

# Detection of *Escherichia coli* ST131 clonal complex (ST705) and *Klebsiella pneumoniae* ST15 among faecal carriage of extended-spectrum $\beta$ -lactamase- and carbapenemase-producing *Enterobacteriaceae*

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## Abstract

**Purpose.** The objective of the present study was to evaluate the prevalence of intestinal colonization with extended-spectrum  $\beta$ -lactamase (ESBL)-producing *Enterobacteriaceae* (ESBL-E) and carbapenemase-producing *Enterobacteriaceae* (CPE) in non-selected hospitalized and non-hospitalized patients from the same geographic area of Madrid.

**Methodology.** A total of 501 fecal samples were screened. Diluted samples in saline were cultured in MacConkey agar plates with ceftazidime, cefotaxime, imipenem and meropenem disks. Colonies growing within the inhibition zone of either disk were selected. Characterization of ESBLs and CPEs were performed by PCR and sequencing. The Wider system was used for the bacterial identification. In addition, clonal analysis was carried out for species predominant among the faecal carriage.

**Key Findings.** Among the 501 patients enrolled, 43 (8.6 %) carried ESBL-E and 8 (1.6 %) patients exhibited CPE. The main intestinal colonizer among ESBL-E was CTX-M-producing *Escherichia coli* isolates in both settings (community and hospital). ST131 clonal complex was the most common among faecal ESBL-producing *E. coli*. All gut carriers of CPE were hospitalized patients, *Klebsiella pneumoniae* being the most prevalent species. Two OXA-48-producing *K. pneumoniae* isolates belonging to ST15 were detected.

**Conclusion.** Present study reveals that faecal carriage of ESBL is common among inpatients and outpatients, whereas carbapenemase producers are only present in the hospital setting. Therefore, active surveillance will be useful for reducing transmission of antimicrobial-resistant bacteria and preventing infection.

## INTRODUCTION

Multidrug-resistant Gram-negative bacteria are a major public health threat. Over the last few years, the production of extended-spectrum  $\beta$ -lactamases (ESBLs) has increased significantly for *Enterobacteriaceae* (such as *Klebsiella pneumoniae* and *Escherichia coli*) (<http://ecdc.europa.eu/en/publications/publications/antimicrobial-resistance-europe-2014.pdf>).

Moreover, the increasing use of carbapenems for the treatment of infections involving multidrug-resistant bacteria has led to a rapid international dissemination of carbapenemase-producing *Enterobacteriaceae* (CPE) (<http://ecdc.europa.eu/en/eaad/Documents/antibiotics-resistance-EU-data-2014.pdf>). Although resistance to carbapenems remains low in Europe, it is increasing in a few countries, especially in southern and southeastern Europe [1–3].

*Enterobacteriaceae* are inhabitants of human intestinal flora [4], and faecal carriers may represent an important reservoir for person-to-person transmission and dissemination of bacteria [4–6]. Furthermore, gut colonization by multidrug-resistant bacteria has been associated with a high risk of developing subsequent clinical infection [7, 8]. Therefore, active surveillance is a key part in preventing the spread of such strains. Early detection of rectal carriers is important for the purpose of controlling infections [7, 9].

As gastrointestinal carriage may serve as a reservoir for CPE and/or ESBL-producing *Enterobacteriaceae* (ESBL-E) transmission, the objective of the present study was to evaluate the prevalence of intestinal colonization, coinciding with infection episodes in our hospital and in other Spanish

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**Keywords:** faecal carriage; carbapenemases; ESBL; *Enterobacteriaceae*; ST705; OXA-48.

**Abbreviations:** CPE, carbapenemase-producing *Enterobacteriaceae*; ESBL, extended-spectrum  $\beta$ -lactamase; ESBL-E, ESBL; MLST, multilocus sequence typing; ST, sequence type.

hospitals [1–3]. During May and June 2014, all faecal samples submitted to our hospital laboratory were examined for ESBL-E and CPE.

## METHODS

### Sample collection

This study was conducted in the Department of Microbiology at Hospital Clínico San Carlos (Madrid, Spain). During May and June 2014, all faecal samples from patients with suspected intestinal infection submitted to our laboratory were collected. A total of 501 samples were analysed 181 were recovered from hospitalized patients and 320 from outpatients. Demographic information was abstracted from the patients' medical records.

Nosocomial-acquired isolates were defined as those acquired at least 48 h after hospital admission. Community-acquired strains were those isolated in the community or within 48 h of hospital admission [10]. The samples were then screened for the presence of ESBLs and carbapenemases.

### Rectal screening protocols

Briefly, each swab containing a sample was placed in 1 ml of sterile 0.9 % saline and then vortexed. Each suspension was cultured on a MacConkey agar plate with four antibiotic discs: cefotaxime (CTX, 30 µg), ceftazidime (CAZ, 30 µg), imipenem (IPM, 10 µg) and meropenem (MEM, 10 µg) (Becton Dickinson). Bacterial growth was evaluated after an overnight (18–24 h) incubation at 37 °C in ambient air. Colonies growing within the inhibition zone of either disc were selected for further identification and phenotypic ESBL and carbapenemase testing.

### Identification and antimicrobial susceptibility testing

Bacterial identification and susceptibility testing confirmation were performed by using a semiautomatic commercial system, Wider System (Francisco Soria Melguizo). Minimum inhibitory concentration breakpoints were defined according to Clinical and Laboratory Standards Institute (CLSI) criteria [11].

### Phenotypic ESBL and carbapenemase detection

Phenotypic detection of ESBLs was carried out using the agar dilution test with cefotaxime, cefotaxime–clavulanic, ceftazidime and ceftazidime–clavulanic as recommended by the CLSI [11].

Carbapenemase production was phenotypically assessed by the modified Hodge test. The presence of metallo-carbapenemases (class B) was screened by the combined disc test with imipenem and EDTA (10 µl, 100 mM), and the combined disc test with meropenem and phenylboronic acid (10 µl, 40 µg µl<sup>-1</sup>) was used to detect the production of class A carbapenemases [12, 13]. OXA-48 production was suspected when these three tests were negatives. The presence of OXA-48 enzyme was determined by PCR.

## Molecular characterization of ESBLs and carbapenemases

PCR was used for detecting the presence of genes encoding relevant β-lactamases. ESBL-E isolates were investigated for screening of *bla*<sub>CTX-M</sub> (groups 1, 2, 8, 9, 25 and 26), *bla*<sub>SHV</sub> and *bla*<sub>TEM</sub> genes as previously described [14–16]. Carbapenem resistance genes (*bla*<sub>KPC</sub>, *bla*<sub>VIM</sub>, *bla*<sub>IMP</sub> and *bla*<sub>OXA-48</sub>) were also identified with specific primers [17]. Sequencing of amplicons (all *bla*<sub>CTX-M</sub> genes of isolates) was carried out by using an ABI PRISM BigDye Primer v3.0 Ready Reaction Cycle Sequencing kit (Applied Biosystems).

### Molecular epidemiology

All ESBL-producing *E. coli* isolates were typed by pulsed-field gel electrophoresis (PFGE) using the PulseNet Europe protocol ([www.pulsenetinternational.org](http://www.pulsenetinternational.org)). PFGE profiles were analysed with FPQuest software version 4.5 (Bio-Rad Laboratories). Cluster analysis of Dice similarity indices based on an unweighted pair group method with arithmetic mean (UPGMA) was used to generate a dendrogram describing the relationships among PFGE profiles. Isolates were considered to belong to the same PFGE group if their Dice similarity index was ≥80 %.

Multilocus sequence typing (MLST) was performed, and the corresponding sequence types (STs) were assigned for *E. coli* isolates (<http://mlst.warwick.ac.uk/mlst/dbs/Ecoli>) and *K. pneumoniae* isolates (<http://bigsd.b.pasteur.fr/klebsiella/klebsiella.html>).

## RESULTS

The overall carrier rate in the study population was 10.2 %. Among the 501 patients enrolled, 43 (8.6 %) carried ESBL-E and 8 (1.6 %) patients exhibited CPE. None of the patients harboured the two types of enzyme. The mean age of the carriage of ESBL-E and CPE was 59.1 years (range, 0–96 years) and 78.5 years (range, 69–93 years), respectively. Among the 501 patients, 22 (4.4 %) male and 21 (4.2 %) female carried ESBL-E, while 4 (0.8 %) male and 4 (0.8 %) female carried CPE.

All gut carriers of CPE were hospitalized patients, while the ESBL-E carriage rate among outpatients was lower (6.25 %) than among inpatients (12.7 %).

All ESBL-E isolates were detected by the initial screening (MacConkey agar plate with four antibiotic discs). Conversely, three of the eight CPE isolates were not identified by the initial screening. These isolates had grown within the inhibition zone of cefotaxime and ceftazidime discs but did not produce an ESBL. Antimicrobial susceptibility was further confirmed by using the Wider system, and these isolates were found to be susceptible to imipenem and meropenem, but ertapenem resistant. PCR for detection of the *bla*<sub>OXA-48</sub> gene was positive for these isolates.

The bacterial identification of the intestinal colonizers is shown in Tables 1 and 2, according to the enzyme detected (ESBL, Table 1; carbapenemases, Table 2). Five different

**Table 1.** Identification of bacterial species and distribution of enzymes among ESBL-E carriers

Species	No. of isolates (%)	SHV	TEM	CTX-M type	CTX-M-1 group		CTX-M-9 group
					CTX-M-1	CTX-M-15	CTX-M-14
<i>E. coli</i>	29 (67.4)	3	1	25	4	10	11
<i>K. pneumoniae</i>	11 (25.6)	0	3	8	0	7	1
<i>Kluyvera cryocrescens</i>	1 (2.3)	0	1	0	0	0	0
<i>Citrobacter amalonaticus</i>	1 (2.3)	0	1	0	0	0	0
<i>Enterobacter aerogenes</i>	1 (2.3)	0	0	1	0	1	0
Total	43 (100)	3	6	34	4	18	12

species were found among the 43 faecal carriage of ESBL, *E. coli* being the most common species (67.4 %) (Table 1). Nearly 80 % (34/43) of the patients who exhibited an ESBL enzyme had CTX-M-type genes, the CTX-M-1 group being the dominant genotype (65 %). Overall, *bla*<sub>CTX-M-15</sub> (*n*=18) was the most frequent ESBL gene detected, followed by *bla*<sub>CTX-M-14</sub> (*n*=12). SHV and TEM enzymes were also identified among ESBL-E.

CPE colonization was detected in eight patients, *K. pneumoniae* being the most common species identified (Table 2). Three carbapenemase genes were detected among CPE: *bla*<sub>VIM-1</sub>, *bla*<sub>KPC-2</sub> and *bla*<sub>OXA-48</sub>.

Because *E. coli* and *K. pneumoniae* species predominated among the faecal carriage of ESBL and CP, respectively, we studied their genetic backgrounds. Of the 29 faecal ESBL-producing *E. coli* isolates, two strains were untypeable by PFGE. These two strains were identified by MLST as belonging to ST705 and ST117, respectively. PFGE analysis of the remaining 27 ESBL-producing *E. coli* isolates is shown in Fig. 1. PFGE revealed six different *E. coli* pulsotypes (P1 to P6). The six PFGE profiles displayed six different STs: ST705 (*n*=12), ST155 (*n*=6), ST371 (*n*=4), ST57 (*n*=3), ST167 (*n*=1) and ST398 (*n*=1) (Fig. 1). Overall, ST131 was the main clonal complex, including 13 isolates (44.8 %); all of them belonged to ST705 and were mainly associated with CTX-M-producing *E. coli*. Seven isolates (24.1 %) were assigned to the ST350 complex, with MLST types ST371 (*n*=4) and ST57 (*n*=3).

MLST analysis of the four CPE isolates displayed three STs: ST15 (*n*=2), ST11 (*n*=1) and ST265 (*n*=1). The two OXA-48-producing strains belonged to ST15, whereas the other two clones had KPC enzyme.

**Table 2.** Bacterial species and carbapenemases identified among CPE carriers

Species	No. of isolates (%)	Enzyme type		
		VIM	KPC	OXA-48
<i>K. pneumoniae</i>	4 (50)	0	2	2
<i>Klebsiella oxytoca</i>	2 (25)	1	0	1
<i>Citrobacter freundii</i>	2 (25)	2	0	0
Total	8 (100)	3	2	3

## DISCUSSION

Screening for intestinal carriage is important for predicting the risk of infection [7, 9], as the gut may serve as a reservoir for CPE or ESBL-E. Tan and Tan [18] demonstrated high rates of infections, such as urinary tract infection, due to ESBL-E in patients who are also intestinal carriers of these pathogens.

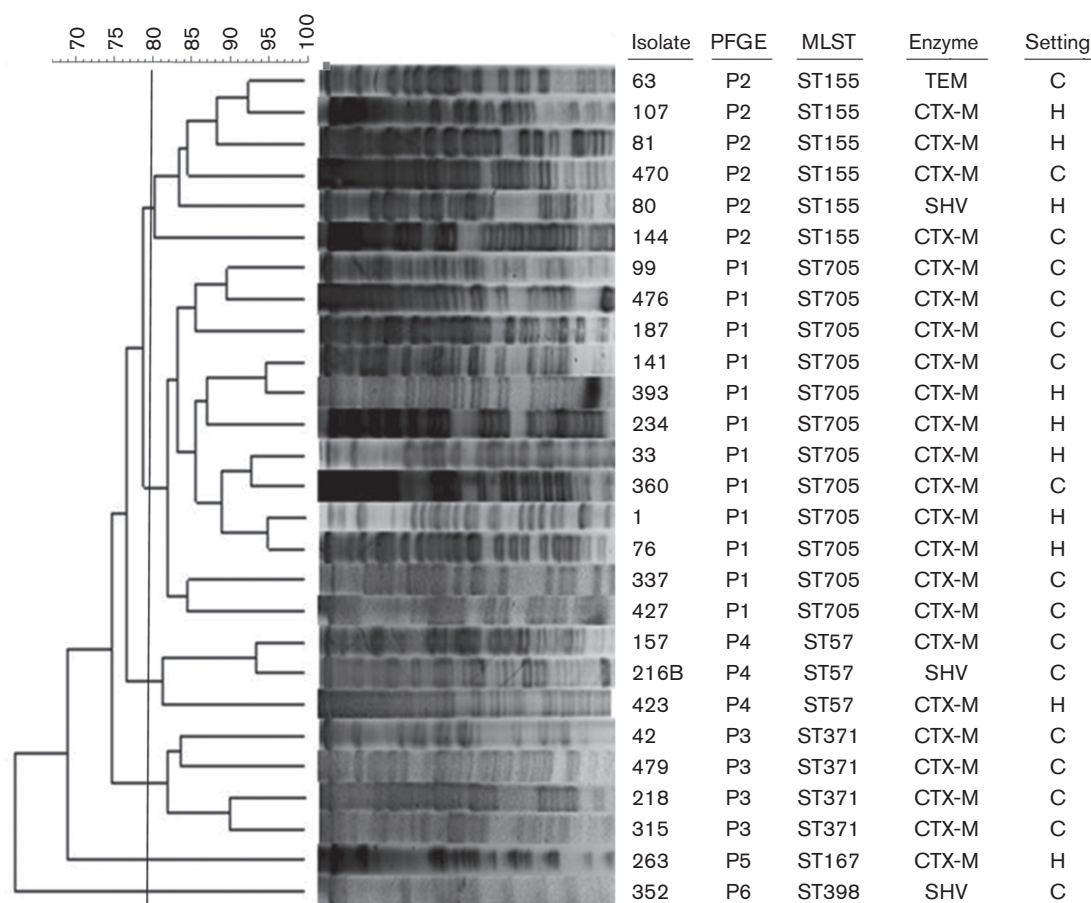
The present study showed a 10.2 % prevalence of intestinal colonization, with *Enterobacteriaceae* harbouring ESBLs or carbapenemases in non-selected hospitalized and nonhospitalized patients from the same geographic area of Madrid.

Overall, ESBL-E isolates were detected in 8.6 % of the patients. Most of the faecal carriage of ESBL-E had more than one underlying condition and multiple courses of antibiotics, such as third-generation cephalosporins, fluoroquinolones and aminoglycosides. Previous studies have also reported faecal carriage rates ranging from 4.6 to 31 % [4, 19–22]. Although the prevalence of faecal carriage of ESBL-E among outpatients (6.25 %) was lower than that among hospitalized patients (12.7 %), several authors have indicated that it is important to look at community patients because they can represent a clear reservoir for ESBL producers, increasing the dispersal of resistance in healthy people [8].

ESBL enzymes are frequently encoded by genes located on different transferable genetic elements, which could make their spread through various enterobacteria possible [23]. Therefore, we have found a diversity of faecal ESBL-E isolates, although *E. coli* was the most dominant species (67.4 %), as previously described [24–27].

The most prevalent ESBL type was CTX-M, comprising 6.8 % of all patients included in our study and nearly 80 % of the patients colonized with ESBL-E. Several surveys about molecular characterization of ESBL producers from the gut have shown CTX-M as the most common type [4, 6, 19, 21, 28, 29]. Moreover, CTX-M-15 type was, as expected over the last years [8, 20, 21, 23, 25, 30, 31], the most frequent ESBL (53 %).

Although the spread of ESBLs is frequently due to the transmission of mobile genetic elements, clonal dissemination of strains has also been described [14]. Interestingly, our clonal



**Fig. 1.** Distribution of genotypes among faecal ESBL-producing *E. coli*. C, community setting; H, hospital setting.

analysis demonstrated that *E. coli* ST705 (clonal complex ST131) was the most represented clone. Our results are in accordance with other authors who have observed the rapid emergence of the ST131 clone among patients colonized with faecal *E. coli* isolates [21, 22, 25, 32]. The uropathogenic ST131 clone is also known to carry CTX-M-15 frequently and has been successful in spreading across the world in a relatively short period of time [2, 14]. In the present study, all of the ST131 *E. coli* strains are linked not only to the CTX-M-15 but also to other ESBL types, as also previously described in Spain [2].

A low percentage of intestinal colonization by CPE was found among the patients enrolled (1.6 %). This rate is similar to other works [26, 33, 34]. CPE colonization was not detected among outpatients.

The carbapenemase genes are carried by plasmids, and therefore, their spread is probably due to both the clonal dissemination of a few specific strains and the transmission of plasmids carrying them [1]. Half of our patients carrying carbapenemases were colonized by *K. pneumoniae*, a frequent species identified among faecal CPE [33, 34]. In a multicentre study performed in Spain, a spread of CPE was

found to be mainly due to successful clones of *K. pneumoniae* of ST11 and ST15 [1]. Surprisingly, we identified three STs for faecal *K. pneumoniae* isolates (ST15, ST11 and ST265). The two strains belonging to ST15 produced OXA-48, whereas the other two clones produced KPC enzyme.

The emergence of CPE could be explained by the fact that all of the colonized patients had varying degrees of exposure to broad-spectrum antibiotics such as third-generation cephalosporins, fluoroquinolones, aminoglycosides and carbapenems. Moreover, most of them were elderly patients with comorbidity conditions.

Gut colonization by multidrug-resistant bacteria has been associated with a high risk for developing subsequent clinical infection [7, 8]. Identification of patients colonized by CPE can be used to control modifiable risk factors and to direct empirical antimicrobial therapy when necessary [7]. In this study, we have addressed progression to infection among the eight patients with CPE rectal carriage. Interestingly, three of these patients were also infected with a metallo- $\beta$ -lactamase-producing isolate, whereas the other five displayed only intestinal colonization. Therefore, active

surveillance will be useful for reducing transmission of anti-microbial-resistant bacteria and preventing infection.

In conclusion, our results support the role of the intestinal tract as a reservoir for ESBL- and carbapenemase-producing isolates, which may increase the risk of dissemination and emphasizes the importance of surveillance studies to improve the understanding of their global epidemiology. The present study reveals a faecal carriage rate with ESBL-E of 8.6 % in non-selected hospitalized and non-hospitalized patients from the same geographic area of Madrid. The main intestinal colonizer was CTX-M-producing *E. coli* isolates in both settings (community and hospital). The ST705 (clonal complex 131) clone was the most common among faecal ESBL-producing *E. coli*. In addition, our findings show a prevalence of CPE carriage of 1.6 %. Two OXA-48-producing *K. pneumoniae* strains belonging to ST15 were identified. Therefore, active surveillance will be useful for controlling and preventing infection.

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#### Conflicts of interest

The authors declare that there are no conflicts of interest.

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