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Nationwide Study of the Prevalence, Characteristics, and Molecular Epidemiology of Extended-Spectrum-β-Lactamase-Producing *Enterobacteriaceae* in France▽

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Among 10,872 isolates of *Enterobacteriaceae* from a nationwide study of 88 French hospitals in 2005, 169 (1.7%) expressed an extended-spectrum β-lactamase. The most prevalent species were *Escherichia coli* (48.5%), *Enterobacter aerogenes* (23.7%), and *Klebsiella pneumoniae* (14.8%). Molecular analysis underlined the polyclonal spread of CTX-M-expressing *E. coli*, primarily isolates of the CTX-M-1 subgroup.

Resistance to extended-spectrum cephalosporins in *Enterobacteriaceae* can be associated with the production of extended-spectrum β-lactamases (ESBL) (11). Since the end of the 1990s, a new family of ESBL, the CTX-M-type β-lactamases, has spread worldwide. To date, over 60 CTX-M-type β-lactamases have been described and divided into five different clusters: CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25 (2, 17, 20). Several local or regional studies indicate the emergence of CTX-M β-lactamases in France, but concurring data for the nation as a whole are lacking (1, 9, 12–16). We have conducted a nationwide prospective study in 88 nonteaching hospitals affiliated with the Collège de Bactériologie-Virologie-Hygiène des Hôpitaux de France network to evaluate both (i) the prevalence of ESBL-producing *Enterobacteriaceae* by using phenotypic confirmatory tests and (ii) the prevalence and molecular epidemiology of CTX-M-harboring *Enterobacteriaceae* (5, 6).

Bacterial isolates. All ESBL-producing *Enterobacteriaceae* isolates were collected from clinical samples during a 1-month period (October 2005). For patients with recurrent infections, only isolates from the first episodes were included. Bacterial identification and ESBL screening were performed by the participating institutions according to the recommendations of the Antibiogram Committee of the French Society of Microbiology (10). The data concerning the total number of *Enterobacteriaceae* isolates and all the ESBL-producing *Enterobacteriaceae* isolates were centralized at the Service d'Hygiène Hospitalière, Centre Hospitalier de Versailles.

Quality controls. The following four *E. coli* isolates were submitted anonymously: a wild type, two isolates harboring an extended-spectrum TEM or CTX-M β-lactamase, and an isolate overexpressing a cephalosporinase. Analyses of the capacity of each participating institution to detect the ESBL phenotype were sent to the central laboratory.

Identification and antimicrobial susceptibility testing. Analysis at the central laboratory confirmed the identification and ESBL production determined by the API 20E and VITEK 2 systems (bioMérieux, Marcy l'Etoile, France), the double-disc synergy test, the MicroScan ESBL plus ESBL confirmation panel (Dade Behring, Sacramento, CA), and the Etest ESBL (AB Biodisk, Piscataway, NJ). Non-β-lactam antimicrobial susceptibility testing was performed by using disk diffusion according to the French guidelines (for nalidixic acid, ciprofloxacin, gentamicin, amikacin, and cotrimoxazole) (10).

PCR amplification. $bla_{\text{CTX-M}}$ genes were amplified by using PCR, as described previously (9). The determination of subgroups was performed using specific primers (Table 1).

Clonality of the isolates. The clonal relationship of the isolates was studied by using pulsed-field gel electrophoresis with the GenePath system and Fingerprinting II software (Bio-Rad, Hercules, CA) in accordance with the manufacturer's recommendations. Clustering was defined when the percent similarity exceeded 80%.

Statistical analysis was performed using a chi-square test. A total of 10,872 *Enterobacteriaceae* isolates were included in this study, and 169 *Enterobacteriaceae* isolates (1.6%) were confirmed by the central laboratory to produce ESBL. As with the quality control isolates, the concordance between the results obtained by the participating centers and those by the referent laboratory was above 90%. The percentage of ESBL-harboring isolates, their distribution, and their clinical origins are reported in Tables 2 and 3. $bla_{\text{CTX-M}}$ genes were identified in 62/82 *E. coli* (76%), 6/25 *K. pneumoniae* (24%), 2/10 *Enterobacter cloacae* (20%), and 0/40 *E. aerogenes* isolates. In *E. coli*, $bla_{\text{CTX-M}}$ genes belonged primarily to the CTX-M-1 (53/62) and CTX-M-9 (7/62) groups. No CTX-M-2-related gene was identified, and only two $bla_{\text{CTX-M}}$ genes could not be characterized by subtype. Resistance to ceftazidime was identified in 49% of the *E. coli* isolates harboring CTX-M group 1. The

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TABLE 1. List of primers used for PCR amplification

Primer target	Primer name	Sequence	Reference
CTX-M consensus	MA1	5′-SCSATGTGCAGYAC CAGTAA-3′	21
	MA2	5′-CCGCRATATGRTTG GTGGTG-3′	
CTX-M-13 subgroup	M13U	5′-GGTTAAAAAATCAC TGCCTC-3′	21
	M13L	5′-TTGGTGACGATTTT AGCCGC-3′	
CTX-M-25 subgroup	M25U	5′-ATGATGACTCAGAG CATTCG-3′	21
	M25L	5′-TGGGTTACGATTTT AGCCGC-3′	
CTX-M-9 subgroup	M9U	5′-ACGGTGACAAAGA GAGTGCA-3′	21
	M9L	5′-CCCTTCGGCGATGA TTCTC-3′	

geographical locations of centers that have isolated CTX-M-harboring *E. coli* in France are reported in Fig. 1.

An analysis of pulsed-field gel electrophoresis profiles showed 10 clusters including 35.4% of the *E. coli* isolates, 6 of which included 63.6% of the *K. pneumoniae* isolates and 3 of which included 71.4% of the *E. aerogenes* isolates (data not shown). Statistical analysis showed that the presence of CTX-M was significantly associated with nalidixic acid or ciprofloxacin resistance ($P < 0.001$) but not with cotrimoxazole, gentamicin, or amikacin resistance ($P = 0.49$, 0.67, and 0.67, respectively).

The last national study of ESBL prevalence in France was performed in 1998, before the rise of *Enterobacteriaceae* producing CTX-M ESBL, when levels of CTX-M ESBL reached 3.2% (4). Only 1.6% of the *Enterobacteriaceae* isolates in our work produced ESBL. This national study does not confirm the increase in the prevalence of ESBL-producing *Enterobacteriaceae* reported in some local or regional settings (3, 13, 20). Such data could have been influenced by the spread of a particular isolate locally. Compared to results in previous studies, the percentages of ESBL-producing isolates decreased for *Proteus mirabilis* (1.31% in this study versus 3.7% in 1998), *E. aerogenes* (21.4% versus 53.5%), and *K. pneumoniae* (3.71% versus 9.4%) but increased for *E. coli* (1.99% versus 0.2%) (4). Until the late 1990s, *E. aerogenes* TEM-3 and TEM-24 clones dominated the species and ESBL group distribution in France (3, 4, 7, 17). CTX-M ESBL appeared in 1989 and then were reported only occasionally (1, 4, 8, 16, 18). Lavigne et al. underline the increase of ESBL prevalence in *E. coli* isolates in 2004 (0.68% versus 0.2% in 1998) from a sample of isolates in southern and central France, which were caused primarily by the rise of the CTX-M-15 subtype (14). Our work confirms these findings at the national level: half of the *E. coli* isolates harboring the CTX-M-1 subgroup show decreased susceptibility to ceftazidime, which indicates the putative presence of CTX-M-15.

For our study, several local outbreaks with the same CTX-M *E. coli* isolate were identified, as reported by different hospitals in the Paris area or in southern France (9, 12, 13, 15, 16). Though *E. coli* CTX-M-15 has spread nationally in the United Kingdom, we failed to identify a single CTX-M clone being so widespread in France (23). Nevertheless, additional studies must be performed to explore the putative spread of the same plasmid in unrelated isolates (19, 20, 22).

A statistical link between CTX-M production and nalidixic acid or fluoroquinolone resistance has been established; this association can be explained at least in part by the high incidence of *qnr* genes in this ESBL type (14, 20).

TABLE 2. Distribution of each species, main clinical origins, and percentage of ESBL-harboring isolates among 10,872 clinically relevant isolates of *Enterobacteriaceae*[a]

Frequency rank[b]	Species	Total isolates		UTI isolates		RTI isolates		BI isolates	
		No.	% ESBL	No.	% ESBL	No.	% ESBL	No.	% ESBL
1	*Escherichia coli*	7,989	1.99	6,295	1.0	119	2.5	666	1.5
2	*Proteus mirabilis*	689	1.31	454	1.8	28	3.6	42	0.0
3	*Salmonella* spp.	70	0	1	0.0	0	0.0	20	0.0
4	*Klebsiella pneumoniae*	647	3.71	413	2.7	45	8.9	65	4.6
5	*Klebsiella oxytoca*	204	2.45	96	4.2	15	0.0	37	2.7
6	*Klebsiella* spp.	10	0	4	0.0	1	0.0	5	0.0
7	*Citrobacter koseri*	112	1.79	71	2.8	5	0.0	10	0.0
8	*Enterobacter cloacae*	392	2.55	152	3.3	49	6.1	70	1.4
9	*Enterobacter aerogenes*	196	21.4	89	25.8	43	18.6	16	37.5
10	*Enterobacter* spp.	16	0	1	0.0	3	0.0	6	0.0
11	*Citrobacter freundii*	120	1.66	79	0.0	7	0.0	8	12.5
12	*Citrobacter* spp.	13	0	4	0.0	1	0.0	2	0.0
13	*Proteus vulgaris*	33	0	19	0.0	4	0.0	3	0.0
14	*Morganella morganii*	185	0	87	0.0	24	0.0	18	0.0
15	*Providencia stuartii*	22	0	13	0.0	3	0.0	2	0.0
16	*Serratia marcescens*	102	1.96	21	4.8	28	3.6	17	0.0
17	Other *Enterobacteriaceae*	63	0	19	0.0	6	0.0	14	0.0
Total		10,872	1.7	7,818	1.5	381	5.2	1,001	1.6

[a] UTI, urinary tract infection; RTI, respiratory tract infection; BI, bloodstream infection.
[b] Species were ranked according to their frequency of isolation among clinical samples, independent of the expression or lack of expression of an ESBL.

TABLE 3. Origins of ESBL-producing *Enterobacteriaceae*

Species	No. of isolates from indicated source						
	Urine	Bloodstream infections	Respiratory tract infections	Suppurations	Intravascular catheter infections	Other	All sources
Escherichia coli	60	9	2	6	2	3	82
Enterobacter aerogenes	18	6	10	5		1	40
Klebsiella pneumoniae	11	3	2	3	2	4	25
Enterobacter cloacae	3	1	2	1	1	2	10
Proteus mirabilis	6						6
Citrobacter koseri	2						2
Citrobacter freundii		1					1
Klebsiella oxytoca						1	1
Salmonella spp.						1	1
Serratia marcescens	1						1
Total	101	20	16	15	5	12	169

The results of our work must be interpreted in light of its limitations. The number of ESBL-producing *Enterobacteriaceae* isolates is quite low; our findings must be confirmed by a larger study. Isoelectric focusing experiments and sequencing of each PCR product would provide more accurate data about the epidemiology of ESBL-producing *Enterobacteriaceae*. We lack extensive analyses of clinical data to establish the ratio of hospital-acquired to community-acquired infections for each ESBL type. Nevertheless, to the best of our knowledge, this work is the largest study of ESBL-producing *Enterobacteria-*

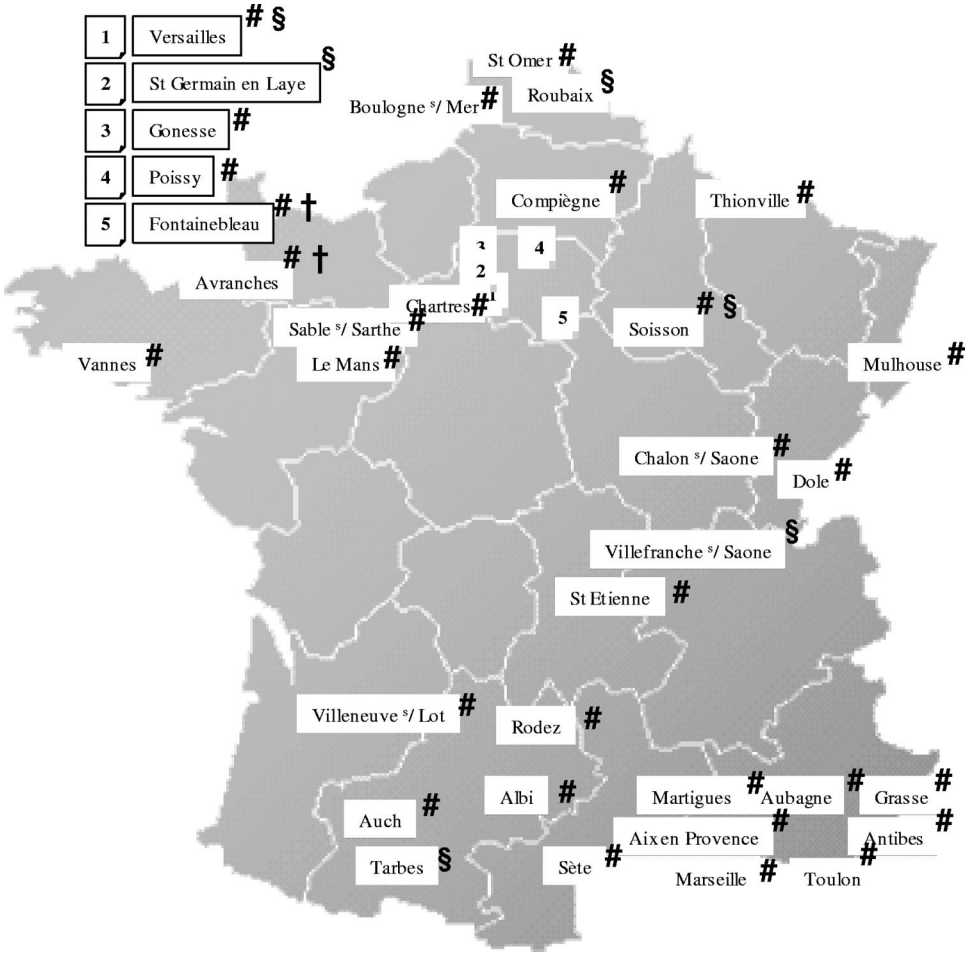

FIG. 1. Geographical locations of the centers that have isolated *Escherichia coli* harboring the following ESBL: CTX-M group 1 (#), CTX-M group 9 (§), other CTX-M groups (†).

ceae epidemiology performed in France. This national study supports the previous local and regional observations that CTX-M-harboring *E. coli* isolates are now the most frequent ESBL-producing *Enterobacteriaceae* in France.

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