Virulence factors and clinical patterns of multiple-clone hypermucoviscous KPC-2 producing *K. pneumoniae*

J.M. Vargas, M.P. Moreno Mochi, J.M. Nuñez, M. Cáceres, S. Mochi, R. del Campo Moreno, M.A. Jure

**A B S T R A C T**

Carbapenemase-producing *Klebsiella pneumoniae* (CRKP) are increasingly reported worldwide being necessary the local epidemiological monitoring. Our aim was to characterize the hypermucoviscous CRKP isolates collected in our hospital during a 6 months period. Carriage of the carbapenemase genes (*bla*KPC, *bla*NDM, *bla*CTX-M, and *bla*OXA-48), extended spectrum β-lactamases (*bla*VIM, *bla*ESBL-M), and the virulence genes (*mexA*, *mexB*, *wcbG*, *wcbL*, *wcaH*, *wcaD*, *iron*, *hly*, and *cnf-1*) were determined by multiplex-PCR. Genetic relationship among the isolates was performed by PFGE and MLST. A total of 35 isolates were recovered, being the urinary and respiratory tract the most common infection sites (34.2%). The *bla*KPC-2 gene was present in all the isolates, coexisting with *bla*CTX-M-2 (45.7%), *bla*SHV-2 (28.6%), and *bla*VIM (14.3%). The capsular serotype K2 corresponded with 68.6% of the isolates. Virulence factors frequency were variable (adhesins [97.1%], siderophores [94.3%] and phagocytosis resistance [96.5%, 80% and 96.5%]). A total of 10 STs were identified although 40% of them clustered on ST25-CC65, and 17% to ST17. The incidence of KPC-2-producing *K. pneumoniae* reported by the hospital was 0.290 per 1000 admissions. In summary, we described an epidemic scenario of multidrug resistant hypermucoviscous KPC-2 producing ST25 *K. pneumoniae* in our institution.

1. Introduction

*Klebsiella pneumoniae* is a member of the ESSEAPNE group (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter species*) and the Gram-negative leading bacterium in hospital-acquired-infections (HAIs) [1, 2]. It was considered to be the most important causal agent of community-acquired infections; but in the early 1970s the epidemiology and infections spectrum dramatically changed when this bacterium was established in the hospital environment. This pathogen has developed increasing resistance to carbapenems, the last resort antibiotics typically used to treat multidrug resistant strains in hospital patients. Regrettfully, new carbapenem resistant *K. pneumoniae* isolates are resistant to almost all available antibiotics and is associated with high rates of mortality [3].

The hypermucoviscous *K. pneumoniae* isolates differ from classical mucosal strains because they present a positive string test. Since these *K. pneumoniae* variant were reported, hypermucoviscosity had been associated with hypervirulent strains; then new evidence has suggested that hypermucoviscosity and hypervirulence are two different phenotypes that should not be used synonymously regardless of whether they can act in synergy under certain circumstances [4].

Pathogenic *K. pneumoniae* strains have the potential to cause a wide variety of infectious diseases, including urinary tract, respiratory tract and blood infections [5]. Some virulent factors have been described codifying for capsule (*mexA*, *mexB*, *wcbG*), hypermucoviscosity-associated gene A specific to K1 capsule serotype (*mexA*, *rpmA*), adhesins (*fnmH, mkdK, lpp*), lipopolysaccharides (*wabG*, *ute*, *yfeM*), iron acquisition systems (*iusA*, *iron*, *entB*) and other virulence factors (*allS*, *huy*, *cnf-1*) that

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**ABSTRACT**

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*Klebsiella pneumoniae* is a member of the ESKAPE group (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter species*) and the Gram-negative leading bacterium in hospital-acquired-infections (HAIs) [1, 2]. It was considered to be the most important causal agent of community-acquired infections; but in the early 1970s the epidemiology and infections spectrum dramatically changed when this bacterium was established in the hospital environment. This pathogen has developed increasing resistance to carbapenems, the last resort antibiotics typically used to treat multidrug resistant strains in hospital patients. Regrettfully, new carbapenem resistant *K. pneumoniae* isolates are resistant to almost all available antibiotics and is associated with high rates of mortality [3].

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enable them to overcome host defenses, although it is not clear the linkage of these genes with antibiotic resistance [6].

Carbapenem hydrolysing β-lactamases have been reported to be increasingly widespread. Ambler molecular class A (KPC), class B (VIM, IMP, NDM) and class D (OXA-48) types are the most frequently found in K. pneumoniae causing serious nosocomial infections [7]. In South America, carbapenemase-producing K. pneumoniae was initially reported in 2006 in Colombia [8] and after in Brazil and Argentina [9, 10]. The hospital-acquired high-risk clones sequence types (ST) ST258 and ST11 were worldwide disseminated [11], whereas the Latin America local epidemiology pointed out to ST11 and ST7437 associated to bla_KPC-2 and bla_KPC-3 production [12, 13]. In reference to our country, Argentina, previous studies demonstrated that the emergence of bla_KPC-2 is also associated with CC258 [14].

The aim of this study was to determine the clinical, epidemiological and molecular patterns of hypermucoviscous carbapenem-resistant K. pneumoniae isolates causing nosocomial infections at a tertiary referral hospital in Tucumán, Argentina.

2. Materials and methods

2.1. Study design

This retrospective study was conducted in a teaching hospital in Tucumán, Argentina (500 beds) with approximately 3000 admissions/day. Over a period of 6 months, from May 1 to October 31, 2014, all patients suffering from K. pneumoniae infections, resistant to carbapenemes and hospitalized for more than 48 hours were studied; patients from other hospitals or with community-acquired infections or without strict infection criteria were not included in this study. After the patients signed an informed consent, the clinical history was accessed and the clinical-epidemiological information was registered: name and surname, age, sex, time of hospitalization prior to isolation, hospital stay, comorbidities, probable site of the acquisition of the infection, type of infection and antibiotic treatment used. Institutional activity data (number of admissions and mean length of stay) of this period were collected by the hospital for the calculation of incidence rates. The ethics committee of the Angel C. Padilla hospital approved the study and authorized the access to clinical information.

2.2. Identification and Antimicrobial Susceptibility Testing

Bacterial identification was confirmed by MALDI-TOF (matrix-assisted laser desorption/ionization time-of-flight) (Microflex LT, Bruker Daltonics, Bremen, Germany), susceptibility patterns were determined by theautomated Vitex 2® system (BioMerieux, Merci l’Etoile, France) and?? by broth microdilution method including ampicillin (AMP), ampicillin/sulbactam (SAM), piperacillin/tazobactam (PTZ), cefalothin (CEF), cefotaxine (CTX), cefazidime (CAZ), cephepine (FEP), meropenem (MER), imipenem (IMP), gentamicin (GEN), amikacin (AKN), colistin (COL), trimethoprim/sulfamethoxazole (TMS) and ciprofloxacin (CIP). Breakpoints were defined following the document M100-S24 of the Clinical and Laboratory Standards Institute [15]. Susceptibility to tigecycline was determined by broth microdilution method and for fosfomycin by agar dilution method with glucose-6-phosphate (25 mg/L in the medium). Breakpoints were defined according to the Committee on Antimicrobial Susceptibility Testing (EUCAST) [16]. Synergy tests with boronic acid and EDTA disks close to the carbapenemes and the modified Hodge test (MHT) were performed for the detection of carbapenemes [17]. K. pneumoniae ATCC700605 and Escherichia coli ATCC 25922 were used as quality control strains for the antibiotic susceptibility tests.

2.3. Detection of the hypermucoviscous phenotype

All carbapenem-resistant K. pneumoniae isolates were grown in nutritive agar (Britania®) enriched with 5% defibrinated blood, Mac Conkey agar (Britania®) and CLED agar (Britania®). Hypermucoviscosity phenotype was defined by the formation of a viscous filament ≥5 mm after stretching a colony with a loop on all the agar plates tested [18, 19].

2.4. Strain selection

All the hypermucoviscous K. pneumoniae strains were selected on the basis of MIC values of ≥2 mg/liter for any of the carbapenemes imipenem, meropenem or ertapenem and Hodge tests positive.

2.5. β-lactamases molecular characterization

DNA extracts were prepared by boiling the bacterial suspensions [20]. Multiplex PCR targeting carbapenemase genes (bla_KPC, bla_NDM, bla_VIM, bla_OXA-48) and extended spectrum β-lactamases-ESBLs: SHV variants including SHV-2 (bla_SHV-2) and CTX-M variants including CTX-M-2 (blaCTX_M-2) were performed [21]. The amplicons were sequenced with ABI3130CL (Applied Biosystems, USA) and the sequences were analyzed on the National Center for Biotechnology Information (NCBI) [22]. The complete CDS of the β-lactamases detected has not been determined and the indicated allelic variant has been obtained from partial sequences.

2.6. Analysis of virulence gene regions

The virulence genes were detected in four-separated multiplex PCR reactions (magA-fimH-Ug-iaA, wabM-rmpA-cnf1-ycfM, hly-iroN-k2A-markD, and km-allS-enb8-wacG) with the following thermal cycling conditions: 5 minutes of pre-denaturation at 95 °C, followed by 30 cycles: 1 minute at 94 °C, 1 minute at 58 °C, 1 minute at 72 °C and 10 minutes of final elongation at 72 °C (Sensoquest Labcycler, Germany) [6].

2.7. Population structure

Molecular typing was performed by pulsed-field electrophoresis (PFGE) and Multilocus Sequence Typing (MLST). Isolates were typed by PFGE of Spel-digested total genomic DNA (TaKaRa, Tokyo, Japan), and the DNA fragments were separated by electrophoresis on 1% Samkeam 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/cm and 14 °C, with alternating pulses at a 120° angle in a 5–20 s pulse time gradient for 19 h. DNA patterns were interpreted according to Tenover et al [23]. 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. Subsequently one isolate for each PFGE pulsotype was submitted to MLST technique following the K. pneumoniae MLST website guidelines [24].

3. Results

A total of 35 patients, which their clinical-epidemiological characteristics are shown on Table 1, infected by carbapenem-resistant K. pneumoniae were identified. Patients were admitted in a range of 3–74 days previous to the carbapenem-resistant K. pneumoniae detection. The sample sources were the respiratory tract (n = 12, 34.2%), urinary tract (n = 12, 34.2%), soft tissue (n = 5, 14.2%), blood (n = 2, 5.7%), cerebrospinal fluid (n = 2, 5.7%) bone (n = 1, 2.8%), and abdominal fluid (n = 1, 2.8%).

All 35 isolates were multidrug resistant, have similar susceptibility profiles (Table 2), and carried the bla_KPC-2 gene. In 16 isolates (45.7%) the bla_KPC-2 gene was also amplified, as well as bla_SHV-2 in 10 isolates (28.6%) and blaCTX_M-2/blaSHV-1 in 5 isolates (14.3%). Virulence factors carriage were as follows: adhesins (97.1%), siderophores (94.3%) and phagocytosis resistance (74.3%) (Table 3). The capsular serotype K2 was identified in 68.6% of the isolates, and in the remaining isolates the
Table 1
Clinical-epidemiological characteristics of 35 patients included in the study.

| Population Characteristics | Patients number (%) |
|----------------------------|---------------------|
| Male                       | 22 (62.8%)          |
| Comorbidities              |                     |
| Diabetes                   | 6 (17.1%)           |
| Neoplata                   | 4 (11.4%)           |
| Chronic renal insufficiency| 3 (8.5%)            |
| Reumathoid disease         | 2 (5.7%)            |
| No Comorbidities           | 20 (57.1%)          |
| Site of acquisition        |                     |
| Intensive care unit        | 16 (45.7%)          |
| Room 3                     | 6 (17.1%)           |
| Surgery                    | 6 (17.1%)           |
| Room 10                    | 1 (2.9%)            |
| Room 11                    | 2 (5.7%)            |
| Intermediate care unit     | 2 (5.7%)            |
| Room 8                     | 1 (2.9%)            |
| Room 7                     | 1 (2.9%)            |
| Type of infection          |                     |
| Urinary tract infection    | 11 (31.4%)          |
| Surgery wound infection    | 5 (14.2%)           |
| Respiratory tract infection| 10 (28.5%)          |
| Intra-abdominal infection  | 5 (14.2%)           |
| catheter-related infections| 1 (2.8%)            |
| Osteoarticular infections  | 2 (5.7%)            |
| Bacteremia                 | 1 (2.8%)            |
| Antibiotics used<sup>a</sup> |                   |
| Amikacin                   | 27 (77.1)           |
| Ciprofloxacin              | 4 (11.4)            |
| Colistin                   | 25 (71.4)           |
| Imipenem                   | 14 (40.0)           |
| Meropenem                  | 11 (31.2)           |
| Piperacillin/tazobactam    | 29 (82.8)           |
| Vancomycin                 | 2 (5.7)             |

<sup>a</sup> All antibiotics mentioned were combined in different therapeutic schemes.

Table 2
Susceptibility testing and minimum inhibitory concentration (MIC) results of 35 CRKP isolates.

| Antimicrobial agent | MIC range (mg/L) | MIC50 (mg/L) | MIC90 (mg/L) | Number of S (%) |
|---------------------|------------------|--------------|--------------|-----------------|
| Ampicillin          | ≥32              | ≥32          | ≥32          | 0 (0)           |
| Ampicillin/β-lactam | ≥32              | ≥32          | ≥128         | 0 (0)           |
| Piperacillin        | 16–128           | 128          | 128          | 0 (0)           |
| Tazobactam          |                  |              |              |                 |
| Cefalotin           | ≥64              | ≥64          | ≥64          | 0 (0)           |
| Cefotaxime          | ≤1–64            | ≥64          | ≥64          | 3 (1)           |
| Cefazidime          | 4–64             | ≥16          | ≥16          | 11 (31)         |
| Ceftibuten          | ≤1–64            | ≥16          | ≥16          | 24 (68)         |
| Meropenem           | ≥16              | ≥16          | ≥16          | 0 (0)           |
| Imipenem            | 8–16             | ≥16          | ≥16          | 0 (0)           |
| Gentamicin<sup>c</sup> | ≤1–16          | ≥16          | ≥16          | 8 (22)          |
| Amikacin            | ≤2–16            | ≥2           | ≤2           | 35 (100)        |
| Colistin            | ≤0.5             | ≤0.5         | ≤0.5         | 35 (100)        |
| Tigecycline<sup>d</sup> | 0.25–1         | 0.5          | 0.5          | 33 (94)         |
| Fosfomycin intravenous | ≤32–64       | ≤32          | ≤32          | 34 (97)         |
| Trimethoprim/β-lactam | ≤20              | ≤32          | ≤32          | 11 (31)         |
| Sulfametoxazole     | ≥320             |              |              |                 |
| Ciprofloxacin       | ≤0.25–32         | 1            | 1            | 0 (0)           |

S Susceptible strains.

<sup>a</sup> Interpreted according to EUCAST clinical breakpoints for E. coli.

4. Discussion

The rapid spread of KPC-producing <i>K. pneumoniae</i> is a major clinical and public health concern and continue epidemiological surveillance is necessary. These broad-spectrum β-lactamas are increasing in new locations worldwide, indicating an ongoing process [25, 26]. The Pan American Health Organization (PAHO) reports that Argentina is one of the countries with the most “pandrug resistant” nosocomial isolations of Latin America [27]. Besides the numerous efforts made at local or national level to control the spread of these species, the rapid dissemination of carbapenem-resistant <i>K. pneumoniae</i> constituted a clinically relevant problem of our region. Tucuman is situated, in the north of Argentine (NOA), within a multi border area limiting with Bolivia, Chile and Paraguay. Since 2006 an active monitoring for carbapenem-resistant <i>K. pneumoniae</i> detection is carried out in our Department.

The present study is focused on the molecular characterization of carbapenem-resistant hypermucoviscous <i>K. pneumoniae</i>. The incidence rate of these strains in our institution was 0.290 per 1000 admissions, higher than the rate registered in Belgium where the average incidence among 9 hospitals was 0.223 per 1000 admissions [28] and even greater than in Germany where it is significantly lower (0.047 per 1000 admissions) [29]. The average time of hospitalization prior to the acquisition of the infection was 30 days, denoting the high hospital stay; the Intensive Care Unit was the most common site of acquisition, in line with previous reports [26, 30, 31, 32, 33]. Unlike other studies, the urinary and respiratory tract was the most common sources of clinical samples (34.2%), followed by soft tissue (14.2%) and blood (5.7%), while other authors reported bacteremia as the leading site of infection [34, 35].

Antimicrobial susceptibility testing confirmed resistance to piperacillin/tazobactam, ciprofloxacin and meropenem in all isolates, higher than the results found in Belgian hospitals: 43.9%, 80.3% and 53% respectively. The resistance proportion for tigecycline and colistin was still lower with only 2 and 1 strain respectively [28].

Focusing on the molecular and genetic characterization, we found Extended spectrum β-lactamas (ESBLs) genes as <i>bla</i>_CTX-M-2 (45.7%), <i>bla</i>_SHV-2 (28.6%) and <i>bla</i>_CTX-M-2/<i>bla</i>_SHV-2 (14.3%). These results differ from that described by Canton et al. reporting of <i>bla</i>_CTX-M-9 in 96% of the isolates, whereas only 1% contained the <i>bla</i>_KPC-15 gene, which is by far the most prevalent CTX-M variant worldwide [36].

KPC-2 is the most prevalent carbapenemase in China, with rare detection of metallo-carbapenemases, the same as other Latin American countries that present <i>bla</i>_KPC-2 and <i>bla</i>_KPC-3 in agreement with our results. However, some other countries may have another dominant carbapenemase; for example, the United Kingdom is likely to have a mixed carbapenemase pattern with VIM and NDM, and NDM types followed by OXA-48-like types were prevalent in India [37, 38].

Molecular typing of our strains showed a clonal dissemination of ST25 and ST17, whereas other Latin America studies reported ST11 and ST437 associated with the spread of <i>bla</i>_(KPC-2 and <i>bla</i>_(KPC-3 [9, 39, 40]. Previous studies located in Argentina described ST258 as the dominant clonal type [14]. The importance of ST25-CGCG5 was previously described by Brise et al. [41].

Since the presence of hypermucoviscous variants of <i>K. pneumoniae</i> in the world, many cases of invasive infections caused by these pathogens were described. Nevertheless, today the terms hypermucoviscous and hypervirulent are different and genes associated with the virulence must be determined [4]. Although in hypervirulent strains the K1 and K2 capsular types were the most exhaustively described, it has been demonstrated that <i>K. pneumoniae</i> producing infection and non-hypervirulent strains can also showed the K2 serotype [42]. Our isolates showed a K2 serotype associated with different genetic lineages in coincidence with Zhao et al. that also demonstrated the K2 serotype in 68.7% of the hypermucoviscosity-positive <i>K. pneumoniae</i> isolates [43].

Isolates containing the <i>magA</i>, <i>cfr1</i>, <i>hly</i> and <i>allS</i> genes were not detected in coincidence with Aksoz et al. In this study, capsule associated genes were <i>wabG</i> (48.5%), <i>ugo</i> (80%), and <i>ycfM</i> (57.1%), encoding...
Table 3
Carbapenem resistance and virulence gene profiles of *K. pneumoniae* strains.

| ST/Capsular Type | Strain       | Clinical sources | Carbapenemase, ESBL | Virulence gene profiles |
|------------------|--------------|------------------|---------------------|-------------------------|
| ST25/K2          | Bone         | KPC-2, CTX-M-2   | ugc, wabG, ycfM, iroN, mrkD, kpn, entB |
| ST25/K2          | Purulent     | KPC-2, CTX-M-2   | ugc, iroN, mrkD, kpn, entB |
| ST25/K2          | Urine        | KPC-2, CTX-M-2   | ugc, ycfM, iroN, mrkD, kpn, entB |
| ST25/K2          | LCR          | KPC-2            | ugc, mrkD, kpn, entB |
| ST25/K2          | Purulent     | KPC-2, CTX-M-2   | ugc, wabG, ycfM, iroN, mrkD, kpn, entB |
| ST25/K2          | Bal          | KPC-2, CTX-M-2   | ugc, ycfM, mrkD, kpn, entB |
| ST25/NT          | Abdominal    | KPC-2, CTX-M-2   | ugc, mrkD, kpn, entB |
| ST17/K2          | Urine        | KPC-2, CTX-M-2   | ugc, ycfM, iroN, mrkD, kpn, entB |
| ST17/K2          | Blood        | KPC-2, CTX-M-2   | ugc, mrkD, kpn, entB |
| ST17/K2          | Bal          | KPC-2, CTX-M-2   | ugc, ycfM, iroN, mrkD, kpn, entB |
| ST17/K2          | Bal          | KPC-2            | ugc, mrkD, kpn, entB |
| ST17/NT          | Blood        | KPC-2, CTX-M-2   | ugc, mrkD, kpn, entB |
| ST629/K2         | Bal          | KPC-2, CTX-M-2   | ugc, ycfM, mrkD, kpn, entB |
| ST629/K2         | Urine        | KPC-2, CTX-M-2   | ugc, mrkD, kpn, entB |
| ST629/K2         | Blood        | KPC-2, CTX-M-2   | ugc, mrkD, kpn, entB |
| ST629/K2         | LCR          | KPC-2, CTX-M-2   | ugc, mrkD, kpn, entB |
| ST629/NT         | Purulent     | KPC-2, CTX-M-2   | ugc, mrkD, kpn, entB |
| ST995/K2         | Urine        | KPC-2, CTX-M-2   | ugc, mrkD, kpn, entB |
| ST147/NT         | LCR          | KPC-2, CTX-M-2   | ugc, mrkD, kpn, entB |
| ST147/NT         | Bal          | KPC-2, CTX-M-2   | ugc, mrkD, kpn, entB |
| ST147/NT         | Bal          | KPC-2            | ugc, mrkD, kpn, entB |
| Clon A/K2        | Urine        | KPC-2, CTX-M-2   | ugc, iutA, ycfM, mrkD |
| Clon B/K2        | Urine        | KPC-2, CTX-M-2   | ugc, iutA, ycfM, mrkD |
| Clon C/NT        | Urine        | KPC-2, CTX-M-2   | ugc, mrkD, iroN, kpn, entB |
| Clon D/K2        | Purulent     | KPC-2, CTX-M-2   | ugc, mrkD, iroN, kpn, entB |

References: genes associated with the resistance to phagocytosis (*uge, ycfM, wabG*), adhesins (*mrkD, kpn*), capsular elements (*magA, k2A*), iron acquisition systems (*entB, iroN*), associated with the cellular wall (*WabG*), and others virulence factors (*allS*). K2: capsular antigen 2. NT: non-typable. BAL: bronchoalveolar lavage. LCR: cerebrospinal fluid. ESBL: extended spectrum β-lactamases.

* The complete CDS of the β-lactamases detected has not been determined and the indicated allelic variant has been obtained from partial sequences.
capsule, lipoprotein, and external membrane protein, respectively. These results are consistent with previous studies reporting wabG (in 88% of isolates), uge (86%), ycfM (80%) and emB (72%) [6]. According to the distribution of virulence genes 16 virulence profiles/35 CRKP strains were defined according with Aksoz et al., who described 29 virulence profiles/34 CRKP strains. This situation evidences the high possibilities of virulence factors combination, forcing the molecular typing at individual isolates level.

The studied strains express two types of fimbrial adhesions; type 1 and type 3 fimbriae [5]. While type 1 fimbriae, encoding fimH, play an important role in urinary tract infections caused by these strains, type 3 fimbriae, encoding mrkD, promote biofilm development [44]. Besides it, siderophores encoding entB, intA and iron binding proteins and they also promote biofilm formation [45,46]. In this study, total fimbrial adhesins (fimH, mrkD and aap) were observed in 34 isolates (97.1%) and siderophores (entB and iron) in 33 isolates (94.2%) similar as were observed by other authors that described total fimbrial adhesins in 42 strains (84%) and siderophores (entB and iron) in 40 isolates (80%). This situation shows that these virulence factors are important for Klebsiella pathogenicity. It is interesting to note that two strains, 23 and 41, have a single virulence factor: kpn and entB, respectively.

In summary, we reported an epidemic scenario of hypermucoviscous blaoximKPC2-producing ST25 and ST17 K. pneumoniae from a single hospital in Tucuman, Argentina. This study reinforces the needed of continuous surveillance to prevent a major dissemination of ST25-CC65.

Declarations

Author contribution statement

Juan Martín Vargas: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper
Maria Paula Moreno Mochi: Performed the experiments; Analyzed and interpreted the data
Juan Manuel Nuñez, Mariel Cáceres, Silvana Mochi: Performed the experiments
Rosa del Campo Moreno: Contributed reagents, materials, analysis tools or data; Wrote the paper
Maria Angela Jure: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

[1] K.E. Holt, H. Wertheim, R.N. Zadoks, S. Baker, C.A. Whitehouse, D. Dance, A. Jenney, T.R. Connor, L.Y. Hsu, J. Severin, S. Brisse, H. Cao, J. Wilksch, C. Gorrie, M.B. Schultz, D.J. Edwards, K.V. Nguyen, T.V. Nguyen, T.T. Dao, M. Mensink, V.L. Minh, N.T. Nha, C. Schulz, K. Kuantman, P.N. Watson, C.E. Moore, R.A. Strugnell, N.R. Thomson, Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in Klebsiella pneumoniae, an urgent threat to public health, Proc. Natl. Acad. Sci. Unit. States Am. 112 (27) (2015) E5574–E5581.

[2] H.W. Boucher, G.H. Talbot, J.S. Bradley, J.E. Edwards, D. Gilbert, L.B. Rice, M. Scheld, B. Spellberg, J. Bartlett, Bad bugs, no drugs: no ESARAFE an update from the Infectious Diseases Society of America, Clin. Infect. Dis. 48 (1) (2009) 1–12.

[3] L.S. Tsouvleakis, A. Markogiannakis, M. Psichogios, P.T. Tasios, G.L. Daikon, Carbenapenemases in Klebsiella pneumoniae and other Enterobacteriaceae: an evolving crisis of global dimensions, Clin. Microbiol. Rev. 25 (4) (2012) 682–707.

[4] J.C. Catalán-Najera, U. Garza-Ramos, H. Barrios–Camacho, Hypermucoviscosity and Hypermucoviscosity: Two Different but Complementary Klebsiella spp. Phenotypes? ISSN: 2150-5594 2150-5608 (Online) Journal Homepage, 2017. http://www.tandfonline.com/loi/kvir20.

[5] R. Podschun, U. Ullmann, Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors, Clin. Microbiol. Rev. 11 (4) (1998) 589–603.

[6] A. Candan, N. Aksoz, Klebsiella pneumoniae: characteristics of carbapenem resistance and virulence factors, Acta Biochim. Pol. 62 (4) (2015) 867–874.

[7] P. Nordmann, T. Naas, L. Poirel, Global spread of carbapenemase-producing enterobacteriaceae, Emerg. Infect. Dis. 17 (10) (2011) 1791–1798.

[8] M.V. Villegas, K. Lolans, A. Correa, C.J. Suarez, J.A. Lopez, M. Vallejo, Colombian Nosocomial Resistance Study Group. First detection of the plasmid-mediated class A carbapenemase KPC-2 in clinical isolates of Klebsiella pneumoniae from South America, Antimicrob. Agents Chemother. 50 (2006) 2880–2882.

[9] J. Monteiro, A. Fernandez Santos, M.D. Asensi, G. Peirano, A.C. Gales, First report of KPC–2-producing Klebsiella pneumoniae strains in Brazil, Antimicrob. Agents Chemother. 53 (2009) 333–334.

[10] F.G. Pasteran, L. Otaegui, G. Guerriero, G. Radice, R. Magni orra, M. Rapoport, D. Faccone, A. Di Martino, M. Galas, Klebsiella pneumoniae carbapenemase-2, Buenos Aires, Argentina, Emerg. Infect. Dis. 14 (8) (2008) 1178–1180.

[11] B. Kitchel, J.K. Rashred, J. Patel, A. Srinivasan, S. Venezia, Y. Carmeli, A. Brodun, C. Giske, Molecular epidemiology of KPC-producing Klebsiella pneumoniae isolates in the United States: clonal expansion of Multilocus sequence type 258, Antimicrob. Agents Chemother. 53 (8) (2009) 3365–3370.

[12] L.N. Andrade, T. Curiao, J.C. Ferreira, M.I. Longo, E.C. Climaco, E. Carneiro, F. Martinez R Baquer, R. Cantó, T. Coque, Dissemination of blakPC-2 by the spread of Klebsiella pneumoniae clonal complex 258 clones (ST258, ST11, ST437) and plasmids (IncFII, IncN, IncL/M) among Enterobacteriaceae species in Brazil, Antimicrob. Agents Chemother. 55 (2011) 3579–3583.

[13] M. Castanheira, J.A. Contello, L.M. Deshpande, R.N. Jones, Expansion of clonal complex 258 KPC-2-producing Klebsiella pneumoniae in Latin American hospitals: report of the SENTRY antimicrobial surveillance program, Antimicrob. Agents Chemother. 56 (2012) 1668–1669.

[14] S.A. Gomez, F.G. Pasteran, D. Faccone, N. Tijet, M. Rapoport, C. Lucero, O. Lastovetska, E. Albornoz, M. Galas, KPC Group, R.G. Melano, A. Cono, A. Petroni, Clonal dissemination of Klebsiella pneumoniae ST258 harbouring KPC-2 in Argentina, Clin. Microbiol. Infect. (2011).

[15] Clinical and Laboratory Standards Institute (CLSI), Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Fourth Informational Supplement. CLSI Document M100–24, CLSI, Wayne, 2014.

[16] European Society of Clinical Microbiology and Infectious Diseases (ESCMID), Clinical Breakpoints Version 4.0, European Committee on Antimicrobial Susceptibility Testing (EUCAST), 2014. http://www.eucast.org.

[17] F. Pasteran, T. Mendez, L. Guerriero, M. Rapoport, A. Corso, Sensitive screening tests for suspected class A carbapenemase production in species of Enterobacteriaceae, J. Antimicrob. Chemother. 47 (6) (2006) 1631–1639.

[18] H.C. Lee, Y.C. Chuang, W.L. Yu, N.Y. Lee, C.M. Chang, N.Y. Ko et al., Clinical implications of hypermucoviscosity phenotype in Klebsiella pneumoniae isolates: association with invasive syndrome in patients with community-acquired bacteremia, J. Intern. Med. 259 (2006) 606–614.

[19] C. Fang, Y. Chuang, C. Shun, C. Jiang, A novel virulence gene in Klebsiella pneumoniae strains causing primary liver abscess and septic metastatic complications, J. Exp. Med. (2004).

[20] N.C. Clark, R.C. Cooksey, B.C. Hill, J.M. Swenson, F.C. Tenover, Characterization of glycopeptide-resistant enterococci from U.S. hospitals, Antimicrob. Agents Chemother. 37 (11) (1993) 2311–2317.

[21] C.I. Dallemé, A. Da Costa, D. Decré, C. Favier, G. Arlet, Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in Enterobacteriaceae, J. Antimicrob. Chemother. 65 (3) (2010 Mar) 490–495.

[22] National Center for Biotechnology Information. http://www.ncbi.nlm.nih.gov/.

[23] F.C. Tenover, R.D. Arbeit, R.V. Goering, P.A. Mickelsen, B.E. Murray, D.H. Persing, F. Pasteran, T. Mendez, L. Guerriero, M. Rapoport, A. Corso, Sensitive screening tests for suspected class A carbapenemase production in species of Enterobacteriaceae, J. Antimicrob. Chemother. 37 (11) (1993) 2311–2317.

[24] C. Giske, Molecular epidemiology of KPC-producing Klebsiella pneumoniae isolates in Argentina, Clin. Microbiol. Infect. (2011).

[25] Antimicrobial Surveillance Program SENTRY, World Health Organization, 2014. http://www.who.int.
[29] M. Kaase, S. Schimanski, R. Schiller, B. Beyreith, A. Thürmer, J. Steinmann, V.A. Kempf, C. Hess, I. Sobottka, I. Fenner, S. Ziesting, I. Burckhardt, L. von Müller, A. Hamprecht, I. Tammer, N. Wantia, R. Becker, T. Holzmann, M. Farritsch, G. Volmer, S.G. Gatermann, Multicentric investigation of carbapenemase-producing Escherichia coli and Klebsiella pneumoniae in German hospitals, Eur. J. Clin. Microbiol. Infect. Dis. (2016).

[30] E. Córdova, M.I. Lespada, N. Gómez, F. Pasterán, V. Oviedo, C. Rodríguez-Izmael, Clinical and epidemiological study of an outbreak of KPC-producing Klebsiella pneumoniae infection in Buenos Aires, Argentina, Enfermedades Infect. Microbiol. Clínica 30 (7) (2012) 376–379.

[31] L.B. Gasink, P.H. Edelstein, E. C. Lautenbach, M. Synnestvedt, N.O. Fishman, Risk factors and clinical impact of Klebsiella pneumoniae carbapenemase-producing K. pneumonia, Infect. Control Hosp. Epidemiol. (2009) 1180–1185.

[32] A. Lucena, L.M. Dalla Costa, K.S. Nogueira, A.P. Matos, A.C. Gales, M.C. Paganini, M.E. Castro, S.M. Raboni, Nosocomial infections with metallo-beta-lactamase-producing Pseudomonas aeruginosa: molecular epidemiology, risk factors, clinical features and outcomes, J. Hosp. Infect. 87 (4) (2014) 234–240.

[33] A. Hoshia, R. Karki, C. Giambi, C. Montano, A. Sisto, A. Bella, et al., Attributable mortality of carbapenem-resistant Klebsiella pneumoniae infections in a prospective matched cohort study in Italy, 2012-2013, J. Hosp. Infect. 92 (2016) 61–66.

[34] M.E. Falagas, I.A. Bliziotis, A. Michalopoulos, G. Sermaides, V.E. Papaioannou, B. Zheng, Y. Dai, Y. Liu, W. Shi, E. Dai, Y. Han, D. Zheng, Y. Yu, M. Li, Molecular epidemiology and risk factors of carbapenem-resistant Klebsiella pneumoniae infections in eastern China, Front. Microbiol. 8 (2017) 1061.

[35] M.E. Falagas, I.A. Bliziotis, A. Michalopoulos, G. Sermaides, V.E. Papaioannou, D. Nikita, N. Choulis, Effect of a policy for restriction of selected classes of antibiotics on antimicrobial drug cost and resistance, J. Chemother. 19 (2) (2007) 178–184.

[36] R. Cantón, J.M. González-Alba, J.C. Galán, CTX-M Enzymes: Origin and diffusion, Front. Microbiol. 2 (3) (2012) 110.

[37] L.S. Munoz-Price, L. Poirel, R.A. Bonomo, M.J. Schwaber, G.L. Daikos, M. Cormican, G. Cornaglia, J. Garau, M. Gniadkowski, K. Hayden, K. Karamanas, D.M. Livermore, J.J. Maya, P. Nordmann, J.B. Patel, D.L. Paterson, J. Pitout, M.V. Villegas, H. Wang, N. Woodford, J.P. Quinn, Clinical epidemiology of the global expansion of Klebsiella pneumoniae carbapenemases, Lancet Infect. Dis. 13 (9) (2013) 785–796.

[38] R. Ranjbar, H. Memariani, R. Sorouri, M. Memariani, Distribution of virulence genes and genotyping of CTX-M-15-producing Klebsiella pneumoniae isolated from patients with community-acquired urinary tract infection (CA-UTI), Microb. Pathog. 100 (2016) 244–249.

[39] L.N. Andrade, T. Curiao, J.C. Ferreira, M.J. Longo, E.C. Climaco, E. Carneiro, R. Martínez, F. Baquero, R. Cantón, T. Coque, Dissemination of mKPC-2 by the spread of Klebsiella pneumoniae clonal complex 258 clones (ST258, ST11, ST437) and plasmids (IncFII, IncN, IncL/M) among Enterobacteriaceae species in Brazil, Antimicrob. Agents Chemother. 55 (2011) 3579–3583.

[40] M. Cantanheiro, J.A. Costello, L.M. Deshpande, R.N. Jones, Expansion of clonal complex 258 KPC-2-Producing Klebsiella pneumoniae in Latin American hospitals: report of the SENTRY antimicrobial surveillance program, J. Antimicrob. Chemother. 56 (2012) 1668–1669.

[41] S. Brisse, C. Fevre, V. Passet, S. Issenhuth-Jeanjean, L. Diancourt, et al., Virulent clones of Klebsiella pneumoniae: identification and evolutionary scenario based on genomic and phenotypic characterization, PLoS One 4 (2009), e4982.

[42] C.H. Lee, J.H. Lee, K.S. Park, J.H. Jeon, Y.B. Kim, C.J. Cha, B.C. Jeong, S.H. Lee, Antimicrobial resistance of hypervirulent Klebsiella pneumoniae: epidemiology, hypervirulence-associated determinants, and resistance mechanisms, Front. Cell. Infect. Microbiol. 21 (7) (2017) 483.

[43] J. Zhao, J. Chen, M. Zhao, X. Qiu, X. Chen, W. Zhang, R. Sun, J.O. Ogutu, F. Zhang, Multilocus sequence types and virulence determinants of hypervirucosity-positive Klebsiella pneumoniae isolated from community-acquired infection cases in Harbin, north China, Jpn. J. Infect. Dis. 69 (5) (2016) 357–360, 21;Epub 2016 Jan 8.

[44] C. Struve, M. Bojer, K.A. Krogfelt, Identification of a conserved chromosomal region encoding Klebsiella pneumoniae type 1 and type 3 fimbrins and assessment of the role of fimbrins in pathogenicity, Infect. Immun. 77 (11) (2009) 5016–5024.

[45] T. May, S. Okahe, Enterobactin is required for biofilm development in reduced-genome Escherichia coli, Appl. Environ. Microbiol. 13 (2011) 3149–3162.

[46] R. El Fertas-Aissani, Y. Messai, S. Alouache, R. Bakour, Virulence proteins and antibiotic susceptibility patterns of Klebsiella pneumoniae strains isolated from different clinical specimens, Pathol. Biol. 61 (2013) 209–216.