ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 2011, p. 3576–3578 Vol. 55, No. 7
0066-4804/11/$12.00 doi:10.1128/AAC.00074-11

Clinical and Molecular Characteristics of Extended-Spectrum-β-Lactamase-Producing *Escherichia coli* Causing Bacteremia in the Rotterdam Area, Netherlands▽

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Received 18 January 2011/Returned for modification 20 March 2011/Accepted 9 April 2011

We investigated the clinical and molecular characteristics of bacteremia caused by extended-spectrum-β-lactamase (ESBL)-producing *Escherichia coli* over a 2-year period (2008 to 2009) in the Rotterdam region (including 1 teaching hospital and 2 community hospitals) of Netherlands. The majority of patients presented with community onset urinary and intra-abdominal infections, with an increase in prevalence during 2009. The majority of *E. coli* isolates produced CTX-M-15, and 4 sequence types (ST38, ST131, ST405, and ST648) predominated. There were significant differences in clinical and molecular characteristics between the 2 community hospitals.

Extended-spectrum β-lactamases (ESBLs) are one of the most common causes of resistance to the oxyimino-cephalosporins. The presence of these enzymes in *Enterobacteriaceae* complicates antibiotic selection, since ESBL-producing bacteria are often multiresistant to various classes of antibiotics, including those that are regularly used for empirical therapy of serious community-associated infections, such as the oxyimino-cephalosporins, aminoglycosides, and fluoroquinolones (8).

Since the late 1990s CTX-M ESBL enzymes have emerged worldwide among *Enterobacteriaceae*, in particular *Escherichia coli*, and have become the most widespread type of ESBL in the world (8). Currently, the most widespread and prevalent type of CTX-M enzyme is CTX-M-15, and *E. coli* strains producing this enzyme often belong to the international uropathogenic sequence type named ST131 (6).

There are no published data available on the clinical and molecular epidemiology of ESBL-producing *E. coli* causing bacteremia in Netherlands, a region that is renowned for its low level of antimicrobial resistance; the national rate resistance to the oxyimino-cephalosporins among *E. coli* isolates from blood was only 3.5% in 2008 but increased to 6.0% in 2009 (Infection Surveillance Information System-Antibiotic Resistance [ISIS-AR] data [www.ISIS-web.nl]) retrieved on 24 March 2011). We investigated the clinical and molecular epidemiology of ESBL-producing *E. coli* isolates causing bacteremia from 2008 to 2009 in two community hospitals and one large university center in the Rotterdam area, Netherlands.

The Erasmus University Medical Center (EMCR), Rotterdam, Netherlands, is a 1,300-bed teaching hospital. It provides general regional and trauma care to the 1.1 million people residing in the city of Rotterdam and adjacent communities. The medical center also provides highly specialized nationwide care, which includes the transplantation of bone marrow and solid organs (i.e., kidney, heart, lung, and liver) and specialized heart surgery and neurosurgery. The Reinier de Graaf Hospital (RGH), Delft, Netherlands, is an 880-bed community teaching hospital that provides general care and highly specialized care to the 300,000 residents of the city of Delft and adjacent communities. The Green Heart Hospital (GHH), Gouda, Netherlands, is a 450-bed community hospital that provides basic regional care to the approximately 200,000 people residing in the community.

ESBL-producing *E. coli* isolates recovered from blood (isolates from repeated sampling of the same patient were not used) between 1 January 2008 and 31 December 2009 were included. A case of ESBL *E. coli* bacteremia was defined as a patient with a systemic inflammatory response (e.g., fever, tachycardia, and leukocytosis) and documented growth of an ESBL-producing isolate in at least one blood culture (10). Hospital-acquired cases were classified as patients that developed infections 48 h after admission to a health care center. Community onset cases were classified as those patients that visited community-based collection sites or nursing homes or those within the first 2 days of admission to an acute care facility. The patients were further classified as having either community-acquired or health care-associated community onset infections (4).

Susceptibility testing was determined with the Vitek 2 instrument (Vitek AMS; bioMérieux Vitek Systems Inc., Hazelwood, MO). Throughout this study, results were interpreted using CLSI criteria for broth dilution (3, 3a). The presence of

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▽ Published ahead of print on 18 April 2011.

TABLE 1. Characteristics of patients with ESBL-producing *E. coli* isolates from blood at EMCR, RGH, and GHH, 2008 to 2009[a]

Characteristic	Value for:		
	EMCR (n = 25)	RGH (n = 7)	GHH (n = 9)
Mean age (yr) (SD)	54 (23)	71 (14)	74 (14)
No. (%) of males	18 (72)	5 (71)	6 (67)
No. (%) with infection type:			
Hospital acquired	14 (56)	1 (14)	3 (33)
Health care associated	9 (36)	5 (71)	5 (56)
Community acquired	2 (8)	1 (14)	1 (11)
No. (%) with clinical presentation of:			
Urosepsis	8 (32)	4 (57)	6 (67)
Pneumonia	2 (8)	1 (14)	1 (11)
Intra-abdominal infection	9 (36)	2 (29)	2 (22)
Other	6 (24)	0 (0)	0 (0)
No. (%) with immunosuppression	6 (24)	0	0
No. (%) taking appropriate antibiotics at presentation	9 (36)	2 (29)	3 (33)

[a] EMCR, Erasmus University Medical Center, Rotterdam, Netherlands; RGH, Reinier de Graaf Hospital, Delft, Netherlands; GHH, Green Heart Hospital, Gouda, Netherlands.

ESBLs was detected in clinical isolates of *E. coli* by using the 2009 CLSI criteria for ESBL screening and confirmation tests. PCR amplification and sequencing of $bla_{\text{CTX-Ms}}$, bla_{OXAs}, bla_{TEMs}, and bla_{SHV} were carried out on the isolates with a GeneAmp 9700 ThermoCycler instrument (Applied Biosystems, Norwalk, CT) using PCR conditions and primers previously described (7).

Genetic relatedness of the ESBL-producing isolates was examined by pulsed-field gel electrophoresis (PFGE) following the extraction of genomic DNA and digestion with XbaI using the standardized *E. coli* (O157:H7) protocol established by the Centers for Disease Control and Prevention, Atlanta, GA (5). Cluster designation was based on isolates showing approximately 80% or greater relatedness, which corresponds to the "possibly related (4- to 6-band difference)" criteria of Tenover et al. (11). All the ESBL-producing isolates were screened for ST131 using a PCR for the *pabB* allele, recently described by Clermont and colleagues (2). Multilocus sequence typing (MLST) was performed using seven conserved housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*) and the MLST databases at the Environmental Research Institute (ERI), University College Cork, website http://mlst.ucc.ie/mlst/dbs/Ecoli. The ESBL-positive isolates were assigned to one of the four main *E. coli* phylogenetic groups (A, B1, B2, and D) by the use of a multiplex PCR-based method (1).

The χ^2 tests with Fisher's exact test in the case of small numbers were used to compare regional differences and differences over time (2008 versus 2009) for categorical data by using the SPSS statistics (version 17) program (IBM Corporation, NY).

During 2008 to 2009, 25 (5%) of 455 *E. coli* isolates from EMCR, 7 (4%) of 193 isolates from RGH, and 9 (5%) of 189 isolates from GHH were positive for ESBL production. A total of 41 residents from the Rotterdam area with incident bloodstream infections due to ESBL-producing *E. coli* isolates were identified; 44% of infections were classified as hospital acquired, 46% as health care-associated community onset, and 10% as community acquired. The characteristics of the patients and differences between the hospitals are shown in Table 1. Patients from EMCR presented less often with urosepsis than patients from the two Dutch community hospitals in Delft and Gouda (32%, versus 57% and 67%, respectively), and infections diagnosed at EMCR were more frequently hospital acquired than infections diagnosed at the two community hospitals (56% versus 14% and 33%, respectively).

Overall, 37 (90%) of the isolates were not susceptible to amoxicillin-clavulanic acid, 25 (61%) were not susceptible to piperacillin-tazobactam, 26 (63%) were not susceptible to ciprofloxacin, 37 (90%) were not susceptible to trimethoprim-sulfamethoxazole (SXT), 13 (32%) were not susceptible to gentamicin, 21 (51%) were not susceptible to tobramycin, and 10 (24%) were not susceptible to amikacin but all remained susceptible to imipenem, meropenem, or ertapenem using the new 2010 breakpoints (3a). Four isolates (all producing CTX-M-14) would have been reported as susceptible to ceftazidime using the 2010 breakpoints (3a).

The characteristics (including phylogenetic groups and sequence types) of the different types of ESBL-producing *E. coli* are shown in Table 2. CTX-M-15 was the most widely distributed ESBL and predominated in all the hospitals. A significant increase in the number of ESBL producers was found when comparing 2008 to 2009 at both Dutch community hospitals (Table 3).

PFGE identified 5 closely related groups of *E. coli* isolates (n = 20) that were designated clones A (n = 12), B (n = 2), C

TABLE 2. Characteristics of *Escherichia coli* isolates and ESBL producers from blood at EMCR, RGH, and GHH, 2008 to 2009

Hospital	Total no. of *E. coli* isolates	No. (%) of ESBL-producing *E. coli* isolates	ESBLs (no. of isolates)	Phylogenetic groups (no. of isolates)	MLST(s), no. (%)
EMCR	455	25 (5)	CTX-M-15 (13), CTX-M-14 (3), CTX-M-2 (2), CTX-M-3 (2), CTX-M-9 (1), CTX-M-27 (1), SHV-5 (1), SHV-12 (2)	A (3), B1 (1), B2 (10), D (11)	ST131, 8 (32); ST38, 1 (4); ST405, 2 (8); ST648, 2 (8)
RGH	193	7 (4)	CTX-M-15 (4), CTX-M-14 (2), TEM-52 (1)	A (3), B1 (1), B2 (1), D (2)	ST131, 1 (14); ST405, 1 (14)
GHH	189	9 (5)	CTX-M-15 (5), CTX-M-14 (1), CTX-M-1 (1), SHV-5 (1), SHV-12 (1)	A (1), B2 (7), D (1)	ST131, 5 (56)

TABLE 3. Prevalence of ESBL-producing *Escherichia coli* isolates from blood at EMCR, RGH, and GHH, 2008 to 2009

Hospital	2008		2009	
	No. of *E. coli* isolates	No. (%) of ESBL producers	No. of *E. coli* isolates	No. (%) of ESBL producers
EMCR	211	9 (4.2)	244	16 (6.6)
RGH	60	1 (1.6)	133	6 (4.5)
GHH	100	3 (3)	89	6 (6.7)

($n = 2$), D ($n = 1$), and E ($n = 3$). Isolates that belonged to clones A and B exhibited >60% but <80% similarity of PFGE profiles. The remaining ESBL-producing isolates ($n = 21$) were not clonally related. All five clones were present at EMCR, Rotterdam, two clones were present at RGH, Delft, and one clone was present at GHH, Gouda.

MLST identified PFGE clones A and B as ST131, C as ST648, D as ST38, and E as ST405; overall 20/41 (49%) of the isolates belonged to one of these sequence types (Table 2). ST131 belonged to phylogroup B2, while ST38, ST405, and ST648 belonged to phylogroup D. ST131 and ST648 produced CTX-M-15; ST38 produced CTX-M-9, while ST405 produced CTX-M-14 and -15. ST131 predominated in GHH: ST131 isolates were responsible for 4 of 6 cases (67%) of community onset urosepsis. Only 1 of 8 patients [13%] from EMCR and 1 of 4 patients [25%] from RGH had urosepsis caused by ST131 isolates. At EMCR, ST131 isolates were more frequently associated with hospital-acquired intra-abdominal infections.

To our knowledge, this is the first study that identified the clinical characteristics and molecular epidemiology of bloodstream infections due to ESBL-producing *E. coli* in Netherlands. Our results showed that ESBL-producing *E. coli* contributed to a substantial and rising proportion of all bloodstream infections from 2008 to 2009 due to *E. coli* in the three Dutch hospitals. As expected, CTX-M-15 was the predominant type of ESBL identified at all locations. However, there were some differences in the clinical presentation between the different hospitals: patients from EMCR presented more often with hospital-acquired intra-abdominal infections than patients from RGH and GHH, who most often presented with community onset urosepsis. This probably reflects the difference in clinical presentations between community- and hospital-acquired infections due to ESBL-producing *E. coli*.

Additionally, although numbers were small, we identified some interesting and significant differences in molecular epidemiology between the two Dutch community hospitals, which are located merely 20 km apart. Most of the isolates isolated at Gouda belonged to the highly virulent phylogenetic group B2 and sequence type ST131 (5/9), while only one of the seven isolates from Delft belonged to ST131. Most of the remaining isolates from Delft belonged to less-pathogenic phylogenetic group A or B1. One of the major risk factors previously described for community-acquired infections due to CTX-M-producing *E. coli* is exposure to the fluoroquinolones (9). At the RGH, Delft, the empirical use of fluoroquinolones for community-associated infections had been restricted since 1993, while the use of fluoroquinolones at the GHH, Gouda, has been restricted only since 2008. Therefore, it is possible that the longer period of unrestricted use of the fluoroquinolones at GHH has played a role in the difference in prevalence of ST131 between these two Dutch community hospitals. It is imperative that future studies be undertaken to investigate the reasons for variations in prevalence of ST131 between the community hospitals. Gaining insight into the local differences at the community level will be important to understand the dynamics of transmission, risk factors, and reservoirs for clonally related CTX-M-producing *E. coli* in community settings.

This work was supported by research grants from the Calgary Laboratory Services RT 73-6350. Gisele Peirano was supported by a postdoctoral research fellowship from the Foreign Affairs and International Trade Canada (DFAIT).

We thank Marco Mol and Nicole Lemmens for their technical support of this study and Ellen Oord en Hennie Dekker for providing the Delft and Gouda isolates.

J.D.D.P. has previously received research funds from Merck and Astra Zeneca. We have no other funding issues to declare.

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