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

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Abstract

To assess the distribution of phylogroups and O25/ST131 in the Netherlands, we performed a real-time polymerase chain reaction (PCR) on a collection of 108 wild-type *Escherichia coli* (WT-EC) and 134 extended-spectrum β-lactamase–producing *E. coli* (ESBL-EC). Phylogroup B2 was predominant, but ESBL-EC were less likely to belong to this phylogroup (48.5%) than were WT-EC (66.7%; p = 0.005). In WT-EC, phylogroups B2 and D seem to be more virulent, having a higher prevalence among midstream urine isolates and blood culture isolates, than in catheter-related urine isolates (83.3% and 87.9% vs. 61.9%; p 0.048). O25/ST131 is associated with ESBL production, being almost absent among phylogroup B2 WT-EC (61.5% vs. 5.6%; p < 0.001).

Keywords: Blood culture, *E. coli*, phylogroups, rectal colonization, ST131, urine

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Introduction

Escherichia coli is an important cause of urinary tract infections and systemic infections in humans [1]. The primary reservoir for infections due to *E. coli* is the patient's own intestinal tract [2]. Factors associated with increased risk of infection are patient- and pathogen dependent. Patient-dependent factors include underlying illnesses, female sex, the use of indwelling catheters, and previous antimicrobial use [3]. Pathogen-dependent factors include differences in virulence between phylogenetic group A, B1, B2 and D [4] *E. coli*. Isolates belonging to phylogroup B2 or D are often extraintestinal pathogenic *E. coli* (ExPEC), causing urinary tract infections and systemic infections [5,6].

Antimicrobial resistance due to production of extended-spectrum β-lactamases in *E. coli* (ESBL-EC) is increasing [7,8]. One subgroup within the ESBL-EC B2 phylogroup, O25/ST131 *E. coli*, has successfully spread worldwide [9] and in the community [10].

Materials and methods

In this study we determined the distribution of phylogroups A, B1, B2, and D, and the prevalence of O25/ST131 in the Netherlands, within a collection of ESBL-EC obtained from rectal colonisation samples, and ESBL-EC and wild-type *E. coli* (WT-EC) obtained from urine and blood cultures. All isolates were collected in our laboratory for medical microbiology, situated in a large teaching hospital in the southern part of the Netherlands. The rectal colonisation samples were obtained from hospitalized patients during routine cross-sectional surveys in November 2012 and 2013. ESBL-EC obtained from urine samples are routinely stored at -80°C, and retrospectively collected between January 2010 and March 2013. A comparable number of WT-EC isolates were collected prospectively between February and August 2013. Isolates obtained from blood cultures are routinely stored at -80°C, and all available ESBL-EC obtained from unique patients were retrospectively collected between January 2010 and March 2013. For every ESBL-EC, the next available WT-EC isolate was included.

Phylogenetic typing was performed on all *E. coli* isolates using real-time polymerase chain reaction (PCR) [11], and an O25/ST131 specific real-time PCR was performed on all phylogroup B2 *E. coli* isolates [12]. Sequence analysis was performed on isolates with inconclusive results in the O25/ST131-specific

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real-time PCR to detect the pathognomonic A and T single nucleotide polymorphism in the pabB gene.

Differences in the prevalence of phylogroups and O25/ST131 were analysed with χ^2 analysis using the Statistical Package for Social Sciences software (SPSS, version 17). Statistical significance was accepted if the chance for coincidence was <5%.

Results

A total of 242 *E. coli* isolates were included in the study. All blood culture isolates, all rectal swab isolates, and all but one of the catheter-related urine isolates (95.2% of WT-EC and 100% of ESBL-EC) were obtained from hospitalised patients, whereas the majority of midstream urine isolates was obtained from general practitioners' patients (40 WT-EC; 74.1% and 34 ESBL-EC; 79.1%). Table 1 shows the age and sex distribution of patients with *E. coli* from the different origins, with patients for whom a midstream isolate was included being more often female and significantly younger than the other patients.

The majority of isolates belonged to phylogroup B2 (56.6%; Fig. 1), and this was the predominant phylogroup in all subcategories. ESBL-EC isolates were less likely to belong to this phylogroup (48.5%) compared with WT-EC (66.7%, p 0.005; 49.0% vs. 66.7% and p 0.010 when excluding the rectal swab isolates from the analysis). The majority of the B2 phylogroup ESBL-EC isolates belonged to clonal complex O25/ST131 (61.5%) vs. a small minority of the B2 phylogroup WT-EC (5.6%; p < 0.001). The prevalence of O25/ST131 in ESBL-EC was lowest in the rectal colonization isolates (18.4%) vs. 37.2%, 36.8% and 29.4% for midstream urine isolates, catheter related urine isolates and blood culture isolates, respectively. However, these differences were not statistically different (p 0.27).

Significant differences in occurrence of phylogroup A, B1 and D between ESBL-EC and WT-EC were only present for phylogroup A, with this phylogroup being more present in ESBL-EC than in WT-EC obtained from blood cultures (26.5% vs 6.1%; p 0.024).

For WT-EC, phylogroups 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% vs. 61.9%; p 0.048). The prevalence of other phylogroups was comparable between the groups of isolates.

Discussion

In the present study, remarkable differences were found between the prevalence of phylogroups and O25/ST131 in ESBL-EC and WT-EC from different origins. Most striking is the difference in prevalence of O25/ST131, being the most prevalent clone in ESBL-EC, and being almost absent in WT-EC. This finding supports the idea that O25/ST131 owes its success to the ESBL phenotype. Among WT-EC, phylogroups A and B1 were found to be less prevalent among midstream urine isolates and human blood culture isolates as compared with WT-EC obtained from catheter-related urine isolates. This finding supports the hypothesis that these phylogroup isolates need devices like catheters to overcome barriers to cause infection. This phenomenon was not observed in ESBL-EC.

Our results regarding the predominance of phylogroup B2 and O25/ST131 in ESBL-EC are in line with the results of Johnson et al. [13]. Furthermore, the findings regarding the higher prevalence of phylogroup A in ESBL-EC than in WT-EC are comparable. 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 ST131 status [14,15].

TABLE 1. Distribution of sex and age

	WT-EC		ESBL-EC	
	Age	Sex	Age	Sex
	Mean (95% CI)	Male (%)	Mean (95% CI)	Male (%)
Rectal colonisation			55.8 (46.4–65.3)	57.9
Urine culture, midstream*	47.4 (40.3–54.4)	14.8	56.8 (48.2–65.4)	25.6
Urine culture, indwelling catheter†	73.3 (66.4–80.2)	47.6	80.3 (76.2–84.3)	52.6
Blood culture	67.6 (62.0–73.1)	48.5	66.4 (60.3–72.5)	58.8

Mean age with 95% confidence interval and percentage of male subject among wild-type *Escherichia coli* (WT-EC) isolates and extended-spectrum β-lactamase–producing *E.coli* (ESBL-EC) isolates.
*Patients with positive midstream urine cultures (both ESBL-EC and WT-EC) were significantly younger than patients with indwelling catheters or with positive blood cultures (p < 0.001 and p < 0.001), and were significantly more often female than the patients from whom other cultures were obtained (p 0.001 for WT-EC and p 0.008 for ESBL-EC).
†Patients with indwelling catheters were significantly older than patients with ESBL-EC in their rectal swabs or *E. coli* in their midstream urine cultures (p 0.001 and p < 0.001, respectively).

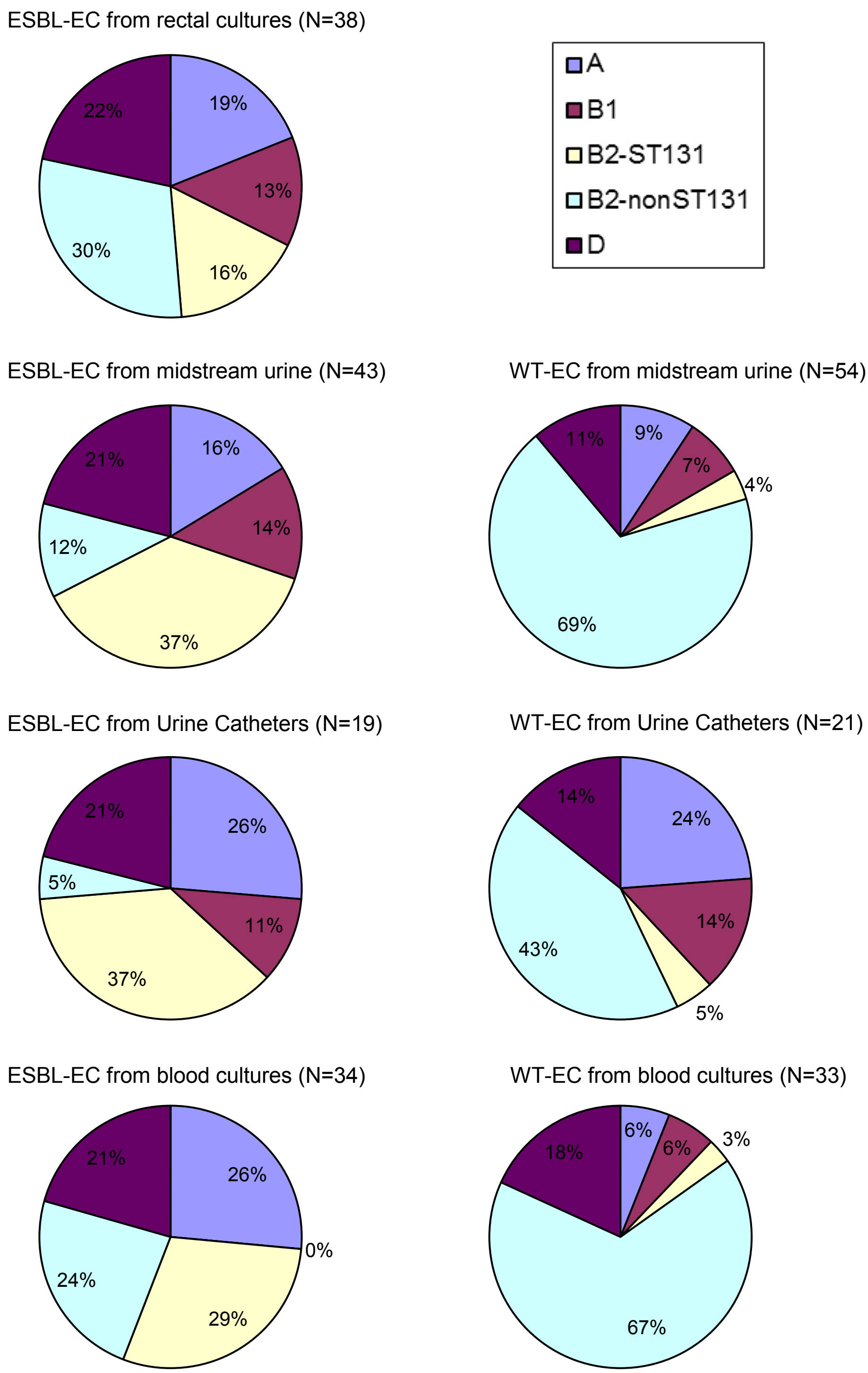

FIG. 1. Distribution of phylogroups and O25/ST131.

In conclusion, our study supports the idea that *E. coli* colonizing the gut are not equally capable of causing infection. For WT-EC, phylogroups B2 and D are more likely to cause infection, whereas other phylogroups need devices such as urinary catheters to overcome barriers and cause infection. Furthermore, our results show a significant association of O25/ST131 with ESBL production, with this strain being almost absent in WT-EC isolates. Overall, phylogroup B2 is less frequently seen in ESBL-EC than in WT-EC, whereas for phylogroup A the opposite was found.

Transparency declaration

Jan Kluytmans received consultancy fees from Pfizer, Biomerieux and 3M. The other authors have nothing to declare.

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