Nationwide survey of extended-spectrum β-lactamase-producing Enterobacteriaceae in the French community setting

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Objectives: The aim of this study was to assess the prevalence of the extended-spectrum β-lactamase (ESBL)-producing enterobacteria (ESBLE) in the French community, during a 2006 survey.

Methods: All enterobacteria isolated from urine samples of patients, exhibiting a decreased susceptibility to broad-spectrum cephalosporins, were analysed for their β-lactamase content (synergy test, isoelectrofocusing, conjugation transfer, PCR amplification and/or cloning experiments and sequencing). Additional co-resistances were investigated by PCR, sequencing and/or cloning. Epidemiological relationship was studied by PFGE for all species and, in addition, for Escherichia coli by the determination of the phylogenetic group, multilocus sequence type (ST) and O25b antigen. Characteristics of CTX-M-producing E. coli carriers were compared with other ESBLE carriers.

Results: Seventy-two ESBLE were collected from 71 patients. Most of them expressed a CTX-M enzyme (n = 42, comprising 40 E. coli), with a predominance of CTX-M-15 (n = 24); 10 CTX-M-15-producing E. coli belonged to the same clone (phylogroup B2, ST131, serotype O25b). The 30 remaining strains possessed a TEM- or SHV-type ESBL. In addition, three strains presented unusual co-resistances such as DHA-1 (n = 2), QnrB4 and ArmA. Risk factors for ESBLE acquisition were substantially less frequent when the ESBL was of the CTX-M type, except for prior antimicrobial therapy. Eighteen percent of the patients were considered to have true community-acquired ESBLE; most of them harboured a CTX-M-producing E. coli.

Conclusions: This first nationwide study reports an ESBLE prevalence of 1.1% in the French community setting in 2006, mainly related to the presence of CTX-M-producing E. coli strains; threatening unusual co-resistances were occasionally observed.

Keywords: ESBLs, enterobacteria, CTX-M, community setting, risk factors

Introduction

Extended-spectrum β-lactamases (ESBLs) are the major cause of resistance to oxyimino-cephalosporins in Enterobacteriaceae. Since the early 2000s, CTX-M-type ESBLs have been increasingly reported and these enzymes have now replaced TEM and SHV as the most common type of ESBL. The CTX-M-β-lactamase-encoding genes appear to have been captured on transferable plasmids from the chromosome of environmental enterobacteria of the Klebsiella spp. To date, due to genetic polymorphism and/or evolution under selection pressure, more than 80 CTX-M-type β-lactamases have been identified, which are divided into five groups based on their amino acid identities (http://www.lahey.org/Studies). CTX-M enzymes, particularly CTX-M-15, have been involved in various epidemiological situations and have disseminated through all continents, especially in Europe, due to epidemic plasmids and/or particular epidemic strains.

A threatening problem is the alarming increase in infections, essentially of the urinary tract, due to CTX-M-producing
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*Escherichia coli* isolated in non-hospitalized persons.6–8 Indeed, these organisms have become widely prevalent in the community setting in some areas of the world, including European countries such as Spain,7 Italy,9 Greece10 and the UK.11 Recent studies suggest that these strains should not be considered as exclusively nosocomial pathogens but, at least in part, as true community ESBL producers. Furthermore, these bacteria seem to have been imported from the community into the hospital setting.1 In 1999, we conducted a survey on antibiotic resistance in Enterobacteriaceae collected in general practice in the French Aquitaine region (area IV; Figure 1), and ESBL producers have been rarely detected in the community setting.12–14 However, in recent years, several surveys have indicated that CTX-M β-lactamases were emerging in French hospitals and healthcare institutions such as nursing homes.15,16 In addition, some French local studies have reported that patients carried CTX-M-producing strains on their hospital admission.17 Nevertheless, concurring data for the nation as a whole, and among patients living at home, were lacking. Accordingly, a nationwide survey focusing on ESBL-producing enterobacteria (ESBLE) isolated from urine samples of community patients was performed in 2006, under the aegis of the Observatoire National de l’Évolution de la Résistance des Bactéries aux Antibiotiques (ONERBA) (http://www.onerba.org).

The aim of this study was to assess the prevalence of ESBLE in the French community population, to characterize ESBLEs and to analyse the epidemiological relationship between ESBLE producers.

Materials and methods

Strains and data collection

During a 2 month period (either March–April or April–May 2006), French private laboratories were solicited by ONERBA to collect enterobacteria with a decreased cefotaxime and/or cefazidime susceptibility (MIC ≥0.5 mg/L or diameter of the inhibition zone ≤25 mm) isolated from urinary tract infections (UTIs) of community patients. The corresponding strains were sent to the Microbiologie Cellulaire et Moléculaire et Pathogénicité (MCMP) Laboratory of the Bordeaux 2 University, together with demographic and clinical information on the infected patients. These included: age, gender, co-morbid diseases, hospitalization during the 6 preceding months, administration of antibiotics during the 3 preceding months, contact with healthcare system or professionals (home care, healthcare worker patient or relatives) and known carriage of ESBLE. In order to assess ESBLE prevalence in the community, we recorded the total number of strains isolated from the UTIs of community patients in the same participating laboratories during the study period. In addition, a random sample of patients with ESBL-negative enterobacteria UTIs (947 patients) were asked about prior history of hospitalization. Statistical comparisons were carried out using the χ² test, with Epi Info software (version 6.04; CDC, USA). A P value <0.05 was considered statistically significant.

Bacterial strains

The identification of ESBLE to the species level was confirmed by the API20E system (bioMérieux, Marcy-l’Étoile, France). Ambiguous or poorly discriminatory results for seven strains (four *Citrobacter koseri*, and one strain each of *Klebsiella pneumoniae*, *Klebsiella oxytoca* and *Citrobacter freundii*) were clarified by molecular methods. The molecular identification consisted of PCR amplification and subsequent sequencing of partial fragments of the 16S rDNA and/or of the *rpoB* gene.18 Amplification and sequencing of species-specific β-lactamases (*blaCTX-M* for *C. koseri*, *blaSHV* for *K. pneumoniae*, *blaOXY* for *K. oxytoca* and *blaCMY-2* for *C. freundii*)19–21 were also carried out.

*E. coli* strains K12, C600 and TOP10 were used as recipient cells for the transfer experiments by conjugation or transformation. Enterobacteria from the collection of the MCMP Laboratory either producing known β-lactamases or exhibiting previously identified genes (i.e. *armA*, *orf513*, ISEcp1 etc.) were added as controls in isoelectrofocusing (IEF) assays and/or PCR amplifications. Plasmid vector pBK-CMV (Stratagene, La Jolla, CA, USA) was used for cloning experiments.

Antimicrobial susceptibility testing

ESBL production was detected by the double-disc synergy test between clavulanic acid and ceftazidime, cefotaxime and cefepime on Mueller–Hinton (MH) agar alone and, when necessary, supplemented with 250 mg/L cloxacillin (http://www.sfm.asso.fr). Antibiotic susceptibility patterns of the ESBL-producing strains and their transconjugants or transformants were determined by the disc diffusion method in MH agar using 27 antibiotic discs (Bio-Rad, Marnes-la-Coquette, France). Strains were classified as susceptible, intermediate or resistant to the tested antibiotics according to the 2008 recommendations of the Comité de l’Antibiogramme de la Société Française de Microbiologie (http://www.sfm.asso.fr). MICs of four β-lactam agents (cefazidime and cefotaxime alone and in combination with 2 mg/L clavulanic acid, imipenem and ertapenem) were determined for all ESBLE by the agar dilution method (http://www.sfm.asso.fr).

β-Lactamase characterization

β-Lactamase content of the ESBL-producing strains and their transconjugants were analysed by IEF.13 The ESBL-encoding genes *blaTEM*, *blaSHV*, *blaOXY-1* and *blaCTX-M* were detected by PCR using specific primers9,17 and further identified by nucleotide sequence analysis of the PCR products. When several enzymes of the same family co-existed, a separation was carried out by PCR amplification and subsequent cloning.13 A multiplex PCR protocol was used to detect the presence of plasmid-mediated AmpC β-lactamase genes.22

Transfer experiments

Conjugation assays were performed by a filter method using either a nalidixic acid- and rifampicin-resistant (NalR RifR) mutant of *E. coli* K12, or an azide-resistant (AζR) mutant of *E. coli* C600 as recipient strains.13 Transconjugants were selected on MH agar containing nalidixic acid or rifampicin (100 mg/L) or azide (300 mg/L), plus ampicillin (100 mg/L) or broad-spectrum cephalosporins (cefazidime, 2 mg/L or cefotaxime, 4 mg/L). When not successful at the first attempt, mating experiments were repeated up to three times.

β-Lactamase gene environment analysis

The genetic environment of the *blaCTX-M* genes was characterized by PCR amplification with primers annealing to ISEcp1 and to *orf513* of ISCR1 together with primers for the *blaCTX-M* genes. For ISEcp1, two experiments were carried out to amplify the regions located upstream or downstream of the *blaCTX-M* genes
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Primers CTXMA_Rev 5' (5'-CTGCACATCGCAAAGCG-3') with IRR1 (5'-GTATTCTGAAGGTCAAG-3') and CTXMB_Rev (5'-accAATGcTATcGGG-3') with IRR2 (5'-ACCTGGGACCTACG-3'). The IS_{Ecp1} element was considered as present if at least one of these two PCR assays was positive. Amplicons for linking IS_{CR1} with \textit{bla}_{CTX-M} were obtained by anchoring one primer at the 5' end of \textit{bla}_{CTX-M} (CTXMA_Rev) and the other one to the 3' end of \textit{orf513} (ORF513FinR, 5'-cgcacgactcccagcc-3'). The \textit{bla}_{DHA-1} gene from \textit{K. oxytoca} was cloned in the \textit{E. coli} TOP10 recipient strain after Sau3AI partial digestion of the conjugative plasmid DNA and ligation in the BamHI-digested pBK-CMV vector. The transformants obtained were selected on a medium containing 2 mg/L ceftazidime.

Analysis of \textit{qnr} and \textit{armA} genes

All ESBL-producing strains were screened by PCR amplifications for the presence of \textit{qnrA}, \textit{qnrB} and \textit{qnrS} (quinolone resistance) genes,

\textsuperscript{23} and the single highly amikacin-resistant strain for \textit{armA} (aminoglycoside resistance).\textsuperscript{24} Amplicons that were obtained were sequenced.

Epidemiological typing

The clonal relationship of the isolates was investigated by PFGE using the CHEF-DRIII system (Bio-Rad) and XbaI-digested (\textit{E. coli}, \textit{Enterobacter aerogenes}, \textit{C. koseri} and \textit{K. pneumoniae}) or SfiI-digested (\textit{Proteus mirabilis}) DNA. Profiles were compared using FFQuest\textsuperscript{TM} software (Bio-Rad). For the \textit{E. coli} strains, the phylogenetic group and subgroup were determined by a multiplex PCR, using a combination of three DNA markers (\textit{chuA}, \textit{yjaA} and \textit{TspE4.2}).\textsuperscript{25,26} In addition, the presence of the O25b antigen was determined using a PCR-based method.\textsuperscript{27} For 10 ESBL-producing \textit{E. coli} exhibiting the O25b antigen, multilocus sequence typing (MLST) was performed using the primers and methodology indicated on the \textit{E. coli} MLST web site (http://web.mpiib-berlin.mpg.de/mlst/dbs/Ecoli).

Results

Clinical data

A total of 25 private laboratories participated in the survey. Their geographic locations were divided into four regions.
likely to have home care (38.2% versus 74.1%, \( P \approx 0.01 \)) and were less likely to have home care (38.2% versus 74.1%, \( P = 0.005 \)), prior ESBL carriage (26.5% versus 48.1%, \( P = 0.08 \)) and prior hospitalization, although the latter difference was not statistically significant (50.0% versus 67.9%, \( P = 0.10 \)). Thirteen patients (10 women and 3 men, median age of 50.8 years) who had no medical contacts (prior hospitalization, home care, healthcare profession and caregivers in their household) were found to carry the ESBL, with those with other ESBL producers were younger (>65 years, 47.5% versus 77.4%, \( P < 0.01 \)) and were less likely to have hospital care (38.2% versus 74.1%, \( P = 0.005 \)).

**ESBL and additional co-resistance characterization**

The 72 ESBL producers are described in Table 2. They included 48 *E. coli* (prevalence rate 0.88%, ranging from 0% to 4.7% according to the laboratory). Among them, 40 expressed a CTX-M enzyme including 23 CTX-M-15. One patient was infected by two ESBL, i.e. one TEM-24-producing *E. aerogenes* and the CTX-M-1-producing *P. mirabilis*. All ESBL exhibited decreased susceptibilities to cefotaxime and/or ceftazidime that were generally restored by clavulanic acid. They remained susceptible to carbapenems (MICs: ertapenem, 0.05 mg/L; imipenem, 0.1 mg/L) [see Table S1, available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/)].

The presence of additional \( \beta \)-lactamases was assessed by combination of IEF, PCR or cloning experiments when necessary. The results are summarized in Table 2, and showed the presence of TEM-1-like, OXA-1-like or both enzymes, preferentially among CTX-M producers. Furthermore, all ESBLE with an acquired cefotaxime resistance restored by clavulanic acid (increase in the inhibition zone diameter = 5 mm) and/or clavulanate–ceftazidime MIC >1 mg/L were subjected to a multiple PCR for the detection of plasmid-mediated AmpC \( \beta \)-lactamases. Among the 23 strains tested (16 *E. coli*, 4 *C. koseri*, 2 *K. pneumoniae* and 1 *K. oxytoca*), 2 (1 *E. coli* CTX-M-15 and 1 *K. oxytoca* SHV-12) gave a positive amplification for the *blaDHA-1* gene.

A total of 86% of the ESBLE were immediately susceptible or resistant to cefotaxime. However, the detection of the *qnrA*, *qnrB* and *qnrS* genes by PCR amplification led to a single positive result. Indeed, only the SHV-12-producing *K. oxytoca* strain was found to carry the *qnrB4* allele, which co-transferred with *blaDHA-1* (discussed subsequently). With regard to aminoglycosides, resistance to gentamicin (29%), amikacin (51%) or both (21%) was associated with a gentamicin–tobramycin–netilmicin, tobramycin–netilmicin–amikacin or gentamicin–tobramycin–netilmicin–amikacin phenotype, respectively. In addition, one of the CTX-M-15-producing *E. coli* exhibited high-level resistance to all aminoglycosides due to the *armA* gene. Resistance to co-trimoxazole (86%), tetracycline (76%) and chloramphenicol (47%) were variable (Table 2). Mating assays allowed the transfer of ESBLs from 54 strains (75%), including 30 CTX-M-producing strains, and among them, 19 CTX-M-15. In general, the additional \( \beta \)-lactamase(s) and other previously cited resistances co-transferred with the ESBL (*blaTEM-1*-like, 37.5%; *blaOXA-1*-like, 61.5%; gentamicin, 78.0%; amikacin, 85.7%; co-trimoxazole, 69.6%; tetracycline, 47.5% and chloramphenicol, 60.9%) (data not shown). Two of the *E. aerogenes* strains were immediately susceptible to cefotaxime (MIC, 16 mg/L), due to a non-inducible chromosomal AmpC cephalosporinase, and one strain harboured a low-level tobramycin resistance phenotype probably associated with the low expression of an *aac(6')-Ib* gene. The four strains of *C. koseri* exhibited a reduced susceptibility or resistance to cefotaxime.

**Table 1. Comparison of characteristics of patients with and without CTX-M-type ESBLs**

<table>
<thead>
<tr>
<th>Clinical data</th>
<th>Recovered data (%)</th>
<th>Patients with ESBLE</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>total</td>
<td>CTX-M-producing <em>E. coli</em> (( n = 40 ))</td>
</tr>
<tr>
<td>Mean age (range)</td>
<td>100</td>
<td>65.9 (1–97)</td>
</tr>
<tr>
<td>Patients &gt;65 years</td>
<td>100</td>
<td>60.6%</td>
</tr>
<tr>
<td>Sex ratio (female/male)</td>
<td>100</td>
<td>1.63</td>
</tr>
<tr>
<td>Co-morbid disease</td>
<td>88.7</td>
<td>71.4%</td>
</tr>
<tr>
<td>Hospitalization &lt;6 months(^a)</td>
<td>90.1</td>
<td>57.8%</td>
</tr>
<tr>
<td>Antibiotic therapy &lt;3 months(^b)</td>
<td>76.1</td>
<td>79.6%</td>
</tr>
<tr>
<td>Home care</td>
<td>85.9</td>
<td>54.1%</td>
</tr>
<tr>
<td>Known ESBLE carriage</td>
<td>85.9</td>
<td>36.1%</td>
</tr>
</tbody>
</table>

\(^a\)Hospital, 63%; clinic, 30%; rehabilitation centre, 3.5%; convalescence centre, 3.5%.

\(^b\)Fluoroquinolone, 35.7%; \( \beta \)-lactam, 32.15%; others, 32.15%.

Only three patients were either healthcare workers or had caregivers in their household.
Table 2. Characteristics of the ESBL

<table>
<thead>
<tr>
<th>Species and ESBL type</th>
<th>E. coli phylogroup</th>
<th>No. of strains (PFGE cluster)b</th>
<th>Geographical area</th>
<th>No. of patients without medical contactsc</th>
<th>Additional β-lactamase(s) or resistance gene</th>
<th>Acquired co-resistancesd</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TEM-1-like</td>
<td>OXA-1-like</td>
</tr>
<tr>
<td>E. coli (48)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CTX-M-15 (23)</td>
<td>B2 (13)</td>
<td>10 (C-I)</td>
<td>I (2), II (2), III (3), IV (3)</td>
<td>1</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>CTX-M-1 (6)</td>
<td>B1 (4)</td>
<td>4 (III, IV)</td>
<td>I (2), II (2)</td>
<td>1</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td>CTX-M-9 (2)</td>
<td>A1 (8)</td>
<td>8 I, II, III, IV (5)</td>
<td>—</td>
<td>1</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>CTX-M-2 (2)</td>
<td>D1 (2)</td>
<td>2 III, IV</td>
<td>—</td>
<td>1</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>CTX-M-3</td>
<td>D2 (2)</td>
<td>1 I</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>CTX-M-27</td>
<td>D2 (2)</td>
<td>1 III</td>
<td>—</td>
<td>1</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>TEM-52 (5)</td>
<td>A1 (3)</td>
<td>3 (II, III)</td>
<td>I, III</td>
<td>1</td>
<td>2</td>
<td>—</td>
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<tr>
<td>E. aerogenes (10)</td>
<td></td>
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<tr>
<td>TEM-24 (9)</td>
<td>—</td>
<td>9 (C-III)</td>
<td>I (2), II (2), III (4)</td>
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<td>—</td>
<td>—</td>
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<tr>
<td>TEM-46</td>
<td>—</td>
<td>1 (C-III)</td>
<td>IV</td>
<td>—</td>
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<td>—</td>
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<td>P. mirabilis (4)</td>
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<tr>
<td>CTX-M-1</td>
<td>—</td>
<td>1 IV</td>
<td>—</td>
<td>1</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>TEM-24 (2)</td>
<td>—</td>
<td>2 IV</td>
<td>—</td>
<td>1</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>TEM-21</td>
<td>—</td>
<td>1 IV</td>
<td>—</td>
<td>—</td>
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<td>—</td>
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<tr>
<td>C. koseri (4)</td>
<td>—</td>
<td>4 (C-IV)</td>
<td>II, IV (3)</td>
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Table 2. Continued

<table>
<thead>
<tr>
<th>Species and ESBL type</th>
<th>E. coli phylogroup</th>
<th>No. of strains (PFGE cluster)b</th>
<th>Geographical area</th>
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<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>TEM-1-like</td>
<td>OXA-1-like</td>
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<td><strong>K. pneumoniae (3)</strong></td>
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<tr>
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<td>I</td>
<td>IV</td>
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<td>TEM-15</td>
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<td>I</td>
<td>IV</td>
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<td>TEM-21</td>
<td>—</td>
<td>I</td>
<td>II</td>
<td></td>
<td>—</td>
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<tr>
<td><strong>C. freundii</strong></td>
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<tr>
<td>TEM-52</td>
<td>—</td>
<td>I</td>
<td>III</td>
<td></td>
<td>—</td>
<td>I</td>
</tr>
<tr>
<td><strong>Providencia stuartii</strong></td>
<td></td>
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<tr>
<td>TEM-46</td>
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<td>I</td>
<td>I</td>
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<tr>
<td><strong>K. oxytoca</strong></td>
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<tr>
<td>SHV-12</td>
<td>—</td>
<td>I</td>
<td>IV</td>
<td></td>
<td>—</td>
<td>I</td>
</tr>
</tbody>
</table>

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*aNumber of strains is indicated in parentheses when >1.
*bThe pulsotype is indicated when considered as similar with a similarity percentage >68%.
*cNumber of patients who had no medical contacts (prior hospitalization, home care, healthcare profession and caregivers in their household).
*dFOX, cefoxitin; OFX, ofloxacin; GEN, gentamicin; AMK, amikacin; SXT, co-trimoxazole; CHL, chloramphenicol; TET, tetracycline.
*eOne strain with the *armA* gene.
*fTwo strains of *E. aerogenes* were intermediately susceptible to cefoxitin (MIC, 16 mg/L), due to a non-inducible chromosomal AmpC cephalosporinase as described previously.16
*gOne strain of *E. aerogenes* harboured a low-level tobramycin resistance phenotype, as described previously.16
*hDecrease in an OmpF-like porin, as indicated by the outer membrane protein analysis (data not shown).
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(MICs, 32–128 mg/L) probably due to a decrease in an OmpF-like porin, as visualized by the outer membrane protein analysis (data not shown).

Epidemiological typing

The 40 CTX-M-producing E. coli mainly belonged to the phylogenetic group A (40%; subgroup A2, 94%), followed by the groups B2 (37.5%; B2a, 100%), D (20%; D8, 87.5%) and B1 (2.5%) (Table 2). Among the 23 CTX-M-15-producing E. coli, the phylogroup B2 was predominant (57%). Considering a cluster defined at a 68% similarity level,5 the 13 E. coli of the subgroup B2 divided into four PFGE patterns, including 10 strains assigned to a similar pulsotype C-I. To corroborate the phylogenetic relationship of these 10 strains, MLST and O25b antigen serotyping were performed and showed that they all belonged to the clone ST131-O25b. The other CTX-M-producing E. coli exhibited totally different pulsotypes, including the 3 remaining B2 CTX-M-15-producing E. coli and the 12 A1 E. coli with CTX-M-15 (8 strains) and CTX-M-1 (4 strains). Among the non-CTX-M-producing E. coli, the phylogroup A1 was also predominant (Table 2), but no relationship could be found between the strains, except for two of the five TEM-52-producing E. coli (profile C-II), despite different geographic origins (areas I and III).

The nine TEM-24-expressing E. aerogenes displayed a similar genetic background, and their PFGE pattern was similar to that of the E. aerogenes strain harbouring the TEM-46 enzyme (profile C-III). Finally, the other strains analysed by PFGE were distinct, except for the four TEM-3-producing C. koseri, which showed either identical profiles (two strains, region IV) or a closely related one (two strains, regions II and IV).

\textit{bla}_{\text{CTX-M}}, \textit{bla}_{\text{DHA-1}} and \textit{qnrB4} genetic environment

The ISEcp1-like and ISCR1 elements were located upstream from 88% (37 strains) and 5% (2 strains \textit{bla}_{\text{CTX-M-9}}) of the 42 \textit{bla}_{\text{CTX-M}} genes, respectively. PCR amplification for ISEcp1-like and ISCR1 was negative for three strains (two \textit{bla}_{\text{CTX-M-14}} and one \textit{bla}_{\text{CTX-M-27}}). The SHV-12-producing \textit{K. oxytoca} strain, which belonged to the phylogenetic group K0 \textit{bla}_{\text{OXY-5.2}} gene, contained the \textit{qnrB4} and \textit{bla}_{\text{DHA-1}} genes 4.1 kb apart in the vicinity of phage shock protein genes, on the 8.161 kb plasmid fragment analysed [Figure S1, available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/)].

Discussion

This first nationwide survey on ESBLE in the French community setting showed an overall prevalence rate of 1.1% from UTIs in 2006. Such prevalence is only slightly lower than that reported in 2005 for 88 French hospitals (1.5%).15 It is similar to those recorded in the community of a neighbouring country, i.e. Spain (1.4% to 1.7%).28 When comparing the 0.3% proportion observed in a regional survey (area IV) in 1999, our findings of 1.4% in the same region in the present survey suggest a significant increase in ESBLE in the French community.12,13

Such an increase in ESBLE could be ascribed to the expansion of CTX-M-producing \textit{E. coli} (prevalence rate, 0.73% in 2006 versus 0% in our regional survey in 1999) in France as in other European countries.7,9–11 Indeed, two-thirds of the ESBLE recovered in the present study were \textit{E. coli} and most of them (83%) expressed CTX-M enzymes. However, the CTX-M-producing \textit{E. coli} formed a very heterogeneous population: part of the strains belonged to the less-virulent commensal phylogenetic groups A and B1, while others belonged to the virulent extra-intestinal phylogenetic groups B2 and D,26,28 and thus expressed seven varieties of CTX-M enzymes. Nevertheless, among the 23 \textit{E. coli} of the subgroup B2, more than half were CTX-M-15-producers. Using a fine resolution tool (PFGE clustering analysis classically defined by a percent similarity exceeding 85%), only four of them appeared to be clonal. However, based on a lower percent similarity,3 six other strains could be considered as related. Furthermore, MLST and serotyping analysis showed that all 10 strains belonged to the ST131-O25b clone disseminated worldwide,24,25 as in the community of all French regions. These results are consistent with a CTX-M dissemination associated with different \textit{E. coli} lineages, and the occasional emergence of particularly well-adapted strains, such as the ST131-O25b clone.26 In our study, two non-\textit{E. coli} strains, i.e. \textit{K. pneumoniae} and \textit{P. mirabilis}, also expressed a group 1 CTX-M enzyme. Such strains might once supplant CTX-M-producing \textit{E. coli}, at least in the hospital environment, as illustrated by the recent dissemination of nosocomial CTX-M-15-producing \textit{K. pneumoniae} in Hungary, representing 97% of all CTX-M producers.30 Following CTX-M-15, CTX-M-1 and CTX-M-14 were the most frequent enzymes as in nearby countries, Italy and Spain.1 A great variety of CTX-M enzymes has been identified in French hospitals, including the remaining CTX-M-2, CTX-M-3, CTX-M-9 and CTX-M-27 ESBLEs characterized here.1,2 CTX-M-27 only differs from the more common CTX-M-14 by the substitution D240G, which confers the advantage of higher levels of ceftazidime resistance, as in CTX-M-15.31

The explosive dissemination of different CTX-M enzymes has been related to the fact that the genes encoding these enzymes are associated with highly transmissible mobile genetic elements.1 In particular, ISEcp1 has been shown to transfer \textit{bla}_{\text{CTX-M}} genes from the bacterial chromosome of \textit{Kluyvera} spp. to conjugative plasmids.1 In agreement with the literature, most of the \textit{bla}_{\text{CTX-M}} genes of our study were linked to an ISEcp1-like element, except the two \textit{bla}_{\text{CTX-M-9}} genes that were embedded in a complex class 1 integron bearing ISCR1. The presence of phage-related elements or insertion/deletion events within ISEcp1 might explain the negative PCR results for three group 9 \textit{bla}_{\text{CTX-M}} genes.3

Our case–control study confirmed that a recent hospitalization (or residence in private healthcare institutions) is a major risk factor for developing community-onset ESBLE-producing bacterial infections.28,32,33 Up to 80% of the patients received a recent antibiotic treatment, confirming that the previous use of fluoroquinolones and \beta-lactams was a risk factor for selection of ESBLE.7,28,33 However, it is noteworthy that except for antimicrobial therapy, all risk factors documented in our study for acquiring ESBLE were less frequent when the ESBLE belonged to the CTX-M family, especially advanced age and home care (P<0.05). In particular, 13 patients (18.3%) were considered as infected by a true community-acquired ESBLE. In 12 of them, the ESBLE was a CTX-M-producing \textit{E. coli}, including 5 belonging to the phylogroup B2 and the remaining ones divided into the group A1 or D2. They carried various CTX-M enzymes, and only one of them belonged to the ST131-O25b clone. Ben-Ami et al.33 also reported that nearly 20% of the
patients infected with ESBLEs at the time of hospital admission had no identifiable risk factors. Altogether, these data reinforce the hypothesis that the CTX-M enzymes have a reservoir other than the hospital. In addition, CTX-M-producing E. coli have been encountered in sick or healthy animals, and in their meat products. These potential ESBL sources or reservoirs probably increase the dispersal of resistance in healthy people.

The non-CTX-M-producing strains, including eight E. coli, essentially produced TEM-type ESBLs (93%, seven varieties of enzymes) and rarely SHV-type ESBLs (7%, 1 SHV-12). Among them, the TEM-24 or TEM-46 positive E. aerogenes belonged to the epidemic clone prevalent in French hospitals at the end of the 1990s. Interestingly, TEM-46 differs from TEM-24 by the single amino acid substitution A237T that confers lower hydrolytic capabilities. Although there is a progressive decline of this clone in the hospital setting, it always represents a stable fraction of the Enterobacter spp. in the community (ca. 4%), but tends to a higher and unusual antibiotic susceptibility. These isolates have been found in community patients with a past history of repeated hospitalizations due to a prolonged digestive carriage. Four TEM-3-producing C. koseri have been identified, which exhibited a cefoxitin resistance usually characteristic of C. freundii. This resistance could be ascribed to the reduced production of an OmpF-like porin. TEM-3-producing E. coli (as one example here) has been among the first ESBL responsible for nosocomial outbreaks in the 1980s. Later, French nosocomial outbreaks caused by TEM-3-producing C. koseri have been reported. Cefoxitin-resistant mutants may have emerged once under therapy and have persisted in patients discharged from hospital as TEM-24-producing E. aerogenes. Finally, other TEM- and SHV-producing strains found in our study included TEM-15, TEM-29, TEM-52 and SHV-12 producers. These strains, mainly those associated with TEM-52, have been described in hospitals in France and in bordering countries such as Italy. We have also previously detected a TEM-15 enzyme in K. pneumoniae in the French community. In addition, TEM-52 and SHV-12 producers have been described in animals and in river waters, indicating that all ESBLs, and not exclusively CTX-M enzymes, are now largely disseminated out of hospitals.

Most of the ESBLs were resistant to the other classes of antibiotics, in particular fluoroquinolones and/or co-trimoxazole, which are commonly prescribed by general practitioners, especially for UTIs. Furthermore, our study has revealed the presence of plasmid-mediated broad-spectrum resistances co-existing with ESBLs, such as the plasmid-mediated cephalosporinase DHA-1 in one CTX-M-15-producing E. coli, and in the SHV-12-producing K. oxytoca. Another CTX-M-15 E. coli harboured the gene armA encoding the 16S rRNA methylase that confers pan-aminoglycoside resistance. Although most of the ESBLs were resistant to fluoroquinolones, a single strain carried a qnr determinant, the qnrB4 allele. However, other plasmid-mediated resistances such as aac(6’)-Ib-cr genes have been reported to confer quinolone resistances. DHA-1, ArmA and QnrB are widespread in Asia, but are rarely encountered in Europe, particularly in France, among nosocomial isolates or occasionally in animal strains. This is the first description in the community setting for ArmA worldwide and for DHA-1 and QnrB4 in France. Indeed, one E. coli strain harbouring qnrB4 and blaDHA-1 and six Italian E. coli carrying a qnrB-like gene have been just reported in the community setting in Switzerland and Italy, respectively. In the K. oxytoca strain of this study, transfer experiments showed that the qnr and blaDHA-1 genes were linked, while blaSHV-12 was carried by another conjugative plasmid. Sequence analysis demonstrated that the fragment encompassing qnrB4 and blaDHA-1 was identical to that found in the plasmid pHS7 of K. pneumoniae isolated in China and, although unmentioned by the authors, in the plasmid pPMDHA of one Parisian strain of K. oxytoca. In the future, it will be interesting to include recent foreign travels as risk factors for acquiring multidrug-resistant bacteria.

In conclusion, this survey has highlighted a significant increase in ESBL in the French community setting, up to 1.1% of the urinary enterobacteria. Analysis of these ESBLs has revealed a great diversity of bacterial species and enzymes. Nevertheless, most were CTX-M-producing E. coli, including a cluster of the pandemic CTX-M-15-expressing clone ST131-O25b. Furthermore, clones harbouring determinants that confer still unusual resistances such as DHA-1, ArmA or QnrB4 were present in these community strains. This worrisome development implies enhanced capabilities for the detection of resistance mechanisms by private as well as public laboratories, in order to implement appropriate infection control practices, and to prescribe adapted chemotherapeutic agents.

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Transparency declarations

None to declare.

Supplementary data

Figure S1 and Table S1 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).
ESBL-producing enterobacteria in the French community

References


