Infections with Nontyphoidal *Salmonella* Species Producing TEM-63 or a Novel TEM Enzyme, TEM-131, in South Africa

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Received 18 November 2003/Returned for modification 13 February 2004/Accepted 7 July 2004

*Salmonella* spp. producing extended-spectrum beta-lactamases (ESBLs) have been reported in many countries, but there is no information on their prevalence in Africa. ESBL-producing *Salmonella enterica* serotype Isangi and *S. enterica* serotype Typhimurium strains have been found in South Africa since 2001. A total of 160 consecutive isolates of *Salmonella* spp. were collected from 13 hospitals located in different cities in South Africa over a 5-month period from December 2002 to April 2003. All strains were screened for production of ESBLs by the double disk diffusion test and for AmpC production by assessing resistance to cefotaxim, *bla*<sub>S</sub>, *bla*<sub>TEM</sub>, *bla*<sub>CTX-M</sub>, and *bla*<sub>SHV</sub>. Nine were sought from all ESBL-positive and ceftoxin-resistant isolates. A total of 15.6% (25 of 160) isolates produced SHV or TEM ESBLs, and 1.9% (3 of 160) produced CMY-2. Nine *S. enterica* serotype Typhimurium, eight *S. enterica* serotype Isangi, and three *S. enterica* serotype Muenchen strains produced either TEM-63 or a derivative of TEM-63 designated TEM-131. Both TEM-63 and TEM-131 have an isoelectric point of 5.6, and their sequences have the following amino acid substitutions compared to the TEM-1 sequence: Leu21Phe, Gln104Lys, Arg164Ser, and Met182Thr. Additionally, TEM-131 has an Ala237Thr substitution. ESBL-producing *Salmonella* spp. have become a significant public health problem in South Africa with particular implications for the treatment of serious nontyphoidal *Salmonella* infections in children, for whom extended-spectrum cephalosporins were the preferred treatment.

Resistance to the extended-spectrum cephalosporins among members of the family *Enterobacteriaceae* has become a growing worldwide problem subsequent to the occurrence of extended-spectrum beta-lactamases (ESBLs) and AmpC-type beta-lactamases (9). Although reports of ESBLs associated with *Salmonella* spp. are relatively rare compared to those for other species in the family *Enterobacteriaceae*, the number of reported cases in this organism has been increasing in recent years. *Salmonella* have been found to express a wide variety of ESBL types, including TEM, SHV, PER, OXA, and CTX-M enzymes (1, 6, 10, 12, 21, 39, 44). Additionally, *Salmonella* strains have been detected which produce plasmid-mediated AmpC-type beta-lactamases (21, 35, 38).

The advent of ESBLs and AmpC beta-lactamases in *Salmonella* species is of considerable therapeutic importance, especially in developing nations where infections with these organisms are numerous. Resistance to ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol is now exceedingly common, necessitating use of fluoroquinolones or extended-spectrum cephalosporins as treatment of extraintestinal infections (13). Widespread fluoroquinolone use in children has been discouraged because of the potential adverse effects on cartilage development. Therefore, extended-spectrum cephalosporins (especially cefotaxim or ceftriaxone) are the mainstay of treatment of serious infections due to nontyphoidal *Salmonella* spp. in children. The production of ESBLs or AmpC beta-lactamases consequently has considerable implications for clinical microbiology laboratories and physicians in areas in which infections with nontyphoidal *Salmonella* spp. are common.

ESBLs have been found in many enterobacterial species in South Africa (7, 14, 22, 36). Since 2000, the Enteric Diseases Reference Unit of the National Institute for Communicable Diseases in South Africa has noted increasing numbers of nontyphoidal *Salmonella* isolates, particularly *S. enterica* serotype Typhimurium and *S. enterica* serotype Isangi, with positive screening tests for ESBLs. The aim of this study was to determine the genetic basis for antibiotic resistance in these isolates and to briefly describe the epidemiology of infections with these organisms.

**MATERIALS AND METHODS**

**Bacterial strains.** *Salmonella* isolates of human origin are sent to the Enteric Diseases Reference Unit of the National Institute for Communicable Diseases in Johannesburg, South Africa, from clinical microbiology laboratories across the country as part of national surveillance for enteric pathogens. A total of 160 consecutive *Salmonella* isolates arriving in this laboratory, collected from thirteen different hospitals in South Africa between December 2002 and March 2003, were selected for further analysis. The identification of the isolates as being of *Salmonella* species was confirmed at the Enteric Diseases Reference Unit by conventional biochemical tests. Serotyping of all isolates was performed, using the method of slide agglutination on the basis of lipopolysaccharide (O) and flagellar (H) antigens and commercially available antisera (Bio-Rad, Marnes-la-Côte, France).

**Antibiotics.** The antibiotics used were amoxicillin-clavulanic acid (AMC), cefotaxim (CTX), ceftriaxone (CRO), ceftoxim (CTX-M), and cefotetan (CFT) (kindly provided by Ciba-Geigy, Basel, Switzerland; Roche, Nutley, NJ). The MICs were determined using the broth microdilution method and the NCCLS reference protocol (18) in 96-well microtiter plates (Sterildisc, Biomerieux, Lyon, France) with an inoculum of 5 x 10<sup>5</sup> CFU ml<sup>-1</sup> and 2-μl volumes per well. The plates were incubated at 37°C for 18 to 20 hours. The MICs were read using a Bio-Tek instrument (model ELX 400; Bio-Tek Instruments, Winooski, VT).

**ESBL detection.** The extended-spectrum beta-lactamase activity was detected by using an ESBL screening test (LabTest Laboratories, Piscataway, NJ). The test is based on the presence of a positive reaction in the reduced disks containing either 30 μg of cefotaxim, 30 μg of ceftriaxone, or both.

**AmpC detection.** The production of AmpC enzymes was detected by using a double-disk diffusion test (21, 35, 38). The test was performed using disks containing 30 μg of cefotaxim and 25 μg of ticarcillin (Biomerieux) and 30 μg of cefotaxim and 30 μg of clavulanic acid (Biomerieux). The reaction was read after 16 to 18 hours of incubation at 37°C. The test was considered positive if a 5- to 10-mm zone of inhibition was observed around either disk.

**Conventional biochemical tests.** The identification of *Salmonella* isolates was performed by using commercially available antisera (Bio-Rad, Marnes-la-Côte, France). The identification of the isolates was confirmed by using the method of slide agglutination on the basis of lipopolysaccharide (O) and flagellar (H) antigens and commercially available antisera (Bio-Rad, Marnes-la-Côte, France). The reference method used for the detection of ESBLs was the method of slide agglutination on the basis of lipopolysaccharide (O) and flagellar (H) antigens and commercially available antisera (Bio-Rad, Marnes-la-Côte, France).
TABLE 1. Nucleotide sequences of the oligonucleotides used for amplification and sequencing

<table>
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<tr>
<th>Primer (reference)*</th>
<th>Sequence (5'–3')</th>
<th>Nucleotide positions</th>
<th>Expected size of amplification product (bp)</th>
</tr>
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<tr>
<td><strong>blaTEM (15)</strong></td>
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<td>Amplification and sequencing</td>
<td>ATGAGTTATCAACATTCTTCGTG</td>
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<tr>
<td>TEM-1 (F)</td>
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<td>840</td>
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<td>TEM-4 (R)</td>
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<td><strong>blaCMY-2 (36)</strong></td>
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<td>Amp3 (F)</td>
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<tr>
<td>Sequencing</td>
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<tr>
<td>Amp (F)</td>
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<tr>
<td>Amp (R)</td>
<td>CAGTACGAGACTCGGCA</td>
<td>2762–2745</td>
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a F, forward primers; R, reverse primers.

The DNA sequence and deduced amino acid sequence of TEM-131, the novel beta-lactamase, has been deposited in GenBank and assigned accession number AY436361.

RESULTS

Epidemiology. From 1999 to 2003, the Enteric Diseases Reference Unit of the National Institute for Communicable Diseases in the Republic of South Africa received annually, from all provinces of the country, between 500 and 1,500 nontyphoidal *Salmonella* strains for serotyping. Prior to 2000, *S. enterica* serotype Isangi was a rare isolate, but in 2002 was second only to *S. enterica* serotype Typhimurium in frequency of isolates.
received by the Enteric Diseases Reference Unit. In 2002, \textit{S. enterica} serotype Isangi accounted for approximately 20% of all isolates received. Furthermore, the majority of \textit{S. enterica} serotype Isangi isolates were found to have positive double disk diffusion test results, implying the presence of ESBLs. Since 2000, isolates of other serotypes (most notably \textit{S. enterica} serotype Typhimurium) have also been found to produce ESBLs.

\textbf{Strains.} A total of 160 consecutive \textit{Salmonella} isolates from the Enteric Diseases Reference Unit, National Institute for Communicable Diseases, South Africa, were selected for further analysis. These isolates were collected from 13 hospitals in South Africa from December 2002 to March 2003. The sources of the isolates were blood cultures (94 isolates), feces (48 isolates), urine (8 cultures), pleural fluid (3 isolates), cerebrospinal fluid (1 isolate), pericardial fluid (1 isolate), and miscellaneous (5 isolates). The serotypes of the strains were as follows: 115 \textit{S. enterica} serotype Typhimurium, 14 \textit{S. enterica} serotype Enteritidis, 10 \textit{S. enterica} serotype Isangi, 6 \textit{S. enterica} serotype Dublin, 5 \textit{S. enterica} serotype Muenchen, 3 \textit{S. enterica} serotype Hadar, 2 \textit{S. enterica} serotype Newport, and 1 each of \textit{S. enterica} serotype Anatum, \textit{S. enterica} serotype Bovismorbificans, \textit{S. enterica} serotype Infantis, \textit{S. enterica} serotype Molade, and \textit{S. enterica} serotype Schwarzengrund. A total of 25 (15.6%) isolates (14 \textit{S. enterica} serotype Typhimurium, 8 \textit{S. enterica} serotype Isangi, and 3 \textit{S. enterica} serotype Muenchen) were screen positive for ESBL production. Two \textit{S. enterica} serotype Typhimurium and the one \textit{S. enterica} serotype Schwarzengrund were cefoxitin resistant. For purposes of further epidemiologic analysis, a further 23 \textit{S. enterica} isolates of serotypes Typhimurium, Isangi, and Muenchen which were ampicillin resistant but not screen positive for ESBL production or cefoxitin resistant were chosen for further analysis.

\textbf{IEF.} Each strain which was screen positive for ESBL production or which was cefoxitin resistant produced one or two beta-lactamases with pI values of 5.4, 5.6, 8.2, and greater than

\begin{table}
\centering
\begin{tabular}{|l|l|l|l|l|}
\hline
Serotype & PFGE type & Isoelectric & Beta-lactamase(s) & Location of origin \\
strain and & & point(s) & & \\
 isolate no. & & & & \\
\hline
Typhimurium & 1 & A1 & 5.4, 8.2 & TEM-1, SHV-12 & Gauteng (hospital 1) \\
& 2 & A1 & 5.4, 8.2 & TEM-1, SHV-12 & Eastern Cape (hospital 5) \\
& 3 & A1 & 5.4, 8.2 & TEM-1, SHV-12 & Gauteng (hospital 2) \\
& 4 & A2 & 5.4, 8.2 & TEM-1, SHV-12 & Gauteng (hospital 1) \\
& 5 & A2 & 5.4, 8.2 & TEM-1, SHV-12 & Gauteng (hospital 3) \\
& 6 & A3 & 5.6 & TEM-131 & Gauteng (hospital 1) \\
& 7 & A3 & 5.6 & TEM-131 & Gauteng (hospital 1) \\
& 8–13 & A3 & 5.6, 8.2 & TEM-131, SHV-5 & Freestate (hospital 7) \\
& 14 & A3 & 5.4 & TEM-1 & Gauteng (hospital 1) \\
& 15 & A4 & 5.6, 8.2 & TEM-131, SHV-5 & Freestate (hospital 7) \\
& 16–19 & A5 & 5.4 & TEM-1 & Gauteng (hospital 3) \\
& 20, 21 & A5 & 5.4 & TEM-1 & Eastern Cape (hospital 5) \\
& 22 & A5 & 5.4 & TEM-1 & Western Cape (hospital 8) \\
& 23–26 & A6 & 5.4 & TEM-1 & Gauteng (hospital 2) \\
& 27 & A7 & 5.4 & TEM-1 & Gauteng (hospital 1) \\
& 28 & A8 & 5.4 & TEM-1 & Gauteng (hospital 1) \\
& 29 & A9 & 5.4 & TEM-1 & Gauteng (hospital 3) \\
& 30 & B & >8.2 & CMY-2 & Western Cape (hospital 8) \\
& 31 & C1 & >8.2 & CMY-2 & Gauteng (hospital 2) \\
& 32 & C2 & 5.4 & TEM-1 & Freestate (hospital 7) \\
& 33 & L & 5.4 & TEM-1 & Freestate (hospital 7) \\
& 34 & N & 5.4 & TEM-1 & Gauteng (hospital 2) \\
& 35 & K & 5.4 & TEM-1 & Gauteng (hospital 4) \\
& 36 & D1 & 5.6 & TEM-63 & Gauteng (hospital 3) \\
& 37 & D2 & 5.6 & TEM-63 & Gauteng (hospital 2) \\
& 38–41 & D3 & 5.6 & TEM-63 & Gauteng (hospital 2) \\
& 42 & E & 5.6 & TEM-131 & Gauteng (hospital 2) \\
& 43 & F & 5.6, 8.2 & TEM-131, SHV-5 & Gauteng (hospital 3) \\
& 44 & M & 5.4 & TEM-1 & Gauteng (hospital 4) \\
& 45 & P & 5.4 & TEM-1 & Gauteng (hospital 2) \\
& 46 & G & 5.6 & TEM-63 & Eastern Cape (hospital 6) \\
& 47–48 & G & 5.6 & TEM-63 & Eastern Cape (hospital 5) \\
& 49 & R & 5.4 & TEM-1 & Western Cape (hospital 8) \\
& 50 & S & 5.4 & TEM-1 & Gauteng (hospital 1) \\
& 51 & H & >8.2 & CMY-2 & Gauteng (hospital 3) \\
\hline
\end{tabular}
\end{table}
8.2 in various combinations (Table 2). Beta-lactamase enzymes with PI values in the range of 5.4 to 5.6 (consistent with the PI range of TEM enzymes) were identified in all isolates screen positive for ESBLs. Enzymes with a PI value of 8.2 (consistent with the PI range of SHV enzymes) were detected in 12 S. enterica serotype Typhimurium and 1 S. enterica serotype Isangi isolates. Two S. enterica serotype Typhimurium and the one S. enterica serotype Schwarzengrund that produced beta-lactamase enzymes with cefoxitin resistance had PI values greater than 8.2 (consistent with the PI range of AmpC-like beta-lactamasases). More than one beta-lactamase was identified in 12 S. enterica serotype Typhimurium and 1 S. enterica serotype Isangi strains.

**Electroporation and plasmid analysis.** Large (>10-kb) plasmids were isolated from all the strains (data not shown). The MICs of the transformants with different plasmids are shown in Table 3.

**PCR for detection of blaTEM, blaSHV, blaCTX-M, and blaCMY resistance genes and sequencing results.** blaTEM amplification was achieved for all the isolates screen positive for ESBL production. Thus, 14 S. enterica serotype Typhimurium isolates, the 8 S. enterica serotype Isangi isolates, and the 3 S. enterica serotype Murenchen isolates found to have blaTEM genes. Among the TEM-type ESBLs, TEM-63 and a novel TEM-type, TEM-131, were identified. The amino acid substitutions of the sequence of TEM-63 compared to the TEM-1 beta-lactamase sequence were Leu21Phe, Glu104Lys, Arg164Ser, and Met182Thr. The new TEM beta-lactamase (TEM-131) differed from TEM-63 by a single substitution (Ala237Thr) (Table 4). This protein has been designated (TEM-131) differed from TEM-63 by a single substitution (Ala237Thr) (Table 4). This protein has been designated (TEM-131) differed from TEM-63 by a single substitution (Ala237Thr) (Table 4). This protein has been designated TEM-131 (http://www.lahey.org/studies/webt.htm).

Three S. enterica serotype Murenchen and six S. enterica serotype Isangi isolates carried the gene for TEM-63, and two S. enterica serotype Isangi and nine S. enterica serotype Typhimurium isolates carried the new TEM-131 gene (Table 2). Seven S. enterica serotype Typhimurium strains and one S. enterica serotype Isangi strain produced the SHV-5 enzyme, and five S. enterica serotype Typhimurium strains produced the SHV-12 enzyme. Under the experimental conditions in this study, we were unable to detect any blaCTX-M genes. Of the cefoxitin-resistant isolates, two S. enterica serotype Typhimurium and one S. enterica serotype Schwarzengrund carried the CMY-2 gene.

### Antibiotic susceptibility testing.

Isolates producing TEM-131 had ceftazidime MICs of >256 µg/ml, cefotaxime MICs in the range of 6 to 64 µg/ml, and cefepime MICs in the range of 4 to 16 µg/ml (Table 5). These ranges were also seen in the transformant E. coli DH5α strains producing this enzyme (Table 3). While the isolates producing TEM-63 also had ceftazidime MICs >256 µg/ml, the cefotaxime MICs were somewhat lower than those observed with strains producing TEM-131 (ceftaxime MICs of 1.5 µg/ml in the TEM-63-producing transformant E. coli DH5α strain compared to 6 µg/ml in the TEM-131–producing strain) (Tables 3 and 5).

As expected, isolates producing CMY-2 had elevated MICs for cefotaxime (range 198 to >256 µg/ml), cefepime (32 µg/ml), and cefoxitin (>256 µg/ml) while retaining susceptibility to the carbapenems.

All but one of the ESBL-producing or CMY-2–producing isolates were resistant to trimethoprim-sulfamethoxazole. All isolates were resistant to chloramphenicol. Although no isolates were ciprofloxacin resistant, all seven S. enterica serotype Typhimurium isolates producing TEM-131 and SHV-5 and all six S. enterica serotype Isangi isolates producing TEM-63 were resistant to nalidixic acid. All isolates were susceptible to carbapenems.

### PFGE.

PFGE results of the S. enterica serotype Typhimurium isolates are presented in Table 2. A total of 29 isolates of S. enterica serotype Typhimurium were possibly related by PFGE (PFGE type A). These isolates were found in four provinces (Gauteng, Eastern Cape, Free State, and Western Cape). SHV-12–producing isolates which were indistinguishable from one another (PFGE type A1) were found in two hospitals in Gauteng and one hospital in the Eastern Cape. These hospitals are more than 1,000 km from one another (Fig. 1). TEM-131–producing isolates which were indistinguishable from one another (PFGE type A3) were found in one hospital in Gauteng and in a hospital in the Free State. TEM-131–producing S. enterica serotype Isangi isolates, unrelated to each other by PFGE, were found in two different hospitals in Gauteng.

TEM-63–producing S. enterica serotype Isangi isolates, possibly related by PFGE (Table 2), were found in two different hospitals in Gauteng province, while isolates of TEM-63–producing S. enterica serotype Murenchen, indistinguishable from one another by PFGE, were found in two different hospitals in the Eastern Cape. The three CMY-2 producers (all different
PFGE types) were found in patients in three different hospitals in Gauteng and in the Western Cape.

**DISCUSSION**

The advent of resistance of nontyphoidal *Salmonella* to extended-spectrum cephalosporin antibiotics is of significant public health importance. The treatment of choice for *Salmonella* meningitis or bacteremia in neonates is cefotaxime, and extended-spectrum cephalosporins are widely used in the treatment of bacteria or osteomyelitis due to nontyphoidal *Salmonella* infections in both infants and older children. We have found that 25 of 160 (15.6%) nontyphoidal *Salmonella* isolates from South Africa produced ESBLs and 3 of 160 (1.9%) produced CMY-2. Furthermore, these isolates were frequently multiply resistant, lacking susceptibility to inexpensive agents such as ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol. Some isolates were also nalidixic acid resistant—infections with such isolates may not respond as well as expected to fluoroquinolones (13).

We identified TEM-63, or a novel TEM enzyme (with the same isoelectric point), TEM-131, in *S. enterica* serotype Muenchen, *S. enterica* serotype Isangi, and *S. enterica* serotype Typhimurium isolates. According to previously published reports, TEM-type beta-lactamases have been rarely found in ESBL-producing salmonellae. The TEM-3 enzyme was found in *S. enterica* serotype Typhimurium in Casablanca (1), TEM-4 was found in *S. enterica* serotype Mbandaka in Tunisia (28), and a TEM-52 strain was found in a nontyphoidal *Salmonella* isolate in Korea (27). It is noteworthy that TEM-63 has been noted in several previous reports of ESBL-producing *Klebsiella, Proteus,* and *Enterobacter* strains in South Africa but never previously in *Salmonella* strains (22). We speculate that TEM-63 is prevalent throughout members of the *Enterobacteriaceae* family across South Africa. It is not certain to us whether the origin of *blaTEM-*63 was in *Salmonella* spp. or whether it originated in other organisms and was then transferred to *Salmonella* spp.

In all, six *S. enterica* serotype Isangi and three *S. enterica* serotype Muenchen isolates produced the TEM-63 enzyme. TEM-131, the novel TEM beta-lactamase, was produced by an additional two *S. enterica* serotype Isangi and nine *S. enterica* serotype Typhimurium strains. The sequence of TEM-63 has four amino acid changes compared with the sequence of TEM-1, and TEM-131 has an additional change compared to TEM-63. This change (alanine to threonine at position 237) also occurs in TEM-5, TEM-24, and TEM-86. Of potential interest is that we observed somewhat higher cefotaxime MICs for TEM-131–producing transformant strains compared to TEM-63–producing transformant strains (Tables 3 and 5). Cefazidime MICs were greatly elevated (>256 μg/ml) for both TEM-63– and TEM-131–producing strains.

One *S. enterica* serotype Isangi and seven *S. enterica* serotype Typhimurium strains produced not only the novel TEM-131 enzyme but also SHV-5. Additionally, five *S. enterica* serotype Typhimurium isolates produced TEM-1 and SHV-12. Although SHV-5 has previously been found in South Africa (14), SHV-12 has not. SHV-type beta-lactamases have been more frequently found in ESBL-producing salmonellae worldwide than TEM-type ESBLs (34). A variety of SHV-type ESBLs have been previously noted in salmonellae (5, 6, 20, 21, 28–30, 39, 40, 43, 44, 46). Under the experimental conditions we used, we did not find any isolates which produced a CTX-M-type beta-lactamase, in contrast to the rising significance of these ESBLs in *Klebsiella* and other species (34). CTX-M–type
ESBLs have been previously found in nontyphoidal *Salmonella* spp. (10).

Two *S. enterica* serotype Typhimurium and one *S. enterica* serotype Schwarzengrund isolates produced CMY-2 beta-lactamase, a beta-lactamase of the AmpC type. CMY-2–producing *S. enterica* serotype Typhimurium has been observed in Taiwan (47), Romania (29), and the United States (16, 45). More notably in North America, *S. enterica* serotype Newport has been found to be a producer of CMY-2 (2, 4). However other serovars which have been found to produce CMY-2 include *S. enterica* serotype Hadar (46), *S. enterica* serotype Senftenberg (26, 39), *S. enterica* serotype Mikawasima (31), and *S. enterica* serotype Montevideo (31). To our knowledge, this is the first report of a CMY-2–producing *S. enterica* serotype Schwarzengrund strain. It is possible that use of primers specific for CMY-2 did not allow us to detect other AmpC beta-lactamases which may have been present in our strains. However, with the exception of one TEM-63–producing *S. enterica* serotype Isangi strain, which showed intermediate resistance to cefoxitin, all other strains were cefoxitin susceptible, making clinically significant AmpC production unlikely in these strains.

South Africa has the highest number of human immunodeficiency virus (HIV)-infected people in the world, with an estimated 5 million infected by the virus (15). Patients with HIV infection have an increased risk of invasive salmonellosis (11, 17–19). More than half of the isolates (59% [94 of 160]) in this series were blood culture isolates. Given the high rate of invasive infection, antibiotic resistance in *Salmonella* isolates in South Africa is potentially of great clinical significance. Ampicillin, trimethoprim-sulfamethoxazole, chloramphenicol, and extended-spectrum cephalosporins are widely utilized therapies for serious *Salmonella* infections and yet are ineffective antibiotic options in the ESBL- and CMY-2–producing isolates we described. Some of the isolates were also nalidixic acid resistant, potentially limiting the effectiveness of fluoroquinolones. Disturbingly, we found ESBL- or CMY-2–producing isolates in four geographically distant provinces. Our molecular epidemiologic analysis shows that some isolates in different provinces were indistinguishable by PFGE (Table 2), indicating a common source or person-to-person spread. Preliminary epidemiologic data suggest that the multiresistant strains actually originated in a nosocomial setting, but we speculate that there has now been spread into the community.

We recommend that resources be utilized so that clinicians in southern Africa can collect relevant sterile site cultures and that clinical microbiology laboratories perform appropriate testing for susceptibility to extended-spectrum cephalosporins. Examples of relevant infections include neonatal *Salmonella* meningitis and *Salmonella* bacteremia or osteomyelitis. We,
REFERENCES


tant ESBL production by isolates that are not resistant to cefoper-


