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Short Communication

CTX-M-15 in combination with *aac(6′)-Ib*-cr is the most prevalent mechanism of resistance both in *Escherichia coli* and *Klebsiella pneumoniae*, including *K. pneumoniae* ST258, in an ICU in Uruguay☆

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ABSTRACT

The objectives of this study were (i) to determine the extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* (ESBL-EcKp) clones circulating in an intensive care unit (ICU) in Uruguay between August 2010 and July 2011, (ii) to characterise the ESBL and plasmid-mediated quinolone resistance (PMQR) genes of the studied isolates and (iii) to determine the virulotype of the clinical isolates. Clinical and gut-colonising ESBL-EcKp from ICU patients were studied. Bacterial identification and antibiotic susceptibility determination were performed using a VITEK®2 system. Detection of ESBL, KPC and PMQR genes was performed by PCR and sequencing. Clonality was assessed by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). In total, 54 ESBL-EcKp isolates (40 *K. pneumoniae* and 14 *E. coli*), with or without PMQR genes, were recovered from 30 of 68 inpatients. Forty-seven isolates were CTX-M-15-producers (36 as a single ESBL and 11 together with CTX-M-14). In addition, four isolates produced CTX-M-14, two produced CTX-M-2 and one produced SHV-5. No carbapenemases were detected either in *E. coli* or *K. pneumoniae* isolates. Among the ESBL-producing isolates, 42 also harboured PMQR genes: 27 *aac(6′)-Ib*-cr; 14 *aac(6′)-Ib*-cr and *qnrB*; and a single isolate carrying only *qnrB*. *K. pneumoniae* ST258, ST48 and ST16 and *E. coli* ST10 and ST405 were detected in 46/54 isolates, including 9 clinical isolates. In conclusion, non-KPC-producing *K. pneumoniae* ST258 harbouring different ESBL and PMQR genes was the main clone disseminated in the ICU. Extensive surveillance measures must be implemented to prevent the emergence of acquired plasmid-encoded bla_{KPC} by ST258 *K. pneumoniae*.

1. Introduction

Antimicrobial resistance constitutes a major problem in public health worldwide [1]. Hospital-acquired infections due to multidrug-resistant Gram-negative bacilli result in higher morbidity and mortality rates, longer hospital stays and higher costs [2].

The main reservoir for multidrug-resistant Gram-negative bacilli appears to be the patient's digestive tract, which usually becomes colonised with such micro-organisms prior to developing an infection [3,4].

The worldwide description of high-risk clones such as CTX-M-15-producing *Escherichia coli* ST131 and/or KPC-producing *Klebsiella pneumoniae* ST258 highlights the dissemination of antibiotic resistance as a global problem [5].

In this study, extended-spectrum β-lactamase (ESBL) and plasmid-mediated quinolone resistance (PMQR) genes in faecal and clinical isolates of *K. pneumoniae* and *E. coli* recovered from inpatients in an intensive care unit (ICU) in Uruguay were sought. The circulation of prevalent clones within the ICU was determined.

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2. Materials and methods

2.1. Study population

Clinical and gut-colonising *K. pneumoniae* and *E. coli* isolates obtained from inpatients in an adult ICU of Hospital de Clínicas (Montevideo, Uruguay) were studied. All patients admitted between August 2010 and July 2011 with a clinically predicted hospital stay >48 h were included in a successive manner. Collection of clinical and surveillance samples has been described previously [4].

2.2. Study design

All patients included in the study were followed until discharge from the ICU or death. The following parameters were recorded: sex; age; diagnosis upon admission; severity of underlying disease; severity of illness; and use of systemic antibiotics for >48 h during the previous 15 days and during ICU stay [4].

2.3. Microbiological processing

Colonisation samples were streaked on MacConkey agar plates (Oxoid Ltd., Basingstoke, UK) supplemented with 1 mg/L cefotaxime (Libra, Montevideo, Uruguay) or 0.125 mg/L ciprofloxacin (ION, Montevideo, Uruguay). To avoid repeat patient data, only the first antibiotic-resistant isolate per patient was included in this study as well as those isolates displaying differences in their resistance phenotype or genotype. However, disc diffusion assays and manual identification were performed for up to five colonies per cefotaxime- and/or ciprofloxacin-supplemented plate.

Colonisation on admission or ICU-acquired infections was defined according to Medina-Presentado et al. [4]. Patients were considered as community-derived when admission to the ICU occurred within 24 h of being hospitalised, whereas patients were regarded as hospital-derived whenever admission to the ICU occurred >24 h after being hospitalised.

2.4. Identification, antibiotic susceptibility testing and extended-spectrum β-lactamase screening

Bacterial identification, antibiotic susceptibility testing, and ESBL and carbapenemase screening were performed with a VITEK®2 system (bioMérieux, Marcy-l'Étoile, France). Susceptibility results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (http://www.eucast.org). All non-duplicate clinical isolates for each patient as well as different colonising isolates were analysed.

2.5. Detection of extended-spectrum β-lactamase, carbapenemases and plasmid-mediated quinolone resistance genes

Positive ESBL screening results were analysed by PCR for the presence of bla_{CTX-M}, bla_{SHV}, bla_{TEM} and bla_{PER-2} and further sequencing [6–9]. Detection of bla_{KPC-2} and bla_{OXA-48} was performed according to with Seija et al. [10]. In addition, resistance mechanisms to fluoroquinolones [*qnrABCDS* and *aac(6′)-Ib*-cr genes] were also sought [8].

2.6. Conjugation assays

Conjugation assays were carried out using *E. coli* J53-2 (rifampicin-resistant) as recipient. Transconjugants were selected on MacConkey agar plates supplemented with 150 mg/L rifampicin (Sigma–Aldrich, Shanghai, China) and 1 mg/L ceftriaxone (Libra) [9].

2.7. Pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST)

Clonality was assessed by PFGE following digestion with the restriction enzyme *Xba*I (Thermo Scientific, Waltham, MA). Band patterns were analysed with BioNumerics v.6.6 (Applied Maths, Sint-Martens-Latem, Belgium) with 2% tolerance and 0% optimisation. Strains were considered as related with ≥85% similarity [8]. Finally, MLST was performed for one clinical isolate per pulsotype following the guidelines in the MLST database (http://bigsdb.web.pasteur.fr/klebsiella/klebsiella.html; http://mlst.warwick.ac.uk/mlst/dbs/Ecoli/).

2.8. Statistics

Numeric variables are expressed with their standard deviation. A χ^2 or Fisher's exact test was performed as appropriate. A *P*-value of <0.05 was considered statistically significant. Relative risks (RRs) and their 95% confidence interval (CI) were calculated using standard methods.

3. Results

3.1. Patients

In total, 68 patients were enrolled in the study (40 male, 28 female); 35 were community-derived and 33 were hospital-derived. The median patient age was 52.5 ± 18.8 years (range 17–85 years). The median Acute Physiology and Chronic Health Evaluation (APACHE) II score at admission was 23 and the mean length of ICU stay was 14.3 days.

Of the 68 enrolled patients, 30 (44.1%) were colonised by extended-spectrum β-lactamase-producing *E. coli* or *K. pneumoniae* (ESBL-EcKp), 24 of whom were still colonised when they were discharged from the study. Nine patients had an infection due to ESBL-EcKp (seven due to *K. pneumoniae* and two due to *E. coli*) (Figs. 1 and 2). Infections with ESBL-EcKp were linked to prior colonisation with such micro-organisms (*P* = 0.01; RR = 11.7, 95% CI 1.4–99.4).

Of the 30 colonised patients, 13 (43.3%) were already colonised at ICU admission. Colonisation by ESBL-EcKp upon admission to the ICU was linked to antibiotic consumption up to 1 month prior to hospitalisation (*P* = 0.002; RR = 9.4, 95% CI 1.9–46.4) and being derived from other hospital facilities (*P* = 0.002; RR = 7.7, 95% CI 2.1–28.1).

3.2. Clonal distribution of isolates and sequence types

Six different *K. pneumoniae* pulsotypes were detected, designated K1–K6. Pulsotypes K4 and K5 belonged to ST258 (detected in 18 patients), pulsotypes K1 and K2 belonged to ST48 (6 patients) and pulsotypes K6 belonged to ST16 (4 patients). Seven clinical *K. pneumoniae* isolates were detected, with three belonging to ST258, three to ST48 and one to ST16 (Fig. 1). The sequence type of K3 was not determined as no clinical isolate was obtained.

On the other hand, *E. coli* isolates were grouped by PFGE into five pulsotypes, designated EI–V. The two clinical isolates studied belonged to pulsotypes I and IV and corresponded to ST10 and ST405, respectively (Fig. 3).

Fig. 2 shows the different patterns of colonisation/infection of the studied *E. coli* and *K. pneumoniae* clones.

Except for *E. coli* clone EIV-ST405, the remaining clones arrived in the ICU with patients already colonised upon admission, with the horizontal dissemination of such clones being a posterior event (Fig. 2).