Chapter 6

Effect of phylotype and O25:ST131 *E. coli*

on rectal carriage and infection

ST131
Chapter 6.1

Prevalence of phylogroups and O25:ST131 in susceptible and extended-spectrum β-lactamase-producing Escherichia coli isolates, the Netherlands

ITMA Overdevest, AMC Bergmans, JJ Verweij, J Vissers, N Bax, E Snelders, JA JW Kluytmans

Submitted
Abstract

*Escherichia coli* can be divided into 4 phylogroups: A, B1, B2 and D. Phylogroup B2 is associated with increased infection risk, and O25:ST131 specifically, is associated with increased virulence and extended-spectrum β-lactamase (ESBL)-production. We compared the prevalence of phylogroups and O25:ST131 in a collection of 108 wildtype *E. coli* and 134 ESBL-producing *E. coli* obtained from human rectal swabs, urine cultures and blood cultures. All isolates were obtained in our laboratory in a teaching hospital in the southern part of the Netherlands between 2010 and 2013. Phylogroup and O25:ST131-status was determined by real-time PCR, and ESBL-production was determined by double disk method according to the Dutch national guideline.

The majority of isolates belonged to phylogroup B2 (56.6%). ESBL-producing *E. coli* were less likely to belong to this phylogroup (48.5%) than were wildtype *E. coli* isolates (66.7%; *P*=0.005). O25:ST131 *E. coli* were almost absent in phylogroup B2 wildtype *E. coli* (5.6%), while being abundant in ESBL-producing *E. coli* (61.5%;*P*<0.001). Phylogroups B2 and D wildtype *E. coli* were more prevalent among midstream urine isolates and human blood culture isolates, than in catheter related urine isolates (83.3% and 87.9% versus 61.9%; *P*=0.048).

Our study supports the hypothesis that phylogroups B2 and D are more likely to cause infections than the other phylogroups, which need devices like urinary catheters to cause infection. Furthermore, O25:ST131 is significantly associated with ESBL-production, and is almost absent among wildtype *E. coli*. 

---

134
Introduction

*Escherichia coli* are an important cause of urinary tract infections and systemic infections in humans. The primary reservoir for infections due to *E. coli* is the patient’s own intestinal tract. Factors associated with increased risk of infection are patient- and pathogen dependent. Patient dependent factors include underlying illnesses, female gender, the use of indwelling catheters, and previous antimicrobial use. Pathogen dependent factors include strain subtype, with certain *E. coli* subgroups being more virulent than others.

*E. coli* can be divided into 3 different groups; intestinal non-pathogenic commensal isolates, intestinal pathogenic isolates, and extra-intestinal pathogenic *E. coli* (ExPEC) isolates. Where intestinal pathogenic isolates cause gastro-enteritis, ExPEC are known to cause urinary tract infections and systemic infections. Phylogenetic analysis has shown that *E. coli* can be subdivided in four phylogenetic groups called A, B1, B2 and D. ExPEC most often belong to group B2 and, to a lesser extent, to group D. Intestinal commensal isolates primarily belong to groups A and B1. Thus, phylogroup B2 is associated with increased risk for infection.

The prevalence of antimicrobial resistance in *E. coli* is rising. Production of extended-spectrum β-lactamases (ESBL) and the corresponding resistance to cephalosporins in *Enterobacteriaceae* has spread significantly over the last few years. The majority of ESBL-producing *Enterobacteriaceae* are *E. coli*. Some reports indicate that ESBL-producing *E. coli* mostly belong to phylogroup B2, but that they seem to be less virulent than their ESBL-negative counterparts. Others report similar aggregate virulence factor scores between ESBL-producing *E. coli* and wildtype *E. coli*. One clone within the B2-phylogroup, O25:ST131 *E. coli*, has successfully spread worldwide, is associated with outbreaks in healthcare settings, and has established itself in the community. This clone is believed to be associated with higher virulence, by producing a biofilm. However, a more recent report indicate that the virulence of O25:ST131 is similar to the virulence of other *E. coli*.

In this study we investigated the distribution of phylogroups A, B1, B2, and D, and the prevalence of O25:ST131 in wildtype *E. coli* and ESBL-producing *E. coli* from rectal colonization samples, urine cultures- and blood cultures.

Methods

Isolate collection

All isolates were collected in our laboratory for medical microbiology, situated in a large teaching hospital in the southern part of the Netherlands. Rectal colonization samples were obtained from hospitalized patients using rectal swab or faecal samples. During
two routine cross-sectional surveys in November 2012 and 2013, all admitted patients were screened for rectal colonization with ESBL-producing *Enterobacteriaceae*. Samples were selectively cultured using Tryptic Soy Broth (TSB) containing 8 mg/l vancomycin and 0.25 mg/l cefotaxime and, after overnight incubation, 10 µl of the broth was inoculated on an EbSA agar plate (Cepheid Benelux, Ledeberg, Belgium), selective for ESBL-producing *Enterobacteriaceae*. Identification of all oxidase-negative Gram-negative bacteria was performed by MALDI-TOF using Vitek-MS (bioMérieux, Marcy l’Étoile, France), susceptibility testing by ViTEK2 (bioMérieux), and ESBL production was confirmed by double disk method according to the Dutch national guideline. All confirmed ESBL-producing *E. coli* isolates were included in this study.

ESBL-producing *E. coli* obtained from urine samples are routinely stored at -80°C, and retrospectively collected between January 2010 and March 2013. Samples were obtained from patients with or without indwelling catheters, from both hospitalized and general practitioners’ patients. A comparable number of wildtype *E. coli* isolates obtained from catheter-related and midstream cultures were collected prospectively between February and August 2013. All isolates were cultured using blood- and uriselect agar (Bio-Rad, Veenendaal, the Netherlands), species identification was performed by MALDI-TOF, and susceptibility was determined by Vitek2 (bioMérieux, Marcy l’Étoile, France). Samples with an increased MIC (>1 g/l) for cefotaxime and/or ceftazidime were tested for ESBL production using the double disk method.

*E. coli* isolates from blood cultures are routinely stored at -80°C, and all available ESBL-producing *E. coli* obtained from unique patients were retrospectively collected between January 2010 and March 2013. For every ESBL-producing *E. coli*, the next available wildtype *E. coli* isolate was included in this study. A collection with similar characteristics was obtained from our laboratory in another teaching hospital 30 kilometres eastwards.

Some selected isolates were lost in storage; this was the case for ESBL-producing *E. coli* from urine cultures and from blood culture samples, as well as for wildtype *E. coli* from blood culture samples. Missing isolates were not replaced by others. Gaps in the frozen collection were random and were just as frequent in ESBL-producing *E. coli* as in wildtype *E. coli*.

### Molecular typing

All *E. coli* isolates were subjected to phylotyping using real-time PCR. An O25:ST131 specific real-time PCR was performed on all phylogroup B2 *E. coli* isolates. Isolates that showed inconclusive results to the O25:ST131 specific real-time PCR, were subjected to sequence-analysis of a part of the pabB gene to detect the O25:ST131-specific A and T SNP.
Statistical analysis

We hypothesise that all *E. coli* phylogroups can colonize the gut, but under normal circumstances only few will cause infection. In the case of indwelling catheters, we assume that virulence plays a minor role for *E. coli* variants to cause urinary tract infection, using the indwelling catheter to overcome the physiological barrier. This may be the case for both wildtype *E. coli* and for ESBL-producing *E. coli*, and should result in differences in prevalence of the four phylogroups and O25:ST131. Differences in prevalence of the different phylogroups were analysed by using the Chi-square test. Statistical significance was accepted if the chance for coincidence was less than 5%. All analyses were performed using the Statistical Package for Social Sciences software (SPSS, version 17).

Results

The collection consisted of 242 *E. coli* isolates of which 134 were ESBL-producing *E. coli* and 108 were wildtype *E. coli*. Thirty-eight ESBL-producing *E. coli* were obtained from human rectal swabs, 43 from midstream urine cultures, 19 from catheter-related urine cultures, and 34 from blood cultures. Fifty-four wildtype *E. coli* were obtained from human midstream urine cultures, 21 from catheter-related urine cultures, and 33 from blood cultures.

Phylotyping PCR showed a positive result for phylogroup A, B1, B2, and D in 40, 22, 137, and 43 of 242 isolates respectively. Using the real-time O25:ST131-specific PCR, the pathognomonic A and T SNPs were detected in 41 of 137 B2 isolates, and were absent 65 isolates. The O25:ST131 PCR showed inconclusive results for 31 (22.6%) of 137 B2 isolates. Of these 31 isolates with inconclusive results, sequence analysis of the pabB gene revealed the O25:ST131-specific A and T SNPs in 2 isolates (6.4%), and revealed absence of the pathognomonic SNPs in the other 29 isolates. Alignment of the pabB sequences revealed heterogeneity in the reverse primer positions used in the O25:ST131-specific A and T SNP real-time PCR which could explain the inconclusive results that were obtained for the non-O25:ST131 isolates.

The majority of midstream urine isolates were from general-practitioners’ patients (40 wildtype *E. coli* isolates; 74.1% and 34 ESBL-producing *E. coli* isolates; 79.1%) whereas all but one of catheter related urine isolates were obtained from hospitalized patients (95.2% of wildtype *E. coli* and 100% of ESBL-producing *E. coli*). All blood culture and rectal swab isolates were also obtained from hospitalized patients. Table 6.1.1 shows the age and sex distribution of patients with *E. coli* from the different origins. Patients with indwelling catheters were significantly older than patients of which rectal swabs or midstream urine cultures were obtained (*P*=0.001 and *P*<0.001, respectively), and patients of which midstream urine cultures were obtained, were significantly younger than patients with indwelling catheters or with positive blood cultures (*P*<0.001 and
This was the case for both ESBL-producing *E. coli* and for wildtype *E. coli*. For wildtype *E. coli* there was no association between phyllogroup and age, whereas for ESBL-producing *E. coli*, patients with non-O25:ST131 phyllogroup B2 *E. coli* were significantly younger than other patients (mean age of 41.4 vs. 67.1 years; *P*<0.001). Patients with *E. coli* in midstream urine cultures were significantly more often female than the patients of which other cultures were obtained (*P*=0.001 for wildtype *E. coli* and *P*=0.008 for ESBL-producing *E. coli*).

<table>
<thead>
<tr>
<th></th>
<th>wildtype <em>E. coli</em></th>
<th>ESBL-producing <em>E. coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>age: Mean (95% CI)</td>
<td>sex: % male</td>
</tr>
<tr>
<td>Rectal colonisation</td>
<td>55.8 (46.4-65.3)</td>
<td>57.9</td>
</tr>
<tr>
<td>Urine culture - midstream</td>
<td>47.4 (40.3-54.4)</td>
<td>14.8</td>
</tr>
<tr>
<td>Urine culture - indwelling catheter</td>
<td>73.3 (66.4-80.2)</td>
<td>47.6</td>
</tr>
<tr>
<td>Blood culture</td>
<td>67.6 (62.0-73.1)</td>
<td>48.5</td>
</tr>
</tbody>
</table>

Mean age with 95% confidence interval and percentage of male subjects among wildtype *E. coli* isolates and ESBL-producing *E. coli* isolates.

Figure 6.1.1 shows the distribution of phyllogroups and O25:ST131 for the different groups of *E. coli* isolates. Overall, the majority of isolates belonged to phyllogroup B2 (56.6%), and this was the predominant phyllogroup in all subgroups. ESBL-producing *E. coli* isolates were less likely to belong to this phyllogroup (48.5%) compared to wildtype *E. coli* (66.7%; *P*=0.005; 49.0% versus 66.7% and *P*=0.010 when excluding the rectal swabs from the analysis). The majority of the B2 phyllogroup ESBL-producing *E. coli* isolates belonged to clonal complex O25:ST131 (61.5%) versus a small minority of the B2 phyllogroup wildtype *E. coli* (5.6%; *P*<0.001). Furthermore, ESBL-producing *E. coli* obtained from blood cultures were more likely to belong to phyllogroup A than did wildtype *E. coli* isolates (26.5% vs. 6.1%; *P*=0.024). There were no significant differences in occurrence of phyllogroup B1 and D when comparing ESBL-producing *E. coli* with wildtype *E. coli*.

For wildtype *E. coli*, phyllogroups B2 and D were more prevalent in midstream urine isolates and in blood culture isolates than in catheter-related urine culture isolates (83.3% and 87.9% versus 61.9%; *P*=0.048). The prevalence of other phyllogroups was comparable between the groups of isolates.

For ESBL-producing *E. coli*, the prevalence of all phyllogroups and O25:ST131 was comparable for the different groups of isolates. Although phyllogroup B1 was absent in blood culture isolates, this was not significantly different from the prevalence in strains isolated from other sources. The prevalence of O25:ST131 was lowest in the rectal colonization isolates (18.4%) versus 37.2%, 36.8% and 29.4% for midstream urine isolates, catheter related urine isolates and blood culture isolates, respectively. Again, these differences were not statistically different (*P*=0.27).
Figure 6.1.1  Distribution of phylogroups and O25:ST131 E. coli.
Discussion

In the present study, remarkable differences were found between the prevalence of phylogroups and O25:ST131 in ESBL-producing *E. coli* and wildtype *E. coli* from different origins. Most striking is the difference in prevalence of O25:ST131, being the most prevalent clone among ESBL-producing *E. coli*, and being almost absent among wildtype *E. coli*. This finding supports the idea that this clone of *E. coli* thanks its success to the ESBL-phenotype. Among wildtype *E. coli*, phylogroups A and B1 were found to be less prevalent among midstream urine isolates and human blood culture isolates, as compared to wildtype *E. coli* obtained from catheter related urine isolates. This finding supports the hypothesis that these phylogroup isolates need devices like catheters to overcome barriers and cause infection. This difference was not observed for ESBL-producing *E. coli*. Of the ESBL-producing *E. coli*, O25:ST131 was most prevalent among urine isolates (both midstream and urinary catheters) and blood cultures isolates, compared to human rectal isolates, although these differences were not statistically different.

Our results are in line with the results of Johnson et al. They also found that phylogroup B2 was the predominant phylogroup in ESBL-producing *E. coli*, and O25:ST131 was the most prevalent clone. Furthermore, they also found phylogroup A to be more prevalent in ESBL-producing *E. coli* than in wildtype *E. coli*. Lee et al. also report the predominance of phylogroup B2 in *E. coli* from urine and blood culture samples. However, they also reported that phylogroup D was the dominant type in faecal samples, which is in contrast with the present study. This difference is probably caused by the fact that the bacterial collection analysed by Lee et al comprised mostly wildtype *E. coli*, whereas our faecal isolates were all ESBL-producing *E. coli*. The strength of real-time PCR for the phylotyping is the speed with which high numbers of isolates can be typed. Furthermore, this method is cost-efficient, easy to use and gives reliable results. These advantages were also true for the O25:ST131 specific real-time PCR, although this PCR showed some inconclusive results. The main limitation of our study is the fact that an O25:ST131-specific real-time PCR was used. Although O25:ST131 constitutes the majority of ST131 isolates, O16 is another serotype associated with equal virulence and ST131 status. Furthermore, the wildtype *E. coli* isolates from urine cultures were obtained prospectively, whereas the other isolates were part of a frozen collection. In addition, some isolates were missing from this collection. However, the absence of isolates occurred randomly and it is unlikely that this has influenced the results significantly.

In conclusion, our study supports the idea that *E. coli* colonizing the gut are not equally capable in causing infection. For wildtype *E. coli*, phylogroups B2 and D are more likely to cause infection, where other phylogroups need devices like urinary catheters to overcome barriers and cause infection. For ESBL-producing *E. coli* this trend was seen for O25:ST131. Furthermore, our results confirm the association of O25:ST131 with
ESBL-production, with this clone being almost absent in wildtype *E. coli* isolates. Overall, phylogroup B2 is less frequently seen in ESBL-producing *E. coli* than in wildtype *E. coli*, while for phylogroup A the opposite is found.
References


Chapter 6.2

Prolonged duration of colonization with *Escherichia coli* O25:ST131 versus other extended-spectrum β-lactamase-producing *E. coli* during an outbreak in a long-term care facility in the Netherlands, 2013-2014

ITMA Overdevest, M Haverkate, J Veenemans, Y Hendriks, C Verhulst, A Mulders, W Couprie, M Bootsma, J Johnson, JAJW Kluytmans

Submitted
Abstract

The extended-spectrum β-lactamase (ESBL)-producing Escherichia coli clone O25:ST131 is pandemic in healthcare settings. The reasons for its success are unknown, but might include more effective transmission and/or longer persistence. Also, whether individuals differ for susceptibility to acquiring ESBL colonization remains unknown. We evaluated an ongoing epidemic of colonization with ESBL-producing Enterobacteriaceae, including E. coli clone O25:ST131, in a long-term care facility (LTCF). During a 14-month-period, 6 repetitive prevalence surveys were performed, using ESBL-selective culture of rectal and faecal samples. Transmission rates, reproduction numbers, and duration of colonization were calculated and compared for ESBL-producing E. coli clone O25:ST131 and other E. coli isolates. Durations of colonization were compared using Kaplan-Meier survival analysis and the likely duration of the outbreak with ESBL-producing E. coli clone O25:ST131 was estimated using mathematical models.

Three LTCF wards experienced a widespread, sustained outbreak with ESBL-producing E. coli clone O25:ST131, whereas 3 other wards experienced smaller, transient outbreaks involving other ESBL-producing strains. Transmission was plausible in 12 residents who acquired ESBL-producing E. coli clone O25:ST131 and 10 residents who acquired other strains. According to survival analysis, the half-life of carriage with ESBL-producing E. coli clone O25:ST131 was 13 months, versus 2-to-3 months for other strains (P<0.001). Per-admission reproduction numbers were 0.66 for ESBL-producing E. coli clone O25:ST131 versus 0.56 for other strains, predicting a mean time of 3-to-4 years for the outbreak strain(s) to disappear from the LTCF under current conditions. Transmissibility rates were comparable for ESBL-producing E. coli clone versus other ESBL-producing E. coli. Prolonged rectal carriage of the O25:ST131 clone explained the persistence of the outbreak. Effective decolonization strategies could have pronounced effects on outbreak duration.
Introduction

The worldwide prevalence of extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae is increasing rapidly.\textsuperscript{1,2} Infections with these and other resistant bacteria are associated with increased morbidity, mortality, and healthcare costs.\textsuperscript{3,4} Enterobacteriaceae colonizing the gut are the most important reservoirs for infection\textsuperscript{5} and can start an outbreak.\textsuperscript{6} In the Netherlands, during cross-sectional measurements, approximately 5\% of hospitalized patients were colonized with ESBL-producing Enterobacteriaceae.\textsuperscript{7}

Initially, outbreaks with ESBL-producing Enterobacteriaceae were hospital-associated. However, more and more outbreaks in long-term care facilities (LTCFs) are reported.\textsuperscript{5,9} Residents of LTCFs are mainly frail, elderly people, who often have medical devices and need medical attention. Among these resident, a low functional status, and thus more medical and nursing dependence, is associated with a greater risk of ESBL carriage.\textsuperscript{10} For their residents, LTCFs emphasize the quality of life, including participation in social activities, over healthcare. Therefore, the amount of interaction between LTCF residents is high in comparison with hospitalized patients, which may be important since the risk of transmission of ESBL-producing Enterobacteriaceae is greater among household contact than among hospital inpatients.\textsuperscript{11} Furthermore, diagnostic sampling frequency in LTCFs is low and infection control measures are not as strict as in hospitals. We assume that, under these conditions, many outbreaks involving rectal carriage of ESBL are detected late or are overlooked.

In June-July 2012, a routine prevalence survey involving 9 LTCFs in the southern Netherlands identified a facility with an unusually high prevalence of rectal ESBL carriage (21\%). Typing showed the presence of one large cluster of ESBL-producing Escherichia coli from sequence type O25:ST131, along with other smaller clusters and unique strains. This prompted further investigations.

The ESBL-producing Escherichia coli from the sequence type O25:ST131 lineage is a worldwide pandemic clone that is a major driver of the current worldwide spread of ESBLs.\textsuperscript{12,14} This clone contains many virulence factors\textsuperscript{15} and is associated with community-acquired infections. Older age and LTCF residence have been implicated as independent risk factors for colonization and infection with ESBL-producing E. coli of sequence type O25:ST131.\textsuperscript{16} O25:ST131 was also the most prevalent clone in a recent study of antimicrobial resistance in another Dutch LTCF.\textsuperscript{17}

Here we evaluated the epidemiology of various ESBL-producing E. coli, including E. coli of sequence type O25:ST131, in a LTCF with an ongoing outbreak of ESBL. Specifically, we tested for inter-individual differences in susceptibility to acquiring ESBL-producing E. coli, and we assessed whether sequence type O25:ST131 strains were more transmissible or more persistent colonizers than other ESBL-producing E. coli, which are theoretical explanations for the pandemic success of this clone.
Methods

Epidemiology

The LTCF comprises 4 semi-separate buildings (A, B, C, and D), each divided into 1-to-3 separate wards (A1-3, B1-2, C1-3 and D). Each ward housed approximately 20 residents and contained 2 kitchens and communal areas. Sanitary facilities were used communally by several residents. Staff members were dedicated to specific wards. The building contains communal recreation and therapy areas where residents from all buildings and wards meet regularly.

During the study period, improved infection control measures, improved emphasis on hand hygiene, and improved cleaning strategies were implemented on all wards. No attempts were made to actively decolonize residents.

Specimen collection

During a 14-month period (March 2013 - April 2014), 6 cross-sectional surveys were performed by culturing faeces or rectal swabs from all residents. Residents admitted during the study were cultured similarly within 1 week after admittance.

To assess for different possible routes of transmission, concurrently with the resident surveys, environmental cultures were obtained 5 times, the hands of all available facility staff were cultured twice, residents' hands were cultured once, and air sedimentation cultures were collected twice near residents colonized with ESBL and near a selection of un-colonized residents.

Identification and detection of resistant strains

Faecal and rectal samples were collected using ESwab (Copan diagnostics, Brescia, Italy). ESwab was also used to culture residents’ hands, emphasizing palms, fingers, nails, and jewellery. For air sedimentation cultures, 5 selective agar plates were placed around the selected residents when they were washing and dressing. Hand cultures were obtained from staff members by having the workers dip and rub their hands in tryptic soy broth (TSB) directly.

For environmental cultures, standardized surfaces of 10x10cm were sampled thoroughly using ESwab medium in the first 2 surveys, and a sterile 10x10cm pad soaked in sterile isotonic saline solution for the next 3 surveys.

The sterile pads used for environmental culturing and all ESwabs were incubated for 16-18h at 37°C in 15 ml of TSB containing 8 mg/l vancomycin and 0.25 mg/l cefotaxime. After incubation, 10 μl of the broth was inoculated on an EbSA agar plate (Cepheid Benelux, Ledeberg, Belgium), selective for ESBL-producing Enterobacteriaceae, which was incubated overnight. The TSB rinsates from staff members’ hands were incubated directly, as were the agar plates used for air sedimentation culture.
Identification of all oxidase-negative, Gram-negative bacteria was performed by MALDI-TOF (bioMérieux, Marcy l’Etoile, France). Susceptibility testing was performed by VITEK2 (bioMérieux, Marcy l’Etoile, France), using EUCAST criteria, and ESBL production was confirmed by a double disk method.18

Typing

All phenotypically confirmed ESBL-producing E. coli underwent phylogroup-defining PCR.19 Group B2 E. coli underwent O25:ST131-specific PCR.20 ESBL-producing E. coli obtained from residents’ colonization cultures, environmental cultures, air sedimentation cultures, and hand cultures underwent genotyping for strain characteristics and ESBL variant. Of residents with repetitive positive colonization cultures with similar ESBL-producing E. coli, only the first isolate underwent genotyping. Similarity was defined as identical identity according to species, phylogroup, and O25:ST131 status, and absence of major susceptibility differences (i.e., susceptible versus resistant) for the 25 antibiotics tested. For strain typing, amplified fragment length polymorphism (AFLP) was used.21 Clusters were defined based on both visual and computerised interpretation of AFLP patterns. For ESBL genotyping, a micro-array was performed according to the manufacturer’s guidelines (Check-MDR CT103, CheckPoints, Wageningen, The Netherlands).22,23

Statistical analysis

Acquisition was defined as detection of an ESBL-producing organism in a previously culture-negative resident. Transmission was defined as acquisition of an ESBL-producing E. coli strain identical according to AFLP profile and ESBL-variant to one already present on the ward where the subject resided prior to the acquisition. Routine prevalence surveys in several LTCFs and a hospital in the same area as the LTCF studied showed little clustering of ESBL-producing E. coli and low prevalence of colonization with O25:ST131 E. coli. Consequentially, it is unlikely for newly admitted residents to be colonized with the same strain as present on the ward they are admitted to. Therefore, transmission was also assumed for residents who were admitted during the study period, stayed on a ward over 14 days before being cultured, and when first cultured yielded an ESBL-producing E. coli strain already present on that ward.

To identify inter-individual differences in susceptibility to acquiring colonization, we assessed whether there were differences between individuals in length of stay before being colonized. We reasoned that if difference in susceptibility are present, residents less susceptible to acquiring colonization should remain un-colonized for a longer length of stay than other residents. Length of stay was grouped into three-month periods in which residents could be ESBL-culture-negative and, consequentially, be at risk for acquisition, or have acquired ESBL-producing E. coli. Differences in acquisition
risk between a length of stay shorter vs. longer than 12 months were assessed by Chi-Square analysis.

Median duration of colonization was calculated from the first positive culture using Kaplan-Meier survival analysis, with loss of colonization as the primary outcome. Differences between ESBL-producing E. coli of sequence type O25:ST131 and other ESBL-producing E. coli were tested with Log-Rank analysis. Transmission rates and corresponding reproduction numbers were calculated for ESBL-producing E. coli of sequence type O25:ST131 and other ESBL-producing E. coli separately, taking into account the ward-level infection pressure and assuming that transmission occurs only at the ward level. Residents were considered to have newly acquired or lost colonization on the day of the culture that detected their changed colonization status. Weighted days at risk (at the ward level) were calculated by multiplying, for each day, the number of positive residents per ward by the number of un-colonized residents on the same ward. Weighted days at risk were summed over all wards, separately for all combinations of AFLP+ESBL-variants. Per day transmission rates were calculated by dividing the number of presumed transmissions by weighted days at risk. A per-admission reproduction number was calculated by multiplying the number of residents on a ward (n=20), by the per-day transmission rates of ESBL-producing E. coli of sequence type O25:ST131 and of other ESBL-producing E. coli and the corresponding mean durations of colonization obtained from the Kaplan-Meier survival analysis.

The time for all ESBL-producing E. coli of sequence type O25:ST131 to disappear from the LTCF was estimated by using a mathematical model that incorporated the per-day transmission rate and a constant decolonization rate equal to the mean duration of colonization obtained from the Kaplan-Meier survival analysis. The model randomly simulated 1 million outbreaks. This was repeated for situations with 1-to-9 colonized residents per ward. Also, the effects on outbreak duration of alterations in the transmission rates and mean duration of colonization on outbreak duration were calculated.

Results

Colonization cultures

During the 14-month study period, the LTCF housed a total of 296 residents, 126 male and 170 female, with an average of 192 residents at the time of the prevalence surveys. During the study period, 125 residents were newly admitted and 120 residents were loss to follow up due to transfer to other facilities, transfer back home, or death. The average age at time of the prevalence surveys was 78 years (range: 46-98 years). The participation rate was 90.6% for intended culturing at the prevalence surveys and at admittance. Only 4 residents declined to participate at all culture points.
In total, 1050 rectal or faecal samples were obtained, of which 188 (17.9%) yielded one or more ESBL-producing *E. coli* isolates; 131 contained ESBL-producing *E. coli* of sequence type O25:ST131 and 57 contained other ESBL-producing *E. coli* isolates. The positive rectal samples were obtained from 69 individual residents (23.3%). Table 6.2.1 shows the number of residents who were colonized at the start of the survey, acquired colonization during the study, or were already colonized when admitted during the study period.

<table>
<thead>
<tr>
<th>Organism category</th>
<th>No. residents colonized with ESBL</th>
<th>At start of the survey (number positive during entire survey)^a</th>
<th>By acquisition (number with presumed by-ward transmission)</th>
<th>When admitted during the study period</th>
<th>Total number colonized at any point</th>
</tr>
</thead>
<tbody>
<tr>
<td>O25:ST131 <em>E. coli</em></td>
<td>24 (10)</td>
<td>14 (12)</td>
<td>3</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Other <em>E. coli</em></td>
<td>11 (1)</td>
<td>17 (10)</td>
<td>5</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>35 (11)</td>
<td>29 (22)^a</td>
<td>8</td>
<td>69^b,c</td>
<td></td>
</tr>
</tbody>
</table>

O25:ST131 *E. coli*: ESBL-producing *E. coli* isolates of sequence type O25:ST131. Other *E. coli*: ESBL-producing *E. coli* isolates of other sequence types. O25:ST131 *E. coli*. ^a^ Only residents that were admitted during the entire study period could be positive during the entire survey, this in contrast to residents that were lost to follow up. ^b^ 2 residents acquired both an ESBL-producing *E. coli* of sequence type O25:ST131 and an other ESBL-producing *E. coli*. ^c^ 3 residents were positive with an ESBL-producing *E. coli* of sequence type O25:ST131 at the start of the survey, and acquired an other ESBL-producing *E. coli* later.

The prevalence of colonization with ESBL-producing *E. coli* was unequally distributed across the LTCF. At study onset, wards B-1, B-2, and C-2 had large clusters with ESBL-producing *E. coli* of sequence type O25:ST131 (29 carriers, all isolates from the same AFLP cluster; by-ward prevalence 39%-45%). Wards A-2, A-3, and C-3 had smaller clusters of other ESBL-producing *E. coli* plus sporadic carriage of non-related isolates (16 carriers; by-ward prevalence 11%-23%). The remaining 3 wards had only sporadic cases of ESBL-producing *E. coli* colonization (2 carriers; by-ward prevalence <5%). During the follow-up period, the outbreak with ESBL-producing *E. coli* of sequence type O25:ST131 continued, and a new outbreak of this clone occurred on ward A-3, whereas the outbreaks of other ESBL-producing *E. coli* disappeared over time. On wards C-1 and D, the prevalence of ESBL colonization remained nil. On ward A-1, a small increase in ESBL-producing *E. coli* colonization was observed in the last survey due to both 2 new introductions and 2 transmissions (Supplementary Figure 6.2.1).

Environmental surveys

Of 485 standardized environmental cultures, 17 (3.5%) yielded ESBL-producing *E. coli*, including 17 (6.5%) of 263 done in the latter 3 surveys using the sterile gauze method, versus none (0%) done in the first 2 surveys using the ESwab method ($P<0.001$). Isolates
from only 9 (53%) of the 17 positive cultures matched isolates obtained from residents on the same ward during the same survey. Three identical environmental ESBL-producing *E. coli* isolates were obtained from ward D, but during the corresponding survey, a prevalence survey was nog performed on this ward. Toilets were the sites most likely to yield any ESBL-producing *E. coli*, and overall ESBL-producing *E. coli* of sequence type O25:ST131 were less often cultured than other ESBL-producing *E. coli* (Table 6.2.2).

Table 6.2.2 Prevalence of surface contamination with extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli*.

<table>
<thead>
<tr>
<th>Surface</th>
<th>Total no. of cultures</th>
<th>No. of cultures positive (row %)</th>
<th>Total</th>
<th>ESBL-ST131</th>
<th>Other ESBL-EC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toilet or potty chair</td>
<td>199</td>
<td>11 (5.5)</td>
<td>3 (1.5)</td>
<td>8 (4.0)</td>
<td></td>
</tr>
<tr>
<td>Sink</td>
<td>95</td>
<td>2 (2.1)</td>
<td>1 (1.1)</td>
<td>1 (1.1)</td>
<td></td>
</tr>
<tr>
<td>Kitchen area</td>
<td>90</td>
<td>3 (3.3)</td>
<td>0</td>
<td>3 (3.3)</td>
<td></td>
</tr>
<tr>
<td>Common living area</td>
<td>100</td>
<td>1 (1.0)</td>
<td>0</td>
<td>1 (1.0)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>484</td>
<td>17 (3.5)</td>
<td>4 (0.8)</td>
<td>13 (2.7)</td>
<td></td>
</tr>
<tr>
<td>Ward related*</td>
<td>484</td>
<td>9 (1.9)</td>
<td>4 (0.8)</td>
<td>5 (1.0)</td>
<td></td>
</tr>
</tbody>
</table>

* Number of isolates which were similar to isolates obtained from residents’ colonization cultures at the same time-period.

Hand and air sedimentation cultures

Of 176 residents, 168 (95.5%) underwent hand culturing. At the time of hand culture, 30 (17.9%) of these residents were colonized with ESBL-producing *E. coli*, and 3 had unknown carriage status. Hand cultures yielded an ESBL-producing organism (in each instance, non-*E. coli*) for only 2 residents, neither of which was known to be colonized with ESBL-producing organisms. For only one of these residents did the cultured strain, a *bla*CTX-M9-producing *Enterobacter cloacae*, correspond with a strain colonizing other ward residents (here, 2).

Air sedimentation cultures were obtained near 52 residents, including all 26 ESBL carriers plus 26 un-colonized patients. Three of these residents, all colonized with ESBL-producing *E. coli* of sequence type O25:ST131, had positive air sedimentation cultures with the same strain they were colonized with. Repeated air sedimentation cultures for these 3 residents, and for 12 other ESBL-carriers, were negative.

Hand cultures from 4 (3%) of 148 cultured staff members yielded ESBL-producing *Enterobacteriaceae*. All 4 individuals worked on ward C-2, an O25:ST131 outbreak ward. However, only one of these individuals carried ESBL-producing *E. coli* of sequence type O25:ST131 on the hands; the other 3 all carried *bla*CTX-M9-producing *Enterobacter cloacae*, another strain present in a colonized ward resident.
Length of stay as protective factor

The risk of acquiring ESBL-producing *E. coli* remained stable for all lengths of stay, and prolonged length of stay did not select for residents less-susceptible to acquiring ESBL-colonization (Figure 6.2.1). For both O25:ST131 *E. coli* and other ESBL-producing *E. coli*, acquisition risk did not differ between residents with a length of stay shorter than 12 months versus longer than 12 months (P=0.13 and P=0.84, respectively).

![Figure 6.2.1. Acquisition of carriage with ESBL-producing *E. coli* at various lengths of stay.](image)

Histogram shows the number of facility residents during the study period (Y-axis) with a length of stay equal or greater than the indicated number of months (X-axis). Blue, residents not colonized with ESBL-producing *E. coli* and therefore at risk for acquisition. Red, residents who acquired ESBL-producing *E. coli* during the indicated time period after admission. Residents already admitted at the start of our survey “entered” the histogram at moment of their first negative culture.
Duration of colonization

During the study, conversion to ESBL-negative was observed for 13 (33%) of 39 carriers with ESBL-producing *E. coli* of sequence type O25:ST131, versus 18 (62%) of 29 carriers with other ESBL-producing *E. coli* (*P*=0.03; residents acquiring colonization in the final prevalence survey were excluded). Survival analysis showed that the half-life of carriage for ESBL-producing *E. coli* of sequence type O25:ST131 was 13 months, compared to 2 to 3 months for other *E. coli* isolates (*P*<0.001; Figure 6.2.2).

Transmission rates

During the study, we documented 12 transmissions involving ESBL-producing *E. coli* of sequence type O25:ST131 and 10 involving other ESBL-producing *E. coli*. The ratio of per-day transmission rates for ESBL-producing *E. coli* of sequence type O25:ST131 versus other ESBL-producing *E. coli* was 0.59 (95% CI: 0.26-1.32), indicating that ESBL-producing *E. coli* spread as readily as ESBL-producing *E. coli* of sequence type O25:ST131. The corresponding reproduction numbers were 0.66 (95% CI 0.25-1.09) for ESBL-producing *E. coli* of sequence type O25:ST131 and 0.56 (95% CI 0.20-1.01) for other ESBL-producing *E. coli*.
Prolonged colonization with O25:ST131 *E. coli*

Estimated outbreak duration

Figure 6.2.3 and supplementary Figure S6.2.2 show the estimated time for all ESBL-producing *E. coli* of sequence type O25:ST131 to disappear from a ward, given the number of colonized residents present, the mean duration of colonization, and the current reproduction numbers. A halving of the average length of colonization, e.g. by active decolonization, was predicted to reduce the expected duration of the outbreak twice as effectively as does halving the transmission rate, e.g. by improved hygiene. In the current situation, with a maximum of 6 colonized resident per ward, the mean expected time for all sequence type O25:ST131 ESBL-producing *E. coli* to disappear from the LTCF is >1000 days or 3-to-4 years. Halving the duration of colonization reduces the average expected time to approximately 400 days (1 year), versus 800 days (2-to-3 years) when the transmission rate is halved.

![Graph showing estimated time for all ESBL-producing Escherichia coli sequences to disappear from a ward in relation to transmission rate and duration of colonization.](image)

**Figure 6.2.3** Estimated time for all ESBL-producing *Escherichia coli* of sequence type O25:ST131 to disappear from a ward in relation to transmission rate and duration of colonization. Dots: mean time for all ESBL-producing *Escherichia coli* of sequence type O25:ST131 to disappear from a ward. Vertical lines: 95% confidence interval. Grey symbols: assume current conditions, without any change in tactics. Blue symbols: assume a 50% reduction of the transmission rate, e.g., by intensified infection control efforts. Red symbols: assume a 50% reduction in the duration of colonization, e.g., by active decolonization.

Discussion

We performed a prospective cohort study of ESBL colonization during an outbreak of intestinal colonization with various ESBL-producing *E. coli*, including ESBL-producing *E. coli* of sequence type O25:ST131, in a LTCF. In spite of the measures taken, the
outbreak with ESBL-producing *E. coli* of sequence type O25:ST131 persisted, while smaller outbreaks of other ESBL-producing *E. coli* resolved over time. In exploring the basis for the sustained outbreak with ESBL-producing *E. coli* of sequence type O25:ST131, we determined that transmission rates did not differ between ESBL-producing *E. coli* of sequence type O25:ST131 and other ESBL-producing *E. coli*, which excluded one possible explanation for the persistence of the outbreak with ESBL-producing *E. coli* of sequence type O25:ST131. Likewise, by examining the environment, staff members and direct resident-to-resident contact as possible transmission routes, we found that ESBL-producing *E. coli* of sequence type O25:ST131 were practically absent from the corresponding cultures, whereas other ESBL-producing *E. coli* were more often detected; thus environmental contamination with ESBL-producing *E. coli* of sequence type O25:ST131 was not explanatory. Instead, we documented more prolonged colonization of individual residents with ESBL-producing *E. coli* of sequence type O25:ST131 with a half-life of approximately 13 months, versus 2-3 months for other ESBL-producing *E. coli* (*P*<0.001). This appeared to sustain the outbreak with ESBL-producing *E. coli* of sequence type O25:ST131, suggesting that outbreak control efforts should focus on reducing duration of colonization rather than reducing transmission.

Against our prior hypothesis, longer length of stay did not select for residents who were less-susceptible for acquisition of ESBL-producing *E. coli*, indicating that residents were equally susceptible during their entire stay and that differences in susceptibility between residents are unlikely. The per-admission reproduction numbers were 0.66 (95% CI: 0.25-1.09) for ESBL-producing *E. coli* of sequence type O25:ST131 and 0.56 (95% CI: 0.20-1.01) for other ESBL-producing *E. coli*, which were comparable. Both were below 1, indicating that the outbreak should end under the current infection control measures.

In the current situation, with residents having a long average length of stay, a strain (ESBL-producing *E. coli* of sequence type O25:ST131) that causes persistent colonization, and infection control measures in place, the estimated time for this outbreak strain to disappear from the LTCF is 3-4 years. This indicates that prolonged periods of increased prevalence do not necessarily mean that infection control measures are ineffective. Improving infection control measures were predicted to influence the duration of this outbreak only minimally, whereas shortening the duration of colonization would have a more pronounced effect. Unfortunately, effective colonization strategies are not abundant. Probiotics\(^2^4\) and donor faeces infusion\(^2^5\) have been used in experimental settings. Selective bowel decontamination regimes are proposed as suitable eradication therapies for ESBL-colonization.\(^{26,27}\) However, others observed only a temporary suppression of ESBL carriage during treatment, with a rapid rebound 1 week after the end of treatment.\(^2^8\)

Few reports of prolonged colonization with ESBL-producing *E. coli* have been published. Alsterlund et al. reported 5 residents who stayed colonized for 41 to 59 months after an
Differences in designating residents versus standardized intervals are these sequence intervals. Our definition was that periods exceeding 12 months (i.e., ~6 months) have been investigated previously. Hilty et al. suggested that E. coli phylogroups B2 and D are more often transmitted within households than are phylogroups A and B1. However, these differences were not statistically significant (P=0.10). Adler et al. found that CTX-M-27 (CTX-M-9 group)-producing E. coli of sequence type ST131 spread more efficiently than the CTX-M15 E. coli of sequence type ST131. Since our cohort included only few CTX-M-9 group-positive O25:ST131 isolates, we could not reliably compare these two ST131 subgroups.

Our analysis has several limitations. One is the underlying assumption that all residents are equally contagious over time, whereas, hypothetically, “super-spreaders” or periods of increased infectiousness may occur. Second, we used a conservative definition for “transmission” that presumed that transmission occurred only between residents on the same ward and disregarded the possibility of plasmid transmission. The resulting transmission number, which might have been underestimates, were used to calculate reproduction numbers, which if too low, could have resulted in underestimation of the average outbreak duration. On the other hand, the method used to type the isolates (AFLP) is not as detailed as, for example, whole genome sequencing. Theoretically this might have led to an overestimation of transmissibility by designating isolates to the same clonal complex which were actually different on whole genome sequencing. However, using AFLP in prevalence surveys in other healthcare facilities in the same area and time period, revealed hardly any clonal relatedness. Therefore, the clonal relatedness in this specific nursing home is likely to represent clonal spread. Another limitation is the setting, i.e. a specific LTCF, during an outbreak that had triggered intensified infection control measures. Transmission rates and duration of colonization might be different in other settings. However, we suspect that the differences in duration of colonization between ESBL-producing E. coli of sequence type O25:ST131 and other ESBL-producing E. coli can be extrapolated reasonably to other settings.

Our study also had notable strengths. Most important is the length of follow-up with standardized intervals at which standardized cultures are taken, and the high participation rate.

In conclusion, we found that ESBL-producing E. coli of sequence type O25:ST131 can colonize residents for prolonged periods, with an estimated half-life of 13 months.
which contrasts the half-life of 2-3 months for other ESBL-producing *E. coli*. Furthermore, calculated transmission rates did not differ between ESBL-producing *E. coli* of sequence type O25:ST131 and other ESBL-producing *E. coli*, and environmental contamination was more abundant for other ESBL-producing *E. coli* than for ESBL-producing *E. coli* of sequence type O25:ST131. Susceptibility was similar for residents with a shorter and longer length of stay. Duration of colonization was therefore the main identified factor contributing to the success of ESBL-producing *E. coli* of sequence type O25:ST131 in this LTCF. We postulate that prolonged colonization also may be the key to success of this clone worldwide. Our models predict that implementing additional infection control measures aimed at limiting the spread of ESBL-producing *E. coli* of sequence type O25:ST131 will only have a minor effect on outbreak duration whereas effective decolonization strategies should have a more profound effect. Therefore, in addition to implementing infection control measures, development of effective decolonization strategies is warranted to combat outbreaks like this worldwide.
References


Supplemental material

Figure S5.1 Structural overview of the LTC with colonization rates over time for the different wards, subgroups, and colonizing pathogens. The y-axis shows the colonization rate, while the x-axis shows the time points. The data suggests that the colonization rate of ESBL-producing E. coli varied over time, with differences in prevalence not automatically implying a loss of colonization or transmission.
Figure S6.2.2  Histograms with estimated duration of the outbreak according to number of colonized residents with ESBL-producing *E. coli* of sequence type O25:ST131.