UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

Colegio de Posgrados

Characterization of mobile genetic elements in carbapenem resistant *Enterobacteriaceae* isolates from Ecuadorian hospitals

Jorge Aníbal Reyes Chacón

Gabriel Trueba Piedrahita Ph.D.
Director de Trabajo de Titulación

Trabajo de titulación de posgrado presentado como requisito
para la obtención del título de Doctor en Microbiología

Quito, 13 de diciembre del 2019

UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

COLEGIO DE POSGRADOS

HOJA DE APROBACIÓN DE TRABAJO DE TITULACIÓN

We recommend acceptance of this thesis in partial fulfillment of the candidate´s requirements for the Degree of Doctor in Microbiology.

Characterization of mobile genetic elements in carbapenem resistant *Enterobacteriaceae* isolates from Ecuadorian hospitals

Jorge Aníbal Reyes Chacón

Gabriel Trueba, Ph.D.
Director del Trabajo de Titulación _____

Roberto Melano, Ph.D.
Co- director del Trabajo de Titulación _____

Ana Aguilar, Ph.D
Miembro del Comité de Tesis _____

Paúl Cárdenas, Ph.D.
Miembro del Comité de Tesis _____

Gabriel Trueba, Ph.D.
Director del programa _____

Stella de la Torre, Ph.D. _____
Decana del Colegio de Ciencias Biológicas y ambientales

Hugo Burgos, Ph.D. _____
Decano del Colegio de Posgrados

Quito, 13 de diciembre del 2019

© Derechos de Autor

Firma: _____

Nombre: Jorge Aníbal Reyes Chacón

Código de estudiante: 00130952

C. I.: 1717730962

Lugar: Quito Fecha: 13 de diciembre del 2019

DEDICATORIA

a Patricia, Esteban y Victoria, mi preciada familia

a Carmen y Jorge, mis preciados padres**.**

AGRADECIMIENTOS

Quiero expresar mis sinceros agradecimientos a Gabriel Trueba por confiar en mí y poder acceder al programa de doctorado. A Roberto Melano por todo el conocimiento brindado, su paciencia y motivación. Ana Aguilar por confiar en mis capacidades desde el inicio de mi Carrera. A Paul por todas las sugerencias acertadas durante mi trabajo.

A mis compañeros Daniel y María Fernanda por su apoyo, motivación y confianza en los momentos más difíciles de la carrera. Las clases de tutoría siempre fueron más divertidas con su presencia.

Mis amigos Santiago Escalante, Pablo Acosta y Eduardo Villacís por sus consejos y constante apoyo

Mis amigos del Instituto Nacional de Investigación en Salud Pública, especialmente a Fernando, Carolina y Rafael.

Mi amada familia, mi esposa Patricia, que fue la luz en los momentos más difíciles, su paciencia y carisma durante este tiempo fueron mi soporte. Mis hijos, Esteban y Victoria, su compañía en los momentos alegres y tristes son inolvidables. El presente trabajo es por ustedes

RESUMEN

Esta tesis se trata sobre la resistencia a los carbapenémicos en el patógeno oportunista *Klebsiella pneumoniae* (RCKPN), la cual fue reportada por primera vez en Ecuador en el 2012 y en otras especies de Enterobacterias resistente a los carbapenémcios (CRE). En el capítulo I incluimos una revisión bibliográfica sobre genes de carbapenemasas y elementos genéticos móviles (MGEs) en America del Sur. En el Capítulo II analizamos las implicaciones clínicas relacionadas a infecciones producidas por CRKPN. En el Capítulo III caracterizamos los MGEs relacionados a gen bla_{KPC-2}, la carbapenemasa más prevalente en el Ecuador. En el capítulo IV describimos el EGM Tn*1999* relacionado al gen bla_{OXA-48}-Like en *Raoultella ornithinolityca.*

Palabras clave: Carbapenemasa, Elemento genético móvil, Enterobacteria.

ABSTRACT

This thesis focuses in carbapenem resistance in the opportunistic pathogen *Klebsiella pneumoniae* (CRKPN), which was first reported in Ecuador in 2012 and other species of carbapenem resistant *Enterobacteriaceae* (CRE). In Chapter I we include a bibliographic review about carbapenemase genes and mobile genetic elements (MGEs) in South America; In Chapter II we analyzed the clinical implications related to infections caused by CRKPN. In Chapter III we characterized the MGEs related to bla_{KPC-2} gene, the most prevalent carbapenemase reported in Ecuador. In Chapter IV we describe the Tn*1999* MGEs related to bla_{OXA-48}-Like present in *Raoultella ornithinolityca.*
Key words: Carbapenemase, Mobile genetic element, *Enterobacteriaceae*

TABLA DE CONTENIDO

ÍNDICE DE TABLAS

ÍNDICE DE FIGURAS

CAPITULO I

Mobile genetic elements associated with carbapenemase genes in South American *Enterobacteriaceae*

Jorge Aníbal Reyes
[1]*Universidad San Francisco de Quito, Colegio de Ciencias Biológicas y Ambientales, Instituto de Microbiología, Quito, Ecuador.*
Postal address: 170901
[2]*Universidad Central del Ecuador, Facultad de Ciencias Químicas, Quito, Ecuador*
Postal address: EC170130

Roberto Melano
[3]*Public Health Ontario, Toronto, Canada*
[4]*University of Toronto, Department of Laboratory Medicine and Pathobiology, Toronto, Canada*

Paul Andrés Cárdenas
[1]*Universidad San Francisco de Quito, Colegio de Ciencias Biológicas y Ambientales, Instituto de Microbiología, Quito, Ecuador*

Gabriel Trueba
[1]*Universidad San Francisco de Quito, Colegio de Ciencias Biológicas y Ambientales, Instituto de Microbiología, Quito, Ecuador*

Corresponding author

Jorge Reyes

Tel. +593 0994551824

jorgereyes83@gmail.com

Postal address: EC170130

Funding information:

Instituto de microbiología, Universidad San Francisco de Quito.

Author Disclosure Statement

No competing financial interests exist

Abstract

Introduction: Carbapenem resistance in members of order *Enterobacteriales* is a growing public health problem causing high mortality in developing and industrialized countries. Their emergence and rapid propagation worldwide were due to both intercontinental spread of pandemic strains and horizontal dissemination via mobile genetic elements (MGE) such as plasmids and transposons.

Objective: To describe MGEs carrying carbapenem resistance genes in Enterobacteriales which have been reported in South America.

Search strategy and selection criteria: A search of the literature in English or Spanish published until 2019 in PubMed and Google Scholar databases was performed for studies of MGE in *Enterobacteriale*s reported in South American countries.

Results: Seven South American countries reported MGE related to carbapenemases. Carbapenemase-producing *Klebsiella pneumoniae* belonging to clonal complex 258 are the most prevalent pathogens reported; others carbapenemase-producing *Enterobacteriacea* such as *Escherichia coli, Serratia marcescens*, and *Providencia rettgeri*, also have been reported. The MGE implicated in the spread of the most prevalent carbapenemase genes are Tn*4401* and non-Tn*4401* elements for bla_{KPC} and IS*Aba125* for bla_{NDM}, located in different plasmid incompatibility groups and bacterial clones.

Conclusion: This review indicates that, like in other parts of the world, the most commonly reported carbapenemases in *Enterobacteriales* from South America are being disseminated in the continent through clones, plasmids, and transposons which have been previously reported in other parts of the world.

Keywords: Mobile genetic elements, carbapenemase, South America.

Running title: Mobile genetic elements in South America

Introduction

Enterobacteriales are the most prevalent opportunistic pathogens in hospital settings, causing, sepsis, pneumonia, soft tissue infections, urinary tract infections, and others. To make this problem worse, the emergence of resistance to ß-lactams, the most commonly used family of antibiotics, has drastically reduced treatment options for those infections.[1] Since Alexander Fleming described the penicillin in 1928, a large number of β-lactams have been reported belonging to five groups: penams, penems, cephems, monobactams, and carbapenems, all of which possess a β -lactam ring. The carbapenems group is the election to treat infections caused by bacteria producing extended-spectrum β-lactamases (ESBLs) or AmpC cephalosporinases.[2]

In recent years the carbapenem-resistant *Enterobacteriaceae* (CRE) have rapidly emerged and disseminated worldwide, this resistance is caused by either production of carbapenemases or a combination of mechanisms such as the presence of different ESBLs (or AmpC) in addition to porin loss. The evidence suggests that clonal dissemination of carbapenemase-producing *Enterobacteriales* (CPE) plays a critical role in hospital outbreaks of life-threatening infections, which has increased mortality, morbidity and hospitalization costs.[3] However, mobile genetic elements (MGE) including plasmids, transposable elements (TEs), and integrons are probably the most important factors implicated with the dissemination of carbapenemase genes among different bacterial species. Transposable elements, including insertion sequences (ISs) and transposons (Tns), are discrete DNA structures, located in plasmids or chromosomes, which can move (through the activity of self-encoded transposases) to new locations in the genome. The ISs are the smallest TEs that can move resistance genes as part of a composite transposon; a gene bounded by two copies of the same or related IS that can move as a single unit.[4,5] In this review, we describe different MGEs associated with carbapenemase genes in *Enterobacteriales* members reported in South American countries.

Methodology

A search of literature published in English or Spanish until August 2019 was performed using PubMed and Google Scholar databases. In a first-round, the keywords 'mobile genetic elements AND carbapenemase resistance, carbapenemase AND *Enterobacteriaceae* or *Enterobacteriales* AND (Argentina OR Brazil OR Bolivia OR Colombia OR Chile OR Ecuador OR Paraguay OR Peru OR Uruguay OR Venezuela)' were used. Abstracts or conference reports were not included unless the article form was unavailable. All selected articles were subjected to a second search round for selection of specific studies using molecular analysis criteria: a) description of clonality based in sequence type (ST) using multilocus sequence typing (MLST) or whole genome sequencing; and b) descriptions of MGEs associated with carbapenemase genes in *Enterobacteriaceae* species, including plasmid incompatibility groups (based on replicon typing or restriction digestion performed with S1 nuclease) and/or description of TE or integrons structures using DNA sequencing.

Results

Argentina

The first report of chromosomally encoded NMC-A carbapenemase was in an *Enterobacter cloacae* isolate recovered from a leukemia patient in 2004.[6] Four years later a KPC-2-producing *K. pneumoniae* strain was reported.[7] Gomez et al. described the Tn*4401a* isoform associated with *bla*$_{\text{KPC-2}}$ harbored in the international clone *K. pneumoniae* ST258. Interestingly, a *bla*$_{\text{KPC-2}}$ gene was associated with non-Tn*4401* elements (NTE) in the non-ST258 clones of *K. pneumoniae* (ST11, ST476, and ST526). In other *Enterobacteriaceae* species, it was harbored in transferable plasmids belonging to IncHI2, IncL/M and IncA/C incompatibility groups.[8] The*bla*$_{\text{NDM-1}}$ gene was located in a Tn*125* composite transposon harbored in plasmids of different sizes, reported in *Providencia rettgeri.*[9] A *bla*$_{\text{IMP-8}}$-harboring plasmid IncA/C1-ST13 was described in *E. coli*, and the carbapenemase gene

was associated with integron class I flanked by two IS*26* elements.[10] Other metallo-ß-lactamases (MBL), such as *bla*$_{\text{VIM-2}}$ and *bla*$_{\text{VIM-11}}$, were associated with integrons In*883*, In*885,* In*346,* In*900* in *E. cloacae,*[11] and *bla*$_{\text{VIM-16}}$ associated with class I integron in *Serratia marcescens*.[12] The *bla*$_{\text{OXA-163}}$ and *bla*$_{\text{OXA-247}}$ genes were reported in *K. pneumoniae,* interesting, their genetic environment comprised the insertion sequence IS*4321* upstream and IS*4*-like insertion downstream.[13]

Colombia

The first description of KPC-2-producing *K. pneumoniae* isolate in South America was reported in Colombia in 2005.[14] Later, a KPC-3- producing *K. pneumoniae* was isolated from an Israeli patient (international spread).[15] Another report showed that the *bla*$_{\text{KPC-2}}$ was most prevalent than *bla*$_{\text{KPC-3}}$ in ST258 and non- ST258 *K. pneumoniae* isolates, and the isoform Tn*4401a* was more prevalent than Tn*4401b*.[16] Other reports showed a close relationship of *bla*$_{\text{KPC-2}}$ with Tn*4401b* isoform (harbored in IncL/M plasmids) in isolates of *K. pneumoniae* ST14, ST338, and ST339.[17] Interestingly, the isoform "b" was also harbored in IncA/C and IncF plasmids in non-*K. pneumoniae* isolates.[18] *bla*$_{\text{NDM-1}}$ was reported in *Escherichia coli* associated with Tn*125* and Tn*5393*, located in an IncA/C plasmids.[19] An outbreak caused by a *K. pneumoniae* ST1043 carrying an IncA/C, *bla*$_{\text{NDM-1}}$-plasmid was also reported.[20]

Brazil

Carbapenem-resistant *K. pneumoniae* ST258 harboring *bla*$_{\text{KPC-2}}$ gene in a Tn*4401*a isoform and located in conjugative plasmids are the most common carbapenemase-producing enterobacterial pathogen in this country. KPC-2-producing *K. pneumoniae* belonging to the clonal complex (CC) 258 (including ST258, ST11, and ST437), has been described in a variety of MGE associations (IncFII, IncN, IncL/M, and untypable plasmids carrying Tn*4401a* or Tn*4401b*, which were successfully disseminated among species of *Enterobacteriales*.[21] Similarly, another study described a KPC-2-producing *K. pneumoniae*

ST11 carrying a Tn*4401* located in a IncW plasmid group.[22] Recent work described two *K. pneumoniae* isolates carrying the *bla*$_{KPC-2}$ gene in NTE IId located in IncQ1 and Col-like plasmids.[23] In one study the authors showed an association of *bla*$_{KPC-2}$ with Tn*4401b* and IncN plasmids in CC11 *K. pneumoniae*.[24] *E. coli, E. cloacae, Enterobacter aerogenes, Citrobacter freundii* have also been reported carrying *bla*$_{KPC-2}$ gene located in Tn*4401*b or Tn*4401*d isoforms on plasmids of different sizes.[25] A new carbapenemase, *bla*$_{BKC-1}$, was reported in *K. pneumoniae* ST1781 and it was classified as a new member of molecular Ambler class A serine carbapenemases in 2015; it was located in a 10-kb no conjugative IncQ plasmid that included a mobilization system, IS*Kpn23*.[26]

Reports of MBL and their MGEs were related to *bla*$_{NDM-1}$ and associated with a truncated IS*Aba125* in *Enterobacter hormaechei*[27] or inside a ~10 kb, composite transposon (two copies of IS*Aba125*) named Tn*125* in *P. rettgeri*.[28] Interestingly, this gene has also been located in the transposon Tn*3000* in *E. coli* and *E. hormaechei*.[29] Other MBLs such *bla*$_{IMP-1}$ gene in *K. pneumoniae* and *P. rettgerii* [30,31] and *bla*$_{IMP-10}$ in *S. marcescens*[32] were linked to class 1 integrons.

A *bla*$_{OXA-370}$ encoding a carbapenemase related to OXA-48, was reported in *E. hormaechei.* This gene was flanked upstream by a Tn*3* family transposase gene *tnpA* (truncated by an IS*5075*-like insertion) and downstream of a Tn*4* family *tnpA* gene (truncated by an IS*15*-like insertion).[33]

Ecuador

A *bla*$_{KPC-2}$ gene was detected in *K. pneumoniae* ST258 and ST25 and associated with Tn*4401a* (Reyes, et al, manuscript in preparation), whereas the*bla*$_{NDM-1}$ gene harbored in IncA/C plasmid, was reported in *K. pneumoniae* ST147.[34] A *bla*$_{OXA48-}$ Like harbored in a Tn*1999* was reported in a clinical isolate *Raoultella ornithinolytica*.[35]

Chile

The Tn*4401a* isoform harboring bla$_{KPC}$ gene was reported in *K. pneumoniae* ST258, ST101, ST25 clones and an NTE variant 1 in *K. pneumoniae* ST11, ST1161, and ST29.[36]

Uruguay

The presence of *bla*$_{KPC-2}$ gene harbored in Tn*4401*a was identified in *K. pneumoniae* ST 258 causing an outbreak.[37]

Venezuela

The *bla*$_{KPC-2}$ located in a Tn*4401*b was reported in *K. pneumoniae* ST11, ST15, ST833, ST1271, ST1857, ST1859 and ST1860 clones.[38] *K. pneumoniae* ST833 was also described carrying *bla*$_{KPC-2}$ and a class 1 integron harboring *bla*$_{VIM-2}$.[39]

Discussion

Although some successful bacterial clones can disseminate resistance vertically (e.g. *K. pneumoniae* belonging to CC258 carrying Tn*4401*/*bla*$_{KPC}$), carbapenemase genes (as well as other antibiotic resistance determinants) are frequently transmitted horizontally between different enterobacterial clones, species, and genera.[40] Furthermore, these genes also disseminate among different plasmids and chromosomes through transposition or recombination events.[41]

In South America, we observed the same patterns reported worldwide (Table 1), which indicates that some clones are being transmitted among South American countries (or elsewhere), raising the need for tracking carbapenemase clone dissemination. There is also evidence of carbapenemase gene mobility between plasmids and clones which potentially facilitates the emergence of new epidemic CRE clones such as *K. pneumoniae* ST11 and the hypervirulent clone ST25 (Table 1). This type of mobility is more difficult to track; plasmids carrying these genes could be identified (by replicon typing or plasmid/whole genome sequencing), but the rearrangements due to recombination and transposition sometimes complicate their traceability.[8,42] A clear example of this evolution is the *bla*$_{KPC-}$

$_2$ gene in *K. pneumoniae* CC258, which has been associated with IncFII derivatives such as pKpQIL [43] and other plasmid incompatibility groups in Argentina, Brazil, Colombia and Ecuador (Table 1) which have been recovered from *K. pneumoniae* CC258, non-CC258 and other members of the *Enterobacteriales* order.[44,45] This variability of plasmids carrying similar *bla*$_{KPC}$ genetic environments suggests active plasmid rearrangements driven by transposons (e.g. Tn*4401*). The *bla*$_{KPC-2}$ dissemination from the original host (*K. pneumoniae*) to other *Enterobacteriales* members is consequence of this evolutionary process: an active transposon harboring antimicrobial resistance genes finding broad (e.g. L/M, N, W) or wide host range plasmids (e.g. IncF, IncH), like it was found in South America (Table 1).[46]

Another approach to partially assess some of these complexities could be analyzing the genetic environment in which these genes are located. A classic example would be Tn*4401*, a Tn*3*-type, 10-kb mobile transposon frequently associated with *bla*$_{KPC}$ genes. Its structure consists of a Tn*3* transposase gene (*tnpA*), a resolvase gene (*tnpR*) and additional insertion sequences (IS*Kpn6* and IS*Kpn7*) delimited by two 39-bp imperfect inverted repeats (IRs) [47]. The isoforms (a to h) are classified based in nucleotide deletions upstream of *bla*$_{KPC}$ gene (affecting its promotor region and the KPC-expression), lacking genes or both: for instance, isoform "a" has a 99 bp deletion; isoform "b" has no deletion; isoform "c", a 215 bp deletion; isoform "d", a 68 bp deletion; isoform "e", a 255 bp deletion; isoform "f", a truncation in *tnp*A, absence of *tnpR*, IS*Kpn7* left part and Tn*4401* IRL-1; isoform "g" is similar to "f" plus a 215 bp deletion (like isoform c);and isoform "h" has a 188-bp deletion (48) (Fig. 1). Non-Tn*4401* elements (NTE) are other genetic environments of *the bla*$_{KPC}$ gene. These are complex DNA structures sharing some Tn*4401* elements such as IS*Kpn6.* Some examples are shown in Fig. 1.[11,49,50] In South America the isoforms Tn*4401a* and *b* were the most commonly found in the regional studies (e.g. in Argentina, Colombia, Brazil, Chile, Ecuador, and Venezuela), but NTEs and the isoforms Tn*4401c* and *d* were also

described in Brazil, respectively, mainly in non-*K. pneumoniae* isolates (Table 1). As it was mentioned before, this lateral dissemination between genera and species would be more related to the type of conjugative plasmid where the transposon 'landed'.

Currently, more than 70 MBLs (chromosomally and plasmid-encoded) have been reported and grouped based on DNA sequence similarity[51]. NDM is one of the most successfully plasmid-disseminated MBL detected in different members of *the Enterobacteriaceae* family around the world and their frequent association with IS*Aba125* suggests a possible *Acinetobacter* spp. origin, a bacterium in which this association is very common.[52] Tn*125*, an IS*Aba125*-based composite transposon, was one of the genetic elements described to be involved in *bla*$_{NDM}$ dissemination, but in *Enterobacteriaceae* Tn*125* has been interrupted or truncated, generating a variety of different genetic contexts for *bla*$_{NDM}$,[53] *K. pneumoniae*, *E. coli*, and *Enterobacter* spp are the predominant carriers of *bla*$_{NDM}$, with heterogeneous clonal backgrounds and multiple acquisitions of *bla*$_{NDM}$ genes across bacterial species, also, different replicon types of *bla*$_{NDM}$-carrying plasmids in the *Enterobacteriaceae* were described, being the most common IncA/C, FIA, FIB, FII and X3[53] (In our bibliographic review we found less frequent descriptions of MBL-producing *Enterobacteriacea*). Reported *bla*$_{NDM-1}$ genes were associated with IS*Aba125* composite transposon Tn*125* (Table 1). There are no extensive descriptions of bacterial clones and plasmid incompatibility groups related to *bla*$_{NDM}$ in South America. In Argentina, one description was found of a *bla*$_{NDM-1}$-harboring, IncB/O plasmid recovered from *P. rettgeri*, in Colombia and Ecuador, *bla*$_{NDM-1}$ was detected on IncA/C plasmids from *E. coli* (Colombia) and *K. pneumoniae* (both countries). More information has to be developed and analyzed to have a clearer picture of *bla*$_{NDM}$ spread in South America. A similar scenario was to *bla*$_{VIM}$ (Argentina-Venezuela) and *bla*$_{IMP}$ (Brazil), both carbapenemase genes associated to class 1 integrons, also described in *Pseudomonas aeruginosa*, evidencing that class I integrons

are efficient genetic platforms to incorporate MBLs genes and disseminated once a transposon or plasmid is involved.[54, 55]

The OXA-enzymes have higher hydrolysis rates against cloxacillin and oxacillin than other β-lactams, they are poorly inhibited by clavulanic acid, tazobactam, and aztreonam, and some can inactivate carbapenems.[56] We found few descriptions of MGEs associated with these carbapenemase genes in South America; in Ecuador, the *bla*$_{\text{OXA-48}}$ -Like was related to Tn*1999* like to previously,[57] however, in Brazil *bla*$_{\text{OXA-347,}}$ a variant of *bla*$_{\text{OXA-48,}}$ showed association with truncated Tn*3* and Tn*4* transposons.

Conclusions

Current evidence enhances the importance of MGE for carbapenemase gene dissemination. Except for Argentina, Brazil, and Colombia, the reports of MGEs are scarce in other South American countries. The *bla*$_{\text{KPC}}$ and *bla*$_{\text{NDM}}$ are the most prevalent carbapenemase genes reported in *Enterobacteriaceales* species are associated with Tn*4401/*NTE and IS*Aba125/*Tn*125* respectively while *bla*$_{\text{VIM}}$ and *bla*$_{\text{IMP}}$ carbapenemase genes are related to class 1 integrons. The location of transposons or integrons in different plasmid incompatibility groups and bacterial clones denotes their capacity in transferability and mobilization. It is possible that intercontinental dissemination of CRE clones was followed by HGT of plasmids carrying carbapenem resistance genes to local bacteria. Any control measure intended to control antibiotic resistance genes dissemination requires the identification of the potential sources of these genes which nowadays is carried out by DNA polymorphisms, unfortunately, carbapenem resistance genes have very little polymorphisms. We argue that the study of MGEs associated with these genes could provide very valuable epidemiological information to detect the potential sources.

References

1. Hawkey P, Warren, R, Livermore D, et al. Treatment of infections caused by multidrug-resistant Gram-negative bacteria: report of the British Society for Antimicrobial Chemotherapy/healthcare Infection Society/British Infection Association Joint Working Party. J Antimicrob Chemother. 2018;73 Suppl3: iii2-iii78.
2. El-gamal MI, Brahim I, Hisham N, et al. Recent updates of carbapenem antibiotics. Eur J Med Chem. 2017;131:185-195.
3. Logan LK, Weinstein RA. The epidemiology of carbapenem-resistant *Enterobacteriaceae*: the impact and evolution of a global menace. J Infect Dis. 2017;215:28-36.
4. Stokes HW, Gillings M R. Gene flow, mobile genetic elements and the recruitment of antibiotic resistance genes into Gram-negative pathogens. FEMS Microbiol Rev. 2011; 35(5):790-819.
5. Trueba G. Why does Evolution Matter ? The Importance of Understanding Evolution. The forces behind the dissemination of bacterial virulence and antibiotic. Cambridge Scholar Publishing. 2014;155-164
6. Radice M, Power P, Gutkind G, et al. First class A carbapenemase isolated from *Enterobacteriaceae* in Argentina. Antimicrob Agents Chemother. 2004; 48(3):1068-1069.
7. Pasteran FG, Otaegui L, Guerriero L, et al. *Klebsiella pneumoniae* Carbapenemase–2, Buenos Aires, Argentina. Emerg infect dis. 2008;14(7):1178.
8. Gomez SA, Pasteran FG, Faccon D, et al. Clonal dissemination of *Klebsiella pneumoniae* ST258 harbouring KPC-2 in Argentina. Clin Microbiol Infect. 2011;17(10):1520-1524

9. Pasteran F, Meo A, Gomez S, et al. Emergence of genetically related NDM-1-producing *Providencia rettgeri* strains in Argentina. J Glob Antimicrob Resist. 2014;2(4):344-345.
10. Elena A, Cejas D, Magariños F, et al. Spread of clonally related *Escherichia coli* strains harboring an IncA/C1 plasmid encoding IMP-8 and its recruitment into an unrelated MCR-1-containing isolate. Antimicrob agents chemother. 2018; *62*(6): e02414-17.
11. De Belder D, Faccone D, Tijet N, et al. Novel class 1 Integrons and sequence types in VIM-2 and VIM-11-producing clinical strains of *Enterobacter cloacae*. Infect Genet Evol. 2017;54:374-378.
12. Nastro M, Monge R, Zintgraff J, et al. First nosocomial outbreak of VIM-16-producing *Serratia marcescens* in Argentina. Clin Microbiol Infect. 2013;19:617–9.
13. Gomez S, Pasteran F, Faccone D, et al. Intrapatient emergence of OXA-247: a novel carbapenemase found in a patient previously infected with OXA-163-producing *Klebsiella pneumoniae*. Clin Microbiol Infect. 2013; *19*(5), E233-E235.
14. Villegas MV, Lolans K, Correa A, et al. First detection of the plasmid-mediated class A carbapenemase KPC-2 in clinical isolates of *Klebsiella pneumoniae* from South America. Antimicrob Agents Chemother. 2006;50(8):2880-2882.
15. Lopez JA, Correa A, Navon-Venezia S, et al. Intercontinental spread from Israel to Colombia of a KPC-3-producing *Klebsiella pneumoniae* strain. Clin Microbiol Infect. 2011;17(1):52-56.
16. Mojica MF, Correa A, Vargas DA, et al. Molecular correlates of the spread of KPC-producing *Enterobacteriaceae* in Colombia. Int J Antimicrob Agents. 2012;40(3):277-279.

17. Cuzon G, Naas T, Truong H, et al. Worldwide diversity of *Klebsiella pneumoniae* that produce β-lactamase *bla*$_{KPC-2}$ gene. Emerg Infect Dis. 2010;16(9):1349.
18. Cuzon G, Naas T, Correa A, et al. Dissemination of the KPC-2 carbapenemase in non-*Klebsiella pneumoniae* enterobacterial isolates from Colombia. Int J Antimicrob Agents. 2013;42(1):59-62.
19. Rojas LJ, Wright MS, De La Cadena E, et al. Initial assessment of the molecular epidemiology of *bla*$_{NDM-1}$ in Colombia. Antimicrob Agents Chemother. 2016;60(7):4346-4350.
20. Escobar Pérez JA, Olarte Escobar NM, Castro-Cardozo B, et al. Outbreak of NDM-1-producing *Klebsiella pneumoniae* in a neonatal unit in Colombia, South America. Antimicrob Agents Chemother. 2013;AAC-01447-12.
21. Andrade LN, Curiao T, Ferreira JC, et al. Dissemination of *bla*$_{KPC-2}$ by the Spread of *Klebsiella pneumonia* Clonal Complex 258 Clones (ST258, ST11, ST437) and Plasmids (IncFII, IncN, IncL/M) among *Enterobacteriaceae* Species in Brazil. Antimicrob Agents Chemother. 2011;55(7):3579-3583.
22. Almeida ACS, de Sá Cavalcanti FL, Vilela MA, et al. *Escherichia coli* ST502 and *Klebsiella pneumoniae* ST11 sharing an IncW plasmid harbouring the *bla*$_{KPC-2}$ gene in an intensive care unit patient. Int J Antimicrob Agents. 2012;40(4):374-376.
23. Cerdeira LT, Lam MMC, Wyres KL, et al. Small IncQ1 and Col-Like Plasmids Harboring *bla*$_{KPC-2}$ and Non-Tn4401 Elements (NTEKPC-IId) in High-Risk Lineages of *Klebsiella pneumoniae* CG258. Antimicrob Agents Chemother. 2019; AAC-02140
24. Zahner V, Carvalho-Assef APD, Asensi MD, et al. Update of the molecular epidemiology of KPC-2-producing *Klebsiella pneumoniae* in Brazil: spread of

clonal complex 11 (ST11, ST437 and ST340). J Antimicrob Chemother. 2012;68:312–6.

25. Tavares CP, Pereira PS, Marques Ede A, et al. Molecular epidemiology of KPC-2–producing *Enterobacteriaceae* (non–*Klebsiella pneumoniae*) isolated from Brazil. Diagn Microbiol Infect Dis. 2015;82(4):326-330.
26. Nicoletti AG, Marcondes MF, Martins WB, et al. Characterization of BKC-1 class A carbapenemase from *Klebsiella pneumoniae* clinical isolates in Brazil. Antimicrob Agents Chemother. 2015;59(9):5159-64.
27. Carvalho-Assef AP, Pereira PS, Albano RM, et al. Detection of NDM-1-, CTX-M-15-, and *qnrB4*-producing *Enterobacter hormaechei* isolates in Brazil. Antimicrob Agents Chemother. 2014;58(4):2475-2476
28. Carvalho-Assef AP, Pereira PS, Albano RM, et al. Isolation of NDM-producing *Providencia rettgeri* in Brazil. J Antimicrob Chemother. 2013;68(12):2956-2957.
29. Campos JC, da Silva MJF, dos Santos PRN, et al. Characterization of Tn*3000*, a transposon responsible for $bla_{\text{NDM-1}}$ dissemination among *Enterobacteriaceae* in Brazil, Nepal, Morocco, and India. Antimicrob Agents Chemother. 2015;*59*(12):7387-7395.
30. Lincopan N, McCulloch JA, Reinert C, et al. First isolation of metallo-β-lactamase-producing multiresistant *Klebsiella pneumoniae* from a patient in Brazil. J Clin Microbio. 2005;43(1):516-519.
31. Lincopan N, Leis R, Vianello MA, et al. Enterobacteria producing extended-spectrum β-lactamases and IMP-1 metallo-β-lactamases isolated from Brazilian hospitals. J Med Microbiol. 2006;55(11):1611-1613.
32. Silva KE, Cayô R, Carvalhaes CG, et al. Coproduction of KPC-2 and IMP-10 in carbapenem-resistant *Serratia marcescens* isolates from an outbreak in a

Brazilian teaching hospital. J Clin Microbiol. 2015;53(7):2324-2328.

33. Sampaio JLM, Ribeiro VB, Campos JC, et al. Detection of OXA-370, an OXA-48-Related Class D β-Lactamase, in *Enterobacter hormaechei* from Brazil. Antimicrob Agents Chemother. 2014;58:3566–3567.
34. Romero-Alvarez D, Reyes J, Quezada V, et al. First case of New Delhi metallo-β-lactamase in *Klebsiella pneumoniae* from Ecuador: an update for South America. Int J Infect Dis. 2017;65:119-121.
35. Reyes JA, Villavicencio F, Villacís JE, et al. First report of a clinical isolate of *bla*OXA-48- carbapenemase producing *Raoultella ornithinolytica* in South America. Rev Argent Microbiol. 2019.
36. Barría-Loaiza C, Pincheira A, Quezada M, et al. Molecular typing and genetic environment of the bla_{KPC} gene in Chilean isolates of *Klebsiella pneumoniae*. J Glob Antimicrob Resist. 2016;4:28-34.
37. Marquez C, Ingold A, Echeverría N, et al. Emergence of KPC-producing *Klebsiella pneumoniae* in Uruguay: infection control and molecular characterization. New Microbes New Infect. 2014;2(3):58-63.
38. Falco Restrepo AD, Nieves MAV, Takiff H. Molecular characterization of KPC-producing *Klebsiella pneumoniae* isolated from patients in a Public Hospital in Caracas, Venezuela. Enferm Infecc Microbiol Clin 2017;35(7):411-416.
39. Falco A, Ramos Y, Franco E, et al. A cluster of KPC-2 and VIM-2-producing *Klebsiella pneumoniae* ST833 isolates from the pediatric service of a Venezuelan Hospital. BMC infect dis 2016;16(1):595
40. Duin D Van, Doi. Y. The global epidemiology of carbapenemase-producing *Enterobacteriaceae*. Virulence. 2017;8(4):460-469
41. Partridge SR, Kwong SM, Firth N, et al. Mobile genetic elements a.ssociated with antimicrobial resistance. Clin Microbiol Rev. 2018;31(4): e00088-17.

42. Conlan S, Park M, Deming C, et al. Plasmid Dynamics in KPC-positive *Klebsiella pneumoniae* during Long-Term Patient Colonization. Mbio 2016;7(3):e00742-16

43. Doumith M, Findlay J, Hirani H, et al. Major role of pKpQIL-like plasmids in the early dissemination of KPC-type carbapenemases in the UK. J Antimicrob Chemother. 2017;72(8):2241-2248

44. Chen YT, Lin JC, Fung CP, et al. KPC-2-encoding plasmids from *Escherichia coli* and *Klebsiella pneumoniae* in Taiwan. J Antimicrob Chemother. 2013;69(3):628-631.

45. Chen L, Chavda KD, Fraimow HS, et al. Complete nucleotide sequences of bla_{KPC-4}-and bla_{KPC-5}-harboring IncN and IncX plasmids from *Klebsiella pneumoniae* strains isolated in New Jersey. Antimicrob Agents Chemother. 2013;57(1):269-276.

46. Rozwandowicz M, Brouwer MSM, Fischer J, et al. Plasmids carrying antimicrobial resistance genes in Enterobacteriaceae. J Antimicrob Chemother. 2018;73(5):1121-1137

47. Naas T, Cuzon G, Villegas MV, et al. Genetic structures at the origin of acquisition of the β-lactamase bla_{KPC} gene. Antimicrob Agents Chemother. 2008;52(4), 1257-1263.

48. Cheruvanky A, Stoesser N, Sheppard AE, et al. Enhanced *Klebsiella pneumoniae* carbapenemase (KPC) expression from a novel Tn*4401* deletion. Antimicrob Agents Chemother. 2017;61(6) AAC-00025-17

49. Shen P, Wei Z, Jiang Y, et al. Novel genetic environment of the carbapenem-hydrolyzing β-lactamase KPC-2 among *Enterobacteriaceae* in China. Antimicrob Agents Chemother. 2009;53(10): 4333-4338.

50. Wolter DJ, Kurpiel PM, Woodford N, et al. Phenotypic and enzymatic

comparative analysis of the novel KPC variant KPC-5 and its evolutionary variants, KPC-2 and KPC-4. Antimicrob Agents Chemother. 2009;53(2): 557-562.

51. Naas T, Oueslati S, Bonnin RA, et al. Beta-lactamase database (BLDB)–structure and function. J Enzyme Inhib Med Chem. 2017;32(1):917-919.
52. Dortet L, Poirel L, Nordmann P. Worldwide dissemination of the NDM-type carbapenemases in Gram-negative bacteria. Biomed Res Int. 2014;2014:249-856.
53. Wu W, Feng Y, Tang G, et al. NDM metallo-β-lactamases and their bacterial producers in health care settings. Clin Microbiol Rev. 2019;32:e00115-18.
54. Diene SM, Rolain JM. Carbapenemase genes and genetic platforms in Gram-negative bacilli: *Enterobacteriaceae*, *Pseudomonas* and *Acinetobacter* species. Clin Microbiol Infec. 2014;20(9):831-838.
55. Queenan, A. M, Bush K. Carbapenemases: the versatile β-lactamases. Clin Microbiol Rev. 2007;20(3):440-458.
56. Oueslati S, Nordmann P, Poirel. Heterogeneous hydrolytic features for OXA-48-like β-lactamases. J Antimicrob Chemother. 2015;70(4): 1059-1063.
57. Mairi A, Pantel A, Sotto A, et al. OXA-48-like carbapenemases producing *Enterobacteriaceae* in different niches. Eur J Clin Microbiol Infect Dis 2018;37(4):587-604

Figure 1. Transposable elements associated with *bla*$_{KPC}$, *bla*$_{OXA-48}$ Like and *bla*$_{NDM}$ carbapenemases found in the *Enterobacteriales* members.

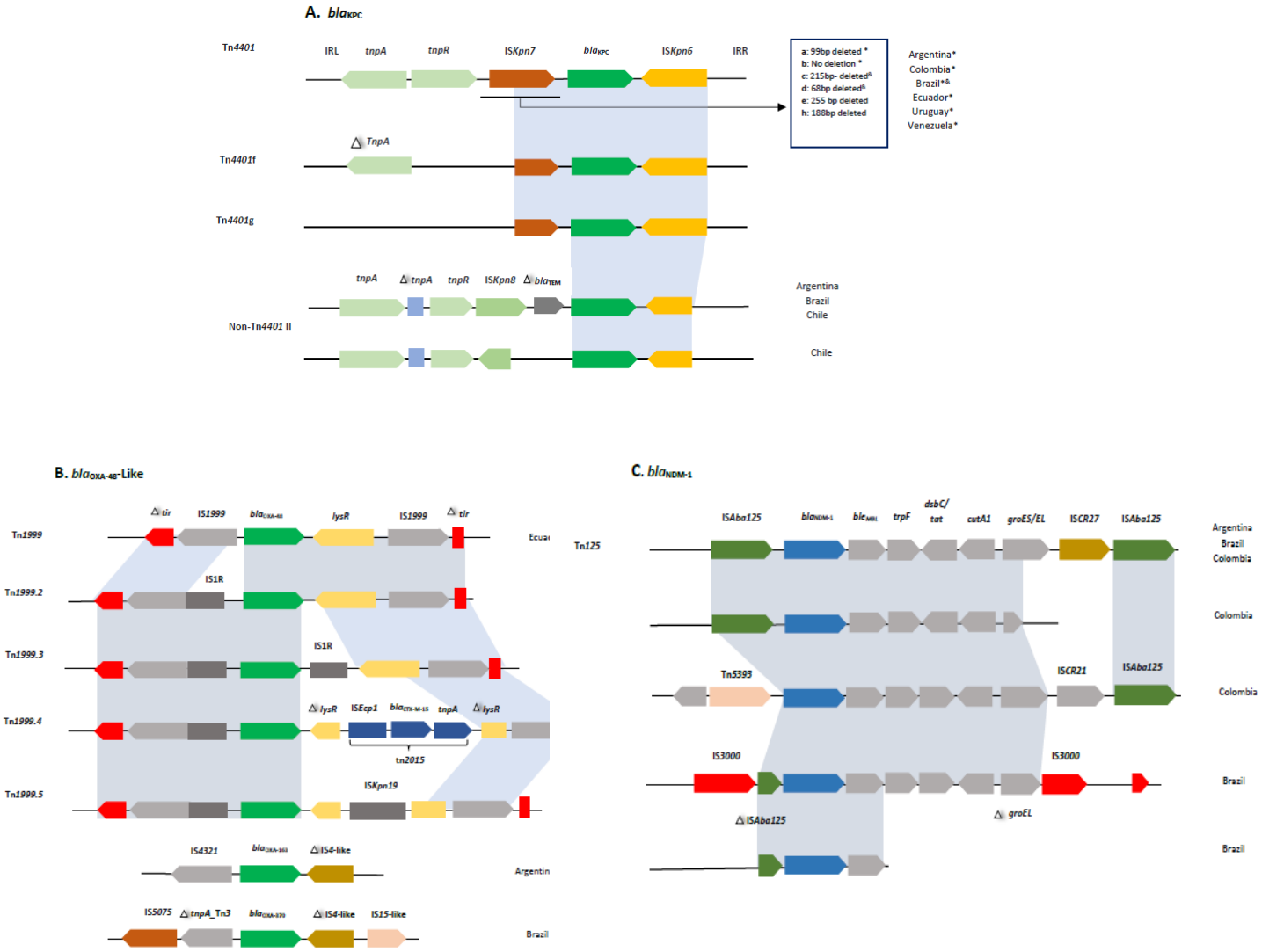


Table 1. Summary of mobile genetic elements reported in carbapenemase-producing *Enterobacteriaceae* in South America

	Isolate	Replicon typing (incompatibility group)	Transposable element	Carbapenemase gene	Reference
Argentina	*K. pneumoniae* ST258	L/M	Tn*4401a*	*bla*KPC-2	8
	C. freundii, E. cloacae *K. pneumoniae* non-258	HI2, L/M, A/C	NTE: variant Ia and Variant Ib	*bla*KPC-2	8
	P. rettgeri *E. coli*	B/O	Tn*125* Class 1 integron	*bla*NDM-1 *bla*IMP-8	9 10
	E. cloacae	Non-typeable	Class 1 integron: In*883*, In*885*, In*346* and In*900*	*bla*VIM-2 and *bla*VIM-11	11
	K. pneumoniae	N/D	IS*4321*- IS*4*	*bla*OXA-48	13
Colombia	*K. pneumoniae* ST14 ST 387, ST388	L/M	Tn*4401b*	*bla*KPC-2	16,17
	K. pneumoniae ST258	N/D	Tn*4401a* or Tn*4401b*	*bla*KPC-2 *bla*KPC-3	16
	K. pneumoniae ST147 and ST14	N/D	Tn*4401a* or Tn*4401b*	*bla*KPC-2	18
	K. pneumoniae ST512	N/D	Tn*4401b*	*bla*KPC-3	17
	S. marcescens	A/C	Tn*4401b*	*bla*KPC-2	18
	E. coli	F	Tn*4401b*	*bla*KPC-2	18
	E. coli	A/C	Tn*125* and Tn*5393*	*bla*NDM-1	19
	K. pneumoniae ST1043	A/C	N/D	*bla*NDM-1	20
Brazil	*K. pneumoniae* ST258, ST11 and ST48	FII	Tn*4401*a	*bla*KPC-2	21

	K. pneumoniae ST327 and ST437 and E. coli *K. pneumoniae*	N Q1	Tn*4401b* NTE	*bla*KPC-2 *bla*KPC-2	21 23
	S. marcescens and *C. freundii*	L/M	N/D	*bla*KPC-2	25
	E. coli, *E. cloacae*, *E. aerogenes*, *C. freundii* and *P. stuartii*	N/D	Tn*4401b* and Tn*4401d*	*bla*KPC-2	25
	K. pneumoniae ST11 and *E. coli* ST502 *K. pneumoniae* ST1781	W IncQ	Tn*4401c* IS*Kpn23*	*bla*KPC-2 *bla*BKC-1	21 26
	Enterobacter hormaeche	FII	IS*Aba125* and Tn*3000*	*bla*ND64-1	27,29
	P. rettgeri *E. coli*	N/D IncX3	Tn*125* Tn*3000*	*bla*ND64-1 *bla*ND64-1	28 29
	K. pneumoniae, *P. rettgeri* *S. marcescens*	N/D N/D	Class I integrons Class I integrons	*bla*IMP-1 *bla*IMP-10	30 32
	E. hormaechei	N/D	Tn*3* truncated – Tn*4* truncated	*bla*OXA-370	33
Chile	*K. pneumoniae* ST258, ST101, ST25	N/D	Tn*4401a*	*bla*KPC-2	36
	K. pneumoniae ST258, ST101, ST25	N/D	NTE: variant 1a	*bla*KPC-2	36

Ecuador	*K. pneumoniae* ST147 *K. pneumoniae* ST248, 25, 42 *Raoultella ornithinolytica*	A/C InFII N/D	N/D Tn*4401a* Tn*1999*	*bla*$_{NDM-1}$ *bla*$_{KPC-2}$ *bla*$_{OXA-48}$	34 -- 35
Uruguay	*K. pneumoniae* ST 258	N/D	Tn*4401a*	*bla*$_{KPC-2}$	37
Venezuela	*K. pneumoniae* ST11, ST15, ST833, ST1271, ST1857, ST1859 and ST1860	N/D	Tn*4401b*	*bla*$_{KPC-2}$	38
	K. pneumoniae ST833	N/D	class 1 integron	*bla*$_{VIM-2}$-*bla*$_{KPC-2}$	39

CHAPTER II

Carbapenem-resistant *Klebsiella pneumoniae*: microbiology key points for clinical practice

Jorge Reyes[1,2*], Ana Cristina Aguilar[1,3], Andrés Caicedo[1,3,4]

[1]Colegio de Ciencias Biológicas y Ambientales, Instituto de Microbiología, Universidad San Francisco de Quito (USFQ), 17-09-01 Quito, Ecuador

[2]Universidad Central del Ecuador, Facultad de Ciencias Químicas, Ecuador.

[3]Universidad San Francisco de Quito (USFQ), Colegio de Ciencias de la Salud (COCSA), Escuela de Medicina, Quito, 17-12-841, Ecuador

[4]Sistemas Médicos (SIME), Universidad San Francisco de Quito (USFQ), Quito, 17-12-841, Ecuador

*Corresponding author:

Jorge Reyes

Instituto de Microbiología, Universidad San Francisco de Quito (USFQ), Diego de Robles y Pampite S/N, 17-09-01 Quito, Ecuador

jorgereyes83@gmail.com

+593 98 44 9 00 22

Abstract

Carbapenemase–producing *Klebsiella pneumoniae* strains (Cp-Kpn) represent a challenge for clinical practitioners due to their increasing prevalence in hospital settings and antibiotic resistance. Clinical practitioners are often overwhelmed by the extensive list of publications regarding Cp-Kpn infections, treatment, characteristics, identification, and diagnosis. In this perspective article, we provide key points for clinical practitioners to consider for improved patient management including identification of risk factors and strategies for treatment. Additionally, we also discuss genetic underpinnings of antibiotic resistance, implementation of an antimicrobial stewardship program (ASP), and use of automated systems for detection of Cp-Kpn. Collectively, implementation of such key points would enhance clinical practices through providing practical knowledge to health professionals worldwide.

Key Words: Carbapenemase–producing *Klebsiella pneumonia; Clinical practitioners, management*

Introduction

K. pneumoniae, described by Edwin Klebs in 1875, is a gram-negative bacterium belonging to the *Enterobacteriaceae* family. This microorganism is part of the healthy microbiome of individuals and colonizes many parts of the body. Despite its role as a healthy component of the microbiome, it can cause severe infections in critically ill patients, newborns, immunocompromised individuals or those with other risk factors in healthcare establishments. Antibiotics such carbapenems are widely used to treat infections, especially those caused by *Enterobacteriaceae*, a producer of extended-spectrum β-lactamase (ESBL); however, the use or misuse of such antibiotics has contributed to the appearance of isolates resistant to carbapenems [1].

Carbapanem-resistant *K. pneumoniae* (Cr-KPN) is a pathogen that affects people worldwide, with prevalence in low, middle and upper income countries. Resistance to carbapenem is mediated by two primary mechanisms. First, Cr-KPN is able to produce β-lactamases with the ability to hydrolyze cephalosporins such *AmpC* cephalosporinase e.g. DHA-1 and CMY-2 or ESBL e.g CTX-M-2 in combination with decreased membrane permeability in the cell wall [2,3]. The second mechanism is mediated by the production of a β-lactamases capable of hydrolyzing most β-lactams antibiotics including carbapenems. According the Ambler classification it belongs to class A (*K. pneumoniae* carbapenemase, KPC), class B or metallo-β-lactamases (MBL) (New Delhi metallo-β-lactamases, NDM) and class D (OXA-48-like carbapenemases) [4]. The NDM carbapenemase was reported from *K. pneumoniae* and *Escherichia coli* in 2009, similar to other member of MBL it requires of zinc for hydrolysis of β-lactam antibiotics and their activity could be inhibited by ethylenediaminetetraacetic acid (EDTA) as chelating agent. [5] KPC–producer *K. pneumoniae* (KPC-Kp) is a pathogen with a high capacity for clonal expansion and exchange of mobile genetic elements (MGEs) promoting increased resistance. KPC-Kp

among their capacities to generate resistance can also persist in human reservoirs and create biofilms, which provide protection from hospital disinfection protocols [6].

Since the first report of KPN-Kp in the United States in 1996 [7], its presence has been evidenced in many other countries including China, Italy, [8], Brasil, Venezuela, Colombia, Ecuador and Argentina [9]. In these countries, KPC-Kp infections have contributed to an increased mortality and substantial costs for health care systems. Detection of KPC-Kp is carried out in clinical microbiology laboratories (CMLs) and is the first step in that physicians take when determining a therapeutic strategy (dose and time of administration) involving active antibiotics; however, accurate and consistent interpretation of CML reports is a long-standing problem across health care systems, particularly in low- and middle-income countries. Clinical practitioners without appropriate training or CMLs removed from medical facilities contributed to the erroneous therapeutic decision-making that facilitates the spread of antibiotic resistance, which ultimately results in adverse outcomes for patients.

The vast existing literature detailing KPC-Kp, its pathogenicity, mechanisms of antibiotic resistance and assays used for its detection in CMLs has not been clearly outlined for physicians in clinical practice. The aim of this perspective article is to provide key points of information for students and clinical practitioners (non-laboratory related) about infections characteristics and CML reports regarding KPC-Kp. By increasing practitioners' understanding and interpretation of CML reports and clinical facilities, such clear, evidence-based information will enhance therapeutic strategies and patient outcomes.

Risk factors and strategies for treating Carbapenemase-producing *Klebsiella pneumoniae* (Cp-Kpn) infections

Nosocomial dissemination of Cp-Kpn results primarily from failure to properly disinfect surfaces and medical equipment. Environments routinely exposed to water and humidity,

such as drains, sinks, faucets, and other locations where liquids are dispensed, are places were pathogens like Cp-Kpn can survive and disseminate, thus increasing the risk of bacterial outbreaks [10,11]. Medical equipment and devices are also common vectors of Cp-Kpn in hospitals [12]. It has been reported that Cp-Kpn can colonize medical equipment such as duodenoscopes and be transmitted to other patients [13]. Healthcare professionals' uniforms and protective clothing such as gown and gloves can also become contaminated with Cp-Kpn after patient examination if not properly used or discarded [14]. The reinforcement of hygiene protocols in healthcare facilities appears to be the most important measure for preventing outbreaks as no direct associations have been found between prevalence of Cp-Kpn outbreaks and differences in institutional infrastructure in low versus high-income countries [8].

In recent years, reports of Cp-Kpn outbreaks, particularly KPC-Kp, have increased worldwide due to the lack of appropriate medical intervention, prolongated hospitalization, presence of comorbidities, and overuse of antibiotics, among other factors [6]. Healthcare-acquired infections are a common and major concern across hospital settings in Greece, the USA, Israel, Spain, China, Colombia, Brazil, and Italy (Xu et al., 2017). Mortality rates among patients infected by Cp-Kpn is approximately 40-50% [15,16]. Prior use of fluoroquinolones, carbapenems or cephalosporins antibiotics, long-term intensive care, chronic renal failure, high APACHE III score and, more recently, the emergency of Cp-Kpn colistin-resistant isolates have all been found to contribute to poor patient outcomes [17–19].

The Centers for Disease Control and Prevention (CDC) has released guidelines for controlling the spread of carbapenem-resistant *Enterobacteriaceae* in healthcare facilities based on the following key points: 1) education and training for healthcare professionals and supportive personnel to reinforce protocols of hand hygiene, 2) patient contact

precautions, 3) minimal use of invasive devices, and 4) environmental cleaning. Active surveillance and continuous testing for quality hospital standards are recommended. Early laboratory identification and notification of bacteria strains is also emphasized as key for prescription and timely antibiotic therapy. Additionally, the implementation of an antimicrobial stewardship program is suggested as this will provide support in the fast identification of colonization or infected status of a patient [20]. In support of this evidence, empirical treatment based on risk factors and prompt decision-making has been shown to help reduce mortality related to Cp-Kpn infections in healthcare institutions [21,22]. Direct communication between CMLs and clinicians facilitates rapid diagnosis in these settings, which leads to faster adoption of targeted therapeutic strategies and effective combination of antibiotics, ultimately improving patient outcomes, especially among critically ill patients [23].

Antibiotic resistance and genetic basis of *bla*$_{KPC}$

The antibiotics imipenem, meropenem, ertapenem, and doripenem are members of the β-lactam family, and most frequently used to treat *Enterobacteriaceae* infections, especially in ESBLs-producing strains. The efficacy of these antibiotics comes from their possession of a reactive β-ring, similar to that of penicillin, cephalosporin and monobactam. Their trans configuration at the C-5--C-6 bond gives these antibiotics increased potency and the capability to inhibit cell wall synthesis by preventing cross-linking of peptidoglycan and transpeptidases. [24]

The mechanisms of carbapenem resistance in *K. pneumoniae* and other *Enterobacteriaceae* strains depend on β-lactamases production, such as ESBLs. ESBLs are encoded in plasmids or by hyperproduction of chromosomally-encoded AmpC cephalosporinases (AmpC) together with the presence of porin alterations in the bacterial wall delaying the diffusion of antibiotics into the bacterial cell; however, the production of enzymes with carbapenemase

activity hydrolyzing β-lactam antibiotics seems the most common mechanism of antibiotic resistance [25]. For example, the enzymes KPC, BKC and SME carbapenemase, which all fall under the category of Class A β-lactamases, contain serine residues in their active site that hydrolyze β-lactam antibiotics [26–28]. The presence of KPC carbapenemase in *K. pneumoniae* and different *Enterobacteriaceae* species isolates, depends, in part, upon whether or not it is located on a mobile genetic element (MGE), such as conjugative plasmids e.g. IncFII, IncL/M, IncA/C [29], and its proximity to Tn*4401* transposons [30] or non-Tn*4401* elements (NTE-KPC) [31]. The presence of plasmid-self transmitting pKpQIL (group incFII plasmid) harboring bla_{KPC} in *K. pneumoniae* complex clonal (CC)258 (an international clone), has been reported to increase bacterial fitness by providing an advantage over isolates harboring different plasmids. Targeting sources of resistance like those found in pKpQIL would decrease the likelihood of bacterial dissemination in nosocomial settings [32].

The role of clinical microbiology in antimicrobial stewardship programs (ASPs) in order to effectively control KPC-Kp

As previously described, the spread of KPC-Kp is an important concern across health care systems in both developed and emerging countries [4]. Implementation of hospital-based ASPs that emphasize the optimization of antibiotic use for controlling multidrug resistant bacteria (MDR) infections and preventing new resistance are needed in order to address increasing bacterial resistance in hospital settings. An ASP program should promote effective antibiotic treatment that avoids use of unnecessary antibiotics, basing its practice on de-escalation therapy and evidence-based guidelines to overcome empirical therapeutic errors and adverse events (*Clostridium difficile* infections) [33]. To optimize the benefits of an ASP in a hospital setting, active participation of clinical microbiologists is a necessary component of multidisciplinary teams of health professionals. Close collaboration between clinical microbiologists, ID physicians, and clinical practitioners would facilitate the

exchange of information, recognition of unusual mechanisms of resistance in pathogens, the application of accurate antimicrobial testing assays, swift communication, and early therapeutic intervention [20].

In CML the phenotypic tests to detect carbapenemase production in *Enterobacteriaceae* are widely used specially in low incoming countries, in some cases the reagents availability and low costs are the main factors implicated in their use. Thus, in isolates with carbapenemase production suspected the use of tests based in inhibitors such boronic acid compounds or EDTA to detect KPC or MBL carbapenemases respectively has become widespread in these laboratories [34]. Recently, the modified carbapenem inactivation method (mCIM) has replaced to modified Hodge test (MHT) in CML due its greater capacity to detect the carbapenemase activity in isolates carbapenem resistant, is easy to perform and their low costs. The use of colorimetric assays such Carba NP or related is a common practice to detect carbapenemases from isolates or blood culture bottles, however, the costs could be a limitation for routine use[35]. In the practice, the phenotypic tests are recommended only for epidemiological or infection control, however the misinterpretation of the results and time of effort without a congruent diagnose (strain identification, resistance and state of patient) could interfere with therapeutic decision-making.

New laboratory tests have been reported to provide information about the detection and analysis specially of KPC carbapenemases production, changing the ways in which hospitals prevent the spread of pathogens. Control of Cp-Kpn infections inside the hospital and communication about their management, particularly in the spread of KPC-Kp, pose a challenge in part because laboratory reports are not easily understood by infectious disease (ID) physicians and clinical practitioners [36].

The use of automated systems for detection of KPC carbapenemase

In CMLs, antibiotic susceptibility testing (AST) can be performed by using a broth dilution test (BD), antimicrobial gradient method (AG), or disk diffusion test (DD), which determines Minimum Inhibitory Concentration (MIC) values through of BD and AG methodologies [37]. Protocols for interpreting these tests are based upon breakpoints recommended by the Clinical and Laboratory Standards Institute (CLSI) or the European Committee on Antimicrobial Susceptibility Testing (EUCAST). This breakpoint allows for greater detection of the number of carbapenemase -producer *Enterobacteriaceae* isolates; however, some of these strains could be classified as carbapenem sensitive, especially if using Meropenem. The level of MIC may depend upon its association with different mechanisms (e.g. other β-lactamases enzymes or the presence of porins) [38]. For this reason, MIC determination must be fast, accurate, and show the full range of MIC values for any antibiotic, especially before the administration of treatment. The Food and Drug Administration (FDA) of the United States has approved several AST automated assays, and they are currently being employed in several reference CMLs. Automation of AST has brought several advantages, including rapid generation of susceptibility test results (3.5–16 h), fewer sample manipulations, and increased accuracy in species identification and of results overall. [39].

Each automatized assay has advantages and disadvantages in the determination and interpretation of MIC values, and shortcomings must be managed carefully when detecting and reporting the mechanisms of antibiotic resistance. The Vitek 2 system, produced by bioMérieux, analyzes several microliters of a given antibiotic after it has been poured into plastic cards. Assays are monitored for turbidity and subsequently interpreted by an Advanced Expert System (AES). Another automatized assay, the Phoenix system (BD Diagnostic Systems) utilizes the BDXpert system, a rule-based software that is capable of

interpreting and give recommendations related to the organism identified by broth microdilution results based in CLIS and EUCAST documents reports. In addition, the assay system includes EpiCenter software, which conducts epidemiological analyses for monitoring multidrug resistant bacteria in hospital settings [40]. The MicroScan assay (Beckman Coulter) is powered by LabPro AlertEX System software and detects atypical results in panels. The Sensititre (Thermo Scientific™) consists of plates with antibiotic dilutions powered by the ARIS™ 2X System and provides automatic, non-extrapolated reads of the MIC values, simultaneously reducing the workload for lab technicians and improving the accuracy of results. Despite each method's unique advantages, utilizing different automated platforms for detecting carbapenem resistance in *K. pneumoniae* harboring bla_{KPC} can lead to discrepancies across studies, which results in frequent reporting of very major errors (VME). VME of true-resistant isolates has led to cases of failure in antibiotic therapy. For example, Tenover F. et al. showed discrepant results for imipenem and meropenem determination in 15 *bla*KPC-positive *K. pneumonia*. After being characterized by isoelectric focusing, all strains were found to be resistant or intermediate to imipenem and meropenem using broth microdilution (BMD) as a gold standard. VME were observed in imipenem determinations, MicroScan: 1 (6.7%) Phoenix: 2 (13.3%), Vitek: 10 (67%), Vitek2: 5 (33%) isolates. Sensititre showed 13 (87%) strains sensitive to imipenem, and 12 (80%) to meropenem [41]. Bratu et al. showed similar results of VME when testing resistance to imipenem in bla_{KPC}-positive *Klebsiella pneumonia* after conducting analyses using the Vitek system (1 isolate) and MicroScan (2 isolates) [42].

To reduce VME, breakpoint updates for carbapenem and other antibiotics were established in 2010 by the CLSI. This modification decreased the occurrence of errors, and several studies have reported a subsequent increase in specificity. Doern et al. showed that the updated CLSI breakpoints resulted in better detection of KPC-producing *K. pneumoniae*

and KPC-producing non-*K. pneumoniae* [43]. Their results were corroborated by Woodford et al. in their study assessing three commercial systems to detect carbapenem resistant isolates, which showed that the sensitivity and specificity values for the presence of carbapenemase were 100%/0% to BD Phoenix, 82 to 85%/6 to 19% in MicroScan, and 74%/38% to Vitek 2. No VMEs were reported in KPC-producing *Enterobacteriaceae* [44]. Pasteran et al, proposed the importance of simultaneously testing two or more carbapenems (impenem and meropenem) in Vitek 2 systems in order to enhance the detection of carbapenemase production in *Enterobacteriacea*e, including those KPC-producing strains [45].

Phenotypic tests for the detection of AmpC inhibition would result helpful in the distinction of AmpC β-lactamases from ESBL and Metallo-β-lactamases (MBL). Tests like the AmpC disk, Gots (Modified Hodge Test, MHT) and three-dimentional test allow its detection when no molecular analysis are available. Important to point out, MHT can provide false negative information about AmpC and EMBL active bacteria being positive for the AmpC Disk Test. The Disk Test would be helping to permeabilize gram-negative cells β-lactamases release. AmpC β-lactamases and MBL can be detected by multiplex AmpC PCR with high consistency [46,47].

During CML analysis, AST can present several challenges that should be carefully considered as they have the potential to influence results. The inoculum effect, the algorithm used to detect carbapenem resistance, and the type of card used to identify the resistance can influence MIC determination [48]. For example, in clinical practice, the fixed and specific range of antibiotic dilutions incorporated in different panels in the automatized assays make it difficult to know whether the MIC values of meropenem or imipenem are out of range; however, customizing cards by incorporating specific antibiotics and their dilution concentrations has enabled microbiologists to overcome this problem. The resulting MIC

values are very important in decision-making regarding therapeutic management of infections by KPC-Kp. This topic has been highlighted by Tumbarello, who showed a low mortality rate in bloodstream infections using meropenem in antibiotic schemes, especially in strains with ≤ 8 mg/L[19]. In their study, Del Bono et al. used the values of pharmacokinetic/pharmacodynamic (PK/PD) targets of meropenem (MEM). These values were not reached in critically-ill patients with bloodstream infections (BSI) due to isolates of KPC-Kp with MEM minimum inhibitory concentrations (MICs) ≥16 mg/L [49]. Additionally, a practical alternative for improving the identification of KPC-Kp could be to perform gradient diffusion methods or customized cards. In summary, the clinical practitioner must know and understand the presence of the VME issues specific to each automatized assay used in the CML, the necessity of testing two or more carbapenems in order to detect resistance, how to interpret the MIC, and how to make appropriate decisions regarding the therapeutic strategy.

Molecular methods and the detection of KPC carbapenemase for therapeutic decision-making

In ASP, quick generation of an AST report is crucial for accurately prescribing antibiotic therapy, especially for patients with sepsis. Despite the advances of automation in CML analysis, DD, BD and AG testing methods all require considerable time (24- 48 hours) to yield results. Performing such assays requires a pure culture of a given pathogen and time to grow and estimate their susceptibility. Several new technologies that draw upon molecular methodologies have been introduced in CMLs in order to overcome the challenges related to time-intensive testing. For example, the molecular antibiogram (MA) is an alternative tool that detects resistance mechanisms clinically relevant to predicting clinical resistance by hydrolysis of antibiotics [50]. Several panel-based molecular diagnoses for MA, approved by the FDA, detect pathogens from samples such as blood culture bottles,

respiratory secretions, stool, and cerebrospinal fluid. In cases of sepsis, the FilmArray Blood Culture Identification (BCID) panel (BioFire Diagnostics, LLC) and Verigene Gram-positive blood culture (BC-GP) and Gram-negative blood culture (BC-GN) tests (Luminex Corporation) can identify a wide range of pathogens and include the detection of *mecA, VanA, VanB, bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{OXA}$, *bla*$_{IMP}$ and *bla*$_{CTX-M}$ genes, all related to MRS, directly from blood-cultured bottles [51].

Several published studies showed clinical and therapeutic advantages for the use of MA in the identification of pathogens and the presence of the most common resistance genes. MA testing panels for Gram-positive and Gram-negative bacteria provide fast CML reports, helping physicians to effectively elect courses of treatment. Nevertheless, increasing the quantity of studies regarding the MA testing panels for KPC-Kp is important for continuing its application in multiple clinical settings. Despite its costliness, the application of MA has been found to decrease mortality rates, the time to de-escalation of antibiotics, and unnecessary antibiotic initiation. ASP has been shown to decrease 1) infections by resistant organisms such as vancomycin resistant enterococci (VRE), 2) patients length of stay (LOS), and 3) costs related to health care [52–57]. As with any other molecular test, the potential for contamination of sample bottles by commensal bacteria could generate uncertainty when clinicians make decisions regarding treatment. To apply MA testing, a protocol with quality-control standards must be implemented for the handling of samples and conducting procedures in order to detect the highest number of pathogens possible and to rule out the possibility of contamination by previous Gram stains. Though ASP has been shown to improve patient mortality rates in cases of sepsis related to KPC-Kp, more evidence of its clinical and therapeutic impact is needed, particularly in hospital settings of low and middle-income countries.

The sensitivity and specificity of molecular assays for detecting *bla*$_{KPC}$ genes from blood cultures are approximately 100% and 98%, respectively [58–60]. Hill et al. evaluated the accuracy of Verigene in Gram-Negative Blood Culture (BC-GN) (Nanosphere, Inc., Northfield, IL) In 54 samples tested, 51 isolates resulted correctly identified and the overall results showed that 31% of the patients could have been identified 33 hours sooner and thus could have received earlier. [61]. Neuner et al. included 877 patients, of which 6 patients were identified as possessing the *bla*$_{KPC}$ gene by employing the Verigene assay. Overall, these results show that the application of ASP combined with MA decreased the time of antimicrobial switch, active therapy administration, and LOS [62].

Despite evidence of MA's potential to improve clinical and therapeutic results in cases of antibiotic resistant bacteria, MA diagnosis related to *bla*$_{KPC}$ determination is not sufficient, and important factors related to its limitations should be taken in consideration. Even if MA could be applied in blood samples, MIC determination is also necessary in order to apply an optimal antibiotic combination. The expression of *bla*$_{KPC}$ in *K. pneumoniae* is not uniform and could stem from DNA regulatory changes upstream of *bla*$_{KPC}$ gene, possibly affecting the MIC values and therapeutic response [63]. Colistin is recommended in combination therapy in patients with KPC-Kp infection; however, this still represents a risk for emerging KPC-Kp colistin resistance, as there is currently no molecular method available to detect it.

Treatment

At the moment, there is no antibiotic scheme regarded as the "gold standard" for KPC-Kp infections. The choice of treatment depends upon the site of serious infection, type of carbapenemase, and the susceptibility profile of the isolate. In critically-ill patients, there is evidence to suggest that combined therapy is preferable to monotherapy. Many reports, including large retrospective cohort studies, have demonstrated that more treatment failures

and higher mortality rates are associated with monotherapy antibiotic schemes consisting of polymyxin (colistin), carbapenems, tigecycline and gentamicin. Receiving combinatory therapies with at least two drugs with tested CML activity has shown greater effectiveness in critically-ill patients. The association of meropenem with other antibiotics is important and beneficial when the KPC-Kp isolate has a MIC of meropenem ≤ 8mg/L [64]; however, in β-lactam antibiotics, is important to maintain antibiotic exposure. Enhancement of fT>MIC could be achieved using either continuous, total daily dose infused over a 24-hour period or prolonged infusions [21].

Ceftazidime is a third generation cephalosporin that can be used in combination with avibactam (β-lactamase inhibitor) if the organism is a KPC or OXA-48 producer. A second antibiotic, such as carbapenem, can be added if both isolates have MICs near the susceptibility breakpoint. Using this antibiotic combination could yield positive results and should be considered when treating serious infections due to these pathogens [65–67]; however, resistance has emerged in KPC-3-producing *K. pneumoniae*, pointing to a failure in combinatory treatment. In this case, a more exhaustive analysis should have been performed during the course of treatment in order to rapidly respond and change the antibiotic combination [68,69]. Recently, the combination of meropenem with vaborbactam (formerly RPX7009), a novel cyclic boronic acid-based beta-lactamase inhibitor, was found to potentiat the activity of meropenem. This combination may be more suitable for treating severe drug resistant gram-negative infections as it shows higher rates of clinical effectiveness. The body of evidence surrounding the combination of meropenem and vaborbactam is still limited compared to ceftazidime-avibactam. Recently, other agents (imipenem-relebactam, plazomicin, and cefiderocol) have begun to be evaluated in clinical trials, and the results from these studies are expected to improve clinical response and its application in ASP [70]. Other potential active drugs for treating KPC-Kp include classic and

new combinations of aminoglycosides, tigecycline, fosfomycin, and the Eravacycline, a fully-synthetic fluorocycline antibiotic, which is used in complicated intra-abdominal infections [71,72]. Uncomplicated and complicated urinary tract infections caused by KPC-Kp are frequently reported in hospital settings; however, there is no evidence about the best antibiotic regimen for its treatment. It may be possible to include aminoglycoside alone or to use on combination with fosfomycin or doxycycline, as it has shown promising results for treating this type of infection [72].

Conclusion

This review provides relevant information for clinical practitioners about the state of the literature on KPC-Kp and current diagnostic tests for its detection. We suggest that healthcare professionals use the key points presented in this review in order to precisely identify and treat KPC-Kp infections. Understanding the genetic underpinnings of antibiotic resistance, its clinical manifestations, and standard diagnostic procedures in CMLs will prevent outbreaks and provide healthcare practitioners and scientists with evidence for understanding KPC-Kp dynamics and epidemiology.

Abbreviations

Cp-Kpn, carbapenemase–producing *K. pneumoniae* strains; ASP, antimicrobial stewardship program; Cr-KPN, carbapanem- resistant *K. pneumoniae*; KPC-Kp, KPC–producer *K. pneumoniae*; KPC, *Klebsiella pneumoniae* carbapenemase; CML, Clinical microbiology laboratories;; ID, infectious disease; MDR, multidrug resistant bacteria; AST, antibiotic susceptibility testing; DD, disk diffusion; BD, broth dilution; AG, antimicrobial gradient; CLSI, Clinical and Laboratory Standards Institute; EUCAST, European

Committee on Antimicrobial Susceptibility Testing; VME, very major errors; MA, molecular antibiogram; LOS, length of stay.

Acknowledgements

The authors would like to thank the School of Medicine of the Universidad San Francisco de Quito (USFQ) for its academic and financial support and for encouraging interdisciplinary collaboration. Thanks, is also given to Sistemas Médicos (SIME-USFQ) for their interest in providing funds for developing research on clinical practice. We would like to thank Clara Cullen and Heather Vellers for their proof reading, help and availability in editing the article. We especially thank to Ana María Gómez for her scientific and medical support. Authors would like to express their gratitude to: Johanna Vicuña, Paola Yépez, Alfredo Terán, Pablo Morejón, Gabriela Aldas, Carolina Escudero, Jonathan Villacís, Manuel Burbano and Mishell Barreno students of the school of Medicine for their initial support and for pointing out the need of microbiology key points for clinical practice regarding Carbapenem-resistant *Klebsiella pneumoniae*.

Contributions

All authors made substantial contributions to conception, design and interpretation of data; they took part in drafting the article and revising it critically for important intellectual content; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. AC put together the team to address the focus of our article.

Competing interests

The authors declare no competing financial and no-financial interests.

Data availability statement

All data generated in this article and derived data supporting the findings of this study are available from the corresponding author on request.

Corresponding author

Instituto de Microbiología, Universidad San Francisco de Quito (USFQ), Diego de Robles y Pampite S/N, 17-09-01 Quito, Ecuador

jorgereyes83@gmail.com

References

1. Lee C-R, Lee JH, Park KS, Kim YB, Jeong BC, Lee SH. Global Dissemination of Carbapenemase-Producing *Klebsiella pneumoniae*: Epidemiology, Genetic Context, Treatment Options, and Detection Methods. *Front Microbiol*. 7. doi:10.3389/fmicb.2016.00895

2. Leavitt A, Chmelnitsky I, Colodner R, Ofek I, Carmeli Y, Navon-Venezia S. Ertapenem resistance among extended-spectrum-beta-lactamase-producing *Klebsiella pneumoniae* isolates. *J Clin Microbiol*. 2009;47(4):969-974. doi:10.1128/JCM.00651-08

3. Wu J-J, Wang L-R, Liu Y-F, Chen H-M, Yan J-J. Prevalence and characteristics of ertapenem-resistant *Klebsiella pneumoniae* isolates in a Taiwanese university hospital. *Microb Drug Resist Larchmt N*. 2011;17(2):259-266. doi:10.1089/mdr.2010.0115

4. Pitout JDD, Nordmann P, Poirel L. Carbapenemase-producing *Klebsiella pneumoniae*, a key pathogen set for global nosocomial dominance. *Antimicrob Agents Chemother*. 2015;59(10):5873-5884. doi:10.1128/AAC.01019-15

5. Yong D, Toleman MA, Giske CG, et al. Characterization of a new metallo-beta-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. *Antimicrob Agents Chemother*. 2009;53(12):5046-5054. doi:10.1128/AAC.00774-09

6. Navon-Venezia S, Kondratyeva K, Carattoli A. Klebsiella pneumoniae: a major worldwide source and shuttle for antibiotic resistance. *FEMS Microbiol Rev*. 2017;41(3):252-275. doi:10.1093/femsre/fux013

7. Yigit H, Queenan AM, Anderson GJ, et al. Novel carbapenem-hydrolyzing beta-lactamase, KPC-1, from a carbapenem-resistant strain of Klebsiella pneumoniae. *Antimicrob Agents Chemother*. 2001;45(4):1151-1161. doi:10.1128/AAC.45.4.1151-1161.2001

8. Munoz-Price LS, Poirel L, Bonomo RA, et al. Clinical epidemiology of the global expansion of *Klebsiella pneumoniae* carbapenemases. *Lancet Infect Dis*. 2013;13(9):785-796. doi:10.1016/S1473-3099(13)70190-7

9. Escandón-Vargas K, Reyes S, Gutiérrez S, Villegas MV. The epidemiology of carbapenemases in Latin America and the Caribbean. *Expert Rev Anti Infect Ther*. 15(3):277-297. doi:10.1080/14787210.2017.1268918

10. Kizny Gordon AE, Mathers AJ, Cheong EYL, et al. The Hospital Water Environment as a Reservoir for Carbapenem-Resistant Organisms Causing Hospital-Acquired Infections-A Systematic Review of the Literature. *Clin Infect Dis Off Publ Infect Dis Soc Am*. 2017;64(10):1435-1444. doi:10.1093/cid/cix132

11. Leitner E, Zarfel G, Luxner J, et al. Contaminated handwashing sinks as the source of a clonal outbreak of KPC-2-producing *Klebsiella oxytoca* on a hematology ward. *Antimicrob Agents Chemother*. 2015;59(1):714-716. doi:10.1128/AAC.04306-14

12. Rock C, Thom KA, Masnick M, Johnson JK, Harris AD, Morgan DJ. Frequency of Klebsiella pneumoniae carbapenemase (KPC)-producing and non-KPC-producing *Klebsiella* species contamination of healthcare workers and the environment. *Infect Control Hosp Epidemiol*. 2014;35(4):426-429. doi:10.1086/675598

13. Gastmeier P, Vonberg R-P. *Klebsiella* spp. in endoscopy-associated infections: we may only be seeing the tip of the iceberg. *Infection*. 2014;42(1):15-21. doi:10.1007/s15010-013-0544-6

14. Saidel-Odes L, Borer A. Limiting and controlling carbapenem-resistant Klebsiella pneumoniae. *Infect Drug Resist*. 2013;7:9-14. doi:10.2147/IDR.S44358

15. Zarkotou O, Pournaras S, Tselioti P, et al. Predictors of mortality in patients with bloodstream infections caused by KPC-producing *Klebsiella pneumoniae* and impact of appropriate antimicrobial treatment. *Clin Microbiol Infect*. 2011;17(12):1798-1803. doi:10.1111/j.1469-0691.2011.03514.x

16. Daikos GL, Tsaousi S, Tzouvelekis LS, et al. Carbapenemase-Producing Klebsiella pneumoniae Bloodstream Infections: Lowering Mortality by Antibiotic Combination Schemes and the Role of Carbapenems. *Antimicrob Agents Chemother*. 2014;58(4):2322–2328. doi:10.1128/AAC.02166-13

17. Schwaber MJ, Klarfeld-Lidji S, Navon-Venezia S, Schwartz D, Leavitt A, Carmeli Y. Predictors of Carbapenem-Resistant *Klebsiella pneumoniae* Acquisition among Hospitalized Adults and Effect of Acquisition on Mortality. *Antimicrob Agents Chemother*. 2008;52(3):1028–1033. doi:10.1128/AAC.01020-07

18. Schwaber MJ, Klarfeld-Lidji S, Navon-Venezia S, Schwartz D, Leavitt A, Carmeli Y. Predictors of Carbapenem-Resistant *Klebsiella pneumonia*e Acquisition among Hospitalized Adults and Effect of Acquisition on Mortality. *Antimicrob Agents Chemother*. 2008;52(3):1028-1033. doi:10.1128/AAC.01020-07

19. Tumbarello M, Trecarichi EM, De Rosa FG, et al. Infections caused by KPC-producing *Klebsiella pneumoniae*: differences in therapy and mortality in a multicentre study. *J Antimicrob Chemother*. 2015;70(7):2133-2143. doi:10.1093/jac/dkv086

20. Facility Guidance for Control of Carbapenem-resistant Enterobacteriaceae (CRE) – November 2015 Update CRE Toolkit | HAI | CDC. https://www.cdc.gov/hai/organisms/cre/cre-toolkit/index.html. Published April 16, 2019. Accessed September 16, 2019.

21. Bassetti M, Giacobbe DR, Giamarellou H, et al. Management of KPC-producing *Klebsiella pneumoniae* infections. *Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis*. 2018;24(2):133-144. doi:10.1016/j.cmi.2017.08.030

22. Rivers E, Nguyen B, Havstad S, et al. Early Goal-Directed Therapy in the Treatment of Severe Sepsis and Septic Shock. *N Engl J Med*. 2001;345(19):1368-1377. doi:10.1056/NEJMoa010307

23. Falagas ME, Lourida P, Poulikakos P, Rafailidis PI, Tansarli GS. Antibiotic treatment of infections due to carbapenem-resistant *Enterobacteriaceae:* systematic evaluation of the available evidence. *Antimicrob Agents Chemother*. 2014;58(2):654-663. doi:10.1128/AAC.01222-13

24. Papp-Wallace KM, Endimiani A, Taracila MA, Bonomo RA. Carbapenems: Past, Present, and Future ▿. *Antimicrob Agents Chemother*. 2011;55(11):4943-4960. doi:10.1128/AAC.00296-11

25. Logan LK, Weinstein RA. The epidemiology of Carbapenem-resistant *Enterobacteriaceae*: The impact and evolution of a global menace. *J Infect Dis*. 2017;215(March):S28-S36. doi:10.1093/infdis/jiw282

26. Bush K, Jacoby GA. Updated functional classification of Beta-lactamases. *Antimicrob Agents Chemother*. 2010;54(3):969-976. doi:10.1128/AAC.01009-09

27. Martins WMBS, Nicoletti AG, Santos SR, Sampaio JLM, Gales AC. Frequency of BKC-1-Producing *Klebsiella* Species Isolates. *Antimicrob Agents Chemother*. 2016;60(8):5044-5046. doi:10.1128/AAC.00470-16

28. Naas T, Oueslati S, Bonnin RA, et al. Beta-lactamase database (BLDB) - structure and function. *J Enzyme Inhib Med Chem*. 2017;32(1):917-919. doi:10.1080/14756366.2017.1344235

29. Chen L, Mathema B, Chavda KD, DeLeo FR, Bonomo RA, Kreiswirth BN. Carbapenemase-producing *Klebsiella pneumoniae*: Molecular and genetic decoding. *Trends Microbiol*. 2014;22(12):686-696. doi:10.1016/j.tim.2014.09.003

30. Naas T, Cuzon G, Villegas MV, Lartigue MF, Quinn JP, Nordmann P. Genetic structures at the origin of acquisition of the β-lactamase blaKPC gene. *Antimicrob Agents Chemother*. 2008;52(4):1257-1263. doi:10.1128/AAC.01451-07

31. Gomez SA, Pasteran FG, Faccone D, et al. Clonal dissemination of Klebsiella pneumoniae ST258 harbouring KPC-2 in Argentina. *Clin Microbiol Infect*. 2011;17(10):1520-1524. doi:10.1111/j.1469-0691.2011.03600.x

32. Doumith M, Findlay J, Hirani H, et al. Major role of pKpQIL-like plasmids in the early dissemination of KPC-type carbapenemases in the UK. *J Antimicrob Chemother*. 2017;72(8):2241-2248. doi:10.1093/jac/dkx141

33. Schuts EC, Hulscher MEJL, Mouton JW, et al. Current evidence on hospital antimicrobial stewardship objectives: a systematic review and meta-analysis. *Lancet Infect Dis*. 2016;16(7):847-856. doi:10.1016/S1473-3099(16)00065-7

34. Pournaras S, Poulou A, Tsakris A. Inhibitor-based methods for the detection of KPC carbapenemase-producing *Enterobacteriaceae* in clinical practice by using boronic acid compounds. *J Antimicrob Chemother*. 2010;65(7):1319-1321. doi:10.1093/jac/dkq124

35. Lima-Morales D de, Ávila H, Soldi T, et al. Rapid Detection of Carbapenemase Production Directly from Blood Culture by Colorimetric Methods: Evaluation in a Routine Microbiology Laboratory. *J Clin Microbiol*. 2018;56(9). doi:10.1128/JCM.00325-18

36. Humphries RM. Clinical laboratory detection of carbapenem-resistant and carbapenemase-producing *Enterobacteriaceae* AU - Miller, Shelley. *Expert Rev Anti Infect Ther*. 2016;14(8):705-717. doi:10.1080/14787210.2016.1206815

37. Reller LB, Weinstein M, Jorgensen JH, Ferraro MJ. Antimicrobial Susceptibility Testing: A Review of General Principles and Contemporary Practices. *Clin Infect Dis*. 2009;49(11):1749-1755. doi:10.1086/647952

38. Fattouh R, Tijet N, McGeer A, Poutanen SM, Melano RG, Patel SN. What Is the Appropriate Meropenem MIC for Screening of Carbapenemase-Producing *Enterobacteriaceae* in Low-Prevalence Settings? *Antimicrob Agents Chemother*. 2015;60(3):1556-1559. doi:10.1128/AAC.02304-15

39. Eigner U, Schmid A, Wild U, Bertsch D, Fahr A-M. Analysis of the comparative workflow and performance characteristics of the VITEK 2 and Phoenix systems. *J Clin Microbiol*. 2005;43(8):3829-3834. doi:10.1128/JCM.43.8.3829-3834.2005

40. Winstanley T, Courvalin P. Expert Systems in Clinical Microbiology. *Clin Microbiol Rev*. 2011;24(3):515–556. doi:10.1128/CMR.00061-10

41. Tenover FC, Kalsi RK, Williams PP, et al. Carbapenem resistance in *Klebsiella pneumoniae* not detected by automated susceptibility testing. *Emerg Infect Dis*. 2006;12(8):1209-1213. doi:10.3201/eid1208.060291

42. Bratu S, Landman D, Haag R, et al. Rapid Spread of Carbapenem-Resistant *Klebsiella pneumoniae* in New York City: A New Threat to Our Antibiotic Armamentarium. *Arch Intern Med*. 2005;165(12):1430-1435. doi:10.1001/archinte.165.12.1430

43. Doern CD, Dunne WMJ, Burnham C-AD. Detection of Klebsiella pneumoniae carbapenemase (KPC) production in non-*Klebsiella pneumoniae Enterobacteriaceae* isolates by use of the Phoenix, Vitek 2, and disk diffusion methods. *J Clin Microbiol*. 2011;49(3):1143-1147. doi:10.1128/JCM.02163-10

44. Woodford N, Eastaway AT, Ford M, et al. Comparison of BD Phoenix, Vitek 2, and MicroScan automated systems for detection and inference of mechanisms responsible for carbapenem resistance in *Enterobacteriaceae. J Clin Microbiol*. 2010;48(8):2999-3002. doi:10.1128/JCM.00341-10

45. Pasteran F, Lucero C, Soloaga R, Rapoport M, Corso A. Can we use imipenem and meropenem Vitek 2 MICs for detection of suspected KPC and other-carbapenemase producers among species o*f Enterobacteriaceae*? *J Clin Microbiol*. 2011;49(2):697-701. doi:10.1128/JCM.01178-10

46. Thomson KS. Extended-Spectrum-β-Lactamase, AmpC, and Carbapenemase Issues. *J Clin Microbiol*. 2010;48(4):1019-1025. doi:10.1128/JCM.00219-10

47. Wong MH, Li Y, Chan EW, Chen S. Functional categorization of carbapenemase-mediated resistance by a combined genotyping and two-tiered Modified Hodge Test approach. *Front Microbiol*. 2015;6. doi:10.3389/fmicb.2015.00293

48. Vading M, Samuelsen O, Haldorsen B, Sundsfjord AS, Giske CG. Comparison of disk diffusion, Etest and VITEK2 for detection of carbapenemase-producing Klebsiella pneumoniae with the EUCAST and CLSI breakpoint systems. *Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis*. 2011;17(5):668-674. doi:10.1111/j.1469-0691.2010.03299.x

49. Del Bono V, Giacobbe DR, Marchese A, et al. Meropenem for treating KPC-producing Klebsiella pneumoniae bloodstream infections: Should we get to the PK/PD root of the paradox? *Virulence*. 2017;8(1):66-73. doi:10.1080/21505594.2016.1213476

50. Arena F, Giani T, Pollini S, Viaggi B, Pecile P, Rossolini GM. Molecular antibiogram in diagnostic clinical microbiology: advantages and challenges. *Future Microbiol*. 2017;12:361-364. doi:10.2217/fmb-2017-0019

51. Ramanan P, Bryson AL, Binnicker MJ, Pritt BS, Patel R. Syndromic Panel-Based Testing in Clinical Microbiology. *Clin Microbiol Rev*. 2018;31(1). doi:10.1128/CMR.00024-17

52. MacVane SH, Nolte FS. Benefits of Adding a Rapid PCR-Based Blood Culture Identification Panel to an Established Antimicrobial Stewardship Program. Burnham C-AD, ed. *J Clin Microbiol*. 2016;54(10):2455–2463. doi:10.1128/JCM.00996-16

53. Pardo J, Klinker KP, Borgert SJ, Butler BM, Giglio PG, Rand KH. Clinical and economic impact of antimicrobial stewardship interventions with the FilmArray blood culture identification panel. *Diagn Microbiol Infect Dis*. 2016;84(2):159-164. doi:10.1016/j.diagmicrobio.2015.10.023

54. Messacar K, Hurst AL, Child J, et al. Clinical Impact and Provider Acceptability of Real-Time Antimicrobial Stewardship Decision Support for Rapid Diagnostics in Children With Positive Blood Culture Results. *J Pediatr Infect Dis Soc*. 2017;6(3):267-274. doi:10.1093/jpids/piw047

55. Walker T, Dumadag S, Lee CJ, et al. Clinical Impact of Laboratory Implementation of Verigene BC-GN Microarray-Based Assay for Detection of Gram-Negative Bacteria in Positive Blood Cultures. *J Clin Microbiol*. 2016;54(7):1789-1796. doi:10.1128/JCM.00376-16

56. Banerjee R, Teng CB, Cunningham SA, et al. Randomized Trial of Rapid Multiplex Polymerase Chain Reaction-Based Blood Culture Identification and Susceptibility Testing. *Clin Infect Dis Off Publ Infect Dis Soc Am*. 2015;61(7):1071-1080. doi:10.1093/cid/civ447

57. Bork JT, Leekha S, Heil EL, Zhao L, Badamas R, Johnson JK. Rapid testing using the Verigene Gram-negative blood culture nucleic acid test in combination with antimicrobial stewardship intervention against Gram-negative bacteremia. *Antimicrob Agents Chemother*. 2015;59(3):1588-1595. doi:10.1128/AAC.04259-14

58. Salimnia H, Fairfax MR, Lephart PR, et al. Evaluation of the FilmArray Blood Culture Identification Panel: Results of a Multicenter Controlled Trial. *J Clin Microbiol*. 2016;54(3):687-698. doi:10.1128/JCM.01679-15

59. Sullivan K V., Deburger B, Roundtree SS, Ventrola CA, Blecker-Shelly DL, Mortensen JE. Pediatric multicenter evaluation of the Verigene gram-negative blood culture test for rapid detection of inpatient bacteremia involving gram-negative organisms, extended-spectrum beta-lactamases, and carbapenemases. *J Clin Microbiol*. 2014;52(7):2416-2421. doi:10.1128/JCM.00737-14

60. Ledeboer NA, Lopansri BK, Dhiman N, et al. Identification of Gram-Negative Bacteria and Genetic Resistance Determinants from Positive Blood Culture Broths by Use of the Verigene Gram-Negative Blood Culture Multiplex Microarray-Based Molecular Assay. *J Clin Microbiol*. 2015;53(8):2460-2472. doi:10.1128/JCM.00581-15

61. Hill JT, Tran K-DT, Barton KL, Labreche MJ, Sharp SE. Evaluation of the nanosphere Verigene BC-GN assay for direct identification of gram-negative bacilli and antibiotic resistance markers from positive blood cultures and potential impact for more-rapid antibiotic interventions. *J Clin Microbiol*. 2014;52(10):3805-3807. doi:10.1128/JCM.01537-14

62. Neuner E, Rivard K, Lam S, et al. Impact of Antimicrobial Stewardship and Rapid Microarray Testing on Patients With Gram-Negative Bacteremia. *Open Forum Infect Dis*. 2016;3(suppl_1). doi:10.1093/ofid/ofw194.11

63. Cheruvanky A, Stoesser N, Sheppard AE, et al. Enhanced *Klebsiella pneumoniae* Carbapenemase Expression from a Novel Tn*4401* Deletion. *Antimicrob Agents Chemother*. 2017;61(6). doi:10.1128/AAC.00025-17

64. Tumbarello M, Trecarichi EM, De Rosa FG, et al. Infections caused by KPC-producing *Klebsiella pneumoniae:* Differences in therapy and mortality in a multicentre study. *J Antimicrob Chemother*. 2014;70(7):2133-2143. doi:10.1093/jac/dkv086

65. Shields RK, Nguyen MH, Chen L, et al. Ceftazidime-Avibactam Is Superior to Other Treatment Regimens against Carbapenem-Resistant *Klebsiella pneumoniae* Bacteremia. *Antimicrob Agents Chemother*. 2017;61(8). doi:10.1128/AAC.00883-17

66. Van Duin D, Lok JJ, et al. Colistin Versus Ceftazidime-Avibactam in the Treatment of Infections Due to Carbapenem-Resistant *Enterobacteriaceae. Clin Infect Dis*. 2017;66(2):163-171. doi:10.1093/cid/cix783

67. Rodriguez-Bano J, Gutierrez-Gutierrez B, Machuca I, Pascual A. Treatment of Infections Caused by Extended-Spectrum-Beta-Lactamase-, AmpC-, and Carbapenemase-Producing *Enterobacteriaceae*. *Clin Microbiol Rev*. 2018;31(2). doi:10.1128/CMR.00079-17

68. Press EG, Haidar G, Doi Y, et al. Clinical Outcomes, Drug Toxicity, and Emergence of Ceftazidime-Avibactam Resistance Among Patients Treated for Carbapenem-Resistant *Enterobacteriaceae* Infections. *Clin Infect Dis*. 2016;63(12):1615-1618. doi:10.1093/cid/ciw636

69. Humphries RM, Yang S, Hemarajata P, et al. First Report of Ceftazidime-Avibactam Resistance in a KPC-3-Expressing Klebsiella pneumoniae Isolate. *Antimicrob Agents Chemother*. 2015;59(10):6605-6607. doi:10.1128/AAC.01165-15

70. Petty LA, Henig O, Patel TS, Pogue JM, Kaye KS. Overview of meropenem-vaborbactam and newer antimicrobial agents for the treatment of carbapenem-resistant *Enterobacteriaceae. Infect Drug Resist*. 2018;11:1461-1472. doi:10.2147/IDR.S150447

71. Abdallah M, Olafisoye O, Cortes C, Urban C, Landman D, Quale J. Activity of eravacycline against *Enterobacteriaceae* and *Acinetobacter baumannii*, including multidrug-resistant isolates, from New York City. *Antimicrob Agents Chemother*. 2015;59(3):1802-1805. doi:10.1128/AAC.04809-14

72. Tzouvelekis LS, Markogiannakis A, Piperaki E, Souli M, Daikos GL. Treating infections caused by carbapenemase-producing *Enterobacteriaceae*. *Clin Microbiol Infect*. 2014;20(9):862-872. doi:10.1111/1469-0691.12697

CAPITULO III

Characterization of *bla*$_{KPC-2}$ – harboring *Klebsiella pneumoniae* isolates and mobile genetic elements from outbreaks in a Hospital in Ecuador.

J. Reyes [1,2*], P. Cárdenas[1], R. Tamayo[3], A. Aguilar[1,4], R. Melano[5,6], G. Trueba[1]

1. Instituto de Microbiología, Colegio de Ciencias Biológicas y Ambientales, Universidad San Francisco de Quito, Quito, Ecuador

2. Facultad de Ciencias Químicas, Universidad Central del Ecuador, Quito, Ecuador

3. Centro de Referencia Nacional de Resistencia a los antimicrobianos "LIP", Quito, Ecuador

4. Escuela de Medicina, Colegio de Ciencias de la Salud (COCSA), Universidad San Francisco de Quito, Quito, Ecuador.

5. Public Health Ontario Laboratory, Toronto, ON, Canada.

6. Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada.

***Corresponding author**

jorgereyes83@gmail.com

Phone: +593 994551824

Address: Quito-Ecuador

Abstract

Aim: To characterize the mobile genetic elements associated to *bla*$_{KPC-2}$ gene using PCR and amplicon sequencing of 62 carbapenem resistant *K. pneumoniae* recovered during a 6-month outbreak in a high complexity hospital from Ecuador.

Results: We found 45 isolates belonging to ST258, 8 to ST25, 6 to ST348, and 3 isolates belonging to 3 different STs (ST42, ST196, and ST1748). In all isolates, the *bla*$_{KPC-2}$ was associated with Tn*4401*a isoform. A total of 61 *K. pneumoniae* isolates harbored pKpQIL plasmid except the ST1438 clone which had a plasmid with a IncM replicon. The DNA sequences contiguous to Tn*4401*a::*bla*$_{KPC-2}$ (in pKpQIL) were identical in ST25, ST348, ST42 and ST196, while all isolates belonging to ST258 presented a deletion in pKpQIL region upstream Tn*4401*a::*bla*$_{KPC-2}$.

Conclusions: This findings highlight the presence of pKpQIL::Tn*4401*a::*bla*$_{KPC-2}$ in a potential clonal expansion of *K. pneumoniae* ST258 and ST25.

Keywords: *Klebsiella pneumoniae* carbapenemase, plasmid, clone.

Running title: *bla*$_{KPC-2}$ – harboring *Klebsiella pneumoniae* isolates and mobile genetic elements from Hospital outbreaks.

Introduction

Since the first description of *Klebsiella pneumoniae* carbapenemase (KPC-1) in 2001 in the United States,[1] 39 types of KPC enzymes linked to more than 100 different sequence types (STs) of *K. pneumoniae* have been reported;[2] the most outstanding is the ST258, a high risk international clone reported around of the world causing outbreaks in hospitals.[3] The *bla*$_{KPC}$ gene is associated with Tn*4401* and non-Tn*4401* transposable elements (NTE) which have moved into different plasmid replicons.[4] However, the *bla*$_{KPC}$-harboring pKpQIL is a self-conjugative IncFIIK2 plasmid most frequently reported in *K. pneumoniae* strains in different countries.[5]

There have been reports of bloodstream infections (BSI) and non-BSI community-acquired infections caused by hypervirulent, hypermucoviscous, clones (hv) of *K. pneumoniae*.[6] In these strains the capsule is the main virulence factor, specially pathogens with capsular serotypes K1 and K2; the presence of carbapenem resistance in these hv *K. pneumoniae* serotypes is an increasing public health problem.[7,8] In Ecuador there have been reports of high risk (ST258) and hv (ST25) clones in hospital outbreaks.[9] Here we describe two outbreaks caused by *K. pneumoniae* ST258 and ST25 in a high complexity hospital in Quito, from July to December 2014. We also characterized the mobile genetic elements associated with the *bla*$_{KPC}$ genes.

Material and methods

Sampling

In 2014 from July to December, an outbreak caused by carbapenem-resistant *K. pneumoniae* (CrKPN) was detected in a high complexity hospital from Quito, Ecuador. We collected 62 isolates from different clinical samples, one isolate per each hospitalized patient in intensive care unit (ICU), internal medicine, neurosurgery, pneumology,

neurology and burn unit departments. All isolates were submitted to the National reference laboratory of the National institute of health "Leopoldo Izquieta Pérez", Quito for molecular testing. Clinical records were used to obtain clinical and demographic data.

Identification, susceptibility test and assays for carbapenemase-activity detection

Bacterial identification and antimicrobial susceptibility testing were performed using the Vitek 2 system, CARD GN AST272. Minimum inhibitory concentrations values were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.[10] The modified carbapenem inactivation method (mCIM) was performed to evaluate the carbapenemase activity in all isolates.[10]

Molecular assays

DNA was released by boiling method in all CrKPN isolates and used in PCR amplification of *bla*$_{KPC,}$ *bla*$_{NDM}$ and *bla*$_{OXA-48}$ –like.[11] The *bla*$_{KPC}$ alleles were identified by PCR and Sanger sequencing (Macrogen, Korea) using primers reported previously [12] (Supplementary Table S1) and analyzed using Mega7 software against the Beta-Lactamase DataBase (BLDB) http://www.bldb.eu/.[2]

Molecular typing was performed amplifying the *pilv-I* gene to distinguish the ST258 clones [13] (Supplementary Table S1). Isolates *pilv-l* negative were typed by multi-locus sequencing typing (MLST) (https://bigsdb.pasteur.fr/klebsiella/klebsiella.html)**.** Their phylogenic relations were performed using Phlyloviz software (http://www.phyloviz.net/).

To confirm the genetic environment of *bla*$_{KPC}$ genes, a previously described, PCR-based approach was followed.[14] PCR products obtained using primers D (Fig. 1) were sequenced (Macrogen, Korea) to determine Tn*4401* isoform. Detection of pKpQIL-like IncFIIK2 plasmids was carried out by amplification of 4 pKpQIL- like backbone target regions as described.[5] In isolates where no amplification was obtained, a set of primers was designed, based on plasmid pKpQIL-234 sequence (accession number KJ146689), using Primer-Blast

software (National Center for Biotechnology Information, available at https://www.ncbi.nlm.nih.gov/tools/primer-blast/).[5] (Supplementary Table S1).

For conjugation experiments, we selected one isolate from each non-ST258 isolates and 20 ST258 isolates to be used as donors; azide-resistant *Escherichia coli* J53 strain was used as recipient, the isolates were grown overnight in tryptic soy broth (TSB) at 37 ℃. One mL of donor culture and 1mL of receipt culture (10^9 CFUs) were added to 2 mL of TSB and incubated overnight at 37℃. The selection of transconjugants was carried out in MacConkey agar supplemented with sodium azide (50 mg/l) and different concentrations of meropenem (0.5 - 0.06 mg/l).[15] The presence of bla_{KPC} in transconjugant was confirmed by PCR and replicons were characterized using the "PBTR 2.0 kit" (Diatheva, Fano, Italy) following the manufacturer's protocols.

All hv suspected clones were subjected to a PCR in order to amplify specific regions coding for K1, K2 and K5 capsular polysaccharides (*cps*).[16]

Results

All 62 carbapenem-resistant *K. pneumoniae* were positive for bla_{KPC-2} gene; isolates recovered from July to December 2014 (n=45) were ST258 (*pilv-I*-positive) and the distribution was: 23 from ICU, 9 from neurosurgery, 7 form the burn unit, 3 from internal medicine, 2 from pneumology and 1 from neurology. Eight isolates recovered in July and September 2014 were ST25 serotype K2 and the distribution was 5 from ICU, 2 from neurosurgery and 1 from the burn unit. Six isolates obtained from October to December were ST348 and the distribution was: 3 from ICU, 1 from neurology and 2 from the burn unit. The remaining 3 isolates belonged to ST196, ST42 and ST1758 and were recovered from patients in internal medicine (Fig. 2 and supplementary Table S2). The most common infections were pneumonia (n=31), skin and soft tissue (n=18), urinary tract (n=7), BSI (5)

and catheter-related bloodstream infection (n=1). In ICU both ST258 and ST25 were related to BSI and pneumonia cases, while ST348 was only related to pneumonia. Table 1 and supplementary Table S2.

The antimicrobial susceptibility test showed that three CrKPN isolates ST25 were also resistant to tigecycline and three ST258 were colistin resistant (non-wild type) both phenotypes recovered from ICU. Additionally, all ST258 isolates were resistant to amikacin. (Supplementary Table S2).

A total of 20 conjugation assays were performed using different antibiotic concentrations for transconjugant selection, however we were successful only when using non-ST258 *K. pneumoniae* strains as donors. IncFII plasmids were identified in transconjugants derived from ST25, ST348, ST42, and ST196 donor isolates; IncM plasmid was obtained in ST1758 -transconjugants.

The plasmid regions amplified in strains of *K. pneumoniae* belonging to ST25, ST348, ST42, and ST 196 were identical to pKpQIL-243 plasmid (accession number KJ146689). However, in all strains belonging to ST258 we found a deletion of ~1000pb region upstream of the Tn*4401* which corresponded to a transposase gene and surrounding DNA regions, this deletion has not been described previously. Both, ST258 and non-ST258 strains harbored the bla_{KPC-2} gene located in the Tn*4401a* isoform. (Fig. 1).

Discussion

Our results indicate that all epidemiologically related *K. pneumoniae* isolates harbored the bla_{KPC-2} gene carried in similar mobile genetic elements (Tn*4401*a and plasmid pKpQIL-like). The fact that most of them (n=54) belonged to two sequence types (ST258, n=45 and ST25, n=8) may indicate the existence of two *K. pneumoniae* outbreaks in this hospital: one caused by ST25 from July to September and the other by ST258 from July to December, especially in ICU and neurosurgery.

KPC-producing *K. pneumoniae* ST258 and ST25 are common in hospitals in Ecuador[9] and other Latin-American countries.[17,18] With respect to ST258, a recent study showed that the high transmissibility of carbapenem non-susceptible *K. pneumoniae* in Europe is associated with intra- and inter-hospital dissemination driven by the spread of four main clonal carbapenemase-positive lineages, one of them the ST258 and derivatives.[19] Reports suggests that ST258 strains can colonize gowns and gloves of health care workers causing health care-associated infections.[20]

All KPC-2-producing *K. pneumoniae* ST25 found in this study were serotyped as K2 and all were recovered from blood and tracheal samples. The capsule is a main virulence factor, producing a mucoviscous exopolysaccharide[21] and a lower induction of reactive oxygen species in neutrophils [22,23] To date, 78 serotypes has been reported, of which serotypes K1, K2, K16, K20, K54, K57, and KN1 are considered the most virulent.[7] However, serotypes K1 and K2 are the most common pathogens recovered from human infections. [24,25,26] It has been reported that *K. pneumoniae* ST23 and ST57 were associated with serotype K1 and ST14, ST25, ST65, ST86, ST375 and ST380 were associated with serotype K2.[27,28] Additionally, ST25 was related to production of KPC enzymes in Argentina, Brazil and Ecuador.[9,17] To the best of our knowledge, this is the first description of *bla*$_{\text{KPC-2}}$- harboring pKpQIL in hypervirulent *K. pneumoniae* ST25 causing BSI and pneumonia as part of a hospital outbreak.

Albeit IncFII is a narrow host range plasmid family, it is also one of the most successful plasmids in *Enterobacteriaceae*, improving the bacterial fitness, carrying antibiotic resistance, and virulence genes.[29] These plasmids are able to overcome plasmid incompatibility by having multi-replicons.[30] The pKpQIL is a IncFIIK2 plasmid widely disseminated in ST258 clones, which originally carried *bla*$_{\text{KPC-3}}$ gene,[31,32] later on replaced by *bla*$_{\text{KPC-2}}$ in ST258 and non-ST258 isolates. To date, it is estimated that ~90% *K.*

pneumoniae clones harbor pKpQIL carrying *bla*$_{KPC-2}$ gene worldwide.[33] The presence of pKpQIL has been associated with *K. pneumoniae* dissemination success in hospital settings[5,34] and also biofilm formation.[35] Interestingly, all ST258 isolates reported here show a deleted region upstream of Tn*4401*a, which has not been described previously and it could further support the notion of clonal dissemination of ST258 in this outbreak. This PCR-based algorithm to determine most prevalent MGEs could be useful to investigate the relationship of isolates causing outbreaks in hospitals. Unfortunately, we were not able to obtain all the clinical information of patients infected with CrKPNs.

Contributions:

JR and GT: conception, design of the study and drafting the article. RM, AA and PC: revising critically for important intellectual content of article and RT: technical support.

Funding information:

This study was funding by Instituto Nacional de Investigación en Salud Pública "Dr. Leopoldo Izquieta Pérez", Quito – Ecuador and Instituto de microbiología, Universidad San Francisco de Quito.

Author Disclosure Statement

No competing financial interests exist.

Supplementary Material

Supplementary Tables S1 and S2

References:

1. Yigit, H., A.M. Queenan, G.J. Anderson, A. Domenech-Sanchez, J.W. Biddle, C.D. Steward, S. Alberti, S. Bush, and F.C. Tenover. 2001. Novel carbapenem-hydrolyzing β-lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. 45:1151-1161.
2. Naas, T., S. Oueslati, , R.A. Bonnin, M. L. Dabos, A. Zavala, L. Dortet, P. Retailleau, and B.I. Iorga,, 2017. Beta-Lactamase DataBase (BLDB) – Structure and Function. J. Enzyme Inhib. Med. Chem. 32:917-919.
3. Pitout, J.D., P. Nordmann, and L. Poirel. 2015. Carbapenemase-producing *Klebsiella pneumoniae*, a key pathogen set for global nosocomial dominance. Antimicrob. Agents Chemother. 59:5873-588.
4. Chen, L., B. Mathema, K.D. Chavda, F.R. DeLeo, R.A. Bonomo, and B.N. Kreiswirth. 2014. Carbapenemase-producing *Klebsiella pneumoniae*: molecular and genetic decoding. Trends in microbiol. 22:686-696.
5. Chen, L., K.D. Chavda, R.G. Melano, M.R. Jacobs, B. Koll, T. Hong, A.D., Rojtman M.H. Levi, R.A. Bonomo, and B.N. Kreiswirth. 2014. Comparative genomic analysis of KPC-encoding pKpQIL-like plasmids and their distribution in New Jersey and New York hospitals. Antimicrob. Agents Chemother. 58:2871-2877.
6. Ko, W.C., D.L. Paterson, A.J. Sagnimeni, D.S. Hansen, A. Von Gottberg, S. Mohapatra, J. M. Casellas, H. Goossens, L. Mulazimoglu, G. Trenholme, K.P. Klugman, J,G. McCormack, and L.Y. Victor. 2002. Community-acquired *Klebsiella pneumoniae* bacteremia: global differences in clinical patterns. Emerging Infect. Dis. 8:160-166
7. Shon, A.S., R.P. Bajwa, and T.A. Russo. 2013. Hypervirulent (hypermucoviscous) *Klebsiella pneumoniae*: a new and dangerous breed. Virulence. 4:107-118.

8. Lee, C.R., Lee, J.H., Park, K.S., Kim, Y.B., Jeong, B.C., and Lee, S.H. 2016. Global dissemination of carbapenemase-producing *Klebsiella pneumoniae*: Epidemiology, genetic context, treatment options, and detection methods. Front Microbiol. 7, 1–30. https://doi.org/10.3389/fmicb.2016.00895
9. Prado-Vivar, M.B., L. Ortiz, J. Reyes, E. Villacis, M. Fornasini, M.E. Baldeon, and P.A. Cardenas. 2019. Molecular typing of a large nosocomial outbreak of *Klebsiella pneumoniae* Carbapenemase-producing bacteria in the biggest third level hospital of Quito, Ecuador. Glob Antimicrob Resist. S2213-7165:30124-9.
10. CLSI. (CLSI supplement M100) in: Performance standards for antimicrobial susceptibility testing. 29th ed. Clinical and Laboratory Standards Institute, Wayne, PA; 2019
11. Poirel L., T.R. Walsh, V. Cuvillier, and P. Nordmann. 2011. Multiplex PCR for detection of acquired carbapenemase genes. Diagn. Microbiol. Infect. Dis. 70:119-123.
12. Schechner, V., K. Straus-Robinson, D. Schwartz, I. Pfeffer, J. Tarabeia, R. Moskovich, I., M.J. Schwaber, Y. Carmeli, and S. Navon-Venezia. 2009. Evaluation of PCR-based testing for surveillance of KPC-producing carbapenem-resistant members of the *Enterobacteriaceae* family. J. Clin. Microbiol. 47:3261-3265.
13. Adler, A., E. Khabra, I. Chmelnitsky, P. Giakkoupi, A. Vatopoulos, A.J. Mathers, A.J. Yeh, C.D. Sifri, G. De Angelis, E. Tacconelli, M.V. Villegas, J. Quinn, and Y. Carmeli. 2014. Development and validation of a multiplex PCR assay for identification of the epidemic ST-258/512 KPC-producing *Klebsiella pneumoniae* clone. Diagn. Microbiol. Infect. Dis. 78:12-15.
14. Gomez, S. A., F.G. Pasteran, D. Faccone, N. Tijet, M. Rapoport, C. Lucero, O. Lastovetska, E. Albornoz, M. Galas, KPC Group., R.G. Melano, A. Corso, and A.

Petronni. 2011. Clonal dissemination of *Klebsiella pneumoniae* ST258 harbouring KPC-2 in Argentina. Clin. Microbiol. Infect. 17:1520-1524.

15. Sidjabat, H.E., F.P. Silveira, B.A. Potoski, K.M. Abu-Elmagd, J.M. Adams-Haduch, D.L. Paterson, and Y. Doi. 2009. Interspecies spread of *Klebsiella pneumoniae* carbapenemase gene in a single patient. Clin. Infect. Dis. 49:1736–1738.
16. Turton, J.F., H. Baklan, L.K. Siu, M.E. Kaufmann, and T.L. Pitt. 2008. Evaluation of a multiplex PCR for detection of serotypes K1, K2 and K5 in *Klebsiella* sp. and comparison of isolates within these serotypes. FEMS Microbiol. Lett. 284:247-252.
17. Cejas, D., A. Elena, D. G. Nuñez, P. S. Platero, A. De Paulis, F. Magariños, C. Alfonso, M.A. Berjer, L. Fernández-Canigia, G. Gutkind, and M. Radice. 2019. Changing Epidemiology of KPC Producing *Klebsiella pneumoniae* in Argentina: Emergence of Hypermucoviscous ST25 and High Risk Clone ST307. J Glob Antimicrob Resist 18: 238-242
18. Ferreira, R. L., B.C.M. da Silva, G.S. Rezende, R. Nakamura-Silva, A. Pitondo-Silva, E. Boni Campanini, M.C.A. Brito, E.M.L. da Silva, C.C.M. Freire, A.F. da Cunha, and M.D.S. Pranchevicius. 2018. High Prevalence of Multidrug-Resistant *Klebsiella Pneumoniae* Harboring Several Virulence and β-Lactamase Encoding Genes in a Brazilian Intensive Care Unit. Front Microbiol. 9:3198.
19. David, S., S. Reuter, S. R. Harris, C. Glasner, T. Feltwell, S. Argimon, K. Abudahab, R. Goater, T. Giani, G. Errico, M. Aspbury, S. Sjunnebo, the EuSCAPE Working Group, the ESGEM Study Group., E.J. Feil, M.G. Rossolini, D. M. Aanensen, and H. Grundmann. 2019. Epidemic of carbapenem-resistant

Klebsiella pneumoniae in Europe is driven by nosocomial spread. Nat Microbiol. 1-11.

20. Rock, C., K.A. Thom, M. Masnick, J.K. Johnson, A.D. Harris, and D.J. Morgan. 2014. Frequency of *Klebsiella pneumoniae* carbapenemase (KPC)-producing and non-KPC-producing Klebsiella species contamination of healthcare workers and the environment. Infect Control Hosp Epidemiol. 35: 426–429.
21. Paczosa, M.K., and J Mecsas. (2016). *Klebsiella pneumoniae*: going on the offense with a strong defense. Microbiol. Mol. Biol. Rev. 80:629-661.
22. Lin, J.C., F.Y. Chang, C.P. Fung, J.Z. Xu, H.P. Cheng, J.J. Wang, L.Y. Huang, and L.K. Siu. 2004. High prevalence of phagocytic-resistant capsular serotypes of *Klebsiella pneumoniae* in liver abscess. Microbes Infect. 6:1191–1198.
23. Sahly, H., H. Aucken, V.J. Benedi, C. Forestier, V. Fussing, D.S. Hansen, I. Ofek, R. Podschun, D. Sirot, D. Sandvang, and J.M. Tomas, U. Ullmann. 2004. Impairment of respiratory burst in polymorphonuclear leukocytes by extended-spectrum beta-lactamase-producing strains of *Klebsiella pneumoniae*. Eur. J. Clin. Microbiol. Infect. Dis. 23:20–26.
24. Fung, C.P., F.Y. Chang, S.C. Lee, B.S. Hu, B.I. Kuo, C.Y. Liu, M. Ho, and L.K. Siu. 2002. A global emerging disease of *Klebsiella pneumoniae* liver abscess: is serotype K1 an important factor for complicated endophthalmitis? Gut. 50: 420–4.
25. Yu V.L., D. S. Hansen, W. C. Ko, A. Sagnimeni, K. P. Klugman, A. Von Gottberg, M.M. Wagener, and V.J. Benedi, International *Klebsiella* Study Group. 2007. Virulence characteristics of *Klebsiella* and clinical manifestations of *K. pneumoniae* bloodstream infections. Emerging Infect. Dis. 13:986-93
26. Yu, W. L., W.C. Ko, K.C. Cheng, C.C. Lee, C.C. Lai, and Y.C. Chuang. 2008. Comparison of prevalence of virulence factors for *Klebsiella pneumoniae* liver

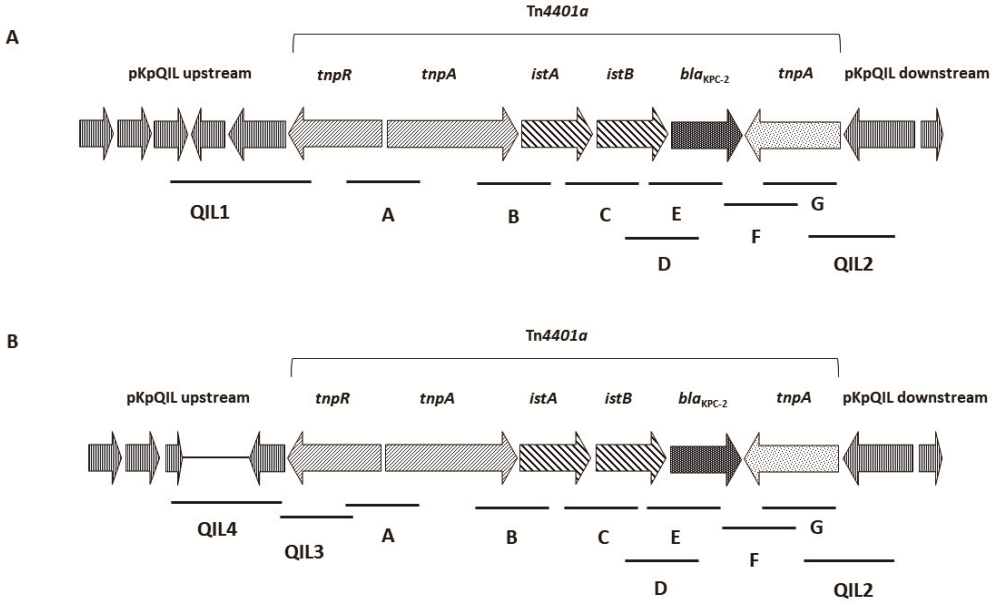
abscesses between isolates with capsular K1/K2 and non-K1/K2 serotypes. Diagn. Microbiol. Infect. Dis. 62:1-6.

27. Guo, Y., S. Wang, L. Zhan, Y. Jin, J. Duan, Z. Hao, J. Lv, X. Qi, L. Chen, B,N. Kreiswirth, L. Wang, and F. Yu. 2017. Microbiological and clinical characteristics of hypermucoviscous *Klebsiella pneumoniae* isolates associated with invasive infections in China. Front Cell Infect Microbiol. 7:24.
28. Harada, S., Y. Ishii, T. Saga, K. Aoki, and K. Tateda. 2018. Molecular epidemiology of *Klebsiella pneumoniae* K1 and K2 isolates in Japan. Diagn. Microbiol. Infect. Dis. 91:354-359.
29. Carattoli, A. 2011. Plasmids in Gram negatives: molecular typing of resistance plasmids. Int. J. Med. Microbiol. 301:654-658.
30. Villa, L., A. García-Fernández, D. Fortini, and A. Carattoli. 2010. Replicon sequence typing of IncF plasmids carrying virulence and resistance determinants. J. Antimicrob. Chemother. 65:2518-2529.
31. Leavitt, A., I. Chmelnitsky, Y. Carmeli, and S. Navon-Venezia. 2010. Complete nucleotide sequence of KPC-3-encoding plasmid pKpQIL in the epidemic *Klebsiella pneumoniae* sequence type 258. Antimicrob. Agents Chemother. 54:4493–4496.
32. García-Fernández, A., L. Villa, C. Carta, C. Venditti, A. Giordano, M. Venditti, C. Mancini, and A. Carattoli. 2012. *Klebsiella pneumoniae* ST258 producing KPC-3 identified in Italy carries novel plasmids and OmpK36/OmpK35 porin variants. Antimicrob. Agents Chemother. 56:2143-2145.
33. Doumith, M., J. Findlay, H. Hirani, K.L. Hopkins, D.M. Livermore, A. Dodgson, and N. Woodford. 2017. Major role of pKpQIL-like plasmids in the early

dissemination of KPC-type carbapenemases in the UK. J. Antimicrob. Chemother. 72:2241-2248.

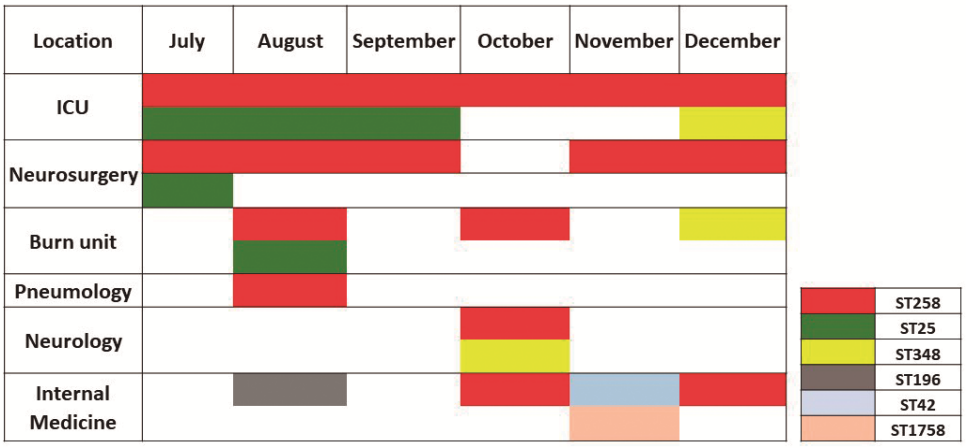
34. Peirano, G., P.A. Bradford, K.M. Kazmierczak, L. Chen, B.N. Kreiswirth, and J.D. Pitout. 2017. Importance of clonal complex 258 and IncFK2-like plasmids among a global collection of *Klebsiella pneumoniae* with bla_{KPC}. Antimicrob. Agents Chemother. 61:e02610-16.
35. Buckner, M.M., H. Saw, R. Osagie, A. McNally, V. Ricci, M.E. Wand, M. Woodford, A. Ivens, M. Webber, and L.J. Piddock. 2018. Clinically relevant plasmid-host interactions indicate that transcriptional and not genomic modifications ameliorate fitness costs of *Klebsiella pneumoniae* carbapenemase-carrying plasmids. MBio. 9:e02303-17.

Figure 1. Genetic environment of *bla*$_{KPC-2}$ and their relationship with IncFII$_{K2}$ backbone genes.



A. Genetic environment of *bla*$_{KPC-2}$ gene in *K. pneumoniae* ST25, ST42, ST348 and ST196, the genetic structure was like to plasmid pKpQIL-234 (accession number KJ146689). **B.** Genetic environment of bla$_{KPC-2}$ gene in *K. pneumoniae* ST258, there is a deletion of ~1000pb region upstream of the Tn*4401*a::*bla*$_{KPC-2}$ insertion.

Figure 2.- Distribution of KPC-2-producing *K. pneumoniae* STs in different hospital locations between July to December.



ST: Sequence type **MIC:** Minimum inhibitory concentration, **N/D**= Non-determined, **KPC**= *Klebsiella pneumoniae* carbapenemase, **PBRT**= PCR-Based Replicon Typing, **MER:** Meropenem and **IMI:** Imipenem

Table 1.- Main characteristics from different *bla*$_{KPC-2}$ gene -harboring *K. pneumoniae* sequence type isolates.

ST	Total	MIC (µg/mL)		KPC type	Transposon	IncFII$_{K2}$		Relation of Tn*4401a::bla*$_{KPC-2}$ with IncFII$_{K2}$		Capsular serotype	Transconjugants PBRT	Specimen
		MER	IMI			*RepA*	*RepFIB*	Upstream	Downstream			
258	45	≥4	≥4	2	Tn*4401*a	Positive	Positive	Deletion	Positive	N/D	N/D	Blood, central venous catheter, tracheal aspirate, sputum, urine and wound
25	8	≥4	≥4	2	Tn*4401*a	Positive	Positive	Positive	Positive	K2	IncFII	Blood, tracheal aspirate and wound
348	6	≥4	≥4	2	Tn*4401*a	Positive	Positive	Positive	Positive	N/D	IncFII	Tracheal aspirate and wound
42	1	≥4	≥4	2	Tn*4401*a	Positive	Positive	Positive	Positive	N/D	IncFII	Wound
196	1	≥4	≥4	2	Tn*4401*a	Positive	Positive	Positive	Positive	N/D	IncFII	Urine
1758	1	≥4	≥4	2	Tn*4401*a	Negative	Negative	Negative	Negative	N/D	IncM	Wound

ST: Sequence type **MIC:** Minimum inhibitory concentration, **N/D**= Non-determined, **KPC**= *Klebsiella pneumoniae* carbapenemase, **PBRT**= PCR-Based Replicon Typing, **MER:** Meropenem and **IMI:** Imipenem

CAPITULO IV

First report of a clinical isolate of *bla*$_{\text{OXA-48}}$- carbapenemase producing *Raoultella ornithinolytica* in South America

Jorge Reyes [a,b] - corresponding author

a. Facultad de Ciencias Químicas. Universidad Central del Ecuador, Quito, Ecuador

b. Instituto de Microbiología, colegio de Ciencias Biológicas y Ambientales, Universidad San Francisco de Quito, Quito, Ecuador. jorgereyes83@mail.com. Postal address 170157

Fernando Villavicencio [c]

c. Centro de Referencia Nacional de Resistencia a los Antimicrobianos Instituto Nacional de Investigación en Salud Pública "Leopoldo Izquieta Pérez", Quito, Ecuador. fervillavicencioz@gmail.com

José E. Villacís [d]

d. Pontificia Universidad Católica del Ecuador, Facultad de Medicina jevillacis@gmail.com

Evelyn Pavón [e]

e. Hospital de especialidades Carlos Andrade Marín. evelyn_gabriela@hotmail.com

Natalia Campoverde [e]

e. Hospital de especialidades Carlos Andrade Marín. natalia_campoverde@yahoo.com

Mauricio Espinel [e]

e. Hospital de especialidades Carlos Andrade Marín. mauricio.espinel@yahoo.com

Byron Nuñez [e]

e. Hospital de especialidades Carlos Andrade Marín. nunez_freile@hotmail.com

Gabriel Trueba [b]

b. Instituto de Microbiología, Colegio de Ciencias Biológicas y Ambientales, Universidad San Francisco de Quito, Quito, Ecuador. gtrueba@usfq.edu.ec

Raoultella spp., a bacterium named after Didier Raoult, is a Gram-negative belonging to the *Enterobacteriaceae* family. There have been described 4 species: *R. planticola, R. ornithinolytica, R. terrigena,* and *R. electrica,* all species are inhabitants of soil and plants. Recently, *R. planticola* and *R. ornithinolytica* have been associated with human infections and resistance to different antibiotics including carbapenems (1). In this report, we describe an infection caused by *bla*$_{\text{OXA-48}}$ producing *R. ornithinolytica*. A 64-year-old male patient was admitted into a 700- bed hospital in Quito, in order to restore intestinal transit after ileocecal resection. Two days after admission the patient presented sepsis characterized by hypotension, fever, and leukocytosis. The patient was directed to intensive care unit where he was treated with Ampicillin/sulbactam for 10 days, subsequent blood cultures were negative however *E. coli* was recovered from tracheal aspirate. The patient was submitted to the surgical department to continue treatment and 10 days late he presented a surgical site infection, *R. planticola* (resistant to imipenem and piperacillin/tazobactam but susceptible to third generation cephalosporins and ciprofloxacin) was isolated and detected using a Vitek® compact 2 system (Biomeriux, France), CARD AST 272 (Table 1). The patient was treated with ciprofloxacin 500mg i.v. q12h and recovered satisfactorily.

Isolate analysis using DNA sequences of *rpoB* and 16S rRNA genes and MALDI-TOF Vitek MS system, (bioMérieux) indicated that the isolate was *R. ornithinolytica* (99.9% identification score). The RAPIDEC CARBA NP® (bioMérieux) assay was positive and polymerase chain reaction (PCR) amplifying the *bla*$_{\text{OXA-48}}$ associated with Tn*1999* was

performed (2). The amplicon was cleaned, using Wizard ® SV gel, and sequenced in Macrogen (South Korea); nucleotide sequences showed the presence of *bla*$_{\text{OXA-48}}$ gene (accession no: MH507508) and Tn*1999* (accession no: MK359485). The amplicon showed 99% nucleotide identity to a Tn*1999* harboring *bla*$_{\text{OXA-48}}$ previously described in *Klebsiella pneumoniae* (accession no: JN626286). We were unable to determine the plasmid incompatibility group (conjugation using *E. coli* J53 as the recipient was unsuccessful), nor could we establish whether the patient was colonized by *R. ornithinolytica* before the surgery. To the best of our knowledge, this is the first description of a clinical isolate of *R. ornithinolytica* harboring *bla*$_{\text{OXA-48}}$ in Ecuador and possibly in South America. Nevertheless, *bla*$_{\text{OXA-48}}$ genes in *Enterobacteriaceae* have been described in Latin American and Caribbean countries (3). In Ecuador, a *bla*$_{\text{OXA-48}}$-like gene was found previously in *K. pneumoniae* (accession number KY609322.1). The *bla*$_{\text{OXA-48}}$-like gene has been found associated with 5 isoforms of Tn*1999* in *Enterobacteriaceae* (4); interestingly, an *R. ornithinolytica* strain containing *bla*$_{\text{OXA-48}}$ associated with Tn*1999.2* was detected in Lebanon (5). The genetic closeness of *Raoultella* spp. and *Klebsiella* spp., may lead to misidentification using biochemical tests e.g., Vitek 2 system, the introduction of *rpoB* and 16S rRNA genes analysis and the new technology based in MALDI-TOF MS allowed us a correct identification to species level of *Raoultella* spp.. Thus, our results underline the accuracy of MALDI-TOF MS in *R. ornithinolytica* identification. This report was approved by the institutional human ethics committee Cod: 02-01-2018-003.

References

Seng P, Boushab B, Romain F, Gouriet F, Bruder N, Martin C, Paganelli F, Bernit E, Le Treut Y, Thomas P, Papazian L, Raoult D, Stein A. Emerging role of *Raoultella ornithinolytica* in human infections : a series of cases and review of the literature. Int J Infec Dis. 2016;45:65–71.

Giani T, Conte V, Di Pilato V, Aschbacher R, Weber C, Larcher C, Rossolini G. *Escherichia coli* from Italy producing OXA-48 carbapenemase encoded by a novel Tn*1999* transposon derivative. Antimicrob Agents Chemother. 2012;56:2211–2213.

Escandón-Vargas K, Reyes S, Gutiérrez S, Villegas MV. The epidemiology of carbapenemases in Latin America and the Caribbean. Expert Rev Anti Infect Ther. 2016;15:277-297.

Mairi A, Pantel A, Sotto A, Lavigne J-P, Touati A. OXA-48-like carbapenemases producing *Enterobacteriaceae* in different niches. Eur J Clin Microbiol Infect Dis. 2018;37:587–604.

Al-Bayssari C, Olaitan AO, Leangapichart T, Okdah L, Dabboussi F, Hamze M, Rolain J. Whole-genome sequence of a *bla*$_{\text{OXA-48}}$-harboring *Raoultella ornithinolytica* clinical isolate from Lebanon. Antimicrob Agents Chemother. 2016;60:2548–2550.

TABLA DE ANEXOS.

Table 1. Antibiotic sensitivity tests, carbapenemase assays and carbapenemase gene detection in an Ecuadorian *Raoultella ornithinolytica* isolate.

Minimum inhibitory concentration (µg/ml) using vitek 2 system									**CIM**	**Rapidec CarbaNP**	**PCR and sequencing**	
Amp/Sul	Pip/Taz	Cfz	Ctx	Imi	Mer	Cip	Ami	Col	Positive	Positive	carbapenemase	MGE
>32	>128	<1	<1	8	2	<0.25	≤ 2	≤ 0.5			$bla_{\text{OXA-48}}$	Tn*1999*

Amp/Sul: Ampicilin-sulbatam, Pip/Taz: Piperacilin-tazobactam, Cfz: Cefazidime, Ctx: Cefotaxime, Imi: Imipenem, Mer: Meropenem, Cip: Ciprofloxacin, Ami: Amikacin, Col: Colistin. CIM: Carbapenem inactivation method. MGE: Mobile genetic element. PCR: polymerase chain reaction.

Supplementary Table S1. Primers used in this study.

Number or letter	**Primer name**	**Sequence**	**Target**	**Size bp**	**Reference**
1	*pliv-I* -F	TGATGCTGATGGCAGACTGA	*pilv-l*	320	Adler *et* al.[13]
	pliv-I-R	TGTAGTCACACCCTGCCCA			
2	UNIKPC F	ATGTCACTGTATCGCCGTCT	bla_{KPC}	894	Schechner et al.[12]
	UNIKPC R	TTACTGCCCCGTTGACGCCC			
A	tnpRA-F	TAGTCGTTGGTCGCATTCAG	*tnpR*/*tnpA*(Tn*3*-group transposon)	1038	Gomez et al. [14]
	tnpRA-R	ATAGCGGCTCAGCACGAAAG			
B	tnpAlstA-F2	GCGGTAAGAAGGAACTGAC	*tnpA*/*istA*	1135	Gomez et al. [14]
	tnpAlstA-R2	TCAGCAACGGGTTGTAGAAG			
C	tnpAlstA-Fseq	GATGTTGACCCAGGAGCAAAC	*istA*/*istB*	1074	Gomez et al. [14]
	lstAB-Rseq	GAGTTTGAGTTGCTGGCACAG			
D	lstAB-Fseq	CTGTGCCAGCAACTCAAACTC	*istB*/bla_{KPC-2}	1046	Gomez et al. [14]
	lstBKPC-Rseq	CAGTGACATCAACGATATTCC			
E	lstBKPC-F	AAGCGAACCTGTTCTTTCAG	*istB*/$bla_{\text{KPC-2}}$	1154	Gomez et al. [14]
	lstBKPC-R	GCCAATCAACAAACTGCTG			

F	KPCtnpA-F	ATACCACGTTCCGTCTGGAC	*bla*$_{KPC-2}$/*tnpA*, IS*Kpn6*	1141	Gomez et al. [14]
	KPCtnpA-R	AGCTACAACGGGTACACAG			
G	tnpA-F	GAACAGCCTCACTACGGAAT	*tnpA*	1284	Gomez et al. [14]
	tnpA-R	GCTACCGCATCTATCTGAG			
3	FIIK-repA-F1	CTTCACGTCCCGTTTTGATT	IncFII *repA* gene	657	Chen et al.[5]
	FIIK-repA-R1	CGCTTCAGCGCTTCTTTATC			
4	K2-repB-F1	CCATTCCGATCCTTTTCTGA	IncFIIK2 *repFIB* gene	395	Chen et al.[5]
	K2-repB-R1	AACGCTACTGTCCAGCCTGT			
QIL1	QIL-F1	ACAGGGAGTGCCAGGAAAG	Junction between Tn*4401 tnpR* and upstream IncFIIK2 backbone gene	2001	Chen et al.[5]
	QIL-R1	TGTATTTGCATGGCGATGAG			
QIL2	QIL-F2	GCCTCAGATAGATGCGGTAGC	Junction between Tn*4401* IS*Kpn6* and downstream Gene	1831	Chen et al.[5]
	QIL-R2	AAGCTGGAGACATGGAATGG			
QIL3	QIL-F3*	TATTGCACAGCCTGACCGC	Junction between Tn*4401 tnpR* and upstream IncFIIK2 backbone gene	1683	This study
	QIL-R3*	GCGCCTATCGCAAGGAGG			
QIL4	BBFK2 F*	TCGGCTGCTCAGGCAAAC	Hypothetical proteins backbone Upstream of Tn*4401*	1717	This study
	BBFK2 R*	CAGCGCCCGGAAAGATGATA			
K1	MagAF1w	GGTGCTCTTTACATCATTGC	*magA* gene	1283	Turton et al.[16]
	MagAR1	GCAATGGCCATTTGCGTTAG			
K2	Wzy-F1	GACCCGATATTCATACTTGACA GAG	*K2*	641	Turton et al.[16]
	Wzy-R1	CCTGAAGTAAAATCGTAAATAG ATGGC			
K5	K5wzxF360	TGGTAGTGATGCTCGCGA	*K5*	280	Turton et al.[16]
	K5wzxR639	CCTGAACCCACCCCAATC			

*These primers were designed in this study.

Supplementary Table S2. Antimicrobial susceptibility test

Month	Number	Code	Sample	Location	ST	SAM	TZP	CAZ	CRO	FEP	ETP	IMI	MER	AMK	GEN	CIP	TGC	CST
JULY	1	14-0477	Wound	ICU	ST258	>=32	>= 128	>=64	>=64	>=64	>=8	8	>=16	>=64	>=16	>=4	2	2
	2	14-0491	Central venous catheter	ICU	ST258	>=32	>= 128	>=64	>=64	>=64	>=8	8	>=16	>=64	>=16	>=4	2	2
	3	14-0508	Blood	ICU	ST258	>=32	>= 128	>=64	>=64	>=64	>=8	8	>=16	>=64	>=16	>=4	2	2
	4	14-0545	Tracheal aspirate	ICU	ST258	>=32	>= 128	16	>=64	>=64	>=8	8	>=16	>=64	>=16	>=4	2	<=0,5
	5	14-0481	Tracheal aspirate	ICU	ST25	>=32	>= 128	16	>=64	4	4	8	>=16	8	>=16	2	2	<=0,5
	6	14-0535	Tracheal aspirate	ICU	ST25	>=32	>= 128	>=64	>=64	>=64	>=8	>=16	>=16	>=64	>=16	1	>=8	<=0,5
	7	14-0530	Blood	ICU	ST25	>=32	>= 128	>=64	>=64	>=64	>=8	>=16	>=16	>=64	>=16	1	>=8	<=0,5
	8	14-0543	Wound	ICU	ST25	>=32	>= 128	>=64	>=64	>=64	>=8	>=16	>=16	>=64	>=16	1	>=8	<=0,5
	9	14-0478	Urine	Neurosurgery	ST25	>=32	>= 128	16	>=64	4	4	8	>=16	8	>=16	2	2	<=0,5
	10	14-0483	Tracheal aspirate	Neurosurgery	ST258	>=32	>= 128	>=64	>=64	>=64	>=8	8	>=16	>=64	>=16	>=4	2	>=16
	11	14-0498	Tracheal aspirate	Neurosurgery	ST25	>=32	>= 128	16	>=64	4	4	8	>=16	8	>=16	2	2	<=0,5
	12	14-0511	Sputum	Neurosurgery	ST258	>=32	>= 128	16	>=64	>=64	>=8	8	>=16	>=64	>=16	>=4	2	<=0,5
AUGUST	13	14-0509	Wound	Burn Unit	ST258	>=32	>= 128	>=64	>=64	>=64	>=8	8	>=16	>=64	>=16	>=4	2	<=0,5
	14	14-0611	Wound	Burn Unit	ST258	>=32	>= 128	>=64	>=64	>=64	>=8	8	>=16	>=64	>=16	>=4	2	<=0,5
	15	14-0633	Tissue	Burn Unit	ST258	>=32	>= 128	>=64	>=64	>=64	>=8	8	>=16	>=64	>=16	>=4	2	<=0,5
	16	14-0634	Wound	Burn Unit	ST258	>=32	>= 128	>=64	>=64	>=64	>=8	8	>=16	>=64	>=16	>=4	2	<=0,5
	17	14-0635	Wound	Burn Unit	ST258	>=32	>= 128	>=64	>=64	>=64	>=8	8	>=16	>=64	>=16	>=4	2	<=0,5
	18	14-0591	Wound	Burn Unit	ST25	>=32	>= 128	16	>=64	4	4	8	>=16	8	>=16	2	2	<=0,5
	19	14-0607	Wound	ICU	ST258	>=32	>= 128	>=64	>=64	>=64	>=8	8	>=16	>=64	>=16	>=4	2	<=0,5
	20	14-0629	Tracheal aspirate	ICU	ST258	>=32	>= 128	>=64	>=64	>=64	>=8	8	>=16	>=64	>=16	>=4	2	<=0,5
	21	14-0619	Urine	Neurosurgery	ST258	>=32	>= 128	16	>=64	>=64	>=8	8	>=16	>=64	>=16	>=4	2	<=0,5

	22	14-0646	Wound	Neurosurgery	ST258	>=32	>= 128	16	>=64	>=64	>=8	8	>=16	>=64	>=16	>=4	2	<=0,5
	23	14-0601	Tracheal aspirate	Pneumology	ST258	>=32	>= 128	16	>=64	>=64	>=8	8	>=16	>=64	>=16	>=4	2	<=0,5
	24	14-0596	Urine	Internal medicine	ST196	>=32	>= 128	16	16	4	>=8	>=16	>=16	<= 2	<= 1	1	1	<=0,5
SEPTEMBER	**25**	14-0651	Blood	ICU	ST258	>=32	>= 128	16	>=64	>=64	>=8	8	>=16	>=64	>=16	>=4	2	<=0,5
	26	14-0653	Tracheal aspirate	ICU	ST258	>=32	>= 128	16	>=64	>=64	>=8	8	>=16	>=64	>=16	>=4	2	<=0,5
	27	14-0672	Tracheal aspirate	ICU	ST258	>=32	>= 128	16	>=64	>=64	>=8	8	>=16	>=64	>=16	>=4	2	<=0,5
	28	14-0751	Tracheal aspirate	ICU	ST258	>=32	>= 128	>=64	>=64	>=64	>=8	>=16	>=16	>=64	>=16	>=4	2	>=16
	29	14-0765	Tracheal aspirate	ICU	ST258	>=32	>= 128	>=64	>=64	>=64	>=8	>=16	>=16	>=64	>=16	>=4	2	>=16
	30	14-0670	Tracheal aspirate	ICU	ST25	>=32	>= 128	16	>=64	4	4	8	>=16	8	>=16	2	2	<=0,5
	31	14-0673	Tracheal aspirate	Neurosurgery	ST258	>=32	>= 128	16	>=64	>=64	>=8	8	>=16	>=64	>=16	>=4	2	<=0,5
	32	14-0705	Urine	Neurosurgery	ST258	>=32	>= 128	16	>=64	>=64	>=8	8	>=16	>=64	>=16	>=4	2	<=0,5
	33	14-0772	Tracheal aspirate	Neurosurgery	ST258	>=32	>= 128	16	>=64	>=64	>=8	8	>=16	>=64	>=16	>=4	2	<=0,5
	34	14-0671	Sputum	Pneumology	ST258	>=32	>= 128	>=64	>=64	>=64	>=8	8	>=16	>=64	>=16	>=4	2	<=0,5
OCTOBER	**35**	14-0764	Wound	Burn Unit	ST258	>=32	>= 128	16	>=64	>=64	>=64	8	>=16	>=64	>=16	>=4	2	<=0,5
	36	14-0848	Urine	Burn Unit	ST258	>=32	>= 128	16	>=64	>=64	>=64	8	>=16	>=64	>=16	>=4	2	<=0,5
	37	14-0768	Blood	ICU	ST258	>=32	>= 128	16	>=64	>=64	>=64	8	>=16	>=64	>=16	>=4	2	<=0,5
	38	14-0854	Wound	ICU	ST258	>=32	>= 128	16	>=64	>=64	>=64	8	>=16	>=64	>=16	>=4	2	<=0,5
	39	14-0851	Tracheal aspirate	Neurology	ST258	>=32	>= 128	16	>=64	>=64	>=64	8	>=16	>=64	>=16	>=4	2	<=0,5
	40	14-0877	Tracheal aspirate	Neurology	ST348	>=32	>= 128	16	>=64	4	>=8	>=16	>=16	<= 2	<= 1	2	2	<=0,5
	41	14-0767	Wound	Internal medicine	ST258	>=32	>= 128	16	>=64	>=64	>=8	>=16	>=16	>=64	>=16	>=4	2	<=0,5
	42	14-0771	Tracheal aspirate	Internal medicine	ST258	>=32	>= 128	16	>=64	>=64	>=8	8	>=16	>=64	>=16	>=4	2	<=0,5
	43	14-0795	Wound	Internal medicine	ST258	>=32	>= 128	16	>=64	>=64	>=8	8	>=16	>=64	>=16	>=4	2	<=0,5
NOVEMBER	**44**	14-0883	Tracheal aspirate	ICU	ST258	>=32	>= 128	16	>=64	>=64	>=8	8	>=16	>=64	>=16	>=4	2	<=0,5

	45	14-0908	Wound	ICU	ST258	>=32	>= 128	16	>=64	>=64	>=8	8	>=16	>=64	>=16	>=4	2	<=0,5
	46	14-0950	Tracheal aspirate	ICU	ST258	>=32	>= 128	16	>=64	>=64	>=8	8	>=16	>=64	>=16	>=4	2	<=0,5
	47	14-0934	Tracheal aspirate	ICU	ST348	>=32	>= 128	16	>=64	>=64	>=8	>=16	>=16	<= 2	<= 1	2	2	<=0,5
	48	14-0910	Sputum	Neurosurgery	ST258	>=32	>= 128	>=64	>=64	>=64	>=8	>=16	>=16	>=64	>=16	>=4	1	<=0,5
	49	14-0937	Wound	Internal medicine	ST1758	>=32	>= 128	16	>=64	>=64	>=8	>=16	>=16	<= 2	>=16	2	1	<=0,5
	50	14-0938	Wound	Internal medicine	ST42	>= 128	>=64	>=64	>=64	4	>=16	>=16	>=64	>=16	>=4	2	<=0,5	<=0,5
DECEMBER	**51**	14-0903	Wound	Burn Unit	ST348	>=32	>= 128	16	>=64	4	>=8	>=16	>=16	<= 2	<= 1	2	2	<=0,5
	52	14-1047	Tracheal aspirate	Burn Unit	ST348	>=32	>= 128	32	>=64	>=64	>=8	>=16	>=16	<= 2	<= 1	2	2	<=0,5
	53	14-0998	Urine	ICU	ST258	>=32	>= 128	>=64	>=64	>=64	>=8	>=16	>=16	>=64	>=16	>=4	2	<=0,5
	54	14-1029	Tracheal aspirate	ICU	ST258	>=32	>= 128	16	>=64	>=64	>=8	8	>=16	>=64	>=16	>=4	2	<=0,5
	55	14-1031	Tracheal aspirate	ICU	ST258	>=32	>= 128	>=64	>=64	>=64	>=8	>=16	>=16	>=64	>=16	>=4	2	<=0,5
	56	14-1049	Tracheal aspirate	ICU	ST258	>=32	>= 128	>=64	>=64	>=64	>=8	>=16	>=16	>=64	>=16	>=4	2	<=0,5
	57	14-1056	Tracheal aspirate	ICU	ST258	>=32	>= 128	>=64	>=64	>=64	>=8	>=16	>=16	>=64	>=16	>=4	2	<=0,5
	58	14-1057	Blood	ICU	ST258	>=32	>= 128	>=64	>=64	>=64	>=8	>=16	>=16	>=64	>=16	>=4	2	<=0,5
	59	14-1043	Tracheal aspirate	ICU	ST258	>=32	>= 128	>=64	>=64	>=64	>=8	>=16	>=16	>=64	>=16	>=4	2	<=0,5
	60	14-1058	Tracheal aspirate	ICU	ST348	>=32	>= 128	16	>=64	>=64	>=8	>=16	>=16	<= 2	<= 1	2	2	<=0,5
	61	14-1059	Tracheal aspirate	ICU	ST348	>=32	>= 128	16	>=64	>=64	>=8	>=16	>=16	<= 2	<= 1	2	2	<=0,5
	62	14-0943	Urine	Neurosurgery	ST258	>=32	>= 128	16	>=64	>=64	>=8	8	>=16	>=64	>=16	>=4	2	<=0,5
			Transconjugants J53 code	Donor isolate		**SAM**	**TZP**	**CAZ**	**CRO**	**FEP**	**ETP**	**IMI**	**MER**	**AMK**	**GEN**	**CIP**	**TGC**	**CST**
			CONJ-535	14-0535	ST25	>=32	>= 128	4	>=64	2	4	>=16	>=16	4	2	<=0.25	<=0,5	<=0,5
Transconjugant			CONJ-596	14-0596	ST196	>=32	>= 128	4	8	<= 1	4	8	>=16	<= 2	<= 1	<=0.26	<=0,5	<=0,5
			CONJ-877	14-0877	ST348	>=32	>= 128	4	8	<= 1	4	8	>=16	<= 2	<= 1	<=0.27	<=0,5	<=0,5
			CONJ-937	14-0937	ST1758	>=32	>= 128	4	32	2	4	>=16	>=16	<= 2	<= 1	<=0.28	<=0,5	<=0,5
			CONJ-938	14-0938	ST42	>=32	>= 128	4	8	<= 1	1	8	>=16	<= 2	<= 1	<=0.29	<=0,5	<=0,5