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Frequency and diversity of Class A extended-spectrum β-lactamases in hospitals of the Auvergne, France: a 2 year prospective study

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***Objectives*: To evaluate the frequency and diversity of extended-spectrum β-lactamases (ESBLs) produced by Enterobacteriaceae and *Pseudomonas aeruginosa* in one French region.**

***Methods*: During 2001–2002, all the non-duplicate isolates of *P. aeruginosa* resistant to ceftazidime and of Enterobacteriaceae intermediate or resistant to ceftazidime and/or cefotaxime and/or aminoglycosides with an AAC(6′) I phenotype were collected in nine hospitals of the area. ESBL isoelectric points were determined, *bla* genes were amplified and sequenced and epidemic isolates were genotyped with ERIC2-PCR.**

***Results*: ESBLs were observed in 297 Enterobacteriaceae (0.8%). The most frequent were TEM-3 like (*n* = 152; 51.2%) and TEM-24 (*n* = 115; 38.7%). Four new enzymes were observed, TEM-112 (pI 5.4), TEM-113 (pI 6.3), TEM-114 (pI 5.9) and TEM-126 (pI 5.4). Other TEMs were TEM-8, TEM-12, TEM-16, TEM-19, TEM-20, TEM-21, TEM-29 and TEM-71. The other ESBLs were SHV-4, SHV-5 and SHV-12, CTX-M-1, CTX-M-3, CTX-M-14 and CTX-M-15. In 37 *P. aeruginosa* (0.7%) only one ESBL was observed, PER-1. Five epidemic strains were detected, *Serratia marcescens* TEM-3 and four observed in several hospitals, *Enterobacter aerogenes* TEM-24, *Citrobacter koseri* TEM-3, *Proteus mirabilis* TEM-3 and *P. aeruginosa* PER-1.**

***Conclusion*: ESBL frequency was lower than in 1998, and CTX-M-type frequency higher (2.1% of ESBLs in 2001, 4.9% in 2002). This long-term survey detected new sporadic enzymes (TEM-112, TEM-113, TEM-114 and TEM-126) and interhospital epidemic strains while avoiding any overestimation of ESBL frequency that may otherwise have occurred because of acute epidemics.**

Keywords: ESBLs, Enterobacteriaceae, *Pseudomonas aeruginosa*, epidemiology

Introduction

During the mid-1980s, extended-spectrum β-lactamases (ESBLs) derived from TEM and SHV appeared in *Klebsiella pneumoniae* and *Escherichia coli*.[1,2] The numerous mutations which occurred led to a great diversity of TEMs (>130) and SHVs (>50). The ESBLs disseminated not only to opportunistic species but also to specific pathogens. During the 1990s, new non-TEM non-SHV ESBL types appeared, the most frequent being CTX-M and PER-1, which were responsible for epidemics.[3–10] Despite this

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great diversity, French epidemiological studies showed the persistence and predominance of a restricted number of enzymes such as TEM-3, TEM-24 or SHV-4.[7] The last French multicentre survey, conducted over 3 months in 1998 in 14 hospitals of 11 regions, showed that ESBL-producing strains among Enterobacteriaceae had increased (3.2%), mainly due to the high prevalence of TEM-24-producing *Enterobacter aerogenes* (53.5%), and that the first CTX-M-3-producing strain had appeared in one centre.[7] To evaluate the frequency and the diversity of ESBLs produced by strains of Enterobacteriaceae and *Pseudomonas aeruginosa* in the French region of the Auvergne, a 2 year survey was conducted at the university hospital, the only centre to have identified the ESBLs, and the seven largest non-teaching hospitals. Together, they constituted a regional surveillance network for detecting new ESBLs and known ESBLs that had not been observed in the Auvergne before.

Materials and methods

The Auvergne is a largely mountainous region in central France with a population of 1.4 million. In seven hospitals antibiotic susceptibility testing was performed on Rapid ATB E and ATB PSE (bioMérieux, Marcy l'Étoile, France) and in one laboratory by the disc diffusion method. From 1 January 2001 to 31 December 2002, all the non-duplicate isolates of *P. aeruginosa* resistant to ceftazidime (MIC > 32 mg/L) and of Enterobacteriaceae intermediate or resistant to ceftazidime and/or cefotaxime (MICs > 4 mg/L) and/or aminoglycosides with an AAC(6′) I phenotype, i.e. amikacin (MIC > 8 mg/L), kanamycin (MIC > 8 mg/L) and tobramycin (MIC > 4 mg/L), because ESBLs were often associated with an AAC(6′),[11] were collected and sent every 3 months to the laboratory of the teaching hospital in Clermont-Ferrand. All isolates from inpatients and outpatients were included, irrespective of where they were sampled or of their implication in infection. Every 6 months, two strains, some of them producing an ESBL, were sent to each laboratory as quality controls. On nine occasions, ESBLs failed to be detected, especially PER-1 produced by a *P. aeruginosa* strain, but all laboratories detected the decrease in antibiotic susceptibility which was the main criterion for the inclusion of the isolate in the study.

Identification of the strains was verified by Rapid ID 32 E for Enterobacteriaceae and ID 32 GN for *P. aeruginosa* (bioMérieux) and antibiotic susceptibility was checked by the disc diffusion method according to the recommendations of the Antibiogram Committee of the French Society for Microbiology (http://www.sfm.asso.fr/). From an overnight non-selective agar medium culture plate, a suspension in Mueller–Hinton broth equivalent to 0.5 McFarland standard (10^8 cfu/mL) was prepared and then diluted to 1/100 and seeded by swabbing onto Mueller–Hinton agar. Antibiotic discs (Bio-Rad, Marnes la Coquette, France) were applied and the inhibitory diameter was read after 18 h of incubation at 35–37°C. Strains were screened for ESBL by the double-disc synergy test[12] and with discs containing 30 μg of cefotaxime or ceftazidime alone and in combination with 10 μg of clavulanate (CDO2 and CDO3; Oxoid SA 69571 Dardilly France). β-Lactamases were characterized by isoelectric focusing, and the genes bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M}$, bla_{PER} were detected by PCR using specific primers as described previously (Table 1).[3] ESBL-producing isolates were classified according to year, centre, species, resistance phenotype, pI and ESBL type. For TEM-type enzymes with pI 6.3 (TEM-3 like) and pI 6.5 (TEM-24 like), and for PER-type enzymes, the *bla* PCR products obtained from the isolates ($n = 57$) of the first sample of each class were selected for direct DNA sequencing to confirm identification of the enzyme. For the other isolates producing TEM-3 or TEM-24 the enzyme type was confirmed with allele-specific PCR (ASPCR) for up to four isolates per year and per centre ($n = 61$).[13] The *bla* genes of all the other isolates were sequenced.

When more than four isolates per year of the same species producing the same ESBL were observed in the same centre, the isolates were genotyped by ERIC2-PCR to study their clonal diversity.[14] *E. aerogenes* isolates were ribotyped according to the procedure of Bingen *et al.*,[15] and in addition they were compared with the clonal strain reported previously.[7,11] When isolates related to the same clonal strain produced an ESBL of the same type and same pI the *bla* genes were sequenced for one isolate. For the other isolates, enzymes were designated TEM-3 like or TEM-24 like. The prevalence of ESBLs was calculated using the number of non-duplicate isolates in each species and each centre.

Table 1. Oligonucleotides used as primers for amplification and/or sequencing

Primer	Sequence	Position	Strand	Reference
ERIC2	5′-AAGTAAGTGACTGGGGTGAGC-3′	NA[a]	NA	14
TEM-A	5′-TAAAATTCTTGAAGACG-3′	−5[b]	Forward	24
TEM-B	5′-TTACCAATGCTTAATCA-3′	1069[b]	Reverse	24
OS5	5′-TTATCTCCCTGTTAGCCACC-3′	23[c]	Forward	4
OS6	5′-GATTTGCTGATTTCGCTCGG-3′	818[c]	Reverse	4
CTX-M A	5′-CGCTTTGCGATGTGCAG-3′	264[d]	Forward	3
CTX-M B	5′-ACCGCGATATCGTTGGT-3′	814[d]	Reverse	3
CTX-M-1A	5′-CTTCCAGAATAAGGAATC-3′	43[d]	Forward	25
CTX-M-1 B	5′-CCGTTTCCGCTATTACAA-3′	950[d]	Reverse	25
CTX-M-9A	5′-CTGATGTAACACGGATTGAC-3′	95[e]	Forward	3
Toho 2 B	5′-TTACAGCCCTTCGGCGAT-3′	1005[e]	Reverse	26
PER-A	5′-TGACGATCTGGAACCTTT-3′	1053[f]	Forward	6
PER-B	5′-AACTGCATAACCTACTCC-3′	204[f]	Reverse	6

[a] Not applicable.
[b] According to Sutcliffe numbering.[23]
Numbers correspond to the position of the first 5′ base of each oligonucleotide sequence with GenBank accession numbers: [c] Y11069 ($bla_{SHV\text{-}6}$); [d] X92506 ($bla_{CTX\text{-}M\text{-}1}$); [e] AJ416345 ($bla_{CTX\text{-}M\text{-}9}$); [f] Z21957 ($bla_{PER\text{-}1}$).

Table 2. Evolution of the distribution of ESBLs according to species [no. ESBL/no. isolates (%)]

Species	France 1990[a]	France 1998[b]	Auvergne 2001–2002
E. coli	5/5324 (0.9)	4/1606 (0.2)	39/24858 (0.2)
P. mirabilis	0/1108 (0.0)	8/215 (3.7)	55/3324 (1.7)
K. pneumoniae	105/730 (13.3)	13/138 (9.4)	14/1625 (0.9)
E. aerogenes	15/612 (2.5)[a]	46/86 (53.5)	105/638 (16.5)
E. cloacae	–[a]	2/30 (6.7)	1/1271 (0.1)
C. koseri	1/197 (0.5)[a]	5/30 (16.7)	60/483 (12.4)
C. freundii	–[a]	0/32 (0.0)	4/499 (0.8)
P. stuartii	0/115 (0.0)[a]	0/15 (0.0)	1/188 (0.5)
K. oxytoca	9/298 (3.0)	0/81 (0.0)	7/1055 (0.7)
S. marcescens	4/336 (1.2)[a]	0/49 (0.0)	10/403 (2.5)
Other	0/648 (0.0)	1/224 (0.4)[c]	1/2017 (0.0)[c]
Total Enterobacteriaceae	139/9368 (1.5)	79/2506 (3.2)	297/36361 (0.8)
P. aeruginosa	ND	ND	37/5304 (0.7)

[a]The 1990 data were collected for *Enterobacter* spp., *Citrobacter* spp., *Serratia* spp. and *Providencia* spp.[27]
[b]Ref. 7; 1990 and 1998 studies were conducted over 3 months.
[c]ESBL-producing isolates were *Salmonella* spp. TEM-4 (1998) and TEM-20 (2001–2002).

Results and discussion

During this 2 year survey, a total of 36361 strains of Enterobacteriaceae and 5304 *P. aeruginosa* were isolated. Of the 1829 resistant strains included in the study, 297 Enterobacteriaceae (0.8%) and 37 *P. aeruginosa* (0.7%) produced an ESBL.

In comparison with 1998 French data, the proportion of ESBL-producing isolates obtained during the survey was lower among Enterobacteriaceae (3.2% versus 0.8%, $P<10^{-7}$) (Table 2).[7] The proportions were stable for *E. coli* (0.2%), increased slightly for *Providencia stuartii* (0.0% versus 0.5%), *Klebsiella oxytoca* (0.0% versus 0.7%), *Serratia marcescens* (0.0% versus 2.5%) and *Citrobacter freundii* (0.0% versus 0.8%) and decreased, but not significantly, for *Citrobacter koseri* (16.7% versus 12.4%) and *Proteus mirabilis* (3.7% versus 1.7%). In contrast, there was a significant decrease in *K. pneumoniae* (9.4% versus 0.9%; $P<10^{-7}$), *E. aerogenes* (53.5% versus 16.5%; $P<10^{-7}$) and *Enterobacter cloacae* (6.7% versus 0.1%; $P<10^{-7}$). Some ESBL producers with low-level resistance to ceftazidime and cefotaxime and susceptible to aminoglycosides may not have been detected in our study. Such isolates used to be rare and were observed mainly in *P. mirabilis*.[16] In 1998, the number of *E. aerogenes* may have been disproportionately inflated by the fact that the survey was short (3 months) and because in some areas, during this period there had been outbreaks.[7]

During the period 2001–2002 (Table 3), the most frequent ESBLs in Enterobacteriaceae were TEM-3 like ($n=152/297$; 51.2%) and TEM-24 like ($n=115/297$; 38.7%). Other previously

Table 3. ESBL distribution among various species [% in 2001–2002 (% in 1998[7])]

Species	TEM-24 like	TEM-3 like	Other TEM type[a]	SHV type[b]	CTX-M type[c]	PER-1
E. coli	1.3 (3.8)	5.1 (0.0)	2.9 (1.3)	0.3 (1.3)	3.7 (0.0)	–[d]
P. mirabilis	1.7(0.0)	15.8 (6.3)	1.0 (3.8)	–	–	–
P. stuartii	–	0.3 (0.0)	–	–	–	–
K. pneumoniae	1.1 (1.3)	2.4 (13.9)	0.3 (0.0)	1.0 (1.3)	–	–
K. oxytoca	0.7 (0.0)	1.7 (0.0)	–	–	–	–
E. aerogenes	34.0 (45.6)	1.0 (8.9)	0.3 (0.0)	0.0 (3.8)	–	–
E. cloacae	–	0.3 (1.3)	–	–	0.0 (1.3)	–
C. koseri	–	20.2 (6.3)	–	–	–	–
C. freundii	–	1.0 (0.0)	0.3 (0.0)	–	–	–
S. marcescens	–	3.4 (0.0)	–	–	–	–
Salmonella spp.	–	–	0.3 (1.3)	–	–	–
P. aeruginosa	–	–	–	–	–	11.1 (ND)
Total[e]	38.7 (50.6)	51.2 (36.7)	5.1 (6.3)	1.3 (5.1)	3.7 (1.3)	11.1 (ND)

ND, not done.
[a]*E. coli* TEM-12, -16, -29, -71, -112, -126; *P. mirabilis* TEM-21, -113; *K. pneumoniae* TEM-19; *E. aerogenes* TEM-114; *C. freundii* TEM-8; *Salmonella* spp. TEM-20.
[b]*E. coli* SHV-12; K. pneumoniae SHV-4, -5.
[c]CTX-M-1, -3, -14, -15.
[d]None.
[e]The percentages refer to the total ESBL-producing Enterobacteriaceae ($n=297$) except for *P. aeruginosa* PER-1 ($n=37$) for which it refers to the total ESBL-producing isolates ($n=334$).

Table 4. Isoelectric point and amino acid substitutions of the four new TEM types and related enzymes

Enzyme	Isoelectric point	Amino acid number[a] and substitutions			
		39	153	238	265
TEM-112[b]	5.4	Gln	Arg	Ser	Thr
TEM-25	5.3	Gln	His	Ser	Met
		39	104	182	238
TEM-113[b]	6.3	Lys	Lys	Thr	Ser
TEM-3	6.3	Lys	Lys	Met	Ser
		39	164	237	240
TEM-114[b]	5.9	Lys	Ser	Thr	Lys
TEM-5	5.6	Gln	Ser	Thr	Lys
		39	179	182	
TEM-126[b]	5.4	Gln	Glu	Thr	
TEM-1a	5.4	Gln	Asp	Met	

[a]Amino acid numbering is according to Ambler *et al.*[28]
[b]GenBank accession numbers: TEM-112, AY589493; TEM-113, AY589494; TEM-114, AY589495; TEM-126, AY628199.

reported TEM-types were TEM-8 ($n=1$), TEM-12 ($n=1$), TEM-16 ($n=1$), TEM-19 ($n=1$), TEM-20 ($n=1$), TEM-21 ($n=2$), TEM-29 ($n=2$) and TEM-71 ($n=2$). In addition, four new TEM types were characterized (Table 4): TEM-112 (pI 5.4; GenBank accession number AY589493), which differs from TEM-1 by the amino acid changes His-153 → Arg and Gly-238 → Ser. Residue 153 is often a basic residue, not conserved in class A β-lactamases, and located at the end of helix H4. Previous reports on TEM-18 (Lys-39, Lys-104) and TEM-56 (Lys-39, Lys-104, Arg-153)[17] suggest that Arg-153 does not modify the catalytic activity of the enzyme. In addition, mutation Thr-265 → Met, which is frequently observed in TEM-derivative ESBLs, has no effect on the hydrolytic activity of the enzyme. The resistance level to broad-spectrum cephalosporins conferred by TEM-112 was similar to that observed with TEM-25. TEM-113 (pI 6.3; GenBank accession number AY589494), differs from TEM-3 by the substitution Met-182 → Thr. This substitution is common in ESBLs (e.g. TEM-20, TEM-43, TEM-52, TEM-63, TEM-72, TEM-87; TEM-91–TEM-95, TEM-106). Molecular modelling has shown that the active site may be stabilized by a new hydrogen bond between the hydroxyl of Thr-182 and the carbonyl of the amide bond of Glu-64.[18] Several studies of crystal structure suggested that the substitution Met-182 → Thr has little effect on enzyme activity but restores stability lost by substitutions near the active site.[19–21] TEM-114 (pI 5.9; GenBank accession number AY589495), was derived from TEM-2 (Lys-39) and harboured the same substitutions, Arg-164 → Ser, Ala-237 → Thr and Glu-240 → Lys, as TEM-5, which was derived from TEM-1. TEM-126 (pI 5.4; GenBank accession number AY628199), derives from TEM-1 by two substitutions. This is the first ESBL mutant of TEM harbouring the substitution Asp-179 → Glu (in addition to the substitution Met-182 → Thr). A substitution at position 179 was reported in SHV-1-derivative ESBLs (SHV-6, SHV-8 and SHV-24). In these enzymes aspartic acid 179 is replaced by a neutral residue (alanine, asparagine, glycine), which disrupts the salt bridge, and Arg-164 increases the flexibility of the omega loop. In the TEM-126 mutant, the disruption of the salt bridge probably did not occur since the aspartic residue was replaced by a glutamic acid residue. Kinetic analysis and molecular modelling are in progress to explain the extended-spectrum activity of this novel mutant.

TEM-3-like enzymes were produced by 10 different species isolates: *C. koseri* ($n=60$), *P. mirabilis* ($n=47$), *E. coli* ($n=15$), *S. marcescens* ($n=10$), *K. pneumoniae* ($n=7$), *K. oxytoca* ($n=5$), *C. freundii* ($n=3$), *E. aerogenes* ($n=3$), *E. cloacae* ($n=1$) and *P. stuartii* ($n=1$). Most TEM-24-like enzymes (87.8%) were produced by *E. aerogenes* isolates ($n=101$), and most ESBLs produced by *E. aerogenes* isolates (96.2%) were TEM-24 like. The other isolates producing TEM-24-like enzymes were *P. mirabilis* ($n=5$), *E. coli* ($n=4$), *K. pneumoniae* ($n=3$) and *K. oxytoca* ($n=2$), which lends support to the previously reported findings of interspecies dissemination of the enzyme.[22]

Among the SHV derivatives, three types were observed: SHV-5 ($n=2$), SHV-4 ($n=1$) and SHV-12 ($n=1$). The other non-TEM, non-SHV ESBLs observed were PER-1 like ($n=37$), CTX-M-3 ($n=4$), CTX-M-14 ($n=3$), CTX-M-15 ($n=3$) and CTX-M-1 ($n=1$). There was no evidence of the production of other ESBLs or of the presence of several ESBLs in the same strain.

In comparison with the 1998 survey,[7] the distribution of ESBLs among Enterobacteriaceae observed during the 2001–2002 period showed an increase in TEM-3-like enzymes (36.7% versus 51.2%, $P=0.02$) and in CTX-M (1.3% versus 3.7%, not significant) and a decrease in TEM-24-like enzymes (50.6% versus 38.7%, not significant), and in SHV (5.1% versus 1.3%, $P=0.04$). The percentages of ESBLs varied considerably between centres, ranging from 0.2% to 3.6% for Enterobacteriaceae isolates and 0.0% to 0.7% for *P. aeruginosa* isolates. This variation was due mainly to local epidemics (documented by different centres) involving the following species and β-lactamases: *S. marcescens*, *C. koseri*, *E. coli* and *P. mirabilis* producing TEM-3, *E. aerogenes* producing TEM-24 and *P. aeruginosa* producing PER-1.

After genotyping, a clonal relationship was confirmed in five species/enzyme groups responsible for epidemics.

(i) *S. marcescens* TEM-3 was limited to one centre. ERIC2-PCR patterns were identical for all the strains.
(ii) *E. aerogenes* TEM-24 was observed in five centres in 2001 and six in 2002. ERIC2-PCR and ribotype patterns were the same for all the strains and identical to those observed in 1998. The isolates were far more frequent in one centre than in the others because of the persistence of the strain in a long-stay care unit.
(iii) *C. koseri* TEM-3 was observed in six centres in 2001 and four in 2002. The epidemic was concentrated in one centre, which was in the east of the region, near Lyon, where an epidemic had occurred 3 years earlier (data not shown). ERIC2-PCR patterns were identical or closely related for all the isolates.
(iv) *P. aeruginosa* PER-1 was observed in two centres in 2001, and one in 2002, where it was epidemic. All the strains had the same or a related genotype profile. *P. aeruginosa* PER-1 isolates are widespread in Turkey[10] and the outbreak may have been related to the immigration of Turkish nationals, since the first strain of our study was isolated from a patient previously hospitalized in Strasbourg (Eastern France), where he might have been in contact with

Turkish patients.[6] This multiresistant strain has been difficult to eradicate.

(v) *P. mirabilis* TEM-3 was observed in four centres in 2001 and five in 2002. ERIC2 profiles were either identical or differed by less than three segments and were considered to be closely related.

(vi) *E. coli* TEM-3 isolates, observed in six centres in 2001 and one in 2002, harboured different genotypes. CTX-M-type enzymes are emerging in the region; they increased from 2.1% in 2001 to 4.9% in 2002, and could give rise to epidemics, as in other countries.[8]

ESBL frequency was considerably lower than in other parts of the world. In Clermont-Ferrand hospital they decreased from about 13 per month in 1988–1989 to four per month during the period of this study.[11] This may have been due to a decrease in the use of rooms with several beds in intensive care units and the use of closed urinary drainage systems. Between the two periods, detection of digestive carriage of ESBL-producing bacteria was implemented, and specific daily alerts were given by the laboratory to the units to allow patients to be isolated when necessary. Antibiotic use was limited and treatment duration was shortened.

The main advantages of a long-term survey are to avoid any overestimation of one species or one enzyme that may otherwise have occurred because of acute epidemics and to detect new sporadic enzymes. On the other hand, because of its length, the survey is exposed to the risk of underestimating the total number of ESBLs, by missing some isolate inclusions. This study showed that, although the frequency of ESBLs is decreasing, these enzymes may still cause epidemics, especially in centres with long-stay care units.

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References

1. Bure, A., Legrand, P., Arlet, G. *et al.* (1988). Dissemination in five French hospitals of *Klebsiella pneumoniae* serotype K25 harbouring a new transferable enzymatic resistance to third generation cephalosporins and aztreonam. *European Journal of Clinical Microbiology and Infectious Diseases* **7**, 780–2.

2. Sirot, D., Sirot, J., Labia, R. *et al.* (1987). Transferable resistance to third-generation cephalosporins in clinical isolates of *Klebsiella pneumoniae*: identification of CTX-1, a novel β-lactamase. *Journal of Antimicrobial Chemotherapy* **20**, 323–34.

3. Bonnet, R., Dutour, C., Sampaio, J. L. M. *et al.* (2001). Novel cefotaximase (CTX-M-16) with increased catalytic efficiency due to substitution Asp-240 → Gly. *Antimicrobial Agents and Chemotherapy* **45**, 2269–75.

4. Cao, V., Lambert, T., Nhu, D. Q. *et al.* (2002). Distribution of extended-spectrum β-lactamases in clinical isolates of Enterobacteriaceae in Vietnam. *Antimicrobial Agents and Chemotherapy* **46**, 3739–43.

5. Casin, I., Hanau-Berçot, B., Podglajen, I. *et al.* (2003). *Salmonella enterica* serovar Typhimurium bla_{PER-1}-carrying plasmid pSTI1 encodes an extended-spectrum aminoglycoside 6′-N-acetyltransferase of type Ib. *Antimicrobial Agents and Chemotherapy* **47**, 697–703.

6. De Champs, C., Poirel, L., Bonnet, R. *et al.* (2002). Prospective survey of β-lactamases produced by ceftazidime-resistant *Pseudomonas aeruginosa* isolated in a French hospital in 2000. *Antimicrobial Agents and Chemotherapy* **46**, 3031–4.

7. De Champs, C., Sirot, D., Chanal, C. *et al.* (2000). A 1998 survey of extended-spectrum β-lactamases in Enterobacteriaceae in France. *Antimicrobial Agents and Chemotherapy* **44**, 3177–9.

8. Gniadkowski, M., Schneider, I., Palucha, A. *et al.* (1998). Cefotaxime-resistant Enterobacteriaceae isolates from a hospital in Warsaw, Poland: identification of a new CTX-M-3 cefotaxime-hydrolyzing β-lactamase that is closely related to the CTX-M-1/MEN-1 enzyme. *Antimicrobial Agents and Chemotherapy* **42**, 827–32.

9. Nordmann, P., Ronco, E., Naas, T. *et al.* (1993). Characterization of a novel extended-spectrum β-lactamase from *Pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy* **37**, 962–9.

10. Vahaboglu, H., Öztürk, R., Aygün, G. *et al.* (1997). Widespread detection of PER-1-type extended-spectrum β-lactamases among nosocomial *Acinetobacter* and *Pseudomonas aeruginosa* isolates in Turkey: a nationwide multicenter study. *Antimicrobial Agents and Chemotherapy* **41**, 2265–9.

11. De Champs, C., Sirot, D., Chanal, C. *et al.* (1991). Concomitant dissemination of three extended-spectrum β-lactamases among different Enterobacteriaceae isolated in a French hospital. *Journal of Antimicrobial Chemotherapy* **27**, 441–57.

12. Sirot, J. (1996). Detection of extended-spectrum plasmid-mediated β-lactamases by disc diffusion. *Clinical Microbiology and Infection* **2**, *Suppl. 1*, 35–9.

13. Mabilat, C. & Courvalin, P. (1990). Development of 'oligotyping' for characterization and molecular epidemiology of TEM β-lactamases in members of the family Enterobacteriaceae. *Antimicrobial Agents and Chemotherapy* **34**, 2210–6.

14. Versalovic, J., Koeuth, T. & Lupski, J. R. (1991). Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. *Nucleic Acids Research* **19**, 6823–31.

15. Bingen, E., Denamur, E. & Elion, J. (1994). Use of ribotyping in epidemiological surveillance of nosocomial outbreaks. *Clinical Microbiology Reviews* **7**, 311–27.

16. Chanal, C., Sirot, D., Romaszko, J. P. *et al.* (1996). Survey of prevalence of extended spectrum β-lactamases among Enterobacteriaceae. *Journal of Antimicrobial Chemotherapy* **38**, 127–32.

17. Neuwirth, C., Labia, R., Siebor, E. *et al.* (2000). Characterization of TEM-56, a novel TEM-derived β-lactamase produced by a *Klebsiella pneumoniae* clinical isolate. *Antimicrobial Agents and Chemotherapy* **44**, 453–5.

18. Farzaneh, S., Chaibi, E. B., Péduzzi, J. *et al.* (1996). Implication of Ile-69 and Thr-182 residues in kinetic characteristics of IRT-3 (TEM-32) β-lactamase. *Antimicrobial Agents and Chemotherapy* **40**, 2434–6.

19. Orencia, M. C., Yoon, J. S., Ness, J. E. *et al.* (2001). Predicting the emergence of antibiotic resistance by directed evolution and structural analysis. *Nature Structural Biology* **8**, 238–42.

20. Wang, X., Minasov, G., Shoichet, B. K. *et al.* (2002). The structural bases of antibiotic resistance in the clinically derived mutant β-lactamases TEM-30, TEM-32 and TEM-34. *Journal of Biological Chemistry* **277**, 32149–56.

21. Wang, X., Minasov, G. & Shoichet, B. K. (2002). Evolution of an antibiotic resistance enzyme constrained by stability and activity trade-offs. *Journal of Molecular Biology* **320**, 85–95.

22. Marchandin, H., Carrière, C., Sirot, D. *et al.* (1999). TEM-24 produced by four different species of Enterobacteriaceae, including *Providencia rettgeri*, in a single patient. *Antimicrobial Agents and Chemotherapy* **43**, 2069–73.

23. Sutcliffe, J. G. (1978). Nucleotide sequence of the amipicillin resistance gene of *Escherichia coli* plasmid pBR322. *Proceedings of the National Academy of Sciences, USA* **75**, 3737–41.

24. Mabilat, C., Goussard, S., Sougakoff, W. *et al.* (1990). Direct sequencing of the amplified structural gene and promoter for the extended-broad-spectrum β-lactamase TEM-9 (RHH-1) of *Klebsiella pneumoniae*. *Plasmid* **23**, 27–34.

25. Dutour, C., Bonnet, R., Marchandin, H. *et al.* (2002). CTX-M-1, CTX-M-3, and CTX-M-14-β-lactamases from Enterobacteriaceae isolated in France. *Antimicrobial Agents and Chemotherapy* **46**, 534–7.

26. Chanawong, A., M'Zali, F. H., Heritage, J. *et al.* (2002). Three cefotaximases, CTX-M-9, CTX-M-13, and CTX-M-14, among Enterobacteriaceae in the People's Republic of China. *Antimicrobial Agents and Chemotherapy* **46**, 630–7.

27. Sirot, D. L., Goldstein, F. W., Soussy, C. J. *et al.* (1992). Resistance to cefotaxime and seven other β-lactams in members of the family Enterobacteriaceae: a 3-year survey in France. *Antimicrobial Agents and Chemotherapy* **36**, 1677–81.

28. Ambler, R. P., Coulson, A. F., Frere, J. M. *et al.* (1991). A standard numbering scheme for the class A β-lactamases. *Biochemical Journal* **276**, 269–70.