

Ceftriaxone-Resistant *Salmonella enterica* Serotype Newport, France

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The multidrug-resistant (MDR) *Salmonella enterica* serotype Newport strain that produces CMY-2 β -lactamase (Newport MDR-AmpC) was the source of sporadic cases and outbreaks in humans in France during 2000–2005. Because this strain was not detected in food animals, it was most likely introduced into France through imported food products.

Third-generation cephalosporins are drugs of choice for treatment of persons with nontyphoidal *Salmonella* infections that require chemotherapy or when fluoroquinolones are contraindicated. A new public health concern is the emergence of third-generation cephalosporin-resistant *Salmonella* isolates (1). Multidrug-resistant (MDR) *Salmonella enterica* serotype Newport isolates that produce CMY-2, a β -lactamase that inactivates third-generation cephalosporins, were first reported in the United States in 1998 (2). These isolates, known as Newport MDR-AmpC, have quickly spread through the United States in cattle and humans (3–5). It has been hypothesized that use of ceftiofur, a third-generation cephalosporin licensed in the United States for use in cattle, could have selected for Newport MDR-AmpC (2–4,7). Several observations and case-control studies suggested beef and milk from dairy cattle were substantial sources of Newport MDR-AmpC infection in humans (6–8).

These isolates seem to be extremely rare in Europe. Two surveys performed in England and Wales (278,308 human *Salmonella* isolates tested, 1992–2003) and Spain (959 human *Salmonella* isolates, 1999–2000) did not detect New-

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port MDR-AmpC (9,10). In St. Petersburg, Russia, only 1 Newport MDR-AmpC isolate was reported among 1,078 *Salmonella* isolates during 2002–2005 (11). In France, a small outbreak (14 cases) of Newport MDR-AmpC was detected in 2003 and linked to consumption of imported horse meat (12). We undertook the present study to acquire more knowledge on circulation of Newport MDR-AmpC in humans, animals, and animal-derived food in France.

The Study

From 2000 through 2005, the French National Reference Centre for *Salmonella* at the Institut Pasteur in Paris reported 829 Newport isolates among 69,759 *Salmonella* clinical isolates. During this period and depending on the year, serotype Newport ranked between 6th and 10th in prevalence among human serotyped isolates. From 2000 through 2005, the Agence Française de Sécurité Sanitaire des Aliments reported 2,160 Newport isolates among 101,791 *Salmonella* isolates collected from animals and food products.

Antimicrobial drug susceptibility testing was performed on 585 human Newport isolates and 342 nonhuman Newport isolates by disk diffusion with 32 antimicrobial drugs (additional information available from fxweill@pasteur.fr). Data for Newport human isolates are shown in the Table. Of 585 isolates tested, 46 (7.9%) were resistant to third-generation cephalosporins. The geographic origin of the isolates was mainly the Paris metropolitan area and northern France (online Appendix Table, available from www.cdc.gov/EID/content/14/6/954-appT.htm). There was a high prevalence of third-generation cephalosporin-resistant isolates during 2000 (15%) and 2003 (17.5% caused by a small outbreak). No third-generation cephalosporin resistance was detected in any of the nonhuman Newport isolates tested.

Experiments were performed on the 46 third-generation cephalosporin-resistant Newport isolates (additional information available from fxweill@pasteur.fr). All but 1 of the Newport isolates were resistant to cefoxitin (online Appendix Table). These isolates showed 4 resistance phenotypes; most (41, 89.1%) were resistant to streptomycin, sulfonamides, chloramphenicol, and tetracycline. PCR and sequencing showed that the 45 isolates resistant to cefoxitin were positive for the *bla*_{CMY-2} gene, and cefoxitin-susceptible isolates contained the extended-spectrum β -lactamase gene *bla*_{CTX-M-1}. Ceftriaxone MICs of Newport MDR-AmpC isolates ranged from 32 mg/L to >256 mg/L, and ceftazidime MICs ranged from 64 mg/L to >256 mg/L. No *bla*_{TEM} genes were detected. Three isolates with additional resistance to aminoglycosides contained a class 1 integron with the 1-kb gene cassette *aadA24* (known to encode resistance to strep-

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Table. Resistance to specific antimicrobial drugs in *Salmonella enterica* serotype Newport from humans in France, 2000–2005*

Drug	% Resistant isolates					
	2000 (n = 100) (N = 109)	2001 (n = 124) (N = 134)	2002 (n = 66) (N = 71)	2003 (n = 126) (N = 138)	2004 (n = 91) (N = 94)	2005 (n = 78) (N = 80)
Amoxicillin	27	9.7	1.5	19.8	8.8	3.8
Ceftriaxone/ceftazidime	15	4	1.5	17.5	2.2	0
Gentamicin	4	1.6	0	1.6	2.2	0
Nalidixic acid	23	7.3	4.5	1.6	4.4	2.6
Ciprofloxacin	0	0	0	0	0	0
Sulfonamides	29	10.5	4.5	19.8	8.8	0
Trimethoprim	10	4	3	1.6	4.4	0
Chloramphenicol	25	9.7	1.5	15.9	8.8	0
Tetracycline	27	11.3	3	19	9.9	3.8

*n, no. of isolates studied; N, no. of isolates received at the French National Reference Centre for *Salmonella* (1 per patient).

tomyacin and spectinomycin) (11). The chloramphenicol/florfenicol resistance gene *floR* was detected in all but 1 CMY-2–producing Newport isolate.

Clonal relatedness of Newport isolates was assessed by multilocus sequence typing (MLST) and PulseNet standard method pulsed-field gel electrophoresis (PFGE) (Figure 1). All 16 Newport MDR-AmpC isolates tested had a common sequence type (ST), ST45. *Xba*I-PFGE identified 10 distinct profiles (similarity 76.7%) among all 45 Newport MDR-AmpC isolates. Single enzyme matches were found for 3 of the profiles (15 isolates) in the US PulseNet national database (www.cdc.gov/pulsenet; online Appendix Table; Figure 2). Two PFGE types (New6 and New8) were divided into 2–4 subtypes because of additional band(s) <100 kb. Isolates from the 2003 outbreak showed 4 similar but distinct PFGE profiles that differed by 1–2 bands, migrated between 60 and 100 kb, and were attributed to plasmid(s) (additional information available from fxweill@pasteur.fr). If only cases with indistinguishable PFGE profiles had been tested, potentially related cases would not have been linked to this outbreak. Therefore, during an outbreak investigation of Newport MDR-AmpC, analysis of plasmid content (either by alkaline lysis or S1 nuclease, depending on size of additional bands) might complete *Xba*I-PFGE profiles for isolates whose profiles differ by 1 or 2 additional bands of low molecular mass.

Alkaline lysis extraction showed that all but 1 of the Newport MDR-AmpC isolates harbored a plasmid >125 kb that hybridized with a *bla*_{CMY-2} probe; the remaining isolate harbored a plasmid of 100 kb (online Appendix Table). Analysis with S1 nuclease showed that these plasmids were 100 kb–370 kb. Up to 3 additional plasmids (3.5 kb–100 kb) that did not have *bla*_{CMY-2} were detected in most isolates (online Appendix Table). Cephalosporin resistance was transferred by electroporation of plasmid DNA to *Escherichia coli* DH10B for all 38 CMY-2–positive isolates tested. When present in the donor strain, resistance to sulfonamides, chloramphenicol, and tetracycline was also transferred. Restriction analysis of plasmids isolated from transformants showed 6 similar restriction profiles

for Newport isolates (R1–R6) (Figure 2, online Appendix Table). R1 was predominant (found in 26 isolates among 35 tested, 74.3%). Newport plasmids R1–R6 and Agona plasmid R8 were shown by PCR to contain variant A/C₂ replicons (13), whereas Typhimurium plasmid R7 contained the I1 replicon.

*Pst*I-digested plasmids analyzed by Southern hybridization with a *bla*_{CMY-2} probe (Figure 2) showed 4 hybridization profiles among Newport isolates. Profile H1 corresponded to plasmid type C described by Carattoli et al. (14). Profiles H2, H3, and H4 differed from H1 by 1 additional band (>10 kb for H2, 3.2 kb for H3, and >18 kb for H4), which indicated that the *bla*_{CMY-2} gene was partially or totally duplicated.

Conclusions

Newport MDR-AmpC isolates have been the source of sporadic cases and small outbreaks in humans in France during 2000–2005. All isolates had the same MLST type, ST45, and highly similar *Xba*I-PFGE profiles. Their plas-

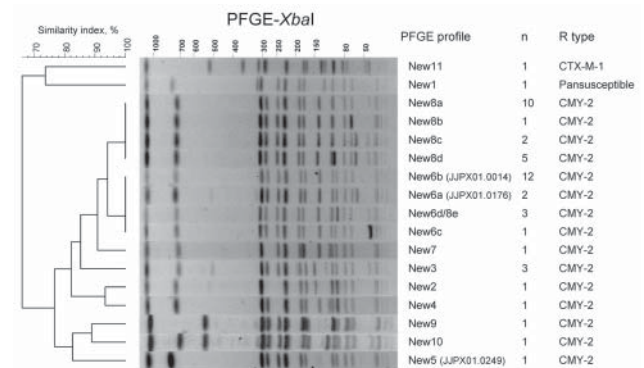


Figure 1. Representative *Xba*I pulsed-field gel electrophoresis (PFGE) profiles of third-generation cephalosporin-resistant *Salmonella* Newport isolates studied. A dendrogram was generated with Bionumerics software (Applied Maths, Sint-Martens-Latem, Belgium). The PFGE profile (and if there were indistinguishable isolates in the PulseNet USA database [www.cdc.gov/pulsenet], the corresponding Centers for Disease Control and Prevention PulseNet profile), the number of isolates, and the β -lactamase genes are indicated.

mids carrying *bla*_{CMY-2} were homogeneous (same incompatibility group A/C₂, a main restriction type R1, and a main hybridization type H1). These results support clonal expansion of 1 Newport strain (or a limited number of genetically related Newport strains) able to acquire and maintain a large *incA/C*₂ MDR plasmid.

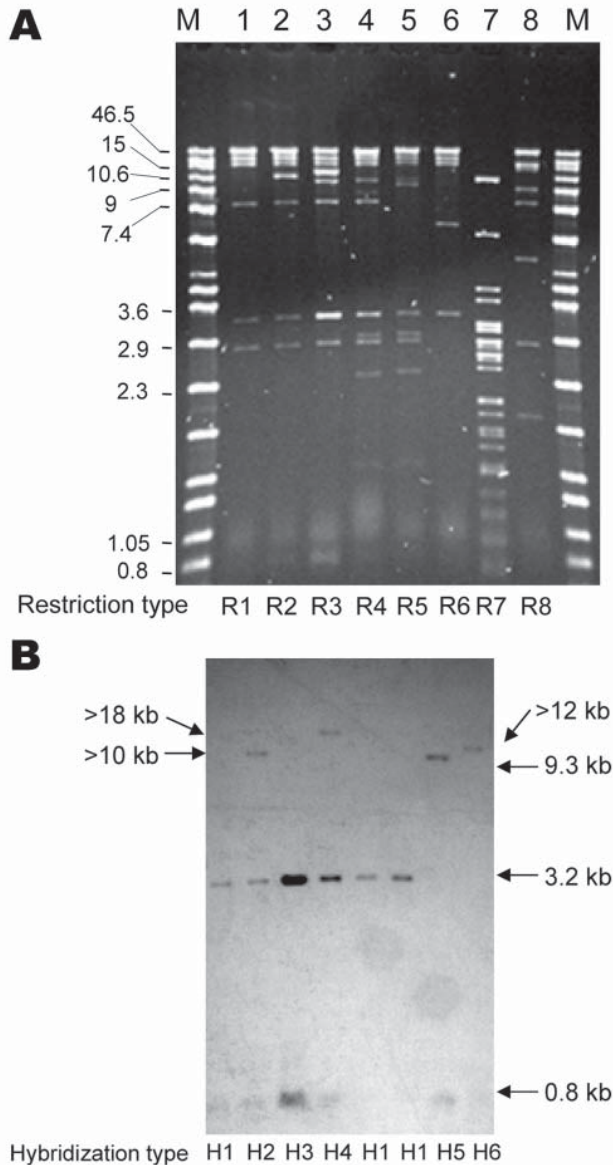


Figure 2. Representative *Pst*I restriction profiles (A) and *bla*_{CMY-2} Southern hybridization (B) of plasmids from *Escherichia coli* DH10B transformants of CMY-2-producing *Salmonella* spp. clinical isolates. Lane M, Raul molecular mass marker (Qbiogene, Illkirch, France). Lane 1, DH10B/00-7490; lane 2, DH10B/03-3349; lane 3, DH10B/03-3367; lane 4, DH10B/00-3525; lane 5, DH10B/00-4165; lane 6, DH10B/03-9969; lane 7, DH10B/03-9243; lane 8, DH10B/02-2049. Values on the left of panel A are in kb. Restriction and hybridization profiles are indicated. The gel is focused on the resolution of high molecular mass bands; smaller bands (in particular, the 0.8-kb band) are not well visualized.

The source of the French isolates remains unknown. However, this strain was not found in French food animals or domestically produced food products (additional information available from fxweill@pasteur.fr). One outbreak during the study period was linked to imported horse meat. Further investigation identified the source as a wholesaler who imported meat from Belgium, the United Kingdom, Hungary, Canada, Brazil, Argentina, Uruguay, and Australia (12). In contrast to Europe, Newport MDR-AmpC has been frequently seen in the United States during the past decade. Furthermore, several characteristics were shared between US and French Newport MDR-AmpC isolates: ST45 (15), PFGE profiles New5, New6a, and New6b (displayed by 15 isolates among the 45 studied), and *bla*_{CMY-2} plasmid hybridization type H1 (14). We can reasonably hypothesize that during 2000–2005 some isolates likely entered France from North America through imported food. Alternatively, they could have come to France and North America from some other country.

Acknowledgments

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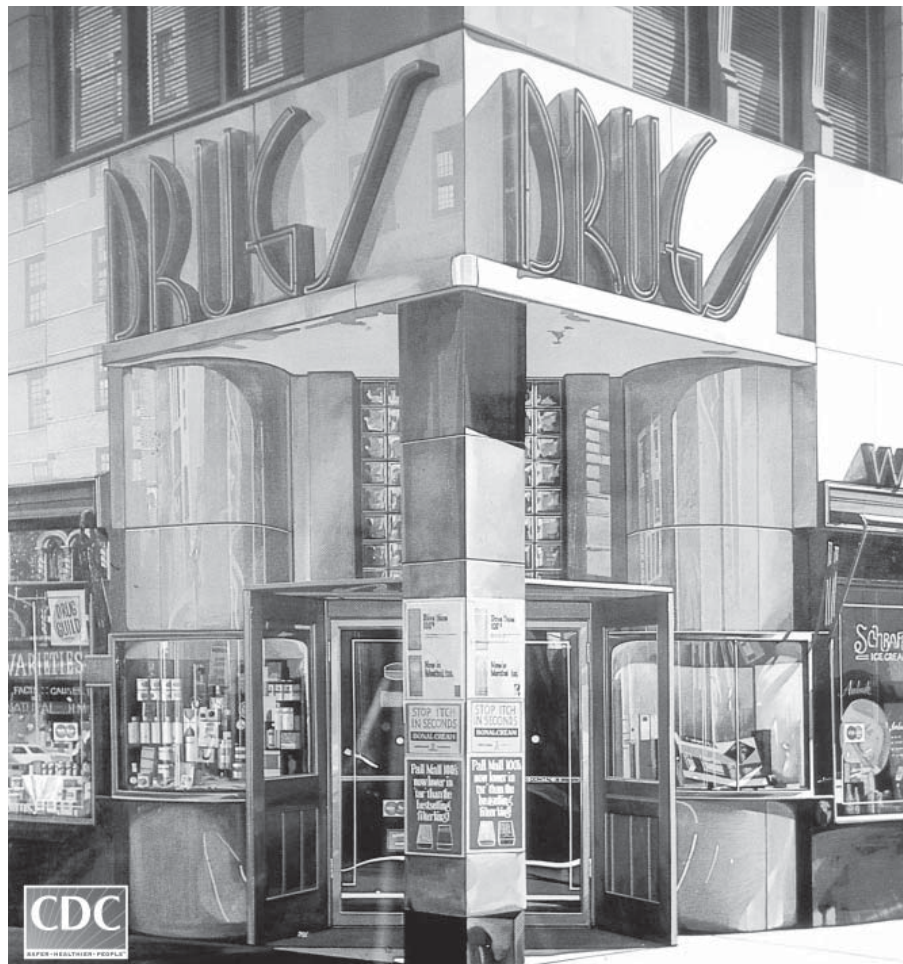
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Appendix Table. Characteristics of *Salmonella* spp. isolates used in this study*

Isolate	Date of isolation	Area of isolation†	Patient age group‡, sex	Source	Antimicrobial drug resistance phenotype§	Class 1 integron size, kb (gene cassette)	PFGE profile¶	MLST type	Plasmid profile#	Plasmid restriction profile**
S. Newport isolates										
00-3525	2000 Jun	75	III-F	Stool	AFoxCazSSuCTe	—	New6b (JJPX01.0014)	ST45	100, 50, 3.5	R4
00-3767	2000 Jun	60	III-F	Stool	AFoxCazSSuCTe	—	New2	ST45	>125, 3.5	R3
00-3784	2000 Jun	75	II-M	Stool	AFoxCazSSpKToGSuCTe	1 (<i>aadA24</i>)	New3	ST45	>125, 7	R1
00-4165	2000 Jul	62	II-M	Stool	AFoxCazSSpKToGSuCTe	1 (<i>aadA24</i>)	New 3	ST45	>125, 7	R5
00-4652	2000 Jul	60	II-F	Stool	AFoxCazSSuCTe	—	New 4	ND	>125, 3.5	R4
00-5089	2000 Jul	2A	II-F	Stool	AFoxCazSSpKToGSuCTe	1 (<i>aadA24</i>)	New 3	ST45	>125, 7	R1
00-6399	2000 Sep	92	V-M	NK	AFoxCazSSuCTe	—	New5 (JJPX01.0249)	ND	>125, 3.5	R1
00-7093	2000 Sep	77	II-F	Stool	AFoxCazSSuCTe	—	New 7	ST45	>125, 90	R1
00-7098	2000 Sep	93	II-F	Stool	AFoxCazSSuCTe	—	New6b (JJPX01.0014)	ND	>125, 90	R1
00-7298	2000 Sep	77	IV-F	Stool	AFoxCazSSuCTe	—	New6b (JJPX01.0014)	ND	>125, 90	R1
00-7325	2000 Sep	75	IV-F	Stool	AFoxCazSSuCTe	—	New6b (JJPX01.0014)	ST45	>125, 90	R1
00-7400	2000 Sep	77	IV-M	Stool	AFoxCazSSuCTe	—	New6b (JJPX01.0014)	ND	>125	R1
00-7490	2000 Sep	89	II-M	Stool	AFoxCazSSuCTe	—	New6b (JJPX01.0014)	ND	>125, 90	R1
00-7777	2000 Oct	77	II-F	NK	AFoxCazSSuCTe	—	New6b (JJPX01.0014)	ND	>125, 90	R1
00-8066	2000 Oct	93	II-F	Stool	AFoxCazSSuCTe	—	New6b (JJPX01.0014)	ND	>125, 90	R1
01-2010	2001 Apr	92	V-F	Stool	AFoxCazSSuCTe	—	New6a (JJPX01.0176)	ND	>125, 60, 3.5	R2
01-2288	2001 Apr	93	IV-F	Blood	AFoxCazSSuCTe	—	New6a (JJPX01.0176)	ST45	>125, 60, 3.5	ND
01-9637	2001 Dec	95	NK-F	Stool	AFoxCazSSuCTe	—	New6b (JJPX01.0014)	ND	>125, 3.5	R1
01-9867	2001 Dec	93	III-F	Stool	AFoxCazSSuCTe	—	New6b (JJPX01.0014)	ND	>125, 3.5	ND
01-10075	2001 Dec	77	II-M	Stool	AFoxCazSSuCTe	—	New6b (JJPX01.0014)	ND	>125, 3.5	ND
02-7891	2002 Oct	78	III-M	Stool	AFoxCazSSuCTe	—	New8a	ST45	>125, 3.5	R1
03-3125	2003 May	62	II-F	Stool	AFoxCazSSuCTe	—	New8b	ND	>125, 70, 3.5	R2
03-3136	2003 May	62	III-F	Stool	AFoxCazSSuCTe	—	New8a	ND	>125, 3.5	R1
03-3179	2003 May	62	III-M	Stool	AFoxCazSSuCTe	—	New8c	ND	>125, 60	R1
03-3184	2003 May	62	IV-F	Stool	AFoxCazSSuCTe	—	New8a	ST45	>125, 3.5	R2
03-3222	2003 May	62	V-F	Blood	AFoxCazSSuCTe	—	New8a	ND	>125, 3.5	ND
03-3224	2003 May	59	IV-M	Stool	AFoxCazSSuCTe	—	New8a	ND	>125, 3.5	ND
03-3225	2003 May	59	IV-F	Stool	AFoxCazSSuCTe	—	New8a	ND	>125, 3.5	R1
03-3243	2003 May	91	III-M	Stool	AFoxCazSSuCTe	—	New8a	ND	>125, 3.5	ND
03-3265	2003 May	62	II-M	Stool	AFoxCazSSuCTe	—	New8d	ND	>125, 100, 60, 3.5	ND
03-3349	2003 May	62	NK-F	Stool	AFoxCazSSuCTe	—	New8d	ST45	>125, 100, 60, 3.5	R2
03-3350	2003 May	62	II-M	Stool	AFoxCazSSuCTe	—	New8c	ND	>125, 60, 3.5	R1
03-3465	2003 May	59	V-M	Stool	AFoxCazSSuCTe	—	New8a	ND	>125, 3.5	ND
03-3519	2003 May	59	II-F	Stool	AFoxCazSSuCTe	—	New8d	ND	>125, 100, 60, 3.5	R1
03-3603	2003 Jun	59	III-M	Stool	AFoxCazSSuCTe	—	New8d	ND	>125, 100, 60, 3.5	R1
03-3642	2003 Jun	92	IV-NK	Stool	AFoxCazSSuCTe	—	New8a	ND	>125, 3.5	ND
03-4620	2003 Jul	62	IV-M	Stool	AFoxCazSSuCTe	—	New8a	ND	>125, 3.5	R1
03-5145	2003 Jul	59	III-M	Stool	AFoxCazSSuCTe	—	New8d	ST45	>125, 100, 60, 3.5	R1
03-6521	2003 Sep	59	IV-M	Stool	AFoxCazSSuCTe	—	New6d/8e	ST45	>125, 100, 3.5	R2
03-6773	2003 Sep	62	IV-F	Stool	AFoxCazSSuCTe	—	New6d/8e	ND	>125, 100, 3.5	R1
03-7268	2003 Sep	62	IV-M	Stool	AFoxCazSSuCTe	—	New6b (JJPX01.0014)	ND	>125, 100, 3.5	R1

03-7338	2003 Sep	62	IV-M	Stool	AFoxCazSSuCTe	–	New6d/8e	ST45	>125, 100, 3.5	R1
03-8748	2003 Nov	11	V-M	Stool	ACroSuTmp	ND	New9	ST118	ND	ND
03-9969	2003 Dec	59	V-F	Stool	AFoxCazSSuTe	–	New6c	ST45	>125, 40, 5	R6
04-4556	2004 Jul	11	V-F	Stool	AFoxCazSSuCTe	–	New9	ND	>125, 3.5	R1
04-9597	2004 Dec	95	V-F	Urine	AFoxCazSSuCTe	–	New10	ST45	>125, 3.5	R1
S. Newport reference strain										
50 K					Pan susceptible	ND	New1	ST31	ND	ND
CMY-2–producing <i>S. Typhimurium</i> isolate										
03-9243	2003 Oct	44	V-F	Stool	AFoxCazSKToGSuTmpCNal	–	STM53	ND	220, 90	R7
CMY-2–producing <i>S. Agona</i> isolates										
02-2049	2002 Apr	93	V-NK	Stool	AFoxCazSKSuTmpCTe	1.2 (<i>dfrA1-orfX</i>)	Ago1 (JABX01.0055)	ND	140	R8
02-2059	2002 Apr	77	V-F	Stool	AFoxCazSKSuTmpCTe	1.2 (<i>dfrA1-orfX</i>)	Ago1 (JABX01.0055)	ND	140	R8

*PFGE, pulsed-field gel electrophoresis; MLST, multilocus sequence typing; ST, sequence type; ND, not determined; NK, not known.

†Numbers are those of Départements (French administrative subdivisions) in the Paris metropolitan area (Départements 60, 75, 77, 78, and 91–95) and northern France (Départements 59 and 62).

‡I, <1 y; II, 1–5 y; III, 6–14 y; IV, 15–64 y; V, ≥65 y.

§A, amoxicillin; Fox, cefoxitin; Caz, ceftazidime; S, streptomycin; Su, sulfonamides; C, chloramphenicol; Te, tetracycline; K, kanamycin; To, tobramycin; G, gentamicin; Sp, spectinomycin; Cro, ceftriaxone; Tmp, trimethoprim; Nal, nalidixic acid.

¶Profiles in parentheses were obtained from the PulseNet USA database (www.cdc.gov/pulsenet).

#Determined by the alkaline lysis method.

**Profiles are shown in Figure 2.