Table. Clinical characteristics of children infected with human Bocavirus*

Age (mo)	Sex	Copathogen	Fever (°C)	Leukocytes (× 10^3/µL)	CRP (mg/L)	SaO_2 (%)	Underlying condition (wks of pregnancy)	Diagnosis	Symptoms†
8	M	RSV	39.0	NA	NA	NA	None	Bronchiolitis	D, C
39	M	RSV	38.5	14.6	13.0	95	Preterm (36)	Asthma	RD, D
12	F	RSV	37.5	15.9	13.6	NA	None	Bronchiolitis	RD, C, O
19	F	None	37.3	15.6	<5.0	91	Preterm (35)	Bronchiolitis	RD
8	M	None	36.8	NA	NA	95	None	Bronchiolitis	D
10	M	None	38.2	12.6	9.6	NA	Preterm (28)	Bronchiolitis	D
9	F	None	38.5	12.7	<5.0	68	Chronic respiratory disease	Acute respiratory distress	RD
14	M	None	38.1	9.0	38.5	93	None	Bronchiolitis	RD, D, C
11	M	None	37.8	9.4	<5.0	96	Preterm (31)	Asthma	D

*CRP, C-reactive protein; SaO_2 saturation of arterial oxygen; RSV, respiratory syncytial virus; NA, not available.
†D, dyspnea; C, cough; RD, respiratory distress; O, otitis.

recovered and were discharged within 1 to 6 days.

The 3.4% incidence of HBoV observed in our study is similar to that (3.1%) reported by Allander et al. (*2*). HBoV was the only infectious agent identified in 6 children, which suggests that it was the causative agent of the disease. However, more studies conducted in children with and without respiratory disease as well as in adults and elderly persons are needed to better assess the pathogenic role of HBoV.

This work was supported by a grant from the Programme Hospitalier de Recherche Clinique of the Montpellier University Hospital (AOI 2003).

Vincent Foulongne,*
Michel Rodière,*
and Michel Segondy*

*Montpellier University Hospital, Montpellier, France

References

1. Juven T, Mertsola J, Waris M, Leimonen M, Meurman O, Roivanen M, et al. Etiology of community-acquired pneumonia in 254 hospitalized children. Pediatr Infect Dis J. 2000;19:293–8.
2. Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A, Anderson B. Cloning of a human parvovirus by molecular screening of respiratory tract samples. Proc Natl Acad Sci U S A. 2005;102:12891–6.
3. Sloots TP, McErlean P, Speicher DJ, Arden K, Nissen MD, Mackay IA. Evidence of human coronavirus HKU1 and human bocavirus in Australian children. J Clin Virol. 2005;35:99–102.

Address for correspondence: Vincent Foulongne, Laboratory of Virology, Montpellier University Hospital, 80 Ave A. Fliche, Montpellier 34295, France; email: v-foulongne@chu-montpellier.fr

Extended-spectrum β-Lactamase–producing *Enterobacteriaceae*, Central African Republic

To the Editor: Since the early 1980s, extended-spectrum β-lactamases (ESBLs) have been the largest source of resistance to broad-spectrum oxyimino-cephalosporins among *Enterobacteriaceae* (*1*). Molecular analysis techniques suggest that many ESBLs are derived from mutations in TEM-1, TEM-2, and SHV-1 β-lactamases and that these ESBLs can hydrolyze the extended-spectrum cephalosporins (particularly ceftazidime) and aztreonam (*1*). Members of a new group of ESBLs have been recently identified (*1*). Among them, CTX-M–type ESBLs are rapidly expanding and are derived from chromosomal class A β-lactamases of *Kluyvera* spp. (*1,2*). The CTX-M enzymes are not related to TEM or SHV enzymes, as they share only 40% identity with these ESBLs (*2*).These ESBLS are usually characterized by a higher level of resistance to cefotaxime than ceftazidime, except for CTX-M-19 (*2*). Most organisms that harbor ESBLs are also resistant to other classes of antimicrobial drugs, such as aminoglycosides, fluoroquinolones, chloramphenicol, and tetracyclines (*1,2*).

Reports concerning the existence of ESBL-producing *Enterobacteriaceae* in sub-Saharan Africa are scarce. We therefore conducted a study in the Central African Republic to determine the frequency of ESBLs in *Enterobacteriaceae* isolated at the Institut Pasteur de Bangui and to characterize their bla_{TEM}, bla_{SHV}, and $bla_{CTX\text{-}M}$ genes.

From January 2003 to March 2005, all *Enterobacteriaceae* isolated from human specimens at the Institut Pasteur de Bangui were screened for ESBLs. Antimicrobial drug susceptibility was determined by using the disk diffusion method (Bio-Rad, Marnes la Coquette, France) on Mueller-Hinton agar (MHA) and interpreted according to the recommendations of the Comité de l'Antibiogramme de la Société

Table. Characteristics of extended-spectrum β-lactamase–producing *Enterobacteriaceae* in Bangui, Central African Republic

Strain†	Patient hospitalized	Results of sequencing			MICs of β-lactams (µg/mL)*							Resistance
		bla_{CTX-M}	bla_{SHV}	bla_{TEM}	AMC	CTX	CAZ	CRO	FEP	CPO	ATM	
K. pneumoniae 022	N		SHV-2a	TEM-1	16	16	16	16	8	8	2	KGT
K. pneumoniae 043	Y		SHV-12	TEM-1	16	16	256	32	8	8	256	KGTNC
K. pneumoniae 106	Y	CTX-M-15		TEM-1	8	256	128	256	64	256	64	None
K. pneumoniae 047	Y		SHV-2a	TEM-1	64	16	16	16	8	16	32	None
E. coli 272	Y	CTX-M-15		TEM-1	32	256	128	256	128	256	128	KGTNC
E. coli 065	Y	CTX-M-15		TEM-1	20	256	128	256	64	256	128	C
E. coli 047	N	CTX-M-15		TEM-1	16	256	32	256	32	128	64	KGTC
E. coli 010	N	CTX-M-15		TEM-1	32	256	128	256	128	256	256	KGT
E. coli 073	N	CTX-M-15		TEM-1	16	256	128	256	128	256	128	KGTC
E. coli 059	Y	CTX-M-15		TEM-1	19	256	128	256	8	256	256	C
E. coli 064	N	CTX-M-15		TEM-1	128	256	128	256	64	256	64	C
E. coli 070	N	CTX-M-15		TEM-1	128	256	128	256	64	256	128	C
E. coli 054	N	CTX-M-15		TEM-1	128	256	32	256	64	256	32	KGTC
E. coli 026	N	CTX-M-15		TEM-1	32	256	64	256	128	256	256	KGTC
E. cloacae 081	Y		SHV-12	TEM-1	32	16	256	16	0.125	1	256	KGTN
E. cloacae 106	Y		SHV-12	TEM-1	128	16	256	16	32	8	256	KGT
E. aerogenes 014	Y	CTX-M-3	SHV-12	TEM-1	128	256	256	256	32	256	128	KGTN

*AMC, amoxicillin + clavulanic acid (2 µg/mL); CTX, cefotaxime; CAZ, ceftazidime; CRO, ceftriaxone; FEP, cefepime; CPO, cefpirome; ATM, aztreonam; K, kanamycin; G, gentamicin; T, tobramycin; N, netilmicin; C, ciprofloxacin.
†On *Klebsiella pneumoniae* strains, polymerase chain reaction and sequencing for bla_{SHV} genes were studied on *Escherichia coli* transconjugant or electroporant.

Française de Microbiologie (CA-SFM) (www.sfm.asso.fr). ESBL-producing *Enterobacteriaceae* were selected by the following criteria: susceptibility to cefoxitin; decreased susceptibility to cefotaxime (30 µg), ceftazidime (30 µg), or cefepime (30 µg) (zone diameter <21 mm); and enhanced susceptibility in the presence of clavulanic acid by the double disk synergy test (*3*). For suspected ESBLs, the MICs of broad-spectrum cephalosporins were determined by using the agar dilution method.

We screened 450 *Enterobacteriaceae* for ESBLs during the study. We isolated and identified 17 (4%) ESBL-producing strains (Table). These strains were associated with urinary tract infection, pneumonia in an AIDS patient, wound infection, vaginal or intestinal colonization, and ear infection. We found that 11 isolates were more resistant to cefotaxime (MIC ≥256 µg/mL) than to ceftazidime (MIC ≤128 µg/mL), which suggests CTX-M–type enzymes. *Enterobacteriaceae* strains that harbor ESBLs were frequently associated with resistance to aminoglycosides and ciprofloxacin (Table).

The conjugal transfer of the resistance determinants was carried out in trypticase soy (TS) broth with rifampin-resistant *Escherichia coli* J53-2 as the recipient. Mating broths were incubated at 37°C for 18 h. Transconjugants were selected on MHA plates containing rifampin (250 µg/mL) and cefotaxime (2.5 µg/mL). If conjugal transfer failed, plasmid DNA was extracted from donors with the Qiagen Plasmid Mini Kit (Qiagen, Courtaboeuf, France); 20 µL of *E. coli* DH10B cells were transformed with plasmid DNA by electroporation according to the manufacturer's instructions (Bio-Rad). Transformants were incubated for 1.5 h at 37°C in TS broth and then plated on MHA plates supplemented with 2.5 µg/mL cefotaxime.

Plasmid-encoded β-lactamase genes were detected on clinical isolates and their tranconjugants or transformants by polymerase chain reaction with oligonucleotide primer sets specific for the bla_{TEM}, bla_{SHV}, and bla_{CTX-M} genes (*4*). PCR assays were performed on total DNA extracted by using the commercial Qiagen DNA Mini Kit. The 3 β-lactamase genes were detected in different clinical isolates (Table). PCR results showed that the strains were harboring ≥2 different types of β-lactamases.

Plasmid-encoded β-lactamase genes were characterized by direct DNA sequencing with PCR primers. The nucleotide sequences were analyzed by the BLASTN (nucleotide basic local alignment search tool) program. For ESBLs, the gene types (SHV-2a, SHV-12, CTX-M-15, and CTX-M-3) were identified from different *Enterobacteriaceae* (Table). Only 1 strain (*Enterobacter aerogenes*) harbored 2 different ESBLs (CTX-M-3 and SHV-12). We identified TEM-1 and CTX-M15 enzymes, which are the most prevalent β-lactamases detected in our strains.

ESBL-producing *Enterobacteriaceae* have been previously described in South Africa (*5*), Kenya (*6*), Senegal (*7*), Cameroon (*8*), Tanzania (*9*), and Nigeria (*10*). As described in these countries, we found that CTX-M-15, SHV-2a, and SHV-12 were the most prevalent enzymes. CTX-M-15, the most recently described ESBL type, is particularly common in Bangui and seems to be

closely related to *E. coli*, as was previously observed in Tanzania (*9*). This finding is also the first report of CTX-M-3 in sub-Saharan Africa.

Multidrug resistance profiles involving non–β-lactam antimicrobial drugs coselected these ESBL-producing isolates. We suggest that the misuse of antimicrobial drugs in the Central African Republic and the migratory flux of regional populations could result in emergence and selection of these ESBL phenotypes in the community. We could not establish a relationship between the different strains isolated in hospitalized and ambulatory patients. Because of the implications for treating such infections, particularly in developing countries, the spread of ESBL-producing *Enterobacteriaceae* merits close surveillance in the Central African Republic.

This work was financed by grants from Institut Pasteur de Bangui, Faculté de Médecine Pierre et Marie Curie, Université Pierre et Marie Curie (Paris VI), and the European Community, contract LSHM-CT 2003-503335.

Thierry Frank,* Guillaume Arlet,†‡ Valerie Gautier,† Antoine Talarmin,* and Raymond Bercion*

*Institut Pasteur de Bangui, Bangui, Central African Republic; †Université Pierre et Marie Curie (Paris VI), Paris, France; and ‡Hôpital Tenon AP-HP, Paris, France

References

1. Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: a clinical update. Clin Microbiol Rev. 2005;18:657–86.
2. Bonnet R. Growing group of extended-spectrum β-lactamases: the CTX-M enzymes. Antimicrob Agents Chemother. 2004;48: 1–14.
3. Jarlier V, Nicolas MH, Fournier G, Phillipon A. Extended broad–spectrum β-lactamases conferring transferable resistance to newer β-lactam agents in *Enterobacteriaceae*: hospital prevalence and susceptibility patterns. Rev Infect Dis. 1988;10:867–78.
4. Eckert C, Gautier V, Saladin-Allard M, Hidri N, Verdet C, Ould-Hocine Z, et al. Dissemination of CTX-M–type β-lactamases among clinical isolates of *Enterobacteriaceae* in Paris, France. Antimicrob Agents Chemother. 2004;48:1249–54.
5. Pitout JDD, Thomson KS, Hanson ND, Ehrhardt AF, Moland ES, Sanders CC. β-lactamases responsible for resistance to expanded-spectrum cephalosporins in *Klebsiella pneumoniae*, *Escherichia coli*, and *Proteus mirabilis* isolates recovered in South Africa. Antimicrob Agents Chemother. 1998;42:1350–4.
6. Kariuki S, Corkill JE, Revathi G, Musoke R, Hart CA. Molecular characterization of a novel plasmid-encoded cefotaximase (CTX-M-12) found in clinical isolates from Kenya. Antimicrob Agents Chemother. 2001;45:2141–3.
7. Weill FX, Perrier-Gros-Claude JD, Demartin M, Coignard S, Grimont P. Characterization of extended–spectrum β-lactamase (CTX-M-15) producing strains of *Salmonella enterica* isolated in France and Senegal. FEMS Microbiol Lett. 2004;238:353–8.
8. Gangoue-Pieboji J, Miriagou V, Vourli S, Tzelepi E, Ngassam P, Tzouvelekis LS. Emergence of CTX-M-15-producing enterobacteria in Cameroon and characterization of a $bla_{\text{CTX-M-15}}$–carrying element. Antimicrob Agents Chemother. 2005; 49:441–3.
9. Blomberg B, Jureen R, Manji KP, Tamim BS, Mwakagile DSM, Urassa WK, et al. High rate of fatal cases of pediatric septicemia caused by gram-negative bacteria with extended-spectrum beta-lactamases in Dar es Salaam, Tanzania. J Clin Microbiol. 2005;43:745–9.
10. Soge OO, Queenan AM, Ojo KK, Adeniyi BA, Roberts MC. CTX-M-15 extended-spectrum β-lactamase from Nigerian *Klebsiella pneumoniae*. J Antimicrob Chemother. Epub 2005 Nov 30.

Address for correspondence: Guillaume Arlet, Service de Bactériologie-Hygiène, Hôpital Tenon, AP-HP, rue de la Chine, 75970 Paris CEDEX 20, France; email: guillaume.arlet@tnn.ap-hop-paris.fr

The opinions expressed by authors contributing to this journal do not necessarily reflect the opinions of the Centers for Disease Control and Prevention or the institutions with which the authors are affiliated.

Novel Recombinant Sapovirus, Japan

To the Editor: *Sapovirus* is the distinct genus within the family *Caliciviridae*; these viruses cause sporadic cases and outbreaks of gastroenteritis in humans worldwide (*1*). The sapovirus genome contains 2 open reading frames (ORFs). ORF1 encodes nonstructural and capsid proteins while ORF2 encodes a small protein (*2*). Sapovirus has a typical "Star of David" configuration by electron microscopic examination. The prototype sapovirus is the Sapporo virus (Hu/SaV/Sapporo virus/1977/ JP), which was originally discovered from an outbreak in a home for infants in Sapporo, Japan, in 1977 (*3*). Sapovirus is divided into 5 genogroups, among which only genogroups I, II, IV, and V are known to infect humans (*4*).

A fecal specimen was collected from a 1-year-old boy with acute gastroenteritis in Osaka, Japan, in March 2005. The viral genome was extracted by using a QIAamp kit (Quigen, Hilden, Germany). By using multiplex reverse transcription–polymerase chain reaction (RT-PCR), 2 groups of diarrheal viruses were identified. The first group included astrovirus, norovirus, and sapovirus; the second group included rotavirus and adenovirus (*5*). Sapovirus polymerase region was also amplified to identify recombinant sapovirus by using primers P290 and P289 (*6*). To eliminate the possibility of co-infection of 2 different sapovirus genotypes, to localize the potential recombination site, and to understand a possible recombination mechanism of recombinant sapovirus, flanking polymerase and capsid regions, with their junction of HU/5862/Osaka/JP, were amplified with primers P290 and SLV5749 to produce a 1,162-bp product (*5,6*). Products were directly sequenced, and capsid- and polymerase-based phylogenetic trees showed recombinant sapovirus.