Emergence and Clonal Spread of KPC-2 Producing Clinical Klebsiella Aerogenes Isolates From a Teaching Hospital in Tucuman, Argentina.

Juan Martín Vargas (juan.martin.vargas@hotmail.com)
Universidad Nacional de Tucuman, Cátedra de Bacteriología, Facultad de Bioquímica, Química y Farmacia https://orcid.org/0000-0002-5209-0883

María Paula Moreno Mochi
Universidad Nacional de Tucuman, Cátedra de Bacteriología, Facultad de Bioquímica, Química y Farmacia

Juan Manuel Nuñez
Departamento de Infectología, Hospital Ángel Cruz Padilla, Tucumán, Argentina

Silvana Mochi
Laboratorio de Microbiología, Hospital Ángel Cruz Padilla

Mariel Cáceres
Laboratorio de Microbiología, Hospital Ángel Cruz Padilla, Tucumán, Argentina

Rosa Campo
Hospital Universitario Ramon y Cajal

María Angela Jure
Universidad Nacional de Tucumán, Cátedra de Bacteriología, Facultad de Bioquímica, Química y Farmacia

Research

Keywords: carbapenem resistant K. aerogenes, outbreak, molecular epidemiology, Klebsiella pneumoniae carbapenemase

Posted Date: September 18th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-73510/v1

Abstract

Introduction

Klebsiella aerogenes is a nosocomial pathogen associated with drug resistance and healthcare-associated infections. We pursued this study to investigate an outbreak of clinical carbapenem-resistant *K. aerogenes*(CRKA) in an argentinian tertiary hospital which persisted for 4 months despite aggressive infection control measures. The primary goals aimed to evaluate the molecular characteristics and the clonal relationships among the CRKA isolates.

Methods

We characterized CRKA isolates by multiplex PCR and PFGE. The information was integrated with clinical and epidemiologic data.

Results

The 14 CRKA strains were disseminated in an adult intensive care unit (50%) and five different wards. In patients who received antimicrobial treatment, 8 staggered to directed treatment, mainly with amikacin(6/8) and/or carbapenemes(5/8). The overall mortality was 42.8%, and the attributed mortality to CRKA infection was 21.4%, strains showed high rates of resistance to most of the antimicrobials without resistance to Amikacin and Tigecycline, and carried the bla_{KPC-2}, bla_{SHV-2} and $bla_{CTXM-15}$ genes. The PFGE indicated 2 distinct groups; 12/14 CRKA isolates

associated with the dominant subgroup A and likely to be primarily responsible for the first isolation and subsequent dissemination in the hospital.

Conclusion

The outbreak characteristics data showed prolonged hospitalization and previous use of broad-spectrum antibiotics as potential risk factors for the acquisition of CRKA.

Introduction

Klebsiella aerogenes (formerly described as *Enterobacter aerogenes*) is a ubiquitous member of the *Enterobacteriaceae* family and a significant nosocomial pathogen associated with drug resistance and a wide variety of infections including pneumonia, bacteremia, urinary tract and surgical site infections[1,2]. *K. aerogenes* infections can arise endogenously (gastrointestinal flora) or be acquired from surroundings in the facility where the patient is admitted (horizontal transmission through colonized healthcare workers, contaminated devices/shared equipment, other patients etc.), with the most critical risk factor for acquiring infection being prolonged broad-spectrum antibiotic administration[3]. Risk factors for *K. aerogenes* infections include prolonged stay at healthcare facilities especially for patients who are immunosuppressed, on mechanical ventilation or harbor foreign devices[3]. Numerous hospital ward

outbreaks in both pediatric and adult populations due to *K. aerogenes* have been described associated to a common source[4] or spread via patient-to-patient transmission[5-7]. A particularly high frequency of hospital ICU outbreaks was continually reported from Western Europe in a period between the 1990s and early 2000s, that were largely attributed to the spread and endemic establishment of a clonal *K. aerogenes* strain harboring the extended-spectrum β-lactamase TEM-24 (bla_{TEM-24})[1,8]. Within the US and other regions across the globe, *Klebsiella aerogenes* has also been reported along with *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Escherichia coli* to be among the frequently isolated carbapenem resistant Enterobacteriaceae[9,10]. *K. aerogenes* strains harboring plasmid-borne serine carbapenemases have been described in the US and worldwide, while metallo-β-lactamases and OXA- 48 have been reported in Europe, Asia and Brazil[1,11]. However, the primary mechanisms underlying resistance to carbapenems in *K. aerogenes* are considered carbapenemase independent and attributed to chromosomal AmpC β-lactamase over-expression and mutations affecting membrane permeability[1].

Considering the importance of *K. aerogenes* in causing healthcare-associated infections we pursued this study to investigate an outbreak of clinical carbapenem-resistant *Klebsiella aerogenes* (CRKA) in an argentinian tertiary hospital which persisted for 4 months despite aggressive infection control measures. The primary goals aimed to evaluate the molecular characteristics and the clonal relationships among the CRKA isolates.

Materials And Methods

Setting, study design and *K. aerogenes* strains

This study was conducted in a teaching hospital in Tucuman, Argentina (500 beds) with approximately 3000 admissions/day, with three 18-bed intensive care unit/s, serving the greater north area of Argentina. A total of 14 *K. aerogenes* strains collected during June-September 2018 were selected for the study and corresponded to a single sequential and non-duplicated CRKA strain isolated during the course of hospitalization. The study was part of Hospital Infection Control Committee activities; as such, no informed consent was needed from the patients. Pertinent clinical and epidemiological information were obtained through review of patient medical records and the laboratory information system and are shown on Table 1.

Microbiologic methods

Clinical samples were processed in the microbiology department of the hospital using routine practice and culture media. Bacterial identification was confirmed by MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight) (Microflex LT, Bruker Daltonics, Bremen, Germany) and antimicrobial susceptibility pattern was determined by the automated Vitek®2 system (BioMerieux, Merci l'Etoile, France) and using standard agar dilution test including ampicillin (AMP), ampicillin/sulbactam (SAM), piperacillin/tazobactam (PTAZ), cephalothin (CEF), cefotaxime (CTX), ceftazidime (CAZ), cefepime (CEP), meropenem (MER), imipenem (IMP), gentamicin (GEN), amikacin (AK),

trimethoprim/sulfamethoxazole (TMS), ciprofloxacin (CIP), tigecycline (TGC) and fosfomycin (FOS); colistin (COL) susceptibility was evaluated with broth microdilution technique. Breakpoints were defined following the document M100-S24 (http://clsi-m100.com/) and according to the European Committee on Antimicrobial Susceptibility Testing (http://www.eucast.org). *K. aerogenes* strains were selected on the basis of MIC (Minimum Inhibitory Concentration) values of ≥2 mg/L for any of the carbapenems imipenem or meropenem.

β-lactamases molecular characterization

Multiplex PCR targeting carbapenemase genes (bla_{KPC}, bla_{NDM}, bla_{VIM} and bla_{OXA-48}) and extended spectrum β-lactamases (ESBLs): SHV variants including SHV-2 (bla_{SHV-2}), CTX-M variants including CTX-M-2 and CTX-M-15 ($bla_{CTX-M-2}$, $bla_{CTX-M-15}$) and TEM variants including TEM-1 and TEM-2 families were performed[12].The amplicons were sequenced with ABI3130CL (Applied Biosystems, USA) and the sequences were analyzed on the National Centre for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov)

Plasmidic resistance to colistin

PCR targeting *mcr-1* gene was performed to evaluate the plasmid-mediated colistin resistance mechanism[13].

Clonal relationships among the CRKA isolates

Molecular typing was performed by pulsed-field electrophoresis (PFGE). Isolates were typed by PFGE of *Spe*I-digested total genomic DNA (TaKaRa, Tokyo, Japan), and the DNA fragments were separated by electrophoresis on 1% SeaKeam®Gold agarose (Lonza, Rockland, ME, United States) in 0.5X TBE (45 mM Tris, 45 mM boric acid, 1.0 mM EDTA; ph 8.0) buffer using the CHEF Mapper XA PFGE system (Bio-Rad, United States) at 6 V/cm2 and 14ºC, with alternating pulses at a 120º angle in a 5–20 s pulse time gradient for 19 h. DNA patterns were interpreted according to Tenover et al[14]. Strains were considered to be the same clone (type) if they showed ≥75% genetic identity, or fewer than three fragment differences on the PFGE profiles.

Results

In a 4-month period in 2018, there was an increased incidence of cultures for carbapenem-resistant *K. aerogenes* (CRKA) disseminated in an adult intensive care unit (50%) and five different wards at the hospital.

A total of 14 patients colonized/infected with CRKA were studied, and their clinical/epidemiological characteristics as demographics and mortality data, unit location, carbapenem exposure, procedures, and surgical histories are shown on Table 1. Patients were admitted in a range of 0-44 days previous to the CRKA detection, in the majority were male patients (93.8%) with an average age of 50 years (23-69 years).

The first identified case (Patient 1/ward 10) had a past medical history significant for intraabdominal infection (Figure 1, Table 1). The temporal association of subsequent positive cultures from patients in the ICU 1 and other different wards at the hospital, prompted an outbreak investigation (Figure 1). In response to the event, infection prevention implemented universal contact precautions, distribution of educational materials pertaining to hand hygiene and equipment disinfection were performed. Infected patients were cohorted to one side of the intensive care unit (ICU1) with dedicated nursing staff and antibiotic stewardship efforts were also reinforced during the study period. Despite interventions, 6 additional ICU1 patients had positive CRKA cultures between June and September 2018 (Figure 1, Table 1). Of the total 14 positive cultures from unique patients, 10 were clinical specimens (one respiratory, three urinary, four soft tissue, one bone and one blood sample) and four were rectal surveillance cultures. Epidemiological investigation of possible risk factors among the patients for developing CRKA infection/colonization was performed. *K. aerogenes* isolates from colonized or infected patients were also included in the study for context and comparison. Cases were not found to be associated with a specific ward. After October 2018, no CRKA were isolated in either clinical or surveillance specimens. Active surveillance in the UCI was discontinued in the end of December 2018 and the outbreak was deemed to have subsided.

The scores of Mc Cabe and Charlson (excluding 2 patients whose information was not available) averaged 2.25 and 3.25, respectively, and indicate a population at medium/high risk of mortality associated with nosocomial complications.

Among the 9 patients who received antimicrobial treatment, 8 staggered to directed treatment, mainly with amikacin (6/8) and/or carbapenemes (5/8). The overall mortality was 42.8% (6/14), and the attributed mortality to CRKA infection was 21.4% (3/14).

According to CLSI (2014) and EUCAST breakpoints, there were high rates of resistance to most of the antimicrobials tested without resistance to Amikacin and Tigecycline. (Table 2).

To confirm the colistin resistance, broth microdilution (the gold standard method) was used to test the isolates, 4 (28,6%) showed resistance (MIC50= 2 µg/ml and MIC range 0,25–16 µg/ml) (Table 2).

All 14 isolates carried the $bla_{\text{KPC-2}}$ as well as the $bla_{\text{SHV-2}}$ and $bla_{\text{CTXM-15}}$ genes. The $bla_{\text{TEM-24}}$ gene was detected in 45% of the isolates and no PCR product was obtained for the other *bla* genes investigated. The *mcr-1* gene was not detected in the colistin resistant isolates.

The PFGE results indicated that the CRKA isolates belonged to 2 distinct PFGE groups (types A and B), with type A being the most dominant including 12 of the *K. aerogenes* isolates (Figure 2). The four *K. aerogenes* isolates resistant to colistin belonged to clonal group A. In our study, the association of KPC-2 with other β-lactamases was evident in all the isolates.

Discussion

The rapid spread of KPC-producing enterobacteria is a major clinical and public health concern and continue epidemiological surveillance is necessary. These broad-spectrum β-lactamases are increasing in new locations worldwide, indicating an ongoing process[15]. The Pan American Health Organization (PAHO) reports that Argentina is one of the countries with the most “pandrug resistant" nosocomial isolations of Latin America [16]. Besides the numerous efforts made at local or national level to control the spread of these bacteria, the rapid dissemination of carbapenem-resistant enterobacteria constituted a clinically relevant problem of our region. Tucuman is situated, in the north of Argentina (NOA), within a multi border area limiting with Bolivia, Chile and Paraguay. Since 2006 an active monitoring for carbapenem-resistant Gram-negative bacteria detection is carried out in our Department.

This study was initiated in order to establish the molecular epidemiology of CRKA strains isolated from patients in our hospital following an outbreak event. During the period between June and September 2018 we informed the Infection prevention team regarding the extent of the transmission event and effectiveness of infection control interventions.

Focusing on the patients a high percentage had comorbidities and risk factors such as prolonged hospitalization and previous use of broad-spectrum antibiotics as potential risk factors for the CRKA acquisition. The average time of hospitalization was 16 days, patients were admitted in a range of 0-40 days previous to CRKA detection denoting the high hospital stay. The Intensive Care Unit was the most common site of acquisition, in line with previous reports[17-19].

According to the revision of clinical histories empirical antimicrobial treatments applied were poor due to multiple reasons: multi-resistance of the strains studied, medical personnel lack of knowledge of the magnitude of the outbreak and limited availability of other therapeutic options (tigecycline and fosfomycin) in the nosocomial pharmacy. These data are in line with Hao et al., who also identified the presence of severe disease and prior use of antimicrobials, primarily cephalosporins and quinolones, as risk factors associated to KPC-2 *K. aerogenes* infection[17].

The soft tissue and the urinary tract (30%) were the most common sources of clinical samples, followed by abdominal liquid (20%), while other authors reported respiratory samples and blood as the prevalent clinical sources[17,20].

Antimicrobial susceptibility testing confirmed resistance to piperacillin/tazobactam, ciprofloxacin and carbapenemes in all isolates, results in line with Hao et al.,[19] who reported similar resistance rates against piperacillin/tazobactam, ciprofloxacin and carbapenemes and higher than 75% to amikacin and gentamicin. None of the isolates presented resistance to tigecycline, results not in line with Tavares et al.,[21] who reported approximately 60% of the *K. aerogenes* isolates as non-susceptible.

In this study, four isolates (28.6%) were found to be resistant to colistin, in coincidence with Tavares et al.,[21] who reported 26,3% of colistin resistance between the *K. aerogenes* isolates also included in the predominant pulse type. Hao et al.,[19] found only one isolate resistant to colistin; there were, indeed, some

reports of colistin resistance in *K. aerogenes* during this time. Since colistin is generally considered a “last-line” antibiotic, this emerging resistance is highly concerning and worth highlighting. To our knowledge, this is the first report of *K. aerogenes* strains resistant to colistin in our region. All polymyxin-resistant *K. aerogenes* isolates belonged to clone A. This is a worrisome fact (it may indicate the adaptation of this clone and possible spread) and mainly warrants a more judicious application of this antimicrobial agent.

In clinical CRKA strains, carbapenem resistance has been associated with either carbapenemase production or coupling of adaptive mutations affecting membrane permeability and AmpC hyperproduction, Szabó et al., in the study of the resistance mechanism in CRKA isolates, indicated that efflux pumps are not involved despite a previous report of their important role in carbapenem resistance and moreover, porin dysfunction is probably not the main mechanism of resistance in these isolates[22]. In Brazil the KPC enzymes have been reported in *E. cloacae* complex and *K. aerogenes* isolates[21,23] the same as in the present study, highlighting the prevalence of bla_{KPC} in *K. aerogenes* isolates. Other authors included bla_{KPC-2}, bla_{KPC-3}, bla_{OXA-48}, bla_{NDM-6} and bla_{NMC-A} genes in these isolates[17].

The ESBL rate in the *K. aerogenes* detected was 100% and corresponds to CTX-M-15 and SHV-2. CTX-M-2is the most common subtype in our country[24] and is found in several species of *Enterobacteriaceae*[25,26]. However, recent studies in coincidence with us, have reported an increase in the number of *Enterobacter* species producing CTX-M-15[24,27].

In the present study, isolates of *K. aerogenes* showed monoclonal spread in line with other authors[1,18,28]. The 12 CRKA strains in the dominant subgroup A were likely to be primarily responsible for the first isolation and subsequent dissemination in the hospital. The outbreak characteristic data showed prolonged hospitalization and previous use of broad-spectrum antibiotics as potential risk factors for the acquisition of CRKA across the hospitalized patients. In conclusion it is extremely important to perform phenotypic and genotypic identification of early genetic resistance mechanisms in these isolates, not only from infections sites but also from colonization, in order to prevent the spread of these MDR isolates, which may present different resistance genes.

Declarations

Funding

There was no influence of the funding organisation on analysis or interpretation of the described data. There review was undertaken as an additional activity parallel to a PhD project.

Acknowledgements

No to declare.

Author information

Affiliation

Laboratorio de Bacteriología Certificado, Cátedra de Bacteriología, Facultad de Bioquímica, Química y Farmacia. Universidad Nacional de Tucumán. San Miguel de Tucumán, Tucumán, Argentina. Ayacucho 471. CP:4000

Juan Martin Vargas (JMV), María Paula Moreno Mochi (MPMM), María Angela Jure (MAJ)

Departamento de Infectología, Hospital Ángel Cruz Padilla. San Miguel de Tucumán, Tucumán, Argentina. Juan Bautista Alberdi 550. CP:4000

Juan Manuel Nuñez (JMN)

Servicio de Microbiología, Hospital Ángel Cruz Padilla. San Miguel de Tucumán, Tucumán, Argentina. Juan Bautista Alberdi 550. CP:4000

Silvana Mochi (SM), Mariel Cáceres (MC)

Instituto Ramón y Cajal de Investigación Sanitaria. Ctra. de Colmenar Viejo, Km. 9100 Madrid, España. CP: 28034

Rosa del Campo (RC)

Authors' contributions

JMV and MAJ conceived the review topic, objectives and write-up. JMN, MPMM, SM and MC participated in the study selection. MAJ and RC reviewed the manuscript critically for its scientific content. All authors reviewed and approved the manuscript.

Ethics approval and consent to participate

This study was reviewed and approved by Angel Cruz Padilla Ethic Committee. All participants signed the consent form, and were informed that their participation was voluntary, anonymous, and they could quit at any time.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

References

1. Davin-Regli A, Pagès J-M. *Enterobacter aerogenes* and *Enterobacter cloacae*; versatile bacterial pathogens confronting antibiotic treatment. Front Microbiol. 2015 6:392. https://doi.org/10.3389/fmicb.2015.00392.
2. Kang CI, Chung DR, Ko KS, et al. Clinical predictors of *Enterobacter* bacteremia among patients admitted to the ED. Am J Emerg Med 2012 30:165–169. https://doi.org/10.1016/j.ajem.2010.09.003
3. Sanders WE, Jr, Sanders CC. *Enterobacter spp.*: pathogens poised to flourish at the turn of the century. Clin Microbiol Rev. 1997 10:220 –241. https://doi.org/10.1128/CMR.10.2.220.
4. Loiwal V, Kumar A, Gupta P, et al. *Enterobacter aerogenes* outbreak in a neonatal intensive care unit. Pediatr Int. 1991 41:157–161.
5. Salso S, Culebras E, Andrade R, et al. Outbreak of TEM-24-producing *Enterobacter aerogenes* in a Spanish hospital. Microb Drug Resist. 2003 9:299 –305. https://doi.org/10.1089/107662903322286517.
6. Piagnerelli M, Kennes B, Brogniez Y, et al. Outbreak of nosocomial multidrug-resistant *Enterobacter aerogenes* in a geriatric unit: failure of isolation contact, analysis of risk factors, and use of pulsed-field gel electrophoresis. Infect Control Hosp Epidemiol. 2000 21:651–653. https://doi.org/10.1086/501704.
7. Davin-Regli A, Saux P, Bollet C, et al. Investigation of outbreaks of *Enterobacter aerogenes* colonisation and infection in intensive care units by random amplification of polymorphic DNA. J Med

   98. 1996 44:89 –98. https://doi.org/10.1099/00222615-44-2-89.
8. Bertrand X, Hocquet D, Boisson K, et al. Molecular epidemiology of *Enterobacteriaceae* producing extendedspectrum beta-lactamase in a French university-affiliated hospital. Int J Antimicrob Agents. 2003 22:128 –133. https://doi.org/10.1016/S0924-8579(03)00098-0.
9. Guh AY, Bulens SN, Mu Y, et al. Epidemiology of carbapenem-resistant *Enterobacteriaceae* in 7 US communities, 2012–2013. JAMA. 2015 314:1479 –1487. https://doi.org/10.1001/jama.2015.12480.
10. Lee HJ, Choi JK, Cho SY, et al. Carbapenem-resistant *Enterobacteriaceae:* prevalence and risk factors in a single community-based hospital in Korea. Infect Chemother. 2015 48:166 –173. https://doi.org/10.3947/ic.2016.48.3.166.
11. Franolic´ I, Bedenic´ B, Beader N, et al. NDM-1-producing *Enterobacter aerogenes* isolated from a patient with a JJ ureteric stent in situ. CEN Case Rep. 2019 8:38–41. https://doi.org/10.1007/s13730-018-0360-z.
12. Dallenne C, Da Costa A, Decré D, et al. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in *Enterobacteriaceae*. J. Antimicrob. Chemother. 2010 Mar;65(3):490–495.

13. Liu YY, Wang Y, Walsh TR, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis. 2016;16(2):161- doi:10.1016/S1473-3099(15)00424-7.
14. Tenover FC, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol. 1995;33(9):2233-
15. Lee CR, Lee JH, Park KS, et al. Global dissemination of carbapenemase-producing Klebsiella pneumoniae: epidemiology, genetic context, treatment options, and detection methods, Front. Microbiol. 2016 895.
16. Antimicrobial Surveillance Program SENTRY, World Health Organization, 2014. who.int
17. Malek A, McGlynn K, Taffner S, et al. Next-Generation-Sequencing-Based Hospital Outbreak Investigation Yields Insight into Klebsiella aerogenes Population Structure and Determinants of Carbapenem Resistance and Pathogenicity. *Antimicrob Agents Chemother*. 2019;63(6):e02577-18. Published 2019 May 24. doi:10.1128/AAC.02577-18
18. Cabral AB, Maciel MAV, Barros JF, et al. Clonal spread and accumulation of β-lactam resistance determinants in Enterobacter aerogenes and Enterobacter cloacae complex isolates from infection and colonization in patients at a public hospital in Recife, Pernambuco, Brazil. *J Med Microbiol*. 2017;66(1):70- doi:10.1099/jmm.0.000398
19. Hao M, Shen Z, Ye M, et al. Outbreak Of *Klebsiella pneumoniae* Carbapenemase-Producing *Klebsiella aerogenes* Strains In A Tertiary Hospital In China. *Infect Drug Resist*. 2019;12:3283- Published 2019 Oct 21. doi:10.2147/IDR.S221279
20. Qin X, Yang Y, Hu F, et al. Hospital clonal dissemination of Enterobacter aerogenes producing carbapenemase KPC-2 in a Chinese teaching hospital. *J Med Microbiol*. 2014;63(Pt 2):222- doi:10.1099/jmm.0.064865-0
21. Tavares CP, Pereira PS, Marques E de A, et al. Molecular epidemiology of KPC-2-producing Enterobacteriaceae (non-Klebsiella pneumoniae) isolated from Brazil. *Diagn Microbiol Infect Dis*. 2015;82(4):326- doi:10.1016/j.diagmicrobio.2015.04.002
22. Szabó D, Silveira F, Hujer AM, et al. Outer membrane protein changes and efflux pump expression together may confer resistance to ertapenem in Enterobacter cloacae. *Antimicrob Agents Chemother*. 2006;50(8):2833- doi:10.1128/AAC.01591-05
23. Jaskulski MR, Medeiros BC, Borges JV, et al. Assessment of extended-spectrum β-lactamase, KPC carbapenemase and porin resistance mechanisms in clinical samples of Klebsiella pneumoniae and Enterobacter spp. *Int J Antimicrob Agents*. 2013;42(1):76- doi:10.1016/j.ijantimicag.2013.03.009
24. Sennati S, Santella G, Di Conza J, et al. Changing epidemiology of extended-spectrum β-lactamases in Argentina: emergence of CTX-M-15. *Antimicrob Agents Chemother*. 2012;56(11):6003-6005. doi:10.1128/AAC.00745-12
25. Cantón R, Gonzalez-Alba JM, Galan JC. CTX-M enzymes: origin and diffusion. *Front Microbiol* 2012;3:110.

26. Gales AC, Castanheira M, Jones RN, et al. Antimicrobial resistance among Gram-negative bacilli isolated from Latin America: results from SENTRY Antimicrobial Surveillance Program (Latin America, 2008-2010). *Diagn Microbiol Infect Dis* 2012;73: 354–360.
27. Seki LM, Pereira PS, de Souza Conceiçao M, et al. Molecular epidemiology of CTX-M producing *Enterobacteriaceae* isolated from bloodstream infections in Rio de Janeiro, Brazil: emergence of CTX-M-15. *Braz J Infect Dis* 2013;17:640–646.
28. Tuon FF, Scharf C, Rocha JL, et al. KPC-producing Enterobacter aerogenes infection. *Braz J Infect Dis*. 2015;19(3):324- doi:10.1016/j.bjid.2015.01.003

Tables

Due to technical limitations, table 1 and 2 is only available as a download in the Supplemental Files section.

Figures

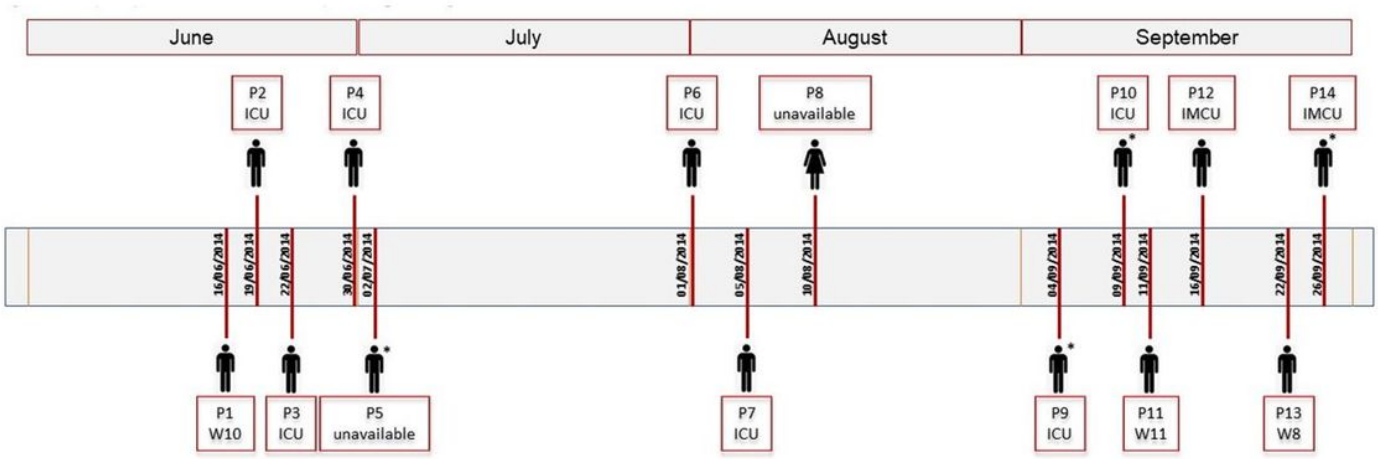

Figure 1

Temporary documentation of KPC-2 producing K. aerogenes. References: P: patient; W: ward; ICU: Intensive care unit; IMCU: intermediate care unit; *Colonized patients

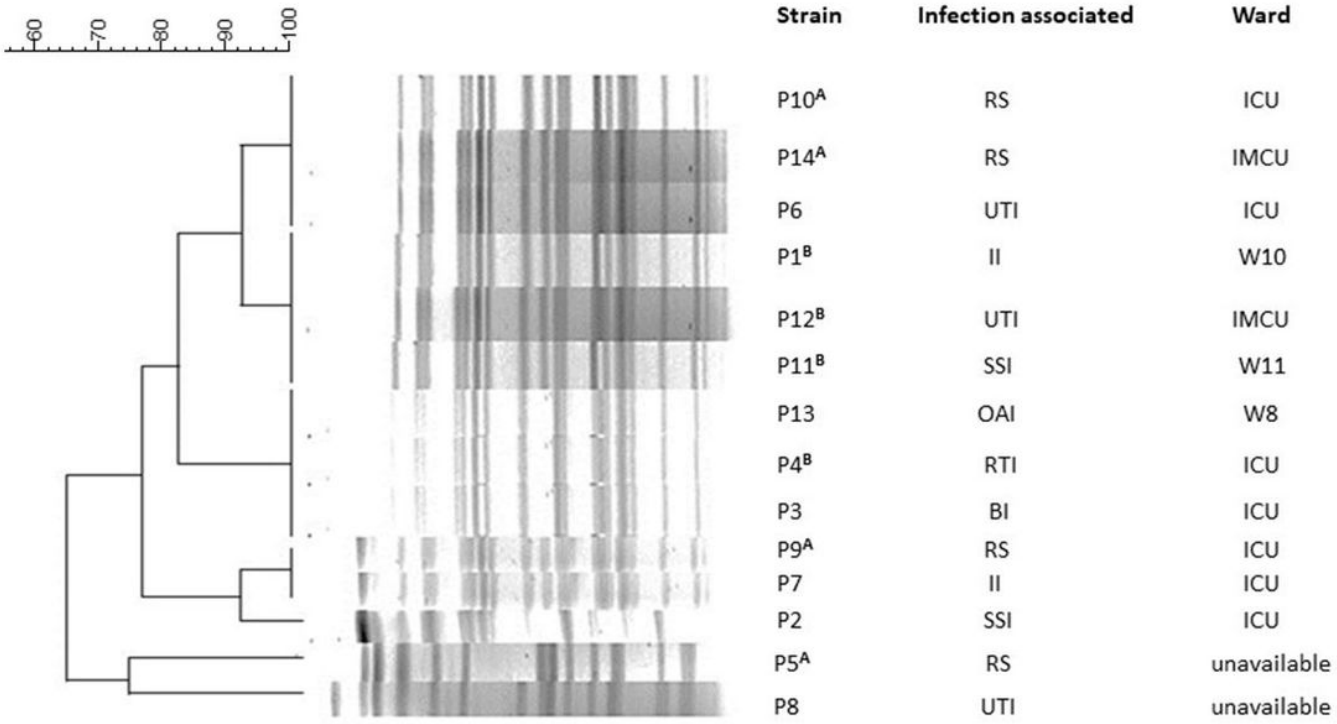

Figure 2

DNA finger printing by PFGE of KPC-2 producing K. aerogenes strains. References: P: patient; ICU: intensive care unit; IMCU: intermediate care unit; RS: rectal swab; II: intraabdominal infection; SSI: surgical site infection; RTI: respiratory tract infection; UTI: urinary tract infection; OAI: osteoarticular infection; BI: blood infection. A: strain recuperated of colonized patients; B: colistin resistant strain.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table12.docx