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CTX-M β-Lactamase-Producing *Escherichia coli* in French Hospitals: Prevalence, Molecular Epidemiology, and Risk Factors[▽]

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In 2004, 65 CTX-M-producing *Escherichia coli* isolates were collected from infected patients in four French hospitals. The $bla_{\text{CTX-M-15}}$ genes were predominant. Pulsed-field gel electrophoresis highlighted a clonal propagation of CTX-M-15-producing strains belonging to phylogenetic group B2, notably in the community. The main risk factors for acquiring these isolates were urinary tract infections or the presence of a urinary catheter in diabetic or renal failure patients.

Recently, CTX-M β-lactamases produced by gram-negative bacteria have been increasingly reported worldwide (6, 9, 13, 18, 23, 34, 37–39, 46, 48), notably in the community (6, 24, 36, 41, 49). According to the National Observatory of Bacterial Resistance to Antibiotics website (http://www.onerba.org), among extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* strains, ESBL-producing *Escherichia coli* increased in prevalence in France from 9.5% in 2001 to 28.1% in 2004. Localized outbreaks of CTX-M-producing *E. coli* have been reported in the north of France (16, 29). However, data on the prevalence of risk factors for and distribution of different CTX-M-type β-lactamases are currently scarce (2, 14, 26). Based on these observations, we conducted a prospective study of CTX-M-producing *E. coli* in two regions of France over a 1-year period.

(This work was presented in part at the 25th Réunion Interdisciplinaire de Chimiothérapie Anti-Infectieuse [RICAI], Paris, France, December 2005 [28]).

To investigate the prevalence of CTX-M β-lactamases and the risk factors associated with CTX-M-producing *E. coli*, a prospective surveillance program was initiated on 1 January 2004 and carried out until 31 December 2004 in three university hospitals (in Clermont-Ferrand, Montpellier, and Nîmes) and one community hospital (in Perpignan) in the south and center of France. All patients in whom ESBL-producing *E. coli* was detected were included. For patients with recurrent infections, only strains from the first episodes were included. The following clinical data were collected prospectively: demographic data; type of clinical ward; diagnosis at admission; isolation site of bacteria; presence of mono- or polymicrobial infection; clinical outcome; underlying diseases and their severity according to the MacCabe score and the Charlson index (10); hospitalization or surgical treatment in the last 12 months; transfer from another hospital, intensive care unit (ICU), or nursing home; antimicrobial treatment in the previous month; nosocomial or community-acquired infection or colonization; and exposure during the present stay before the isolation of bacteria from urinary catheters. Patients were deemed to have community-acquired disease if the first culture found positive for ESBL-producing *E. coli* was obtained within 48 h of admission. In this category, we distinguished between patients who had frequent association with the health care system and patients who had never been hospitalized.

The genus and species were determined biochemically with the Vitek 2-ID-GNB identification card (bioMérieux, Marcy-l'Etoile, France). Susceptibility to antimicrobial agents was tested by using the disk diffusion assay on Mueller-Hinton agar. ESBL production was screened with the double-disk synergy test (19). Strains were classified as susceptible, intermediately resistant, or resistant to the antibiotics tested according to the recommendations of the Antibiotic Susceptibility Testing Committee of the French Society for Microbiology (47). Isoelectric focusing was performed with polyacrylamide gels as previously described (14). The genes bla_{TEM}, bla_{SHV}, and $bla_{\text{CTX-M}}$ were detected by PCR using specific primers as previously reported (5, 14, 15, 33) and further identified by sequencing the PCR products. A macrorestriction analysis of chromosomal DNA was performed according to previously published procedures and analyzed with GelCompar computer software (Applied Math, Kortrijk, Belgium) as previously described (26). Phylogenetic grouping of CTX-M-producing *E. coli* isolates was determined by a PCR-based method developed by Clermont et al. (11). Continuous variables were compared by using Fisher's exact test. Qualitative variables were compared by the chi-square test; odds ratios and 95% confidence intervals were calculated. A P value of ≤ 0.05 was con-

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TABLE 1. Distribution of ESBL-producing *Escherichia coli* strains described in four French hospitals

Hospital (no. of beds)	No. of strains (n = 112)	Prevalence (%) of strains producing:		CTX-M types produced (no. of strains [n = 65])	Other ESBL types produced (no. of strains [n = 47])
		ESBLs[a]	CTX-M[b]		
Montpellier (2,923)	71	2.40	1.39	CTX-M-15 (24), CTX-M-14 (8), CTX-M-1 (7), CTX-M-2 (1), CTX-M-27 (1)	TEM-24 (15), TEM-3 (4), TEM-15 (1), TEM-19 (1), TEM-21 (1), TEM-106 (1), SHV-5 (5) SHV-2 (1), SHV-4 (1)
Nîmes (1,700)	19	0.81	0.34	CTX-M-15 (5), CTX-M-1 (2), CTX-M-14 (1)	TEM-24 (6), TEM-3 (2), TEM-19 (1), TEM-52 (1), TEM-129 (1)
Clermont-Ferrand (2,068)	12	0.35	0.29	CTX-M-15 (5), CTX-M-1 (3), CTX-M-14 (2)	TEM-24 (2)
Perpignan (922)	10	1.13	0.68	CTX-M-15 (3), CTX-M-14 (2), CTX-M-1 (1)	TEM-24 (3), SHV-2 (1)

[a] The number of ESBL-producing *E. coli* strains divided by the number of total *E. coli* strains.
[b] The number of CTX-M-producing *E. coli* strains divided by the number of total *E. coli* isolates.

sidered to reflect statistical significance. Logistic regression was performed to determine the variables and interactions that were significantly associated with the risk of infection with CTX-M-producing *E. coli*. Variables were selected in a stepwise backward process (30). All statistical tests were performed using JMP software (version 3.2.2; SAS Institute, Inc.).

During the study period, 112 ESBL-producing *E. coli* strains were isolated from 111 patients. The prevalence of ESBL production among the *E. coli* isolates was <3% (Table 1). The prevalence of CTX-M-producing *E. coli* among the total number of *E. coli* isolates was 0.68%, and the occurrence of CTX-M-producing *E. coli* among ESBL-producing *E. coli* isolates was 58%. The distribution of the different types of ESBLs recovered during this study according to the geographical origin of the corresponding strains is shown in Table 1. CTX-M-15 was the most prevalent CTX-M type produced in our study (Table 1). Among CTX-M-producing strains, two isolates (strains MECA13 and PEC2) were resistant to cefoxitin (MIC between 64 and 128 μg/ml), and four strains (MECT, MECB5072, CF1110, and CF1229) were intermediate to cefoxitin (MIC between 8 and 32 μg/ml). All isolates were susceptible to imipenem. Resistance was observed with tobramycin (63.1%), gentamicin (52.3%), amikacin (27.7%), co-trimoxazole (72.3%), and tetracyclines (66.2%). Of the CTX-M-producing *E. coli* isolates, 73.8% were resistant to quinolones (nalidixic acid) versus 51.1% of the TEM- and SHV-producing *E. coli* isolates ($P < 0.01$). Similarly, 66.2% of CTX-M-producing isolates were resistant to ciprofloxacin versus 42.6% of the TEM- and SHV-producing isolates ($P < 0.01$).

Pulsed-field gel electrophoresis revealed a high level of genomic diversity for both TEM-type and SHV-type ESBL-producing *E. coli* isolates. No identical pulsotypes were observed for CTX-M-producing isolates except for those producing CTX-M-15. Indeed, pulsed-field gel electrophoresis revealed three different groups of closely related restriction patterns among these CTX-M-15-producing isolates. The most important group was found in Montpellier Hospital (cluster C_I, 19 isolates). Two others were found in Nîmes Hospital (cluster C_{II}, four strains) and in Perpignan Hospital (cluster C_{III}, two strains) (Fig. 1). Twenty-two strains belonging to the three clusters were isolated from urinary samples (C_I, 16/19 strains; C_{II}, 4/4 strains; C_{III}, 2/2 strains). Twelve strains had a community origin (C_I, 11/19 strains; C_{II}, 0/4 strains; C_{III}, 1/2 strains). Five patients infected with these strains were associated with the health care system (C_I, 4/19 patients; C_{II}, 0/4 patients; C_{III}, 1/2 patients), and seven patients had never been hospitalized (C_I, 7/19 patients; C_{II}, 0/4 patients; C_{III}, 0/2 patients). No clustering of patients could be demonstrated. The clonal isolates harbored similar ESBL-encoding plasmids, and these plasmids yielded similar restriction patterns after digestion with HindIII (27).

The majority of *E. coli* strains were isolated from urinary tract specimens (64 strains, 57.1%), most notably CTX-M-producing isolates (44 strains, 67.7%). Among these isolates analyzed, phylogenetic group B2, which is the source of most uropathogenic *E. coli* clones (12), included 45.5% of the strains (2.3% were subgroup $B2_2$, and 43.2% were subgroup $B2_3$). Phylogenetic group D, which is also but to a lesser extent a source of uropathogenic *E. coli*, included 36.4% of the strains (31.9% were subgroup D_1, and 4.5% were subgroup D_2). Phylogenetic groups A and B1 represented 13.6% (4.5% were subgroup A_0, and 9.1% were subgroup A_1) and 4.5% of the strains, respectively. The strains comprising Montpellier cluster C_I and Nîmes cluster C_{II} belonged to the B2 group. The two isolates of Perpignan cluster C_{III} belonged to the D1 group.

A univariate analysis comparing patients with CTX-M-producing *E. coli* isolates and patients with *E. coli* isolates producing other ESBLs is shown in Table 2. CTX-M-producing *E. coli* isolates were involved mainly in infections (colonization/infection rate, 0.23), especially with urinary tract infections (UTIs) (67.8%). Of the patients with UTIs, 43.8% had received antibiotic therapy in the last month. Interestingly, 22 of 65 CTX-M-producing bacteria had community origins ($P < 0.01$). The multivariate analysis selected diabetes mellitus, renal disease, UTI, gynecological surgery, and the presence of a urinary catheter as independent factors associated with an increased risk of isolation of CTX-M-producing *E. coli* ($P < 0.01$) (Table 3). Moreover, a high proportion of these infections had a community origin ($P < 0.01$). When only patients infected with a CTX-M-producing clone were considered, the risk factors identified by multivariate analysis were cardiovas-

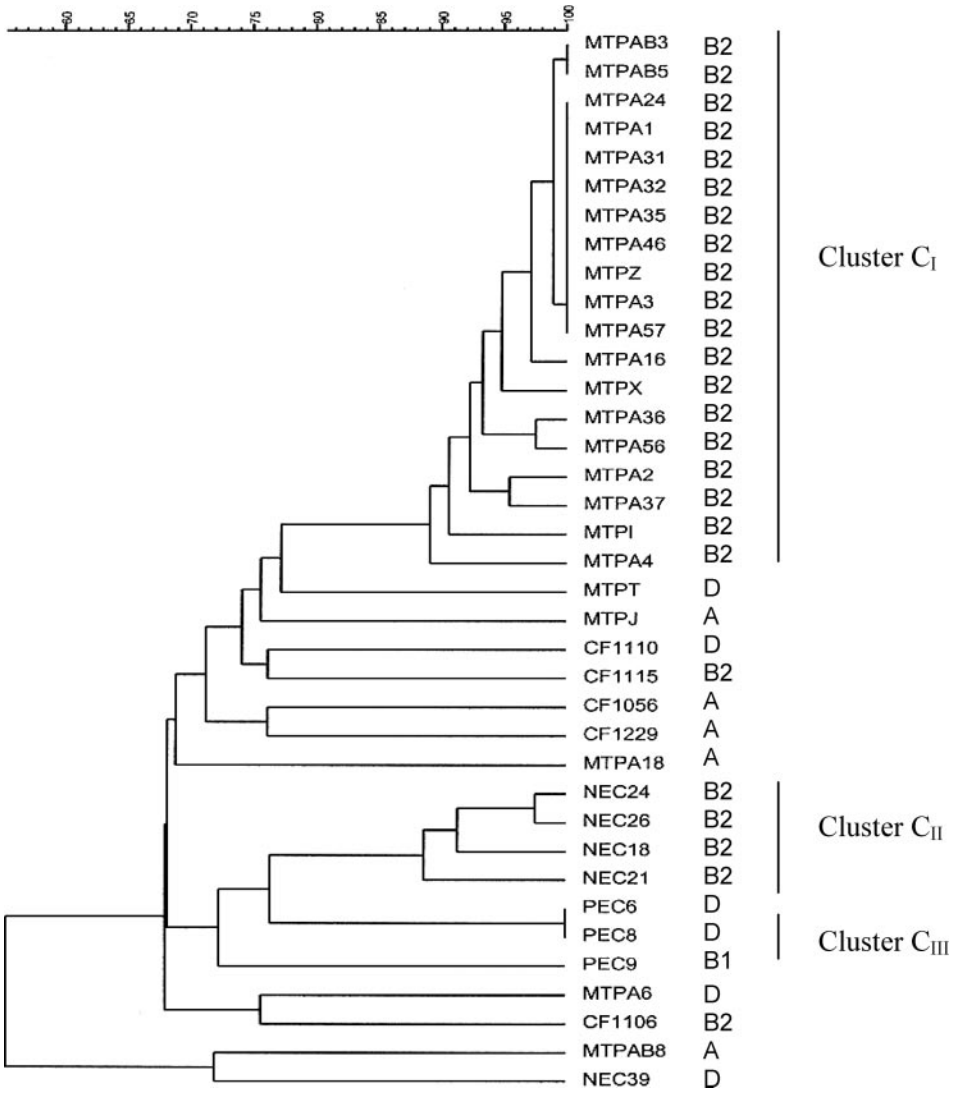

FIG. 1. Dendrogram of XbaI-digested genomic DNAs and phylogenetic groups of all CTX-M-15-producing *E. coli* isolates from four hospitals in France. Strains were clustered with the unweighted-pair group method using average linkages. The scale indicates the percentage of genetic similarity.

cular disease, urinary incontinence, the presence of a urinary catheter, and polymicrobial infection ($P < 0.01$) (Table 3).

This report documents the recent increase in ESBL-producing *E. coli* in France (to a prevalence of 28.1%, as reported by the National Observatory of Bacterial Resistance to Antibiotics) and, for the first time, the important role of CTX-M-producing strains in this evolution. However, we noted a geographic imbalance in the rates of CTX-M producers (Table 1), probably due to the heterogenous populations among the different hospitals. These CTX-M-producing strains have emerged and spread in most parts of the world (6, 9, 13, 18, 23, 34, 37–39, 46, 48). During this period, the proportion of CTX-M strains among ESBL-producing *E. coli* isolates has dramatically increased from 38.2% to 87% (8, 38, 41, 43, 44, 46). The CTX-M-15 enzyme seems to be the most common, as previously described (3, 8, 17, 22, 25, 26, 29, 31, 34, 38). Length of hospital stay, severity of illness, time in the intensive care unit, intubation and mechanical ventilation, urinary or arterial catheterization, and previous exposure to antibiotics have been described as the main risk factors associated with acquiring ESBL-producing strains (4). In this study, other risk factors were associated with CTX-M-producing *E. coli* infection, like renal disease, diabetes mellitus, and surgery of the genitourinary tract. Usually, infections caused by ESBL-producing *E. coli* have a nosocomial origin. We observed that, in comparison with strains producing other ESBLs (6.4%) as noted previously (2, 40, 41, 43, 45, 49), 33.8% of the CTX-M-producing strains were isolated in outpatients. Among the 22 outpatients infected by CTX-M-producing *E. coli*, 5 had been hospitalized during the last year, one of the main risk factors for CTX-M-β-lactamase acquisition in the outpatients (45). This study revealed a highly diverse population structure of ESBL-producing strains, with only 25 clonally related CTX-M-15-producing strains grouped in three unrelated clusters. Among these 25 clonal strains, only one cross-contamination could be identified in a medicine ward of Montpellier University Hospital. Data

TABLE 2. Univariate analysis of potential risk factors associated with the isolation of CTX-M-producing *E. coli*

Characteristic[b]	Value for *E. coli* isolates producing:[a]		OR (95% CI)[d]	*P* value
	CTX-M	TEM or SHV		
No. of strains	65	47		
Age, median yr	72	72	—	—
Male/female	27/37	26/21		
Sex ratio	0.73	1.24	—	—
Comorbid disease/condition				
Charlson index	4.1 [0–10]	3.4 [0–11]	—	0.01
Cardiovascular diseases	28 (43.8)	23 (48.9)	—	—
Myocardial infarction	5 (7.8)	4 (8.5)	—	—
Heart failure	7 (10.9)	6 (12.8)	—	—
Renal disease	25 (39.1)	5 (10.6)	5.1 (2.4–12.0)	<0.001
Diabetes mellitus	29 (45.3)	7 (14.9)	3.4 (1.7–7.0)	<0.001
COPD	14 (21.9)	11 (23.4)	—	—
Hematologic or solid malignancy	16 (25.0)	9 (19.1)	—	—
Neutropenia	10 (15.6)	5 (10.6)	—	—
HIV	1 (1.6)	(0)	—	—
Immunosuppressor	13 (20.3)	6 (12.8)	—	—
Solid organ transplant	4 (6.3)	(0)	0.0 (0.0–1.21)	0.03
Hepatic diseases	8 (12.5)	3 (6.4)	—	—
Cirrhosis	5 (7.8)	2 (4.3)	—	—
Peptic ulcer	1 (1.6)	4 (8.5)	—	—
Alcoholism	5 (7.8)	2 (4.3)	—	—
Neurological disease	7 (10.9)	3 (6.4)	—	—
Cerebrovascular accident	4 (6.3)	5 (10.6)	—	—
Hemiplegia	6 (9.4)	13 (27.7)	0.3 (0.1–0.9)	0.02
Urinary incontinence	12 (18.8)	11 (23.4)	—	—
Dementia	7 (10.9)	7 (14.9)	—	—
Autoimmune diseases	1 (1.6)	2 (4.3)	—	—
Bedridden	6 (9.4)	11 (23.4)	0.3 (0.1–1.1)	0.06
MacCabe scores				
0	24 (37.4)	25 (53.2)	—	—
1	28 (43.8)	16 (34.0)	—	—
2	12 (18.8)	6 (12.8)	—	—
Mortality rate	12 (18.8)	6 (12.8)	—	—
Colonization/infection	12/53 (0.23)	19/28 (0.68)	3.0 (1.5–6.2)	0.001
Nosocomial/community origin	33/22 (1.5)	44/3 (14.6)	0.1 (0.1–0.3)	<0.0001
Acquisition delay (days)	3	5	—	—
Hospitalization delay (days)	20	22	—	—
Wards				
Medicine	25 (39.1)	12 (25.5)	—	—
Surgery	12 (18.8)	11 (23.4)	—	—
ICU	17 (26.5)	12 (25.5)	—	—
Geriatric	2 (3.1)	5 (10.6)	—	—
Recovery	2 (3.1)	6 (12.8)	0.2 (0.0–1.3)	0.06
Emergency	6 (9.4)	1 (2.2)	—	—
Presence of:				
Urinary catheter	26 (40.6)	12 (25.5)	2.7 (1.1–7.0)	0.02
Mechanical ventilation	5 (7.8)	7 (14.9)	—	—
Tracheotomy	(0)	8 (17.0)	0.0 (0–0.2)	<0.0001
Parenteral nutrition	7 (10.9)	3 (6.4)	—	—
Central venous catheter	16 (25.0)	9 (19.1)	—	—
Sources				
Urinary[c]	44 (67.8)	20 (42.6)	2.6 (1.4–4.8)	0.002
Cutaneous	1 (1.5)	10 (21.3)	0.2 (0.1–0.6)	<0.0001
Blood[c]	2 (3.1)	2 (4.3)	—	—
Respiratory tract	3 (4.6)	2 (4.3)	—	—

Continued on following page

TABLE 2—*Continued*

Characteristic[b]	Value for *E. coli* isolates producing:[a]		OR (95% CI)[d]	*P* value
	CTX-M	TEM or SHV		
Pus	14 (21.5)	12 (25.5)	—	—
Catheter	1 (1.5)	(0)	—	—
Bone[c]	(0)	1 (2.1)	—	—
Antecedents				
Hospitalization <1 yr	40 (62.5)	30 (63.8)	—	—
ICU <1 yr	20 (31.3)	18 (38.3)	—	—
Transfer from another hospital	23 (35.9)	15 (31.9)	—	—
Surgery	20 (31.3)	13 (27.7)	—	—
Gynecologic	7 (10.9)	(0)	2.1 (0.0–5.2)	0.002
Urologic	12 (18.5)	3 (6.4)	—	—
Prior antibiotic therapy <1 mo	28 (43.8)	18 (38.3)	—	—
Fluoroquinolones	14 (21.9)	3 (6.4)	—	—
β-Lactams	17 (26.6)	14 (29.8)	—	—
B-S cephalosporins	8 (12.5)	6 (12.8)	—	—
Carbapenems	2 (3.1)	3 (6.4)	—	—
Polymicrobial infections	21 (32.3)	14 (29.8)	—	—
Enterococcus spp.	12 (18.5)	5 (10.6)	—	—
Candida spp.	4 (6.2)		—	—
Proteus mirabilis	(0)	3 (6.4)	—	—

[a] Values in parentheses indicate interquartile ranges or percentages.
[b] All characteristics except number of strains, age, sex ratio, Charlson index, MacCabe scores, and mortality data are given in number (%) of patients. COPD, chronic obstructive pulmonary disease; HIV, human immunodeficiency virus; ICU, intensive care unit; B-S, broad-spectrum.
[c] Factors associated exclusively with infections.
[d] OR, odds ratio; CI, confidence interval; —, not significant.

collected for the remaining isolates suggested that the clonal dissemination of CTX-M-15-producing strains is not associated with the spread of the strains in the hospital, since no relationships between patients (temporal, geographical, or other association) have been found. Moreover, seven unrelated patients hospitalized at the Montpellier Hospital acquired clonally related CTX-M-15-producing *E. coli* from the community. No seasonal variation was found in our study. The sparse dissemination of these bacteria suggested a probable food or water source, the source most common to all the outpatients, as previously suggested (1, 35). Patients with urinary tract diseases or foreign materials (e.g., infection, catheter, or incontinence) more frequently developed infections with clonally CTX-M-producing *E. coli*, even in the presence of a small number of patients ($n = 25$). Finally, these data showed the emergence of three different clones of CTX-M-15-producing *E. coli* strains, including strains of probable community origin, contrasting with previous reports describing localized, nosocomial outbreaks (16, 17, 29, 32, 46, 49).

TABLE 3. Multivariate analysis of risk factors associated with increased risk of acquisition of ESBL-producing *E. coli*

Patient group and risk factor	OR[a]	*P* value
Patients with CTX-M-producing *E. coli*		
Renal disease	8.4	0.0037
Diabetes mellitus	5.2	0.0231
Urinary tract infection	17.9	0.0030
Community-acquired infection	26.7	<0.0001
Surgery	7.1	0.0281
Gynecological surgery	6.9	0.0087
Urinary catheter	4.1	0.0437
Patients with clonal CTX-M-producing *E. coli*		
Cardiovascular disease	5.9	0.0153
Urinary incontinence	8.9	0.0028
Urinary catheter	5.3	0.0211
Polymicrobial infection	5.4	0.0205
Patients with other ESBL-producing *E. coli*		
Cutaneous samples	16.2	0.0064
Nosocomial infection	23.3	<0.0001
Tracheotomy	14.8	0.0001

[a] OR, odds ratio.

The majority of CTX-M-producing strains recovered during this study were isolated from UTIs. The phylogenetic distribution showed a majority of strains in non-B2 phylogenetic groups (24/44 strains), as previously reported (7), except for CTX-M-15 strains, which belonged mainly to the B2 group (20/30 strains), as recently described (42). Branger et al. demonstrated that *E. coli* strains belonging to non-B2 phylogenetic groups have a greater incidence of antimicrobial resistance, express significantly fewer virulence factors, and more frequently invade compromised hosts (7). In this study, the population described was frequently immunocompromised by a particular alteration of the urinary tract, the main risk factor identified by the multivariate analysis (20, 21, 42). The frequency of CTX-M strains in weakened patients and the incidence of these strains in the community invite further study of the epidemiologic evolution of these strains and dissemination of this information to the medical community.

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