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Prevalence of third-generation cephalosporin-resistant Enterobacterales colonization on hospital admission and ESBL genotype-specific risk factors: a cross-sectional study in six German university hospitals

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Objectives: To assess the admission prevalence of third-generation cephalosporin-resistant Enterobacterales (3GCREB) and to assess whether risk factors vary by β-lactamase genotype.

Methods: Adult patients were recruited within 72 h of admission to general wards of six university hospitals in 2014 and 2015. Rectal swabs were screened for 3GCREB and isolates were analysed phenotypically and genotypically. Patients were questioned on potential risk factors. Multivariable analyses were performed to identify risk factors for 3GCREB colonization and for specific β-lactamases.

Results: Of 8753 patients screened, 828 were 3GCREB positive (9.5%). Eight hundred and thirteen isolates were available for genotyping. CTX-M-15 was the most common ESBL (38.0%), followed by CTX-M-1 (22.5%), CTX-M-14 (8.7%), CTX-M-27 (7.5%) and SHV-ESBL (4.4%). AmpC was found in 11.9%. Interestingly, 18 *Escherichia coli* isolates were AmpC positive, 12 of which (67%) contained AmpC on a gene of plasmid origin [CMY ($n = 10$), DHA ($n = 2$)]. Risk factors for 3GCREB colonization varied by genotype. Recent antibiotic exposure and prior colonization by antibiotic-resistant bacteria were risk factors for all β-lactamases except CTX-M-14 and CTX-M-27. Travel outside Europe was a risk factor for CTX-M-15 and CTX-M-27 [adjusted OR (aOR) 3.49, 95% CI 2.88–4.24 and aOR 2.73, 95% CI 1.68–4.43]. A previous stay in a long-term care facility was associated with CTX-M-14 (aOR 3.01, 95% CI 1.98–4.59). A preceding hospital stay in Germany increased the risk of CTX-M-15 (aOR 1.27, 95% CI 1.14–1.41), while a prior hospital stay in other European countries increased the risk of SHV-ESBL colonization (aOR 3.85, 95% CI 1.67–8.92).

Conclusions: The detection of different ESBL types is associated with specific risk factor sets that might represent distinct sources of colonization and ESBL-specific dissemination routes.

Introduction

Third-generation cephalosporin-resistant Enterobacterales (3GCREB) are emerging worldwide and have been rated by the WHO as high-priority pathogens among resistant bacteria.[1] In this context, bacteria that have acquired resistance from plasmid-encoded, mobile ESBL-producing resistance genes, such as CTX-M, are of special concern.[2,3] Third-generation cephalosporin resistance can also be mediated by AmpC β-lactamases. In the European population, the prevalence of ESBL-producing Enterobacterales colonization in the community ranges from 6% to 11%.[4–7] In Germany, 3GCREB prevalence in hospitalized patients has been reported to be as high as 13% in some regions.[8,9] Antibiotic exposure, a stay in a long-term care facility and treatment of gastro-oesophageal reflux disease have been identified as important risk factors for colonization with 3GCREB.[8] Travel to high-prevalence countries has been reported to result in colonization with 3GCREB in up to 72% of returning travellers who were previously negative.[10,11]

Globally, the CTX-M-1 group dominates the ESBL landscape. However, regional differences are apparent, though their origin is unclear.[2] These differences may be due to a set of risk factors specific to each region. From the ATHOS admission prevalence project, a previous analysis on the prevalence of 3GCREB at hospital admission in Germany in 2014 was published. We found the CTX-M-1 group, the CTX-M-9 group, SHV and AmpC to be the most common resistance mechanisms in colonized patients.[8] Here, we use the extended, complete ATHOS admission prevalence dataset from 2014 and 2015 in order to determine whether different ESBL genotypes are associated with specific patient risk factors.

Materials and methods

Study design and setting

This analysis includes datasets from two cross-sectional admission prevalence surveys from the ATHOS project, one from 2014[8] and the other from 2015. Both were performed in six large university hospitals in Germany. Each participating hospital recruited at least 500 patients per year.

Patients, risk factor questionnaire and data collection

Included were adult patients (≥18 years) admitted to general wards who had given their informed consent; patients from ICUs, dermatology, obstetrics, ophthalmology, otorhinolaryngology and psychiatry were excluded. Each ward was represented by as many patients as they had beds. In total, at least 500 patients per centre were recruited. Rectal swabs were obtained within the first 3 days of hospital stay (admission day = Day 1). The primary outcome was the 3GCREB admission status (positive or negative). The secondary outcome was the specific β-lactamase genotype status (positive or negative).

The patients answered a questionnaire on potential risk factors, including gender, age, current antibiotic exposure (as it may alter the ability to detect ESBL), colonization with any MDR organism (MDRO) at any timepoint (indicating previous healthcare contact), and occupational and private contact with animals. In addition, antimicrobial exposure, foreign travel (inside or outside the EU), a stay at a rehabilitation centre or long-term care facility (LTCF), hospitalization in Germany, in other European countries or elsewhere, and medical management of gastro-oesophageal reflux disease by proton pump inhibitors and antacids during the previous 6 months were documented. Information was retrieved through standardized interviews conducted by study nurses. The questionnaire was published with the previous ATHOS prevalence publication.[8]

Phenotypic and molecular characterization

Phenotypic detection of 3GCREB was performed with ChromID ESBL plates (bioMérieux, Nürtingen, Germany), MALDI-TOF MS or the Vitek 2 GN ID card and susceptibility testing with Vitek 2 (bioMérieux). ESBL and AmpC production was determined by phenotypic methods as published previously.[8] Additionally, all isolates were molecularly characterized. Expression of $bla_{\text{CTX-M-1 group}}$, $bla_{\text{CTX-M-2 group}}$, $bla_{\text{CTX-M-9 group}}$, bla_{TEM} and bla_{SHV} was first assessed as previously described.[8] Isolates that were positive for $bla_{\text{CTX-M-1 group}}$, $bla_{\text{CTX-M-2 group}}$ or $bla_{\text{CTX-M-9 group}}$ in the first step were characterized by an additional PCR and sequencing of the ORF using primers described elsewhere.[12] AmpC-producing *Escherichia coli* were investigated for the presence of plasmid-mediated AmpC as previously described.[13] Presence of bla_{KPC}, bla_{VIM}, bla_{IMP}, $bla_{\text{OXA-48}}$ and bla_{NDM} was assessed by multiplex PCR in isolates with meropenem MICs >0.25 mg/L.[14]

Ethics

The study was approved by the local ethics committees (approval number EA4/018/14).

Statistics

The prevalence of 3GCREB on admission was expressed as the number of patients positive for 3GCREB per 100 patients included in the study. In the descriptive analysis, numbers and percentages were calculated; differences were tested using the χ^2 test. To evaluate risk factors for colonization on admission, multivariable regression analyses were performed. Some 3GCREB isolates were unavailable for genotyping. These patients were analysed as positive in the 3GCREB analysis, but were analysed as negative in the genotype-specific analyses. The same was applied when the ESBL genotype could not be established with certainty. Because individual patients could present more than one resistance genotype (either in one or several 3GCREB), such patients met the outcome criteria for more than one genotype-specific analysis, and appeared in each appropriate analysis as positive. Interactions between covariates were not included. Age was separated into categories (≤45, 46–55, 56–65, 66–75, >75 years). Questionnaire parameters were categorized as 'no' (= reference), 'yes' or 'unknown'. In the case of ESBL-specific analyses, binary categorization was used for some variables ('yes' versus 'no'/'unknown'). The analyses were based on generalized estimating equation (GEE) models, which account for clustering effects in the different university hospitals by using an exchangeable correlation structure. They were performed as follows. For each outcome, all questionnaire parameters were included in a full model. Non-significant covariates were excluded stepwise backward if the *P* value of the Type III test was ≥0.05. The models obtained were adjusted by centre, age and gender. In this way, adjusted ORs (aORs) with 95% CI were calculated. All analyses were performed using SPSS 22 (IBM SPSS Statistics, Somer, NY, USA) and SAS 9.4 (SAS Institute, Cary, NC, USA).

Results

Descriptive statistics

We included 8753 patients (Figure 1), of whom 48.1% were female ($n = 4213$). The median age was 62 years (IQR 50–73) (Table 1). Age differed considerably among ESBL types. By median, patients with CTX-M-15 isolates were the youngest (60 years, IQR 47–73) and patients with AmpC producers were the oldest (67 years, IQR 60–75) (Table S1, available as Supplementary data at *JAC* Online). In total, 828 patients were identified as 3GCREB carriers, a prevalence of 9.5% (Figure 1). The prevalence differed between the centres, ranging from 6.4% to 10.9% ($P = 0.003$), and was

higher in men than in women (10.5% versus 8.3%, $P < 0.001$). Patients with CTX-M-1 and AmpC were distributed unequally over the centres and age groups, while CTX-M-15 and SHV-ESBL 3GCREB were distributed unequally between genders. Notably, 71% of SHV-ESBL-positive patients were male (Table S2).

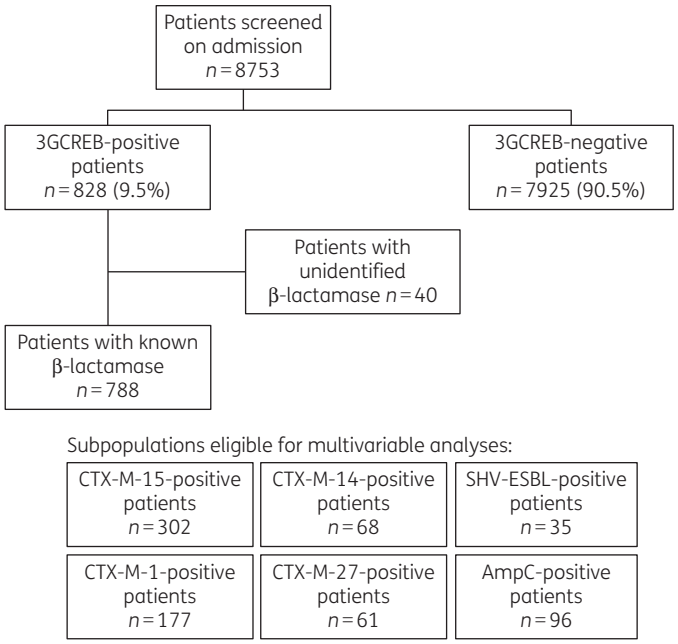

Figure 1. Flow chart of patients screened.

Carbapenem-resistant isolates were detected in eight patients (0.1%).

Phenotypic and molecular 3GCREB epidemiology

Twenty-six patients carried more than one 3GCREB (0.3%), resulting in 856 isolates. The majority were *E. coli* (79.4%), followed by *Klebsiella pneumoniae* (6.8%), *Enterobacter* spp. (5.8%) and *Citrobacter* spp. (4.9%). Most were resistant only to third-generation cephalosporins (57.1%); 41.9% displayed additional ciprofloxacin resistance. Only 0.9% of 3GCREB isolates were carbapenem resistant (Table 2).

Among the 813 3GCREB isolates available for genotyping, the predominant ESBL types were CTX-M-15 (38.0%) and CTX-M-1 (22.5%). CTX-M-14 was found in 8.7%, CTX-M-27 in 7.5%, AmpC in 11.9% and SHV-ESBL in 4.4% of isolates (Table S3). Seven of the eight carbapenem-resistant isolates were available for analysis; among these, two had OXA-48, one had NDM-1, three had VIM-1 and one had IMP-8 carbapenemase (Table S3).

Multivariable analysis of overall 3GCREB status

Multivariable analysis identified several risk factors for 3GCREB colonization. Patients with a recent travel history outside Europe were 2.44 times more likely to be colonized with 3GCREB (95% CI 2.27–2.63). Patients with a self-reported previous MDRO diagnosis had a 2.20 times higher chance of being colonized (95% CI 1.94–2.50). Current and previous antimicrobial exposure increased the chance of colonization by a factor of 1.37 and 1.77, respectively. Medication with proton pump inhibitors or antacids for gastro-oesophageal reflux disease enhanced the chance of

Table 1. Descriptive statistics of patient data according to 3GCREB status

		3GCREB positive		
Patient demographics	3GCREB negative, *n* (%)	*n* (%)	prevalence (%)	*P* value
Hospital				
Centre 1	3598 (45.4)	415 (50.1)	10.3	0.003
Centre 2	890 (11.2)	109 (13.1)	10.9	
Centre 3	907 (11.4)	92 (11.1)	9.2	
Centre 4	920 (11.6)	85 (10.3)	8.5	
Centre 5	805 (10.2)	72 (8.7)	8.2	
Centre 6	805 (10.2)	55 (6.6)	6.4	
Gender				
male	4062 (51.3)	478 (57.7)	10.5	<0.001
female	3863 (48.7)	350 (42.3)	8.3	
Age (years)				
≤45	1514 (19.1)	144 (17.4)	8.7	0.385
46–55	1362 (17.2)	129 (15.6)	8.7	
56–65	1672 (21.1)	185 (22.3)	10.0	
66–75	1929 (24.3)	204 (24.6)	9.6	
>75	1448 (18.3)	166 (20.0)	10.3	
Year				
2014	3960 (50.0)	416 (50.2)	9.5	0.881
2015	3965 (50.0)	412 (49.8)	9.4	

Differences among categories tested by χ^2 test.

Table 2. Distribution of resistance to ciprofloxacin and meropenem among 3GCREB

Species	Isolates, *n* (%)			Total, *n* (%)
	3GCREB + CIP S + MEM S	3GCREB + CIP I/R + MEM S	3GCREB + CIP I/R + MEM I/R	
E. coli	367 (42.9)	311 (36.3)	2 (0.2)	680 (79.4)
K. pneumoniae	25 (2.9)	31(3.6)	2 (0.2)	58 (6.8)
K. aerogenes	9 (1.1)	1 (0.1)	0	10 (1.2)
K. oxytoca	5 (0.6)	1 (0.1)	1 (0.1)	7 (0.8)
Enterobacter spp.	41 (4.8)	8 (0.9)	1 (0.1)	50 (5.8)
Citrobacter spp.	37 (4.3)	3 (0.4)	2 (0.2)	42 (4.9)
Hafnia alveii	3 (0.4)	1 (0.1)	0	4 (0.5)
Proteus spp.	2 (0.2)	3 (0.4)	0	5 (0.6)
Total	489 (57.1)	359 (41.9)	8 (0.9)	856 (100.0)

CIP, ciprofloxacin; MEM, meropenem; S, susceptible; I, intermediate; R, resistant.

3GCREB by 1.1 (95% CI 1.05–1.22). Men had a 1.29 times higher chance of being colonized than women (95% CI 1.16–1.44) (Table 3).

Multivariable analyses of different ESBL genotypes

To identify potential ESBL-specific risk factors, we performed multivariable analyses for each of the more abundant ESBL genotypes and adjusted the self-reported risk factors for centre, age and gender (Table 3; Table S4 includes categories 'no' and 'unknown'). Previous MDRO detection and antibiotic exposure during the preceding 6 months increased the chance of colonization for both CTX-M-15 and CTX-M-1 ESBL. Other risk factors for colonization with CTX-M-15 were travel outside Europe (aOR 3.49, 95% CI 2.88–4.24) and a hospital stay in Germany (aOR 1.27, 95% CI 1.14–1.41). In contrast, these factors did not influence colonization with CTX-M-1 ESBL.

The sole risk factor for CTX-M-14 was a stay in an LTCF (aOR 3.01, 95% CI 1.98–4.59). Travel outside Europe was the sole risk factor for CTX-M-27 colonization (aOR 2.73, 95% CI 1.68–4.43).

Risk factors for SHV-ESBL colonization were previous detection of any MDRO and a recent hospital stay abroad, but only in a European country. The latter increased the chance of SHV-ESBL colonization 3.85-fold. AmpC β-lactamase colonization was only influenced by previous MDRO detection and prior antibiotic exposure (aOR 2.74, 95% CI 1.98–3.79 and aOR 3.05, 95% CI 2.08–4.47, respectively).

Discussion

Overall

The 3GCREB prevalence on admission did not change from 2014 to 2015 (9.5% and 9.4%, respectively). Other European studies have reported a similar ESBL Enterobacterales admission prevalence (7.7%–12.7%),[5,9,15,16] which could be even higher in patients with haematological malignancies (5.8%–23.1%).[17] Similar to results reported by other studies, *E. coli* was the most frequent 3GCREB (79.4%) among our patients. However, a lower proportion of *K. pneumoniae* (6.8%) was found than in other investigations (11.1%–20.6%).[9,15] Only 0.1% of the participating patients were colonized with carbapenem-resistant Enterobacterales, which is in the range reported by other northern European prevalence studies.[6,7,10,11] On admission, 41.9% of the 3GCREB gut colonizers were also fluoroquinolone resistant (Table 2). This is less than found in clinical isolates taken from patients of the participating centres on admission (Days 1–3) or later during a hospital stay (60.1% and 46.2%, respectively).[18]

Predominant ESBL types

Among the different resistance mechanisms, CTX-M-type β-lactamases prevailed. CTX-M-15 and CTX-M-1 were the most common, similar to other studies from Germany.[7,9] At 0.5%, CTX-M-9 was found less frequently than in other studies, which have reported up to 20.7%.[9,15,16] However, 8.7% of the ESBLs we detected were CTX-M-14 and 7.5% were CTX-M-27, both of which are members of the CTX-M-9 group. We detected AmpC β-lactamases in 11.9% of the isolates. This is a higher percentage than reported by British colleagues[16] (7.3%) and a lower percentage than reported by Dutch researchers[19] (17.6%). However, these differences might be the result of the different study populations and additionally different screening methodology, e.g. in the Dutch study an agar without any AmpC inhibitors was used for screening, which may explain the overall higher number of AmpC isolates detected. We found AmpC β-lactamases in *Enterobacter* spp., *Citrobacter* spp., *E. coli* and *Klebsiella aerogenes*. In *E. coli* isolates, AmpC was molecularly characterized; 12 of 18 isolates (67%) were of plasmid origin [CMY-2 ($n=5$), CMY-42 ($n=5$) and DHA ($n=2$)], a much higher pAmpC/AmpC ratio than found in the Dutch community prevalence study (17%, 7/42 *E. coli* isolates).[19]

Multivariable analyses

The unequal distribution of specific ESBL types over the participating centres (Table S2) is also reflected in ESBL-specific multivariable analyses (Table 3). The highest aORs for CTX-M-1 and CTX-M-27 detection were seen in Centre 1, while the highest chance of CTX-M-15 and SHV-ESBL was seen in Centre 2 and for CTX-M-14 and AmpC in Centre 3. This may suggest regional differences in ESBL distribution, but also hints at specific patient subgroups

Table 3. Multivariable risk factor analyses for outcome of 3GCREB colonization and for specific ESBL genotypes

Parameter	Category	3GCREB (*n* = 828)	CTX-M-1 (*n* = 177)	CTX-M-15 (*n* = 302)	CTX-M-14 (*n* = 68)	CTX-M-27 (*n* = 61)	SHV-ESBL (*n* = 35)	AmpC (*n* = 96)
Age (years)	≤45	**0.75 (0.57–1.00)**	**0.44 (0.35–0.54)**	1.16 (0.81–1.66)	1.06 (0.57–1.98)	1.01 (0.50–2.03)	0.71 (0.25–2.04)	**0.25 (0.15–0.42)**
	46–55	**0.72 (0.62–0.84)**	**0.48 (0.38–0.61)**	1.06 (0.80–1.40)	1.63 (0.98–2.73)	0.75 (0.44–1.29)	1.13 (0.53–2.41)	**0.36 (0.20–0.64)**
	56–65	0.86 (0.71–1.05)	0.93 (0.71–1.22)	0.94 (0.59–1.49)	1.33 (1.07–1.67)	1.07 (0.58–1.98)	0.74 (0.37–1.47)	0.93 (0.62–1.42)
	66–75	**0.84 (0.76–0.93)**	**0.60 (0.48–0.73)**	0.94 (0.73–1.22)	1.20 (0.96–1.50)	0.84 (0.32–2.18)	0.60 (0.32–1.11)	0.97 (0.60–1.57)
	>75[a]	1	1	1	1	1	1	1
Gender	male	**1.29 (1.16–1.44)**	**1.3 (1.18–1.43)**	1.23 (0.98–1.54)	1.19 (0.72–1.97)	1.24 (0.92–1.67)	**2.34 (1.42–3.86)**	1.26 (0.90–1.75)
	female[a]	1	1	1	1	1	1	1
Previous MDRO	yes	**2.20 (1.94–2.50)**	**1.86 (1.26–2.76)**	**2.04 (1.26–3.29)**			**4.55 (2.92–7.07)**	**2.74 (1.98–3.79)**
Antibiotic use[b]	yes	**1.77 (1.55–2.03)**	**1.84 (1.45–2.34)**	**1.46 (1.21–1.76)**				**3.05 (2.08–4.47)**
Travel outside Europe[b]	yes	**2.44 (2.27–2.63)**		**3.49 (2.88–4.24)**		**2.73 (1.68–4.43)**		
Travel outside Europe binary[b]	yes		**0.53 (0.35–0.81)**					
Long-term care stay[b]								
Long-term care stay binary[b]	yes	**1.36 (1.01–1.69)**			**3.01 (1.98–4.59)**			
Hospital stay Europe[b]	yes						**3.85 (1.67–8.92)**	
Hospital stay Germany binary[b]	yes			**1.27 (1.14–1.41)**				
Pets	yes			**0.79 (0.66–0.95)**				
PPI and antacids[b]	yes	**1.13 (1.05–1.22)**						
Current antibiotic use	yes	**1.37 (1.26–1.49)**		**1.36 (1.25–1.49)**	**1.72 (1.25–2.38)**		**0.45 (0.18–1.09)**	**1.62 (1.15–2.29)**
Centre	Centre 1[a]	1	1	1	1	1	1	1
	Centre 2	**1.06 (1.02–1.09)**	**0.55 (0.53–0.58)**	**1.51 (1.38–1.65)**	0.98 (0.9–1.06)	**0.88 (0.86–0.89)**	**1.60 (1.44–1.77)**	**1.29 (1.18–1.41)**
	Centre 3	**0.85 (0.80–0.89)**	**0.26 (0.26–0.27)**	**1.19 (1.08–1.31)**	**1.32 (1.28–1.36)**	**0.88 (0.83–0.93)**	0.90 (0.77–1.05)	**2.47 (2.36–2.58)**
	Centre 4	**0.80 (0.77–0.83)**	**0.47 (0.45–0.48)**	**1.13 (1.03–1.24)**	0.94 (0.84–1.05)	**0.92 (0.90–0.94)**	**0.86 (0.79–0.93)**	**1.71 (1.57–1.85)**
	Centre 5	**0.72 (0.69–0.75)**	**0.50 (0.48–0.53)**	**0.81 (0.76–0.87)**	**1.20 (1.08–1.34)**	**0.28 (0.25–0.30)**	1.19 (0.96–1.48)	**1.62 (1.45–1.81)**
	Centre 6	**0.62 (0.58–0.66)**	**0.25 (0.24–0.27)**	0.94 (0.84–1.04)	**0.47 (0.43–0.51)**	0.95 (0.87–1.03)	**0.44 (0.24–0.80)**	**1.68 (1.48–1.91)**

Each column represents one multivariable analysis; outcomes (= colonization with 3GCREB or specific β-lactamase genotype) are stated in the header; only significant parameters remained in the models.
ORs adjusted for centre, age and gender including 95% CIs were estimated. Statistically significant categories are shown in bold.
Data including categories 'no' and 'unknown', as well as *P* values, can be found in Table S4.
Unless otherwise stated, category 'no' is the reference. For binary parameters, the categories 'no' and 'unknown' were combined to be used as reference.
[a]Reference category.
[b]In previous 6 months.

in the participating centres, an indication supported by the age difference in patients colonized with different ESBL (Table S1).

CTX-M-1 and CTX-M-15 both belong to the CTX-M-1 group. However, they did not share the same risk factor pattern. The chance of detection of both was increased by a history of MDRO and recent antibiotic exposure, but CTX-M-15 was associated primarily with travel outside Europe (OR 3.49, 95% CI 2.88–4.24). This is seconded by a British study assessing ESBL-specific risk factors.[16] CTX-M-15 is highly prevalent in India and South-East Asia,[2] and previously negative travellers often return as carriers.[6,10,11] In our dataset, CTX-M-15-positive patients were the youngest, very likely to have travelled outside Europe and the least likely to have stayed in an LTCF compared with patients colonized with other β-lactamases (Table S2).

CTX-M-1 colonization was only associated with previous MDRO detection and antibiotic exposure, as was AmpC colonization. However, the impact of previous antibiotic exposure was higher for AmpC colonization (aOR 3.05, 95% CI 2.08–4.47 versus aOR 1.84, 95% CI 1.45–2.34). AmpC was detected mostly in *Enterobacter*, *Citrobacter* or *Morganella*, where it is naturally occurring and usually chromosomally located.[19] It can be hyperproduced upon exposure to many β-lactam antibiotics. Accordingly, we found the highest ratio of previous and current antibiotic therapy in patients with AmpC-producing isolates (63.5% and 31.3%, respectively) (Table S2). In contrast, CTX-M-type ESBLs are usually not chromosomally encoded and not naturally present in Enterobacterales or the gastrointestinal microbiome. This might explain why previous antibiotic exposure was not a risk factor for CTX-M-14 or CTX-M-27. It remains unclear why antibiotic exposure is a risk factor for CTX-M-1 group ESBLs. Possibly, CTX-M-1 provides a survival benefit in the hospital setting (e.g. higher tenacity).

A UK study assessing risk factors for ESBL genotypes found travel outside Europe to be a risk factor for CTX-M-15 and the CTX-M-9 group. Unfortunately, the microarray used could not differentiate between the different CTX-M-9 group types. Our analysis showed that they differ with respect to travel outside Europe. Recent travel outside Europe was a risk factor only for CTX-M-27 and did not influence CTX-M-14 colonization. CTX-M-27 is an emerging ESBL, which has been captured by the highly virulent ST131 *E. coli*.[2] This emerging ST131 clade, C1-M27, has been hypothesized to have driven a change in the ESBL landscape.[2,20] It has been shown to be more easily transmissible and patients colonized showed prolonged colonization compared with other ESBLs.[21,22] In Europe, it has been described in human samples from 2014 onwards.[23–25] Here, we provide evidence of CTX-M-27 emergence in Germany (7.5% of 3GCREB isolates compared with 0%–3.8% in isolates collected in other studies from 2009 to 2014).[7,26] In addition, we were able to show that travel outside Europe is by far the most important risk factor for CTX-M-27 *E. coli* colonization (aOR 2.73, 95% CI 1.68–4.43). Patients affected were more likely to have travelled outside of Europe than other ESBL-positive patients (except CTX-M-15) and were the least likely to have pets, to have been hospitalized or to have had a rehabilitation centre stay during the 6 months prior to admission (Table S2).

In Germany, CTX-M-14 was still slightly more prevalent than CTX-M-27 (8.7% of the isolates of hospitalized patients) in our and other studies.[7,26,27] A study from New Zealand found colonization by distinct ESBL types in distinct demographic groups. CTX-M-14 was more likely to be found in patients without prior hospitalization.[28] Similarly, we did not find prior hospitalization to be a risk for CTX-M-14. However, a prior stay in an LTCF did present a risk.

Limitations

First, we are not able to provide data on the number of patients that refused to participate and therefore cannot calculate a refusal rate. Also, we did not gather any data from these patients. Therefore, we cannot assess a potential bias in screening. Second, the most important limitation is probably the self-reporting of risk factors, such as antibiotic exposure, as this underlies a recall bias. We did not include antibiotic prescription data with substance or duration due to accessibility issues for data from ambulatory care. Third, data on foreign travel represent primarily a change in environment. Apart from self-reported hospital stays in foreign countries, we lack data on contact with foreign healthcare systems. Fourth, our screening approach using rayon swabs might not have been optimal but was used for reasons of feasibility, as it had already been established in all participating institutions. Fifth, no pre-enrichment of samples was performed, which has been shown to increase sensitivity.[29,30] Sixth, ChromID ESBL agar has a high sensitivity for detection of ESBLs and most carbapenemases. However, some AmpC-producing isolates (with lower MICs/without hyperproduction) and ESBL-negative OXA-48-producing isolates are suppressed, which could have influenced the findings and resulted in underestimation of these β-lactamases. Seventh, PCR and Sanger sequencing could not differentiate between some closely related CTX-M-variants (e.g. CTX-M-14 and CTX-M-17/-18, which differ from CTX-M-14 by a single nucleotide at the 3′ end of the ORF). Lastly, all participating centres are large tertiary care hospitals with likely higher numbers of patients at risk/pre-treated. Therefore, the ability to generalize to small hospitals or the general population is limited.

Conclusions

Our analyses showed that different ESBL types have distinct risk factor sets. The data suggest that different habits and medical histories might influence the patients' risk of colonization with specific ESBL genotypes. Knowledge about specific ESBL genotypes and their association with the different risk factors could help to better understand the dynamics of ESBL dissemination. Based on the present data, future studies should be performed to analyse the background of these ESBL-specific risk factors in more detail, e.g. the association of CTX-M-14 with LTCF should be investigated in a larger LTCF-based study. Additionally, studies analysing transmission events with different ESBL types should be initiated, e.g. analysing whether CTX-M-1 isolates or plasmids are more easily transmitted under antibiotic selection pressure compared with other ESBLs.

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Author contributions

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Supplementary data

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

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