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Global Distribution of O Serotypes and Antibiotic Resistance in Extraintestinal Pathogenic *Escherichia coli* Collected From the Blood of Patients With Bacteremia Across Multiple Surveillance Studies

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Background. Extraintestinal pathogenic *Escherichia coli* (ExPEC) is the leading cause of bacteremia worldwide, with older populations having increased risk of invasive bacterial disease. Increasing resistance to first-line antibiotics and emergence of multidrug-resistant (MDR) strains represent major treatment challenges. ExPEC O serotypes are key targets for potential multivalent conjugate vaccine development. Therefore, we evaluated the O serotype distribution and antibiotic resistance profiles of ExPEC strains causing bloodstream infections across 4 regions.

Methods. Blood culture isolates from patients aged ≥60 years collected during 5 retrospective *E. coli* surveillance studies in Europe, North America, Asia-Pacific, and South America (2011–2017) were analyzed. Isolates were O serotyped by agglutination; O genotyping was performed for nontypeable isolates. Antimicrobial susceptibility testing was also conducted.

Results. Among 3217 ExPEC blood culture isolates, the most ubiquitous O serotype was O25 (n = 737 [22.9%]), followed by O2, O6, O1, O75, O15, O8, O16, O4, O18, O77 group, O153, O9, O101/O162, O86, and O13 (prevalence of ≥1%). The prevalence of these O serotypes was generally consistent across regions, apart from South America; together, these 16 O serotypes represented 77.6% of all ExPEC bacteremia isolates analyzed. The overall MDR frequency was 10.7%, with limited variation between regions. Within the MDR subset (n = 345), O25 showed a dominant prevalence of 63.2% (n = 218).

Conclusions. Predominant O serotypes among ExPEC bacteremia isolates are widespread across different regions. O25 was the most prevalent O serotype overall and particularly dominant among MDR isolates. These findings may inform the design of multivalent conjugate vaccines that can target the predominant O serotypes associated with invasive ExPEC disease in older adults.

Keywords. ExPEC; *Escherichia coli*; antibiotic resistance; serotype; multidrug resistance.

Extraintestinal pathogenic *Escherichia coli* (ExPEC) is a major human pathogen with the capacity to colonize, infect, and invade any body tissue, which can lead to invasive ExPEC disease (or invasive *E. coli* disease [IED]) and death [1]. ExPEC is the most common cause of bacteremia in adults worldwide and is a leading cause of sepsis and subsequent hospitalization or death in the United States [2–5]. In addition, ExPEC is the leading cause of urinary tract infections, the second most common cause of neonatal meningitis [6], and one of the most common causative pathogens of nosocomial and healthcare-associated infections [7, 8].

The risk of developing IED, including bacteremia and sepsis, increases with age [9, 10]. A systematic literature review found that the overall incidence of *E. coli* bacteremia in adults was 48 cases per 100 000 person-years, increasing to ≥100 cases per 100 000 person-years in 55–75-year-olds, and ≥300 cases per 100 000 person-years in 75–85-year-olds [10]. An epidemiological study of inpatient and hospital-based outpatient visits in US hospitals between 2009 and 2016 found that invasive *E. coli* infections had a prevalence of 0.50 events per 1000 visits and 1.82 events per 1000 patients [11]. Global morbidity and mortality rates attributed to ExPEC are considerable and increase annually [2, 10], partly owing to the emergence of multidrug-resistant (MDR) ExPEC strains, such as O25B sequence type (ST) 131 [12].

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An important virulence factor for ExPEC survival is the O antigen, the distal-end polysaccharide structure of lipopolysaccharide located on the cell surface of *E. coli*. The production of O antigen–specific antibodies can provide protection against infection in vivo through promotion of opsonophagocytosis and direct bacterial killing [13], making the O antigen a suitable target for a prophylactic vaccine [1, 13, 14].

Although >180 different O serotypes have been identified [15, 16], studies in the United Kingdom and France in recent years suggested that the most prevalent O serotypes associated with ExPEC bloodstream infections (BSIs) were O25B (a subtype of the O25 serotype), O6, and O2 [17–19]. Similarly, a study in the Netherlands found that the most common O serotypes were O25, O8, O2, O6, and O15 [20]. A successful prophylactic vaccine will need to show broad coverage for prevalent IED-associated O serotypes, including those associated with MDR infections. A global picture and continuous monitoring of O serotype distribution is needed to guide O serotype selection for a multivalent glycoconjugate ExPEC vaccine. Therefore, we characterized the O serotype distribution and antibiotic resistance profiles of ExPEC bacteremia isolates from patients aged ≥60 years hospitalized in Europe, North America, Asia-Pacific, and South America.

METHODS

Study Design

This pooled, retrospective surveillance study collected ExPEC bacteremia isolates from 5 *E. coli* surveillance studies conducted between January 2011 and December 2017 (see Supplementary Table 1). The purpose of these studies was to develop a globally representative collection of ExPEC blood culture isolates for future analysis. Blood isolates (1 sample per patient) were collected from hospitalized patients with ExPEC bacteremia, aged ≥60 years, from 4 regions (Supplementary Table 2), either by obtaining these directly from the hospital University Medical Center Utrecht or by using existing surveillance networks. ExPEC strains together with accompanying patient demographics were then characterized using various assays. Demographic data included patient sex, age, location, and inpatient/outpatient status; no patient-identifiable information was collected for this study. The designation of inpatient or outpatient status was based on the patient's location at the time of blood sample acquisition, as provided by the participating health facilities (Table 1), rather than where the patient with IED was treated. Blood culture isolates were identified as *E. coli* in either hospital or central laboratories using Bruker matrix–assisted laser desorption ionization time-of-flight mass spectrometry.

O Serotyping

ExPEC isolates were O serotyped and reported based on the agglutination method [21]. For isolates that were nontypeable by agglutination, O genotyping was conducted based on whole-genome sequencing with identification of unique O serotype-specific sequences of *wzy*, *wzx*, *wzt*, and *wzm* genes following guidelines and using reference sequences described elsewhere [15, 16]. O serotype grouping information can be found in the Supplementary Materials. For isolates that showed no match or multiple matches with known O serotypes, the O genotype was designated as "nondetermined." O serosubtyping for O25A/B was performed by polymerase chain reaction (PCR), targeting the O antigen-specific glycosyltransferase genes (*wbuB* [O25A] and *wbbL* [O25B]). ST131 subtyping was performed by PCR, targeting an ST131-specific region downstream of the RNA-directed DNA polymerase (EC2.7.7.49). (Supplementary Table 3). For a subset of isolates,

Table 1. Patient Demographics and Characteristics

	Region				
Characteristic	Europe (n = 1110)	North America (n = 977)	Asia-Pacific (n = 869)	South America (n = 261)	TSP (N = 3217)
Collection sites, no.	71	42	18	7	138
Sex, no. (%)					
Female	517 (47.3)	558 (57.3)	466 (53.6)	137 (52.7)	1678 (52.5)
Male	575 (52.7)	415 (42.7)	403 (46.4)	123 (47.3)	1516 (47.5)
Unknown	18	4	0	1	23
Age, median (range), y	77 (60–101)	76 (60–97)	76 (60–104)	72 (60–104)	76 (60–104)
Age group, no. (%)					
60–74 y	446 (40.2)	461 (47.2)	400 (46.0)	151 (57.9)	1458 (45.3)
≥75 y	664 (59.8)	516 (52.8)	469 (54.0)	110 (42.1)	1759 (54.7)
Patient status, no. (%)[a]					
Inpatient	766 (69.0)	772 (79.0)	488 (56.2)	216 (82.8)	2242 (69.7)
Outpatient	248 (22.3)	196 (20.1)	381 (43.8)	45 (17.2)	870 (27.0)
Unknown	96 (8.7)	9 (0.9)	0	0	105 (3.3)

Abbreviation: TSP, total study population.

[a]Status based on the patient's location at the time of blood sample acquisition. Inpatients included those in general/medicine/surgery wards or intensive care units; outpatients, those seen in the clinic/office, emergency room, or nursing home/rehabilitation.

multilocus sequence typing was performed using mlst software, v2.15.2 [22], based on whole-genome sequences [23]. O serotypes with a prevalence of ≥1% (by agglutination and genotyping of nontypeable isolates) in ≥1 region were reported, with focus on the most prevalent (≥1%) O serotypes in the total study population (TSP).

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing used broth microdilution. Antibiotic panels varied between studies, with a total of 21 antibiotics from 10 antimicrobial drug classes being tested (see Supplementary Materials for all antibiotics). Antibiotic resistance was determined using minimal inhibitory concentration values and susceptibility criteria defined by either the Clinical and Laboratory Standards Institute [24] or European Committee on Antimicrobial Susceptibility Testing [25], depending on the standard practices in the different studies.

To account for variation between the 5 studies in the antibiotic panels, we reported resistance to representative antibiotics in the following 5 classes: aminoglycosides (tobramycin), cephalosporins (ceftazidime), fluoroquinolones (ciprofloxacin or levofloxacin), β-lactam/β-lactamase inhibitors (piperacillin-tazobactam), and sulfonamides (trimethoprim-sulfamethoxazole; sulfonamides were not examined in the CAPITAL 2011 study). Based on these 5 classes (and representative antibiotics), MDR was defined as isolates that were resistant to ≥3 of the 5 antibiotic classes listed [26]. Resistance to last-resort antibiotics, carbapenems (doripenem, ertapenem, imipenem, and meropenem) and colistin were also examined, using Clinical and Laboratory Standards Institute breakpoints for minimum inhibitory concentration values.

Statistical Methods

No formal sample size calculation was performed for the number of patients or isolates. Non–*E. coli* isolates, as determined by genome-based multilocus sequence typing analysis [27] of agglutination nontypeable isolates [21–23], were excluded from the analysis. Although the studies were conducted in 20 countries and isolates were collected from patients of all ages between 2002 and 2017, the analysis included only patients aged ≥60 years, from countries with ≥50 isolates collected between 2011 and 2017 (14 in total).

RESULTS

Overall Patient Demographics

In total, 3217 *E. coli* bloodstream isolates were collected from 138 sites in 4 regions: Europe (n = 1110), North America (n = 977), Asia-Pacific (n = 869), and South America (n = 261) (Supplementary Table 2). Overall, 1516 (47.5%) isolates were collected from men, and 1678 (52.5%)% were collected from women. The median age of the total population was 76 years (range, 60–104 years) (Table 1). Most isolates (n = 2242 [69.7%]) isolates were collected from inpatients, with another 870 isolates from outpatients. Patient hospitalization status and the time of blood collection were unknown for 105 of the isolates collected.

O Serotype Prevalence and Distribution

Based on agglutination O serotyping plus O genotyping for isolates nontypeable by agglutination, the most prevalent (≥1%) O serotypes in the TSP were O25, O2, O6, O1, O75, O15, O8, O16, O4, O18, O77 group, O153, O9, O101/O162, O86, and O13 (Figure 1 and Table 2; see Supplementary Materials for details

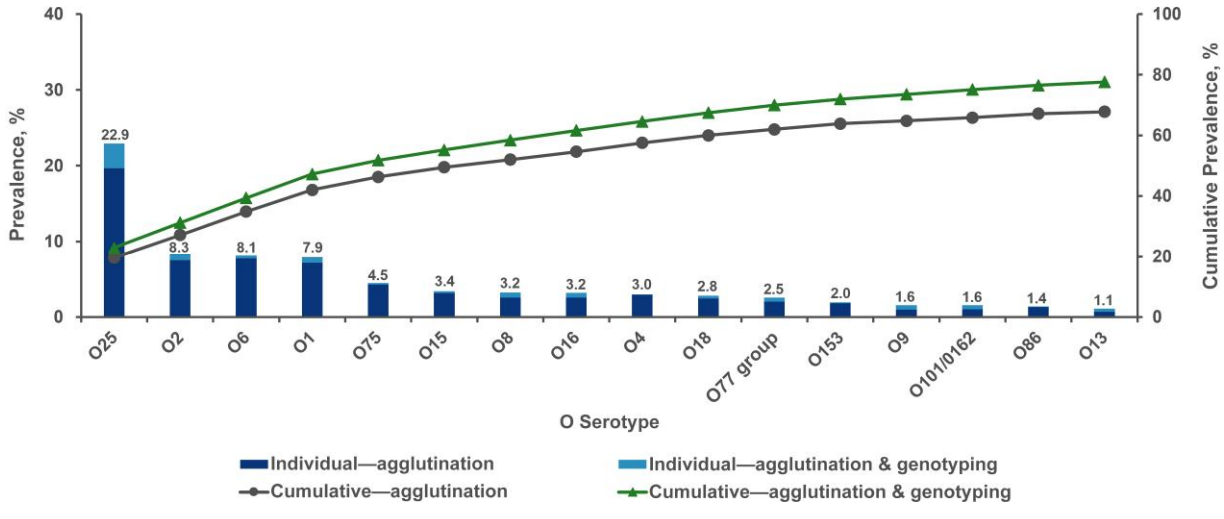

Figure 1. The most prevalent (≥1%) O serotypes in the total study population, based on individual/cumulative agglutination and individual/cumulative agglutination and genotyping. Individual/cumulative agglutination and genotyping includes the O serotype designation by the whole-genome sequencing–based genotyping method for isolates that are nontypeable by the agglutination method; individual/cumulative agglutination excludes the whole-genome sequencing–based predictions.

Table 2. Prevalence of O Serotypes in Different Regions

	Isolates, No. (%; Cumulative %)[a]				
Serotype	Europe (n = 1110)	North America (n = 977)	Asia-Pacific (n = 869)	South America (n = 261)	TSP (N = 3217)
O25	230 (20.7; 20.7)	260 (26.6; 26.6)	201 (23.1; 23.1)	46 (17.6; 17.6)	737 (22.9; 22.9)
O2	79 (7.1; 27.8)	104 (10.6; 37.3)	78 (9.0; 32.1)	7 (2.7; 20.3)	268 (8.3; 31.2)
O6	109 (9.8; 37.6)	83 (8.5; 45.8)	56 (6.4; 38.6)	13 (5.0; 25.3)	261 (8.1; 39.4)
O1	55 (5.0; 42.6)	87 (8.9; 54.7)	95 (10.9; 49.5)	18 (6.9; 32.2)	255 (7.9; 47.3)
O75	55 (5.0; 47.6)	28 (2.9; 57.5)	55 (6.3; 55.8)	7 (2.7; 34.9)	145 (4.5; 51.8)
O15	40 (3.6; 51.2)	25 (2.6; 60.1)	37 (4.3; 60.1)	8 (3.1; 37.9)	110 (3.4; 55.2)
O8	55 (5.0; 56.1)	17 (1.7; 61.8)	23 (2.6; 62.7)	9 (3.4; 41.4)	104 (3.2; 58.4)
O16	36 (3.2; 59.4)	35 (3.6; 65.4)	27 (3.1; 65.8)	5 (1.9; 43.3)	103 (3.2; 61.6)
O4	34 (3.1; 62.4)	34 (3.5; 68.9)	19 (2.2; 68.0)	9 (3.4; 46.7)	96 (3.0; 64.6)
O18	40 (3.6; 66.0)	24 (2.5; 71.3)	25 (2.9; 70.9)	2 (0.8; 47.5)	91 (2.8; 67.5)
O77 group[b]	32 (2.9; 68.9)	29 (3.0; 74.3)	13 (1.5; 72.4)	8 (3.1; 50.6)	82 (2.5; 70.0)
O153	18 (1.6; 70.5)	15 (1.5; 75.8)	8 (0.9; 73.3)	22 (8.4; 59.0)	63 (2.0; 72.0)
O9	32 (2.9; 73.4)	6 (0.6; 76.5)	7 (0.8; 74.1)	6 (2.3; 61.3)	51 (1.6; 73.5)
O101/O162	22 (2.0; 75.4)	8 (0.8; 77.3)	8 (0.9; 75.0)	12 (4.6; 65.9)	50 (1.6; 75.1)
O86	14 (1.3; 76.7)	12 (1.2; 78.5)	11 (1.3; 76.3)	8 (3.1; 69.0)	45 (1.4; 76.5)
O13[c]	9 (0.8; 77.5)	7 (0.7; 79.2)	18 (2.1; 78.4)	0	34 (1.1; 77.6)
O107/O117	14 (1.3; 78.7)	4 (0.4; 79.6)	8 (0.9; 79.3)	4 (1.5; 70.5)	30 (0.9; 78.5)
O7	9 (0.8; 79.5)	11 (1.1; 80.8)	3 (0.3; 79.6)	7 (2.7; 73.2)	30 (0.9; 79.4)
O21	9 (0.8; 80.4)	12 (1.2; 82.0)	6 (0.7; 80.3)	2 (0.8; 73.9)	29 (0.9; 80.3)
O11	6 (0.5; 80.9)	6 (0.6; 82.6)	7 (0.8; 81.1)	8 (3.1; 77.0)	27 (0.8; 81.2)
O44	12 (1.1; 82.0)	6 (0.6; 83.2)	4 (0.5; 81.6)	3 (1.1; 78.2)	25 (0.8; 81.9)
O102	7 (0.6; 82.6)	8 (0.8; 84.0)	9 (1.0; 82.6)	0	24 (0.7; 82.7)
O20	2 (0.2; 82.8)	3 (0.3; 84.3)	11 (1.3; 83.9)	5 (1.9; 80.1)	21 (0.7; 83.3)
O83	13 (1.2; 84.0)	2 (0.2; 84.5)	6 (0.7; 84.6)	0	21 (0.7; 84.0)
O68	5 (0.5; 84.4)	8 (0.8; 85.4)	1 (0.1; 84.7)	6 (2.3; 82.4)	20 (0.6; 84.6)
O46/O134	1 (0.1; 84.5)	10 (1.0; 86.4)	8 (0.9; 85.6)	0	19 (0.6; 85.2)
O24	3 (0.3; 84.8)	4 (0.4; 86.8)	1 (0.1; 85.7)	3 (1.1; 83.5)	11 (0.3; 85.5)
O119	0	0	0	5 (1.9; 85.4)	5 (0.2; 85.7)
Other[d]	169 (15.2; NA)	129 (13.2; NA)	124 (14.3; NA)	38 (14.6; NA)	460 (14.3; NA)

Abbreviations: NA, not available; TSP, total study population.

[a]O serotypes were determined by agglutination and genotyping of isolates that were nontypeable by agglutination using the whole-genome sequencing–based genotyping method. O serotypes with the highest frequency were reported (cutoff, O serotype prevalence of ≥1% in ≥1 of the 4 regions), in descending order based on the TSP.

[b]O77 group includes O serotypes O17, O73, O77, and O106 for agglutination and also O44 for agglutination and genotyping.

[c]O13 includes O serotypes O13, O129, and O135.

[d]"Other" includes isolates nontypeable by agglutination and genotyping.

about O13 and the O77 group). These O serotypes accounted for 77.6% of ExPEC bacteremia isolates analyzed; the prevalence of O serotypes was generally consistent across regions with limited regional variation, except South America. The 10 most prevalent O serotypes in the TSP were associated with 67.5% of all ExPEC bacteremia cases analyzed (Figure 1).

Serotype O25

Serotype O25 was the most prevalent O serotype (22.9%) in the TSP and was more than twice as prevalent as the next most prevalent O serotype in each region (Table 2). O25 prevalence was highest in Italy (39.6%) and lowest in the Netherlands (9.7%) (Figure 2). O25 was the most prevalent serotype across all countries studied, except for the Netherlands and France, where serotype O2 predominated at a prevalence of 11.3% and 12.6%, respectively (data not shown). PCR-based analysis of the O25 subset showed that 96.9% belonged to subtype O25B, with the remaining 3.1% belonging to O25A. Among O25B isolates, 93.3% were ST131 (Supplementary Table 4).

Other Prevalent Serotypes

After O25, the most abundant serotypes were O2 (8.3%), O6 (8.1%), and O1 (7.9%) (Table 2), which were associated with ST95 (O1, O2) and ST73 (O6) (Supplementary Table 5). These 3 O serotypes were common in all regions, although with some differences in ranking. Compared with the other regions, O serotype distribution appeared to vary more in South America, where O153 (8.4%) was the second most prevalent O serotype after O25 (17.6%), and the contribution of serotype O2 was markedly lower.

Antibiotic Resistance

Across the TSP, the highest levels of resistance were reported to sulfonamides and fluoroquinolones (35% and 32%,

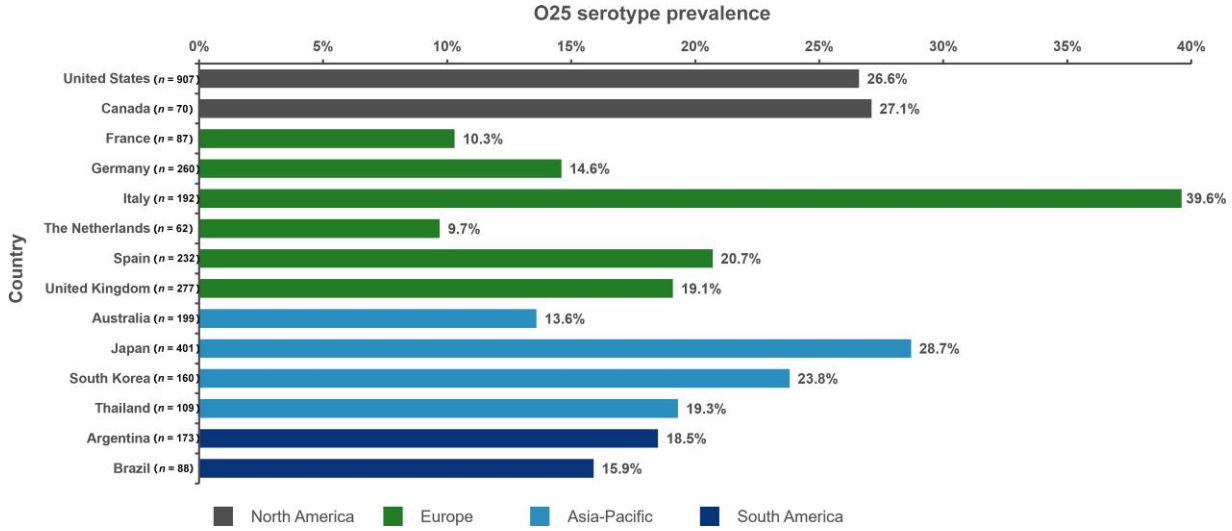

Figure 2. Prevalence of O25 O serotypes by participating country, along with total number of isolates for that country. O serotypes were determined by agglutination and genotyping of isolates that were nontypeable by agglutination using the whole-genome sequencing–based genotyping method

respectively) (Table 3). Resistance to extended-spectrum third- and fourth-generation cephalosporins was 9%. Last-resort antibiotics colistin and carbapenems showed resistance levels of 0.7% and 0.1–0.2%, respectively. Of the colistin-resistant isolates, 31.6% were serotype O25 (6 of 19); of the carbapenem-resistant isolates, this proportion was 42.9% (3 of 7; Supplementary Table 6). For colistin-resistant isolates, the predominance of O25 was followed by O1, O8, O6, O107/O117,

Table 3. Prevalence of Antibiotic Resistance

Class of Antibiotic	Isolates, No. (%)				
	Europe (n = 1110)	North America (n = 977)	Asia-Pacific (n = 869)	South America (n = 261)	TSP (N = 3217)
Resistance[a]					
Sensitive	553 (49.8)	539 (55.2)	440 (50.6)	94 (36.0)	1626 (50.5)
MDR	137 (12.3)	79 (8.1)	98 (11.3)	31 (11.9)	345 (10.7)
Resistance to single class[a]					
Aminoglycosides (tobramycin)	138 (12.4)	90 (9.2)	120 (13.8)	31 (11.9)	379 (11.8)
β-Lactam/β-lactamase inhibitors (piperacillin-tazobactam)	64 (5.8)	40 (4.1)	32 (3.7)	17 (6.5)	153 (4.8)
Extended-spectrum 3rd- or 4th-generation cephalosporin (ceftazidime)	109 (9.8)	66 (6.8)	84 (9.7)	24 (9.2)	283 (8.8)
Fluoroquinolones (ciprofloxacin or levofloxacin)	325 (29.3)	319 (32.7)	299 (34.4)	90 (34.5)	1033 (32.1)
Sulfonamides (trimethoprim-sulfamethoxazole)	409 (36.8)	242 (33.3)	258 (29.7)	132 (50.6)	1041 (35.1)
Resistance to last-resort antibiotics					
Polymyxins[b]					
Colistin	9 (0.9)[c]	6 (0.8)[d]	4 (0.5)	0	19 (0.7)[e]
Carbapenems					
Doripenem	0[c]	2 (0.2)	1 (0.1)	0	3 (0.1)[f]
Ertapenem	0[c]	3 (0.4)[d]	2 (0.2)	1 (0.4)	6 (0.2)[e]
Imipenem	0[c]	3 (0.3)	1 (0.1)	0	4 (0.1)[f]
Meropenem	0	3 (0.3)	1 (0.1)	0	4 (0.1)

Abbreviations: MDR, multidrug resistant; TSP, total study population.

[a]The degree of drug resistance was based on susceptibility to representative antibiotics in the following 5 classes of antimicrobial drugs: aminoglycosides, β-lactam/β-lactamase inhibitors, cephalosporins, fluoroquinolones, and sulfonamides.

[b]Polymyxin-B is excluded from the table because no breakpoints have been established for this antibiotic and the minimum inhibitory concentration is translated as not applicable.

[c]N = 1048.

[d]N = 726.

[e]N = 2904.

[f]N = 3155.

O23, O153, O7, O16, O18, and O2. For carbapenem-resistant isolates, O25 predominance was followed by O102, O183, O4, and O9. Resistance patterns were generally consistent across the individual regions, although notably, in South America, ExPEC bacteremia isolates showed up to 50.6% resistance to sulfonamides. Of the 3217 ExPEC isolates collected, 345 (10.7%) were MDR (Table 3). The percentage of MDR isolates in each region ranged from 8.1% in North America to 12.3% in Europe (Table 3).

O Serotype Prevalence Among MDR Strains

Among 345 MDR isolates, O25 was the dominant O serotype (n = 218 [63.2%]). O25 predominance in the MDR ExPEC isolates population was followed at a considerable distance by the following O serotypes, which had a prevalence of 1.2%–4.6% in the TSP (order of decreasing prevalence): O1, O101/O162, O8, O102, O20, O153, O9, O15, and O75 (Supplementary Table 7).

DISCUSSION

ExPEC-associated global morbidity and mortality rates continue to rise because of the increasing prevalence of ExPEC MDR strains, as well as aging populations with high rates of comorbid conditions and hospitalization, healthcare-associated infections, and bacteremia/sepsis [1, 2]. Despite the high disease burden and associated costs, the clinical importance of IED in public health surveillance systems is still routinely undervalued. For example, *E. coli* is yet to be included in the US Centers for Disease Control and Prevention's (CDC's) Active Bacterial Core surveillance network for invasive bacterial diseases [28], despite being the number 1 pathogen associated with sepsis in a 2020 study encompassing 17 430 adults with culture-positive sepsis across 104 US hospitals [4] and in a study of 1078 adult patients with sepsis from hospitals in the CDC's Emerging Infections Program in 10 states [5]. Few recent studies have described the prevalence and distribution of ExPEC O serotypes associated with IED, including bacteremia [17–20]. Such data are key for developing effective prophylactic vaccines that could prevent a significant proportion of IED.

Consistent with previous findings [18, 29, 30], this study supports evidence that O25, and more specifically O25B, is the most prevalent ExPEC O serotype worldwide. In addition, we identified serotypes O2, O6, O1, O75, O15, O8, O16, O4, O18, O77 group, O153, O9, O101/O162, O86, and O13 as other prominent ExPEC O serotypes observed in older adults with BSIs. Based on agglutination and genotyping of isolates nontypeable by agglutination, approximately 77.6% of ExPEC bacteremia cases analyzed were caused by these 16 O serotypes, of which O25 alone was responsible for 22.9% of cases. Except for South America, regional variation was minimal. In a 2021 study by Lipworth et al [18] that analyzed *E. coli* bloodstream isolates collected in the United Kingdom, O serotypes O1A, O2, O4, O6A, O8, O15, O16, O18A, O25B, and O75 made up 72% of 3278 bloodstream *E. coli* isolates analyzed; this aligns with our findings.

The continued increase in antibiotic resistance among gram-negative bacteria, including *E. coli,* is a global emergency [31–33] and is one of the main drivers of prophylactic vaccine development alongside ExPEC disease burden. Furthermore, antibiotic resistance, especially to fluoroquinolones, has been shown to contribute to septicemia hospitalization and mortality rates [32]. Resistance to the most prescribed antibiotic classes for IED was examined in our study. Resistance rates to sulfonamides and fluoroquinolones were highest of all the antibiotic classes studied (35% and 32%, respectively). Resistance to extended-spectrum third- and fourth-generation cephalosporins was approximately 9%. Consistent with reports in previous studies, resistance to carbapenems was rare [18, 33]. Lipworth et al [18] reported that of only 2 carbapenem-resistant isolates, neither had an O serotype identified as among the 10 occurring most frequently in this study (one was O17, which is part of the O77 group; the other, O19). In our study, 42.9% of carbapenem-resistant isolates carried the O25 antigen.

Many O25 strains are clonally related to ST131. O25B ST131 presents a growing challenge for clinicians due to its association with multidrug resistance, its increasing prevalence worldwide, and ability to cause localized outbreaks of IED [1, 12, 31]. Therefore, it was unsurprising that most O25B isolates in this study (93.3%) were ST131. The virulent *E. coli* clones ST95 and ST73, which are also highly associated with BSI [34], were observed among the most prevalent O serotypes in this study as well.

Across the 4 regions included in this study, approximately 1 in 10 isolates were classified as MDR, and >60% of MDR isolates were of serotype O25. This overall proportion of MDR isolates in our study was lower than that reported in Lipworth et al [18], where 44% of isolates (1434 of 3278) collected in Oxfordshire, United Kingdom, were classified as MDR (resistance to ≥3 antibiotic classes). This variability could be due to regional differences in antibiotic use, differences in antibiotics tested, and/or the methods used to ascertain antibiotic susceptibility across studies. Lipworth et al [18] used disc diffusion with British Society for Antimicrobial Chemotherapy breakpoints [35] for samples obtained before and during 2013 and the Becton Dickinson Phoenix platform with European Committee on Antimicrobial Susceptibility Testing breakpoints [25] for samples taken after.

Overall, our findings contribute to a growing body of evidence that antibiotic resistance poses an increasingly urgent threat to successful IED treatment and management. Our

data, showing the distribution and antibiotic resistance profiles of *E. coli* O serotypes across Europe, North and South America, and Asia-Pacific may contribute to the development of effective, targeted multivalent prophylactic vaccines for ExPEC.

Limitations of the current study include its exploratory nature and the relatively small number of isolates collected in some countries from a limited number of sites, complicating data analysis at the country-specific level. In addition, participating centers could have used different criteria for blood culture collection, sample transport, and storage procedures. Only one *E. coli* isolate was collected per patient; therefore, it is possible that the collected strain was not the single causative agent for the BSI. Clinical data on signs and symptoms of IED were not collected, and selection bias cannot be excluded; in some centers, the isolates may have been obtained only from severely ill patients. Moreover, *E. coli* linked to BSIs that were treated in an outpatient setting would not have been included. Differences observed in O25 serotype prevalence might have been influenced by date of collection; for example, isolates from the Netherlands were collected between 2011 and 2012. Because data were collected from multiple countries and regions, sampling bias could have occurred.

Study strengths include the large total sample size with multiregional representation of isolates, limitation of isolates to those from older adults who experience the greatest burden of ExPEC IED and are likely to be the initial target of a prophylactic vaccine, and alignment of results with other publications from individual countries or centers showing the predominance of O25 as well as other IED-associated O serotypes.

In conclusion, the most prevalent O serotypes among ExPEC bacteremia isolates were O25, O2, O6, O1, O75, O15, O8, O16, O4, O18, O77 group, O153, O9, O101/O162, O86, and O13, together comprising 77.6% of all isolates collected. The most prevalent O serotype among all bacteremia and MDR bacteremia isolates was O25, accounting for 22.9% and 63.2% of these isolates, respectively. O serotype prevalence was generally consistent across all regions, except South America, where some differences were observed. One in 10 isolates was resistant to ≥3 classes of antibiotic. A prophylactic vaccine targeting ExPEC, which targets prevalent O serotypes and is effective in older adults, could have a clinically significant impact in preventing IED, including bacteremia and sepsis.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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