Bacteraemia caused by third-generation cephalosporin-resistant *Escherichia coli* in France: prevalence, molecular epidemiology and clinical features

A. Courpon-Claudinon[1,2], A. Lefort[3,4], X. Panhard[5], O. Clermont[2], Q. Dornic[5], B. Fantin[3,4], F. Mentré[5], M. Wolff[6], E. Denamur[2], C. Branger[1,2], on behalf of the COLIBAFI group*

1) *AP-HP, Hôpital Louis Mourier, Service de Microbiologie-Hygiène, Colombes,* 2) *INSERM U722 and Université Paris 7, Faculté de Médecine, Site Xavier Bichat,* 3) *AP-HP, Hôpital Beaujon, Service de Médecine interne, Clichy,* 4) *EA3965, Université Paris 7, Faculté de Médecine, Site Xavier Bichat,* 5) *INSERM U738 and Université Paris 7, Faculté de Médecine, Site Xavier Bichat and* 6) *AP-HP, Hôpital Bichat-Claude Bernard, Service de Réanimation Médicale et Infectieuse, Paris, France*

Abstract

Escherichia coli is one of the major pathogens responsible for bactaeremia. Empirical antibiotherapy of these infections usually relies on third-generation cephalosporins (3GCs). Thus, the occurrence and epidemiology of 3GC-resistant strains have to be monitored. The French prospective multicentre study COLIBAFI collected 1081 strains of *E. coli* responsible for bacteraemia in 2005. In the present work, the prevalence of resistance to 3GCs was evaluated, and the implicated molecular mechanisms were characterized by specific PCR and sequencing. Phylogenetic grouping, O-typing, pulsed-field gel electrophoresis and virulence factor analysis were used to investigate the genetic background of the 3GC-resistant (3GC-R) strains. Clinical features of the patients with documented data (n = 1051) were analysed. Decreased susceptibility to 3GCs was observed in 41 strains (3.8%): 19, 18 and four had extended-spectrum β-lactamase (ESBL), AmpC cephalosporinase and OXA-type penicillinase phenotypes, respectively. Pulsed-field gel electrophoresis revealed that the 3GC-R strains constitute a diverse population. All but one of the strains with an ESBL phenotype produced a CTX-M-type enzyme, and six of them belonged to the widespread intercontinental clone O25b:H4-ST131. AmpC phenotype strains harboured various chromosomal *ampC* promoter and coding region mutations and/or the bla_{CMY-2} plasmidic gene. 3GC-R strains carried fewer virulence factors and were more co-resistant to other antibiotics than 3GC-susceptible (3GC-S) strains. Infections with 3GC-R strains were mostly community-acquired and, as compared with those caused by their 3GC-S counterparts, were more severe. Underlying chronic disease and prior use of antibiotics were independent risk factors for development of a 3GC-R strain bacteraemia. The fact that the molecular support of 3GC resistance is mainly plasmid-mediated represents a potentially epidemic threat.

Keywords: β-Lactamases, bloodstream infection, epidemiology, *Escherichia coli*, multiresistance
Original Submission: 4 February 2010; **Revised Submission:** 7 June 2010; **Accepted:** 13 June 2010
Editor: R. Cantón
Article published online: 23 July 2010
Clin Microbiol Infect 2011; **17:** 557–565
10.1111/j.1469-0691.2010.03298.x

Corresponding author: A. Courpon-Claudinon, Hôpital Louis Mourier, Service de Microbiologie-Hygiène, 178 rue des Renouillers, 92701 Colombes, France
E-mail: aurorecourpon@gmail.com

*Members of the COLIBAFI group are listed in the Acknowledgements.

Introduction

Bacteraemias represent a major cause of death in industrialized countries such as Europe and the USA, with large increases in incidence and mortality being seen over the past 20 years [1]. For *Escherichia coli*, a leading pathogen implicated in these infections [2], an increase in the prevalence of β-lactam resistance, especially concerning the third-generation cephalosporins (3GCs), has been observed recently, according to the annual report of the European Antimicrobial Resistance Surveillance System (http://www.rivm.nl/earss). Three kinds of β-lactamase are commonly responsible for 3GC resistance: extended-spectrum β-lactamases (ESBLs), OXA-type penicillinases (or oxacillinases) and AmpC cephalosporinases (chromosomal or plasmid-mediated). Among ESBLs, the CTX-M β-lactamases have now become most prevalent [3,4].

As 3GCs form part of empirical antimicrobial chemotherapy in severe infections, the prevalence of resistance in *E. coli* is important to evaluate because of the risk of treatment failure. In 2005, a large, prospective multicentre study, COLIBAFI (http://www.colibafi.net), was conducted to identify the factors of severity associated with *E. coli* bacteraemia. The present ancillary study aimed to investigate the prevalence and molecular epidemiology of resistance to 3GCs of the COLIBAFI collection, and to analyse clinical features of patients infected by these resistant strains.

Materials and Methods

Study protocol and bacterial strains

The prospective multicentre study COLIBAFI was conducted in 15 hospitals in different areas in France: Paris (eight hospitals), Angers, Brest, Caen, Dijon, Nantes, Rennes and Tours. All except one were university hospitals. During the year 2005, all cases of *E. coli* bacteraemia, defined on the basis of the isolation of *E. coli* from one or more sets of blood culture bottles, were collected by the local bacteriology laboratory. Only patients receiving vasopressors before the bacteraemia or patients already included in the study for a previous episode were not considered for inclusion. Overall, 1099 adults were included. Forty-eight patients were excluded either because the *E. coli* isolate was not available (n = 18) or because clinical data were lacking (n = 30). Thus, the microbiological study was conducted on 1081 strains, and the clinical study concerned 1051 patients. Bacterial identification was performed with the API20E system (bioMerieux, Marcy l'Etoile, France). Antimicrobial susceptibility was determined by the disk diffusion method on Mueller–Hinton agar, and interpreted according to the 2005 guidelines of the Antibiogram Committee of the French Society for Microbiology (CA-SFM) (http://www.sfm.asso.fr). The strains were sent to a central laboratory (INSERM U722) with clinical features of the patients and antimicrobial susceptibility data. Clinical characteristics, collected by a tandem of senior investigators (an infectious disease clinician and a bacteriologist) included age, sex, underlying chronic disease, immunosuppression, antibiotherapy received within 2 weeks before the bacteraemia, community-acquired or nosocomial infection, portal of entry and clinical outcome. Bacteraemia episodes were defined as community-acquired if the first positive blood culture was obtained <48 h following hospital admission. The full description of the cohort will be published elsewhere (A. Lefort, X. Panhard, O. Clermont, P. L. Woerther, C. Branger, F. Mentré, B. Fantin, M. Wolff, E. Denamur and the COLIBAFI group; personal communication). Strains with decreased susceptibility to cefotaxime and/or ceftazidime according to the 2005 guidelines of the CA-SFM were selected. Strains with decreased susceptibility to cefoxitin were also considered, as this can be a helpful marker with which to detect AmpC production [5].

Antimicrobial susceptibility testing of the selected strains

All selected strains were tested for ESBL production by the double-disk synergy test [6]. For the strains with a negative test result, MICs of cefoxitin, cefotaxime, ceftazidime and cefepime were determined by the Etest diffusion method (AB Biodisk, Solna, Sweden).

Molecular characterization of β-lactamases

For strains with a positive double-disk synergy test result, characterization of ESBLs was performed by specific PCR amplification and sequencing [7].

For strains with a negative double-disk synergy test result, the chromosomal *ampC* gene, its promoter and its attenuator were amplified and sequenced with primers Int-B2 and Int-HN [8]. Mutations were studied by comparison with the published *ampC* gene sequence of *E. coli* K-12 (GenBank accession number NC_000913). Plasmid-mediated AmpC cephalosporinase was detected with a multiplex PCR method [9] and identified by sequencing.

The strains were also screened for the presence of an OXA-type β-lactamase by PCR [10], and this was followed by sequencing to identify the bla_{OXA} gene.

Strain genetic background analysis

Phylogenetic grouping of the *E. coli* strains was determined by a PCR-based method [11]. The strains were screened for 17 genes encoding putative virulence factors (*sfa/foc*, *iroN*, *iutA*, *iha*, *papC*, *papG* (II and III alleles), *hlyC*, *cnf1*, *hra*, *sat*, *ire*, *usp*, chromosomal *ompT*, *ibeA*, *fyuA*, *irp2* and *traT*) by PCR [12]. For each strain, a virulence score was defined as the sum of virulence factors present over the 17 tested. Twenty-five O-types were determined with a molecular approach based on allele-specific PCR [13] in the 3GC-resistant (3GC-R) strains (see Table S1 for a list of the primers used). They include the O-types most frequently found in extra-intestinal pathogenic [13] and ESBL-producing [14,15] strains. An allele-specific PCR of the *pabB* gene was used to detect strains belonging to the O25b:H4-ST131 clone [16]. Pulsed-field gel electrophoresis was performed with a CHEF DRII System (BioRad, Marnes-la-Coquette, France) using genomic DNA digested with *XbaI* [17]. A dendrogram was constructed using the Dice similarity coefficient, and the UPGMA algorithm was used to cluster the strains.

Statistical analysis

The 1081 strains were considered for analysis concerning microbiological data. Resistance to other antibiotics, phylogenetic group and virulence factor score were compared between 3GC-R strains (n = 41) and the 3-GC-susceptible (3GC-S) strains (n = 1040). A second comparison was performed between the ESBL-producing strains (n = 19) and the AmpC phenotype-expressing strains (n = 18). The clinical characteristics of 1051 patients were studied with the available data. As above, two comparisons were made.

Comparisons of discrete variables were performed with Fisher's exact test, and comparisons of continuous variables were performed using Wilcoxon–Mann–Whitney tests. All tests were two-sided, with a type I error of 0.05. Risk factors for 3GC resistance were also studied by backward multivariate logistic regression. The statistical analysis was performed using SAS software version 9.1.

Results and Discussion

Overall, 41 of 1081 strains (3.8% (95% CI 2.7–5.1%)) showed decreased susceptibility to at least one of the 3GCs. This rate is close to the percentage of 3% described for bacteraemias in France in 2005 by the surveillance network ONERBA (http://www.onerba.org). Thus, because of the large number of strains included and the participation of several hospitals in different areas, the COLIBAFI study seems to be representative of the situation regarding antimicrobial resistance of *E. coli* in France.

ESBLs and AmpC, both equally represented, are the main causes of resistance to 3GCs

Strains with an ESBL phenotype. The double-disk synergy test detected 19 ESBL-producing *E. coli* strains, 18 of which belonged to the CTX-M group and only one to the TEM family (TEM-52) (Table 1). This result shows that the great majority of ESBL-producing *E. coli* strains responsible for bacteraemia carry a $bla_{CTX\text{-}M}$ gene, confirming the observation of the worldwide dissemination of this type of ESBL since the 2000s [3], both in the hospital environment and in the community [4,18], with *E. coli* as a major host. To date, few observations have been made on the prevalence of this mechanism of resistance in such serious infections. In Spain, two recent studies, conducted between October 2004 and January 2006, concerning community-onset and nosocomial bacteraemia caused by EBSL-producing *E. coli*, found prevalences of CTX-M enzymes of, respectively, 87% and 81%; the enzymes were predominantly of the CTX-M-14 type [19,20]. However, they noted the increasing prevalence of the CTX-M-15 type, in relation to the worldwide spread of the ST131 clone. In the COLIBAFI study, two enzymes were predominant: CTX-M-15 (50%) and CTX-M-14 (30%).

Strains with an AmpC phenotype. Eighteen strains had reduced susceptibility to cefoxitin (MIC >32 mg/L) and to at least one of the 3GCs tested, but produced no ESBL or OXA-type penicillinase. This phenotype was compatible with the overproduction of the chromosomal AmpC enzyme resulting from mutations in the *ampC* gene and/or acquisition of a plasmid-mediated AmpC.

Plasmid-mediated AmpCs, all of the CMY-2 type, were detected in five strains (28%). The predominance of this type of enzyme has been described in Europe [21,22] and in North America [5,23].

The promoter region of the *ampC* gene of these strains was investigated. In 11 strains, an association of five mutations (in positions −88, −82, −42, −18 and −1) was found (Table 2). The –42 (C → T) transition is known to increase the strength of the promoter by creating a new perfect consensus sequence, TTTACA, separated by 17 bp from a new −10 sequence created by the −18 (G → A) transition [24]. Strains lacking this pattern displayed other mutations known to enhance the strength of the promoter: the transversion –32 (T → A) in the –35 box [24], or single-nucleotide insertions between the –35 box and the –10 box. Only two strains (08–152 and 10–020) had a promoter region strictly identical to that of *E. coli* K-12, but they both harboured a plasmid-mediated AmpC. Finally, four strains with an MIC for cefepime ≥4 mg/L had additional mutations in the attenuator region (in position +20 or +23) (Table 2), which might increase transcription [25]. All of these findings are consistent with a recent description of the importance of these mutations in the promoter region of the *ampC* gene [22].

The extension of the hydrolysis spectrum of AmpC to cefepime has been related to modifications in several specific locations of the protein, and especially the H-9 helix and the H-10 helix [8,26–28]. Indeed, in our study, five strains with cefepime MICs >1 mg/L had amino acid substitutions in these regions (Table 2). The S287N substitution might have a greater impact than the S287C substitution on the increase in the catalytic efficiency of AmpC towards 3GCs [8], and specifically in *E. coli* strains belonging to phylogroup A [28], as observed in our study (Tables 1 and 2). The L293P substitution found in strain 08-121 (cefepime MIC of 16 mg/L) has never been described to date in a clinical isolate of *E. coli.* However, it has already been found in an *in vitro* mutant of the *Enterobacter cloacae* P99 reference strain (cefepime MIC of 8 mg/L) [26] and in a clinical isolate of *Enterobacter aerogenes* (cefepime MIC of 32 mg/L) selected during cefepime treatment [29].

TABLE 1. Characteristics of the *Escherichia coli* isolates resistant to third-generation cephalosporins

Strain ID	β-Lactam resistance type	Phylogroup[a]	O-type[b]	VF score[c]	Co-resistance[d]
02-007	CTX-M-9	B2		5	S, K, G, T, N, A, Te, Mi, Na, Pe, Cp, Ch, Sxt
01-066	CTX-M-14	B2	O4	10	S, Te, Mi, Sxt
02-008	CTX-M-14	B2[e]	O25b	8	Na, Pe, Cp
05-029	CTX-M-14	A		2	Te, Na, Pe, Cp, Sxt
08-024	CTX-M-14	A		8	S, Te, Mi, Sxt
12-028	CTX-M-14	A		2	K, G, T, N, A, Te, Mi, Na, Pe, Cp, Ch, Sxt
11-078	CTX-M-14	D	O16	10	Te, Mi, Na, Pe, Cp, Sxt
02-018	CTX-M-1	A	O78	5	S, K, Te, Mi, Na, Pe, Cp, Ch, Sxt
12-176	CTX-M-1	A		2	Te, Mi, Sxt
05-058	CTX-M-15 + OXA-1	A		1	K, G, T, N, Te, Mi, Na, Pe, Cp, Ch, Sxt
11-080	CTX-M-15 + OXA-1	A		0	K, G, T, N, Te, Mi, Na, Pe, Cp, Ch, Sxt
11-104	CTX-M-15 + OXA-1	A		2	K, G, T, N, Te, Mi, Na, Pe, Cp, Sxt
05-032	CTX-M-15 + OXA-1	B2[e]	O25b	8	K, G, T, N, Mi, Na, Pe, Cp
06-024	CTX-M-15 + OXA-1	B2[e]	O25b	5	S, K, T, N, A, Te, Mi, Na, Pe, Cp, Sxt
13-011	CTX-M-15 + OXA-1	B2[e]	O25b	7	K, G, T, N, Na, Pe, Cp, Sxt
13-027	CTX-M-15	B2[e]	O25b	4	Mi, Na, Pe, Cp
13-038	CTX-M-15	B2[e]	O25b	8	Mi, Na, Pe, Cp, Ch
11-005	CTX-M-15	A		3	K, G, T, N, Te, Mi, Na, Pe, Cp, Sxt
06-008	TEM-52	A		3	S, K, Te, Mi, Na, Pe, Cp, Ch, Sxt
10-020	CMY-2	A		3	Te, Mi, Na, Pe, Cp, Ch, Sxt
04-004	AmpC	A		5	S, Te, Mi, Sxt
06-021	AmpC	A	O21	4	S, Te, Mi, Ch, Sxt
08-092	AmpC	A		7	S, Te, Mi, Sxt
12-140	AmpC	A		6	S, K, Te, Mi, Sxt
13-030	AmpC	A		9	S, K, Te, Mi, Ch, Sxt
12-133	AmpC	B1		0	
12-169	AmpC	B2	O7	11	S, Te, Mi, Sxt
05-008	AmpC	B2	O6	15	Na
08-152	AmpC + CMY-2	A		5	S, Te, Mi, Na, Pe, Cp, Sxt
12-003	AmpC + CMY-2	A		1	S, Te, Mi, Na, Pe, Cp, Ch, Sxt
12-015	AmpC + CMY-2	B1		4	K, G, T, Te, Mi, Na, Pe, Cp, Sxt
12-052	AmpC	A		8	S, K, Te, Mi, Na, Sxt
06-006	AmpC	A		6	Mi, Na, Pe, Cp, Ch
06-062	AmpC	D		5	Te, Mi, Na, Pe, Cp, Sxt
07-107	AmpC	A		6	Mi, Na, Pe, Cp
05-063	AmpC + CMY-2	A		5	S, K, Te, Mi, Na, Pe, Cp, Sxt
08-121	AmpC	A		5	S, K, Te, Mi, Na, Pe, Ch, Cp, Sxt
02-033	OXA-1	D		5	Te, Na, Pe, Cp, Ch
07-102	OXA-1	B2		16	S, K, Te, Mi, Ch
08-110	OXA-1	A		8	S, K, Te, Mi, Na, Pe, Cp, Ch, Sxt
11-153	OXA-1	A		4	S, K, G, T, Te, Mi, Na, Pe, Cp, Ch, Sxt

[a]Determined as in [11].
[b]Determined as in [13], among a pool of 25 O-types (see Materials and Methods and Table S1). The absence of results indicates that the isolates do not belong to any of the O-types searched for.
[c]VF, virulence factor.
[d]S, streptomycin; K, kanamycin; G, gentamicin; T, tobramycin; N, netilmicin; A, amikacin; Te, tetracycline; Mi, minocycline; Na, nalidixic acid; Pe, pefloxacin; Cp, ciprofloxacin; Ch, chloramphenicol; Sxt, trimethoprim–sulphonamides.
[e]Strains belonging to the O25b:H4-ST131 clone.

Strains with an OXA phenotype. Four strains with decreased susceptibility to cefoxitin and showing selective hydrolysis of cefepime produced an OXA-type penicillinase encoded by a bla_{OXA-1} gene (Table 1). This gene was also found in strains producing a CTX-M enzyme. Indeed, the bla_{OXA-1} gene can be found alone or with the $bla_{CTX-M-15}$ gene on the same plasmid [3,30,31].

3GC-R strains constitute a diverse population that is more co-resistant and has fewer virulence factors than 3GC-S strains

Pulsed-field gel electrophoresis revealed that the 3GC-R strains constitute a diverse population. Six CTX-M-producing strains, of phylogroup B2, were found in a cluster distant from the others (Fig. 1), and belonged to the recently described widespread intercontinental clone O25b:H4-ST131 [14,16,32]. This clone usually includes strains harbouring the $bla_{CTX-M15}$ gene, but recent studies have shown that other types of ESBL can be found [16,31].

The 3GC-R strains had heterogeneous co-resistance profile (Tables 1 and 3). They were significantly more resistant than 3GC-S strains to several major antibiotics: ciprofloxacin ($p < 0.0001$), gentamicin ($p < 0.0001$), amikacin (p 0.01), and co-trimoxazole ($p < 0.0001$). Ciprofloxacin resistance was found in 28 of 41 strains, with a significant predominance in the ESBL group vs. the AmpC group (p 0.04). Gentamicin resistance was found in ten of 41 strains: six were CTX-M-15 producers. Co-trimoxazole was inactive in 75.6% of the cases with no difference between ESBL– and AmpC–producing strains. All of these findings concerning multiresistance in ESBL-producing *E. coli* strains highlight the difficulty in finding an

TABLE 2. Comparison of sequences of the *ampC* gene and its promoter for 18 isolates of *Escherichia coli* exhibiting an AmpC phenotype with the *E. coli* K-12 sequence as reference; MICs of cephalosporins and the presence of a plasmid-mediated cephalosporinase are also detailed for each strain

	MIC (mg/L)					Promoter with attenuator (from +17 to +37) Nucleotide at position[a,b]:															
Strain	FOX	CAZ	CTX	FEP	Plasmid-mediated AmpC	−88	−82	−73	−62 −61	<u>−42</u>	<u>**−32**</u>	−28	<u>−18</u>	**−14 −13**	**−13 −12**	−1	**+5**	**+6**	**+20**	**+23**	**+58**
K-12	2	0.06	0.06	0.06		C	A	C	TC	C	T	G	G	TT	TA	C	C	C	C	G	C
10-020	>256	>256	8	0.75	CMY-2																
05-008	64	8	1	0.125				T							T[d]			T			
12-169	64	6	1.5	0.19				T			A	A									
04-004	64	24	2	0.19		T	G			T			A			T					T
06-021	>256	32	2	0.25		T	G			T			A			T					T
08-092	64	32	1.5	0.125		T	G			T			A			T					T
12-133	64	12	1.5	0.19		T	G			T			A			T					T
12-140	256	48	2	0.25		T	G			T			A			T					T
13-030	96	48	2	0.5		T	G			T			A			T					T
08-152	256	>256	4	0.5	CMY-2																
12-003	>256	>256	8	0.75	CMY-2	T	G						A			T					T
12-015	>256	>256	8	0.75	CMY-2	T	G						A			T					T
12-052	32	>256	4	1		T	G			T			A			T					T
06-062	>256	>256	12	4										G[c]	T[c]		T				
06-006	96	>256	8	4		T	G		A[c]	T			A			T			A		T
07-107	96	>256	8	4		T	G			T			A			T			A		T
05-063	96	>256	8	6	CMY-2	T	G			T			A			T			A		
08-121	192	>256	12	16		T	G			T			A			T				A	T

	Coding region Amino acid at position[a,b]:																												
Strain	23	76	89	94	129	141	143	153	175	193	194	215	220	232	235	239	241	244	245	247	274	**282**	<u>**287**</u>	**288**	**292**	**293**	296	300	351
K-12	Q	D	T	P	S	A	A	S	Q	P	A	A	A	R	Q	K	L	N	E	T	L	S	S	D	A	L	R	A	D
10-020																													
05-008									K	S												I						P	A
12-169					G															I									
04-004			A								P				R	N	R		D					G			H		A
06-021			A								P	V			R	N	R		D					G			H		A
08-092			A								P				R	N	R		D					G			H		A
12-133		E											T	C	R	N	R		D			I							A
12-140			A								P				R	N	R		D					G			H		A
13-030			A								P				R	N	R		D					G			H		A
08-152	K		A	S									T	C	R	N	R		D					G					T
12-003			A								P				R	N	R		D					G			H		A
12-015						T					P				R	N	R		D					G			H		A
12-052			A								P				R	N	R		D				C	G			H		A
06-062			A				T	T										T			Q	I			V				T
06-006			A								P				R	N	R		D				N	G			H		A
07-107			A								P				R	N	R		D				N	G			H		A
05-063			A								P				R	N	R		D				N	G			H		A
08-121			A								P				R	N	R		D					G		P	H		A

FOX, cefoxitin; CAZ, ceftazidime; CTX, cefotaxime; FEP, cefepime.

[a]Mutations that are underlined have an effect experimentally demonstrated by the strength of the promoter of the *ampC* gene, or by the extension of the hydrolysis spectrum of the cephalosporinase.

[b]The −35 and −10 boxes, as well as the H-9 helix and H-10 helix, are indicated in bold.

[c]Inserted base.

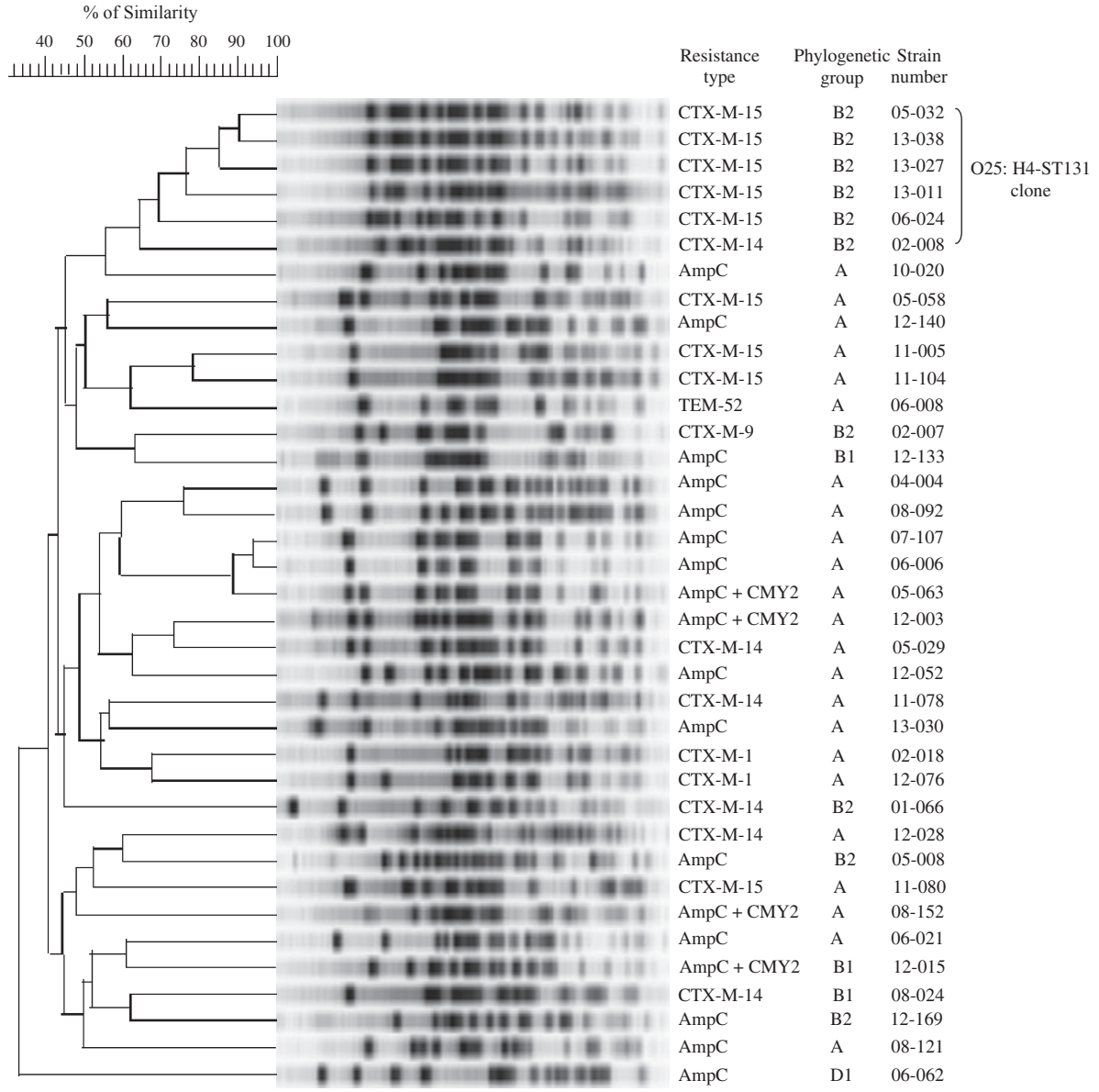

FIG. 1. Dendrogram showing the estimated genetic relationships among the *Escherichia coli* strains harbouring an extended-spectrum β-lactamase or AmpC phenotype. The dendrogram was generated by applying the UPGMA algorithm to *Xba*I patterns.

appropriate antimicrobial therapy to cure these severe infections [33].

Phylogroup A was the most common (61%) among the 3GC-R strains, and less common among the 3GC-S strains ($p < 0.0001$), where phylogroups B2 or D are predominant (Table 3). This result agrees with several observations showing that resistant strains of *E. coli* belong to phylogroups that are usually considered to have low virulence [7,34], i.e. A, B1 and D [35]. Indeed, 3GC-R strains carried significantly fewer virulence factors than 3GC-S strains ($p < 0.0001$). These results also confirm that the trade-off between resistance to fluoroquinolones and virulence [7,36] is also found in *E. coli* strains isolated from bacteraemias. Finally, with the exception of the strains belonging to the ST131 clone, only six strains were O-typed (Table 1), indicating that the 3GC-R strains of COLIBAFI do not express the O-types usually described in *E. coli* strains involved in extra-intestinal infections [13].

Prior use of antibiotics and underlying chronic disease \are risk factors for development of 3GC-resistant *E. coli* bacteraemia

The majority of the patients developed a community-acquired infection. Several factors were found to be different

TABLE 3. Statistical comparison of microbiological data of *Escherichia coli* strains of the COLIBAFI study

Variable	3GC-S vs. 3GC-R strains			ESBL-producing vs. AmpC-producing strains		
	3GC-S (*n* = 1040)	3GC-R (*n* = 41)	p_1	ESBL (*n* = 19)	AmpC (*n* = 18)	p_2
Antimicrobial resistance, *n* (%)						
Ciprofloxacin	101 (9.7)	28 (68.3)	<0.0001	16 (84.2)	9 (50)	0.04
Gentamicin	39 (3.8)	10 (24.4)	<0.0001	8 (42.1)	1 (5.6)	0.02
Amikacin	11 (1)	3 (7.32)	0.01	3 (15.8)	0 (0)	0.23
Co-trimoxazole	291 (28)	31 (75.6)	<0.0001	15 (79)	14 (77.8)	1
Phylogenetic group, *n* (%)						
A	221 (21.3)	25 (61)	<0.0001	10 (52.6)	13 (72.2)	0.08
B1	46 (4.4)	2 (4.9)		0	2 (11.1)	
B2	549 (52.8)	11 (26.8)		8 (42.1)	2 (11.1)	
D	224 (21.5)	3 (7.3)		1 (5.3)	1 (5.6)	
VF score, mean ± SD	8.2 ± 4.3	5.6 ± 3.6	<0.0001	4.9 ± 3.1	5.8 ± 3.5	0.43

3GC-S, susceptible to third-generation cephalosporins (3GCs); 3GC-R, resistant to 3GCs; ESBL, extended-spectrum β-lactamase; p_1, p-value for the comparison between 3GC-S and 3GC-R strains; p_2, p-value for the comparison between ESBL-producing and AmpC-producing strains; VF score, virulence factor score; SD, standard deviation.

between the 3GC-S and the 3GCR groups (Table 4). The following risk factors were identified by multivariate analysis: underlying chronic disease (OR 2.20 (95% CI 1.15–4.20), p 0.0176), prior use of antibiotics (OR 2.58 (95% CI 1.30–5.13), p 0.0069) and an unidentified or non-standard portal of entry (OR 2.04 (95% CI 1.06–3.93), p 0.0331).

Bacteraemias with 3GC-R isolates were more frequently associated with death than bacteraemias with 3GC-S isolates (p 0.0024) (Table 4). The full study of risk factors associated with death in the COLIBAFI study will be presented elsewhere (A. Lefort, X. Panhard, O. Clermont, P. L. Woerther, C. Branger, F. Mentré, B. Fantin, M. Wolff, E. Denamur and the COLIBAFI group; personal communication).

Within the 3GC-R group, male sex was predominant in the ESBL group, which is consistent with previous published data [33]. Immunosuppression (p 0.036) and prior antibiotic treatment (p 0.0041) seemed to be more important risk factors for developing an infection with ESBL-producing *E. coli* than for developing an infection with an AmpC-over-producing strains. Bacteraemia was no more lethal in the ESBL group than in the AmpC group (Table 4).

In conclusion the COLIBAFI study showed that, in France in 2005, 3.8% of *E. coli* strains responsible for bacteraemia had decreased susceptibility to 3GCs. This study relied on a prospective and multicentre approach, making it well representative of French epidemiology. Moreover, the statistical comparison between the 3GC-R group and the 3GC-S group seems to be the best study design with which to investigate factors associated with antimicrobial resistance [20]. However, the low number of 3GC-R strains may have led to an underestimation of the presence of confounding bias. ESBL-producing strains and AmpC producers were equally represented. Resistance was plasmid-mediated in 68.3% of the cases, representing a potentially epidemic

TABLE 4. Clinical characteristics of patients presenting with an *Eschrichia coli* bacteraemia

Variable	Patients infected with:					
	3GC-S isolate (*n* = 1012)	3GC-R isolate (*n* = 39)	p_1	ESBL-producing isolate (*n* = 19)	AmpC-producing isolate (*n* = 16)	p_2
Age (years), mean ± SD	66.8 ± 17.6	71.2 ± 13.2	0.20	69.8 ± 14.0	74.8 ± 12.9	0.21
Male sex	424 (41.9)	23 (59.0)	0.046	13 (68.4)	8 (50.0)	0.32
Underlying chronic disease	311 (30.7)	19 (48.7)	0.022	10 (52.6)	6 (37.5)	0.50
Immunosuppression	380 (37.6)	17 (43.6)	0.50	11 (57.9)	3 (18.8)	0.036
Prior use of antibiotic	162 (16.0)	14 (35.9)	0.0032	10 (52.6)	1 (6.3)	0.0041
Origin of infection						
Community-acquired	819 (80.9)	30 (76.9)	0.54	15 (79.0)	13 (81.3)	1
Nosocomial	193 (19.1)	9 (23.1)		4 (21.0)	3 (18.7)	
Portal of entry						
Urinary tract	583 (57.6)	15 (38.5)	0.021	5 (26.3)	9 (56.3)	0.09
Intra-abdominal	133 (13.1)	5 (12.8)	1.00	3 (15.8)	2 (12.5)	1
Others or unknown	303 (29.9)	19 (48.7)	0.020	11 (57.9)	5 (31.3)	0.18
Death	124 (12.25)	12 (30.77)	0.0024	5 (26.3)	4 (25.0)	1

Data are no. and percentages of patients, unless otherwise indicated.
3GC-S, susceptible to third-generation cephalosporins (3GCs); 3GC-R, resistant to 3GCs; ESBL, extended-spectrum β-lactamase; p_1, p-value for the comparison between the 3GC-S group and the 3GC-R group; p_2, p-value for the comparison between ESBL-producing and AmpC-producing strains; SD, standard deviation.

threat. These 3GC-R strains, mostly community-acquired, were significantly more frequent in patients with underlying chronic disease and were associated with a more severe outcome. Prior use of antibiotics and immunosuppression are greater risk factors for infection by ESBL-producing strains than for infection by AmpC overproducers. Adequate antimicrobial therapy of ESBL-producing strains relies on carbapenems, whereas cefepime could usually be used to treat infections with AmpC producers. The recent revision of the MIC breakpoints for cephalosporins by the European Committee on Antimicrobial Susceptibility Testing (EUCAST: http://www.escmid.org) and the CA-SFM will have an impact on the susceptibility categorization of cefepime. The initial breakpoint was lowered from 4 mg/L to 1 mg/L, which might decrease the number of strains categorized as susceptible among the AmpC-producing strains. This could lead to an increase in the use of carbapenems, with the threat of contributing to the emergence and spread of carbapenem-resistant *E. coli* [37].

Acknowledgements

We gratefully acknowledge the contributions of all members of the COLIBAFI group.

Investigators: Michel Wolff, Loubna Alavoine, Xavier Duval, David Skurnik, Paul-Louis Woerther and Antoine Andremont (CHU Bichat-Claude Bernard, Paris, France); Etienne Carbonnelle, Olivier Lortholary and Xavier Nassif (CHU Necker-Enfants Malades, Paris, France); Sophie Abgrall, Françoise Jaureguy and Bertrand Picard (CHU Avicenne, Bobigny, France); Véronique Houdouin, Yannick Aujard, Stéphane Bonacorsi and Edouard Bingen (CHU Robert Debré, Paris, France); Agnès Meybeck, Guilène Barnaud and Catherine Branger (CHU Louis Mourier, Colombes, France); Agnès Lefort, Bruno Fantin, Claire Bellier, Frédéric Bert and Marie-Hélène Nicolas-Chanoine (CHU Beaujon, Clichy, France); Bernard Page, Julie Cremniter and Jean-Louis Gaillard (CHU Ambroise Paré, Boulogne Billancourt, France); Bernard Garo, Séverine Ansart, Geneviève Herry-Arnaud and Didier Tandé (CHU Brest, Brest, France); Jean Claude Renet, René Ze Bekolo, Renaud Verdon and Roland Leclercq (CHU Caen, Caen, France); Claire de Gialluly, Jean Marc Besnier, Laurent Mereghetti and Roland Quentin (CHU Tours, Tours, France); Achille Kouatchet, Alain Mercat and Marie Laure Joly-Guillou (CHU Angers, Angers, France); Catherine Dalebroux, Pascal Chavanet and Catherine Neuwirth (CHU Dijon, Dijon, France); Camille Colliard, Martin Dary, Gilles Potel and Jocelyne Caillon (CHU Nantes, Nantes, France); Françoise Leturdu, Jean Pierre Sollet and Gaëtan Plantefève (CH Argenteuil, Argenteuil, France); Agnès de Patureaux, Pierre Tattevin and Pierre Yves Donnio (CHU Rennes, Rennes, France).

Clinical Research Unit: Estelle Marcault and Florence Tubach (CHU Bichat-Claude Bernard, Paris, France).

Biostatistics: Xavière Panhard, Ludovic Lassel, Quentin Dornic and France Mentré (AP-HP, Hôpital Bichat, UF de Biostatistiques, Paris, France).

Bacterial genotyping: Erick Denamur, Olivier Clermont, Christine Amorin and Jeremy Glodt (INSERM U722, Université Paris 171, Paris, France).

We thank Marie-Claire Hipeaux for technical assistance.

This work was presented in part at the 49th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), San Francisco, CA, USA, 2009.

Funding

This work was partially supported by the 'Programme Hospitalier de Recherche Clinique' AOR 04053EI and the 'Réseau de Recherche Clinique' INSERM RBM 03-58.

Transparency Declaration

No conflicts of interest to declare.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. List of the primers used for the PCR O-typing.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

References

1. Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. *N Engl J Med* 2003; 348: 1546–1554.
2. Diekema DJ, Beekmann SE, Chapin KC, Morel KA, Munson E, Doern GV. Epidemiology and outcome of nosocomial and community-onset bloodstream infection. *J Clin Microbiol* 2003; 41: 3655–3660.
3. Livermore DM, Canton R, Gniadkowski M *et al.* CTX-M: changing the face of ESBLs in Europe. *J Antimicrob Chemother* 2007; 59: 165–174.
4. Pitout JD, Laupland KB. Extended-spectrum beta-lactamase-producing Enterobacteriaceae: an emerging public-health concern. *Lancet Infect Dis* 2008; 8: 159–166.

5. Mulvey MR, Bryce E, Boyd DA *et al.* Molecular characterization of cefoxitin-resistant *Escherichia coli* from Canadian hospitals. *Antimicrob Agents Chemother* 2005; 49: 358–365.
6. Jarlier V, Nicolas MH, Fournier G, Philippon A. Extended broad-spectrum beta-lactamases conferring transferable resistance to newer beta-lactam agents in *Enterobacteriaceae*: hospital prevalence and susceptibility patterns. *Rev Infect Dis* 1988; 10: 867–878.
7. Branger C, Zamfir O, Geoffroy S *et al.* Genetic background of *Escherichia coli* and extended-spectrum beta-lactamase type. *Emerg Infect Dis* 2005; 11: 54–61.
8. Mammeri H, Poirel L, Fortineau N, Nordmann P. Naturally occurring extended-spectrum cephalosporinases in *Escherichia coli*. *Antimicrob Agents Chemother* 2006; 50: 2573–2576.
9. Perez-Perez FJ, Hanson ND. Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. *J Clin Microbiol* 2002; 40: 2153–2162.
10. Steward CD, Rasheed JK, Hubert SK *et al.* Characterization of clinical isolates of *Klebsiella pneumoniae* from 19 laboratories using the National Committee for Clinical Laboratory Standards extended-spectrum beta-lactamase detection methods. *J Clin Microbiol* 2001; 39: 2864–2872.
11. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl Environ Microbiol* 2000; 66: 4555–4558.
12. Johnson JR, Clermont O, Menard M, Kuskowski MA, Picard B, Denamur E. Experimental mouse lethality of *Escherichia coli* isolates, in relation to accessory traits, phylogenetic group, and ecological source. *J Infect Dis* 2006; 194: 1141–1150.
13. Clermont O, Johnson JR, Menard M, Denamur E. Determination of *Escherichia coli* O types by allele-specific polymerase chain reaction: application to the O types involved in human septicemia. *Diagn Microbiol Infect Dis* 2007; 57: 129–136.
14. Clermont O, Lavollay M, Vimont S *et al.* The CTX-M-15-producing *Escherichia coli* diffusing clone belongs to a highly virulent B2 phylogenetic subgroup. *J Antimicrob Chemother* 2008; 61: 1024–1028.
15. Deschamps C, Clermont O, Hipeaux MC, Arlet G, Denamur E, Branger C. Multiple acquisitions of CTX-M plasmids in the rare D2 genotype of *Escherichia coli* provide evidence for convergent evolution. *Microbiology* 2009; 155: 1656–1668.
16. Clermont O, Dhanji H, Upton M *et al.* Rapid detection of the O25b-ST131 clone of *Escherichia coli* encompassing the CTX-M-15-producing strains. *J Antimicrob Chemother* 2009; 64: 274–277.
17. Branger C, Bruneau B, Lesimple AL *et al.* Epidemiological typing of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* isolates responsible for five outbreaks in a university hospital. *J Hosp Infect* 1997; 36: 23–36.
18. Canton R, Novais A, Valverde A *et al.* Prevalence and spread of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in Europe. *Clin Microbiol Infect* 2008; 14 (suppl 1): 144–153.
19. Rodriguez-Bano J, Picon E, Gijon P *et al.* Risk factors and prognosis of nosocomial bloodstream infections caused by extended-spectrum-beta-lactamase-producing *Escherichia coli*. *J Clin Microbiol* 2010; 48: 1726–1731.
20. Rodriguez-Bano J, Picon E, Gijon P *et al.* Community-onset bacteremia due to extended-spectrum beta-lactamase-producing *Escherichia coli*: risk factors and prognosis. *Clin Infect Dis* 2010; 50: 40–48.
21. Brinas L, Lantero M, de Diego I, Alvarez M, Zarazaga M, Torres C. Mechanisms of resistance to expanded-spectrum cephalosporins in *Escherichia coli* isolates recovered in a Spanish hospital. *J Antimicrob Chemother* 2005; 56: 1107–1110.
22. Haldorsen B, Aasnaes B, Dahl KH *et al.* The AmpC phenotype in Norwegian clinical isolates of *Escherichia coli* is associated with an acquired ISEcp1-like ampC element or hyperproduction of the endogenous AmpC. *J Antimicrob Chemother* 2008; 62: 694–702.
23. Deshpande LM, Jones RN, Fritsche TR, Sader HS. Occurrence of plasmidic AmpC type beta-lactamase-mediated resistance in *Escherichia coli*: report from the SENTRY Antimicrobial Surveillance Program (North America, 2004). *Int J Antimicrob Agents* 2006; 28: 578–581.
24. Caroff N, Espaze E, Gautreau D, Richet H, Reynaud A. Analysis of the effects of -42 and -32 ampC promoter mutations in clinical isolates of *Escherichia coli* hyperproducing AmpC. *J Antimicrob Chemother* 2000; 45: 783–788.
25. Jaurin B, Grundstrom T, Edlund T, Normark S. The *E. coli* beta-lactamase attenuator mediates growth rate-dependent regulation. *Nature* 1981; 290: 221–225.
26. Vakulenko SB, Golemi D, Geryk B *et al.* Mutational replacement of Leu-293 in the class C *Enterobacter cloacae* P99 beta-lactamase confers increased MIC of cefepime. *Antimicrob Agents Chemother* 2002; 46: 1966–1970.
27. Mammeri H, Poirel L, Nordmann P. Extension of the hydrolysis spectrum of AmpC beta-lactamase of *Escherichia coli* due to amino acid insertion in the H-10 helix. *J Antimicrob Chemother* 2007; 60: 490–494.
28. Mammeri H, Galleni M, Nordmann P. Role of the Ser-287–Asn replacement in the hydrolysis spectrum extension of AmpC beta-lactamases in *Escherichia coli*. *Antimicrob Agents Chemother* 2009; 53: 323–326.
29. Barnaud G, Benzerara Y, Gravisse J *et al.* Selection during cefepime treatment of a new cephalosporinase variant with extended-spectrum resistance to cefepime in an *Enterobacter aerogenes* clinical isolate. *Antimicrob Agents Chemother* 2004; 48: 1040–1042.
30. Boyd DA, Tyler S, Christianson S *et al.* Complete nucleotide sequence of a 92-kilobase plasmid harboring the CTX-M-15 extended-spectrum beta-lactamase involved in an outbreak in long-term-care facilities in Toronto, Canada. *Antimicrob Agents Chemother* 2004; 48: 3758–3764.
31. Pitout JD, Gregson DB, Campbell L, Laupland KB. Molecular characteristics of extended-spectrum-beta-lactamase-producing *Escherichia coli* isolates causing bacteremia in the Calgary Health Region from 2000 to 2007: emergence of clone ST131 as a cause of community-acquired infections. *Antimicrob Agents Chemother* 2009; 53: 2846–2851.
32. Nicolas-Chanoine MH, Blanco J, Leflon-Guibout V *et al.* Intercontinental emergence of *Escherichia coli* clone O25:H4-ST131 producing CTX-M-15. *J Antimicrob Chemother* 2008; 61: 273–281.
33. Melzer M, Petersen I. Mortality following bacteraemic infection caused by extended spectrum beta-lactamase (ESBL) producing *E. coli* compared to non-ESBL producing *E. coli*. *J Infect* 2007; 55: 254–259.
34. Corvec S, Prodhomme A, Giraudeau C, Dauvergne S, Reynaud A, Caroff N. Most *Escherichia coli* strains overproducing chromosomal AmpC beta-lactamase belong to phylogenetic group A. *J Antimicrob Chemother* 2007; 60: 872–876.
35. Johnson JR, Goullet P, Picard B, Moseley SL, Roberts PL, Stamm WE. Association of carboxylesterase B electrophoretic pattern with presence and expression of urovirulence factor determinants and antimicrobial resistance among strains of *Escherichia coli* that cause urosepsis. *Infect Immun* 1991; 59: 2311–2315.
36. Johnson JR, van der Schee C, Kuskowski MA, Goessens W, van Belkum A. Phylogenetic background and virulence profiles of fluoroquinolone-resistant clinical *Escherichia coli* isolates from the Netherlands. *J Infect Dis* 2002; 186: 1852–1856.
37. Oteo J, Delgado-Iribarren A, Vega D *et al.* Emergence of imipenem resistance in clinical *Escherichia coli* during therapy. *Int J Antimicrob Agents* 2008; 32: 534–537.