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# One decade of point-prevalence surveys for carriage of extended-spectrum beta-lactamase-producing enterobacterales: whole genome sequencing based prevalence and genetic characterization in a large Dutch teaching hospital from 2013 to 2022

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## Abstract

**Objectives** To determine the prevalence, trends, and potential nosocomial transmission events of the hidden reservoir of rectal carriage of extended-spectrum beta-lactamase-producing Enterobacterales (ESBL-E).

**Methods** From 2013 to 2022, yearly point prevalence surveys were conducted in a large Dutch teaching hospital. On the day of the survey, all admitted patients were screened for ESBL-E rectal carriage using peri-anal swabs and a consistent and sensitive selective culturing method. All Enterobacterales phenotypically suspected of ESBL production were analysed using whole genome sequencing for ESBL gene detection and clonal relatedness analysis.

**Results** On average, the ESBL-E prevalence was 4.6% (188/4,119 patients), ranging from 2.1 to 6.6% per year. The ESBL-prevalence decreased on average 5.5% per year. After time trend correction, the prevalence in 2016 and 2020 was lower compared to the other year. Among the ESBL-E, Escherichia coli (80%) and CTX-M genes (85%) predominated. Potential nosocomial transmission events could be found in 5.9% (11/188) of the ESBL-E carriers.

**Conclusions** The ESBL-E rectal carriage prevalence among hospitalized patients was 4.6% with a downward trend from 2013 to 2022. The decrease in ESBL-E prevalence in 2020 could have been due to the COVID-19 pandemic and subsequent countrywide measures as no nosocomial transmission events were detected in 2020. However, the persistently low ESBL-E prevalences in 2021 and 2022 suggest that the decline in ESBL-E prevalence goes beyond the COVID-19 pandemic, indicating that overall ESBL-E carriage rates are declining over time. Continuous monitoring of ESBL-E prevalence and transmission rates can aid infection control policy to keep antibiotic resistance rates in hospitals low.

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**Keywords** ESBL, Prevalence, Rectal carriage, Molecular typing, Nosocomial transmission

## Introduction

In Enterobacterales, extended-spectrum beta-lactamases (ESBL) cause resistance to most beta-lactam antibiotics, including third generation cephalosporins. Infections with ESBL producing Enterobacterales (ESBL-E) have been associated with higher morbidity and mortality rates as well as greater costs compared to infections with susceptible Enterobacterales [1–3]. The prevalence of ESBL-E is increasing worldwide [4–6], and the WHO lists ESBL-E as high-priority pathogens [7]. The prevalence of ESBL-E in the Netherlands is among the lowest worldwide [5, 8]. However, not much is known about trends in ESBL-E asymptomatic rectal carriage after 2016.

ESBL-E infections are often preceded by asymptomatic rectal carriage [9, 10], which can be widespread before outbreaks are noticed [11]. Therefore, knowledge of the reservoir of asymptomatic carriage can provide important information about the risks of nosocomial transmission and guide infection control policy [3].

In the present study, we describe the ESBL-E prevalence over the course of 10 years based on a series of annual point prevalence surveys (PPS) undertaken in a Dutch teaching hospital from 2013 to 2022. Secondly, we determine the ESBL-producing Enterobacterales species and the presence of different ESBL genes over time using whole-genome sequencing for genotypic characterisation. Lastly, we identify possible nosocomial transmission events based on whole genome cluster analysis.

## Methods

### Study design

This is a prospective cross-sectional study.

### Setting

The study was performed in the Amphia hospital, a teaching hospital in Breda, the Netherlands. Nine one-day PPS were performed between 2013 and 2022, excluding the year 2018. The surveys were carried out between October – December in 2013–2017 and 2022 and in June in 2019–2021. More details of the hospital and infection control measures can be found in the supplementary [methods](#).

### Participants

The screening encompassed all inpatients, including the dialysis department and the day-care clinic. Patients admitted at the psychiatric ward or emergency department were excluded.

## Sample collection and selective culturing

Culturing of the obtained peri-anal swabs was done according to the protocol previously described by Willemssen et al. [12]. In short, after the swab was plated on a blood agar plate to serve as a growth control, the liquid Amies eluent was inoculated in selective tryptic soy broth, containing cefotaxime (0.25 mg/L) and vancomycin (8 mg/L) (TSB-VC) and subsequently plates on EbSA agar plates (AlphaOmega, 's-Gravenhage, Netherlands) after one day of incubation. Species identification of all morphologically distinct oxidase-negative Enterobacterales was performed using MALDI-TOF MS (2013–2020 bioMérieux, Marcy l'Etoile, France; 2021–2022 Bruker Daltonics, Bremen, Germany), followed by susceptibility testing via VITEK2 AST (bioMérieux, Marcy l'Etoile, France). Phenotypic confirmation of ESBL production was performed of isolates exhibiting a MIC of >1 mg/L for ceftazidime and/or cefotaxime using the combination disc method for cefotaxime, ceftazidime, and/or cefepime with or without clavulanic acid (MastGroup, Bootle, UK) in accordance with the EUCAST protocol (7).

## Molecular typing

One isolate per species per patient was sequenced using Nextera XT chemistry on a MiSeq sequencer (Illumina, San Diego, CA, USA). Error-correction and de novo genome assembly processes were executed using CLC genomic workbench 21.0 (Qiagen, Germantown, MD, USA). The following quality control criteria were used: coverage  $\geq 20$ ; number of scaffolds  $\leq 1,000$ ;  $N50 \geq 15,000$  bases; maximum scaffold length  $\geq 50,000$  bases; used reads  $> 90\%$  and expected genome size 90–115% of the reference. ESBL genes were identified using Abricate v1.0.1 based on the AMRFinderPlus database with a coverage and identity of at least 90%. Multi-Locus Sequence Typing (MLST) and clonal relatedness was analysed using Ridom SeqSphere+ version 8.0 (Ridom GmbH, Munich, Germany). Clonal relatedness between all isolates was determined based on pairwise comparisons of alleles within species-specific whole genome MLST (wgMLST) schemes. Missing values were excluded from the pairwise comparisons. Clonally related isolates were defined as two isolates with the number of allelic differences below the cut-off of clonal relatedness. The following cut-offs based on percentage of different alleles were used: *Escherichia coli* 0.0095; *Klebsiella pneumoniae* 0.0045; *Citrobacter* spp. 0.0030; *Enterobacter cloacae* complex 0.0035 [13].

### Data collection and analysis

Patients were considered ESBL-E positive when at least one of the isolates contained an ESBL gene (defined as such in the NCBI curated database (October 2023)). Cultures with a negative growth control or error in the culture procedure were excluded. Patient demographics were systematically gathered and subsequently anonymized upon integration with the culture data. Patient demographics collected were age, gender, treating specialty, admission date and hospital admission location. When patients transferred during the day of the PPS, only the first culture and corresponding demographics were included. The length of stay was defined as at the time from the start of admission to the time of testing.

### Epidemiological analysis

When clonally related isolates were identified in two patients admitted to the same ward simultaneously, nosocomial transmission was deemed likely. Nosocomial transmission was considered possible, when clonally related isolates were detected in patients admitted at the same time to different wards within the same hospital location or when clonally related isolates were detected in patients admitted to the same ward not at the same time, but within a 60-day window from each other [14]. When clonally related isolates were identified in patients where the conditions for likely or possible nosocomial transmission were not met, nosocomial transmission was deemed unlikely.

### Statistical analysis

The prevalence of ESBL-E carriage was calculated for each year and used as outcome variable in logistic

regression analyses using Statistical Package for Social Science software (IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.). The time trend over the years was calculated using logistic regression analysis correcting for potential confounders: continuous linear yearly time trend, patient’s age (years), length of stay (days), and ICU admission (yes/no). Those were chosen a-priori based on an association with ESBL-E carriage in previous studies [3, 12]. Statistical significance was accepted at a  $p$ -value < 0.05 for the time trend over the years. Additionally, for each year a multiple logistic regression model on the ESBL prevalence was trained with the ESBL carriage status as dependent variable and with the above-mentioned potential confounders. For each year, the ESBL-E prevalence was predicted based on the estimated logistic model of the total of the other eight years, given the average values of the confounders in the left-out year. The difference between this predicted ESBL prevalence and the observed prevalence was calculated and tested for statistical significance.  $P$ -values below 0.0064 per year were considered statistically significant, coinciding with a total experiment wise two-sided significance level alpha of 0.05 across the nine years of which eight were considered mutually independent ( $\alpha_{test} = 1 - (1 - 0.05)^{\frac{1}{8}}$ ). A more detailed description of the statistical analysis can be found in the supplementary methods.

### Results

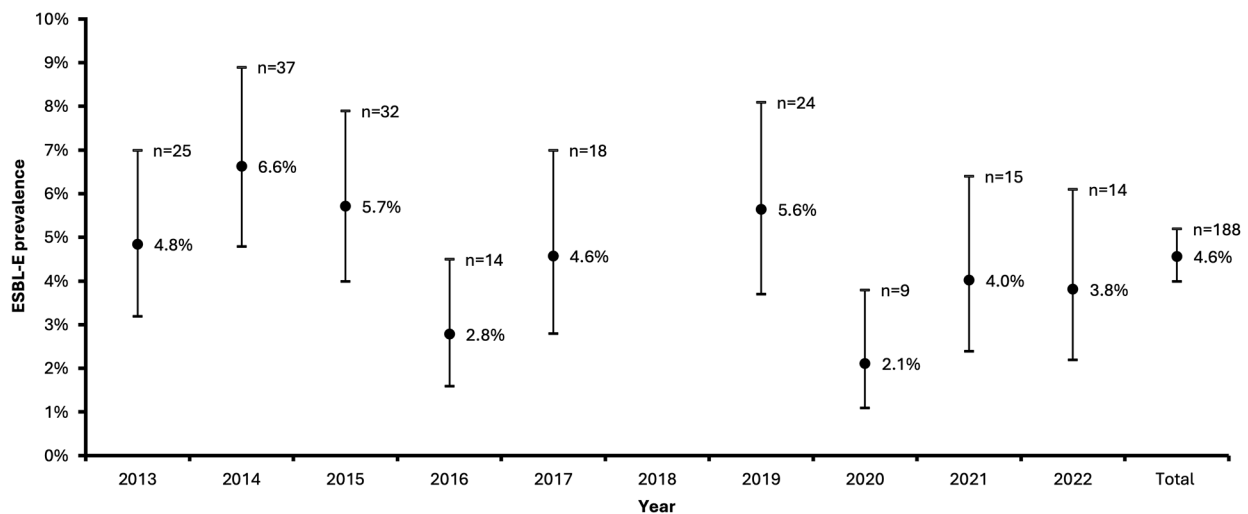
During the nine PPS, 5,494 patients were eligible for ESBL-E screening, of which 4,165 (75.8%) underwent screening resulting in 4,119 (75.0%) evaluable cultures (Table 1). A total of 46 cultures were excluded. Baseline

**Table 1** Baseline characteristics of screened patients in the nine conducted point prevalence surveys

Year	2013	2014	2015	2016	2017	2019	2020	2021	2022	Total
Period	Nov	Oct	Oct	Nov	Dec	June	June	June	Nov	
Hospitalised patients, <i>n</i>	626	653	655	613	597	664	583	559	544	5494
Patients screened, <i>n</i> (%)	516 (82%)	568 (87%)	565 (86%)	512 (84%)	397 (69%)	425 (64%)	430 (74%)	380 (68%)	372 (68%)	4165 (76%)
Excluded cultures, <i>n</i>	0	10	5	10	4	0	5	7	5	46 (1%)
Evaluable cultures, <i>n</i> (%)	516 (82%)	558 (86%)	560 (86%)	502 (82%)	393 (66%)	425 (64%)	425 (73%)	373 (67%)	367 (68%)	4119 (75%)
Age (years), median, (IQR)	65.5 (50–75.8)	66 (50–76)	67 (47.3–76)	67 (54–78)	71 (58.5–80)	68 (53–76)	68 (56–78)	71 (57–78)	70 (58–78)	68 (53–77)
Length of stay (days), median, (IQR)	2 (1–6)	2 (0–6)	2 (0–5)	3.5 (1–8)	4 (1–9)	2 (0–7)	3 (1–7.5)	4 (1–8)	3 (1–8)	3 (1–7)
Sex, <i>n</i> (%) female	250 (48%)	290 (52%)	290 (52%)	271 (54%)	172 (44%)	204 (48%)	221 (52%)	174 (47%)	180 (49%)	2052 (50%)
Medical specialty, <i>n</i> (%)										
Intensive care	18 (4%)	13 (2%)	18 (3%)	22 (4%)	18 (5%)	19 (5%)	20 (5%)	20 (5%)	14 (4%)	162 (4%)
Internal medicine	107 (21%)	105 (20%)	106 (19%)	108 (22%)	55 (14%)	87 (21%)	87 (21%)	71 (19%)	102 (28%)	828 (20%)
General surgery	81 (16%)	83 (15%)	85 (15%)	80 (16%)	70 (18%)	75 (18%)	80 (19%)	58 (16%)	38 (10%)	650 (16%)
Other	310 (60%)	357 (64%)	351 (63%)	292 (59%)	250 (64%)	244 (57%)	238 (56%)	224 (60%)	213 (58%)	2479 (60%)

**Table 2** ESBL prevalence over time, including bacterial species and identified ESBL genes of the ESBL-E

Year	2013	2014	2015	2016	2017	2019	2020	2021	2022	Total
Period	Nov	Oct/Nov	Oct	Nov	Dec	June	June	June	Nov	
Evaluable cultures, n	516	558	560	502	393	425	425	373	367	4119
ESBL positive patients based on phenotype, n (%)	28 (5.4%)	39 (7.0%)	33 (5.9%)	16 (3.2%)	21 (5.3%)	25 (5.9%)	9 (2.1%)	18 (4.8%)	17 (4.6%)	206 (5.0%)
ESBL positive patients based on genotype, No.	25 (4.8%)	37(6.6%)	32 (5.7%)	14 (2.8%)	18 (4.6%)	24 (5.6%)	9 (2.1%)	15 (4.0%)	14 (3.8%)	188 (4.6%)
Patients with > 1 ESBL producing isolate, No.	4	0	1	0	0	0	2	0	1	8
<b>ESBL producing isolates</b>										
Genetically confirmed ESBL isolates, No.	30	37	34	14	18	24	11	15	15	198
<i>Escherichia coli</i> , No.	25	29	30	12	15	18	10	10	9	158 (79.8%)
<i>Klebsiella pneumoniae</i> , No.	5	4	2	2	2	2	0	2	3	22 (11.1%)
<i>Enterobacter cloacae</i> complex, No.	0	3	2	0	1	4	0	3	2	15 (7.6%)
Other species, No.	0	1	0	0	0	0	1	0	1	3 (1.5%)
<b>ESBL genes</b>										
Isolates with > 1 ESBL gene, No.	3	4	3	0	1	2	1	1	2	17 (8.6%)
Total ESBL genes identified, No.	33	41	37	14	19	26	12	16	17	215
CTX-M family, No.	30	36	32	11	15	21	10	14	14	183 (85.1%)
SHV family, No.	2	5	4	2	2	3	1	1	3	23 (10.7%)
TEM family, No.	0	0	1	1	2	2	0	0	0	6 (2.8%)
Other, No.	0	0	0	0	0	0	1	0	0	1 (0.5%)



**Fig. 1** ESBL-E prevalence from 2013 to 2022, excluding 2018, with adjusted Wald 95% confidence interval. The numbers of ESBL-E found per year are presented above the corresponding prevalence data

characteristics of the included patients are shown in Table 1.

**ESBL-E prevalence**

In total, 214 Enterobacterales suspected of ESBL production were found in 206 patients. However, in 18 of these isolates the phenotypical ESBL test was considered false positive as no ESBL gene was identified (Table S1). This resulted in an ESBL-E rectal carriage prevalence of 5.0% based on phenotypical tests for ESBL production and

in a prevalence of 4.6% (n=188) based on WGS data (Table 2).

**Factors associated with ESBL-E prevalence**

The ESBL-E prevalence ranged from 2.1 to 6.6% over the years (Fig. 1). The ESBL-prevalence decreased on average 5.5% per year (unadjusted odds ratio 0.945, 95% CI 0.899–0.994, p=0.028; adjusted odds ratio 0.945, 95% CI 0.901–0.990, p=0.017). A prolonged length of stay was associated with a 1.9% increased probability of

ESBL-E carriage on average per extra day of hospitalization (unadjusted odds ratio 1.017, 95% CI 1.004–1.030,  $p=0.011$ ; adjusted odds ratio 1.019, 95% CI 1.006–1.032,  $p=0.005$ ). Age and ICU admission had no significant effect on ESBL-E carriage: a 10 year higher age was on average associated with a 1% higher ESBL-E prevalence (odds ratio 1.01, 95% CI 0.951–1.073, ns) and ICU-admission was on average associated with a 26% lower ESBL-E prevalence (odds ratio 0.74, 95% CI 0.32–1.71, ns). Post-hoc testing revealed that the ESBL-E prevalence in 2016 and 2020 was notably lower compared to other years and this observation persisted after adjusting for time trend and possible confounders (Table S2). In 2016 the ESBL-E prevalence was 2.8%, while a prevalence of 5.1% was predicted based on the other years ( $p=0.0026$ , significance accepted at  $<0.0064$ ). Similarly, in 2020 the observed prevalence was 2.1% with a predicted prevalence of 4.2% based on the other years ( $p=0.0072$ , significance accepted at  $<0.0064$ ).

#### Identified ESBL producing species and sequence types

Eight of the 188 ESBL-E carrying patients carried two or more different isolates resulting in 198 ESBL-positive isolates. In total, 79.8% ( $n=158$ ) of the identified ESBL-E were *E. coli*, followed by 11.1% ( $n=22$ ) of *K. pneumoniae* (Table 2). Among the *E. coli* isolates, ST131 was the most common sequence type detected ( $n=36$ , 22.8%). For *K. pneumoniae* isolates, no predominant MLST was observed (Table S3).

#### Identified ESBL genes

Collectively, the 198 ESBL-E harboured 215 ESBL genes, with a maximum of two ESBL genes per isolate, resulting in 9.7% ( $n=19$ ) of the ESBL-E isolates harbouring two ESBL genes. Most of the identified genes belonged to the CTX-M family ( $n=182$ , 84.7%), with CTX-M-15 being the most prevalent ( $n=85$ , 40.0%) (Table S3).

#### Clonal relatedness

Based on wgMLST analysis, 36 of the 198 ESBL-E isolates (18.2%) were clonally related to at least one other ESBL-E isolate. Of those, 25 were *E. coli*, corresponding to 15.8% of all 158 *E. coli* isolates found. The others were 7 of the 15 *E. cloacae* isolates (46.7%) and 4 of the 22 *K. pneumoniae* (18.2%). In 2020 no related isolates were found that were clonally related to any other ESBL-E isolate, and in 2016 only one isolate was clonally related to another isolate, which was from a different year (Table 3). Apart from the years 2016 and 2020–2022, clonal relatedness was detected between isolates derived from the same year as well as cross-annual (Table 3). ESBL-E isolates were never clonally related to more than one isolate detected in the same year. However, cross-annual analysis revealed an *E. coli* isolate clonally related to nine other isolates. For *E. cloacae* this maximum was two other isolates and for *K. pneumoniae* no cross-annual clonality was found.

#### Nosocomial transmission events

A total of 38 out of 12,739 pairwise comparisons (consisting of 12,403 comparisons between the 158 *E. coli* isolates, 231 comparisons between the 22 *K. pneumoniae* isolates and 105 comparisons between the 15 *E. cloacae* isolates), revealed clonally related ESBL-E isolates from different patients. In 27 (71.1%) clonally related ESBL-E pairs no epidemiological link was found between the patients (Table S4), while in 11 pairs a link could be found deeming nosocomial transmission likely ( $n=5$ ) or possible ( $n=6$ ) (Table 4). There were two possible or likely nosocomial transmission events per year between patients enrolled in the same year in 2013, 2014, 2015, and 2017, and one per year in 2019 and 2021 (Table 4). Potential nosocomial transmission events were found in 8 of the 31 pairwise comparisons (25.8%) involving patients with clonally related *E. coli* isolates. As opposed to 20% (1/5) and 100% (2/2) of the comparisons between

**Table 3** Identified clonally related isolates each year within the same year and the total number of clonally related isolates including cross-annual clonal relatedness

Year	ESBL-E isolates, No.	Total clonally related isolates within the same year, No. (%)	Total clonally related isolates incl. cross-annual, No. (%)
2013	30	4 (13.3%)	6 (20.0%)
2014	37	4 (10.8%)	6 (16.2%)
2015	34	4 (11.8%)	7 (20.6%)
2016	14	0	1 (7.1%)
2017	18	4 (22.2%)	4 (22.2%)
2019	24	2 (8.3%)	7 (29.2%)
2020	11	0	0
2021	15	2 (13.3%)	2 (13.3%)
2022	15	0	3 (20.0%)
Total	198	n.v.t.	36 (18.8%)



**Table 4** Pairwise comparisons between two patients with clonally related ESBL-E isolates for which nosocomial transmission was deemed possible or likely based on the epidemiological link between those patients

Year of collection	Species	MLST	ESBL genes	Epidemiological link	Likelihood nosocomial transmission
2013	<i>Klebsiella pneumoniae</i>	ST107	CTX-M-14	Same time, same location, same ward	Likely
2013	<i>Escherichia coli</i>	ST131	1 isolate CTX-M-32 1 isolate CTX-M-27	Same time, other location of the hospital	Possible
2014	<i>Escherichia coli</i>	ST38	CTX-M-15	Same time, same location, different ward	Possible
2014	<i>Klebsiella pneumoniae</i>	ST4283	CTX-M-15	Same time, same location, same ward	Likely
2015	<i>Escherichia coli</i>	ST616	CTX-M-15	Same time, other location of the hospital	Possible
2015	<i>Escherichia coli</i>	ST131	CTX-M-27	Same time, same location, different ward	Possible
2017	<i>Escherichia coli</i>	ST131	CTX-M-27	Same time, other location of the hospital	Possible
2017	<i>Escherichia coli</i>	ST131	CTX-M-15	Same time, same location, same ward	Likely
2017/2019	<i>Escherichia coli</i>	ST131	CTX-M-15	Same time, same location, different ward	Possible
2019	<i>Enterobacter cloacae</i> complex	ST50	SHV-12	Same time, same location, different ward	Likely
2021	<i>Escherichia coli</i>	ST44	CTX-M-15	Same time, same location, same ward	Likely

patient with clonal *E. cloacae* and *K. pneumoniae* isolates respectively. Overall, 5.9% (11/188) of ESBL-E rectal carriage cases could be attributed to potential nosocomial transmission, with 2.7% (5/188) considered likely.

## Discussion

We found an ESBL-E rectal carriage prevalence of 4.6% ranging from 2.1 to 6.6% based on WGS, measured in nine point prevalence surveys from 2013 to 2022. The ESBL-E prevalence declined on average 5.5% each year. Potential nosocomial transmission events were rarely observed in this study as only 5.9% of ESBL-E cases could be attributed to nosocomial transmission.

The observed decrease in ESBL-E prevalence over time is in line with NethMap and EARS-NET reports on the ESBL-E prevalence in the Netherlands and Europe, respectively, showing a stable or (slightly) decreasing prevalence of third generation cephalosporin resistance over the years in clinical isolates [8, 15]. Consistent with other studies, *E. coli*, and particularly ST131, predominated [4–6, 11, 12, 16, 17]. Among the ESBL genes, CTX-M genes and more specifically CTX-M-15, were most abundant [11, 12, 16]. Interestingly, we found that ST131 comprised only 22.8% of the *E. coli* isolates, but 80.6% of the clonally related pairwise comparisons between *E. coli* and 62.5% of the potential nosocomial transmission events. This indicates either relatively more genetic similarity between ST131 isolates or enhanced transmissibility of *E. coli* ST131. The latter could be a partial explanation of the remarkably fast global expansion of ESBL-producing ST131 of which the mechanism is still unknown [18].

Furthermore, we may have underestimated the number of nosocomial transmission events as indirect epidemiological links might be missed. For clonally related *K. pneumoniae* isolates, we could always identify a direct

epidemiological link between patients. This was different for clonally related *E. coli* and *E. cloacae* complex isolates, for which a direct epidemiological link was only present in 25.8% and 20%, respectively. It could be that we missed patients that link these epidemiologically unrelated patients together or that patients share epidemiological links outside the hospital, indicating community transmission of *E. coli* and *E. cloacae* complex - and not of *K. pneumoniae* - which is detected in the hospital. This is in concordance with another study that identified transmission in the healthcare setting as important driver for ESBL producing *K. pneumoniae*, but not for spread of ESBL producing *E. coli* [19]. Missed indirect links between *E. coli* and *E. cloacae* isolates within the hospital rather than community transmission would imply that the community ESBL-E carriage prevalence is lower than in the hospital. However, the ESBL-E carriage prevalence observed in a Dutch study from 2014 to 2016 was similar to the in-hospital prevalence that we observed ( $\approx 5\%$ ) [20]. This makes missed indirect in-hospital links unlikely and would support community transmission as major player in *E. coli* carriage dynamics.

The lowest prevalence rates were observed in 2016 and 2020. Of particular interest is the year 2020, coinciding with the onset of the COVID-19 pandemic in the Netherlands, prompting the implementation of nationwide measures from March 2020 onwards to mitigate SARS-CoV-2 transmission. Subsequent extra attention for standard precautions to minimize pathogen transmission across patients could explain the observed decrease in ESBL-E prevalence in 2020. This hypothesis is supported by the fact that no clonally related isolates and thus potential nosocomial transmission events were detected in 2020. Additionally, the nationwide measures to combat COVID-19 included travel restrictions, implemented a few months before the PPS in 2020 was conducted. It

could also have resulted in lower ESBL-E prevalence rates compared to previous years as recent travel abroad is also established as a risk factor for ESBL-E colonization [11, 16]. Unfortunately, our dataset lacks comprehensive information regarding patients' travel history and antibiotic usage. While the COVID-19 measure could explain the decline in 2020, the persistently low ESBL-E prevalences in 2021 and 2022 suggest the decline in ESBL-E prevalence belong to the COVID-19 pandemic.

Beside leaking data on the patients' travel history and antibiotic usage, limitations of our study are the lack of data on the year 2018 and the variation in the timing of PPS across different years. Some were conducted in June (2019–2021) and others in October–December (2013–2017, 2022). Despite this, our analysis did not reveal a difference in ESBL-E prevalence between seasons. While seasonal effects on ESBL carriage are not well-established, we considered the possibility of increased summer travel and higher incidences in enteric infectious diseases influencing prevalence [20, 21]. To investigate this, we conducted a PPS in both June and November 2021, but found no significant difference in ESBL-E rectal carriage prevalence (data not shown).

A notable strength of the present study is the consistent use of sensitive and selective ESBL-E culture methods each year. In 2021 the revised Dutch guideline for detection of highly resistant micro-organisms states that the use of enrichment broths for detection of ESBL carriage is favoured, because this increases the sensitivity of the ESBL-E cultures substantially [22, 23]. In this study, enrichment broths were already used from the study's inception in 2013 ensuring reliable data comparison over the years.

Over a ten-year period, PPS provided continuous monitoring for the ESBL-E prevalence and transmission rates in our hospital informing and refining our infection control policies. Notably, when the hospital transitioned to a building with exclusively single-bed rooms, contact precaution measures for ESBL-E carriers were lifted, under the condition that periodic assessment of ESBL-E carriage rates was conducted. This strategy ensured that significant ESBL-E outbreaks were not overlooked and validated that standard precautions were sufficient to contain ESBL-E in a hospital environment consisting of single rooms with private sanitary facilities. This may also apply to multiple-bed rooms, as evidence indicates that multiple-bed rooms are non-inferior to single-bed rooms in containing ESBL-E when contact precautions for known ESBL-E carriers are implemented [24]. Nevertheless, further research is required to confirm this hypothesis. Annual PPS not only track the impact of changes in infection control policies, but also provide yearly insights into the efficacy of the hospital's infection control measures and facilitate the early detection of potential

ESBL-E outbreaks. Nonetheless, the implementation of prospective WGS on selected ESBL-E isolates could offer even more immediate data on potential outbreaks [14].

## Conclusions

We observed a low ESBL-E prevalence with a declining trend over time with the COVID-19 pandemic and subsequent measures as possible explanations for the observed decrease in 2020. However, the persistently low ESBL-E prevalences in 2021 and 2022 indicate that the overall ESBL-E prevalence is declining over the years. Continuous monitoring of ESBL-E prevalence and transmission rates can aid infection control policy to keep antibiotic resistance rates in hospitals low.

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13756-024-01460-y>.

Supplementary Material 1

Supplementary Material 2

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## Author contributions

Conceptualization—KH, VW, JK, and JS; methodology—KH, VW, WB, PM and JS; validation—KH, VW, WB and JS; formal analysis—KH, VW, MT and PM; investigation—KH and VW; resources—VW, WB, MR, JV, JM and JS; data curation—KH and MT; writing—original draft—KH, VW and JS; writing—review and editing—all authors; visualization—KH; supervision—VW and JS; project administration—KH, VW, WB, MR, JV, JM and JS; funding acquisition—VW, WB, JK, MR and JS.

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## Data availability

Genomic sequences that support the findings of this study have been deposited in the European Nucleotide Archive with the primary accession code PRJEB78530.

## Declarations

### Ethics approval and consent to participate

This study was conducted as part of the hospital's standard infection control policy. Ethical and medical approvals were not deemed necessary for this non-invasive procedure, as it aligned with Dutch regulations pertaining to research involving human subjects. Patients were provided the option to decline participation (opt-out) if they chose.

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing interests.

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