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Drivers of extended-spectrum β-lactamase (ESBL)-producing Enterobacterales colonization among residents of long-term care facilities: a European multicentre prospective cohort study

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SUMMARY

Background: Extended-spectrum β-lactamase (ESBL)-producing Enterobacterales (ESBL-PE) are highly prevalent in long-term care (LTCF) settings. In order to estimate the acquisition rate of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* in LTCF settings, and identify clinical and environmental risk factors, a multi-centre, prospective cohort study was conducted in six LTCFs in Germany, France, Spain and the Netherlands.
Methods: Longitudinal screening of residents was performed over 32 weeks, collecting epidemiological and clinical data and environmental samples. The primary outcome was the rate of new acquisition of ESBL-PE among LTCF residents. Molecular epidemiology was studied using whole genome sequencing, and risk factor analysis was undertaken using logistic and Poisson regression models.

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Colonization
Molecular epidemiology

Results: In total, 299 residents provided 1958 samples during follow-up. The prevalence of ESBL-PE colonization at baseline was 16.4%, and the incidence of acquisition was 0.79 per 1000 resident-days, both with high variability between LTCFs. Age ≥80 years, vascular disease and antibiotic consumption within the preceding year were risk factors for baseline colonization. Lack of hand sanitizers and a low nurse:resident ratio were associated with colonization. The presence of medical devices was associated with risk of acquisition. Vascular disease, hemiplegia, antibiotic consumption, and non-availability of private bathrooms were associated with carriage of multiple sequence types (STs). The prevalence of ESBL-PE among environmental samples was 2%, exclusively in LTCFs with high prevalence among residents. Genetic analysis showed a high prevalence of ST10 *E. coli* and ST405 *K. pneumoniae* at two study sites.
Conclusion: Infection prevention interventions, including availability of hand sanitizers, the number of nurses per resident, and antimicrobial stewardship, constitute important measures to control ESBL-PE in LTCFs. Genome-based surveillance could guide targeted interventions.

Introduction

Extended-spectrum β-lactamase (ESBL)-producing Enterobacterales (ESBL-PE) are considered a critical priority in the 2018 World Health Organization list of antibiotic-resistant pathogens [1]. The 2019 epidemiological report of the European Antimicrobial Resistance Surveillance System Network showed a persistent high rate of third-generation cephalosporin resistance for *Escherichia coli* (mostly ESBL) between 2015 and 2019 [2]. Furthermore, colonization with ESBL-PE constitutes a considerable risk of infection [3,4], leading to increased piperacillin-tazobactam and carbapenem usage, which, in turn, contributes substantially to the burden of antimicrobial resistance [5].

Approximately half of healthcare-associated infections (HCAIs) in Europe are estimated to be among long-term care facility (LTCF) residents, estimated number of 4.4 (cumulative 95% confidence interval (cCI) 2.0—8.0) million each year, with at least 130,000 LTCF residents having at least one HCAI on any given day [6]. In 2017, a systematic review of the burden of ESBL-PE in LTCFs estimated a pooled prevalence of 18% [7]. High colonization and infection rates have been attributed to multiple causes including immunosuppression, advanced age, comorbidities, use of catheters, and extensive use of antibiotic agents [7]. Due to the complex care required for LTCF residents, prevention of transmission in this setting is challenging. Moreover, the possible vertical and horizontal transmission of ESBL genes adds substantially to the burden [8].

Although the transmission of ESBL-PE has been studied extensively in hospitals, data in community settings are scarce and heterogeneous. Comprehensive understanding of the reservoirs, vehicles, and determinants for colonization and transmission of ESBL-PE is important to implement preventive measures and infection control strategies, and to reduce the endemic nature of ESBL-PE in LTCFs. Current evidence is limited by the scarcity of longitudinal screening, use of typing methods, lack of systematic study of environmental contamination, antimicrobial use, and other potential epidemiological determinants of transmission. Prospective multinational cohort studies are lacking, and international comparisons are based on a meta-analysis of heterogeneous point-prevalence studies [7].

Given the pivotal role of ESBL-PE in morbidity and mortality, and the high potential for dissemination of resistance, the present longitudinal European multi-centre prospective cohort study aimed to estimate the acquisition rate of ESBL-producing *E. coli* and *Klebsiella pneumoniae* in LTCF settings, identify clinical and environmental drivers of acquisition, and investigate molecular epidemiology of ESBL-PE using whole genome sequencing (WGS).

Methods

Study design and follow-up

A prospective, observational multi-centre cohort study of ESBL-PE colonization and new acquisition was conducted among residents of six LTCFs in four European cities (Tübingen, Germany; Besançon, France; Seville, Spain; Utrecht, the Netherlands). All LTCF residents were eligible to participate and were informed about the study. Informed consent was obtained from either the resident or legal guardian.

The study was carried out in two consecutive 12-week follow-up periods, with an 8-week interval. Epidemiological and clinical data at the individual resident level as well as LTCF characteristics in the residents' environment were collected at baseline and during follow-up. LTCF characteristics such as average age and length of stay of residents, bed occupancy, nurse:resident ratio, living assistance, common area and dining arrangements, and infection control measures were documented. The nurse:resident ratio was defined as the number of nurses working per three shifts or over a 24-h period divided by the number of occupied beds over the same time period [9].

All participants were screened at the beginning of each follow-up period, and at 1, 4 and 12 weeks following the initial visit using faecal samples (rectal swabs, perianal swabs or stool samples). Environmental samples from surfaces with frequent contact and from public areas such as sinks, showers, toilets, door handles and light switches were taken with swabs during each follow-up period within the LTCF. All data were collected using structured clinical reporting forms in compliance with the ICH E6 guideline for good clinical practice and regulatory institutional guidelines, and transferred into a centralized,

secured online data collection system (REDCap) [10]. The study was approved by the respective institutional review boards.

Microbiological methods

Samples were cultured on selective media, and isolates identified as *E. coli* or *K. pneumoniae* with a confirmed ESBL-producing phenotype were cryoconserved at -80 °C until further processing for WGS. Genomes of strains showing an ESBL-producing phenotype were fully sequenced using the short-read sequencing technique on a NextSeq platform (Illumina, San Diego, CA, USA). A detailed description is provided in the online supplementary material.

Statistical analysis

The primary outcomes of interest were point prevalence of ESBL-PE among LTCF residents at baseline, and rate of acquisition (incidence rate) of ESBL-PE among LTCF residents. The baseline point prevalence of ESBL-PE colonization was defined as the proportion of screened residents who tested positive for ESBL-PE colonization at baseline. The incidence rate was defined as the number of residents who tested positive for ESBL-PE colonization divided by the total time at risk of the residents who were ESBL-PE negative at baseline. The time at risk for each resident was defined as the time from the first sample until the first ESBL-PE-positive sample or last follow-up sample, whichever was earliest.

Risk factors for ESBL-PE colonization at baseline were studied using mixed logistic regression analysis, and the measures have been reported as odds ratios (ORs). Mixed Poisson regression models with fixed effects at the centre level were used to study factors associated with ESBL-PE acquisition, and incidence rate ratios (IRRs) of ESBL-PE colonization have been reported. Antibiotic treatment and hospitalization were included as time-dependent covariates.

As secondary analyses, the molecular epidemiology of ESBL-PE isolates was studied using genomic sequencing results and phylogenetic analysis. Sequence type (ST) switching was defined as detection of a different ST in follow-up samples of a resident, and multiple ST carriage was defined as detection of two or more STs in a resident in the same sample. Associations between resident characteristics and switching/carriage were studied using Chi-squared test. All statistical analyses were performed using R Version 4.0.2. and STATA Version 15.1.

Results

Cohort description and ESBL-PE colonization

In total, 306 residents agreed to participate, and 299 residents were included from six LTCFs (Supplementary Figure 2). The cohort was predominantly female (N=216, 72.2%) with a median age of 83.5 (interquartile range 79–90) years. Length of stay ranged from 24 to 52 months. The cohort and LTCF characteristics are presented in Table I and Supplementary Table 1, respectively.

Overall, 1958 faecal samples were collected from 299 residents, with 166 (55.5%) residents providing all samples (Supplementary Table 2, Supplementary Figure 3). Forty-nine residents were colonized at first screening, with a prevalence of 16.4% [95% confidence interval (CI) 12.4–21.1%], ranging from 0% (95% CI 0–8%) in Utrecht to 42% (95% CI 31–54%) in Seville (Supplementary Table 3). Acquisition was assessed in 250 residents with a negative baseline sample, contributing to 41,563 resident-days at risk; 33 residents (11.7%) became positive during follow-up, ranging from 2 (4.4%) in Utrecht to 23 (48.9%) in Seville. The incidence rate of ESBL-PE was 0.79 (95% CI 0.55–1.22) per 1000 resident-days, varying from 0.26 (95% CI

Table I
Characteristics of the long-term care facility (LTCF) residents (N=299)

Resident characteristic	Frequency	Percentage
Age (years), median (IQR)	83.5 (79–90)	
Gender		
Female	216	72.2
Male	83	27.8
Cohabitation (two people living in the same room)	160	54
Access to private bathroom	96	32
Assistance with personal hygiene	283	94.7
Mobility/wheelchair usage	147	49.2
Incontinence	191	63.9
Medical history		
Invasive medical devices	29	9.7
Urinary catheter	21	7.0
Central venous catheter	2	1
Mechanical ventilation	1	0
Percutaneous feeding tube	3	1
Stoma	4	1
Comorbidities		
Diabetes mellitus	67	22.4
Vascular disease	222	74.2
Pulmonary disease	40	13.4
Renal failure	32	10.7
Liver disease	12	4
Hemiplegia	25	8
Malignancy	22	7
Cognitive state – capable of giving consent	123	41
Surgical procedures in preceding 12 months	21	7
Data on previous antibiotic consumption available for 280 residents[a]	142/280	50.7
Number of different antibiotics[a]		
1	73	26.1
2	38	13.6
3	14	5
>3	17	6.1
Duration of antibiotic consumption (days)[a]		
≤2	16	5.7
3–7	91	32.5
≥8	29	10.4

IQR, interquartile range.
[a] Refers to the subgroup of 280 patients.

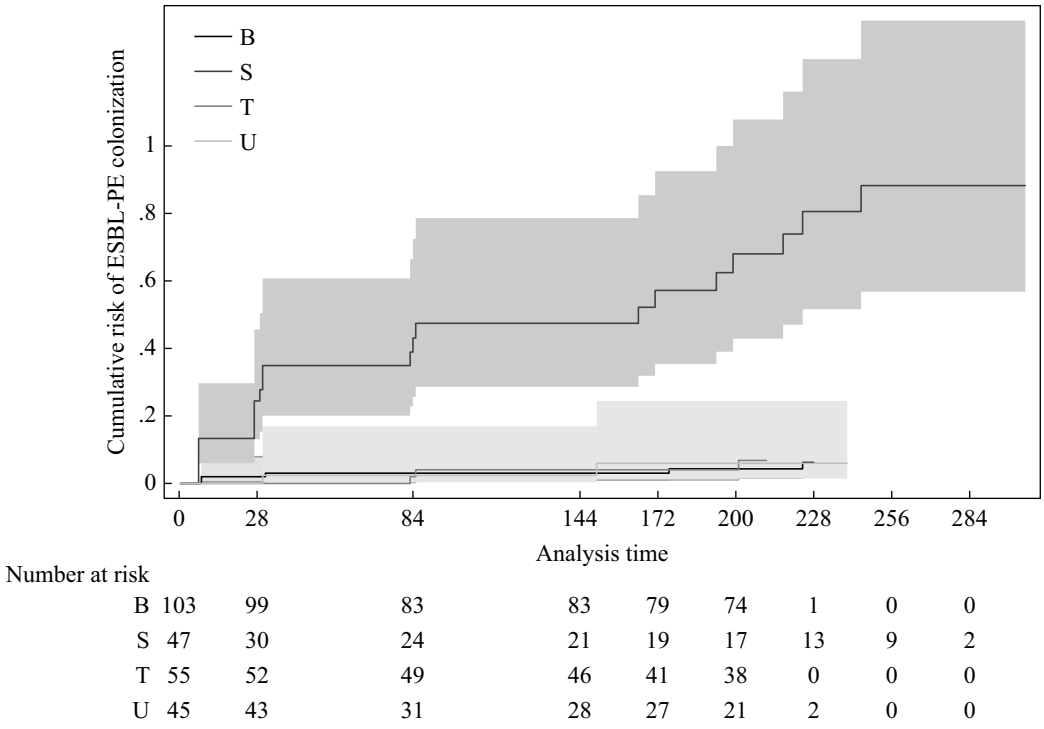

Figure 1. Cumulative incidence of extended-spectrum β-lactamase-producing Enterobacterales (ESBL-PE) colonization among long-term care facility (LTCF) residents in the four countries during the study period. T, Tübingen; B, Besançon; S, Seville; U, Utrecht.

0.09—0.61) in Besançon to 4.01 (95% CI 2.54—6.01) in Seville (Figure 1, Supplementary Table 3).

Resident characteristics and ESBL-PE

Residents aged ≥80 years (OR 2.64, 95% CI 1.12—6.22; *P*=0.03) and those with vascular disease (OR 3.67, 95% CI 1.41—9.55; *P*=0.008) had a significantly higher baseline rate of ESBL-PE colonization. The association with vascular disease remained significant even after adjusting for age and number of comorbidities (Supplementary Tables 4 and 5). The unadjusted IRR for acquisition of ESBL-PE colonization (Supplementary Table 6) showed that the presence of invasive medical devices in the preceding year was significantly associated with ESBL-PE acquisition (IRR 3.33, 95% CI 1.16—9.58; *P*=0.026).

LTCF characteristics and ESBL-PE

The number of beds in LTCFs ranged from 50 to 264. All LTCFs provided either single- or double-bedded rooms: the proportion of single rooms varied between 33% and 93%. The nurse:resident ratio ranged from 0.06 to 0.29. Hand sanitizer dispensers were located in public rooms/restrooms of five LTCFs, and in resident rooms in two LTCFs; one LTCF did not provide hand sanitizers in either location (Supplementary Table 7). Screening for ESBL-PE was not carried out routinely. The nurse:resident ratio had a significant negative correlation with ESBL-PE baseline colonization (*r*=-0.88, 95% CI 0.99—0.23; *P*=0.05). LTCFs with a low nurse:resident ratio had higher rates of colonization with ESBL-PE, including baseline and acquisition during the study period. Similarly, LTCFs providing individual room sanitizers and public sanitizers (0.04 and 0.11) had the lowest rates of ESBL-PE colonization (both baseline and acquisition) compared with LTCFs with public sanitizers alone (0.17, 0.19 and 0.55) and the LTCF without sanitizers (0.79; *P*<0.001).

Antibiotic treatment and ESBL-PE

Of 280 residents with available data on previous antibiotic treatment, 142 (50.7%) had taken at least one course of antibiotics in the year preceding the study. At baseline, 17 (5.7%) residents were on antibiotics, of whom seven (41.2%) were ESBL-PE colonized. Overall, 104 residents (104/299, 34.8%) received 164 courses of antibiotic treatment, with a rate of 2.85 (95% CI 2.44—3.32) antibiotic therapies per 1000 resident-days (Supplementary Tables 8—10). Among 232 residents with data on both previous and current antibiotic treatments, the lowest colonization rate was among residents who had not taken any antibiotics in the preceding year (5/85, 6%) and the highest colonization rate was among residents who had taken antibiotics during the preceding year (19/75, 19%; hazard ratio 4.17, 95% CI 1.56—11.17; *P*=0.004) (Supplementary Table 11).

Clinical outcomes and mortality

In total, 71 (23.8%) residents had 118 outpatient visits and 37 (12.0%) residents had 46 hospitalizations during the entire study period, with no significant difference between colonized and uncolonized patients. The overall mortality rate was 13.4% (95% CI 9.7—17.8%), including 40 deceased residents. The

mortality rate was 15.9% (13/82) among ESBL-PE positive residents and 12.4% (27/217) among ESBL-PE-negative residents (P=0.44).

Molecular epidemiology

In total, 261 (13.3%) ESBL-*E. coli* (ESBL-Ec) and 71 (3.6%) ESBL-*K. pneumoniae* (ESBL-Kp) were isolated, corresponding to 84 ESBL-Ec and 48 ESBL-Kp with a unique ST-bla_{ESBL} combination after eliminating duplicates from follow-up. A broad range of STs were identified (Supplementary Figure 4). The three most prevalent STs in *E. coli* were ST131 (N=41, 48.8%), ST10 (N=17, 20.2%) and ST69 (N=4, 4.8%), and the three most prevalent STs in *K. pneumoniae* were ST405 (N=28, 58.3%), ST307 (N=6, 12.5%) and ST571 (N=4, 8.3%). In both *E. coli* (N=43, 51.2%) and *K. pneumoniae* (N=38, 79.2%), $bla_{CTX\text{-}M\text{-}15}$ was predominant (Supplementary Figure 4A–D). *E. coli* harboured predominantly ESBL-encoding genes of the CTX-M-family, such as $bla_{CTX\text{-}M\text{-}27}$, $bla_{CTX\text{-}M\text{-}14}$, $bla_{CTX\text{-}M\text{-}1}$ and $bla_{CTX\text{-}M\text{-}55}$. *K. pneumoniae* strains harboured ESBL-encoding genes of the SHV family, frequently in combination with $bla_{CTX\text{-}M\text{-}15}$ (Supplementary Figure 5).

The maximum-likelihood phylogeny reflected the different multi-locus STs, with the exception of *E. coli* ST131 with isolates clustering on different branches (Supplementary Figure 5A). ESBL-Ec ST131 isolates were found in all LTCFs harbouring $bla_{CTX\text{-}M\text{-}15}$, $bla_{CTX\text{-}M\text{-}27}$, $bla_{CTX\text{-}M\text{-}14}$ and $bla_{SHV\text{-}12}$. In one LTCF from Spain, ST10/$bla_{CTX\text{-}M\text{-}15}$ *E. coli* was detected in faecal swabs of 16 study participants as well as two surface samples (Supplementary Figure 6A).

ESBL-Kp strains were only found in samples originating from France and Spain, with 48 non-duplicate isolates belonging to nine different STs. LTCF 1 from Spain showed the highest rate of ESBL-Kp (N=36). Multiple ST405/$bla_{CTX\text{-}M\text{-}15}$ *K. pneumoniae* isolates could be observed in LTCF 2 in Spain, involving seven residents and three surface samples (Supplementary Figure 6B), peaking in the second observation period. In these LTCFs, a high prevalence of different STs of ESBL-PE (47/81, 58%) was still detectable after excluding isolates with ST10 or ST405.

Environmental samples

ESBL-PE was detected in 14 (2.0%) of 707 environmental samples, all from two LTCFs in Spain (14/144, 9.7%). Seven were ESBL-Ec isolates and seven were ESBL-Kp isolates, with $bla_{CTX\text{-}M\text{-}15}$ (57.1%) being predominant. Three (42.9%) ESBL-Kp isolates belonged to ST405 and were retrieved from LTCF 2. ST307 (N=2, 28.6%), ST571 (N=1, 14.3%) and ST2809 (N=1, 14.3%) were also detected in *K. pneumoniae* isolates. ST131 was detected in three (42.9%) ESBL-producing *E. coli* isolates, and ST10/$bla_{CTX\text{-}M\text{-}15}$ *E. coli* was found in two (28.6%) samples from LTCF 1. The other two isolates (28.6%) were ST69/$bla_{CTX\text{-}M\text{-}55}$ *E. coli* isolates.

Multiple ST carriage and switching of STs

Of 82 residents with at least one positive sample, 20 (24.4%) carried multiple STs. Residents with vascular disease had a higher rate of multiple ST carriage (Supplementary Table 12). Among 62 residents with at least two positive samples, 35 (56.5%) had an ST switch (Supplementary Table 13). Residents with no access to private bathrooms (34/51, 66.7%) had a higher rate of ST switching compared with those who had a private bathroom (1/11, 9.1%; P<0.001). However, ST switches mainly occurred in the two LTCFs in Spain with a high prevalence of ESBL-PE, and only four ST switches were found in other countries. In five residents, the ESBL-encoding gene remained the same, while subsequent samples showed ST switching. In five residents, a change of species could be observed with the same ESBL gene in subsequent samples. In one resident, both phenomena occurred (Supplementary Table 14).

Discussion

To the authors' knowledge, this is the first prospective multi-national study on ESBL-PE colonization in LTCFs combining human and environmental sampling, and following-up residents for 32 weeks. Baseline ESBL-PE colonization among LTCF residents varied from 0% in the Netherlands to 42% in Spain, and the ESBL-PE acquisition rate varied between 4% in the Netherlands to 49% in Spain. Modifiable risk factors for ESBL-PE colonization and acquisition were found to be antibiotic prescription at the resident level, availability of hand sanitizers, and number of nurses per resident at the facility level. These results highlight the role of ESBL-PE colonization in LTCFs, and underline the importance of repeated screening when assessing epidemiological scenarios, especially in order to implement antibiotic stewardship programmes and infection control measures.

The geographical variation of colonization rates among LTCF residents at different centres partially reflects the pattern seen in the general population. The European Centre for Disease Control and Prevention (ECDC) reported the highest rates of third-generation cephalosporin-resistant *K. pneumoniae* (28%) and *E. coli* (13%) in Spain, followed by France (25% and 8%, respectively), Germany (10% and 9%, respectively) and the Netherlands (10% and 7%, respectively) [11]. Previous studies reporting ESBL carriage in LTCF residents have recorded varying prevalence estimates: the prevalence of ESBL Enterobacterales in 12 Amsterdam LTCFs varied between 0% and 34% [12], another study among 18 LTCFs around Besançon showed a prevalence of 0–44% [13]. Prevalence variations were seen from 0% to 47%, even in different wards of the same LTCF [14], indicating that LTCF as well as resident characteristics are major determinants. The present data showed variability not only in different LTCFs, but also in the percentage of positive carriers at each sampling within the same LTCFs (37–51% Seville, 3–9% Besançon, 9–16% Tübingen, 0–3% Utrecht), which might be attributed to different strains associated with varying carriage durations [15].

Lower nurse:resident ratio and lack of hand sanitizers in either public spaces or residents' rooms were associated with high colonization rates in this study. Interestingly, LTCFs with these risk factors were the only centres with positive environmental samples. High background prevalence rates coupled with gaps in nursing care and hygiene could have paved the way to the high prevalence as well as acquisition rates, and the two STs (ST10 and ST405), which may indicate possible local outbreak situations. *E. coli* ST10 was the second most prevalent ST found in all isolates, and was present in 37% of the isolates found in LTCF 1 and in a few surface samples restricted to this LTCF, particularly around the study baseline. This LTCF had no

hand sanitizers, no access to private bathrooms, the lowest nurse:resident ratio, and the highest prevalence of ESBL-PE (79%). With substantial evidence for hand hygiene in the prevention of transmission of infections [16] and significant improvement of healthcare outcomes with more nursing hours [17,18], this finding reinforces the role of infection control in preventing the spread of ESBL-PE in healthcare settings. The other cluster with 20 ST405 *K. pneumoniae* isolates was related to another possible outbreak situation in Spanish LTCF 2, in seven participants and three surface samples, peaking in the second sampling period. This facility had hand sanitizers available only in the public toilets and public rooms, had the second lowest nurse-resident ratio, and the second highest prevalence of ESBL-PE (55%).

The most prevalent ST, found in all LTCFs with diverse ST distribution, was *E. coli* ST131 harbouring $bla_{\text{CTX-M-15}}$ in accordance with other studies showing the worldwide spread of ST131 [2]. The Spanish LTCFs with high incidence rates also exhibited high rates of multiple ST carriage or ST switching. The association of ST switching with lack of private bathrooms may reflect transmission dynamics, making access to individual bathrooms a possible intervention. However, carriage of multiple STs at a certain timepoint cannot be excluded, as the laboratory set up did not allow for systematic screening for co-colonization of multiple STs in a single sample, but only included colonies with different morphologies in the analysis. This limitation of the study may have led to underestimation of ST switching. Multiple bla_{ESBL} genes were only identified in a few isolates, almost exclusively in *K. pneumoniae* and frequently in combination with $bla_{\text{CTX-M-15}}$ (Supplementary Figure 3C,D), as seen in studies from Portugal [19] and Japan [20]. This could signal more exchange of mobile genetic elements by *K. pneumoniae* than *E. coli*, which is supported by other studies attributing a driving role to *K. pneumoniae* in the exchange of resistant genes [21].

The present results highlight the importance of repeated screening and careful implementation of basic hygienic standards, as defined by a technical report by ECDC [22]. Particularly in high-incidence settings, molecular analysis can reveal transmission dynamics that might otherwise be overlooked. The importance of adherence to hand hygiene regulations has been emphasized in the context of LTCFs for numerous infectious agents, and has also been applied to outbreak situations with multi-drug-resistant bacterial pathogens [23,24]. Even with the basic information on distribution and availability of hand sanitizers, an association with colonization could be seen; previous studies in hospital settings have emphasized the importance of availability and visibility of hand sanitizers to hand hygiene compliance [25,26].

Among the epidemiological and clinical risk factors studied, age $\geq$80 years and vascular disease were associated with colonization. These findings corroborate with a cohort study among four Italian LTCFs, which found that age $\geq$86 years was a risk factor for ESBL-PE colonization [27]. The association of age with infection has been well documented [28], and senescence of gut microbiota and the immune system facilitating the establishment of pathogens seem to be a plausible explanation [29].

Heterogeneity in the distribution as well as the assessment of baseline comorbidities among various LTCF studies for ESBL-PE colonization restricts the availability of current evidence. Although this study found that vascular disease was independently associated with ESBL-PE colonization after adjusting for age and comorbidities, most studies focusing on risk factors did not investigate vascular disease. A Japanese single-centre study reported that stroke was associated with oral ESBL-PE colonization in LTCF residents [30]. However, further investigation was limited by the use of a broad definition of vascular disease in the present study. This study identified the presence of medical devices within the preceding year as another risk factor for ESBL-PE acquisition, corroborating previous studies [31—33], being possible evidence of dependency and need of assistance. This indicates that targeted infection control programmes should be evaluated and implemented for this specific risk.

The prospective nature of this study enabled the authors to determine a strong association between antibiotic treatment and ESBL-PE colonization. However, various combinations of antibiotic treatments, along with non-identical gaps in screening samplings and highly varying centre-specific incidence rates, made it difficult to study the independent and individual effects of antibiotics on ESBL-PE colonization. Unlike other studies, the present study found no clear correlation of ESBL-PE colonization with hospitalizations and/or ambulatory clinical visits [28].

Interestingly, the environmental screening identified ESBL-PE in a very low percentage of samples at one high endemic LTCF. This is discordant with a previous study of ESBL-PE in US LTCFs [34] that reported prevalence of 16% and environmental contamination of 6—11%. In the present study, a disproportionate number of *K. pneumoniae* were found in environmental samples (50%) compared with colonization in the residents (21%); however, the small number (N=7) impeded further analysis and indicated contamination of the environment from residents rather than relevant transmission through environmental contact. The higher prevalence of *K. pneumoniae* in environmental samples has been documented previously [35,36], with the present data adding to the evidence that hospital environmental contamination is more frequent in instances of faecal carriage or infection with ESBL-producing *K. pneumoniae* than ESBL-producing *E. coli*. However, the use of swabs without disinfectant neutralizer may have resulted in under-reporting of environmental contamination in the present study.

This study has some limitations. A major drawback is the limited number of residents colonized in the LTCFs. In particular, the small number of residents with *K. pneumoniae* limits the possibility to analyse the results by bacterial species. Although heterogeneity among LTCFs was detected, the study represents 'real-life' data that were collected using a homogenous study protocol allowing colonization rates and dynamics in a longitudinal screening to be studied. On the other hand, analysis of risk factors is confounded by highly heterogeneous LTCF characteristics, and was further hindered by a large variation in prevalence between the different LTCFs, with the characteristics of highly prevalent LTCFs dominating the data. Especially in the LTCFs with high prevalence, a high rate of missing samples over the course of longitudinal screening complicated the interpretation of ESBL-PE dynamics.

The prevalence of ESBL-PE in LTCFs should be assessed by periodic point-prevalence studies in highly endemic LTCFs. The added value of genomic sequencing, especially in these settings, to detect outbreaks and transmission dynamics, and implement appropriate countermeasures should be explored

further. Implementation of antibiotic stewardship and infection control programmes seems to play a pivotal role to control the spread of these bacteria in LTCFs. During times of financial constraint, particularly in LTCFs, cost-effectiveness studies of minimum nurse staffing requirements should be carried out urgently.

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Author contributions

SG: resources, investigation, methodology, supervision, writing — original draft. JG: microbiological/WGS analyses — investigation, data curation, resources, supervision, methodology, software, visualization, writing — review and editing. BPG: data curation, formal analysis, software, visualization, writing — original draft. NC: data curation, resources, project administration, writing — original draft. SB: data curation, resources, project administration, writing — original draft. TT: formal analysis, investigation, software, visualization, writing — review and editing. TDV: data curation, supervision, writing — review and editing. DM: data curation, supervision, writing — review and editing. ES: data curation, supervision, writing — review and editing. MD-V: data curation, supervision, writing — review and editing. IBA: microbiological/WGS analyses — investigation, resources, writing — review and editing. SP: microbiological/WGS analyses — investigation, supervision, methodology, writing — review and editing. JAJWK: funding acquisition, investigation, supervision, methodology, writing — review and editing. DH: funding acquisition, investigation, supervision, methodology, writing — review and editing. JR-N: funding acquisition, investigation, supervision, methodology, writing — review and editing. ET: conceptualization, funding acquisition, resources, investigation, supervision, writing — review and editing.

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All the authors stated above meet the required criteria of the International Committee of Medical Journal Editors.

Conflict of interest statement

SG, SP and BPG receive funding from the German Centre for Infection Research. DH receives funding from Région Bourgogne Franche-Comté and ANR. MD-V, ESR, ET, DM, JG, NC, SB, SP, TDV, TT and JAJWK declare no further competing interests.

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Ethical approval

This study was approved by the Ethics Committee of the University of Tübingen in February 2018 (818/2017 B01).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhin.2024.12.010.

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