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ARTICLE

Increase in hospital-acquired bloodstream infections caused by extended spectrum β-lactamase-producing *Escherichia coli* in a large French teaching hospital

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Abstract Our goal was to determine the characteristics and the mode of acquisition of healthcare-associated bacteraemia due to CTX-M-producing *Escherichia coli* in a 1,800-bed hospital. Sixteen extended-spectrum β-lactamase (ESBL)-producing *E. coli* strains were collected between 2001 and 2006 from patients with bloodstream infections. The incidence density of these infections increased from 0.002 to 0.02 per 1,000 days of hospitalisation during the study period. Most of the strains (87%) produced a CTX-M-type enzyme associated with TEM-1 (86%), OXA-30 (50%), AAC(3)-II (57%), AAC(6′) (50%) and QnrS1 (7%). When present (n=8), the $bla_{\text{CTX-M-15}}$ gene was always located downstream of the insertion sequence IS*Ecp1*. Co-resistance was generally observed: fluoroquinolones (81%), trimethoprim-sulfamethoxazole (62%) and/or aminoglycosides (69%). Although the strains were found to be genetically unrelated, most of the cases were hospital-acquired (69%) or healthcare-associated (25%), underlining the need for infection control measures to limit the spread of ESBL-producing *E. coli* in hospital settings.

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Introduction

The first two CTX-M-type extended-spectrum β-lactamases (ESBLs) were isolated in 1989 in Europe and in Argentina respectively [1, 2]. The number of CTX-M-type variants described has increased steadily since 1995 [3]. Since the early 2000s, CTX-M-type enzymes have spread worldwide among *Escherichia coli* strains [4, 5] and the threat of severe infections such as bloodstream infections caused by ESBL-producing *E. coli* have become a reality [6]. When bacteraemia is caused by ESBL producers, there is a delay in initiating an appropriate antibiotic treatment and high mortality rates are reported [6, 7]. Indeed, CTX-M production is frequently associated with resistance to fluoroquinolones [6–10], and empirical treatment by third-generation cephalosporins or fluoroquinolones, two groups of antibiotics often used for bloodstream infections suspected of being caused by *E. coli*, is inadequate in this context. Studies have shown that CTX-M-producing *E. coli* have spread in the community [11, 12], constituting a wide reservoir for these organisms. Since the circumstances and the consequences of the emergence of ESBL *E. coli* in severe infections are still not well understood, despite several studies [6, 7, 13, 14], this work was performed with the objective of analysing the clinical and epidemiological features of these infections over a period of 66 months, as well as the phenotypic, molecular and virulence aspects.

Materials and methods

Study design and patients

The study was conducted at Pitié-Salpêtrière (PS) hospital, Paris, France, a 1,800-bed teaching hospital that began to survey ESBL Enterobacteriaceae in the mid 1980s [15]. The LIS database of the Clinical Microbiology Laboratory was retrospectively analysed for the period January 2001 to June 2006 to identify patients who had had an episode of bacteraemia caused by a strain of *E. coli* with an ESBL-compatible pattern during their stay in PS hospital, i.e. with a positive double disk synergy test, a test that has been systematically applied since 1985 to each strain of enterobacteria in this laboratory [15, 16]. If several blood cultures from the same patient were positive for an *E. coli* strain with an ESBL-compatible pattern, the first isolate was selected for further study. Upon review of the patients' records, the following data were collected: age, sex, hospital location, date of admission and length of stay in hospital, date of collection of positive specimen(s), primary site of infection, admission to any hospital during the previous year, use of an intravascular device, urinary catheter or mechanical ventilation before bacteraemia, previous antibiotherapy during the hospital stay, antibiotic treatment received for ESBL *E. coli* bacteraemia and outcome.

Nosocomial infection was defined as an infection that occurred >48 h after admission to the hospital in a patient with no sign of infection at admission. Bacteraemia that occurred within the first 48 h after admission was further classified as healthcare-associated if any of the following criteria were present: >48-h hospital admission during the previous 90 days, chronic haemodialysis, chronic treatment by intravenous medication or home wound care in the previous 30 days, or residence in a nursing home or long-term care facility. Otherwise, the cases were considered to be community-acquired [17].

Bacterial strains and susceptibility assays

The strains included were re-identified by the API 20E system (bioMérieux, Marcy l'Etoile, France) and susceptibility to antimicrobial agents was checked using the disk diffusion method and interpreted according to the guidelines of the Antibiogram Committee of the French Society for Microbiology (CA-SFM) (www.sfm.asso.fr). The double disk synergy test was performed on MH agar plates with disks of cefotaxime, ceftazidime, cefepime, aztreonam on one side and amoxicillin-clavulanic acid-containing disks on the other [16]. The synergy between cephalosporin and clavulanic acid was quantified using Etests CT/CTL and TZ/TZL (AB Biodisk, Solna, Sweden), combining cefotaxime or ceftazidime with clavulanate, performed on MH agar plates. The ESBL Etest was considered positive according to the manufacturer's protocol.

Beta-lactamase characterisation and screening of resistance genes

The strains included were screened using the PCR primers given in Table 1 for the presence of the following β-lactamase genes: *bla* $_{\text{TEM}}$, *bla* $_{\text{SHV}}$, *bla* $_{\text{CTX-M}}$, *bla* $_{\text{OXA-30}}$, as well as other genes of resistance to fluoroquinolones, *qnrA*, *qnrB* and *qnrS*, and to aminoglycosides *aac(3)II* and *aac (6')-Ib*. Amplicons were then sequenced using the Big Dye Terminator cycle sequencing ready reaction kit (Applied Biosystems, Foster City, CA, USA) in an ABI Prism 310 DNA sequencer (Applied Biosystems). The sequences obtained were compared with the sequences available (GeneBank).

Genetic environment of $bla_{\text{CTX-M-15}}$

The genetic environment of $bla_{\text{CTX-M-15}}$ was characterised by PCR with the primers listed in Table 1. The region located upstream of the $bla_{\text{CTX-M-15}}$ gene was amplified with forward primers hybridising to the insertion sequence IS*Ecp1* and with the $bla_{\text{CTX-M}}$ reverse primer; amplicons were sequenced on both strands.

Molecular typing

The strains included were also evaluated for genetic relatedness using Pulsed-Field Gel Electrophoresis (PFGE) with *Xba* I (New England Biolabs, Ipswich, MA, USA) digestion of genomic DNA. DNA fragments were separated with a CHEF-DRII system (Biorad) through a 1% agarose gel in 0.5X Tris-borate-EDTA buffer. Conditions for migration were as follows: temperature, 14°C; voltage, 5V/cm; pulses, 5–40 s for 21 h. Gels were stained using ethidium bromide and photographed under UV illumination. Fingerprints were first interpreted visually, then with the Gelcompar software (Applied Maths, Sint-Martens-Latem, Belgium) according to the criteria suggested by Tenover et al. [18].

Phylogenetic grouping

The phylogenetic group was determined for each of the strains included with markers *yjaA* and *chuA*, and the DNA fragment TSPE4.C2 [19]. Those markers were screened by PCR as described above, with the primers listed in Table 1. The results were interpreted according to Clermont et al. [19].

Table 1 Nucleotide sequences of primers used in the extended-spectrum β-lactamase (ESBL) *Escherichia coli* study

Primer	Nucleotide sequence 5′→3′	Gene/location
TEM-A	GAG TAT TCA ACA TTT CCG TGT C	*bla* $_{TEM}$
TEM-B	TAA TCA GTG AGG CAC CTA TCT C	
SHV-bis	ATG CGT TAT ATT CGC CTG TGT ATT	*bla* $_{SHV}$
SHV-rev	GCG TTG CCA GTG CTC GAT CAG CGC	
M-13 upper	GGT TAA AAA ATC ACT GCG TC	*bla* $_{CTX-M=1group}$
M-13 lower	TTG GTG ACG ATT TTA GCC GC	
M-9 upper	ATG GTG ACA AAG AGA GTG CA	*bla* $_{CTX-M=9group}$
M-9 lower	CCC TTC GGC GAT GAT TCT C	
M-25 upper	ATG ATG ACT CAG AGC ATT CG	*bla* $_{CTX-M=2group}$
M-25 lower	TGG GTT ACG ATT TTC GCC GC	
OXA1A	CAC AAT ACA TAT CAA CTT CG	$bla_{OXA-1group}$
OXA1-2	TAG TGT GTT TAG AAT GGT GA	
ChuA.1	GAC GAA CCA ACG GTC AGC AT	*chuA*
ChuA.2	TGC CGC CAG TAC CAA AGA CA	
YjaA.1	TGA AGT GTC AGG AGA CGC TG	*yjaA*
YjaA.2	ATG GAG AAT GCG TTC CTC AAC	
TspE4C2.1	GAG TAA TGT CGG GGC ATT CA	TSPE4.C2
TspE4C2.2	CGC GCC AAC AAA GTA TTA CG	
AAC(6)ID	CAT GAC TGA GCA TGA CCT T	*aac(6′)-Ib*
AAC(6)IU	GAA GGG TTA GGC ATC ACT	
AAC3F	CAA TAA CGG AGG CAA TTC G	*aac(3)-II*
AAC3R	GAT TAT CAT TGT CGA CGG	
qnrA	TCAGCAAGAGGATTTCTCA	*qnrA*
	GGCAGCACTATTACTCCCA	
qnrB	AGCGGCACTGAATTTAT	*qnrB*
	GTTTGCTGCTCGCCAGTC	
qnrS	GGA AAC CTA CAA TCA TAC ATA	*qnrS*
	GTC AGG ATA AAC AAC AAT ACC	
ISEcp1A	GCA GGT CTT TTT CTG CTC C	IS*Ecp1 tnpA*, forward
PROM+	TGC TCT GTG GAT AAC TTG C	IS*Ecp1* promoter, reverse
PROM-	GCA GTC TAA ATT CTT CGT G	IS*Ecp1* right part, reverse
CTX-MA2	CCG CA TAT GT TGG TGG TG	bla_{CTX-M}, reverse

Results

During the period January 2001 to June 2006, 18 episodes of bacteraemia caused by a strain of *E. coli* with an ESBL-compatible pattern were identified. ESBL production was confirmed by the double disk synergy test and by ESBL Etests for 16 of the 18 strains. For the 2 remaining strains, the result of the synergy test between β-lactam and clavulanate was not compatible with ESBL production when quantified using ESBL Etests. In these 2 strains, bla_{OXA-30}, but no ESBL, was detected and the two corresponding episodes were subsequently excluded.

Overall, the 16 included cases represented 1.5% of *E. coli* bacteraemia episodes for the study period. However, this proportion increased from 0.4 to 4.3% between 2001 and the first semester of 2006, as shown in Fig. 1 (Chi-squared

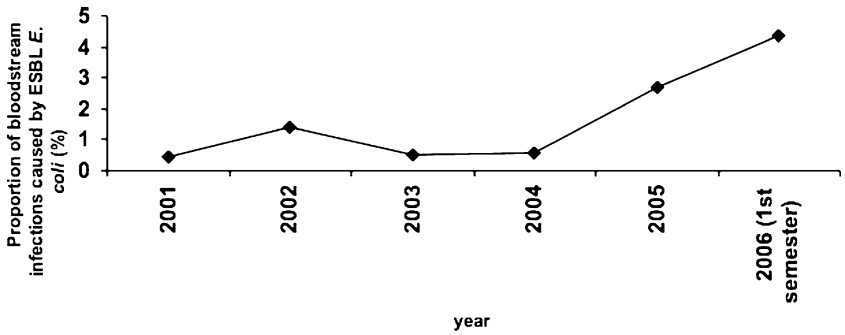

Fig. 1 Evolution of the proportion of bloodstream infections caused by an extended-spectrum β-lactamase (ESBL)-producing strain among bloodstream infections caused by *Escherichia coli*

test for trend: 7.28, p=0.007). The incidence density of bacteraemia caused by ESBL-producing *E. coli* increased from 0.002 to 0.02 per 1,000 days of hospitalisation.

As shown in Table 2, the median age was 52 (range 26–69, mean 51.5±11.1 years). Seven patients had been hospitalised at least once during the year before their stay in PS Hospital. The mean and the median of the duration of stay in PS hospital before the sampling of positive blood culture were 23±30 and 10 days respectively. Before this sampling, the majority of the patients had stayed in the intensive care unit for at least 5 days (9 out of 16, 56%), had had at least one invasive procedure (13 out of 16, 81%), mainly a urinary catheter (n=11), an intravascular device (n=12) or mechanical ventilation (n=6), or had received previous antibiotic therapy (11 out of 16, 69%), mainly third generation cephalosporin (n=6) or fluoroquinolone (n=8). The primary source of ESBL *E. coli* infection was established in 13 patients: urinary tract (n=8), respiratory tract (n=3), catheter-associated infection (n=1), mediastinitis (n=1) and infection of the ascitic fluid (n=1). On the basis of the data collected, 11 out of the 16 cases were considered to be hospital-acquired, 4 as health-care-associated and only 1 as community-acquired.

Table 2 Demographic and clinical characteristics of the 16 cases of ESBL *Escherichia coli* bacteraemia

Characteristic	Value or number of patients
Age, mean ± SD	51.5±11.1
Male:female ratio	10:6
Admission to any hospital during the previous year	7
Mean (± SD) length of hospital stay before first positive blood culture	22.9±30.4 days
(median, range)	10.5 (0–99) days
Hospitalisation in the ICU at the time of the first positive blood culture	9
Prior invasive procedure	13
Urinary catheter	11
Vascular catheter	9
Implantable port	3
Mechanical ventilation	6
Primary site/origin of infection	
Urinary tract	8
Lung	3
Intravascular catheter-related infection	1
Surgical site	1
Peritoneum	1
Not established	2
Prior use of antibiotics during the hospital stay	11
Third generation cephalosporins	6
Fluoroquinolones	8
Imipenem	4
Acquisition of ESBL *E. coli* bloodstream infection	
Nosocomial	11
Health care-associated	4
Community-acquired	1
Antimicrobial therapy with imipenem	
Before the results of the drug susceptibility pattern	5
After the results of the drug susceptibility pattern	
Imipenem alone	1
Imipenem + amikacin	9
Imipenem + gentamicin	2
Imipenem + fluoroquinolone	1
Imipenem + fluoroquinolone + aminoglycoside	1
Imipenem + amikacin + fosfomycin	1
30-day mortality	3

ICU, Intensive Care Unit

Five (31%) patients were empirically treated with imipenem shortly after sampling, but before the results of susceptibility tests. All but one patients were treated with imipenem after the diagnosis (the latter patient died before the results were available), in association with aminoglycosides in 13 cases. Three of the 16 patients (19%) died within 30 days of the diagnosis: 2 from septic shock and 1 from post-sternotomy mediastinitis. Death was directly attributed to ESBL *E. coli* bacteraemia in these three cases.

The results of susceptibility tests and content in ESBL-encoding genes for the 16 strains are shown in Table 3. A $bla_{\text{CTX-M}}$ gene was detected in 14 (87%) strains: either $bla_{\text{CTX-M-15}}$ (n=8), $bla_{\text{CTX-M-1}}$ (n=3), $bla_{\text{CTX-M-9}}$ (n=2) or $bla_{\text{CTX-M-14}}$ (n=1). The two remaining strains produced TEM-52. CTX-M-15 was always associated with another β-lactamase: OXA-30 and TEM-1 (n=4), OXA-30 alone (n=1), or TEM-1 alone (n=3). Five of the six strains producing CTX-M-1, CTX-M-9 or CTX-M-14 also produced TEM-1 and OXA-30 (n=2) or TEM-1 alone (n=3). The two strains producing TEM-52 did not carry any other *bla* genes. The majority of the strains (13 out of 16) were resistant to fluoroquinolones, but only one carried a *qnr* gene, *qnrS1* in a CTX-M-15-producing isolate. Almost all (11 out of 13) CTX-M-producing strains carried either both *aac(3)-II* and *aac(6')-Ib* genes (4 CTX-M-15-, 1 CTX-M-1- and 1 CTX-M-9-producing strains) or *aac(3)-II* alone (1 CTX-M-9- and 1 CTX-M-14-producing strain). CTX-M-1, -9 and -14 were always associated with resistance to tetracycline and sulfonamides, whereas CTX-M-15 strains were mainly susceptible to both drugs or resistant to tetracycline alone.

The insertion sequence IS*Ecp1* was identified at 48 bp upstream of the $bla_{\text{CTX-M}}$ gene in all CTX-M-15-producing strains. In three strains (03-1, 06-4 and 06-5), PCR amplification was obtained with IS*Ecp1* PROM+ primer, but not with IS*Ecp1*A primer, suggesting the presence of a disrupted IS element.

Table 3 Characteristics of the 16 ESBL-producing *Escherichia coli*

ESBL	Strain[a]	Other resistance genes				Resistance pattern			Phylogenetic group
		bla (β-lactamase)	*qnr*	*aac(6')-Ib*	*aac(3)-II*	Quinolone		Other traits[d]	
						NA[b]	CIP[c]		
CTX-M-15	02-2	TEM-1, OXA-30	Negative	Positive	Positive	Positive	Positive	None	$B2_3$
	02-3	TEM-1, OXA-30	Negative	Positive	Positive	Positive	Positive	Te, Sul	A_1
	03-1	TEM-1, OXA-30	Negative	Positive	Positive	Positive	Positive	Te	$B2_3$
	05-2	TEM-1	Negative	Negative	Negative	Negative	Negative	None	$B2_3$
	05-3	TEM-1	Negative	Negative	Negative	Positive	Positive	Te	A_1
	06-1	TEM-1	Negative	Negative	Negative	Positive	Positive	None	D_2
	06-4	TEM-1, OXA-30	*qnrS1*	Negative	Negative	Positive	Positive	Te	$B2_3$
	06-5	OXA-30	Negative	Positive	Positive	Positive	Positive	Te	A_1
CTX-M-1	04-1	None	Negative	Negative	Negative	Negative	Negative	Te, Sul	D_2
	05-5	TEM-1	Negative	Negative	Negative	Negative	Negative	Te, Sul	A_1
	06-3	TEM-1, OXA-30	Negative	Positive	Positive	Positive	Positive	Te, Sul	D_2
CTX-M-9	01-1	TEM-1, OXA-30	Negative	Positive	Positive	Positive	Positive	Te, Sul	D_2
	05-1	TEM-1	Negative	Positive	Positive	Positive	Positive	Te, Sul	D_2
CTX-M-14	05-4	TEM-1	Negative	Negative	Positive	Positive	Positive	Te, Sul	$B2_3$
TEM-52	02-1	None	Negative	–	–	Positive	Positive	Sul	B1
	06-2	None	Negative	–	–	Positive	Positive	Sul	A_1

[a] The first two digits indicate year, the third digit indicates the rank of ESBL-producing *E. coli* within each year
[b] NA, nalidixic acid; positive, MIC > 8 mg/L
[c] CIP, ciprofloxacin; positive, MIC > 0.5 mg/L
[d] Te, tetracycline; Sul, sulfonamides

According to the criteria commonly used [18], the PFGE patterns obtained with the *Xba* I-restricted DNA were unrelated, even for the CTX-M-15-producing isolates (data not shown). Distribution into phylogenetic groups was as follows: 5 isolates belonged to each of the $B2_3$, D_2 and A_1 groups, whereas the last isolate belonged to the B1 group (Table 3). Concerning specifically the 8 CTX-M-15-producing strains, 4 belonged to group $B2_3$, 3 to group A_1 and 1 to group D_2. In contrast, only 1 of the 6 other CTX-M-producing strains belonged to the $B2_3$ group.

Discussion

Several recent studies have investigated ESBL *E. coli* bacteraemia, but this is the first study to focus on the emergence of ESBL-producing *E. coli* bloodstream infections in France, where they were virtually absent before 2000, as shown by national surveillance (www.onerba.org). At PS hospital, the largest teaching hospital in France, the yearly number of such infections increased from 1 case in 2001 to 5 cases in 2005, and 5 cases for the first semester of 2006, leading to a 10-fold increase in the proportion of ESBL strains among *E. coli* bacteraemia cases (from 0.4 to 4.3%), and in the incidence rate of ESBL *E. coli* bacteraemia cases (from 0.002 to 0.02 per 1,000 days of hospitalisation). This phenomenon has also been observed in southern Spain by Rodríguez-Baño et al., who reported an increase from 6 cases in 2001 to 16 cases in 2004 in a 950-bed teaching hospital [6]. The increase in infections due to ESBL *E. coli* in our hospital is consistent with the increasing proportion of strains resistant to third generation cephalosporins in *E. coli* bacteraemia cases assessed in France during the same period through the European Antibiotic Resistance Surveillance System (EARSS, *www.rivm.nl/earss*), from <1 to 2% between 2002 and 2006. Despite a clear increase, the proportion of strains resistant to third generation cephalosporins among *E. coli* isolated from blood culture remained in 2006 markedly lower in our study than in other European countries, e.g. 8.8% in Spain in 2001–2005 and 13% in the UK in 2003–2005 [6, 7].

In our study, most of the strains (14 out of 16, 87%) produced a CTX-M-type ESBL, a rate somewhat higher than in other studies on ESBL *E. coli* bacteraemia during the same period: 70% in southern Spain and 37% in Italy [6, 9]. Moreover, the results of the two latter studies contrast with ours due to the fact that CTX-M-15 was absent in southern Spain or weakly represented in Rome (3%) among CTX-M-type ESBLs [6, 9]. The high proportion of CTX-M as a whole, and more precisely of CTX-M-15, we report here is consistent with the place of these emerging β-lactamases among ESBL *E. coli* isolated from clinical samples in France during the first half of 2000s, ranging between 40 and 76% [10, 20, 21]. CTX-M-

15 differs from CTX-M-3 due to an Asp-240-Gly substitution that increases its catalytic efficiency against ceftazidime [22]. This feature likely confers an epidemiological advantage to CTX-M-15 in a hospital setting, where ceftazidime is largely used (www.esac.ua.ac.be).

Not surprisingly, almost all (13 out of 14) the CTX-M-producing *E. coli* carried at least one other β-lactamase-encoding gene, mainly TEM-1, OXA-30 or both. Half of the CTX-M-15-producing strains carried both genes, a combination already encountered in a single plasmid [23–25]. Our results also show high rates of co-resistance to potentially active drugs, e.g., fluoroquinolones (81%), trimethoprim-sulfamethoxazole (62%) and aminoglycosides (69%), as already found in other studies on ESBL *E. coli* bacteraemia [6–9, 20, 21]. In this study, the AAC(3)-II and AAC(6′) determinants were identified in half of the CTX-M-producing strains. However, only 3 of the 8 CTX-M-15-producing strains carried the combination of the four determinants OXA-30, TEM-1, AAC(3)-II and AAC(6′) that has been described in the 92-kb plasmid pC15-1a in association with the tetracycline resistance gene *tetA* [23], whereas the other strains exhibited three distinct combinations of these four genes, suggesting high genomic variability. As described in several studies, the $bla_{\text{CTX-M-15}}$ gene was always located 48 bp downstream of the insertion sequence IS*Ecp1,* which was disrupted in several strains [20, 23, 26–28].

Plasmid-mediated resistance to quinolones (*qnrS1*) was detected in only one CTX-M-15-producing strain (12%). The proportion of *qnr*-carrying strains in France was 8% of CTX-M *E. coli* in 2004, and 3.3% of ESBL-producing Enterobacteriaceae in 2004 [29, 30]. The *qnr* genes found in these two studies were all *qnrA*. The gene *qnrS1* was found in some ESBL-producing Enterobacteriaceae in 2002–2005 in Paris, but always associated with CTX-M-1 [31]. The same gene has also been found in association with CTX-M-14, CTX-M-24 [32], CTX-M-9 and recently with CTX-M-15 in an *Enterobacter cloacae* isolate [33].

The primary sites of *E. coli* bacteraemia were mainly urinary tract infections (50%) in our study, as in a few others [6, 7], contrasting with other studies that reported lower proportions of this type of infection. These differences could be due to the different frequency of a urinary catheter in the study population, a major risk factor for UTI and bacteraemia [8, 9, 13, 14]. Pulmonary infections (19%) were the second most common primary sites of infections, similar to rates previously reported [8, 13], but higher than those in other studies reporting proportions of only 0–5% [6, 7, 9]. The high frequency of pulmonary infections in our study can be explained by the high frequency of mechanical ventilation (37%).

In our study, the median duration of stay in hospital before blood culture positive for ESBL *E. coli* was 10 days. Rodríguez-Baño et al. reported a median duration of stay of 26 days and found that a previous long hospital stay was a risk factor for ESBL-producing isolates in patients with nosocomial bacteraemia [34]. In our study, most patients (81%) had at least one invasive procedure, mainly insertion of urinary (69%) and intravascular catheters (56%), reported to be risk factors for ESBL-producing isolates in patients with bacteraemia [34, 35]. The majority of the patients included in our study (68%) had received previous antibiotic therapy before bacteraemia, mainly third generation cephalosporins (37%) or fluoroquinolones (50%), molecules found in other studies to be associated with ESBL-producing isolates in patients with bloodstream infections [13, 34–36]. ESBL-producing *E. coli*, more precisely CTX-M-producing strains, have often been described as community-acquired [11, 12]. In this study, we found that cases of bacteraemia with these organisms were rarely strictly community-acquired, and only 1 patient (6%) had neither a known previous stay in hospital nor any invasive procedures. This proportion of community-acquired bacteraemia cases among bacteraemia episodes due to ESBL-producing strains in our study is lower than those reported by Rodríguez-Baño et al. and Kang et al. (19% and 37% respectively) [6, 9, 14]. The other cases were either hospital-acquired (69%) or healthcare-associated (25%), showing the importance of hospital acquisition and invasive procedures in the occurrence of ESBL *E. coli* bacteraemia. Moreover, more than half of the patients were hospitalised in the ICU at the time of the positive blood culture, a rate higher than those reported in other studies [7, 8]. The mortality rate (19%) was similar to those reported elsewhere [6, 13, 14], but lower than that (61%) reported by Melzer et al. [7], who found a delay of at least 1 day before the appropriate antibiotic treatment was begun.

E. coli is divided into four main phylogenetic groups: A, B1, B2 and D [19, 37]. Virulent extra-intestinal strains mainly belong to the phylogenetic group B2, and, to a lesser extent, to group D [38], whereas commensal strains mainly belong to groups A or B1. In our study, only a third of the strains belonged to group B2, whereas another third belonged to group A. These results contrast with those reported in Tunisia, where 83% of ESBL *E. coli* isolated from blood culture belonged to the group B2 [39]. The discordance between the severity of the illness and the low-virulence groups can be explained by the iatrogenic origin of those bacteraemia episodes, since among the 6 cases that occurred due to a A or B1 strain, 2 had ventilator-associated pneumonia, 2 an iatrogenic urinary tract infection, 1 a catheter-associated infection, and the last one several invasive procedures.

Although mainly hospital-acquired, the 16 strains studied were unrelated on the basis of resistance patterns,

phylogenetic groups and PFGE patterns, indicating a wide diversity. Therefore, clonal diffusion of one or few strains was not the cause of the nosocomial acquisition in our study, whereas outbreaks of clonally-related ESBL *E. coli* were reported in other healthcare facilities [23, 40].

In conclusion, ESBL-producing *E. coli* is an emerging cause of bloodstream infections in France, and involves mainly CTX-M-15. This emergence corresponds to a rapid change in the epidemiology of those infections. CTX-M-type enzymes have spread within the communities, favouring admission to hospitals of patients with severe community-acquired infections due to CTX-M-15-producing *E. coli*. However, our report demonstrates that these organisms are also causing severe hospital-acquired infections, and that infection control measures commonly applied to other multiple resistant organisms could contribute to limiting their spread into hospital settings.

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