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Colonisation with ESBL-producing and carbapenemase-producing *Enterobacteriaceae*, vancomycin-resistant enterococci, and meticillin-resistant *Staphylococcus aureus* in a long-term care facility over one year

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Abstract

Background: This study examined colonisation with and characteristics of antimicrobial-resistant organisms among residents of a long-term care facility (LTCF) over one year, including strain persistence and molecular diversity among isolates of extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*.

Methods: Sixty-four residents of a LTCF were recruited (51 at baseline, 13 during the year). Data on dependency levels, hospitalisations, and antimicrobial prescribing were collected. Nasal and rectal swabs and catheter urine specimens were examined quarterly, using chromogenic agars, for ESBL-producing *Enterobacteriaceae*, carbapenemase-producing *Enterobacteriaceae* (CPE), vancomycin-resistant enterococci (VRE), and meticillin-resistant *S. aureus* (MRSA). All ESBL-producing *E. coli* (ESBL-EC) were characterised by pulsed-field gel electrophoresis (PFGE) and PCR to assess for sequence type (ST) ST131, its resistance-associated *H*30 and *H*30-Rx subclones, and bla_{CTX-M}, bla_{TEM}, bla_{SHV}, and bla_{OXA-1}.

Results: The overall number of residents colonised, by organism, was as follows: ESBL-EC, 35 (55%); MRSA, 17 (27%); ESBL-producing *K. pneumoniae* (ESBL-KP), 5 (8%); VRE, 2 (3%) and CPE, 0 (0%). All 98 ESBL-EC isolates were *H*30-Rx ST131, with $bla_{CTX-M-group\ 1}$. By PFGE, a group of 91 ESBL-EC (from 33 participants) had ≥ 85% similar profiles and resembled UK epidemic strain A/ international pulsotype PFGE812. Sequential ESBL-EC from individual residents were closely related. Six ESBL-KP isolates, from five participants, had $bla_{CTX-M-group\ 1}$ and by PFGE were closely related. Colonisation with ESBL and MRSA was associated with location within the LTCF and previous exposure to antimicrobials.

Conclusions: Among LTCF residents, colonisation with ESBL-EC and MRSA was common. All ESBL-EC were *H*30-Rx ST131, consistent with clonal dissemination.

Keywords: Extended-spectrum beta-lactamase (ESBL), Carbapenemase-producing *Enterobacteriaceae* (CPE), Vancomycin-resistant enterococci (VRE), Meticillin-resistant *S. aureus* (MRSA), Long-term care facilities

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Background

Antimicrobial resistance has been identified as a key public health challenge [1]. Amongst the major acquired antimicrobial-resistant organisms (AROs) are extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*, carbapenemase-producing *Enterobacteriaceae* (CPE), vancomycin-resistant enterococci (VRE), and meticillin-resistant *Staphylococcus aureus* (MRSA).

ESBL-producing *E. coli* (ESBL-EC) have become increasingly common throughout the world. In Ireland, ESBL-EC represented 10% of all *E. coli* bloodstream infections in 2013, compared with 3% in 2006 [2]. Spread of CTX-M beta-lactamases is linked with specific epidemic clonal groups such as *E. coli* sequence type ST131 (typically of serotype O25b:H4). Although ST131 has been identified from as early as 1967, ST131 isolates containing ESBLs, predominantly CTX-M-15, emerged mainly in the 2000s [3,4]. There is significant genomic diversity within ST131, with over 170 distinct PFGE patterns/pulsotypes (>94% similar *Xba*I PFGE profiles) recognised, some of which are associated with particular sources and antimicrobial resistance patterns [3].

The *H*30 ST131 subclone, so named for its carriage of allele 30 of *fimH* (type 1 fimbrial adhesin gene), reportedly expanded and disseminated rapidly after 2000 to become the most common subclone among clinical *E. coli* isolates globally [5,6]. *H*30 is closely associated with fluoroquinolone resistance [5,6]. ESBL-EC, most of which carry $bla_{CTX-M-15}$, belong mainly to a subset within *H*30 known as *H*30-Rx, which is strongly associated with sepsis [5,7].

Analogous to ST131 among ESBL-EC, several widely disseminated clonal groups of ESBL-producing *K. pneumoniae* (ESBL-KP) have also been identified, including *K. pneumoniae* ST11, ST15, ST16, ST23, and ST48 [8-12]. Recently, emerging carbapenem resistance has been reported in both *E. coli* and *K. pneumoniae,* further limiting options for antimicrobial therapy for these organisms [13,14].

Since the first *Klebsiella pneumoniae* carbapenemase (KPC)-producers were reported in Ireland in 2009, and the first KPC-producing *E. coli* in 2011, the numbers of CPE detected are increasing [15,16]. Likewise, VRE has caused an increasing proportion of all enterococcus bloodstream infections in Ireland in recent years [17]. By contrast with the above antimicrobial-resistant organisms, MRSA as a proportion of all *S. aureus* bloodstream infection has declined markedly, although strains isolated remain predominantly the hospital-associated strains that have been prevalent for decades [17,18].

There is increasing evidence that long-term care facilities (LTCFs) are important reservoirs for AROs, including reports of widespread colonisation and outbreaks [19-23]. This phenomenon is likely to increase in importance, since the proportion of the European Union population aged ≥ 65 years is now 16% and will increase to 29.3% (152.6 million) by 2060 [24]. This is likely to further increase the population residing in LTCFs, currently at 19,800 in Ireland (2012) and approximately 3.7 million in the EU (2010) [24,25].

Risk factors for acquisition of AROs such as old age, urinary catheterisation, antimicrobial consumption, and hospitalisation are common among residents of LTCFs [26-30]. Although there are a number of studies of prevalence of AROs in LTCFs, there are few longitudinal studies regarding the stability of colonisation over time, especially in relation to molecular characteristics of colonising organisms [31]. The aims of this study were: (i) to determine the baseline prevalence of colonisation; (ii) to monitor residents' colonisation status at quarterly intervals over one year; (iii) to identify risk factors for colonisation; (iv) to characterise the antimicrobial susceptibility of the AROs; and (v) to define the molecular characteristics of all ESBL-producing *Enterobacteriaceae* isolates.

Methods

Setting

The study was based in a newly built 100-bed LTCF that had 88 residents at the start of the study. There are four discrete care areas, with residents grouped by dependency level. Based on the Barthel Index, residents in care areas 1 and 2 are generally highly dependent for assistance for the 10 activities for daily living care (daily care), whereas those in care areas 3 and 4 require only occasional assistance with daily living (intermittent care). To simplify the models for statistical analysis, the four care areas were collapsed into two groups. Each of the four care areas has 21 single and two double en-suite rooms, a dining area, and a day room. There are two lifts and two sets of stairs.

Ethical approval

Ethical approval was granted by the Galway University Hospital Ethics Committee. Written consent was obtained at the outset and participants could withdraw from the study at any time.

Design and data collection

The study period was July 2012-August 2013. For each participating resident, data collected included gender, age on admission, date of admission, from where the resident was admitted, presence of an indwelling urinary catheter, systemic antimicrobial treatment, and hospitalisation in the previous 12 months, and previous ESBL, CPE, VRE, and MRSA result recorded before study commencement. At each quarterly interval additional data were recorded, including recent antimicrobial treatment in the LTCF, number of residents in the same room,

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hospitalisation, presence of a wound/ulcer, Barthel Index score (dependency Index), and location within the LTCF. However, data regarding antimicrobial treatment received during intercurrent hospitalisation were not accessible. Antimicrobials were classified as: (1) narrow spectrum beta-lactams, (2) broad spectrum beta-lactams, (3) quinolone/fluoroquinolones, (4) nitroimidazole, (5) nitrofurantoin, and (6) others. An antimicrobial day was defined as any day on which a resident received an antimicrobial.

Clinical sample collection

At quarterly intervals nasal and rectal swabs were collected from each participating resident, and urine samples were obtained from catheterised participants.

Laboratory detection

Rectal swabs were cultured on chromogenic agar for detection of ESBL-producing *Enterobacteriaceae* and vancomycin resistant enterococci (ID™ ESBL agar and chrom ID™ VRE agar (bioMérieux, Marcy l'Etoile, France)). The Centers for Disease Control and Prevention (CDC) method was applied to detect CPE [32,33]. Nasal swabs were cultured on chrom ID™ MRSA agar (bioMérieux, Marcy l'Etoile, France). In each case suspect colonies were subcultured for identification by standard methods and susceptibility testing was performed in accordance with EUCAST disk diffusion methods [34].

Molecular analysis

All ESBL-EC and ESBL-KP isolates were tested by PCR for bla_{CTX-M}, bla_{TEM}, bla_{SHV}, and bla_{OXA-1} as described previously [35,36]. *E. coli* were tested for ST131-specific single-nucleotide polymorphisms (SNPs) in *pabB* by PCR and, for isolates testing positive, additional SNP-based PCR reactions were performed to identify the *H*30 and *H*30-Rx subclones [5,37,38]. *Xba*I pulsed-field gel electrophoresis (PFGE) analysis was done according to the Pulse-Net protocol [39]. PFGE profiles were analysed using the Dice coefficient with clustering by the unweighted pair group method with arithmetic averaging (UPGMA). For reference, *E. coli* ST131 isolates representing UK Strains A, C, and D, and international pulsotypes PFGE788, PFGE797, PFGE800, PFGE806, PFGE812, PFGE837, PFGE842, PFGE903, PFGE905, PFGE906, PFGE945, PFGE968, PFGE987, and PFGE1140 were included in the *E. coli* PFGE analysis [3,40]. Similarly, representative *K. pneumoniae* isolates for STs ST14, ST15, ST16, ST23, ST35, ST37, ST45, ST48, ST101, ST147, ST161, ST258, ST280, ST307, ST392, ST429, and ST1236 were included in the *K. pneumoniae* PFGE analysis.

Environmental sampling

In August 2013, after commencement of the longitudinal study, environmental sampling was performed to detect ESBL-EC, ESBL-KP, VRE, and MRSA. Samples were taken from five en-suite bedrooms and two shared toilet facilities. The criteria for the selection of the five bedrooms was based on the selection of bedrooms of residents who tested positive for carriage of ESBL-EC, VRE and MRSA (n = 1), ESBL-EC and ESBL-KP (n = 1), ESBL-EC only (n = 1), ESBL-KP only (n = 1), and one occupant who did not have detectable colonisation with any of the target organisms (n = 1). The five bedrooms selected were based in two care areas and one common bathroom was selected from each of these two care areas. Eleven sites in each bedroom and seven sites in each shared toilet facility were tested. Selected sites included door handles entering and exiting areas, floor surface at base of toilet, handles beside showers, toilet flushers, toilet seats, tap handles, railings beside toilets, bed-frames, bed-side lockers, and on-call buttons.

Sites were swabbed using Copan ESwabs (BS ISO 18593:2004) and inoculated into peptone water overnight. Ten microlitres of peptone water was plated onto chromID™ MRSA, chromID™ESBL agar and chromID™VRE (bioMerieux) for detection of MRSA, ESBL, and VRE respectively. Identification of suspect isolates was by standard methods and susceptibility testing was interpreted by EUCAST methods and criteria [34].

Statistical analysis

For ESBL and MRSA colonisation univariate comparisons were made to identify associations with patient characteristics.

Generalised Estimating Equations (GEE) models with an exchangeable correlation structure were used to investigate the longitudinal effects taking dependency between repeated measurements in the same individual into account (repeated individual measures at 0, 3, 6, 9 and 12 months). A forward selection procedure was used to estimate the relation between ESBL/MRSA colonization and specific or general antimicrobial use. Potential confounding effects included in the models were previous colonisation, care area, hospitalisation, age, gender and other patient characteristics. Age and gender were kept in the final model, however other variables were only included if found to be a confounder. Interaction terms were checked but omitted if not significant. P-values <0.05 were considered statistically significant. Results are presented as adjusted odds ratios (OR) (adjusted for potential confounders) with corresponding 95% confidence intervals (CI). Analyses were done using SPSS for Windows version 20 and STATA/IC version 13.0.

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Results

Participating residents

Sixty-four LTCF residents agreed to participate in this study, including 51 (58% of 88 initial residents) at baseline and 13 (45% of 29 subsequently admitted residents) later in the year. The 51 baseline enrolees had a 76-month median prior length of stay in the LTCF. Thirty-four of these participants remained in the study for the entire study year whereas 17 withdrew early (15 due to ill health or death and 2 refused repeated sampling); however, all but 3 of the 17 had at least two rectal and nasal samples taken prior to withdrawal. All 13 newly admitted residents who were enrolled partway through the study year were followed until the end of the study. Of these participants, 11 entered the LTCF from hospital, one from home, and one from another LTCF. Key participant characteristics are summarised in Table 1. During the study period, 10 baseline participants and one new participant were hospitalised (for from 2 to 40 hospital days each). Additionally, 28 baseline and 5 new participants received antimicrobials while in the LTCF (for from 3 to 203 antimicrobial days each).

Detection and characterisation of ESBL-producing *E. coli*

As summarised in Table 2, ESBL-EC were detected in 35 (55%) of the 64 participants, including 28 (80%) by the first rectal swab, and seven (20%) by subsequent rectal swabs. For the 24 positive ESBL-EC participants with at least two available follow-up (3 or more total) swabs, 75% remained positive for ≥ 6 months and 42% remained positive for ≥ 1 year. Most (71%) of the ESBL-EC-colonised participants were from (high-dependency) care areas 1 and 2. Approximately half (51%) of baseline participants remained un-colonised throughout, and when new admissions were taken into account, 45% of residents were not colonised.

The 98 ESBL-EC isolates were recovered from rectal (n = 95) and urine samples (n = 3) and were uniformly resistant to ciprofloxacin, and susceptible to cefoxitin, ertapenem, meropenem, and gentamicin. They all belonged to the *H*30-Rx ST131 subclone and carried $bla_{CTX-M \ group \ 1}$. Eighty-five (87%) also contained bla_{OXA-1}, whereas none contained bla_{TEM} or bla_{SHV} (Table 3). All 85 bla_{OXA-1}-positive isolates were co-amoxiclav-resistant, whereas all bla_{OXA-1} negative isolates were co-amoxiclav-susceptible.

In a PFGE dendrogram that included each colonised resident's first ESBL-EC isolate (n = 35) and selected reference isolates, the study isolates clustered with the three UK and international reference ST131 strains at 72% profile similarity (Figure 1). The *H*30-Rx ST131 isolates were divided into two clusters (clusters A and B), each with ≥ 85% profile similarity. Cluster A comprised 91 isolates from 33 participants and included reference isolates for UK strain A and international PFGE812. Cluster B comprised six isolates from two participants and UK strain D.

Detection and characterisation of ESBL- producing *K. pneumoniae*

At baseline, none of the participants tested positive for ESBL-KP; however, five subsequently tested positive, including three from a high dependency area and two from a low dependency area. The positive tests occurred at study months 3, 6, 9, and 12, in 2, 0, 2, and 2 participants, respectively (Table 2). Of the five ESBL-KP-colonised individuals, four were also colonised with ESBL-EC, therefore in total there were 36 residents colonised with ESBL-producing *Enterobacteriaceae*. None of the five ESBL-KP-colonised individuals had previously been identified as an ESBL carrier and only one had been hospitalised or received antimicrobials within three months of first being detected positive. That individual was newly admitted to the LTCF in May 2013 with ESBL-KP already present in the LTCF. All six ESBL-KP isolates were resistant to ciprofloxacin and gentamicin, and susceptible to piperacillin/tazobactam, cefoxitin, ertapenem, and meropenem. The six isolates were 91% similar by PFGE (Figure 2) and positive for $bla_{CTX-M \ group \ 1}$, bla_{SHV}, and bla_{OXA-1} (Table 3). The two isolates collected in October 2012 from different participants were indistinguishable by PFGE. The four subsequent isolates,

Table 1 Demographics and clinical details of the 64 long-term care facility participants

Factor	Description	N	% of 64
Gender	Male	34	53
	Female	30	47
Admission group	Hospital	6	9
	Long-term care facility	19	30
	Home	39	61
Diabetic	Yes	16	25
Care area	1&2	30	47
	3&4	34	53
Urinary catheter	Yes	9	14
Wound/ulcer	Yes	8	13
Antimicrobials[a]	In year previous	31	48
Antimicrobials[b]	During study	33	52
Hospitalisation[c]	In year previous	11	17
Hospitalisation[d]	During study	37	58
	Mean (SD)	**Min-max**	**Median**
Age	80 (10.52)	37-98	80
Initial barthel index	33 (33.17)	0-100	17.5

[a-d]Details were only accessible for participants in the long-term care facility during the time period, therefore no information on prior hospitalisation and antimicrobial usage was included for new admissions.

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Table 2 Results for all participants tested from 0 to 12 months for colonisation with the targeted antimicrobial resistant organisms

Resistance phenotype	Proportion colonised (%)					
	July 2012[f]	October 2012[f]	January 2013[f]	May 2013[f]	August 2013[f]	Any time point
ESBL-EC[a]	20/51 (39%)	21/51 (41%)[7]	14/50 (28%)	21/49 (43%)[7]	19/45 (42%)[7]	35/64 (55%)
ESBL-KP[b]	0/51 (0%)	2/51 (4%)	0/50 (0%)	2/49 (4%)	2/45 (4%)	5/64 (8%)
CPE[c]	0/51 (0%)	0/51 (0%)	0/50 (0%)	0/49 (0%)	0/45 (0%)	0/64 0%)
VRE[d]	0/51 (0%)	0/51 (0%)	1/50 (2%)	1/49 (2%)	1/45 (2%)	2/64 (3%)
MRSA[e]	8/51 (16%)	9/51 (18%)	7/50 (14%)	7/49 (14%)	4/45 (9%)	17/64 (27%)
Any of above	24/51 (47%)	25/51 (49%)	17/50 (34%)	23/49 (47%)	20/45 (44%)	39/64 (61%)

[a]ESBL-EC; Extended-spectrum beta-lactamase producing-*Escherichia coli*.
[b]ESBL-KP; Extended-spectrum beta-lactamase-producing-*Klebsiella pneumoniae*.
[c]CPE; Carbapenemase-producing *Enterobacteriaceae*.
[d]VRE; Vancomycin-resistant enterococci.
[e]MRSA; Meticilin-resistant *Staphylococcus aureus*.
[f]Number of participants tested at each interval varied, accounting for participants who died, withdrew from the study, or newly joined the study.
[g]One resident had an ESBL-EC positive urine and rectal culture.

collected from three participants in May and August 2013 (one patient was positive in both May and August), were indistinguishable from one another by PFGE but differed from the previous isolates by 3 bands (Figure 2). In comparison with a collection of ESBL-KP isolates from other regions of Ireland (unpublished data, D. Morris) the present isolates exhibited 86% PFGE profile similarity to reference isolates from *K. pneumoniae* clonal complex (CC) CC43 (i.e., ST48 and ST123).

Detection and characterisation of CPE, VRE, and MRSA

Carbapenemase-producing *Enterobacteriaceae* were not detected and vancomycin-resistant *E. faecium* were detected in only two participants. MRSA were detected in 17 (27%) of the 64 participants, including 11 (65%) by the first nasal swab, and six (35%) by subsequent nasal swabs. The 17 MRSA colonised residents comprised 13 baseline participants; 8 of 13 positive on the first round of screening, and four new participants (Table 2). Of the 17 (27% of 64) total participants colonised with MRSA

at some point, six (35%) were positive for MRSA on two or more subsequent tests after their first positive test. Five (29%) of 17 MRSA-positive participants had been recognised as such prior to their first positive study test based on clinical samples from wound (n = 2) or nasal/ body swabs (n = 3).

Co-colonisation with multiple antimicrobial-resistant organisms

Co-colonisation with different AROs was common, with 14/36 (39%) ESBL-producing *Enterobacteriaceae*-positive participants also positive for MRSA, compared with 3/28 (11%) non-ESBL-colonised participants (P = 0.008). The two VRE-colonised participants were also colonised with ESBL-EC and MRSA. Of the 14 participants' positive for both ESBL-producing *Enterobacteriaceae* and for MRSA, 10 were high dependency with a Barthel Index score of 25 or less indicating immobility and no level of ability/requiring major help to perform activities of daily living. The remaining four participants had Barthel Index Scores

Table 3 Correlation of pulsed-field gel electrophoresis (PFGE) clusters with *bla* type, sequence type, and subclone among 124 antimicrobial-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates from long-term care facility residents

Organism	PFGE cluster	No. of isolates	*bla* genes, no. of isolates				ST^a and subclone (no. of isolates)
			$bla_{CTX-M-group-1}$ [b]	bla_{TEM}	bla_{SHV}	bla_{OXA-1}	
E. coli	EcA[c]	91	91	0	0	79	ST131 *H*30-Rx(91)
E. coli	EcA1[d]	2	1	0	0	0	ST131 *H*30-Rx (1)
E. coli	EcB[e]	6	6	0	0	6	ST131 *H*30-Rx (6)
K. pneumoniae	KpA[f]	6	6	0	6	6	ST1236/ST48 (6)[g]

[a]ST = sequence type (from multilocus sequence typing). Inferred from PCR-based assays for *E. coli* and by PFGE similarity to reference strains of known sequence type (ST) for *K. pneumoniae* strains.
[b]Although other bla_{CTX-M} genes were tested for including $bla_{CTX-M-group-2}$, $bla_{CTX-M-group-8}$, $bla_{CTX-M-group-9}$, and $bla_{CTX-M-group-25}$, none were detected other than $bla_{CTX-M-group-1}$.
[c]EcA = ESBL-EC (n = 91) represented in PFGE Cluster A and similar to representative of UK Strain A and international PFGE812.
[d]EcA1 = ESBL-EC (n = 1) demonstrating 83% similarity to strains in PFGE Cluster A.
[e]EcB = ESBL-EC (n = 6) represented in PFGE Cluster B.
[f]KpA = ESBL-KP PFGE cluster similar to ST1236.
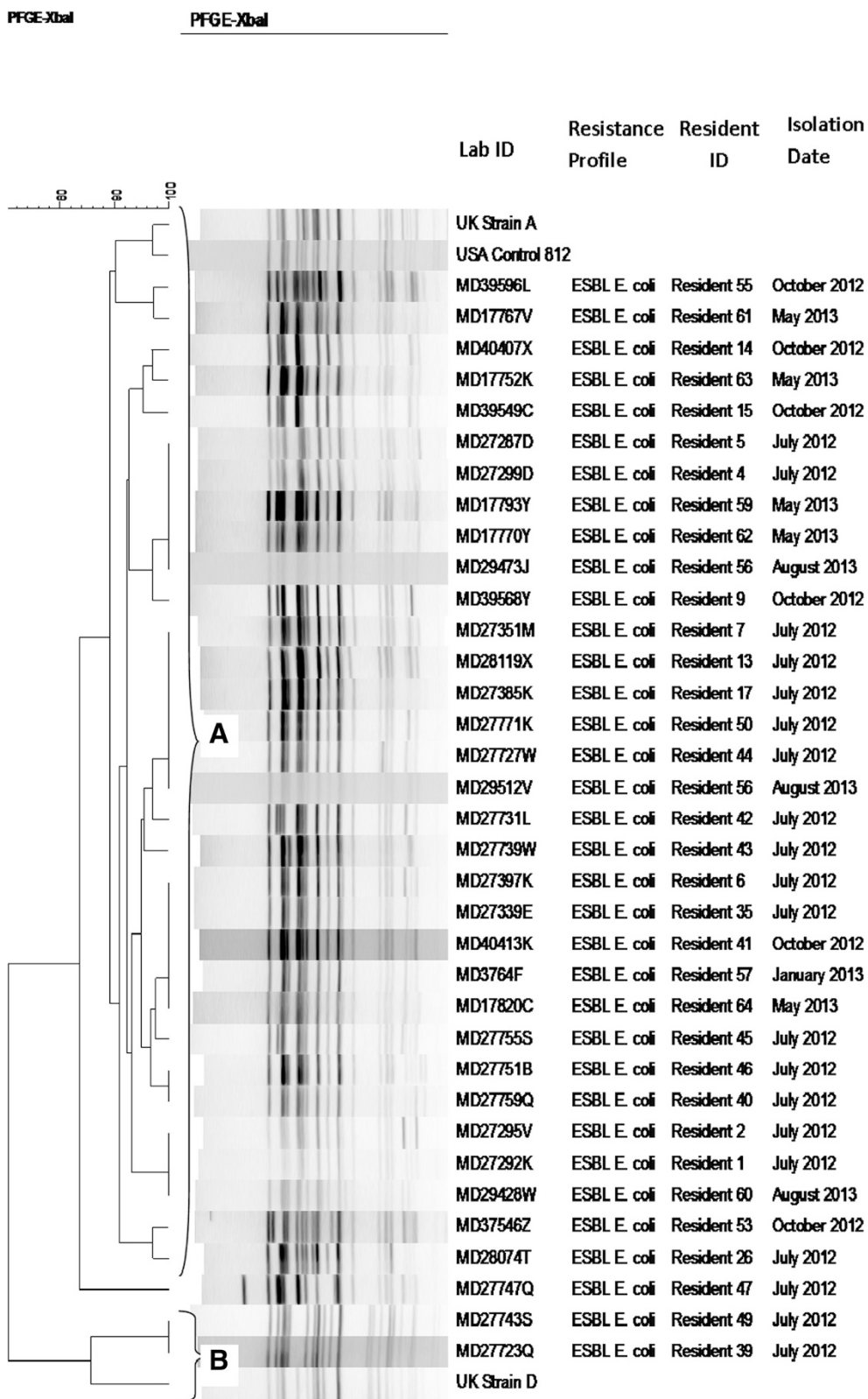[g]ST1236 is a single-locus variant of ST48; both are part of clonal complex 43.

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	Lab ID	Resistance Profile	Resident ID	Isolation Date
PFGE-XbaI				
	UK Strain A			
	USA Control 812			
	MD39596L	ESBL E. coli	Resident 55	October 2012
	MD17767V	ESBL E. coli	Resident 61	May 2013
	MD40407X	ESBL E. coli	Resident 14	October 2012
	MD17752K	ESBL E. coli	Resident 63	May 2013
	MD39549C	ESBL E. coli	Resident 15	October 2012
	MD27287D	ESBL E. coli	Resident 5	July 2012
	MD27299D	ESBL E. coli	Resident 4	July 2012
	MD17793Y	ESBL E. coli	Resident 59	May 2013
	MD17770Y	ESBL E. coli	Resident 62	May 2013
	MD29473J	ESBL E. coli	Resident 56	August 2013
	MD39568Y	ESBL E. coli	Resident 9	October 2012
	MD27351M	ESBL E. coli	Resident 7	July 2012
	MD28119X	ESBL E. coli	Resident 13	July 2012
	MD27385K	ESBL E. coli	Resident 17	July 2012
A	MD27771K	ESBL E. coli	Resident 50	July 2012
	MD27727W	ESBL E. coli	Resident 44	July 2012
	MD29512V	ESBL E. coli	Resident 56	August 2013
	MD27731L	ESBL E. coli	Resident 42	July 2012
	MD27739W	ESBL E. coli	Resident 43	July 2012
	MD27397K	ESBL E. coli	Resident 6	July 2012
	MD27339E	ESBL E. coli	Resident 35	July 2012
	MD40413K	ESBL E. coli	Resident 41	October 2012
	MD3764F	ESBL E. coli	Resident 57	January 2013
	MD17820C	ESBL E. coli	Resident 64	May 2013
	MD27755S	ESBL E. coli	Resident 45	July 2012
	MD27751B	ESBL E. coli	Resident 46	July 2012
	MD27759Q	ESBL E. coli	Resident 40	July 2012
	MD27295V	ESBL E. coli	Resident 2	July 2012
	MD27292K	ESBL E. coli	Resident 1	July 2012
	MD29428W	ESBL E. coli	Resident 60	August 2013
	MD37546Z	ESBL E. coli	Resident 53	October 2012
	MD28074T	ESBL E. coli	Resident 26	July 2012
	MD27747Q	ESBL E. coli	Resident 47	July 2012
	MD27743S	ESBL E. coli	Resident 49	July 2012
B	MD27723Q	ESBL E. coli	Resident 39	July 2012
	UK Strain D			

Figure 1 (See legend on next page.)

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Figure 1 Dendrogram of *Xba*I pulsed-field gel electrophoresis (PFGE) profiles for *Escherichia coli* isolates. One representative of each PFGE type per subject was included in the dendrogram, which includes extended-spectrum β-lactamase-producing *E. coli* (ESBL-EC) (n = 35) isolates, plus reference strains representing UK Strains A and D, and international pulsotype labelled USA Control 812. Reference strains with ≥ 85% profile similarity to study isolates are shown. The dendrogram was generated using the unweighted pair group method with arithmetic mean (UPGMA) algorithm based on Dice similarity coefficients. Two PFGE clusters **(A and B)** were defined based on ≥ 85% profile similarity. Isolates labelled "ESBL *E. coli*" represent ESBL-EC.

ranging from 30 – 100, with only 1 participant being able to perform all activity of daily living independence from any help.

Environmental sampling

Four bedrooms and two common bathrooms were sampled based on the occupants' colonisation status and care area; one with ESBL-EC, VRE and MRSA, one with ESBL-EC and ESBL-KP, one with ESBL-EC only, one with ESBL-KP only, and one occupant did not have detectable colonisation with any of the target organisms. MRSA was recovered from 12/69 (17%) environmental swabs, and from 5/7 (71%) rooms sampled. The only two rooms in which MRSA was not detected included one shared toilet facility and the bedroom of a resident in whom colonisation with ESBL-producing *Enterobacteriaceae*, VRE, and MRSA was not detected. Among the five MRSA-positive rooms, MRSA was recovered at least once from each of the 11 types of surfaces sampled, except for tap handles, railing beside the toilet, and the on-call button. ESBL-producing *Enterobacteriaceae* and VRE were not detected in environmental cultures.

Longitudinal analysis of ESBL and MRSA colonisation

For ESBL-producing *Enterobacteriaceae*, receipt of any antimicrobial in the previous 3 months more than doubled the odds of colonisation (odds ratio 2.4 (1.5-3.8)).

In a more detailed model that included antimicrobial class, use in the previous three months of broad-spectrum beta-lactam antimicrobials (OR 2.1 (1.2-3.4)) or nitrofurantoin (OR 3.5 (1.1-11.3)) was independently associated with ESBL colonisation. Care area was also an independent risk factor, with participants in (high-dependency) areas 1 and 2 having a nearly four-fold increased odds of ESBL colonisation (OR 3.9 (1.6-9.7)).

For MRSA, receipt of any antimicrobials in the previous three months conferred a 3.1-fold (1.1-9.1) higher odds of testing MRSA-positive. Care area was also independently associated with MRSA colonisation, with participants in care areas 1 and 2 having a 6.9-fold (1.6-29.3) higher odds of testing MRSA-positive. Additional independent correlates of MRSA colonization included previous MRSA positivity (OR 17.7 (3.8-83.1)) and age (OR 1.12 (1.0-1.2)). No specific antimicrobial class was independently associated with MRSA colonisation.

Discussion and conclusions

This is the first study to our knowledge that provides longitudinal data on colonisation with AROs and risk factors for colonisation in a LTCF. The need for such studies has been recognised as urgent in a recent editorial on *E. coli* ST131 [31]. Our study yielded nine key findings. First, ESBL-EC were detected in more than 50% of participants of the study LTCF. Second, MRSA
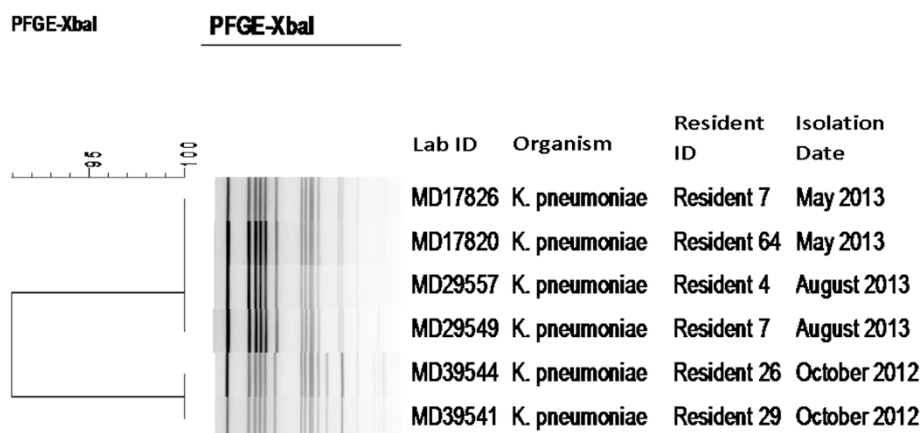

Figure 2 Dendrogram of *Xba*I pulsed-field gel electrophoresis (PFGE) profiles for *Klebsiella pneumoniae* isolates. PFGE dendrogram of the six ESBL-producing *Klebsiella pneumoniae* isolates, from 5 participants from October 2012 – August 2013. The dendrogram was generated using the unweighted pair group method with arithmetic mean (UPGMA) algorithm based on Dice similarity coefficients. All profiles are ≥ 91% similar.

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were detected in more than 25% of participants, and co-colonisation of ESBL-EC and MRSA was common. Third, care area (corresponding with level of dependency) and antimicrobial use were risk factors for carriage of ESBL-EC and MRSA. Fourth, all ESBL-EC isolates belonged to the *H*30-Rx ST131 subclone. Fifth, PFGE divided the *H*30-Rx subclone into two clusters, one of which included UK Strain A and a representative of the international PFGE812 group, the other included UK strain D. Sixth, all ESBL-EC and ESBL-KP contained $bla_{CTX-M\ group\ 1}$. Seventh, once subjects acquired ESBL-EC they tended to remain colonised with the same strain. Eight, we observed that although ESBL-producing *Enterobacteriaceae* were recovered from a much higher proportion of participants than MRSA, ESBL-producing *Enterobacteriaceae* was not detected in the environment, whereas detection of MRSA was common. Finally, 39% of residents did not become colonised with ESBL-EC or MRSA at any time, despite sharing the same LTCF with colonised residents from up to a year. These findings have significant implications for our understanding of the relationship between LTCF and the problem of acquired resistance to antimicrobial agents.

Several studies have assessed the prevalence of colonisation with ESBL-EC among LTCF participants. Our findings are broadly consistent with those of a previous study in Northern Ireland. However, our observed colonisation prevalence with ESBL-EC (56%) is higher than that study's (41%) [20], and is much higher than noted in previous international LTCF surveillance studies, including studies from 2008–2010 and 2003–2012 from Sweden identified ESBL- EC in just 1/268 (0.004%) and 2/1131 (0.002%) residents respectively. Similarly, studies performed in 2010 and 2011 from Melbourne identified ESBL-EC in 14/119 (12%) and 12/115 (10%) of LTCF residents respectively [41-44].

MRSA colonisation was detected at some time in 27% of participants, which is similar to a previous study from Northern Ireland (23% of residents in 45 LTCFs), but higher than an earlier study performed in six LTCFs in Ireland between 1995 and 1996 (9% and 10% respectively) [23,45]. It is possible that 27% represents an underestimate of colonisation, since practical constraints limited our sampling to nasal swabs. A low prevalence of MRSA colonisation among LTCF residents was reported from Sweden, the Netherlands, and Denmark [46-48]. However, these countries have a low proportion of MRSA bloodstream infections compared with Ireland [17], which suggests that MRSA is less prevalent overall as a healthcare-associated organism in those countries compared with Ireland.

Co-colonisation with ESBL-producing *Enterobacteriaceae* and MRSA was common (39%). Although VRE colonisation was uncommon (3%), it likewise was associated with ESBL-EC and MRSA colonisation. Elsewhere, VRE colonisation of LTCF residents was not detected in Belgium, but has been reported in Australia (2%), Israel (10%), and in the USA (4 to 45%) [42,49-52]. Similarly, Donskey and colleagues (2003) found that, in a Department of Veterans Affairs medical centre (which includes an acute care facility and a separate nursing home), colonisation with MRSA and ceftazidime-resistant Gram-negative bacilli was more prevalent among VRE-positive than VRE-negative patients [53]. Thus our findings of co-colonisation are consistent with existing literature.

Colonisation with ESBL-producing *Enterobacteriaceae* and MRSA were associated with residence in particular care areas within the LTCF and with antimicrobial consumption. Specifically, participants in care areas 1 and 2 were 3.9 odds more likely to be ESBL colonised, and 6.9 odds more likely to be MRSA colonised. The association with care area may plausibly reflect the interaction of a number of factors associated with providing care for the most dependent residents, including greater resident vulnerability, and more frequent contact with healthcare workers. This reflects the challenges of containing spread of AROs while providing appropriate social, psychological, and physical care for highly dependent residents in a LTCF.

Participants also had a 2.4-fold higher odds of ESBL colonisation and 3.1-fold higher odds of MRSA colonisation if they received antimicrobial in the three months before sampling. Fifty-two percent of participants received antimicrobials during the one-year study period. Broad spectrum beta-lactam antimicrobials (included amoxicillin, co-amoxiclav, and cefuroxime) were the most frequently consumed antimicrobials, with amoxicillin-clavulanic acid accounting for the majority. This is consistent with the European Surveillance of Antimicrobial Consumption point prevalence studies, which identified beta-lactam antimicrobials and penicillins as the most common antimicrobials prescribed in Irish LTCFs [54]. The 2013 HALT survey for Ireland reported 9% and 11% respectively as the median overall antimicrobial use prevalence in general nursing homes (n = 103) and mixed care facilities (n = 26), both with length-of-stay > 12 months [55]. Likewise, in 722 European LTCFs the most commonly prescribed agents were amoxicillin-clavulanic acid (12.7%), nitrofurantoin (10.4%), trimethoprim (9.9%), amoxicillin (7.3%), and ciprofloxacin (6.9%) [24]. A higher point prevalence of antimicrobial prescribing (16%) has been reported in LTCFs in other European studies [56,57]. Benoit *et al.* [58] reported 42% of residents in 73 LTCFs as receiving antimicrobials in the previous six months in the USA.

Given that third-generation cephalosporins and fluoro-quinolones were used infrequently in this facility, it appears that selective pressure from these agents is not

likely to be a major factor in the high prevalence of colonisation with ESBL-producing *Enterobacteriaceae* or MRSA. However, we note that in this LTCF the dominant ESBL-*Enterobacteriaceae* and MRSA are resistant to broad-spectrum beta-lactams, the most commonly consumed antimicrobials in the LTCF; thus, the overall association of colonisation with antimicrobial consumption is not surprising from a microbiological perspective.

It was surprising that nitrofurantoin was independently associated with ESBL colonisation (OR 3.4 (1.1-11.3)), since it is generally considered to have limited selective impact on gut flora [59]. However, record review indicated that 8/9 participants who received nitrofurantoin prior to sampling had already been identified as ESBL-positive before the nitrofurantoin was prescribed, based on clinically-submitted urine samples. Since nitrofurantoin use in these patients was most likely guided by prior susceptibility test reports, and since nitrofurantoin is one of few oral antimicrobials active against ESBL-EC, this association can be explained on that basis.

The finding that all ESBL-EC isolates represented the ST131 subclone contributes to the evidence that dissemination of ST131 is a global public health problem in LTCFs. Banerjee *et al.* [60] reported that, among consecutive *E. coli* clinical isolates from Rochester, Minnesota, ST131 accounted for 80/299 (27%) of isolates from healthcare or community-associated infections, but for fully 28/37 (76%) of isolates from LTCFs. Rooney *et al.* and Dhanji *et al.* reported ST131 representing 99% of ESBL-EC collected from residents of LTCFs in Northern Ireland [20,61]. The proportion of ST131 we encountered among faecal ESBL-EC was similar to these reports, and higher than in other international studies of LTCF residents [20,60,61]. The predominance of ST131 in LTCFs suggests that such facilities may serve as reservoirs for dissemination of ST131. This problem is likely to be exacerbated by growing number of LTCF residents, which currently is approximately 19,800 (2012) in Ireland, 3.7 million in the EU (2010), and 1.5 million in the United States (2009) [24,25,31].

Genomic diversity within ST131 has been described previously based on sequencing the *fimH*, *gyrA*, and *parC* genes, PFGE, and whole genome analysis [3,6,7]. The *H*30 subclone and its *H*30-Rx subset have been identified as the major lineages of *E. coli* associated with antimicrobial-resistant infections. Colpan *et al.* [38] found that the *H*30 subclone as a whole accounted for ≥ 95% of fluoroquinolone-resistant and ESBL-producing ST131 isolates from US veterans. Price *et al.* [7] and Banerjee *et al.* [5] reported a high proportion of ESBL-EC as belonging to the *H*30-Rx subset; 91% and 92% respectively [5,7]. Similarly, in the present study all ESBL-EC belonged to the *H*30-Rx subclone. The *H*30-Rx subclone has a reported association with sepsis, suggesting that virulence may be contributing to its epidemiologic success [5,7].

Johnson *et al.* [3] and Colpan *et al.* [38] identified various pulsotypes within ST131, with PFGE968, PFGE800, and PFGE812 being the most prevalent [3,38]. Here, 91/98 (93%) of the *H*30-Rx isolates formed a single PFGE cluster, at the ≥ 85% similarity level. This group includes the ST131 UK epidemic strain A and a representative of international PFGE812 (Figure 1). The latter two reference strains represent, respectively, the most prevalent ST131 variant in the UK [40,62] and the most prevalent European-associated ST131 pulsotype in a large private PFGE library (unpublished data, J. R. Johnson) [3]. Thus, the predominance of this clonal group within the study LTCF is part of the international dissemination of the *H*30Rx ST131 subclone. However, six of the remaining eight *H*30-Rx isolates, from two participants, formed a distinct PFGE cluster, Cluster B (Figure 1). It is of interest that this variant was not entirely displaced by the predominant variant; thus, two variants of the *H*30-Rx subclone were circulating in the LTCF.

Our finding of high prevalence of bla_{CTX-M} group 1 is consistent with a previous reports from Banjeree *et al.* [5] and Price *et al.* [7] demonstrating a high prevalence (91%) of $bla_{CTX-M-15}$ within the *H*30-Rx subclone [5,7]. Additionally, we found that most isolates (87%) also carried bla_{OXA-1}, which was associated with co-amoxiclav resistance, reflecting inefficient inhibition of OXA enzymes by clavulanic acid [63]. This finding may be relevant to the success of this clonal group given the frequent consumption of co-amoxiclav.

Although few, the ESBL-KP in our study were 91% similar by PFGE (Figure 2). All carried $bla_{CTX-M\ group\ 1}$ and bla_{OXA-1} and were resistant to co-amoxiclav. The six ESBL-KP strains were 86% similar to other Irish isolates belonging to *K. pneumoniae* clonal CC43 (i.e., ST48 and ST123) This is consistent with clonal dissemination of ESBL-KP in Ireland, similar to that described elsewhere [64]. Various clonal groups of *K. pneumoniae* have been reported worldwide, such as CC43, which is commonly associated with resistance enzymes such as TEM-3, SHV-12, and CTX-M-15 [10,12]. We detected ESBL-KP for the first time in this LTCF three months after commencing the study. Given the propensity for dissemination of such strains of *K. pneumoniae* [65], it seems surprising that after nine months of follow-up only five participants had evidence of colonisation.

The duration of persistence of colonisation with ESBL-producing *Enterobacteriaceae* has previously been reported. The median time to clearance of ESBL-producing *Enterobacteriaceae* after hospital discharge was 6.6 months in a French study [66] and 98 days (i.e., approximately 3 months) in a study from Thailand [67]. A Swedish study by Titelman *et al.* [68] concluded that clearance of ESBL-producing *Enterobacteriaceae* has not occurred in many patients (43%) at 12 months after infection and

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that persistence is associated with phylogroup B2 *E. coli* carrying $bla_{\text{CTX-M group 9}}$. This study also demonstrated that failure to detect the organism on sampling does not reliably establish clearance [68]. However, these studies did not address residents of LTCFs.

We found that spontaneous clearance of colonisation of *E. coli* carrying $bla_{\text{CTX-M group 1}}$ was uncommon; 75% of ESBL-EC colonised participants who had two or more subsequent samples after their initial positive swab remained positive. Moreover, despite the presence within this LTCF of two different PFGE variants of ESBL-EC ST131, the variant identified in a given resident was stable over time. Thus, participants tended to remain colonised with the same ESBL-EC strain for an extended period. Furthermore, two initially ESBL-EC-colonised participants in whom ESBL-EC ST131 was not detected at 3 or 6 months reverted to being positive at 9 and 12 months, in both cases with isolates that were indistinguishable from or ≥ 95% similar to the previous isolates by PFGE. This is consistent with persistence, or auto-reinfection. As no environmental contamination with ESBL-producing *Enterobacteriaceae* was detected, auto-reinfection does not appear to be from an environmental reservoir but more likely from resident-resident interactions or resident-health care worker interactions. This may also indicate the need for caution in accepting failure to detect colonisation as confirmation of clearance.

Persistent colonisation was also common for MRSA. Of the 17 MRSA-colonised participants 73% of those who had two or more subsequent samples remained positive. Consistent with standard practice in LTCF in Ireland this LTCF had no programme of routine resident MRSA decolonisation.

Regarding environmental contamination, MRSA was recovered much more commonly from the environment than was ESBL-EC, despite being recovered from a smaller proportion of participants, which is consistent with previous findings [69]. The greater frequency of environmental detection of MRSA compared with ESBL-*E. coli* may reflect more intense shedding of MRSA or better environmental survival. The environmental detection of MRSA compared with no ESBL-EC environmental contamination may reflect more intense shedding of MRSA or better environmental survival.

Interestingly, a large number of residents did not become colonised with ESBL-EC or MRSA throughout the year. This occurred despite an absence of restrictions on social interactions for residents colonised with MRSA or ESBL-*Enterobacteriaceae*, and frequent environmental contamination with MRSA. It is clear that, as the inverse of risk factors for colonisation, those who do not become colonised were less likely to have consumed antimicrobial agents and to reside in the high-dependency areas. However, other factors may contribute to colonisation

resistance. This phenomenon clearly merits further study.

The study has several limitations. First, although initial study enrolment was high and retention was good, both were incomplete, with represents one of many challenges in conducting a longitudinal LTCF-based study. Second, antimicrobial exposure may have been underestimated, since antimicrobial use during hospital admission was not captured. Third, although the observed clonality of the ESBL-EC and ESBL-KP study isolates supports dissemination within this LTCF, it is not possible from the current analysis to differentiate definitively between acquisition of ESBL-producing organisms within the LTCF vs. during episodes of hospitalisation or via other exposures. In future studies, emerging higher-resolution typing methods such as whole-genome sequencing could be used to better clarify transmission pathways [70].

Despite those limitations this study represents a significant contribution to research in this area given the lack of longitudinal studies in LTCFs and there are several novel and important findings. Antimicrobial prescribing in LTCFs is an immediately modifiable risk factor for colonisation with AROs. We demonstrate the clonality and temporal stability of ESBL-producing *Enterobacteriaceae* within a single LTCF. Although multiple ST131 variants were detected, all belong to the *H*30-Rx subclone [5,7]. Colonised participants tended to retain the same pulsotype over an extended period. We report the first identified ESBL-KP in an Irish LTCF. We note that although present in 5 participants this organism has not disseminated to the same extent as ESBL-EC (n = 35). Further studies are needed to clarify the acquisition, retention, and dissemination of antimicrobial-resistant clones within LTCFs and to establish if there are other characteristics of such individuals, e.g., the gut microbiota, that may contribute to such colonisation resistance.

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