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.

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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 oximino-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 Kluvyera 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 ceftotaxime 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 Enterobactereaceae 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 β-lactamases.

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

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Table. Clinical characteristics of children infected with human Bocavirus*

<table>
<thead>
<tr>
<th>Age (mo)</th>
<th>Sex</th>
<th>Copathogen</th>
<th>Fever (°C)</th>
<th>Leukocytes (× 10^3)/μL</th>
<th>CRP (mg/L)</th>
<th>SaO₂ (%)</th>
<th>Underlying condition (wks of pregnancy)</th>
<th>Diagnosis</th>
<th>Symptoms†</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>M</td>
<td>RSV</td>
<td>39.0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>None</td>
<td>Bronchiolitis</td>
<td>D, C</td>
</tr>
<tr>
<td>39</td>
<td>M</td>
<td>RSV</td>
<td>38.5</td>
<td>14.6</td>
<td>13.0</td>
<td>95</td>
<td>Preterm (36)</td>
<td>Asthma</td>
<td>RD, D</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>RSV</td>
<td>37.5</td>
<td>15.9</td>
<td>13.6</td>
<td>NA</td>
<td>None</td>
<td>Bronchiolitis</td>
<td>RD, C, O</td>
</tr>
<tr>
<td>19</td>
<td>F</td>
<td>None</td>
<td>37.3</td>
<td>15.6</td>
<td>&lt;5.0</td>
<td>91</td>
<td>Preterm (35)</td>
<td>Bronchiolitis</td>
<td>RD</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>None</td>
<td>36.8</td>
<td>NA</td>
<td>NA</td>
<td>95</td>
<td>None</td>
<td>Bronchiolitis</td>
<td>D</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>None</td>
<td>38.2</td>
<td>12.6</td>
<td>9.6</td>
<td>NA</td>
<td>Preterm (28)</td>
<td>Bronchiolitis</td>
<td>D</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>None</td>
<td>38.5</td>
<td>12.7</td>
<td>&lt;5.0</td>
<td>68</td>
<td>Chronic respiratory disease</td>
<td>Acute respiratory distress</td>
<td>RD</td>
</tr>
<tr>
<td>14</td>
<td>M</td>
<td>None</td>
<td>38.1</td>
<td>9.0</td>
<td>38.5</td>
<td>93</td>
<td>None</td>
<td>Bronchiolitis</td>
<td>RD, D, C</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>None</td>
<td>37.8</td>
<td>9.4</td>
<td>&lt;5.0</td>
<td>96</td>
<td>Preterm (31)</td>
<td>Asthma</td>
<td>D</td>
</tr>
</tbody>
</table>

*CRP, C-reactive protein; SaO₂, saturation of arterial oxygen; RSV, respiratory syncytial virus; NA, not available.
†D, dyspnea; C, cough; RD, respiratory distress; O, otitis.

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Française de Microbiologie (CASFM) (www.sfm.asso.fr). ESBL-producing Enterobacteriaceae were selected by the following criteria: susceptibility to cefoxitin; decreased susceptibility to cefotaxime (30 µg), cefazidime (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 transconjugants or transformants by polymerase chain reaction with oligonucleotide primer sets specific for the blaTEM, blaSHV, and blaCTX-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-lactamas 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

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

<table>
<thead>
<tr>
<th>Strain†</th>
<th>Patient hospitalized</th>
<th>Results of sequencing</th>
<th>MICs of β-lactams (μg/mL)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>K. pneumoniae 022</td>
<td>N</td>
<td>SHV-2a</td>
<td>TEM-1</td>
</tr>
<tr>
<td>K. pneumoniae 043</td>
<td>Y</td>
<td>SHV-12</td>
<td>TEM-1</td>
</tr>
<tr>
<td>K. pneumoniae 106</td>
<td>Y</td>
<td>CTX-M-15</td>
<td>TEM-1</td>
</tr>
<tr>
<td>K. pneumoniae 041</td>
<td>Y</td>
<td>SHV-2a</td>
<td>TEM-1</td>
</tr>
<tr>
<td>E. coli 272</td>
<td>Y</td>
<td>CTX-M-15</td>
<td>TEM-1</td>
</tr>
<tr>
<td>E. coli 065</td>
<td>Y</td>
<td>CTX-M-15</td>
<td>TEM-1</td>
</tr>
<tr>
<td>E. coli 047</td>
<td>N</td>
<td>CTX-M-15</td>
<td>TEM-1</td>
</tr>
<tr>
<td>E. coli 010</td>
<td>N</td>
<td>CTX-M-15</td>
<td>TEM-1</td>
</tr>
<tr>
<td>E. coli 073</td>
<td>N</td>
<td>CTX-M-15</td>
<td>TEM-1</td>
</tr>
<tr>
<td>E. coli 059</td>
<td>Y</td>
<td>CTX-M-15</td>
<td>TEM-1</td>
</tr>
<tr>
<td>E. coli 064</td>
<td>N</td>
<td>CTX-M-15</td>
<td>TEM-1</td>
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<tr>
<td>E. coli 070</td>
<td>N</td>
<td>CTX-M-15</td>
<td>TEM-1</td>
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<td>E. coli 054</td>
<td>N</td>
<td>CTX-M-15</td>
<td>TEM-1</td>
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<td>E. coli 026</td>
<td>N</td>
<td>CTX-M-15</td>
<td>TEM-1</td>
</tr>
<tr>
<td>E. cloacae 081</td>
<td>Y</td>
<td>SHV-12</td>
<td>TEM-1</td>
</tr>
<tr>
<td>E. cloacae 106</td>
<td>Y</td>
<td>SHV-12</td>
<td>TEM-1</td>
</tr>
<tr>
<td>E. aerogenes 014</td>
<td>Y</td>
<td>CTX-M-3</td>
<td>SHV-12</td>
</tr>
</tbody>
</table>

†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 bladspv, genes were studied on Escherichia coli transconjugant or electroporant.
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.

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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.