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SHORT REPORT
Different *Escherichia coli* B2-ST131 clades (B and C) producing extended-spectrum β-lactamases (ESBL) colonizing residents of Portuguese nursing homes

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SUMMARY

ESBL-producing *Enterobacteriaceae* and particularly *Escherichia coli* ST131 isolates producing CTX-M enzymes are commonly found colonizing the intestine of nursing home (NH) residents, but ST131 subclonal structure has been scarcely explored in this vulnerable population. Our goal was to perform a pilot study to assess the faecal carriage rate and epidemiological features of ESBL- and/or carbapenemase-producing *Enterobacteriaceae* (ESBL-E and CPE, respectively) among NH residents. For this purpose, faecal samples from residents at 4 different NHs in the North of Portugal (representing 9·5% of the residents' population, July 2014) were screened for ESBL-E and/or CPE by phenotypic and genotypic methods. Clonal structure and plasmid typing of ESBL-producing *E. coli* (ESBL-*Ec*) was performed by PCR and sequencing. Four ESBL-*Ec* isolates (2 CTX-M-15/2 CTX-M-14) were found in 20% of the samples, all belonging to the pandemic clonal lineage B2-ST131-O25b:H4. Two different clades were identified, the C2/H30-Rx-virotype C producing CTX-M-15 and an atypical B/H22-like-virotype D5 (producing CTX-M-14 and fluoroquinolone-resistant), firstly described in Portugal. This pilot study highlights the role of NH residents as a source of different ST131 clades, besides emphasizing the importance of *E. coli* B2-ST131 subtyping in different clinical settings, and understanding the transmission dynamics of the different variants.

Key words: CTX-M, faecal carriage, *fimH*, ST131 clades, virotypes.

Nursing home (NH) residents are known to be reservoirs of multidrug-resistant (MDR) bacteria, mainly due to their frequent hospitalizations, recurrent use of invasive medical devices and high antibiotic consumption [1]. Variable rates of intestinal colonization by extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* (ESBL-E) (6–41%) have been reported in European countries, while carbapenemase-producing *Enterobacteriaceae* (CPE) have not yet been identified [2–5]. CTX-M-producing *Escherichia coli* Sequence Type (ST) 131 clone dominates by far the population of MDR *Enterobacteriaceae* colonizing the intestine of NH residents [3], but detailed analysis of ST131 subclonal structure has been scarce. In Portugal, ESBL-E (and particularly CTX-M-15-producing *E. coli* ST131 or *Klebsiella pneumoniae* ST15 clones) are endemic for several years in the clinical setting [6–8], whereas CPE (mainly

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KPC-3-producing *K. pneumoniae*) are quickly penetrating in our geographic region since the end of 2015, especially on susceptible populations [9]. The aim of this work was to perform a pilot study to assess the current faecal carriage rate of ESBL-E or CPE among NH residents in Portugal, and the clonal and subclonal structure of these isolates.

Fresh rectal swabs from 20 residents at four NHs located in the North of Portugal (5–6 km distance between them) were collected in July 2014 and analysed. Five residents *per* NH (ten females, ten males) were recovered, representing 9·5% of the total residents' population. Eighty-five per cent of residents were ⩾65 years old (mean age of 75 years), 70% were previously hospitalized and all of them received antibiotic treatment during the 3 months preceding sampling (Supplementary Table S1). Samples were suspended in 2 ml of saline and screened for *Enterobacteriaceae* resistant to third-generation cephalosporins and/or carbapenems by seeding 0·2 ml of the suspension on CHROMagar™ Orientation plates supplemented with vancomycin (4 mg/l) plus ceftazidime (1 mg/l) or ertapenem (0·25 mg/l), respectively, and further incubation (37 °C/24 h) [10]. Presumptive *Enterobacteriaceae* isolates (oxidase negative, each different morphotype *per* plate) were selected for further studies. ESBLs and/or carbapenemases were identified by the DDST and Blue-Carba test, respectively, followed by polymerase chain reaction (PCR) and sequencing [10]. Susceptibility testing to non-β-lactam antibiotics was performed by the disk diffusion method (http://www.eucast.org/clinical_breakpoints/) and presumptive *E. coli* ESBL producers were identified by species-specific PCR [10]. The clonal structure of ESBL-producing *E. coli* was analysed by identification of *E. coli* phylogenetic groups and MLST (http://mlst.ucc.ie/mlst/dbs/Ecoli) [10]. Subclonal typing of B2-ST131 isolates was performed by PCR or PCR and sequencing of markers for ST131 serogroups (O25b:H4, O16:H5), clades (A, B, C1, C2) and *fimH*$_{TR}$ allele, and virulence genes (*ibeA*, *iroN*, *sat*, *afa/draBC*, *papG allele II/III*, *cnf1*, *hlyA*, *cdtB*, *K1*) [10, 11]. Plasmid analysis included replicon typing and subtyping (IncF plasmids) by PCR and sequencing (http://pubmlst.org/plasmid/primers/incF.shtml).

Intestinal colonization by ESBL-E was detected in 4/20 (20%; 95% confidence interval (CI) 5·7–43·7) of the residents (Table 1), a colonization rate similar to that (24·5%) reported previously in our country in a larger sample from residents at NHs and long-term care facilities (LTCFs) [8]. These NH are managed

Table 1. *Epidemiological data of ESBL-producing E. coli isolates identified in faecal samples from NH residents in Portugal*

ESBL-type	Nursing home	Local of the previous hospitalization	Gender/ age[a]	PhG[b]-ST[c]	Serotype	ST131 clade	*fimH*	Virotype	Plasmid Inc groups (IncF subtyping)[d,e]	Resistance to Non-β-Lactams[e,f]
CTX-M-15 ($n = 2$)	NH4	–	F/73	$B2_3$-ST131	O25b:H4	C2	30	C	N + X4	CIP, NAL, STR, SUL, TET, TMP
		H1	M/81							
CTX-M-14 ($n = 2$)	NH3	H1	F/94	$B2_3$-ST131	O25b:H4	B	161[g]	D5	I1 + (HI2) + ColE (F2:A-:B1)	CIP, NAL, (STR), TET
	NH4	–	M/72							

[a] F, Female, M, Male.
[b] PhG, *E. coli* phylogenetic group.
[c] ST, Sequence Type.
[d] IncF plasmids were identified using the FAB formula (FII, FIA, FIB) as proposed in http://pubmlst.org/plasmid/
[e] Variability among isolates is shown in parenthesis.
[f] CIP, ciprofloxacin; NAL, nalidixic acid; STR, streptomycin; SUL, sulphonamides; TET, tetracycline; TMP, trimethoprim.
[g] *fimH*161, one SNP to *fimH*22.

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by the same institution, share the nursing team and are served by the same hospital (H1). However, the asymmetry in the colonization rates observed (varying from 0% in NH1/NH2, 20% in NH3 and 60% in NH4) might be explained by the higher number of bedridden residents in NH3 and NH4 at sampling, which are at a higher risk of acquisition of MDR bacteria.

The four NH residents positive for ESBL-E had recognized risk factors for ESBL-E carriage, such as previous antibiotic exposure and hospitalizations, but there was no significant statistic association between colonization and demographic (age, gender) or clinical (previous antibiotic treatment or hospitalization) data (Supplementary Table S1). Besides the low sample size, the absence of CPE is noteworthy but might not reflect the current situation since sampling occurred before the burden of CPE producers in clinical settings [9, 12].

All the ESBL-E were identified as *E. coli* producing CTX-M-15 ($n = 2$; two samples) or CTX-M-14 ($n = 2$; 2 samples) from different residents (Table 1). The species and the ESBL-types detected in our study are in line with the recent epidemiological trends in Portuguese hospitals [6], and with those observed in NHs from different European countries [3, 4, 8]. All ESBL-producing *E. coli* belonged to the pandemic B2-ST131-O25b:H4 clone and different clades thereof (C2/H30-Rx and B/H22-like). For both of them, the previous hospitalization of residents in the same hospital suggests nosocomial acquisition (Table 1). The C2/H30-Rx clade producing CTX-M-15 ($n = 2$) was identified in two residents from the same institution (NH4). It belonged to virotype C (*sat*), presented a MDR pattern and harboured only N and X4 plasmid replicons, instead of the typical IncF plasmids (Table 1) [13]. In fact, this clade corresponds to the most worldwide disseminated within *E. coli* B2-ST131 including in Portugal (Novais Â, unpublished results) [3]. Interestingly, B2-ST131-H30 virotypes A and B, previously associated with NH residents, were not detected in our sample [3]. Isolates from the less common clade B/H22-like (*fimH*161, differing in one SNP from *fimH*22) were identified in residents from NH3 and NH4, belonged to virotype D5 (*ibeA*, *iroN*, *cnf1, hlyA*), were MDR and produced CTX-M-14, and carried a higher diversity of plasmid replicons [I1, HI2, ColE, and an F2:A-:B1 virulence plasmid (resembling pAPEC-O2-ColV, GenBank accession number AY545598)] (Table 1). This clade (B/*fimH*22), firstly described in our country, is usually linked to community-acquired infections, but infrequently to fluoroquinolone resistance or ESBL production as reported in this study, which deserves further monitoring [3, 13]. This study, together with previous data, highlights circulation of different ST131 clades in diverse clinical and non-clinical settings in our country: (i) clade C2/H30-Rx in different Portuguese hospitals, NHs and LTCFs (this study, data not shown); (ii) clades C1-M27 and C1-nM27 in hospitals and healthy volunteers [10]; and (iii) an atypical clade B in NHs (this study).

In summary, this pilot study among NH residents in our country pointed-out the role of this setting as a source of different CTX-M-producing *E. coli* B2-ST131 clades (CTX-M-15-clade C2/H30-Rx and CTX-M-14-clade B/H22-like). Our data underscore the importance of B2-ST131 subtyping in different settings and further evaluation of transmission dynamics of the different subclones.

SUPPLEMENTARY MATERIAL

The supplementary material for this article can be found at https://doi.org/10.1017/S0950268817002266.

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DECLARATION OF INTEREST

None to declare.

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