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National point prevalence study on carriage of multidrug-resistant microorganisms in Dutch long-term care facilities in 2018

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Objectives: Long-term care facilities (LTCFs) may act as a reservoir of ESBL-producing Enterobacterales (ESBL-E) and carbapenemase-producing Enterobacterales (CPE) for hospitals and the general population. In this study, we estimated the prevalence and molecular epidemiology of rectal carriage with ESBL-E and CPE in residents of Dutch LTCFs between March 2018 and December 2018.

Methods: LTCFs were geographically selected across the country. For each LTCF, a random sample of residents were tested for ESBL-E and CPE in 2018. To identify risk factors for high carriage prevalence and/or individual carriage, characteristics of LTCFs and of a subset of the tested residents were collected. WGS was conducted on isolates from LTCFs with an ESBL-E prevalence of >10% and all CPE isolates to identify institutional clonal transmission.

Results: A total of 4420 residents of 159 LTCFs were included. The weighted mean ESBL-E prevalence was 8.3% (95% CI: 6.8–10.0) and no CPE were found. In 53 LTCFs (33%), where ESBL-E prevalence was >10%, MLST using WGS (wgMLST) was performed. This included 264 isolates, the majority being *Escherichia coli* ($n = 224$) followed by *Klebsiella pneumoniae* ($n = 30$). Genetic clusters were identified in more than half (30/53; 57%) of high ESBL-positive LTCFs. Among the *E. coli* isolates, $bla_{\text{CTX-M-15}}$ (92/224; 41%) and $bla_{\text{CTX-M-27}}$ (40/224; 18%) were the most prevalent ESBL-encoding genes. For *K. pneumoniae* isolates, the most common was $bla_{\text{CTX-M-15}}$ (23/30; 80%).

Conclusions: The estimated prevalence of ESBL-E rectal carriage in Dutch LTCFs is 8.3% and resistance is observed mainly in *E. coli* with predominance of $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-27}}$. ESBL-E prevalence in LTCFs seems comparable to previously reported prevalence in hospitals and the general population.

Introduction

The prevalence of antimicrobial resistance (AMR) in clinical isolates is on the rise, imposing an increasing public health threat in Europe. In the light of their emergence and activity against a number of β-lactam antibiotics, ESBL-producing Enterobacterales (ESBL-E) and carbapenemase-producing Enterobacterales (CPE) are MDR organisms (MDROs) of particular concern.[1] In the Netherlands, the number of infections caused by MDROs is still relatively low. To illustrate, in 2019, ESBL prevalence was found to be 6%–9% in Enterobacterales isolates from diagnostic samples of hospitalized patients.[2] Long-term care facilities (LTCFs) have been considered high-risk areas for MDRO transmission due to an older, frail population, with healthcare-associated infection rates matching acute settings in Europe.[3] High-level exposure to

antimicrobials,[4] frequent use of invasive devices[5] and (frequent) hospital admission[6] have been suggested as risk factors associated with MDRO acquisition in the elderly care population. However, evidence from LTCF settings remains scarce. A recent systematic review identified studies with small sample sizes and LTCFs across countries differing in case mix, age and care provided, hampering legitimate pooling of findings.[6] Insights into the prevalence of MDROs among residents of LTCFs is further hampered by the fact that microbiological diagnostics following suspected infection are infrequently performed.[2]

Also for the Netherlands, existing studies on ESBL-E carriage in LTCFs are largely confined to a single facility and/or regional settings.[7–10] The largest national study to date found an ESBL-producing *Escherichia coli* prevalence of 4.2% (95% CI: 3.3–6.0) among LTCF residents. These study findings likely represent an underestimation, while using urine and/or incontinence samples.[11] Indeed, higher ESBL carriage rates of 8.2% (range: 5.3–18.8) were identified among perianal swabs and faeces samples of hospitalized LTCF residents,[8] as well as subregional studies finding a mean of 10.9% (range: 0–20.6) and 14.5% (range 0–34) of ESBL carriage in a southern region[9] and urban regions, respectively.[12]

In the context of potential (regional) transmission between LTCFs and hospitals,[13] we aim to provide for the first time, to the best of our knowledge, a national representative estimate of the prevalence of ESBL-E and CPE in Dutch LTCFs. We explore risk factors, according to Dutch standards, for high prevalence (>10%) of ESBL-E and CPE on an institutional level, as well as risk factors for ESBL-E and CPE carriage on an individual, LTCF-resident level. Finally, we present a genetic characterization of a subsample of MDROs to identify the most common ESBL-encoding genes. Hence we intend to provide evidence for clonal transmission within LTCFs, as well as allow for comparison of prevalence and molecular epidemiology between LTCF, hospital and community settings.

Methods

Study design

We conducted a cross-sectional point prevalence study from March 2018 to December 2018 to determine the prevalence of rectal ESBL-E and CPE carriage among residents of LTCFs geographically spread across the Netherlands.

Selection of LTCFs and LTCF residents

LTCFs were recruited via the 10 Regional Care Networks (RCNs) for antibiotic resistance participating between March 2018 and December 2018. RCNs were requested to take a convenience sample of 30 eligible LTCFs in their region. To be eligible, LTCFs had to have a minimum of 30 beds, covering different types of care (i.e. long-term psychogeriatric or somatic care, or geriatric rehabilitation) and be supervised by an elderly care physician. Each participating LTCF was requested to create a list of residents and to include a random sample of 40 residents. In anticipation of a response rate of 50%, 80 residents per LTCF were invited. LTCFs were instructed to use a random number generator according to their preference; for convenience, the study protocol provided a guidance example using Excel. If an LTCF had fewer than 80 beds, all participants were invited. The responsible elderly care physician and the managing board of the individual LTCFs approved participation.

Data collection

Sample collection within each LTCF took place on one or, if necessary, a maximum of three consecutive sampling days if not all participants could be swabbed on the same day. Perirectal swabs were collected by one of the local LTCF nurses. Swabs were analysed for the presence of ESBL-E and CPE by local medical microbiological laboratories. To identify clustering of residents within LTCFs, all ESBL and CPE isolates were sent to the National Institute for Public Health and the Environment (RIVM) for genotyping in cases where an LTCF had: (1) an ESBL-E prevalence of >10%, i.e. higher than the highest ESBL rate found in Dutch community carriage rate studies;[14–18] (2) two or more residents with ESBL-producing *Klebsiella pneumoniae* (as *K. pneumoniae* has been associated with cross-transmission more frequently than *E. coli*, albeit in ICU settings);[19] or (3) one or more CPE carriers (to ensure timely identification and prevention of CPE cross-transmission, which to date has been sporadic in the Netherlands).

All participating LTCFs received a web-based survey to collect data on LTCF characteristics (Table 1). Furthermore, we aimed to collect detailed data on resident characteristics from 10% of the aimed sample size of 300 LTCFs (Table 2).

Microbiology and genotyping

Bacterial isolates

Perirectal swabs were inoculated in routinely used non-selective broth and cultured overnight at 37°C at the local microbiology laboratories. ESBL-selective CHROMagar™ plates (Rambach, Paris, France) were inoculated with the primary culture, whether this was turbid or not, and incubated overnight at 37°C. A maximum of five morphologically distinct colonies per swab were used for species identification using MALDI-TOF and were tested for ESBL and/or CPE phenotype according to the Dutch guideline 'Laboratory detection of highly resistant microorganisms'.[20] For ESBL, this entailed assessing the cefotaxime (or ceftriaxone) MIC and/or ceftazidime MIC and selecting isolates with MICs above 1 mg/L. For species in which AmpC β-lactamases rarely occur, the ESBL phenotype was confirmed by assessing resistance to cefotaxime and ceftazidime in the presence or absence of clavulanic acid. In the case of species in which AmpCs are common, the ESBL phenotype was confirmed by assessing resistance to cefepime in the presence or absence of clavulanic acid. Isolates with a meropenem MIC >0.25 mg/L or an imipenem MIC >1 mg/L were suspected to represent CPE and were subjected to the carbapenem inactivation method (CIM) test to assess carbapenemase production.[21]

Sequencing and genotyping

Next-generation sequencing (NGS) was performed using a standardized protocol.[22] To identify the presence of resistance genes, ResFinder 2.1[23] was used at a threshold of 90% identity and 60% minimum matching length. MLST and whole-genome MLST (wgMLST) of *E. coli* and *K. pneumoniae* isolates were performed using SeqSphere 3.5.0 (Ridom GmbH, Münster, Germany) using in-house wgMLST schemes.[21] All genotyping data were imported into a BioNumerics database for further analyses (BioNumerics v7.6, Applied Maths, Sint-Martens-Latem, Belgium). The wgMLST allelic profiles were compared in advanced cluster analyses as categorical data, ignoring missing genes. Based on analyses of multiple isolates obtained from the same patient and on isolates from patients in proven transmission events, we considered *K. pneumoniae* differing in no more than 20 wgMLST genes as the same strain and for *E. coli* we used a 25 wgMLST gene cut-off. For multiple indistinguishable isolates obtained from the same resident, only one isolate was included in further analyses.

Table 1. Facility-level characteristics of the LTCFs that participated in this study ($N = 144$)

LTCF characteristics	All LTCFs $N = 144$	LTCFs with <5% prevalence $N = 46$[h]	LTCFs with >10% prevalence $N = 37$[i]	Univariate OR <5% vs >10%	Multivariate OR <5% vs >10%
Number of residents included in study, median (IQR)[a]		30 (26–36)	30 (25–37)	—	—
>50 beds (vs ≤50, reference), *n* (%)[b]	116 (81)	42 (91)	32 (86)	—	
Type of long-term care provided, *n* (%)					
Revalidation[c]	21 (15)	6 (13)	10 (30)	2.5 (0.8–8.0)	1.5 (0.4–5.4)
Primary care nursing[b]	5 (3)	2 (4)	2 (5)	—	—
Somatic[c]	73 (51)	24 (52)	26 (70)	2.2 (0.9–5.5)	1.6 (0.6 4.7)
Psychosomatic[b]	127 (89)	42 (91)	31 (84)	—	—
Other[b]	11 (8)	3 (7)	1 (3)	NA	NA
IPC committee present, *n* (%)				NA	NA
No or unknown	10 (7)	0 (0)	3 (8)		
Yes	134 (93)	46 (100)	34 (92)		
IPC contact if no outbreak (per year), *n* (%)[b] (<5% vs >10%: 1 missing value; ≤10% vs >10%: 4 missing values)[d]				—	—
No IPC contact or unknown	7 (5)	2 (4)	3 (8)		
<4	8 (6)	4 (9)	1 (3)		
4–6	96 (69)	29 (63)	25 (68)		
>6	29 (21)	11 (24)	8 (22)		
IPC contact if outbreak (per week), *n* (%)[b] (<5% vs >10%: 11 missing values; ≤10% vs >10%: 15 missing values)[d]				—	—
No IPC contact or unknown	7 (5)	9 (20)	5 (14)		
≥1	43 (34)	16 (35)	14 (38)		
<1	44 (34)	11 (24)	5 (14)		
Depending on situation	35 (27)	10 (22)	13 (35)		
Contact with infection preventionist, *n* (%)				NA	NA
No or unknown	8 (5)	2 (4)	2 (5)		
Yes	136 (94)	44 (96)	35 (95)		
Infection preventionist contacted, *n* (%)[c]				—	—
No or unknown	29 (22)	8 (18)	9 (26)		
Yes	100 (78)	36 (82)	26 (74)		
Reason for contacting infection preventionist, *n* (%)[e] (≤10% vs >10%: 1 missing value)					
Outbreak[b]	122 (85)	37 (80)	34 (92)	2.8 (0.8–13.2)	2.0 (0.5–10.4)
Training[c]	91 (64)	34 (72)	24 (65)	—	—
Audits[c]	81 (57)	25 (54)	22 (60)	—	—
IPC meeting[b]	43 (30)	17 (37)	14 (38)	—	—
Questions[b]	15 (10)	6 (13)	2 (5)	—	—
ESBL carriers known, *n* (%)[c, f]				—	—
No or unknown	68 (47)	24 (52)	15 (41)		
Yes	76 (53)	22 (48)	22 (60)		
Number of ESBL carriers known, median (IQR)[a, f, g] (<5% vs >10%: 12 missing values; ≤10% vs >10%: 15 missing values)	1 (0–2)	1 (0–2)	2 (0–3)	1.3 (1.0–1.7)	1.2 (1.0–1.6)
CPE carriers known, *n* (%)[f]				NA	NA
No or unknown	142 (99)	46 (100)	37 (100)		
Yes	2 (1)	0 (0)	0 (0)		
Number of CPE carriers known, median (IQR)[g] (<5% vs >10%: 12 missing values; ≤10% vs >10%: 15 missing values)	0 (0–0)	0 (0–0)	0 (0–0)	NA	NA
MDRO outbreak, *n* (%)[b, d, f]				—	—

Continued

Table 1. *Continued*

LTCF characteristics	All LTCFs $N = 144$	LTCFs with <5% prevalence $N = 46$[h]	LTCFs with >10% prevalence $N = 37$[i]	Univariate OR <5% vs >10%	Multivariate OR <5% vs >10%
No or unknown	139 (97)	45 (98)	34 (92)		
Yes	5 (4)	1 (2)	3 (8)		
MDRO outbreak type, *n* (%)[f]				NA	NA
None	139 (97)	45 (98)	34 (92)		
ESBL	3 (2)	1 (2)	1 (3)		
MRSA	1 (1)	0 (0)	1 (3)		
Other	1 (1)	0 (0)	1 (3)		
How MDRO carriers registered, *n* (%)[e]					
Individual client dossier[b]	135 (94)	41 (89)	35 (95)	—	—
Label in digital system[c]	37 (26)	16 (35)	9 (24)	—	—
Dedicated MDRO register[b]	7 (5)	3 (7)	2 (5)	—	—
RCN, *n* (%)[b]				NA	NA
Noord Nederland (01)	12 (8)	3 (7)	2 (5)		
EuregioZwolle (02)	5 (3)	1 (2)	0 (0)		
GAIN (03)	19 (13)	3 (7)	4 (11)		
Utrecht (04)	11 (8)	3 (7)	4 (11)		
Noord-Holland West (05)	11 (8)	4 (9)	4 (11)		
Noord-Holland Oost-Flevoland (06)	6 (4)	3 (7)	2 (5)		
Holland West (07)	29 (20)	11 (24)	9 (24)		
Zuidwest NL (08)	24 (17)	6 (13)	8 (22)		
Noord-Brabant (09)	22 (15)	9 (20)	4 (11)		
LINK (10)	5 (3)	3 (7)	0 (0)		

NA, no test performed due to singularity (zero or low number of observations in one or more of the possible categories). For definition of MDRO see https://www.rivm.nl/documenten/wip-richtlijn-brmo-vwk.
[a]Assessed with Mann–Whitney *U*-test.
[b]Assessed with Fisher's exact test.
[c]Assessed with chi-squared test.
[d]To keep data from these LTCFs in the model analyses, all missing observations were imputed with 'No or unknown'.
[e]Non-exclusive categories.
[f]Up to 1 year prior to study.
[g]For those that filled out 'unknown', we imputed 0 ESBLs/CPEs known.
[h]Two LTCFs with a prevalence of <5% did not fill out the survey.
[i]Four LTCFs with a prevalence of >10% did not fill out the survey.

Statistical analysis

Power

To determine an absolute increase or decrease of 1%, assuming a baseline prevalence of 8% with 80% power, and 95% confidence on repeat of the study, sample size calculations identified a need for the inclusion of 12 000 LTCF residents. To determine a cross-sectional prevalence of 7%, 10% and 13%, significantly lower numbers of residents (1276, 865 and 643, respectively) were needed. Furthermore, to distinguish LTCFs with low and high prevalence, we estimated that a minimum of 20 residents per LTCF were required to estimate the prevalence on an individual LTCF level.

Prevalence estimates

Crude prevalences of ESBL-E and CPE nationally and for individual LTCFs separately were calculated by dividing the number of ESBL-E-positive and CPE-positive residents by the total number of residents. Additionally, a weighted national prevalence was estimated using the 'survey' package in R v3.5.1, allowing a finite population correction to account for the fraction of residents sampled from the total population within each LTCF and a potential cluster effect. A facility ESBL prevalence of >10% was considered 'high' and <5% was considered 'low'.

Statistical analysis risk factors

Risk factors for high ESBL-E prevalence (LTCF level; <5% versus >10%) were assessed by chi-squared test or Fisher's exact test for categorical variables, and by *t*-test and Mann–Whitney *U*-test for continuous variables using data from all LTCFs that provided complete data. Mean and SD were estimated for normally distributed continuous variables; median and IQR were estimated for non-normally distributed variables. Variables with a *P* value of <0.2 in the univariate analysis were included in a multivariate logistic model. A sensitivity analysis was conducted, comparing LTCFs having high ESBL-E prevalence (>10% prevalence) with LTCFs having ≤10% prevalence.

To investigate risk factors for individual ESBL-E carriage we fitted a logistic regression model including all five putative risk factors collected for the individual-resident level. A random intercept was included to account for clustering on an LTCF level. Logistic regression analyses were done using the 'stats' package and the 'lme4' package was used for the multi-level logistic regression analyses in R v3.6.1.

Table 2. Characteristics of residents of LTCFs that participated in this study for whom detailed information was available, by ESBL-E test result (*N* = 949)

Characteristics of residents (*N* = 949)	ESBL-E negative (*N* = 891)	ESBL-E positive (*N* = 58)	OR	aOR
Age, years (1 missing value)				
<75	100 (11)	7 (12)	Ref	
75–84	283 (32)	19 (33)	1.0 (0.4–2.5)	1.5 (0.5–4.1)
85–90	277 (31)	15 (26)	0.9 (0.3–2.3)	1.3 (0.4–3.5)
≥90	230 (26)	17 (29)	1.2 (0.5–3.0)	2.1 (0.8–6.2)
Gender (7 missing values)				
Female	611 (69)	34 (59)	Ref	—
Male	273 (31)	24 (41)	1.4 (0.8–2.5)	1.7 (0.9–3.2)
Admission indication (2 missing values)				
Psychogeriatic	588 (66)	19 (33)	Ref	—
Revalidation	31 (3)	1 (2)	1.2 (0.1–13.8)	1.6 (0.1–18.8)
Somatic	261 (29)	37 (64)	5.0 (2.7–9.5)	4.8 (2.5–9.2)
Other	9 (1)	1 (2)	7.5 (0.7–85.3)	8.0 (0.7–96.1)
Known ESBL carrier[a]				
No or unknown	882 (99)	52 (90)	Ref	—
Yes	9 (1)	6 (10)	16.2 (4.9–53.7)	19.4 (4.7–79.1)
Antibiotic exposure on day of study (11 missing values)				
No or unknown[b]	860 (97)	53 (91)	Ref	—
Yes	31 (3)	5 (9)	2.35 (0.8–6.6)	1.6 (0.5–4.9)

Ref, category used as reference group.
[a]Up to 1 year prior to study.
[b]Missing observations were imputed with 'No or unknown'.

Ethics

The medical ethics committee of the Amsterdam Medical Centers, location VUmc approved the study design (METC 2017.567). All residents, or their legal representative, provided written informed consent before participation. They were informed that a positive test result might imply the need for individual infection control measures. Sample collection was non-anonymous but data were processed and analysed in a pseudonymized manner by the researchers. Individual- and LTCF-level test results were only disclosed to the responsible elderly care physician of the respective LTCF.

Results

A total of 4420 residents from 159 enrolled LTCFs participated in the study. Of the LTCFs, 77% (123/159) had 20 residents or more and 91% (144/159) of LTCFs provided LTCF characteristics. The participating LTCFs had a median of 84 beds (IQR: 55–140), with most beds occupied by residents with a need for psychogeriatric [52 (32–88)] or somatic [21 (0–41)] care. The vast majority of LTCFs reported having an infection prevention and control (IPC) committee in place (134/144; 93%) and/or had contact with an infection prevention specialist (136/144; 94%). Over half of LTCFs (76/144; 53%) reported having had a known ESBL-E carrier within the 12 months prior to the study (Table 1). Two LTCFs reported known carriage of CPE within the 12 months before the study. Data on individual resident characteristics were received from 949/4420 residents (21%) residing in 32 LTCFs. The mean age of residents was 85 years (SD = 8.3) and 68% (645/942) were female; 64% (607/947) of the participants were psychogeriatric residents and 31% (298/947) were somatic care residents. Thirty-six of 938 residents (4%) had received antibiotics on the day of study.

National prevalence of ESBL-E and CPE in Dutch LTCFs

Among the 4420 participating residents, 358 (8.1%) carried ESBL-E. None of the residents carried CPE (Figure 1). This equalled an adjusted ESBL-E prevalence of 8.3% (95% CI: 6.8–10.0). Prevalence rates of individual LTCFs varied from 0% to 34%; 32% (51/159) and 42% (66/159) of LTCFs had a high (>10%) and low (<5%) ESBL-E prevalence, respectively. This was similar when limiting the sample to LTCFs that included at least 20 residents in their sample (high: 41/123; 33% and low: 48/123; 39%).

LTCF characteristics and possible risk factors for high ESBL-E prevalence in Dutch LTCFs

Based on our multivariate analysis none of the explored potential risk factors at the LTCF level was associated with a high prevalence of ESBL-E. Neither did a sensitivity analysis, comparing LTCFs having ≤10% (instead of <5%) with >10% prevalence, identify statistically significant risk factors for high ESBL-E prevalence (Table S1, available as Supplementary data at *JAC* Online).

Possible risk factors for ESBL-E carriage among LTCF residents

Among the 949 residents who provided individual characteristics, the prevalence of ESBL-E carriage was 6% (58/949). In the

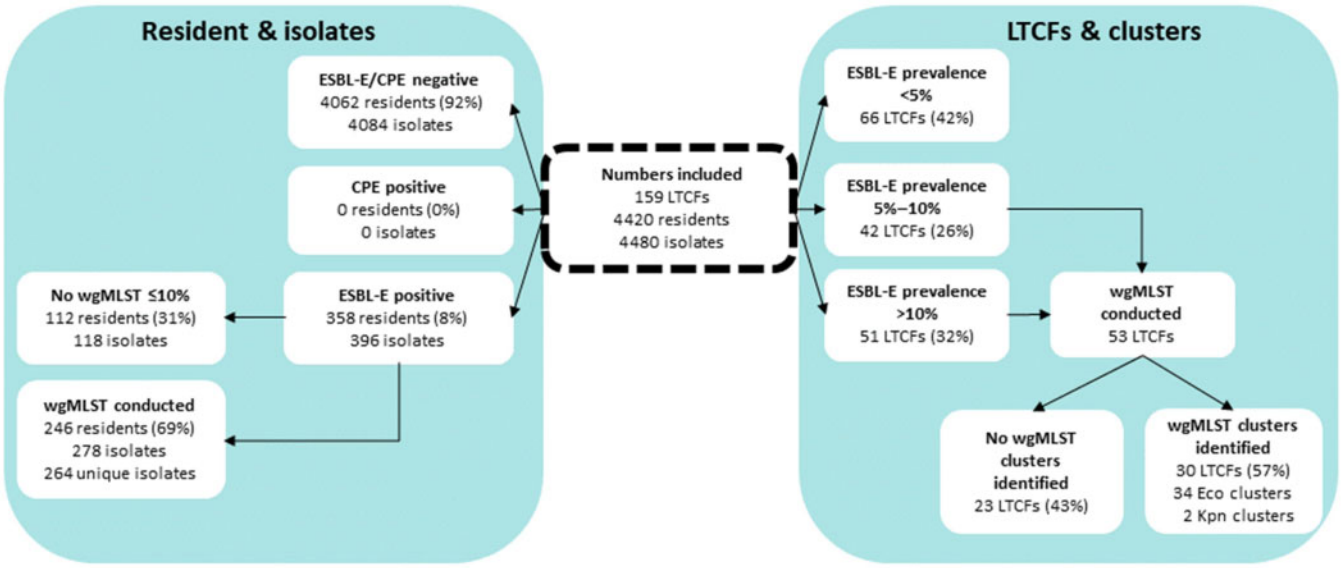

Figure 1. Summary of ESBL-E and CPE test results and clustering shown at resident level ($N = 4420$) and facility level ($N = 159$). This figure appears in colour in the online version of *JAC* and in black and white in the printed version of *JAC*.

multivariate analysis, residents with an LTCF admission indication for somatic care were more likely to carry ESBL-E [adjusted OR (aOR): 4.8; 95% CI: 2.5–9.2, Table 2], as well as residents with previous detection of ESBL-E carriage up to 1 year prior to the study, albeit with a large uncertain magnitude (aOR: 19.4; 95% CI: 4.7–79.1).

Genetic relationship between ESBL-E isolates

The medical microbiological laboratories identified 396 ESBL-E isolates from 358 residents in 53 LTCFs, with 264 (66.7%) of these isolates being genotyped. The main reason for genotyping was an ESBL-E prevalence of >10% (51/53; 96%) (Figure 1). *E. coli* (224/264; 85%) and *K. pneumoniae* (30/264; 11%) were the primary species identified (Figure 2), with four residents carrying both bacterial species.

Genetic clusters were detected in more than half (30/53; 57%) of LTCFs, comprising 35 within-LTCF clusters, or 36 clusters when isolates were allowed to cluster between LTCFs. The vast majority (34/36; 94%) comprised *E. coli* clusters (Eco clusters) with an average size of 3 isolates (range 2–10; Table S2). Furthermore, two *K. pneumoniae* clusters (Kpn clusters) were identified (Table S3). In nine Eco clusters, isolates originated from multiple LTCFs (mean: 3 LTCFs; range 2–4; Figure 3). The two isolates in Kpn cluster 02 were from a single LTCF, whereas the three isolates in Kpn cluster 01 were cultured from three residents and two LTCFs (Table S3).

Distribution of ESBL genes

Twelve distinct ESBL genes were identified in *E. coli* and mostly a single ESBL gene was present per isolate. In 14/244 (6%) *E. coli* isolates, no ESBL gene was found (Figure 2). The $bla_{\text{CTX-M-15}}$ gene was the predominant ESBL gene, present in 40% (89/224) of the *E. coli* isolates (Figure 2) and 38% (13/34) of Eco clusters (Figure 4, Table S4). The other most frequently found genes were $bla_{\text{CTX-M-27}}$ (16%), $bla_{\text{CTX-M-1}}$ (11%) and $bla_{\text{CTX-M-14}}$ (10%).

In *K. pneumoniae* we found five ESBL genes and eight ESBL gene combinations, with no ESBL gene being found in one isolate. In *K. pneumoniae*, $bla_{\text{CTX-M-15}}$, either as a single ESBL gene or in combination with bla_{SHV} genes, was the dominant ESBL gene, present in 77% (23/30) of the *K. pneumoniae* isolates (Figure 2). ESBL genes were not found in one of the *K. pneumoniae* isolates in two genetic clusters and in two other clusters no ESBL genes were identified in any of the isolates.

Discussion

In this national cross-sectional study on ESBL-E and CPE prevalence in Dutch LTCFs, we found an adjusted ESBL-E overall prevalence of 8.3% (95% CI: 6.8–10.0) and no CPE carriers. The ESBL-E prevalence ranged from 0% to 34% between LTCFs, with, for the Netherlands, a high prevalence (>10%) in one-third of the LTCFs. We did not identify risk factors for high LTCF-level prevalence, but on individual-resident level, having an ESBL-E identified up to 1 year before study inclusion or admission to somatic care was associated with higher risk of ESBL-E carriage.

Our prevalence estimate is in line with the 6%–9% prevalence of ESBL-E among hospitalized patients (excluding ICUs) based on routine diagnostic samples,[2] but suggests ESBL-E carriage among Dutch LTCF residents is—accounting for CIs—just above estimates found in the largest ESBL study in the Dutch general community (5%; 95% CI: 3.4–6.6).[18] Moreover, the predominant ESBL gene in both *E. coli* and *K. pneumoniae* isolates, $bla_{\text{CTX-M-15}}$, was similar to those found in community and hospital settings. *E. coli* producing $bla_{\text{CTX-M-15}}$ is now considered the most common cause of community-onset urinary tract infections in Europe and elsewhere.[24] Similarly, in the Netherlands, two large prevalence studies among inhabitants living in a poultry-rich area[25] and patients obtaining clinical diagnostics,[26] have shown predominance of $bla_{\text{CTX-M-15}}$ in third-generation cephalosporin-resistant Enterobacterales.

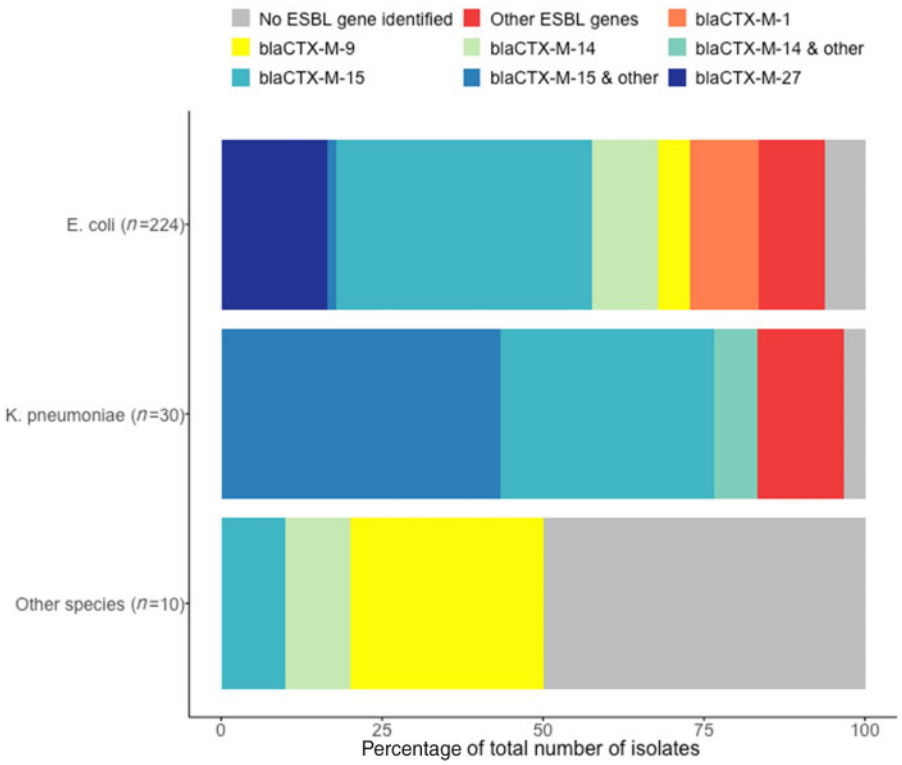

Figure 2. Distribution of species and ESBL genes identified among the ESBL-E-positive isolates using NGS data. Other single ESBL genes for *E.coli*: bla_{SHV-12} ($n=6$); $bla_{TEM-M-52B}$ ($n=6$); $bla_{CTX-M-55}$ ($n=5$); $bla_{CTX-M-3}$ ($n=3$); $bla_{CTX-M-32}$ ($n=2$); $bla_{CTX-M-14b}$ ($n=1$); and $bla_{CTX-M-164}$ ($n=1$). ESBL genes combined with $bla_{CTX-M-15}$ for *E. coli*: $bla_{CTX-M-27}$ ($n=3$). Other single ESBL genes for *K. pneumoniae*: bla_{SHV-12}; bla_{SHV-40}; $bla_{SHV-187}$; and $bla_{TEM-52B}$ ($n=1$ for each). ESBL genes combined with $bla_{CTX-M-14}$ for *K. pneumoniae*: bla_{SHV-13} ($n=1$); and $bla_{SHV-187}$ ($n=1$). ESBL genes combined with $bla_{CTX-M-15}$ for *K. pneumoniae*: bla_{SHV-40} ($n=4$); $bla_{SHV-187}$ ($n=3$); bla_{SHV-27} ($n=2$); bla_{SHV-69} ($n=2$); bla_{SHV-65} ($n=1$); and bla_{SHV-13} ($n=1$). Other species were *Citrobacter freundii* ($n=3$), *Enterobacter cloacae* complex ($n=3$), *Proteus hauseri* ($n=2$), *Citrobacter youngae* ($n=1$) and *Klebsiella aerogenes* ($n=1$). This figure appears in colour in the online version of *JAC* and in black and white in the printed version of *JAC*.

With prevalence rates and ESBL gene types comparable between hospital and LTCF settings, it remains unclear whether LTCF residents act as a potential reservoir for ESBL-E transmission as suggested for other European countries, e.g. Northern Ireland[27] and Italy.[28] Data on the hospital admission history of our study participants could have provided further insight into the interconnectedness of transmission events, but such data were not collected as part of this study. In particular, hospital and long-term care settings frequently exchange individuals, allowing for the potential for introduction and subsequent transmission of resistant bacteria.[13] Whether this could also explain clusters of genetically similar isolates from multiple LTCFs remains to be explored. A recent Dutch study among 12 LTCFs confined to one urban area showed that despite higher overall ESBL-E prevalence of 14.5% (range: 0–34), similar to our study, there was evidence for transmission within LTCFs, with one cluster involving multiple LTCFs.[12] Moreover, unnoticed transmission across regions and settings has been identified for CPE in the Netherlands,[29] with shared healthcare exposure as a proposed hypothesis.

Identification of ESBL-E carriage up to 1 year before study inclusion and being admitted for somatic care were identified as risk factors for ESBL-E carriage among LTCF residents. The first is in line with other studies summarized by Flokas and colleagues[6] and is likely explained by long-term carriage of ESBL-E. As shown by Wielders and colleagues,[17] ESBL-E isolates remain detectable for 5 months in half, and for 8 months in one-third of ESBL-E carriers in the open population, suggesting our ESBL-E-positive residents could have been known carriers. We did not find an association between antibiotic exposure and ESBL-E risk, contrasting with previous studies, but this may be a consequence of our limited information on antibiotic use (i.e. exposure on the day of study).

Our study has several other limitations. As we took a convenience sample of LTCFs, this may have resulted in the inclusion of LTCFs that are more concerned with IPC. Our multivariate analysis comparing LTCFs experiencing high ESBL prevalence with low ESBL-prevalence LTCFs was based on small numbers in the respective groups, resulting in an underpowered analysis. Therefore, we can not make hard conclusions on which LTCF characteristics, including our measured IPC indicators (e.g. IPC committee in place) are associated with low prevalence rates, nor which ones are well-measured proxies for IPC performance. Primary reasons for non-participation of LTCFs were lack of time and staff capacity. An important strength of our study is that with 4420 individuals sampled this study comprises the largest MDRO study in LTCFs conducted in the Netherlands. This point prevalence estimate could be established with high power and certainty, despite not reaching our intended sample size of 12 000 residents, while sample size calculations were aimed at allowing estimation of

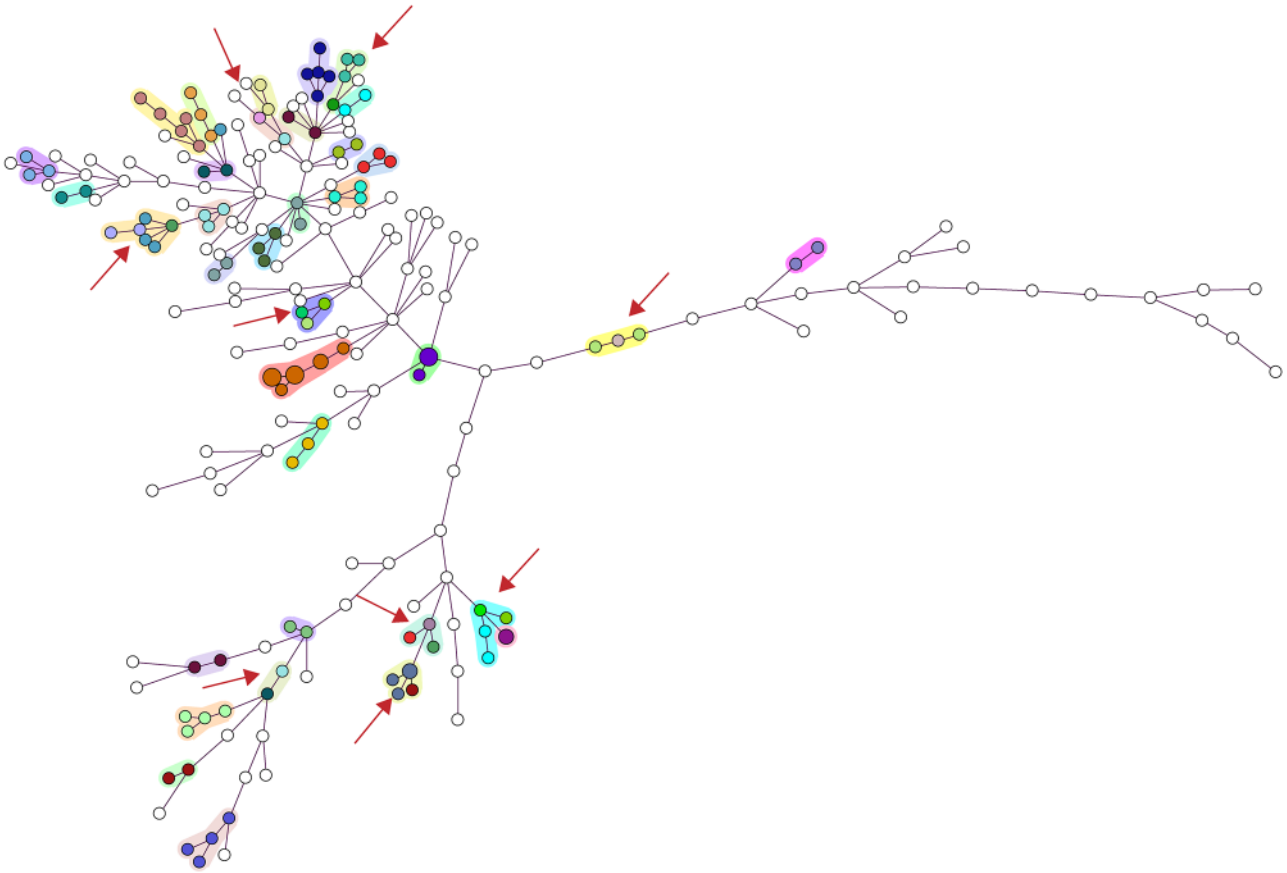

Figure 3. Clustering of ESBL-producing *E. coli* isolates in LTCFs with high prevalence. Minimum spanning tree of wgMLST of 224 *E. coli* isolates obtained from 201 residents. Each circle represents a wgMLST ST and the size of the circle denotes the number of isolates with that particular wgMLST ST. The connections between types denote the distance in number of genes, with logarithmic scaling of the connections. The coloured halos indicate the genetic clusters. Distinct colours within halos correspond to LTCFs from which the isolates within the genetic clusters were obtained. The arrows indicate the genetic clusters in which isolates were obtained from multiple LTCFs. This figure appears in colour in the online version of *JAC* and in black and white in the printed version of *JAC*.

trends over time and comparing LTCFs with low and high prevalence. In addition, our study gives, for the first time to the best of our knowledge, insight into the molecular background of ESBL-E in LTCFs on a nationwide level.

In conclusion, we find the prevalence rates and molecular epidemiology in humans to be comparable in LTCF and hospital settings, and only marginally higher than in the general community. Recent studies show that bacterial populations and the prevalence of resistance genes between human and animal reservoirs differ, and suggest human-to-human transmission to be the most likely source of ESBL-E acquisition, e.g. related to household transmission,[30–32] although estimated reproduction numbers for community transmission[31] and even ICU transmission[19] of ESBL-E are low. Therefore, following the work presented here, we now have detailed insight into the ESBL-E prevalence and dynamics in all of the most important niches of the Netherlands. To continue monitoring trends in ESBL-positive microorganisms and other MDROs, well-designed surveillance systems, based on routine community and clinical sampling, remain of utmost importance to monitor and control AMR epidemiology. These should be complemented with periodic point prevalence studies, proportional in terms of cost to the scale of the AMR burden in the Netherlands, to assess any increasing trends in LTCF and community settings, and better understand MDRO epidemiology.

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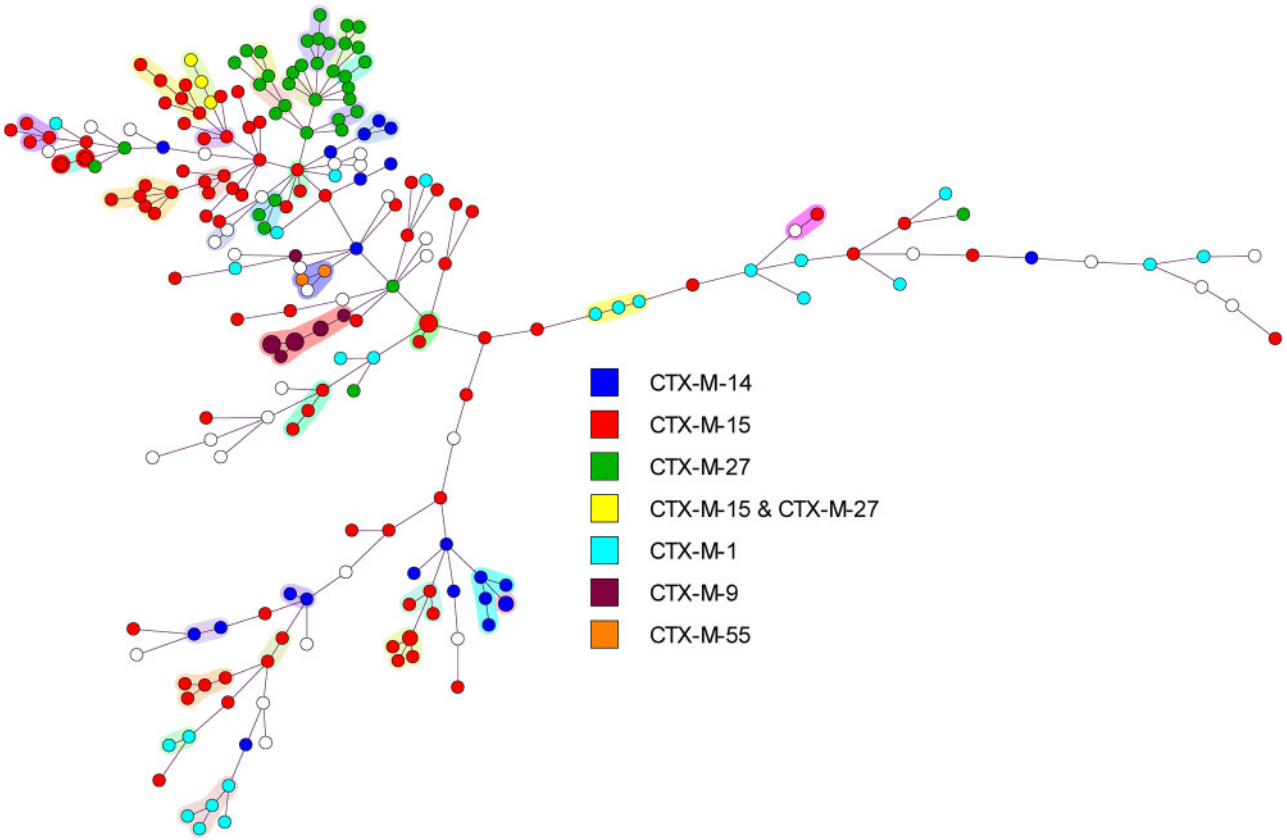

Figure 4. Distribution of ESBL genes among *E. coli* from LTCFs with high prevalence. Relationship between the presence of ESBL genes and the genetic clusters in 224 *E. coli* isolates. The colours of the circles represent the ESBL genes from which the isolates within the genetic clusters were obtained. The halos surrounding the circles denote the genetic clusters in the colours used in Figure 3. For other information on this figure, see the legend of Figure 3. This figure appears in colour in the online version of *JAC* and in black and white in the printed version of *JAC*.

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Transparency declarations

None to declare.

Author contributions

M.J.M.B., S.G.F., S.C.d.G., C.M.P.M.H., E.v.K., M.v.d.L. and L.M.S. were involved in study design. E.v.K. and J.v.W. coordinated and implemented the study and oversaw data collection on a national level with close involvement of S.G.F., S.C.d.G., C.M.P.M.H., M.v.d.L. and L.M.S. Members of the PPO study group comprised the regional study coordinators, hired by the RCN, and were involved in the implementation of the study in their respective region, entailing the inclusion of LTCFs and regional data collection. Statistical analyses were conducted by E.v.K. and C.C.H.W., including insightful discussions with S.C.d.G. Molecular analyses were performed by L.M.S. E.v.K. wrote the manuscript with significant contributions from M.J.M.B., S.G.F., S.C.d.G, C.M.P.M.H., A.T. and L.M.S.

Supplementary data

Tables S1 to S4 are available as Supplementary data at *JAC* Online.

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