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Virulence Factors of *Escherichia coli* Isolates That Produce CTX-M-Type Extended-Spectrum β-Lactamases

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This study determined the phylogenetic groups and virulence factors of 37 *Escherichia coli* isolates producing types of CTX-M compared with those of 19 isolates producing different types of extended-spectrum β-lactamases (ESBLs) in a well-defined North American population. Most CTX-M-14 producers (97%) were from phylogenic group D; 67% of the CTX-M-15 producers were from group B2. A single CTX-M-14-producing strain belonged to clonal group A. There were significant prevalence differences for individual virulence factors among CTX-M producers and nonproducers; however, aggregate virulence factor scores were similar. CTX-M producers more commonly caused repeat urinary tract infections. Our results indicate that CTX-M type predicts phylogenetic background, and the virulence potential of ESBL-producing *E. coli* isolates is a complex issue, requiring further study and ongoing surveillance.

Enterobacteriaceae, especially *Klebsiella* spp. producing extended-spectrum β-lactamases (ESBLs), such as SHV and TEM types, have been recognized since the 1980s as a major cause of hospital-acquired infections (3). However, during the late 1990s and 2000s, *Enterobacteriaceae* (mostly *Escherichia coli*) producing novel ESBLs, the CTX-M enzymes, have been identified, from both the hospital and community settings (26). Surveys from several countries, including Canada (25), Spain (27), and the United Kingdom (31) show that CTX-M-producing *E. coli* strains isolated from community sites, exhibited coresistance to trimethoprim-sulfamethoxazole, tetracycline, gentamicin, and ciprofloxacin, further limiting therapeutic options.

Phylogenetic analyses have shown that *E. coli* strains fall into four main phylogenetic groups (A, B1, B2, and D) (10). Virulent extraintestinal strains derive mainly from group B2 and, to a lesser extent, group D, whereas most commensal strains derive from groups A and B1 (11). Most virulence factors are concentrated predominantly either within group B2 or jointly within groups B2 and D, although certain factors are concentrated significantly in group D but not in group B2, while others are distributed across the population without a significant concentration in either group B2 or group D. These diverse patterns suggest more complex evolutionary histories for the various virulence factors present in *E. coli*. Fluoroquinolone-resistant *E. coli* isolates causing urinary tract infections (UTIs) most often belong to the low-virulence phylogenetic groups A and B1 (12) or group D (14). Such resistant strains typically exhibit a decreased prevalence of certain virulence factors and a seemingly reduced invasive capacity (30). We studied a well-defined population from North America to determine the virulence-associated traits of *E. coli* isolates from community and hospital source specimens. Specifically, isolates producing types of CTX-M β-lactamases were compared, according to phylogenetic group and virulence factor profile, with *E. coli* isolates producing different types of well-characterized ESBLs.

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MATERIALS AND METHODS

Patient population and definitions. The Calgary Health Region provides all publicly funded healthcare services to the more than 1 million people residing in the cities of Calgary and Airdrie and numerous adjacent surrounding communities covering an area of 37,000 km^2 (6). Acute care is provided principally through one pediatric hospital and three large adult hospitals. A centralized laboratory (Calgary Laboratory Services) performs the routine clinical microbiology services for both the community clinics and hospitals within the Calgary Health Region. Community onset infections were diagnosed in individuals who did not have a hospitalization in the preceding three months and were either outpatients or admitted patients whose first positive cultures were obtained within 48 h of hospital admission. Other hospitalized patients and patients from nursing homes were deemed to have nosocomial infections. A case of *E. coli* UTI was defined as a patient with a clinical suspicion of UTI (as indicated on the requisition), whose clean-catch urine yielded $>10^5$ CFU per ml of urine. Repeat infection was defined by ≥3 episodes of culture-documented UTI (as defined above) during the 3-year study period.

Bacterial strains. Fifty-six *E. coli* isolates collected from various community (n = 37 [66%]) and hospital sites (n = 19 [34%]) within the Calgary Health Region during 2000–2002, producing the following ESBLs, were included in the study: CTX-M-14 (n = 27), CTX-M-15 (n = 9), CTX-M-3 (n = 1), TEM types (n = 8), SHV types (n = 10), and VEB-1 (n = 1). These isolates were randomly selected from a larger collection of all ESBL-producing *E. coli* strains isolated during the study period (25). The great majority of strains (45/56 [80%]) were isolated from urine samples and were previously characterized by susceptibility testing, identification of β-lactamases, and molecular typing (pulsed-field gel electrophoresis [PFGE]) (Table 1) (24). DNA relatedness was calculated based on the Dice coefficient. Isolates were considered to be genetically related if the Dice coefficient correlation was 80% or greater, which corresponds to the "possibly related" (four- to six-band difference) criterion of Tenover et al. (29).

Phylogenetic analysis and virulence genotyping. All isolates were assigned to one of the four main *E. coli* phylogenetic groups (A, B1, B2, and D) by the use

TABLE 1. Characteristics and phylogenetic background of 56 extended-spectrum beta-lactamase-producing *Escherichia coli* isolates from community and hospital sites in the Calgary Health Region

Type of ESBL produced by organism (no. of organisms)	PFGE pattern[a]	No. of isolates (% of total)[b]						
		Isolated from urine	Repeat infections	Resistant to ciprofloxacin	Phylogenetic group			
					A	D	B1	B2
CTX-M-14 (11)	CTXM14A	11 (100)	4 (36)	10 (91)	0	11 (100)	0	0
CTX-M-14 (8)	CTXM14AR	5 (63)	4 (50)	8 (100)	0	8 (100)	0	0
CTX-M-14 (8)	CTXM14NR	5 (63)	1 (12)	6 (75)	0	7 (88)	0	1 (12)
CTX-M-15 (9)	NRB	6 (67)	2 (22)	9 (100)	2 (22)	1 (11)	0	6 (67)
CTX-M-3 (1)	NRC	1	1	0	1	0	0	0
TEM (8)	NRD	7 (88)	1 (12)	3 (38)	2 (25)	2 (25)	1 (12)	3 (38)
SHV (10)	NRE	8 (80)	0	2 (20)	1 (10)	6 (60)	1 (10)	2 (20)
VEB-1 (1)	NRF	1	0	1	1	0	0	0

[a] The CTXM14A isolates formed a cluster with >80% similar PFGE profiles. The CTXM14AR isolates exhibited >60% similarity of profiles to CTXM14A and each other, while the CTXM14NR isolates were more distantly related to these two groups. The remaining strains (PFGE groups NRB, NRC, NRD, NRE, and NRF) were not clonally related, i.e., exhibited <80% similar PFGE profiles.
[b] Percentage not shown for groups containing a single isolate.

of a modified version of the multiplex PCR-based method of Clermont et al. (7). Published primers for *chuA* and *yjaA* that amplified DNA fragments of 279 bp and 211 bp, respectively, were used. In addition, primers newly designed for enhanced identification of TSPE4.C2 were TspE4II′ F 5′-AGTAATGTC GGGGCATTCAG-3′ and TspE4II′ R 5′-TCGCGCCAACAAAGTATTACG-3′; these amplified a 151-bp fragment. All isolates were also screened for the five key virulence markers of extraintestinal pathogenic *E. coli* (ExPEC) isolates, including *papA* and *papC* (P fimbriae structural subunit and assembly; these were analyzed collectively), *sfa*/*foc* (S and F1C fimbriae), *afa*/*dra* (Dr-binding adhesins), *iutA* (aerobactin receptor), and *kpsM* II (group 2 capsules). The presence of ≥2 of these defined an isolate as ExPEC. Extended virulence profiles for an additional 34 ExPEC-associated genes were determined by established PCR-based assays (16–18, 28). A specific PCR assay for *E. coli* "clonal group A" was performed on phylogenetic group D isolates (15).

Statistical methods. Fisher's exact test was used to compare group categorical data using Stata 8.0 (Stata Corp., College Station, TX). Comparisons involving virulence factor scores were assessed using the Mann-Whitney *U* test. The threshold for statistical significance was a *P* value of <0.05.

RESULTS AND DISCUSSION

The CTX-M-14 producers were responsible for a community-wide clonal outbreak of UTIs in the Calgary Health Region catchment's area during 2000 and 2001 (24). PFGE identified two closely related groups of *E. coli* isolates producing CTX-M-14 (designated CTXM14A [$n = 11$] and CTXM14AR [$n = 8$] [i.e., related to A]), plus a well-removed third group, designated CTXM14NR ($n = 8$) (i.e., not related to A). The CTXM14A isolates formed a cluster with >80% similar PFGE profiles. The CTXM14AR isolates exhibited >60% similarity of profiles to CTXM14A and each other, which indicates that CTXM14AR is related to CTXM14A, although the significance is doubtful. The CTXM14NR isolates were more distantly related to these two groups (Table 1) (24). The remaining strains (PFGE groups NRB, NRC, NRD, NRE, and NRF) were not clonally related, i.e., exhibited <80% similar PFGE profiles (Table 1). The NRA, NRB, NRC, NRD, NRE, and NRF groups were heterogeneous and not related to each other or CTX-M-14 clusters. The PFGE groups are different from the phylogenetic groups, which are based on the multiplex PCR. A greater proportion of CTX-M-positive isolates (CTX-M-14, CTX-M-15, and CTX-M-3) than CTX-M-negative ESBL isolates (33/37 [89%] versus 6/19 [32%], respectively [$P < 0.0001$]) was resistant to ciprofloxacin.

Of the total 56 ESBL-producing *E. coli* isolates, 35 (63%) were derived from phylogenetic group D, 12 (21%) from group B2, 2 (4%), from group B1, and 7 (13%) from group A (Table 1). With the exception of one isolate from B2, all of the CTX-M-14 producers (26/27 [96%]) were from group D. Thus, CTX-M-14 producers were more likely to belong to group D than other ESBL-producers, including those producing non-14 CTX-M variants (26/27 [96%] versus 9/29 [31%] [$P < 0.0001$]). In contrast, of the nine CTX-M-15 producers, six were from group B2, two were from group A, and only one was from group D (1/9 [11%] CTX-M-15 producers from group D versus 26/27 [96%] CTX-M-14 producers [$P < 0.0001$]). Group B2 was significantly more common among CTX-M-15 producers than among other ESBL-producing organisms (6/9 [66.7%] versus 6/47 [13%] [$P < 0.0001$]) (Table 1). The single CTX-M-3 producer was from group A. As for the CTX-M-negative strains, the predominant phylogenetic groups, by ESBL type, were as follows: for TEM producers, group B2 (38%); for SHV producers, group D (60%); and for the single VEB-1 strain, group A (Table 1). A single CTX-M-14-producing strain (Cal3) from group D exhibited the specific clonal group A *fumC* polymorphism.

Several virulence factors differed in prevalence among the organisms that produced CTX-M enzymes compared to producers of TEM, SHV, and VEB-1 ESBLs (Table 2). CTX-M producers were more commonly positive for *afa*/*dra* (Dr-binding adhesins), *iha* (putative adhesin-siderophore receptor), *sat* (secreted autotransporter toxin), and *kpsM* II. In contrast, non-CTX-M producers significantly more often exhibited *ireA* (iron-regulated element) and *cvaC* (colicin [microcin] V). On balance, aggregate virulence factor scores were similar among CTX-M producers and nonproducers, with median scores of 8.5 and 8.7, respectively ($P = 0.10$). Thirty-three of 37 (89%) CTX-M producers and 9/19 (47%) nonproducers were ExPEC isolates ($P < 0.0001$). Isolate Cal3 exhibited the stereotypical virulence factor profile observed among clonal group A isolates from around the world (21). We also compared isolates originating from the community to those from nosocomial sites but found no statistical differences among phylogenetic groups and virulence factors.

The CTX-M producers were more likely to have caused repeat UTIs (12/37; 23%) than were strains not producing

TABLE 2. Virulence factors exhibiting significant prevalence differences according to extended-spectrum beta-lactamase type among 56 ESBL-producing *Escherichia coli* isolates from the Calgary Health Region

Factor[a]	Prevalence of factor (% of total)			*P* value
	CTX-M-14[b] (*n* = 27)	CTX-M-15[b] (*n* = 9)	CTX-M negative (*n* = 19)	
afa/*dra*	18 (67)	0	4 (21)	<0.001
cvaC	0	0	5 (26)	0.005
iha	26 (96)	6 (67)	6 (32)	<0.001
ireA	0	0	4 (21)	0.02
iutA	27 (100)	6 (67)	17 (89)	0.009
kpsM II	27 (100)	7 (78)	7 (37)	<0.001
malX	2 (7)	6 (67)	6 (32)	0.001
ompT	4 (15)	6 (67)	8 (42)	0.007
sat	27 (100)	7 (78)	4 (21)	<0.001
traT	26 (96)	5 (56)	13 (68)	0.004
usp	0	6 (67)	6 (32)	<0.001

[a] *afa*, Dr-binding adhesins; *cvaC*, microcin ColV; *iha*, adhesin-siderophore receptor; *ire*, iron-regulated element; *iutA*, aerobactin receptor; *kpsM* II, group 2 capsular polysaccharide synthesis; *malX*, pathogenicity island marker; *ompT*, outer membrane protein; *sat*, secreted autotransporter toxin; *traT*, surface exclusion serum survival associated; *usp*, uropathogenic-specific protein.

[b] The strain producing CTX-M-3 was not included in the analysis.

CTX-M enzymes (1/19; 5%) ($P = 0.04$). The only virulence factor significantly associated with an increased risk for repeat UTIs was *iha* (12/13 [92%] were positive for *iha* compared to 26/43 [60%] causing nonrepeat UTIs [$P = 0.04$]).

A recent study of hospital-acquired *E. coli* isolates producing various types of ESBLs from different parts of France showed that whereas the preponderance of the SHV- and TEM-producing strains (approximately 60% and 37%, respectively) were from group B2, the greatest proportion of CTX-M producers (40%) was from group D (4). In that study, isolates from group B2 exhibited numerous virulence factors but were usually susceptible to fluoroquinolones, while the group D strains lacked virulence factors but were resistant to fluoroquinolones (4). Notably, the CTX-M-producing strains in that study did not include CTX-M-15 producers. Our results support some of these findings, in that we found phylogenetic group D to predominate overall among ESBL-producing *E. coli* isolates from the Calgary Health Region, especially those producing CTX-M-14, the most common CTX-M variant (Table 1). Likewise, the majority (24/27 [89%]) of CTX-M-14 producers were resistant to ciprofloxacin (Table 1). The fact that the majority of our CTX-M-14 producers were clonally related could in part explain the high prevalence of phylogenetic group D among the strains producing CTX-M-14. However, our findings differ from those of the French study in that a substantial fraction of our CTX-M producers were from group B2; these were almost all CTX-M-15 producers.

Clonal group A is a multidrug-resistant clonal group of *E coli* that was responsible for community outbreaks of UTIs in several areas of the United States during the late 1990s and early 2000 (21). The Calgary strain (Cal3) belonging to clonal group A was isolated from the urine of a 5-year-old girl that presented to the emergency department during 2000 with the diagnosis of pyelonephritis. This isolate was resistant to ampicillin, chloramphenicol, tetracycline, and trimethoprim-sulfamethoxazole but was susceptible to the aminoglycosides and fluoroquinolones, as is typical for clonal group A (21). To our knowledge, this is the first report of an ESBL-producing *E. coli* isolate belonging to clonal group A and the first evidence of clonal group A in Canada.

There has been a recent dramatic increase in reports describing bacteria producing CTX-M-15 from different parts of the world, including North America (2), Europe (5, 8, 22), Asia (19), the Middle East (23), and Africa (1, 9) Therefore, results obtained with strains producing CTX-M-15 were of interest (Table 1). This is similar to the findings of Leflon-Guibout et al., who investigated clonally related *E. coli* isolates producing CTX-M-15 which caused outbreaks of UTIs in a geriatric hospital in France (20). Our results and the studies from France are in contrast to previous publications indicating that fluoroquinolone-resistant *E. coli* isolates causing UTI most often derive from low-virulence phylogenetic groups A and B1 (13). The appearance of fluoroquinolone resistance and ESBL production among group B2 isolates with multiple virulence factors is a concerning development that deserves close attention. This might also in part explain the worldwide appearance of CTX-M-15-producing organisms.

Notable limitations of this study include small numbers and limited clinical and epidemiological data. Accordingly, we could not explore relationships among patients, such as family contacts to assess the possibility of a common source or transmission between individuals within the community. To address these deficits, we have initiated a large prospective study at our center to more extensively explore the role of several risk factors and to increase the sample size within the different subgroups.

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