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Short communication

O-serotype distribution of *Escherichia coli* bloodstream infection isolates in critically ill patients in The Netherlands

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ABSTRACT

Objectives: Invasive infections by extra-intestinal pathogenic *Escherichia coli* (ExPEC) strains are increasing. We determined O-serogroups of *E. coli* isolates from ICU patients having bloodstream infections (BSI) and the potential coverage of a 10-valent O-polysaccharide conjugate vaccine currently in development for the prevention of invasive ExPEC disease.
Methods: We studied *E. coli* BSI among patients admitted to a tertiary ICU in the Netherlands between April 2011 and November 2016. O-serogroups were determined *in vitro* by agglutination and whole genome sequencing.
Results: Among 714 ICU patients having BSI, 70 (10%) had an *E. coli* BSI. Among 68 (97%) isolates serogrouped, the most common serogroups were O25 (n = 11; 16%), O8 (n = 5; 7%), O2 (n = 4; 6%), O6 (n = 4; 6%), and O15 (n = 4; 6%). The theoretical coverage of a 10-valent ExPEC vaccine was 54% (n = 37).
Conclusions: A multi-valent ExPEC O-polysaccharide conjugate vaccine in development could potentially aid in the prevention of *E. coli* BSI in Dutch ICU patients.

1. Introduction

Extraintestinal pathogenic *Escherichia coli* (ExPEC) is a common pathogen causing bloodstream infections (BSI), frequently associated with antimicrobial resistance (1). Its incidence in hospitals across Europe is increasing, which is largely driven by an aging population [1,2]. Furthermore, *E. coli* is among the most frequently isolated pathogens among sepsis patients in the intensive care unit (ICU) [3].

As the development of new antibiotics has not kept up with the global increase in antimicrobial resistance, preventive strategies, such as vaccines, are needed. A multivalent ExPEC glycoconjugate vaccine, targeting 10 specific O-antigens located on the distal end of the lipopolysaccharide (LPS) of *E. coli* is currently under development. Its 4-valent predecessor was demonstrated to be both safe and immunogenic in subjects with recurrent urinary tract infections, as well as healthy adults [4,5].

Although more than 180 different O-serogroups have been described in *E. coli,* most ExPEC infections can be attributed to a smaller subset of O-serogroups. Previously, serogroups O2, O6, and O25 were reported to be the most common among invasive *E. coli* isolates obtained from urine and blood in the UK [6]. However, O-serogroup distributions among invasive isolates may change over time and may differ according to age, source of infection, and geographical and clinical setting [6–8]. Furthermore, little is known about the O-serogroup distribution among invasive *E. coli* isolates in ICU patients. In this report we describe patient and disease characteristics and O-serogroup distribution in ICU patients with *E. coli* BSI in the Netherlands.

2. Methods

We identified all first occurrences of BSI caused by *E. coli* in a cohort of critically ill patients. Patients had been consecutively

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admitted to the mixed-ICU of a tertiary care hospital in the Netherlands, between April 2011 and November 2016. Data collection was part of the Molecular Diagnosis and Risk Stratification of Sepsis (MARS) study for which ethical approval was provided by the Medical Ethics Committee of the University Medical Center Utrecht, including an opt-out consent method (IRB No. 10-056C) [9]. Most likely sources of *E. coli* BSI had been recorded prospectively by researchers for each new antimicrobial therapy prescription. Previously reported risk-factors for *E. coli* BSI were retrieved, including the presence of solid tumors or hematological malignancies, chronic dialysis, chronic renal failure, underlying urological pathophysiology (including calculi, obstruction, and retention),

Table 1
Characteristics of 70 patients and concordant strains with an *E. coli* bloodstream infection.

Variable	Community-acquired (n = 34)	Nosocomial (n = 36)	P-value
Patient and disease characteristics			
Age (median IQR)	64 (56, 73)	65 (53, 71)	0.66
Male (n %)	18 (52.9)	24 (66.7)	0.24
APACHE IV score at admission (median, IQR)	102 (74, 132)	95 (80, 124)	0.36
Charlson comorbidity score (median, IQR)	2 (0, 3)	2 (0, 3)	0.51
SOFA score on the day of the BSI (median, IQR)	10 (8, 14)	9 (6, 13)	0.41
Septic shock on the day of the BSI (n, %)	24 (71)	24 (67)	0.72
Polymicrobial BSI (n, %)	8 (24)	6 (17)	0.47
Risk factors for E. coli BSI			
Solid tumor disease (n, %)	11 (32)	12 (33)	0.93
Hematologic Malignancy (n, %)	5 (15)	4 (11)	0.65
Chronic dialysis (n, %)	1 (3)	2 (6)	0.59
Chronic renal failure (n, %)	6 (18)	8 (22)	0.63
Underlying urological pathophysiology (including calculi, obstruction, and retention) (n, %)	5 (15)	2 (6)	0.20
Recurrent UTI's (3 UTIs in the last year) (n, %)	1 (3)	0 (0.0)	0.30
Urinary catheter device (in the past two weeks, greater than48hrs) (n, %)	5 (15)	30 (83)	<0.0001
Chronic catheter	5 (100)	1 (3)	
Solid organ transplant (n, %)	1 (3)	4 (11)	0.18
Recent abdominal surgery (<30 days) (n, %)	1 (3)	14 (39)	<0.0001
1 or more risk factors present (n, %)	19 (56)	26 (72)	0.46
Source of infection:			0.61
- Intra-abdominal infection (n, %)	11 (32.4)	13 (36)	
Secondary peritonitis	3 (27)	10 (78)	
Biliary tract	7 (64)	1 (8)	
Other (translocation, primary peritonitis)	1 (9)	2 (15)	
- Urinary tract infection (n, %)	12 (35)	7 (19)	
- Pneumonia (n, %)	2 (6)	2 (6)	
- Skin or wound infection (n, %)	3 (9)	6 (17)	
- Other (n, %)	6 (18)	8 (22)	
Outcomes			
Recurrent *E. coli* BSI short term (<1 month) (n, %)	1 (3)	1 (3)	0.97
Recurrent *E. coli* BSI long term (<2 year) (n, %)	5 (15)	1 (3)	0.07
Total ICU length of Stay (median IQR)	3 (1, 12)	9 (3, 24)	0.02
30-day mortality (n, %)	10 (29)	14 (39)	0.40
1-year mortality (n %)	17 (50)	21 (58)	0.48
Strain characteristics			
Antimicrobial non-susceptibility (n, %)			
Gentamycin and/or tobramycin	4 (12)	6 (17)	0.56
Ceftriaxone	2 (6)	10 (28)	0.02
ESBL-production	2 (6)	9 (25)	0.03
Ciprofloxacin	5 (15)	9 (25)	0.28
Trimethoprim/sulfamethoxazole	16 (47)	14 (39)	0.49
Meropenem	0 (0)	0 (0)	NA
Colistin	0 (0)	1 (3)	0.33
O-serogroups (n, %)[a]			0.75
25	6 (18)	5 (15)	
15	3 (9)	1 (3)	
2	2 (6)	2 (6)	
6	2 (6)	2 (6)	
16	1 (3)	2 (6)	
8	3 (9)	2(6)	
75	1 (3)	2 (6)	
1	0 (0)	1 (3)	
18	0 (0)	1 (3)	
4	1 (3)	0 (0)	
17	2 (6)	1 (3)	
101	0 (0)	2 (6)	
78	2 (6)	0 (0)	
Non -typeable	1 (3)	2 (6)	
Other serogroups[b]	10 (29)	11 (32)	
4-valent vaccine coverage	10 (29)	10 (29)	1.0
10-valent vaccine coverage	19 (56)	18 (53)	0.81

APACHE = Acute Physiology and Chronic Health Evaluation, ICU = Intensive Care Unit, ESBL = Extended spectrum beta-lactamase, SOFA = Sequential Organ Failure Assessment. WGS = Whole-genome sequencing. Serogroups included in the 10-valent vaccine that is in development are shown in bold.

[a] Serogroup percentage are based on the 68 available strains, i.e. 34 community-acquired and 34 nosocomial.

[b] Other serogroups include: 162, 153, 117, 111, 107, 86, 77, 73, 68, 58, 45, 44, 23, 21, 13, 9 and 3. Non-typeable strains were either negative or multiple positive.

recurrent urinary tract infections (UTI) (defined as the occurrence of 3 or more UTIs in the previous year), presence of a urinary catheter device (in the past two weeks for more than 48 h), a previous solid organ transplant, and recent abdominal surgery (<30 days) [10,11]. Sepsis was defined as a sequential organ failure assessment (SOFA) score of 2 or more and septic shock was defined as the presence of sepsis and the need for vasopressors and a lactate >2 mmol/L [12]. BSI events were categorized as community-acquired if they occurred within 48 h of hospital admission, otherwise they were considered to have nosocomial onset. ICU-acquired BSI had an onset of 72 h after ICU admission. We report the recurrence of an *E. coli* BSI for up to 2 years following the primary event.

A single clinical *E. coli* isolate was analyzed for each patient. BD BACTEC™ (Becton and Dickinson Microbiology System, Sparks, MD, USA) blood culture bottles were incubated using a BD BACTEC™ blood culture system with automatic microbial growth signaling under 35 °C. Subsequently MALDI-ToF MS was used for pathogen identification. *E. coli* isolates were subsequently stored at −80 °C for later serotyping. Cultures yielding multiple species were considered polymicrobial (except for contamination caused by coagulase-negative staphylococci). Antimicrobial susceptibility testing was performed using phenotypic methodology, and the reference minimum inhibitory concentrations (MIC) provided by EUCAST. Individual *E. coli* isolates were categorized as susceptible or non-susceptible, which included isolates with intermediate susceptibility.

O-serotyping of *E. coli* isolates was conducted at the Pennsylvania State University (University Park, PA, USA) and Janssen Research and Development (Raritan, NJ, USA) by agglutination using O-antisera [13]. Due to an incomplete or absent LPS structure, *E. coli* strains can either respond to two or more antisera (i.e. multiple positive result), or not respond at all (i.e. negative result) in agglutination assays. *E. coli* isolates not typeable by agglutination were subjected to whole-genome sequencing (WGS) to allow for O-serotyping at the genetic level. The prediction of O-serogroup from WGS was performed using O-serotyper v0.1, developed by Janssen Vaccines and Prevention. This tool uses the EcOH database to screen assembled contigs for allelic variants in O-antigen *rfb* cluster to infer *E. coli* O-serogroups using the *wzy*/*wzx* genes of published genomes with known *rfb* clusters [14–16]. Among the serogrouped isolates vaccine coverage was estimated by calculating the percentage of *E. coli* isolates with an O-serogroup included in the 10-valent O-polysaccharide conjugate vaccine that is currently in development. This vaccine includes the following serogroups: O25, O6, O2, O1, O75, O8, O15, O18, O16, and O4 [17]. While its 4-valent predecessor included: 025, O6, O2 and 01 [4]. Differences between community-acquired and nosocomial BSI were analyzed using the Wilcoxon rank sum, Chi-square or Fisher's exact test as appropriate. A p-value < 0.05 was considered statistically significant.

3. Results

Of 9,660 admitted patients in the MARS cohort, 714 had a positive culture (excluding results suggesting contamination), and 70 (10%) had *E. coli* BSI of whom 34 (49%) were community-acquired, 36 (51%) were nosocomial, and 11 were ICU-acquired (Table 1). Patients with nosocomial *E. coli* BSI more frequently had indwelling urinary catheters and recent abdominal surgery, but other known risk factors for *E. coli* BSI were evenly distributed among patients with community-acquired and nosocomial infections. Median SOFA score was 10 (IQR 7, 13) at the day of *E. coli* BSI, and 24 of 70 patients (34%) died within 30 days after BSI.

Nosocomial *E. coli* BSI were most frequently attributed to an intra-abdominal infection (n = 13; 36%), in particular secondary peritonitis (n = 10), whereas UTI was the most common source for community-acquired *E. coli* BSI (n = 12; 35%). Recurrence of *E. coli* BSI within one month occurred in two patients (one with community-acquired and one with nosocomial BSI). An additional five patients with community-acquired *E. coli* BSI (15%) and one patient with nosocomial *E. coli* BSI (3%) had a recurrent BSI within two years (Table 1; p = 0.07). Overall, antibiotic resistance was more prevalent among nosocomial BSI strains (Table 1). Of all strains, 12 (17% (95% CI 8%–26%) were resistant to ceftriaxone and all but one produced extended-spectrum beta-lactamase. All strains were susceptible to meropenem and one was resistant to colistin.

Serogroups could be determined for 68 of 70 *E. coli* isolates examined (97%); 58 strains autoagglutinated and 10 were non-typeable using standard serology. Of these 10 non-typeable strains,

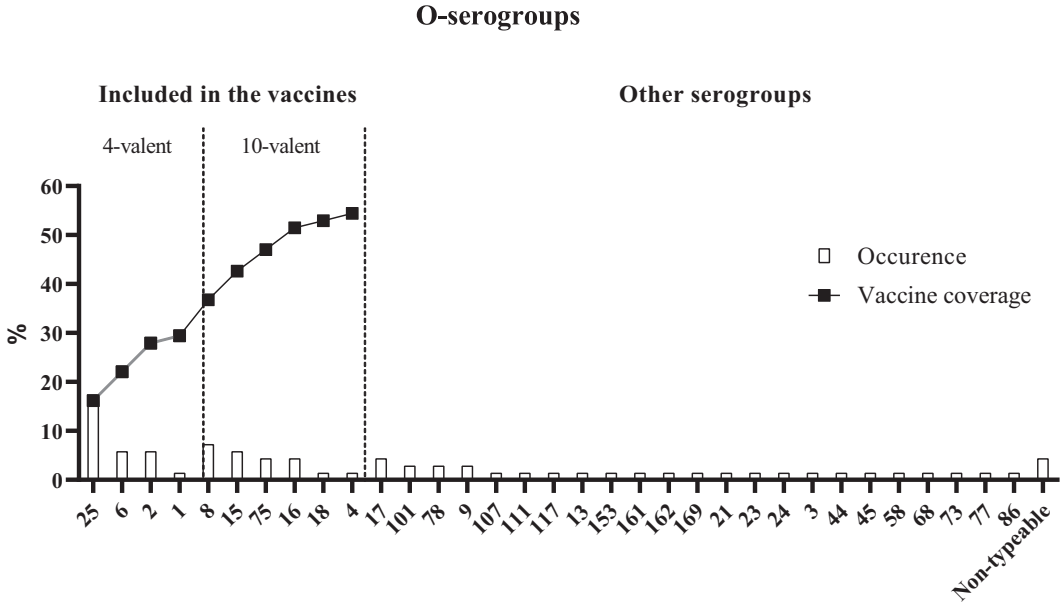

Fig. 1. O-serogroups of the *E. coli* bloodstream isolates (n = 68). Based on agglutination and whole-genome sequencing vaccine coverage was 54%.

9 were available for WGS, which yielded 7O-serogroups, and 2 remained non-typeable. Therefore, in all there were 65 known O-serogroups (96%), and 3 non-typeable strains (4%) (Table 1). Overall, serogroup O25 was most prevalent (11 isolates, 16%), followed by O8 (5 isolates, 7%), O2 (4 isolates, 6%), O6 (4 isolates, 6%), and O15 (4 isolates, 6%) (Table 1). In this study the overall theoretical coverage of the 4-valent vaccine was 29% for both community-acquired and nosocomial *E. coli* BSI (p = 1.0, Fig. 1 and Table 1). The theoretical coverage for the 10-valent vaccine that is in development was 54% overall; 56% for community-acquired and 53% for nosocomial *E. coli* BSI, (p = 0.81, Fig. 1 and Table 1).

4. Discussion

In this cohort of *E. coli* isolates associated with BSI in 70 critically ill patients in a Dutch ICU the theoretical O-serogroup coverage of a 10-valent *E. coli* vaccine that is in development was 54%. The coverage was similar for both community-acquired and nosocomial infections. Vaccine effectiveness however, is impacted by more factors than theoretical coverage alone. For example, the vaccinees age and immune status play an important role as well as the focus and burden of the target infection within the target population [18]. In a post-hoc exploratory analyses, Huttner and colleagues demonstrated that although the number of vaccine serogroup UTIs did not differ significantly between the vaccine and placebo groups, the number of UTIs caused by any *E. coli* O-serogroup was significantly reduced in the vaccine group receiving an experimental 4-valent ExPEC O-polysaccharide conjugate vaccine [4]. It was suggested that cross-reactivity between O-serogroups and unspecific immune response boosting may improve O-polysaccharide conjugate vaccine effectiveness, although, until today, no further evidence supporting this hypothesis has been provided. The presence of these mechanisms and the potential vaccine effectiveness should be further explored.

The overall prevalence of third generation cephalosporin resistance (i.e. ceftriaxone) was 17% (95% CI 8%–26%) in this cohort, which is comparable to European surveillance data on blood and spinal fluid isolates (12–13.1%) [19]. The observed prevalence is higher than the average prevalence among *E. coli* blood culture isolates in the Netherlands [20], but reflects the prevalence in a critically ill patient population in an ICU of a tertiary care hospital that are likely exposed to multiple antibiotics during the course of treatment.

The rising incidence of invasive *E. coli* infections has prompted policy makers to target this infection for prevention. For instance, the UK's current national action plan aims to reduce Gram-negative BSI (including *E. coli* BSI) by 50% in the next 5 years. As a considerable proportion of invasive *E. coli* infections is community-acquired and predominantly occurring in older adults, generalized senior vaccination could be an effective measure [21]. To optimize vaccine strategies, additional risk-based approaches need to be explored. In the current study, 72.2% of patients with nosocomial infection and 55.9% of patients with community-acquired infections had at least one identifiable risk factor. Therefore, identifying patients with relevant comorbidities could be a starting point in selecting at risk populations for the evaluation of prevention strategies.

This study has several limitations. Three strains could not be retrieved for serogrouping by agglutination assay or WGS and calculations were based on the serogrouped strains alone. Also, we did not examine sequence types (e.g. ST131) and O-antigen subtypes (e.g. O25AB). Furthermore, our results are not generalizable beyond the Dutch ICU population. Finally, our limited sample size precludes robust conclusions on risk factors for invasive ExPEC disease or O-serogroup epidemiology. Further research is therefore needed for risk stratification of patients at risk for *E. coli* bloodstream infection.

In conclusion, the multi-valent ExPEC O-polysaccharide vaccine that is currently in development had a theoretical O-serogroup coverage of 54% for *E. coli* isolates associated with BSI in Dutch ICU patients. Vaccine strategies to prevent *E. coli* BSI in critically ill patients should be further explored.

Ethics approval and consent to participate

The institutional review board approved an opt-out consent procedure (protocol number 10-056C).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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