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Prevalence, genetic diversity of and factors associated with ESBL-producing Enterobacterales carriage in residents of French nursing homes

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SUMMARY

Objective: To determine the prevalence and genotypic characteristics of extended-spectrum β-lactamase-producing Enterobacterales (ESBLE) and carbapenemase-producing Enterobacterales (CPE) in nursing homes (NHs) in a French region. Risk factors associated with their carriage were also investigated.

Methods: A point-prevalence survey was proposed from November 2017 to June 2018 to NHs in the study region. Volunteer residents were screened for ESBLE and CPE carriage. *Escherichia coli* and *Klebsiella pneumoniae* isolates were genotyped using multi-locus sequence typing, pulsed-field gel electrophoresis (PFGE) and phylogrouping (for *E. coli* alone). Collective and individual data were analysed by random-effects logistic regression.

Results: The study was conducted in 18 NHs and included 262 patients. Fifty-two patients (19.8%) carried at least one ESBLE, corresponding to 56 isolates (42 *E. coli*, 11 *K. pneumoniae* and three others), while no CPE was detected. The majority (27/42) of ESBL *E. coli* belonged to phylogroup B2, and ST131 was over-represented in this subset (21/27). PFGE analysis revealed ST131 cross-transmission within NHs. Regarding ESBL *K. pneumoniae*, nine of 11 isolates belonged to ST663, and PFGE suggested diffusion of the clone in six NHs. Significant individual risk factors for colonization by ESBLE were: use of a shared bathroom, previous antibiotic use and recent history of hospitalization. Significant collective protective factors were proper compliance with glove use and support of the NH by a healthcare facility.

Conclusion: This study shows that NHs in the study region are an important reservoir of ESBLE, whereas no residents were CPE carriers. The control of ESBLE in NHs should focus on antibiotic stewardship and excreta management policies.

Introduction

Antimicrobial resistance is a worldwide problem and is projected to surpass cancer as the leading cause of death by 2050 [1]. Of particular interest, extended-spectrum β-

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lactamase-producing Enterobacterales (ESBLE) has become a major European public health concern in the last two decades [2]. For instance, the incidence of infections caused by ESBLE has increased dramatically in French hospitals, and *Escherichia coli* has become the most common species among ESBLE (60%), before *Klebsiella pneumoniae* (25%) [3]. There is rising concern regarding nursing homes (NHs) which are considered as reservoirs of ESBLE, because the residents frequently require medical care and antimicrobial treatments [4]. In addition, the recent emergence of carbapenemase-producing Enterobacterales (CPE) represents a significant worldwide threat to public health, and some studies from southern Europe have reported significant prevalence of CPE carriage in residents of NHs or long-term care facilities [5,6]. However, the prevalence of ESBLE and CPE carriage among residents of French NHs remains poorly described. In this context, the objective of this study was to assess the prevalence of ESBLE and CPE carriage in residents of NHs of Franche-Comté, a region in eastern France. In addition, the molecular characteristics of these multi-drug-resistant isolates were investigated, as well as collective and individual risk factors associated with their carriage.

Methods

Study design, setting and participants

A cross-sectional prevalence survey was performed from November 2017 to June 2018 in NHs in Franche-Comté. A two-stage random sampling method was used. Fifty NHs were selected at random from the 135 NHs eligible to participate in the study. Between 20 and 35 residents, depending on housing capacity, were selected at random from each of these 50 NHs. Residents who were unable to express their agreement to participate were excluded. Individual residents who agreed to participate in the study were informed of the study protocol by the medical coordinator. Their oral approval was noted in their medical record. The study was approved by the ethical committee 'Comité d'Etude Clinique' of Besançon University Hospital, Besançon, France (Reference: 2017-A00276-47).

Microbiological analysis

Rectal bacterial samples were collected using a rectal swab or fresh stool (faecal swab, Copan Diagnostics, Brescia, Italy). Samples were analysed in the hygiene laboratory at Besançon University Hospital within 72 h of collection. Samples were streaked on to ChromiD ESBL and ChromID CARBA SMART agar (bioMérieux, Marcy-l'Etoile, France), and incubated aerobically at 35 ± 2°C for 24 h in accordance with the manufacturer's instructions. Isolates obtained were identified to species level using matrix-assisted laser desorption/ionization-time of flight mass spectrometry (Microflex LT, Bruker Daltonik GmbH, Bremen, Germany) in accordance with the manufacturer's recommendations. For all ESBL-suspected Enterobacterales isolates, the presence of ESBL was confirmed using a double-disk synergy test in accordance with the recommendations of the European Committee on Antimicrobial Susceptibility Testing (http://www.eucast.org/clinical_breakpoints/). ESBL genes were identified by polymerase chain reaction and sequencing. After screening all samples with consensus primers targeting bla_{CTX-M}, more specific amplifications were performed, beginning with primers targeting different groups of CTX-M (bla_{CTX-M} group 1 bla_{CTX-M} group 9, bla_{CTX-M} group 2). Next, samples were tested for the presence of bla_{SHV} or bla_{TEM} genes [7]. All ESBL-producing *E. coli* isolates were typed by phylogrouping as described previously [8]. Multi-locus sequence typing (MLST) was performed on all ESBL-producing *E. coli* and *K. pneumoniae* isolates in accordance with protocols described previously [9]. The MLST website (http://pubmlst.org/) was used for the assignment of allele numbers and sequence types (STs). Pulsed-field gel electrophoresis (PFGE) was used to assess clonal diversity within each ST using *Xba*I digestion. Pulsotypes (PTs) were defined in accordance with international recommendations [10]. For a given bacteria, isolates which shared similar PTs and were recovered from the same NH were thought to be cross-transmitted.

Data collection

Two trained infection control practitioners collected all data using standardized questionnaires for collective and individual variables.

Facility questionnaire

Numerical variables collected included: a French index to evaluate the average level of dependency of NH residents (GMP), housing capacity and number of physicians participating in care of the residents. Categorical variables collected included: sectorization (specific staff allocated to units of the NH during day and night shifts), whether physicians had a list of recommended antibiotics and antimicrobial stewardship guidelines, whether the NH was linked to a larger healthcare institution, existence of an in-house pharmacy, hygiene protocols regarding ESBLE and CPE or route of excreta disposal, whether the NH benefits from the intervention of an infection control nurse from the regional mobile team, availability of items related to hygiene (bedpan washer disinfectors, near-patient alcohol-based hand rub, single-use gloves and aprons) and frequency of use, staff training in standard precautions, and hand hygiene and information to residents regarding hand hygiene.

Resident questionnaire

Individual data collected were: age, sex, a French index to evaluate the level of dependency (GIR, varying from 1 to 6, with an index close to 1 indicating a high level of dependency), duration of stay, private or shared bedroom, bed rest, route of excreta disposal (private bathroom, shared bathroom, commode chair, use of incontinence products), medical history including diabetes, immunodeficiency, urinary and faecal incontinence, presence of invasive medical devices, antibiotic treatment in the preceding 6 months, and hospitalization in the preceding 12 months.

Statistical analysis

Descriptive statistics were displayed for both NHs and individual residents. Univariate comparison between groups was performed using Pearson's Chi-squared test or Fisher's exact test for categorical variables. Numerical variables were compared using Student's *t*-test or the Mann–Whitney U test, as appropriate. Multi-variate analysis was conducted using a random-effects logistic regression model, taking into account the clustered structure of data (residents nested within NHs). The binary outcome variable was ESBLE positivity on stool

sample. Explanatory variables were selected using stepwise regression through backward elimination procedure. All explanatory variables that showed a *P*-value <0.10 on univariate analysis were considered for the multi-variate analysis. Analyses were conducted using Stata Version 14.1 (Stata Corp., College Station, TX, USA). A *P*-value <0.05 was considered to indicate significance.

Results

Participation

Of the 50 NHs randomized, 18 (36.0%) agreed to participate in the study. In these NHs, 776 residents met the inclusion criteria. After randomization and recording their agreement to participate, 262 residents, with complete data and samples, were included, representing an average of 14.5 residents per NH (range 3–33). The flow chart of the study is presented in Figure 1. Data collected on NHs and residents are displayed in Tables I and II, respectively.

Microbiological analysis

Fifty-two of the residents [19.8%, 95% confidence interval (CI) 12.5–27.1] carried at least one ESBLE, corresponding to 56 isolates (42 *E. coli*, 11 *K. pneumoniae*, two *Citrobacter farmer*i and one *Morganella morganii*), while no CPE was detected. The overall prevalence of ESBLE in NHs varied from 0 to 43.8%; four NHs had no ESBLE carriers amongst the five to 17 residents tested, while one NH had seven carriers amongst the 16 residents tested.

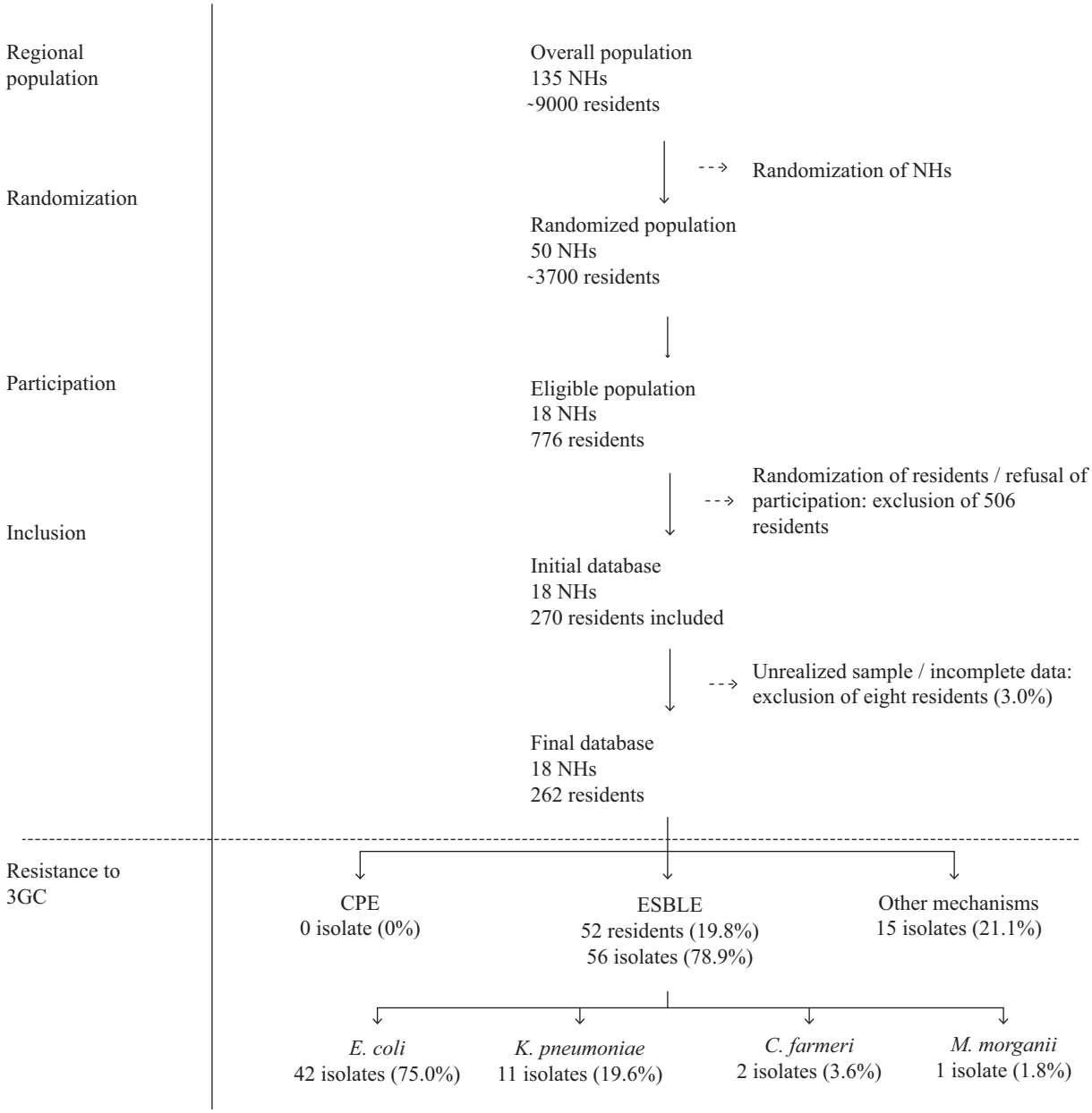

Figure 1. Study flow chart. NH, nursing home; ESBLE, extended-spectrum beta-lactamase-producing Enterobacterales; CPE, carbapenemase-producing Enterobacterales; 3GC, third-generation cephalosporins; *E. coli*, *Escherichia coli*; *K. pneumoniae*, *Klebsiella pneumoniae*; *C. farmeri*, *Citrobacter farmeri*; *M. morganii*, *Morganella morganii*.

Table I
Characteristics of nursing homes (NHs) (*N*=18)

	N (%)	Median (range)
GMP		729.5 (568–840)
Housing capacity		78.9 (24–236)
Number of eligible residents per NH		30.0 (9–197)
Number of residents who agreed to participate per NH		15.5 (3–33)
Number of physicians		9.5 (1–31)
Sectorization of care during the day	11 (61.1)	
Sectorization of care during the night	6 (33.3)	
Existence of a preferential list of ATB	8 (44.4)	
Existence of an institutional ATB policy	13 (72.2)	
NH linked to a larger healthcare institution	8 (44.4)	
Existence of an in-house pharmacy	9 (50.0)	
Intervention of an infection control nurse from the regional mobile team	13 (72.2)	
Existence of a route for waste disposal	18 (100)	
Existence of protocols regarding ESBLE	11 (61.1)	
Existence of protocols regarding CPE	11 (61.1)	
Bedpan washer disinfector	6 (33.3)	
ABHR available close to the patient	18 (100)	
Single-use gloves available	18 (100)	
Single-use apron available	18 (100)	
Staff training in universal hygiene precautions	15 (83.3)	
Staff training in hand hygiene	16 (88.9)	
Information to resident regarding hand hygiene	11 (61.1)	

GMP, a French index to assess the average level of dependence of NH residents; ATB, antibiotics; ESBLE, extended-spectrum beta-lactamase-producing Enterobacterales; CPE, carbapenemase-producing Enterobacterales; ABHR, alcohol-based hand rub.

Table II
Characteristics of patients according to their carrier status (*N*=262)

	ESBLE carriers *N*=52 *N* (%) or median (range)	Non-ESBLE carriers *N*=210 *N* (%) or median (range)
Sex		
Female	40 (76.9)	163 (77.6)
Male	12 (23.1)	47 (22.4)
Age (years)	88 (62–100)	89 (62–100)
GIR <3	37 (71.2)	118 (56.2)
Duration of stay (days)	1030 (20–4802)	779.5 (19–5790)
Private bedroom	43 (82.7)	156 (74.3)
Route of excreta disposal		
Private bathroom	27 (51.9)	143 (68.1)
Shared bathroom	8 (15.4)	56 (26.7)
Commode chair	15 (28.9)	54 (25.7)
Incontinence pads	47 (90.4)	170 (81.0)
Medical history		
Immunodeficiency	4 (7.7)	12 (5.7)
Diabetes	6 (11.5)	29 (13.8)
Urinary incontinence	46 (88.5)	153 (72.9)
Faecal incontinence	31 (59.6)	100 (47.6)
Bed rest	5 (9.6)	16 (7.6)
Previous carriage of ESBLE or CPE (*N*= 206)	4/37 (10.8)	5/169 (3.0)
Exposure to an invasive medical device	3 (5.8)	13 (6.2)
Digestive tract device	0	1 (0.5)
Urinary tract device	1 (3.1)	7 (3.3)
Prior exposure to ATB in preceding 6 months (*N*=253)	30/50 (60.0)	76/203 (37.4)
Fluoroquinolones	2 (4.0)	8 (3.9)
Amoxicillin	10 (20.0)	18 (8.9)
Amoxicillin-clavulanate	5 (10.0)	19 (9.4)
3GC	10 (20.0)	21 (10.3)
Carbapenems	0	0
Other	10 (20.0)	31 (15.3)
Previous hospitalization in preceding 12 months	24 (46.2)	50 (23.8)

GIR, a French index for level of dependency; ESBLE, extended-spectrum beta-lactamase-producing Enterobacterales; CPE, carbapenemase-producing Enterobacterales; ATB, antibiotics; 3GC, third-generation cephalosporins.

Four residents carried two ESBLE (three *E. coli* and *K. pneumoniae*, one *E. coli* and *M. morganii*). The majority (27/42) of ESBL *E. coli* belonged to phylogroup B2, and the others were distributed in phylogroup D (*N*=8), phylogroup A (*N*=4) and phylogroup B1 (*N*=3). ST131 was over-represented in phylogroup B2 (21/27 isolates). The 21 ST131 isolates were recovered from five NHs (between one and nine cases per NH), and PFGE analysis revealed ST131 cross-transmission within NHs. In two NHs, all of the isolates (*N*=4 and *N*=6, respectively) belonged to the same PFGE pattern. In the NH where nine ST131 isolates were identified, isolates clustered in four PFGE patterns (with six isolates sharing the same PFGE pattern). It should be noted that no PFGE patterns of ST131 were shared by residents from different NHs. All the ESBLs identified in ST131 isolates were of CTX-M type, with a predominance of CTX-M-27 (*N*=9) and CTX-M-15 (*N*=8). In phylogroup D, CC69 and ST362 were identified for three and two isolates, respectively, with no evidence of cross-transmission. Regarding ESBL-producing *K. pneumoniae*, nine of 11 isolates were ST663, CTX-M-15 producers and belonged to the same PFGE pattern, but were detected in residents from six different NHs. Genotyping data are summarized in Table III.

Statistical analysis

The results of univariate and multi-variate analysis are presented in Table IV. The risk of carrying ESBLE was increased by use of a shared bathroom [odds ratio (OR) 2.32, 95% CI 1.17–4.57, *P*=0.015], history of antibiotic use in the preceding 6 months (OR 2.32, 95% CI 1.20–4.49, *P*=0.012) or hospitalization in the preceding 12 months (OR 2.04, 95% CI 1.03–4.03,

Table III
Summary table of the genotypic and phenotypic characteristics of bacterial isolates

Isolate no.	NH no.	Resident no.	Species	Phylogroup	ST	CC	ESBL	PFGE no.
1	1/19	1/19/002	*E. coli*	B2	1618	73	CTX-M-17	
2	6/01	6/01/007	*E. coli*	B2	131	131	CTX-M-27	5
3	6/01	6/01/008	*E. coli*	B2	131	131	CTX-M-27	5
4	6/01	6/01/015	*E. coli*	B2	131	131	CTX-M-27	5
5	6/01	6/01/016	*E. coli*	B2	131	131	CTX-M-27	5
6	7/63	7/63/004	*E. coli*	B2	141	141	CTX-M-14	
7	4/26	4/26/007	*E. coli*	B1	708	469	CTX-M-1	8
8	4/26	4/26/008	*E. coli*	B1	708	469	CTX-M-1	8
9	4/26	4/26/011	*E. coli*	D	362		CTX-M-1	
10	4/26	4/26/019	*E. coli*	B2	372		CTX-M-1	
11	7/64	7/64/002	*E. coli*	B2	73	73	CTX-M-3	
12	7/64	7/64/004	*E. coli*	B1	443		CTX-M-15	
13	8/1	8/01/086	*E. coli*	D	648		CTX-M-15	
14	8/2	8/02/001	*E. coli*	D	69	69	CTX-M-1	
15	8/2	8/02/007	*E. coli*	B2	131	131	CTX-M-1	6
16	9/1	9/01/003	*E. coli*	A	23	23	CTX-M-14	
17	9/1	9/01/006	*E. coli*	D	362		CTX-M-14	
18	9/1	9/01/007	*E. coli*	A	23	23	CTX-M-14	
19	8/3	8/03/004	*E. coli*	B2	131	131	CTX-M-1	
20	8/3	8/03/005	*E. coli*	D	69	69	CTX-M-1	
21	8/3	8/03/010	*E. coli*	D	106	69	CTX-M-1	
22	9/3	9/03/001	*E. coli*	B2	131	131	CTX-M-27	3
23	9/3	9/03/002	*E. coli*	B2	131	131	CTX-M-27	3
24	9/3	9/03/005	*E. coli*	B2	131	131	CTX-M-15	4
25	9/3	9/03/008	*E. coli*	B2	131	131	CTX-M-27	3
26	9/3	9/03/013	*E. coli*	B2	131	131	CTX-M-27	3
27	9/3	9/03/014	*E. coli*	B2	131	131	CTX-M-27	3
28	9/3	9/03/019	*E. coli*	B2	131	131	CTX-M-15	2
29	9/3	9/03/023	*E. coli*	B2	131	131	CTX-M-27	3
30	9/3	9/03/026	*E. coli*	B2	131	131	CTX-M-1	7
31	9/4	9/04/005	*E. coli*	B2	104		CTX-M-14	
32	9/4	9/04/008	*E. coli*	B2	127		CTX-M-15	
33	9/4	9/04/011	*E. coli*	D	59	59	CTX-M-14	
34	9/4	9/04/014	*E. coli*	A	23	23	CTX-M-14	
35	9/4	9/04/016	*E. coli*	A	23	23	CTX-M-14	
36	9/5	9/05/001	*E. coli*	D	38	38	CTX-M-15	
37	9/5	9/05/012	*E. coli*	B2	131	131	CTX-M-15	1
38	9/5	9/05/013	*E. coli*	B2	131	131	CTX-M-15	1
39	9/5	9/05/016	*E. coli*	B2	131	131	CTX-M-15	1
40	9/5	9/05/018	*E. coli*	B2	131	131	CTX-M-15	1
41	9/5	9/05/024	*E. coli*	B2	131	131	CTX-M-15	1
42	9/5	9/05/026	*E. coli*	B2	131	131	CTX-M-15	1
43	1/54	1/54/003	*K. pneumoniae*		663		CTX-M-15	2
44	1/54	1/54/008	*K. pneumoniae*		663		CTX-M-15	2
45	8/1	8/01/068	*K. pneumoniae*		663		CTX-M-15	2
46	8/2	8/02/001	*K. pneumoniae*		663		CTX-M-15	2
47	8/2	8/02/002	*K. pneumoniae*		663		CTX-M-15	2
48	8/2	8/02/014	*K. pneumoniae*		663		CTX-M-15	2
49	9/3	9/03/023	*K. pneumoniae*		663		CTX-M-15	2
50	9/4	9/04/003	*K. pneumoniae*		663		CTX-M-15	2
51	9/4	9/04/016	*K. pneumoniae*		3455		CTX-M-14	3
52	9/5	9/05/018	*K. pneumoniae*		307		CTX-M-15	1
53	9/5	9/05/009	*K. pneumoniae*		663		CTX-M-15	2
54	9/2	9/02/004	*C. farmeri*					
55	9/2	9/02/016	*C. farmeri*					
56	9/4	9/04/006	*M. morgannii*					

NH, nursing home; ST, sequence type; CC, clonal complex; ESBL, extended-spectrum beta-lactamase; PFGE, pulsed-field gel electrophoresis; *E. coli*, *Escherichia coli*; *K. pneumoniae*, *Klebsiella pneumoniae*; *C. farmeri*, *Citrobacter farmeri*; *M. morganii*, *Morganella morganii*.

Table IV
Factors associated with extended-spectrum beta-lactamase-producing Enterobacterales carriage from random-effects logistic regression analysis

	Univariate analysis		Multi-variate analysis	
	OR	95% CI	OR	95% CI
Collective variables				
Housing capacity 50–100 residents	4.71	1.00–22.29		
Sectorization of care at night	0.42	0.19–0.92		
NH linked to a larger healthcare institution	0.43	0.21–0.90	0.41	0.19–0.87
Existence of an in-house pharmacy	0.49	0.23–1.03		
Existence of a preferential list of ATB	0.47	0.22–1.02		
Systematic use of single-use gloves	0.39	0.16–0.96	0.25	0.11–0.58
Staff training in hand hygiene	4.59	0.93–22.6		
Individual variables				
GIR <3	2.04	0.99–4.18		
Use of shared bathroom	2.06	1.05–4.04	2.32	1.18–4.57
Urinary incontinence	2.70	1.05–6.93		
Prior exposure to ATB in preceding 6 months	2.38	1.25–4.52	2.32	1.20–4.49
Amoxicillin	2.40	1.00–5.79		
3GC	2.33	0.97–5.56		
Previous hospitalization in preceding 12 months	2.56	1.31–4.98	2.04	1.03–4.03

GIR, a French index for level of dependency; ATB, antibiotics; 3GC, third-generation cephalosporins; NH, nursing home; OR, odds ratio; CI, confidence interval.

P=0.041). Protective factors were systematic use of single-use gloves whenever expected (OR 0.25, 95% CI 0.11–0.58, P=0.001) and the NH being linked to a larger healthcare institution (OR 0.41, 95% CI 0.19–0.87, P=0.020).

Discussion

This study shows that NHs in the study region are an important reservoir of ESBLE, with almost 20% carriage amongst residents. This figure is in line with the pooled prevalence of ESBLE reported by Flokas *et al*. in their systematic review (i.e. 18% in European NHs) despite considerable geographical variability [11]. Huge variability was observed within NHs included in this study, mainly due to intra-NH outbreaks of pandemic clonal group ST131. This survey revealed occult outbreaks of ST131-specific pulsotypes in three NHs, involving C1-CTX-M-27 and C2-CTX-M-15 clades. The latter is the predominant ST131 cluster, responsible for the worldwide spread of CTX-M-15 [12], and was first detected in the study region in 2006 [13]. The C1-CTX-M-27 clade emerged in 2010 at the regional university hospital [13]. This cluster, initially described in Japan in 2004 [14], has spread in Europe where it has been notably identified in children [15] and adult inpatients [16]. To the best of the authors' knowledge, this report is the first to detect a C1-CTX-M-27 cluster in French NHs.

The ST131 clonal group, which had a carriage rate of 8% in this study, has been frequently associated with elderly people and NHs, with carriage rates varying from 4% to 20% of residents [17,18]. The specific success of ESBL ST131 over other ESBL-producing *E. coli* clones in NHs may be due to prolonged colonization. Indeed, Overdevest *et al*. demonstrated that half-time carriage was significantly longer for ESBL ST131 (13 months) than for other ESBL-producing *E. coli* (2–3 months) in Dutch NHs [19].

PFGE results of ESBL-producing *K. pneumoniae* strongly suggest spread of isolates of ST663 in NHs from a common reservoir. This ST has only been reported in Spanish hospitals associated with OXA-48 production [20]; it is a single-locus variant of ST405, which was responsible for an outbreak in the university hospital in the study region (X. Bertrand, personal data). Consequently, it is very likely that this strain originated from the university hospital and was imported into NHs via colonized patients. It should be noted that one of the residents was a carrier of the emerging pandemic ST307 lineage. A recent phylogenetic analysis using whole-genome sequencing showed that this emerged in the 1990s, and has spread globally in association with a conserved plasmid containing the $bla_{\text{CTX-M-15}}$ ESBL gene and several other resistance genes [21]. ST307 can also acquire and disseminate carbapenemases [21]. None of the residents were CPE carriers; this suggests that the study region is still an area with very low incidence of CPE [22].

Risk analysis in the current study indicated that previous hospitalization, previous antibiotic exposure and the use of a shared bathroom were associated with high probability of ESBLE carriage. Acute care hospitals and NHs seem to act as communicating vessels for ESBLE, as mentioned by Latour *et al*. [23], and history of previous hospitalization as well as previous antibiotic use were frequently identified as risk factors for ESBLE carriage in NHs [11]. Excreta management has also been identified in person-to-person transmission of ESBLE [24]. The present analysis stresses the importance of private toilets for residents.

This study revealed a significant protective impact of the proper use of single-use gloves, underlining the need for proper practices for excreta management. However, the relevance of the use of gloves was not evaluated, and misuse of gloves, such as overuse, can increase the risk of cross-transmission via contaminated gloved hands [25]. Other risk factors, such as history of invasive devices and underlying comorbidities, which have been reported to be associated with ESBLE carriage in NH residents [11] were not found in the present study, probably due to an insufficient sample size. In accordance with previous studies [16], level of mobility and gender were not associated with ESBLE carriage in this study.

This study had several limitations. First, the study design (point-prevalence survey) did not take into account the dynamics of ESBLE epidemiology. Second, the low proportion of NHs that agreed to participate in the study limits the number of residents included, and potentially hinders the identification of risk factors associated with ESBLE carriage.

In conclusion, this study shows that NHs in the study region are an important reservoir of ESBLE, special attention should be given to pandemic ST131 *E. coli* and *K. pneumoniae* clones, and ESBLE control in NHs should focus on antibiotic stewardship and excreta management policies.

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Conflict of interest statement
None declared.

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References

[1] O'Neil J. Review on antimicrobial resistance. Antimicrobial resistance: tackling a crisis for the health and wealth of nations. 2014. Available at: https://amr-review.org/sites/default/files/AMR%20Review%20Paper%20-%20Tackling%20a%20crisis%20for%20the%20health%20and%20wealth%20of%20nations_1.pdf.

[2] Cassini A, Plachouras D, Monnet DL. Attributable deaths caused by infections with antibiotic-resistant bacteria in France — Authors' reply. Lancet Infect Dis 2019;19:129—30.

[3] Carbonne A, Arnaud I, Maugat S, Marty N, Dumartin C, Bertrand X, et al. National multidrug-resistant bacteria (MDRB) surveillance in France through the RAISIN network: a 9 year experience. J Antimicrob Chemother 2013;68:954—9.

[4] Willemsen I, Nelson J, Hendriks Y, Mulders A, Verhoeff S, Mulder P, et al. Extensive dissemination of extended spectrum beta-lactamase-producing Enterobacteriaceae in a Dutch nursing home. Infect Control Hosp Epidemiol 2015;36:394—400.

[5] Ruiz-Garbajosa P, Hernandez-Garcia M, Beatobe L, Tato M, Mendez MI, Grandal M, et al. A single-day point-prevalence study of faecal carriers in long-term care hospitals in Madrid (Spain) depicts a complex clonal and polyclonal dissemination of carbapenemase-producing Enterobacteriaceae. J Antimicrob Chemother 2016;71:348—52.

[6] Nucleo E, Caltagirone M, Marchetti VM, D'Angelo R, Fogato E, Confalonieri M, et al. Colonization of long-term care facility residents in three Italian provinces by multidrug-resistant bacteria. Antimicrob Resist Infect Control 2018;7:33.

[7] Brechet C, Plantin J, Sauget M, Thouverez M, Talon D, Cholley P, et al. Wastewater treatment plants release large amounts of extended-spectrum beta-lactamase-producing *Escherichia coli* into the environment. Clin Infect Dis 2014;58:1658—65.

[8] Clermont O, Christenson JK, Denamur E, Gordon DM. The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. Environ Microbiol Rep 2013;5:58—65.

[9] Diancourt L, Passet V, Verhoef J, Grimont PA, Brisse S. Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. J Clin Microbiol 2005;43:4178—82.

[10] Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 1995;33:2233—9.

[11] Flokas ME, Alevizakos M, Shehadeh F, Andreatos N, Mylonakis E. Extended-spectrum beta-lactamase-producing Enterobacteriaceae colonisation in long-term care facilities: a systematic review and meta-analysis. Int J Antimicrob Agents 2017;50:649—56.

[12] Ben Zakour NL, Alsheikh-Hussain AS, Ashcroft MM, Khanh Nhu NT, Roberts LW, Stanton-Cook M, et al. Sequential acquisition of virulence and fluoroquinolone resistance has shaped the evolution of *Escherichia coli* ST131. MBio 2016;7.

[13] Sauget M, Cholley P, Vannier A, Thouverez M, Nicolas-Chanoine MH, Hocquet D, et al. Trends of extended-spectrum beta-lactamase-producing *Escherichia coli* sequence type 131 and its H30 subclone in a French hospital over a 15-year period. Int J Antimicrob Agents 2016;48:744—7.

[14] Matsumura Y, Pitout JD, Gomi R, Matsuda T, Noguchi T, Yamamoto M, et al. Global *Escherichia coli* sequence type 131 clade with blaCTX-M-27 gene. Emerg Infect Dis 2016;22:1900—7.

[15] Birgy A, Levy C, Nicolas-Chanoine MH, Cointe A, Hobson CA, Magnan M, et al. Independent host factors and bacterial genetic determinants of the emergence and dominance of *Escherichia coli* sequence type 131 CTX-M-27 in a community pediatric cohort study. Antimicrob Agents Chemother 2019;63:pii:e00382-19

[16] Ghosh H, Doijad S, Falgenhauer L, Fritzenwanker M, Imirzalioglu C, Chakraborty T. blaCTX-M-27-encoding *Escherichia coli* sequence type 131 lineage C1-M27 clone in clinical isolates, Germany. Emerg Infect Dis 2017;23:1754—6.

[17] Arvand M, Moser V, Pfeifer Y. Prevalence of extended-spectrum-beta-lactamase-producing *Escherichia coli* and spread of the epidemic clonal lineage ST131 in nursing homes in Hesse, Germany. J Antimicrob Chemother 2013;68:2686—8.

[18] Rodrigues C, Machado E, Fernandes S, Peixe L, Novais A. Different *Escherichia coli* B2-ST131 clades (B and C) producing extended-spectrum beta-lactamases (ESBL) colonizing residents of Portuguese nursing homes. Epidemiol Infect 2017;145:3303—6.

[19] Overdevest I, Haverkate M, Veenemans J, Hendriks Y, Verhulst C, Mulders A, et al. Prolonged colonisation with *Escherichia coli* O25:ST131 versus other extended-spectrum beta-lactamase-producing *E. coli* in a long-term care facility with high endemic level of rectal colonisation, the Netherlands, 2013 to 2014. Euro Surveill 2016;42:21. https://doi.org/10.2807/1560-7917.ES.2016.21.42.30376.

[20] Oteo J, Hernandez JM, Espasa M, Fleites A, Saez D, Bautista V, et al. Emergence of OXA-48-producing *Klebsiella pneumoniae* and the novel carbapenemases OXA-244 and OXA-245 in Spain. J Antimicrob Chemother 2013;68:317—21.

[21] Wyres KL, Hawkey J, Hetland MAK, Fostervold A, Wick RR, Judd LM, et al. Emergence and rapid global dissemination of CTX-M-15-associated *Klebsiella pneumoniae* strain ST307. J Antimicrob Chemother 2019;74:577—81.

[22] Emery A, Jeanvoine A, Bailly P, Gbaguidi-Haore H, Hocquet D, Bertrand X. Management of carbapenemase-producing Enterobacteriaceae in a low incidence area: a six-year experience in a university hospital. Infect Control Hosp Epidemiol 2019;40:936—8.

[23] Latour K, Huang TD, Jans B, Berhin C, Bogaerts P, Noel A, et al. Prevalence of multidrug-resistant organisms in nursing homes in Belgium in 2015. PLoS One 2019;14:e0214327.

[24] N'Guyen TTH, Bourigault C, Guillet V, Buttes AGD, Montassier E, Batard E, et al. Association between excreta management and incidence of extended-spectrum beta-lactamase-producing Enterobacteriaceae: role of healthcare workers' knowledge and practices. J Hosp Infect 2019;102:31—6.

[25] Picheansanthian W, Chotibang J. Glove utilization in the prevention of cross transmission: a systematic review. JBI Database System Rev Implement Rep 2015;13:188—230.