# Intestinal carriage of extended-spectrum beta-lactamase–producing Enterobacteriaceae at admission in a Portuguese hospital

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## Abstract

To evaluate the prevalence of extended-spectrum β-lactamase (ESBL)–producing Enterobacteriaceae fecal carriers at admission in a Portuguese hospital and to determine the epidemiology and antimicrobial resistance patterns of ESBL-producing isolates. During a 2-month period, rectal swabs were collected at hospital admission from 151 at-risk patients. In addition, 48 rectal swabs were obtained from weekly screenings of 37 patients hospitalized for > 48 h. All ESBL/carbapenemase-producing isolates were tested for antimicrobial susceptibility and characterized by PFGE and MLST. The prevalence of ESBL producers at hospital admission was 17% and 24% among at-risk patients hospitalized for > 48 h, while the prevalence of carbapenemase producers was 3% in both cases. Most of the isolates were *Escherichia coli* (54%) and *Klebsiella pneumoniae* (41%). The most common ESBL identified was CTX-M-15 ($n = 17/34$; 50%), followed by CTX-M-27 ($n = 10$; 29%), CTX-M-33 ($n = 4$; 12%),  SHV-12  ($n = 2$), and CTX-M-55 ($n = 1$). The 20 *E. coli* isolates were distributed into 16 PFGE types and nine sequence types (ST), with 60% of the isolates belonging to ST131. The 15 *K. pneumoniae* were grouped into 12 PFGE types and nine STs, with three STs (ST17, ST449, ST147) corresponding to 60% of the isolates. A high proportion of isolates showed resistance to ciprofloxacin (86%), trimethoprim-sulfamethoxazole (68%), tobramycin (57%), and gentamicin (43%). All isolates remained susceptible to fosfomycin. A high prevalence of ESBL-producing Enterobacteriaceae was found at hospital admission among at-risk patients and > 50% of the isolates showed resistance to first-line antibiotics for the treatment of lower urinary tract infections, leaving fosfomycin as an alternative.

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## Introduction

The emergence of extended-spectrum β-lactamase (ESBL)–producing Gram-negative bacteria and their subsequent spread is considered a global threat. Until the 1990s, ESBL were mainly found among *Klebsiella* spp. and *Enterobacter* spp., almost exclusively in hospitals, particularly in intensive care units (ICU), and were encoded by derivatives of the $bla_{TEM}$ and $bla_{SHV}$ penicillinase genes [1]. However, in the late 1990s, the scenario basically changed with ESBL producers emerging in the community, mainly among *Escherichia coli* causing urinary tract infections (UTI). Meanwhile, there has been a replacement of TEM and SHV-type ESBLs by CTX-M-type enzymes [2, 3].

The prevalence of invasive *E. coli* isolates showing resistance to broad-spectrum cephalosporins is still increasing in Europe, reaching 14.9% in 2017 [4]. Furthermore, a recent meta-analysis showed that overall, 14% of healthy individuals are colonized with ESBL-producing

Enterobacteriaceae and 6% of these cases are in southern Europe [5].

In Portugal, the prevalence of *E. coli* invasive isolates with resistance to broad-spectrum cephalosporins was estimated at 15.6% in 2017 [4]. Several studies reported the occurrence of ESBL producers in Portugal, with a predominance of CTX-M-15 not only among *K. pneumoniae* nosocomial isolates [6] but also in *E. coli*, both from the community and hospital settings [7]. However, there was no single prospective study evaluating ESBL-producing Enterobacteriaceae fecal carriers at hospital admission in the country, although such a study would be essential to provide a better guidance in the empiric antibiotic stewardship and infection control measures.

The aims of the present study were to prospectively evaluate the prevalence of gut carriage by ESBL-producing Enterobacteriaceae at admission in a Portuguese hospital and to determine the epidemiology and antimicrobial resistance of ESBL-producing isolates.

## Materials and methods

### Study design

This prospective study was conducted in a private 123-bed hospital in Lisbon, Portugal, during a 2-month period (from December 1 2018 to February 2 2019). During this period, all consecutive admitted patients presenting at least one risk factor for colonization/infection by ESBL producers were screened. These included patients transferred from another hospital or from a nursing home, patients with history of hospitalization or stay in a nursing home during the last 12 months, patients with previous infection with ESBL-producing Enterobacteriaceae, patients with chronic renal failure under dialysis, oncologic patients with active disease, patients with AIDS, and patients tracheostomized during the previous year, all being thereafter defined as "at-risk" patients. All such patients were rectally screened within the first 48 h of admission. In addition, patients at the ICU (eight beds) and Medicine Intermediate Care Unit (five beds) were screened once a week during the hospital stay. All samples were obtained from routine screenings already implemented in the hospital as infection control measures; therefore, no specific ethical concern required approval.

At hospital admission, information was obtained concerning the origin of the patient (home, nursing home, or another hospital) and previous hospitalization during the previous 12 months.

### Bacterial isolates

The swabs were incubated overnight at 37 °C in Tryptic Soy Broth (Becton, Dickinson & Co, NJ, USA) for enrichment.

The next day, a volume of 25 μl of each broth was inoculated onto two selective media: (i) CHROMagar ESBL (Frilabo, Maia, Portugal) to select for ESBL producers and (ii) ChromID Carba Smart selective medium (bioMérieux, La Balme-les-Grottes, France) to select for carbapenem-resistant isolates. The isolates were identified at the species level using the API20E system (bioMérieux).

### Susceptibility testing

Antimicrobial susceptibility testing was performed using the disc diffusion method on Mueller-Hinton (MH) agar plates (Becton, Dickinson & Co, NJ, USA) for amoxicillin, amoxicillin/clavulanic acid, temocillin, ceftazidime, cefotaxime, cefepime, cefoxitin, aztreonam, ertapenem, imipenem, amikacin, gentamicin, tobramycin, fosfomycin, trimethoprim-sulfamethoxazole (SXT), ciprofloxacin, and tigecycline, following EUCAST recommendations.

Identification of carbapenemase producers was assessed by using the Rapidec Carba NP test (bioMérieux) [8], while that of ESBL producers relied on results of the ESBL NDP test [9].

### Molecular analysis

Identification of ESBL [10] and carbapenemase genes [11] was performed by PCR, as described previously, followed by sequencing of the amplicons. Standard PCR conditions were used to amplify the *qnrA*, *qnrB*, and *qnrS* quinolone resistance genes [12].

The clonal relationship of the isolates was evaluated for the entire collection by pulsed-field gel electrophoresis (PFGE) as described previously [13]. Multilocus sequence typing (MLST) was performed for a representative strain of each PFGE type, and sequence types (STs) were assigned using the MLST databases for *K. pneumoniae* and *E. coli* (https://cge.cbs.dtu.dk/services/MLST-2.0/).

### Conjugation experiments and plasmid analysis

Mating-out assays were performed using the azide-resistant *E. coli* J53 as the recipient. *E. coli* J53 and $bla_{KPC-3}$ or $bla_{OXA-181}$-positive donors were separately inoculated overnight into Luria Bertani (LB) broth (5 mL) and incubated. The samples were subsequently mixed at a ratio of 10:1 (donor/recipient) for 5 h and 100 μl of this mix was deposited onto 22-μm filters and incubated overnight at 37 °C onto LB agar plates. After the incubation, filters were resuspended in NaCl 0.85% and 100 μl of this mixture was plated onto LB agar plates supplemented with ticarcillin (100 μg/ml) and azide (100 μg/ml). Susceptibility testing was performed for all *E. coli* transconjugants, and positivity for $bla_{KPC-3}$ or $bla_{OXA-181}$ was assessed by PCR.

Plasmids were classified according to their incompatibility group using the PCR-based replicon typing (PBRT) method as described previously [14].

## Results

During the study period, 151 patients presenting with at least one risk factor for ESBL colonization/infection were admitted to the hospital and rectal swabs from all patients were obtained within the first 48 h. In addition, a total of 48 rectal swabs were obtained from weekly screenings of 37 patients hospitalized for more than 48 h (25 in the ICU and 12 in the Medicine Intermediate Care Unit). The rectal swabs were mainly obtained from men (131/199; 66%) and the mean age of the patients was 74.2 years.

From a total of 199 swabs obtained, 37 ESBL-producing and/or carbapenem-resistant isolates were recovered, out of which 20 (54%) were *E. coli*, 15 (41%) were *K. pneumoniae*, and two were *Enterobacter cloacae* (Table 1).

A total of 34 isolates produced an ESBL, five isolates produced a single carbapenemase, and a single isolate co-produced two carbapenemases. The prevalence of ESBL producers at hospital admission among at-risk patients was 17% (25 isolates out of 151 admissions) and 24% among patients hospitalized for > 48 h (9 isolates out of 37 patients), while the prevalence of carbapenemase producers was 3% in both cases (5/151 and 1/37, respectively). The most common ESBL identified was CTX-M-15 ($n = 17/34$; 50%), followed by CTX-M-27 ($n = 10$; 29%), CTX-M-33 ($n = 4$; 12%), SHV-12 ($n = 2$), and CTX-M-55 ($n = 1$). Of note, CTX-M-27 and SHV-12 were exclusively found among *E. coli*.

Five *K. pneumoniae* isolates produced a carbapenemase, namely KPC-3 ($n = 2$) and OXA-181 ($n = 3$). In addition, a single isolate co-produced both the KPC-3 and GES-5 carbapenemases. Three isolates were co-producers of an ESBL (CTX-M-15) and a carbapenemase (KPC-3 or OXA-181). Of note, the six patients colonized by a carbapenemase producer had been hospitalized during the previous year, which is known to constitute a significant risk factor.

Three patients were colonized simultaneously by different isolates (Table 1); two patients carried an ESBL-producing *E. coli* (CTX-M-27) in addition to an ESBL-producing *K. pneumoniae* (CTX-M-15), and a single patient carried an ESBL-producing *E. coli* (SHV-12), an ESBL-producing *K. pneumoniae* (CTX-M-15), and a carbapenemase-producing *K. pneumoniae* (OXA-181). Two of these patients had been transferred from another hospital and the other one was living in a nursing home and had been hospitalized in this hospital during the previous year.

Two out of three patients colonized by an ESBL, or carbapenemase-producing isolate at admission who were

screened one week later during their hospital stay, maintained the carriage state.

PBRT performed on respective *E. coli* transconjugants producing a carbapenemase revealed that the $bla_{OXA-181}$ gene was always located on an IncX3 plasmid, the $bla_{KPC-3}$ gene either on IncN and IncFII plasmid types, and the $bla_{GES-5}$ gene on a ColE1 plasmid.

Antimicrobial susceptibility testing showed that all isolates were susceptible to fosfomycin. Considering the different antibiotics tested, there was no significant difference between isolates recovered at admission or > 48 h of admission (Fig. 1). Most of the ESBL-producing isolates showed resistance to ciprofloxacin (86%), SXT (68%), tobramycin (57%), and gentamicin (43%).

The plasmid-mediated quinolone resistance gene *qnrS* was identified in eight isolates, all being resistant to ciprofloxacin (including the three isolates producing OXA-181 and a single isolate producing KPC-3), while *qnrA* and *qnrB* genes were absent in our collection.

PFGE analysis showed a high clonal diversity, distributing the 20 *E. coli*, the 15 *K. pneumoniae*, and the two *E. cloacae* isolates into 16, 12, and two PFGE types, respectively (Table 1). MLST analysis showed that the 16 *E. coli* PFGE types corresponded to nine STs. Interestingly, 60% (12/20) of the *E. coli* isolates could be grouped in a single ST (ST131). The 12 *K. pneumoniae* PFGE types belonged to nine STs (Table 1), out of which three (ST17, ST449, and ST147) included 60% of the isolates.

## Discussion

To our knowledge, this is the first prospective study evaluating the prevalence of intestinal carriers of ESBL-producing Enterobacteriaceae at hospital admission in Portugal among at-risk patients. We report a high rate of ESBL fecal carriage at admission (17%), highlighting the high incidence of gut colonization with ESBL producers in the community among individuals presenting risk factors for colonization/infection by multidrug-resistant bacteria. Our results highlight that the prevalence of ESBL producers in the community among at-risk patients exposed to healthcare facilities is likely high in the country in contrast to results obtained from a fecal screening conducted in 2013–2014 that revealed a very low (2%) occurrence of ESBL producers among randomly selected healthy adults from different regions in Portugal [15]. Furthermore, the rate found in the present study (17%) is considerably higher than the ones reported in other European countries. For instance, a recent study showed that 9% (360/4006) of the patients admitted in a hospital in London, UK, were gut carriers of ESBL-producing Enterobacteriaceae [16]. In Madrid, Spain, the prevalence of carriers among 10,643 patients admitted to a university

**Table 1** Characteristics of the 37 ESBL- and carbapenemase-producing Enterobacteriaceae isolates

| Species | Isolate | Patient | PFGE type | ST | ESBL | Carbapenemase | Plasmid type[a] | qnrS | AMX | AMC | TEM | CZD | CTX |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E. coli (n = 20) | 156E | 156 | A | 131 | CTX-M-15 | | | – | R | R | S | R | R |
| | 8E | 8 | B | 131 | CTX-M-15 | | | – | R | R | S | I | R |
| | 26E | 26 | C | 131 | CTX-M-15 | | | – | R | I | S | I | R |
| | 97E | 97 | D | 44 | CTX-M-15 | | | – | R | R | S | R | R |
| | 146E | 146 | E | 131 | CTX-M-15 | | | + | R | S | S | I | R |
| | 184E | 184 | F | New1 (CC131) | CTX-M-15 | | | – | R | S | S | S | R |
| | 144E | 144 | G | 131 | CTX-M-27 | | | – | R | S | S | I | R |
| | 160E | 160 | G | 131 | CTX-M-27 | | | – | R | S | S | R | R |
| | 124E | 124 | H1 | 131 | CTX-M-27 | | | – | R | S | S | I | R |
| | 130E | 130 | H2 | 131 | CTX-M-27 | | | – | R | S | S | S | R |
| | 135E | 135 | H3 | 131 | CTX-M-27 | | | – | R | S | S | S | R |
| | 28E | 28 | I1 | 131 | CTX-M-27 | | | – | R | S | S | I | R |
| | 51E | 51 | I2 | 131 | CTX-M-27 | | | – | R | I | S | I | I |
| | 75E | 75 | J | New2 (CC10) | CTX-M-27 | | | + | R | R | S | S | R |
| | 95E | 95 | K | 2179 | CTX-M-27 | | | – | R | S | S | S | R |
| | 99E | 99 | L | 648 | CTX-M-27 | | | – | R | R | S | S | S |
| | 18E | 18 | M | 131 | CTX-M-33 | | | – | R | R | S | R | R |
| | 54E | 54 | N | 117 | CTX-M-55 | | | – | R | S | S | R | S |
| | 116E | 116 | O | 1158 | SHV-12 | | | – | R | S | S | R | S |
| | 69E | 69 | P | New3 (CC10) | SHV-12 | | | – | R | I | S | R | S |
| K. pneumoniae (n = 15) | 69E | 69 | A1 | 17 | CTX-M-15 | | | – | R | R | S | R | R |
| | 142OXA | 142 | A2 | 17 | CTX-M-15 | OXA-181 | IncX3 | + | R | R | R | I | R |
| | 143OXA | 143 | A2 | 17 | CTX-M-15 | OXA-181 | IncX3 | + | R | R | R | R | R |
| | 69OXA | 69 | B | 17 | | OXA-181 | IncX3 | + | R | R | R | S | S |
| | 51E | 51 | C | 30 | CTX-M-15 | | | – | R | R | S | R | R |
| | 75E | 75 | D | 15 | CTX-M-15 | | | – | R | R | S | R | R |
| | 122E | 122 | E | 13 | CTX-M-15 | | | + | R | R | S | R | R |
| | 19E | 19 | F | 2982 | CTX-M-15 | | | – | R | S | S | R | R |
| | 66E | 66 | G | 307 | CTX-M-15 | | | – | R | I | S | R | R |
| | 134 | 134 | H | 147 | CTX-M-15 | KPC-3 | IncFII | – | R | R | S | R | R |
| | 62 | 62 | I | 147 | | KPC-3/GES-5 | IncN/ColE1 | – | R | R | S | R | R |
| | 42 | 42 | J | 449 | | KPC-3 | IncN | + | R | R | R | S | R |
| | 2E | 2 | K | 449 | CTX-M-33 | | | – | R | R | S | R | R |
| | 1E | 1 | K | 449 | CTX-M-33 | | | – | R | R | S | R | R |
| | 5E | 5 | L | 3031 | CTX-M-33 | | | + | R | I | S | R | R |
| E. aerogenes (n = 2) | 71CAR | 71 | A | NA | CTX-M-15 | | | – | R | R | S | R | R |
| | 93E | 93 | B | NA | CTX-M-15 | | | – | R | R | S | R | R |

**Table 1** (continued)

| Species | CEF | FOX | ATM | ETP | IPM | AKN | GMI | TOB | FOS | SXT | CIP | TIG |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *E. coli* (*n* = 20) | R | S | R | S | S | S | R | R | S | R | R | S |
| | R | S | R | S | S | S | R | R | S | S | R | S |
| | R | S | R | S | S | S | R | R | S | R | R | S |
| | R | S | R | S | S | S | R | R | S | S | R | S |
| | R | S | R | S | S | S | S | S | S | R | R | S |
| | R | S | R | S | S | S | S | S | S | S | R | S |
| | R | S | I | S | S | S | S | S | S | R | R | S |
| | R | S | I | S | S | S | S | S | S | S | R | S |
| | R | S | I | S | S | R | S | S | S | R | R | S |
| | R | S | I | S | S | S | S | S | S | S | R | S |
| | I | S | I | S | S | S | S | S | S | R | R | S |
| | R | S | R | S | S | S | S | I | S | S | R | S |
| | R | S | I | S | S | I | S | S | S | R | R | S |
| | I | S | S | S | S | S | S | S | S | R | S | S |
| | R | S | R | S | S | S | S | R | S | S | R | S |
| | S | S | R | S | S | S | S | S | S | R | R | R |
| *K. pneumoniae* (*n* = 15) | R | R | R | S | S | S | R | R | S | R | R | R |
| | R | R | R | R | S | S | S | R | S | R | R | S |
| | R | S | R | R | I | S | S | R | S | R | R | R |
| | S | S | S | R | S | S | R | R | S | R | R | S |
| | R | S | R | S | S | S | R | R | S | R | R | R |
| | R | S | R | S | S | I | R | S | S | S | R | R |
| | R | S | R | S | S | S | R | R | S | R | R | S |
| | I | S | R | S | S | S | S | S | S | R | S | R |
| | R | S | R | R | I | S | I | I | S | R | R | R |
| | R | R | R | R | R | I | I | R | S | R | R | S |
| | R | S | R | I | I | I | I | R | S | R | R | S |
| | R | S | R | S | S | S | R | R | S | R | R | S |
| | R | S | R | S | S | S | S | R | S | R | R | S |
| | R | S | R | S | S | S | S | S | S | R | S | S |
| | S | S | S | S | S | S | R | R | S | S | R | S |
| *E. aerogenes* (*n* = 2) | R | R | R | R | R | S | R | R | S | R | R | S |
| | R | R | R | S | S | S | R | I | S | R | S | S |

*PFGE*, pulsed-field gel electrophoresis; *ST*, sequence type determined by multilocus sequence typing; *CC*, clonal complex; *NA*, not applicable

*AMX*, amoxicillin; *AMC*, amoxicillin/clavulanic acid; *TEM*, temocillin; *CZD*, ceftazidime; *CTX*, cefotaxime; *CEF*, cefepime; *FOX*, cefoxitin; *ATM*, aztreonam; *ETP*, ertapenem; *IMP*, imipenem; *AKN*, amikacin; *GMI*, gentamicin; *TOB*, tobramycin; *FOS*, fosfomycin; *SXT*, trimethoprim-sulfamethoxazole; *CIP*, ciprofloxacin; *TIG*, tigecycline
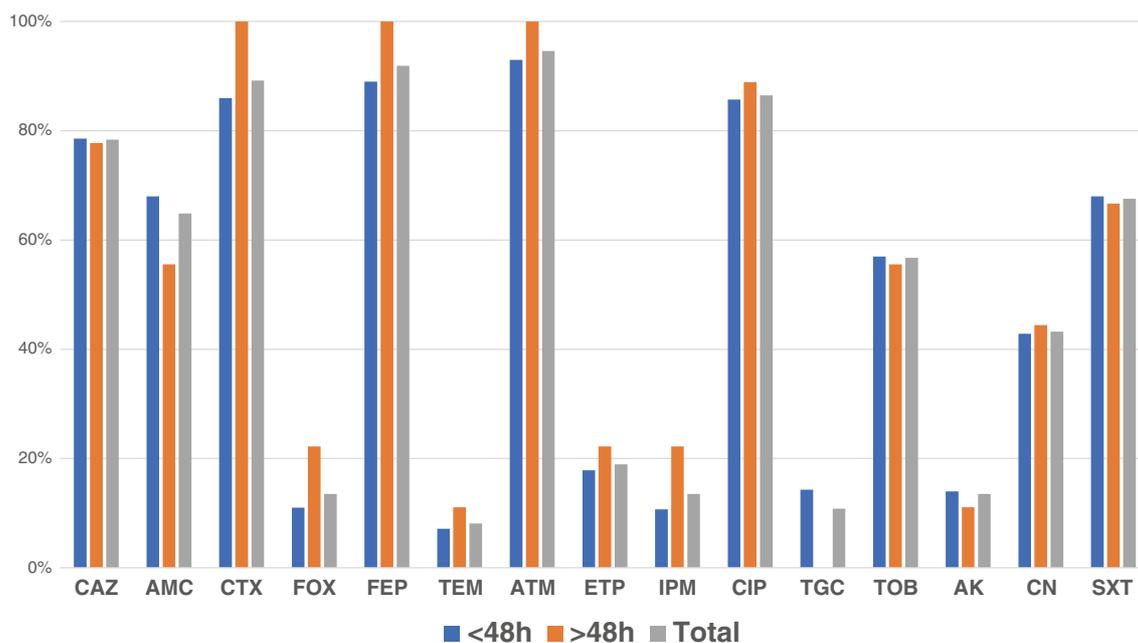
[a] Plasmid carrying the carbapenemase gene

**Fig. 1** Antimicrobial resistance among the 37 ESBL- and carbapenemase-producing Enterobacteriaceae isolates

hospital was 7.7% [17]. The rates were also lower in multi-center studies, such as a large admission prevalence study conducted in six German hospitals (9.5%; 416/4376) [18] and in a study involving four hospitals in the Netherlands (8.2%; 111/1351) [19]. However, contrary to most of these studies that performed a universal screening at hospital admission, our study focused exclusively on patients presenting risk factors for colonization with ESBL producers, which might explain the higher colonization rates. Considering we investigated a private hospital, the colonization rates might be underestimated due to a low representation of lower social classes that may live in environments where carriage of ESBL producers might be higher (for instance as a consequence of poorer hygiene conditions).

*E. coli* was the most commonly identified ESBL producer recovered at hospital admission (54%), while CTX-M-15 (50%) and CTX-M-27 (29%) were the most frequent ESBLs. Several European studies reported similar findings with *E. coli* rates varying between 68 and 89% and ESBLs of the CTX-M-1 and the CTX-M-9 groups varying between 53 and 89% and 6–21%, respectively [16–20]. CTX-M-15 was first reported in Portugal in 2005 in a hospital in Lisbon [21] and was subsequently reported as the main ESBL among Enterobacteriaceae isolates recovered in several health care facilities from various regions [6, 22, 23] and also in nursing homes and long-term care facilities [24]. It is actually accepted that CTX-M-15 is the most common ESBL determinant in *E. coli* worldwide. Noteworthy, here, we identified a CTX-M-15-producing ST44 *E. coli* isolate, as recently identified in healthy pigs in Portugal, suggesting a possible zoonotic origin [25].

Recent surveillance studies showed that CTX-M-27 that we found in this work in almost 30% of the isolates is currently emerging in certain parts of the world, namely in Asia and Europe [26], including different hospitals and community settings in Portugal [15].

CTX-M-33, which is a CTX-M-15 derivative, has been recently shown to confer reduced susceptibility to carbapenems (mainly meropenem) and to β-lactam/β-lactamase inhibitor combinations [27]. Interestingly, this infrequent enzyme (only reported in a single clinical *E. coli* in Greece in 2002 [28], in a single *Aeromonas dhakensis* isolate from fish in South Korea in 2014 [29] and in a *K. pneumoniae* isolate colonizing the gut of a patient admitted to the same Portuguese hospital in 2015 [27]), was found here among three *K. pneumoniae* isolates and in a single *E. coli* ST131 isolate, highlighting the dissemination of this ESBL gene in that hospital.

Our study showed that *E. coli* ST131 constitutes a major clone in the community in Portugal, harboring different ESBL encoding genes (CTX-M-15, CTX-M-27, and CTX-M-33). *E. coli* ST131 is considered the most significant high-risk clone among ESBL-producing *E. coli* since due to its predominance in many countries across the developed world, its association with multidrug resistance and virulence, and its ability to readily colonize and transmit among human hosts [30].

First-line antibiotics for the treatment of uncomplicated lower UTI include SXT, β-lactams, fluoroquinolones, nitrofurantoin, and fosfomycin [31, 32]. We found a high prevalence of ESBL-producing Enterobacteriaceae at hospital admission and more than half of the isolates showed resistance to SXT and ciprofloxacin. Therefore, the choice of empiric

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drugs to treat UTI in the community should be cautious, leaving fosfomycin as a safe alternative.

In conclusion, considering the high prevalence of ESBL-producing Enterobacteriaceae at hospital admission among patients presenting risk factors, namely recent exposition to healthcare facilities, routine screenings at hospital admission should be widely implemented in other Portuguese hospitals, enabling cohort precautions and limiting further spread of such multidrug-resistant isolates.

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