

Duration of Colonisation With Extended-spectrum Beta-lactamase-producing *Escherichia Coli*. Results of an Open Cohort Study With Dutch Nursing Home Residents (2013 – 2019)

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Research

Keywords: ST131, ESBL, colonisation, E. coli, survival curve

Posted Date: December 31st, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-136458/v1>

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Abstract

Background

Escherichia coli sequence type ST131 is a recently emerged worldwide pandemic clonal group. Antibiotic resistance, virulence factors or colonisation fitness are mentioned among other as possible factors contributing to the worldwide success. In this study, we assessed the duration of rectal ESBL-producing *E. coli* colonisation in the residents, and compare duration of colonisation for ESBL-ST131 versus ESBL-non-ST131.

Methods

Rectal or faecal samples were obtained from residents of nursing home A between 2013 and 2019 and nursing home B between 2017 and 2019, with repeated point prevalence surveys at intervals of three to six months. Extended-spectrum β -lactamase (ESBL)-producing strains of *E. coli* were identified on selective culture and selective enrichment broth, and examined by antimicrobial susceptibility testing. In nursing home A multilocus sequence typing (MLST) and cluster analyse was performed by respectively O25:ST131-specific PCR and amplified fragment length polymorphism (AFLP). In nursing home B whole genome sequencing data were used to determine MLST and to perform a cluster analyse. Kaplan Meier survival analysis was performed to calculate the median time of rectal colonisation of ESBL-EC with a Log-Rank analysis to test for differences between ESBL-ST131 and ESBL-non-ST131.

Results

A total of 144 residents were included: 84 residents (58%) with ESBL-ST131 rectal colonisation and 60 residents (42%) with ESBL-non-ST131 rectal colonisation. Survival analysis showed a median colonisation length of 13 months for ESBL-ST131 (95%CI: 7,2 – 18,7) versus 8,3 months (95%CI: 2,8 – 13,8) for ESBL-non-ST131 ($p = 0,028$). Remarkably, in the subgroup ST131 the median colonisation length was significantly longer in female than in males: 25,7 months versus 8,1 months ($p = 0,013$).

Conclusion

Here we found a prolonged colonisation duration of ESBL-ST131 compared to ESBL-non-ST131 in residents of Dutch nursing homes. Prolonged colonisation duration complicates the controlling and ending an ESBL-ST131 outbreak, especially in long stay settings such as nursing homes.

Background

Background/rationale

Escherichia coli (*E. coli*) sequence type (ST) 131 is an extraintestinal pathogenic *E. coli* (ExPEC) that has emerged recently (1,2), and is nowadays the predominant *E. coli* lineage among ExPEC isolates worldwide (3). Moreover, ST131 is associated with the worldwide spread of the CTX-M-15 extended

spectrum β -lactamase (ESBL) resistance gene (3). ESBL producing ST131 (ESBL-ST131) is a major contributor to hospital- and community-acquired infections, such as urinary tract infection and bloodstream infections (4,5). Whereby ESBL-ST131 infections are most common among elderly and ESBL-ST131 carriage is particularly prevalent in nursing homes and long-term care facilities (6,7).

Despite many studies examining the epidemiology of ESBL-ST131, the reason why this clone achieved such considerable success in such a short time span is still unclear. Antibiotic resistance, virulence factors or colonisation fitness are mentioned among other as possible factors contributing to this success (3).

In 2016, Overdeest et al. evaluated an ESBL-ST131 outbreak in a Dutch nursing home (nursing home A) between March 2013 and April 2014, whereby six point prevalence surveys were performed at intervals three months by culturing faeces or rectal swabs from all residents (8). The study showed a prolonged colonisation duration of residents with ESBL-ST131, with a median colonisation duration of 13 months compared to two to three months for other ESBL-producing *E. coli* (ESBL-non-ST131) ($p < 0.001$).

The point prevalence surveys were continued until June 2019 in nursing home A. In this study, we evaluated the duration of ESBL-producing *E. coli* (ESBL-EC) colonisation in nursing home A over the period of 6 years (March 2013 to June 2019). In addition, we included a ST131 outbreak in a second Dutch nursing home (nursing home B) from the period March 2017 to June 2019.

The objective of this study was to assess the duration of rectal ESBL-producing *E. coli* colonisation in residents of two Dutch nursing homes, and compare duration of colonisation for ESBL-ST131 versus ESBL-non-ST131.

Methods

Detection of the ST131 outbreak and setting

As part of standard infection control measure, a prevalence survey was performed in the two nursing homes (nursing home A in 2012 (7), nursing home B in 2016) (Fig. 1). Both surveys showed a high prevalence of rectal ESBL colonisation of 20,6% (7) in nursing home A and 13,9% in nursing home B. Strain typing showed the presence of different clusters of ESBL-ST131, among other smaller clusters and unique strains of other sequences types in both nursing homes. Outbreak measures were implemented, including repetitive prevalence surveys at intervals of three to six months by culturing faeces or rectal swabs from all residents. In June 2019, the prevalence surveys were ended in both nursing homes.

Nursing home A is located on one location consisting of four semi-separate buildings with a total of nine wards. Nursing home B consists of five different locations with a total of 15 wards. In two wards (on two locations), an ST131 outbreak was detected, which were included in this study. In both nursing homes, wards housed about 20 residents and contained communal areas. Sanitary facilities were shared by

several residents each. Nursing staff was dedicated to specific wards. The locations contained communal recreation and therapy areas where residents from all wards met regularly.

Study design and study population

We conducted an open cohort study with repeated point prevalence surveys from March 2013 to June 2019 in nursing home A and from March 2017 to June 2019 in nursing home B in the Netherlands. Residents with at least one ESBL-EC positive rectal or faecal swab in a prevalence survey in the study period were eligible for inclusion. Residents acquiring colonisation in the final prevalence survey were excluded.

Definitions

Rectal colonization with ESBL-EC was defined as detection of ESBL-EC in at least one rectal swab or faecal sample. A resident was considered no longer colonised (loss of colonisation) when one rectal swab or stool sample no longer yielded ESBL-EC or when strain typing showed a different cluster or sequence type than found in the previous ESBL-EC positive culture of the resident. Residents were only included in the study once.

Variables

Data concerning gender, date of birth, day of discharge from nursing home or day of death were obtained from the nursing homes records.

Detection of ESBL-producing *E. coli*

The intended sampling schedule consisted of a quarterly screening in all residents of the wards involved, in nursing home A and B. Rectal colonisation of ESBL-EC was determined by culture of rectal or faecal samples (Eswab, Copan, Italy). Swabs were inoculated on extended-spectrum β -lactamase screening agar (EbSA) plate (AlphaOmega, 's-Gravenhage, Netherlands) and 5% Sheep blood agar (growth control). The remaining Eswab fluid was transferred in 5 mL tryptic soy broth containing cefotaxime (0.25 mg/L) and vancomycin (8 mg/L) (TSB-VC). After 18–24 hours incubation (35–37 °C), the TSB-VC was subcultured on an EbSA plate. For all Gram-negative rods growing on the EbSA, species identification and susceptibility testing was performed by MALDI-TOF (bioMérieux, Marcy l'Etoile, France) and VITEK 2 (bioMérieux, Marcy l'Etoile, France), respectively. Phenotypic ESBL production was confirmed by double disk method (9)

Genotyping and strain typing

Nursing home A

All phenotypically confirmed ESBL-EC underwent an O25:ST131-specific PCR (10). ESBL genotyping was performed using a micro-array (CheckPoints, Wageningen, the Netherlands) (11,12) and strain typing by using amplified fragment length polymorphism (AFLP) (13). An AFLP cluster was assigned based on both visual and computerised interpretation of AFLP patterns.

Genotyping and strain typing were performed for the first ESBL-EC from each resident and for and subsequent ESBL-EC strains that were not similar to the first strain. Similarity was defined as identical species, identical phylogroup and O25:ST131 status and absence of major differences in susceptibility (susceptible vs. resistant) for the all antibiotics tested.

Nursing home B

All phenotypically confirmed ESBL-EC were sent to the University Medical Center Groningen, The Netherlands, for whole-genome sequencing (WGS) and subsequent downstream data analysis multilocus sequence typing (MLST), Whole-genome MLST (wgMLST) (core and accessory genome) and resistome analysis was performed at Microvida, location Amphia, The Netherlands. Genomic DNA was extracted using the Ultraclean Microbial DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, US) following the manufacturer's instructions. The DNA library was prepared using the Nextera XT v2 kit (Illumina, San Diego, CA, USA) according to the manufacturer's instructions and then run on the MiSeq (Illumina) for generating paired-end 250-bp or 300-bp reads (Table 1). De novo assembly was performed by CLC Genomics Workbench version 10.0.3, 10.1.1, 11.0, 11.0.1 or 12.0 (QIAGEN, Hilden, Germany) (Table 1) after quality trimming ($Q_s \geq 20$) with optimal word sizes (Kmer size 30). Assembled genomes were typed using MLST and wgMLST with SeqSphere version 6.0.2 (Ridom GmbH, Münster, Germany). Clusters were defined using wgMLST% identity via distance matrix, with cluster cut-off criteria described by Kluytmans et al. (14). Assembled genomes were uploaded to the webtool ABRicate on the Galaxy platform version 1.0.1 (<https://usegalaxy.eu>) using the Resfinder database (last update September 10, 2019) for identifying the acquired resistance genes with default settings (minimal coverage 60%; %ID75%).

Table 1
Summary of the Miseq reagents kit and CLC Genomics workbench versions used in the study.

Prevalence date	Mar-17	Jun-17	Oct-17	Jan-18	Apr-18	Jul-18	Nov-18	Mar-19	Jun-19
Miseq reagent kit	2 × 250	2 × 250	2 × 300	2 × 150; 2 × 250	2 × 250	2 × 250	2 × 250	2 × 250	2 × 250
CLC Genomics Workbench version	10.0.3	10.0.3	10.1.1	11.0	11.01	11.01	12.0	12.0	12.0

Genotyping and strain typing were performed for every detected ESBL-EC strain throughout the study period. When more than one (morphologically different) ESBL-EC was detected in a culture, genotyping and strain typing were only performed for strains with major differences in susceptibility.

Statistical analysis

Data analysis was performed using statistical package for social science (SPSS) version 25. Comparison of categorical variables between the ESBL-ST131 and ESBL-non-ST131 group was performed with chi-square test or Fisher's exact test. Kaplan Meier survival analysis was performed to calculate the median time of rectal colonisation of ESBL-EC with a Log-Rank analysis to test for differences between ESBL-ST131 and ESBL-non-ST131. For testing the effect of the few available variables (age, gender, nursing home) on time of carriage in residents, log rank tests were also performed. P-value < 0.05 was considered as statistically significant.

For each resident, the time of rectal colonisation of ESBL-EC was calculated as the time from the first ESBL-EC positive sample until the last ESBL-EC positive sample plus the half-time between the last ESBL-EC positive culture and the first ESBL-EC negative culture (Fig. 2a) or the first ESBL-EC positive culture with a different sequence type or cluster type (Fig. 2b). Both situations were labelled as an 'event' in the Kaplan Meier survival analysis.

In residents whose last sample was still ESBL-EC positive (no loss of colonisation) at the end of the study period, the time of rectal colonisation of ESBL-*E. coli* was calculated as the time from the first ESBL-EC positive sample until the last ESBL-EC positive sample (Fig. 2c). Residents who died or were discharged before the end of the study, the time of rectal colonisation of ESBL-*E. coli* was calculated as the time from the first ESBL-EC positive sample until time of death or discharge (Fig. 2d). These cases were labelled as 'censored' in the Kaplan Meier survival analysis.

Results

Between March 2013 and June 2019, 23 point prevalence surveys were performed in nursing home A and nine point prevalence surveys in nursing home B at intervals of three months to six months by culturing faecal or rectal swabs from all residents ($n = 1059$ unique residents). In total, 155 residents had at least one ESBL-EC positive sample in a prevalence survey between (Fig. 3). Of these, eleven residents were excluded from the study because O25:ST131-specific PCR was not performed ($n = 5$), result of the O25:ST131-specific PCR or WGS MLST was inconclusive ($n = 2$), resident acquired ESBL-EC in the final prevalence survey ($n = 6$) or the resident carried an ESBL-ST131 and ESBL-non-ST131 in the first sample ($n = 1$). For one resident, wgMLST cluster analysis showed 18 different ST131 strains in nine rectal samples of the resident. Further analysis of the WGS data showed that the hypermutation of these ST131 strains were due to a defect in *mutA* gene which involves in the mismatch repair mechanism (15). Consequently, determining duration of colonisation at strain level was not possible and the resident was excluded from the study.

Therefore, 144 residents were included: 84 residents (58%) with ESBL-ST131 rectal colonisation and 60 residents (42%) with ESBL-non-ST131 rectal colonisation (S1-2). In the case of 77/144 residents (53%) the resident was already colonised with ESBL-EC in the first sample of the surveys: 45/84 residents (53,6%) with ESBL-ST131 and 32/60 residents (53,3%) with ESBL-non-ST131 colonisation. Gender and age at time of first ESBL-EC positive sample for both groups are depicted in Table 2.

Table 2
Age on the day of the first ESBL-EC positive rectal sample and gender characteristics of the residents

Characteristics	ESBL-ST131	ESBL-non-ST131	Total	<i>p</i>
Age in years, median (IQR)	82 (76,2–88,0)	81 (72,2–86,0)	82 (75,0–87,0)	0,413
Female gender, <i>n</i> (%)	48 (57,1)	34 (56,7)	82 (56,9)	0,955

Antibiotic susceptibility testing and molecular characterization of ESBL genes

In total, 553 ESBL-EC strains were isolated from the 144 residents. Antibiotic susceptibility testing showed that all ST131-EC strains ($n = 368$) were resistant to ciprofloxacin (100%), in contrast to non-ST131-EC ($n = 185$) - whereby 44 strains (23,8%) obtained from 21 residents were resistant to ciprofloxacin. With regards to co-trimoxazole, 63 ST131-EC strains (17,1%) obtained from 22 residents were resistant versus 110 non-ST131-EC strains (59,4%) obtained from 45 residents.

No resistance to colistin, meropenem or imipenem was observed in any of the strains.

The predominant ESBL genotype in the ESBL-ST131 group was *bla*CTX-M-15 ($n = 72$; 85,7%), followed by *bla*CTX-M-9 ($n = 9$; 10,7%), *bla*CTX-M-27 ($n = 2$; 2,4%) and *bla*CTX-M-1 ($n = 1$; 1,2%). The ESBL genes in the non-ESBL-ST131 group were more diverse, the predominant ESBL genotype was also *bla*CTX-M-15 ($n = 16$; 26,7%), followed by *bla*CTX-M-9 ($n = 13$; 21,7%), *bla*CTX-M-1 ($n = 11$; 18,3%), *bla*CTX-M-3 ($n = 9$; 15,0%), TEM ($n = 4$; 6,7%), SHV ($n = 4$; 6,7%) and one CTX-M-14 ($n = 2$; 3,3%) CTX-M-32 ($n = 1$; 1,7%)

Duration of colonisation

During the study period, loss of colonisation of ESBL-EC or a different sequence type and/or cluster type than in previous cultures, was observed in 39 ST131-EC carriers (46,4%) and in 33 non-ST131-EC carriers (55,0%) ($p = 0,31$) (Fig. 4). The median colonisation length for ST131-ESBL was 13 months (95%CI: 7,2–18,7), in contrast to 8,3 months (95%CI: 2,8–13,8) ($p = 0,028$) for non ST131 (Fig. 5).

Additionally, a further analysis was based on the stratification of the ESBL-ST131 and ESBL-non-ST131 groups for age, gender and nursing home. In the subgroup ST131, median colonisation length was significantly longer in female than in males: 25,6 months [95% CI, 0,6–50,7] versus 8,1 months [95%CI, 5,9–10,3] ($p = 0,013$) (S3-5). For age and nursing home, no statistical differences were found in the subgroup analysis.

Discussion

In 2016, Overdeest et al. evaluated the colonisation durations of ESBL-EC in nursing home A for the period 2013–2014 (14 months), and found a prolonged colonisation duration of residents with ESBL-

ST131, with a median of 13 months compared to two to three months for other ESBL-producing *E. coli* (ESBL-non-ST131) ($p < 0.001$) (8). In this study, we evaluated the duration of rectal ESBL-producing *E. coli* colonisation in residents of nursing home A for a study period of six years (2013–2019) and included a second Dutch nursing home (nursing home B) with a study period of two years. (2017–2019). In concordance with Overdeest et al. we found a prolonged colonisation of residents with ESBL-ST131, with a median of 13 months compared to eight months for ESBL-non-ST131 ($p = 0,028$). Remarkably, in the subgroup ST131 the median colonisation length was significantly longer in female than in males: 25,6 months versus 8,1 months ($p = 0,013$).

On a resident level, prolonged colonisation is a risk for the reason that colonisation can proceed to (extraintestinal) infections (16) for an extended time, and extraintestinal infections due to *Escherichia coli* cause considerable morbidity, mortality, and increased health care costs (17).

At the institutional level, prolonged colonisation is a risk as colonised residents can contribute to transmission for an extended times. And once ESBL-ST131 is introduced and spread in a nursing home, it will take a lot of effort and time for ESBL-ST131 to disappear from a nursing home setting partly because of the prolonged duration of colonisation (8).

There are a few other studies that investigated duration of colonisation for ESBL-ST131 versus other ESBL-EC. Van Duijkeren et al. found that ESBL-EC colonisation persisted for > 8 months in one-third of the ESBL-positive persons and found that prolonged colonisation was statistically significantly associated with the detection of phylogenetic group B2 and ST131, among others (18). Titelman et al. showed that colonisation with *E. coli* was still apparent after 12 months in 64% ($n = 26$), and 40% ($n = 14$) of carrying *E. coli* ST131 or other STs, respectively ($p = 0.12$) (19). Ismail et al. investigated the incidence and duration of colonisation of ciprofloxacin-resistant *E. coli* in nursing homes in Michigan. The study showed that ST131 strains in residents were carried for significantly longer duration when compared to non-ST131 strains (10 months versus 3 months) (20).

The exact reason for the prolonged ESBL-ST131 colonisation is still unclear, but several studies have demonstrated that ESBL-ST131 has enhanced intestinal colonisation capabilities over other *E. coli*, which are mediated by type-1 fimbriae. Type-1 fimbriae are adhesion organelles that facilitate adherence to mucosal surfaces (21) via interaction with mannosylated receptors (22). Sarkar et al. investigated clinical ESBL-ST131 isolates and type 1 fimbriae null mutants for colonisation of human intestinal epithelia and in mouse intestinal colonisation (22). The study demonstrated that ST131 strains adhered and invaded human intestinal epithelial cells more than commensal *E. coli* strains. Moreover, they showed that ST131 strains can overcome host colonisation resistance to establish persistence within the intestines, and that type-1 fimbriae enhanced long term colonisation. Type-1 fimbriae also have critical role in biofilm formation by ST131 strains. *In vitro* studies of Sarkar et al. (23) and of Kudinha et al. (24) suggested that ST131 strains are more capable at biofilm production than other *E. coli* strains. Gibreel et al. investigated the metabolic profiles of 300 *E. coli* strains using Vitek2 Advanced Expert System and showed that ST131 strains were significantly more likely to have a higher metabolic potential than strains of other

sequence types (25). These traits (improved biofilm production and high metabolic potential) probably enhance the ability of ST131 to establish and maintain intestinal colonisation (3,26).

The large difference of median colonisation length between female and male (25,6 months versus 8,1 months) in the subgroup ST131 was an unexpected finding. This finding could not be explained by differences in age distribution within the subgroup, nor by the number of residents who died during the study period. **(S3)**. A possible explanation could be a difference in the presence of known risk factors for (prolonged) ESBL colonisation between the sexes, such as antibiotic use, proton pump inhibitor use or variables associated with higher need for care (18,27). Unfortunately, such clinical information was not acquired in this study. Further studies into this observed difference are warranted.

Another limitation of this study is the setting. The study was performed in two Dutch nursing homes in an outbreak situation, which may reduce generalizability for other settings and/or patient populations. The major strength of our study includes the standardised cultures taken at defined intervals and most importantly the long follow-up period up to six years in nursing home A and 2 years in nursing home B.

Conclusion

Understanding the dynamics of ESBL-ST131 in residents in nursing homes is crucial to identify effective infection control measures. Here we found a prolonged colonisation duration of ESBL-ST131 compared to ESBL-non-ST131. A consequence is that once ESBL-ST131 is introduced and spread in a nursing home, it will take a lot of effort and time for ESBL-ST131 to disappear from the setting. More stringent control measures for ESBL-ST131 may therefore be warranted.

List Of Abbreviations

AFLP Amplified Fragment Length Polymorphism

E. coli *Escherichia coli* sequence type ST131

ExPEC extraintestinal pathogenic *E. coli*

ESBL extended spectrum β -lactamase

ESBL-EC extended spectrum β -lactamase producing *Escherichia coli*

ST131 sequence type ST131

MLST multilocus sequence typing

wgMLST whole-genome MLST

WGS whole-genome sequencing

Declarations

Ethics approval and consent to participate

The data of patients used in this study were part of routine clinical practices in The Riethorst Stromenland and Thebe Long-care facilities and their anonymous use is beyond the scope of the Medical Research Involving Human Subjects Act.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

Funding

None

Authors' contributions

VW, TdG, YH, LK, AM, BvdW and JK performed the investigation and management of the outbreak. VW and JK did the data analysis. VW wrote the first draft of the manuscript, TdG, YH, LK, AM, BvdW and JK reviewed, provided critical feedback and contributed to subsequent draft. All authors approved the final version for publication.

Acknowledgments

We would like to acknowledge the healthcare workers and colleagues of Microvida Laboratory for Microbiology who contributed to the investigation and the control of this outbreak.

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Figures

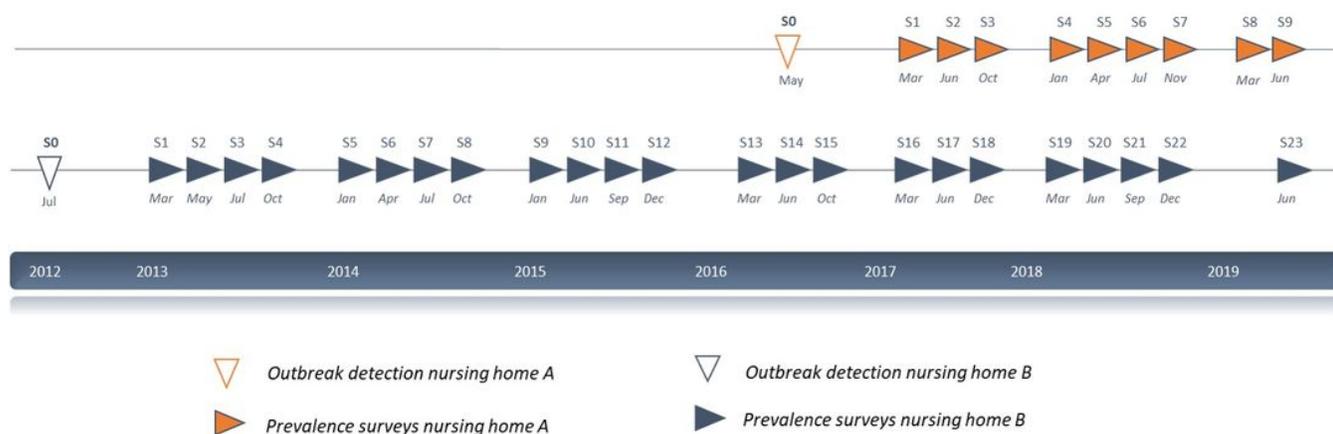


Figure 1

Overview of the outbreak detection and subsequent prevalence studies in nursing home A and nursing home B.

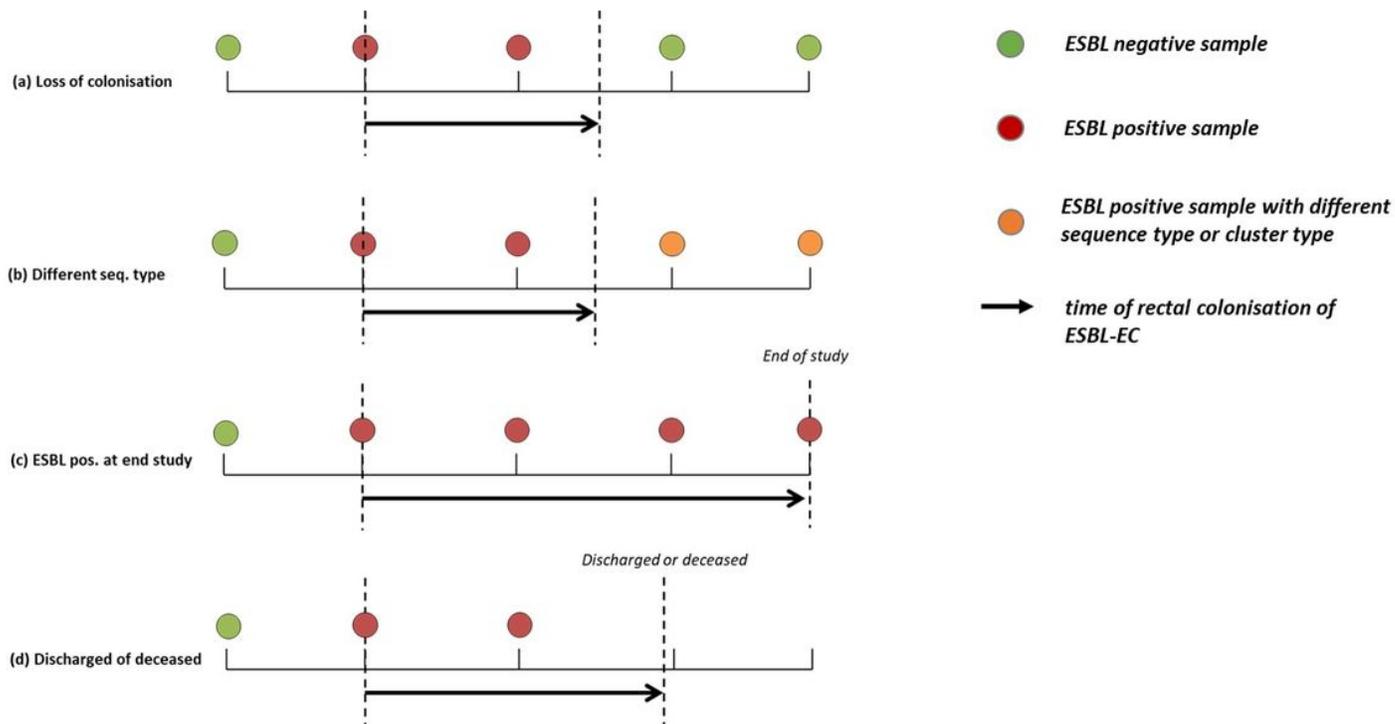


Figure 2

schematic representation of determination colonisation duration in different situations.

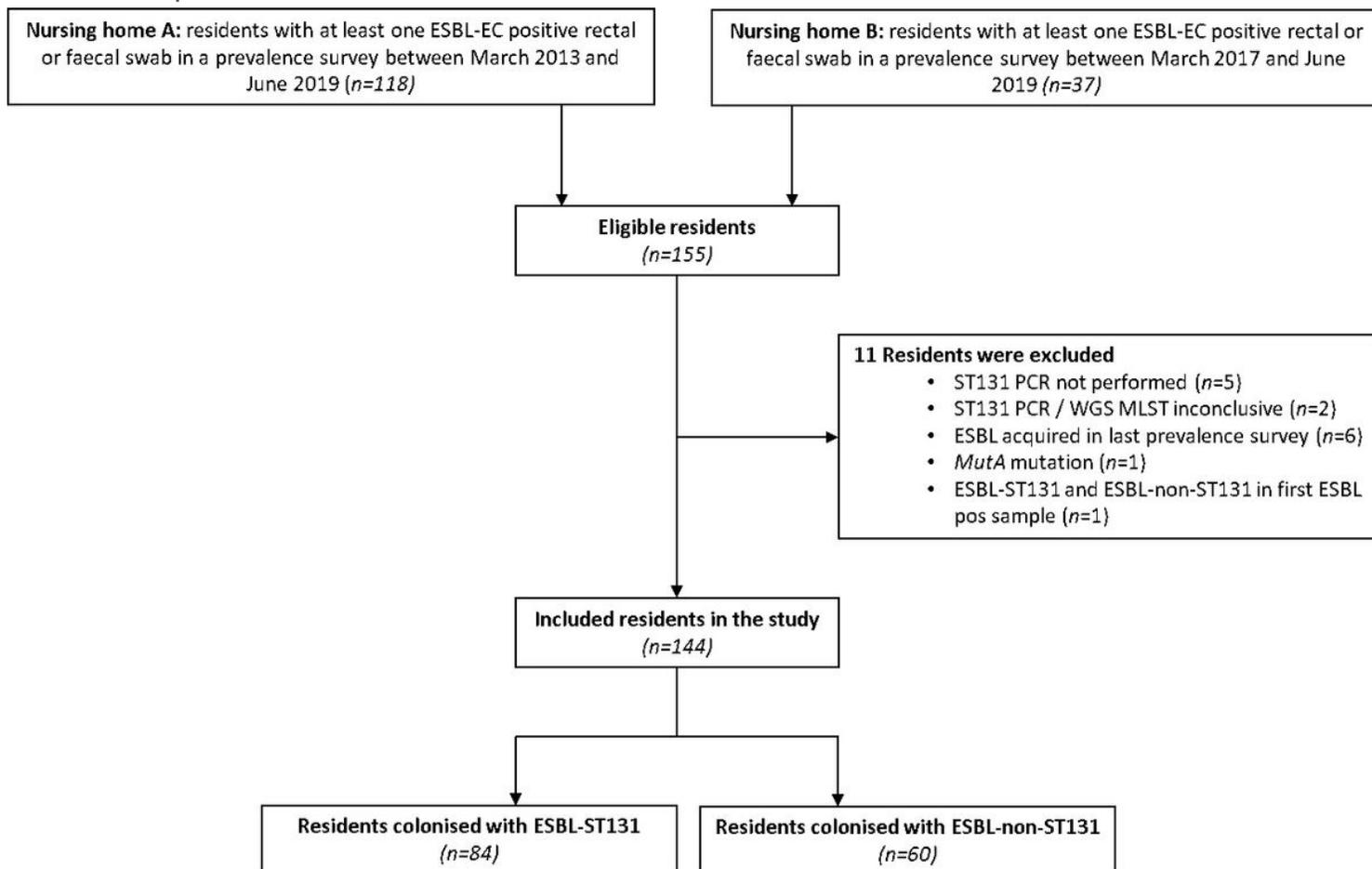


Figure 3

Flowchart for inclusion

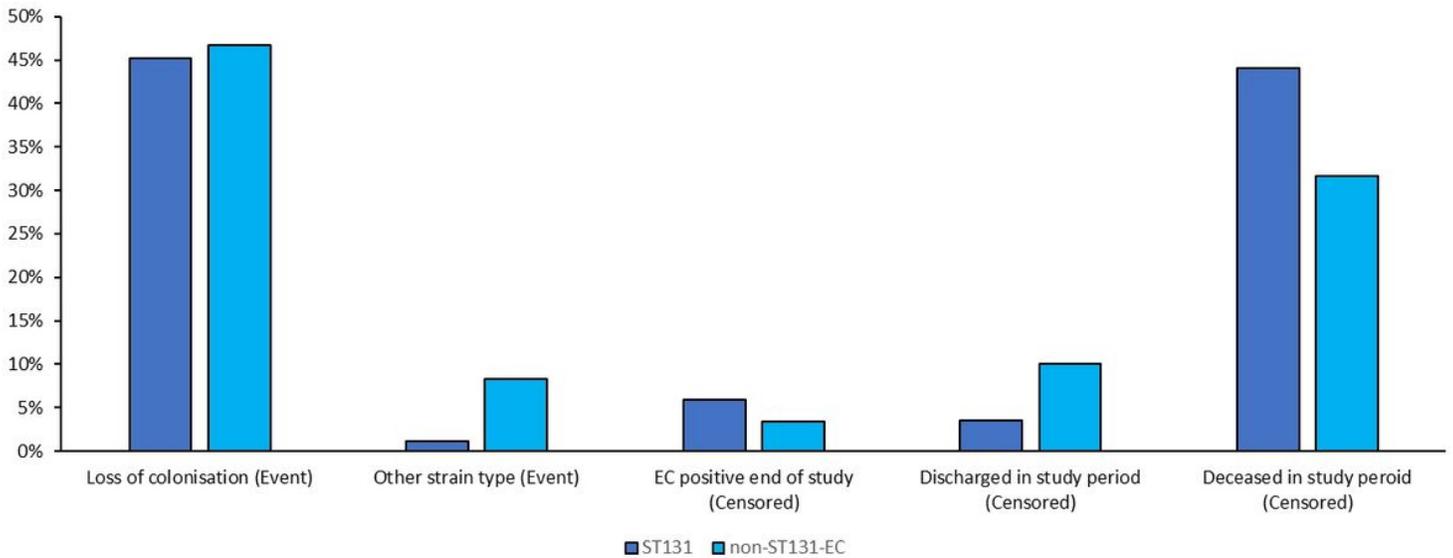
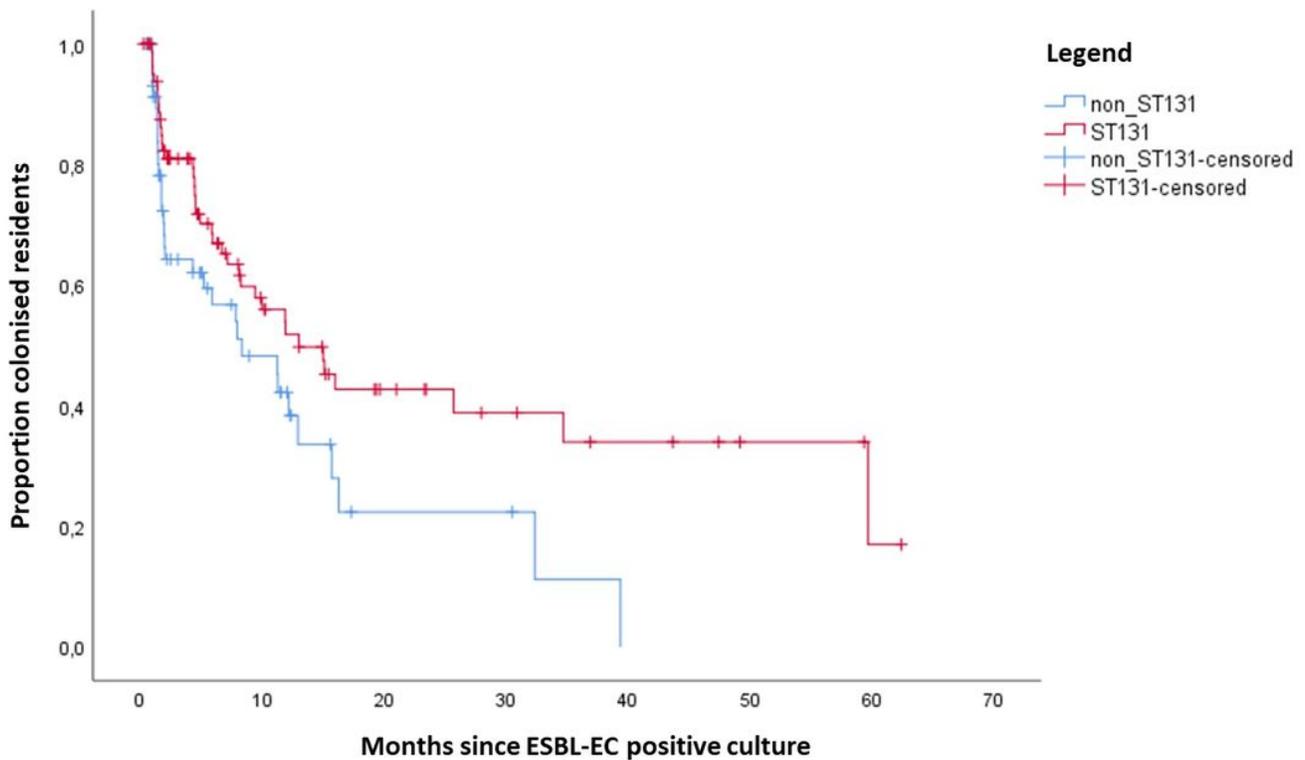


Figure 4

overview of the proportion and reason of events and censoring for the Kaplan Meier analyses for the ESBL-ST131 and ESBL-non-ST131 group. There were no significant differences between the groups ESBL-ST131 and ESBL-non-ST131 within the different groups of reasons for event/censoring.



ST131	84	29	14	9	6	3	1
Non-ST131	60	16	3				

Figure 5

Kaplan-Meier curves of survival probability of residents colonised with ESBL-producing *E. coli*, by ST131 and non-ST131. Values below indicate the number of residents per group.

Supplementary Files

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- [2020.12.07SupplementarymaterialsST131.docx](#)