerged at the hospital that lasted for approximately 14 months with an unknown number of cases. From 2012–2013, a total of 800 cases were identified as KPC-positive *K. pneumoniae* by conventional biochemistry and phenotypic methods to detect resistance to carbapenems and were sent for storage and further molecular studies at INSPI. From such a time period, a sample size of 100 isolates (from 100 randomly chosen patients) was selected from the following departments: intensive care unit (ICU) ($n = 24$); surgery departments ($n = 18$); emergency ($n = 12$); internal medicine ($n = 7$); traumatology ($n = 6$); pulmonology ($n = 6$); burns unit ($n = 4$); neurology ($n = 4$); transplant unit ($n = 3$); otorhinolaryngology ($n = 1$); psychiatry ($n = 1$); oncology ($n = 1$); and other services ($n = 13$).

2.2. Ethical approval

All stored isolates were de-identified and were coded at INSPI. The Bioethics Committee of Universidad de las Américas (Quito, Ecuador) approved the study.

2.3. Bacterial strains and microbiological and molecular methods

2.3.1. Antimicrobial susceptibility testing

Klebsiella pneumoniae from samples were identified by conventional methods (Gram stain, culture and API 20E). Antimicrobial susceptibility was determined by the disk diffusion method for amoxicillin/clavulanic acid, piperacillin/tazobactam, ceftazidime, cefotaxime, cefoxitin, imipenem, meropenem, nalidixic acid, polymyxin B, tetracycline and tigecycline. All antibiotics tested (except polymyxin B and tigecycline) were interpreted according cut-off values of the Clinical and Laboratory Standards Institute (CLSI) 2015. For polymyxin B and tigecycline, cut-off values from the US Food and Drug Administration (FDA) were used.

2.3.2. Multilocus sequence typing (MLST)

Molecular epidemiological relationships were determined using MLST for clinical *K. pneumoniae* isolates. DNA was extracted using a bead beating method (Precellys® 24; Bertin Technologies SAS. Montigny-le-Bretonneux, France). PCR was performed to detect seven housekeeping genes (*gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB* and *tonB*) according to the protocol described on the *K. pneumoniae* MLST website (http://www.pasteur.fr/mlst). Confirmation of PCR amplification was performed by electrophoresis and visualisation of amplicon bands. PCR products were further cleaned using AMPure beads (Beckman Coulter, Brea, CA, USA) and were Sanger sequenced at Macrogen Inc. (Seoul, South Korea).

2.3.3. Molecular analysis of antimicrobial resistance genes

PCR of the extracted DNA was used for detection of the bla_{KPC} carbapenemase gene using previously reported primers (product size, 1000 bp) [18]. The PCR product was later cleaned and was sequenced as previously described at Macrogen Inc. Nucleotide sequences were analysed using software from the Institut Pasteur MLST database (http://www.pasteur.fr/mlst; accessed 9 July 2018).

2.3.4. Multilocus sequence typing and analysis of bla_{KPC}

Epidemiological phylogenetic relationships were analysed by MLST and the allelic profile, sequence type (ST) and bla_{KPC} genes were assigned using online databases at the Institut Pasteur (https://bigsdb.pasteur.fr/cgi-bin/bigsdb/bigsdb.pl?db=pubmlst_-klebsiella_isolates; accessed 15 October 2019). Isolates were considered to be the same clone (type) if they showed 100% genetic identity on each allele; STs and bla_{KPC} variants that had not been described previously in the database were assigned as novel.

2.3.5. Analysis of allelic diversity

The profiles of DNA sequences of seven MLST loci obtained allowed the use of a sequence-based typing method to generate allelic profiles and their associated epidemiological data using PHYLOViZ (http://www.phyloviz.net/; accessed 9 July 2018), which uses the eBURST 3 algorithm [19].

2.4. Statistical analysis

Being a descriptive molecular study, the sample size was fixed to 100; however, 18 samples were excluded from the final analysis owing to issues in DNA extraction/PCR amplification/DNA sequencing. Thus, statistical analysis was performed only for the remaining 82 samples (from 82 patients) for which it was possible to obtain complete molecular and epidemiological results. Descriptive statistics such as frequencies and percentages were calculated for categorical variables. For continuous variables, central tendency statistics with corresponding measures of dispersion were calculated. Comparison of categorical data was performed by χ^2 test. All statistical tests were two-tailed and a P-value of 0.05 was considered statistically significant. Clinical data were analysed using the statistical software package SPSS v.12.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Clinical characteristics of study population

Isolates came from tracheal secretion ($n = 36$), urine ($n = 8$), sputum ($n = 7$), wound secretion ($n = 6$), pressure ulcer ($n = 5$) and other secretions ($n = 34$); records were not available for 4 isolates. The majority of participants were male (58; 58%) and were aged >50 years of age (Supplementary Table S1).

Table 1
Antimicrobial susceptibility of *Klebsiella pneumoniae* outbreak isolates ($n = 100$).

Antimicrobial group/agent (disk content)	Interpretive breakpoints (mm)[a]			Frequency (%)		
	S	I	R	S	I	R
Penicillins						
Amoxicillin/clavulanic acid (20/10 μg)	≥18	14–17	≤13	0	0	100
Piperacillin/tazobactam (100/10 μg)	≥21	18–20	≤17	1	1	98
Cephems						
Cefotaxime (30 μg)	≥26	23–25	≤22	0	0	100
Ceftazidime (30 μg)	≥21	18–20	≤17	1	1	98
Cefoxitin (30 μg)	≥18	15–17	≤14	5	23	72
Carbapenems[b]						
Imipenem (10 μg)	≥23	20–22	≤19	16	9	75
Meropenem (10 μg)	≥23	20–22	≤19	3	15	82
Tetracyclines						
Tetracycline (30 μg)	≥15	12–14	≤11	2	39	59
Tigecycline (15 μg)	≥19	15–18	≤14	56	7	37
Quinolones						
Nalidixic acid (30 μg)	≥19	14–18	≤13	27	9	64
Lipopeptides						
Polymyxin B (300 μg)	≥12	–	≤11	74	0	26

S, susceptible; I, intermediate; R, resistant.
[a] All antibiotics tested (except polymyxin B and tigecycline) were interpreted according cut-off values of the Clinical and Laboratory Standards Institute (CLSI) 2015. For polymyxin B and tigecycline, cut-off values from the US Food and Drug Administration (FDA) were used.
[b] Isolates belonging to ST25 were resistant to imipenem and meropenem.

Table 2
Clinical characteristics of cases/samples of the outbreak with multidrug-resistant *Klebsiella pneumoniae* according to sequence type (ST)[a].

Service	N (%)								
	ST25	ST258	ST1393	ST348	ST451	ST859	ST11	ST151	Total
ICU	9 (50.0)	8 (44.4)	1 (5.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	18 (100)
Emergency	6 (60.0)	4 (40.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	10 (100)
Internal medicine	5 (71.4)	1 (14.3)	1 (14.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	7 (100)
Other wards[b]	16 (35.6)	23 (51.1)	1 (2.2)	1 (2.2)	1 (2.2)	1 (2.2)	1 (2.2)	1 (2.2)	45 (100)
Data not available	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (100)

ICU, intensive care unit.
[a] Eighteen isolates (18%) were excluded from the analysis because they presented errors in sequencing data for one or more alleles and therefore it was not was possible to determine the ST.
[b] Other wards includes traumatology, pulmonology, burns unit, cardiology, transplant unit, psychiatry, cardiothoracic, plastic surgery, vascular surgery, neurosurgery, cardiac surgery, oncology and general surgery.

Details of the epidemiological characteristics are not available because the data from patient records were not collected or were not performed in this study.

3.2. Antimicrobial susceptibility patterns of Klebsiella pneumoniae isolates

Antimicrobial susceptibility profiles for all of the isolates are summarised in Table 1. All *K. pneumoniae* isolates were resistant to cefotaxime and amoxicillin/clavulanic acid. Antimicrobial resistance rates to piperacillin/tazobactam (98%), ceftazidime (98%), imipenem (75%) and meropenem (82%) were also high. The rate of susceptibility of the *K. pneumoniae* isolates to polymyxin B, tigecycline and nalidixic acid was 74%, 56% and 27%, respectively (Table 1).

3.3. Molecular analyses

Of the 100 isolates, 82% were similar to previously reported genotypes registered in the database of the Institut Pasteur and the remaining 18% were not possible to identify. From the STs identified, the most commonly identified were ST25 ($n = 38$), ST258 ($n = 36$) and ST1393 ($n = 3$). ST11, ST151, ST859, ST348 and ST451 were each identified once in the study. Eighteen isolates were excluded from the analysis as a result of low-quality sequencing results from one or more of the seven housekeeping genes.

ST258 was common in the ICU, emergency department and internal medicine with a prevalence of 44.4% ($n = 8$), 40% ($n = 4$) and 14.3% ($n = 1$) in the three indicated units, respectively. Regarding ST25 isolates, the prevalence was 50.0% ($n = 9$), 60.0% ($n = 6$) and 71.4% ($n = 5$) in the ICU, emergency and internal medicine departments, respectively (Table 2). ST258 and ST25 were more common in people aged >50 years.

3.4. Sequence typing of bla_{KPC} genes

Among the 82 KPC-producing *K. pneumoniae* isolates sequenced, the bla_{KPC-5} gene was present in 36 isolates each (43.9%) of the ST25 and ST258 isolates as well as in isolates of ST11 ($n = 1$), ST151 ($n = 1$), ST348 ($n = 1$), ST451 ($n = 1$), ST859 ($n = 1$) and ST1393 ($n = 2$). The bla_{KPC-4} gene was identified in 2.4% (2/82) ST25 isolates, while the bla_{KPC-9} gene was found one ST1393 isolate.

3.5. Relationships of allelic profiles

Eight previously reported STs were identified, with most sequence alignments not showing insertion or deletion or partial match. By allelic relationship analysis using PHYLOViZ, it was possible to determine that from the node of the most commonly encountered ST (ST25), all other STs emerged. Thus, ST258 and ST859 are variants from ST11; and ST1393, ST348, ST451 and ST151 are variants derived from ST25.

It was interesting that ST258 and ST11 emerged from the same node, showing a phylogenetic relationship with ST25 (Supplementary Fig. 1).

4. Discussion

Nosocomial infections have been a topic of interest in hospitals in recent years [20]. Improper use of antibiotics, including carbapenems, has contributed to the development of multidrug-resistant bacteria [21]. In the current study, MLST of 82 *K. pneumoniae* isolates identified eight STs. The most frequent clones observed were ST25 ($n = 38$) and ST258 ($n = 36$), and the majority came from surgical services, intensive care and emergency units. Interestingly, the distribution of STs in the emergency service was similar to that found in other hospital services. The current results could indicate that patients who arrived at the hospital with a diagnosis of nosocomial infection were infected with STs present within the hospital. As a consequence, it is likely that these STs are circulating in several healthcare units in Quito. The present results highlight the importance of combining epidemiological, clinical and molecular data to better understand the means of transmission of nosocomial pathogens at the hospital level.

Klebsiella pneumoniae, a member of the Enterobacteriaceae, is one of the most prevalent pathogens causing community- and hospital-acquired infections [22,23]. Several factors can contribute to increased nosocomial infections in the ICU, including the severity of patient conditions with potential immunosuppression, use of invasive procedures, and frequent use of antibiotics [24]. One of the limitations of this study was that it was not possible to obtain the record data from the isolates to analyse the conditions described before.

The most common KPC-producing *K. pneumoniae* strains are those of the clonal group 258 [25]. *Klebsiella pneumoniae* ST258 is a hybrid clone composed of 80% of the ST11 genome and 20% of the ST442 genome [26]. It is perhaps the most common genotype around the world and is likely to be responsible for the global spread of KPC [27]. ST258 has been reported in a large number of countries, including the USA, Canada, Italy, Colombia, Chile and Brazil [28]. In agreement with the current results, among isolates obtained from diverse healthcare facilities in the USA, 89% of strains that were detected as ST258 were isolated from tracheal secretions, blood, urine and invasive devices [28].

The most common KPC-producing *K. pneumoniae* strains reported in the literature are those of clonal groups 258 and 25 [25]. The most common genotype of *K. pneumoniae* identified in current outbreak was ST25 clone. These results differ from those found in a large study conducted in Latin America (Argentina, Colombia, Costa Rica, Ecuador, El Salvador, Nicaragua, Paraguay and Peru) in 2016, where out of 143 *K. pneumoniae* isolates detected, only one ST25 was found [29]. The MLST database has not registered ST25 from North America or Africa, however there are several reports from Asia, Europe and South America [30]. Together these results could indicate an increasing dissemination of ST25 isolates in Ecuador and the region.

The second most common genotype of *K. pneumoniae* identified in current outbreak was ST258 clone. *Klebsiella pneumoniae* ST258 is a hybrid clone comprised of 80% of the ST11 genome and 20% of the ST442 genome [26]. It is perhaps the most common genotype around the world and is likely to be responsible for the global spread of KPC [27]. ST258 has been reported in a large number of countries, including the USA, Canada, Italy, Colombia, Chile and Brazil [28]. In agreement with the current results, among isolates obtained from diverse healthcare facilities in the USA, 89% of strains that were detected as ST258 were isolated from tracheal secretions, blood, urine and invasive devices [28].

Another *K. pneumoniae* genotype with increasing frequency in some countries (such as Brazil and China) is ST11, a variant of the *tonB* allele from ST258, that was isolated from one case from a wound secretion in the present series. This genotype was found to be the most prevalent clone of *K. pneumoniae* producing KPC in China [31]. In Brazil, ST11 was mentioned as the next dominant clone responsible for the spread of carbapenemase genes [32]. Because epidemiological data for the patients were not available, it cannot be excluded that these STs were introduced into the hospital as epidemiologically independent events and did not belong to the outbreak. In addition, ST348 was another single case described in current outbreak that was isolated from a tracheal secretion. This genotype has been reported recently in sporadic cases of infection with KPC-producing *K. pneumoniae* in Portugal [33]. In the current study, three more single cases of STs were found, namely ST151, ST451 and ST859; there are no other studies on that topic.

A case of KPC-producing *K. pneumoniae* in Ecuador was reported for the first time in 2010, isolated from a patient from Azogues and confirmed by PCR as KPC-2; since that time there has been no study regarding the spread of carbapenemase [16]. The present data show a total of three variants of KPC, with the KPC-5 variant being the most common. KPC-5 differs from KPC-2, which is the most common variant worldwide, by one amino acid substitution (Pro103→Arg) [16,26]. Most of the ST258 isolates in the current study produced KPC-5, in contrast to most studies which found that this genotype is usually the KPC-2 variant [34]. KPC-5 was first found in *Pseudomonas aeruginosa* in Puerto Rico in a nosocomial outbreak [18]. Two isolates had the KPC-4 variant, which was recorded for the first time in the UK in an *Enterobacter cloacae* isolate [35], and one isolate had KPC-9. Previous studies have reported KPC-9 in secretions from rectal bleeding in *K. pneumoniae* ST258 and *Escherichia coli* [36].

According to the molecular analysis, combined analysis of MLST data and bla_{KPC} genotype indicated the presence of different clones circulating in the hospital. As a consequence, bla_{KPC-5} was the most common variant in different STs. Owing to this frequent and uncommon emergence, horizontal spread of the bla_{KPC-5} gene by plasmid acquisition among different clones may be assumed. It would be important to perform a deeper clonal analysis using whole-genome sequencing to provide more complete information regarding functional (such as antimicrobial resistance and virulence) and metabolic genes in addition to MLST data [37].

In conclusion, this outbreak at the largest tertiary-care hospital in Quito, Ecuador, had a polyclonal origin, with similar genotypes reported in Colombia, Argentina, Brazil, North America and Asia. In addition, the frequent emergence of bla_{KPC-5} genes in different STs indicates possible horizontal transmission that requires further analysis in detail. It would be important to carry out similar molecular epidemiological studies in other areas of the country to gain a better idea of the epidemiology of the spread of KPC-producing *K. pneumoniae*. The development of this and other studies will provide a better understanding of the aetiological origin and spread of multidrug-resistant *K. pneumoniae* strains.

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Competing interests

None declared.

Ethical approval

This study was approved by the Institutional Review Board of Universidad de las Américas (Quito, Ecuador).

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jgar.2013.06.001.

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