ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 2004, p. 4263–4270 Vol. 48, No. 11
0066-4804/04/$08.00+0 DOI: 10.1128/AAC.48.11.4263–4270.2004

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

Tersia Kruger,[1] Dora Szabo,[2,3] Karen H. Keddy,[1]* Kathleen Deeley,[2] Jane W. Marsh,[2] Andrea M. Hujer,[4] Robert A. Bonomo,[4] and David L. Paterson[2]*

Enteric Diseases Reference Unit, National Institute for Communicable Diseases (NHLS), and University of the Witwatersrand, Johannesburg, Republic of South Africa[1]; Division of Infectious Diseases, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania[2]; Institute for Medical Microbiology, Semmelweis University, Budapest, Hungary[3]; and Research Service, Louis Stokes Cleveland Veterans Affairs Medical Center, Cleveland, Ohio[4]

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 noted 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 cefoxitin. bla_{SHV}, bla_{TEM}, bla_{CTX-M}, and bla_{CMY-2} were sought from all ESBL-positive and cefoxitin-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, Glu104Lys, 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. Salmonellae 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 cefotaxime 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-

* Corresponding author. Mailing address for David Paterson: UPMC Division of Infectious Diseases, Suite 3A Falk Medical Building, 3601 5th Ave., Pittsburgh, PA 15213. Phone: (412) 648-6478. Fax: (412) 648-6399. E-mail: patersond@msx.dept-med.pitt.edu. Mailing address for Karen Keddy: Enteric Diseases Reference Unit, National Institute for Communicable Diseases, NHLS, P.O. Box 1038, 2000 Johannesburg, Republic of South Africa. Phone: 27-11-489-9151. Fax: 27-11-489-9357. E-mail: karen.keddy@nhls.ac.za.

TABLE 1. Nucleotide sequences of the oligonucleotides used for amplification and sequencing

Primer (reference)[a]	Sequence (5′–3′)	Nucleotide positions	Expected size of amplification product (bp)
bla_{TEM} (15)			
Amplification and sequencing			
TEM-1 (F)	ATGAGTATTCAACATTTCCGTG	1–22	
TEM-4 (R)	TTACCAATGCTTAATCAGTGAG	861–840	840
Sequencing			
TEM-2 (R)	TTCTGTGACTGGTGAGTACT	324–305	
TEM-3 (R)	GAGTAAGTAGTTCGCCAGTT	595–576	
bla_{SHV} (15)			
Amplification and sequencing			
SHV-1 (F)	ATGCGTTATATTCGCCTGTG	1–20	846
SHV-3 (R)	GTTAGCGTTGCCAGTGCTCG	865–846	
Sequencing			
SHV-2 (R)	CGTTTCCCAGCGGTCAAGG	489–471	
bla_{CTX-M} (7)			
Amplification			
CTX-MA	CGCTTTGCGATGTGCAG	264–280	550
CTX-MB	ACCGCGATATCGTTGGT	814–798	
bla_{CMY-2} (36)			
Amplification and sequencing			
Amp3 (F)	ATGATGAAAAAATCGTTATGCTGC	1924–1947	1,145
Amp2 (R)	TTATTGCAGCTTTTCAAGAATGCGCCA	3069–3043	
Sequencing			
Amp (F)	ATAACCACCCAGTCACGC	2132–2149	
Amp (R)	CAGTAGCGAGACTGCGCA	2762–2745	

[a] F, forward primers; R, reverse primers.

Coquette, France), according to the Kauffman-White scheme for *Salmonella* serotyping (25, 37). Double disk diffusion testing was performed according to the method of Jarlier et al. (23), with ceftriaxone, cefotaxime, ceftazidime, and aztreonam disks 30 mm (center to center) away from a disk containing amoxicillin-clavulanic acid. Further analysis of the isolates was undertaken in Pittsburgh and Cleveland.

Antibiotic susceptibility. Susceptibility tests were performed using the Kirby-Bauer disk diffusion method and following NCCLS guidelines (32). Antimicrobials used were ampicillin, aztreonam, cefixime, cefepime, cefoxitin, cefotaxime, cefpodoxime, ceftazidime, ceftriaxone, amoxicillin-clavulanic acid, ertapenem, imipenem, meropenem, norfloxacin, nalidixic acid, ofloxacin, levofloxacin, gatifloxacin, ciprofloxacin, moxifloxacin, tetracycline, chloramphenicol, streptomycin, and trimethoprim-sulfamethoxazole.

The MICs of cefotaxime, cefotaxime plus clavulanic acid, ceftazidime, ceftazidime plus clavulanic acid, cefepime, cefoxitin, nalidixic acid, ciprofloxacin, trimethoprim-sulfamethoxazole, imipenem, and meropenem were determined by E-test for strains with positive double disk diffusion tests (as an indicator of ESBL production) or cefoxitin resistance (as an indicator of possible plasmid-mediated AmpC beta-lactamases). The strips were used according to the manufacturer's instructions (AB Biodisk, Solna, Sweden).

Escherichia coli ATCC 25922 was used as the reference strain for antimicrobial susceptibility testing (32).

IEF. Analytical isoelectric focusing (IEF) was performed in duplicate on all isolates with positive double disk diffusion test results or cefoxitin resistance by the use of methods that have been previously described (34). Enzyme activity was detected by placing filter paper soaked in nitrocefin (Becton Dickinson, Sparks, Md.) (500 μg/ml) over the focused gel.

PFGE. Pulsed field gel electrophoresis (PFGE) analysis was performed according to the Centers for Disease Control and Prevention PulseNet protocol (41). Briefly, genomic DNA was isolated and digested with XbaI (New England Biolabs, Beverly, Mass.). PFGE was performed with a CHEF III system (Bio-Rad, Hercules, Calif.) and the following run parameters: for block I, a switch time of 3 to 65 s and a run time of 17 h; for block II, a switch time of 15 to 30 s and a run time of 6 h. Dendrograms were created with Molecular Analyst (Bio-Rad) by using the Dice coefficient, unweighted pair group method with arithmetic means (UPGMA), and a position tolerance of 1.3%. Relatedness of the isolates was also determined by the criteria of Tenover et al. (42).

Plasmid profiles. Plasmids were extracted and electrophoresed by the method of Kado and Liu (24). Transformation assays were performed by electroporation (Gene Pulser; Bio-Rad) with *E. coli* strain DH10B (Bio-Rad) as the recipient. Transformants were cultivated on nutrient agar containing ampicillin (100 μg/ml).

PCR screening. A 10-μl aliquot of an overnight culture of the test isolate was diluted 1:10 with water and boiled for 15 min. PCR amplification was then performed with 10 μl of this dilution as the DNA template. The primer sets are shown in Table 1. Four primer pairs were used: bla_{TEM-1} (14), bla_{SHV-1} (14), bla_{CTX-M} (8), and bla_{CMY-2} (33, 34). PCR conditions were as previously described (8, 14, 33).

Sequencing. The primers used for DNA sequencing are shown in Table 1. Numbering follows the scheme of Ambler et al. (3). The nucleotide sequences of the amplified products were determined using ABI3700 and ABI3100 genetic analyzers at a core facility at the University of Pittsburgh. Data collection and analysis were performed using Lasergene DNASTAR sequencing analysis software.

Nucleotide sequence accession number. 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

TABLE 2. Molecular epidemiology of ESBL- and non-ESBL-producing isolates of *S. enterica* serotype Typhimurium, *S. enterica* serotype Isangi, *S. enterica* serotype Muenchen, and *S. enterica* serotype Schwarzengrund

S. enterica strain and isolate no.	PFGE type	Isoelectric point(s)	Beta-lactamase(s)	Location of origin
Serotype 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)
Serotype Isangi				
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)
Serotype Muenchen				
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)
Serotype Schwarzengrund				
51	H	>8.2	CMY-2	Gauteng (hospital 3)

received by the Enteric Diseases Reference Unit. In 2002, *S. enterica* serotype Isangi accounted for approximately 20% of all isolates received. Furthermore, the majority of *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 *S. enterica* serotype Typhimurium) have also been found to produce ESBLs.

Strains. A total of 160 consecutive *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 *S. enterica* serotype Typhimurium, 14 *S. enterica* serotype Enteritidis, 10 *S. enterica* serotype Isangi, 6 *S. enterica* serotype Dublin, 5 *S. enterica* serotype Muenchen, 3 *S. enterica* serotype Hadar, 2 *S. enterica* serotype Newport, and 1 each of *S. enterica* serotype Anatum, *S. enterica* serotype Bovismorbificans, *S. enterica* serotype Infantis, *S. enterica* serotype Molade, and *S. enterica* serotype Schwarzengrund. A total of 25 (15.6%) isolates (14 *S. enterica* serotype Typhimurium, 8 *S. enterica* serotype Isangi, and 3 *S. enterica* serotype Muenchen) were screen positive for ESBL production. Two *S. enterica* serotype Typhimurium and the one *S. enterica* serotype Schwarzengrund were cefoxitin resistant. For purposes of further epidemiologic analysis, a further 23 *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.

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

TABLE 3. Antibiotic susceptibility (MIC values [in micrograms per milliliter]) of the *E. coli* DH10B strain without (Tf−) and with (Tf+) plasmid

Antibiotic(s)	*E. coli* DH10B (Tf−)	*E. coli* DH10B (Tf+) producing:			
		TEM-1 and SHV-12	TEM-63	TEM-131	TEM-131 and SHV-5
Cefotaxime	0.94	16	1.5	6	16
Cefotaxime plus, clavulanic acid	<0.25	0.19	0.125	0.25	0.25
Ceftazidime	0.5	>256	>256	>256	>256
Ceftazidime plus clavulanic acid	<0.25	0.75	1.5	1.5	1.5
Cefepime	0.064	4	3	2	4
Cefoxitin	4	4	4	4	4
Imipenem	0.38	0.38	0.38	0.38	0.38
Meropenem	0.023	0.032	0.032	0.032	0.032

TABLE 4. Sequence analysis of the bla_{TEM} genes from the isolates with phenotypic evidence of ESBL production

Enzyme	Amino acid at position:				
	21	104	164	182	237
TEM-1	L	E	R	M	A
TEM-63	F	K	S	T	
TEM-131	F	K	S	T	T

The one-letter amino acid code used is as follows: A, alanine; E, glutamic acid; F, phenylalanine; K, lysine; L, leucine; M, methionine; R, arginine; S, serine; T, threonine.

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 isolate. 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-lactamases). 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 bla_{TEM}, bla_{SHV}, bla_{CTX-M}, and bla_{CMY} resistance genes and sequencing results. bla_{TEM} 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 Muenchen isolates were found to have bla_{TEM} genes. Among the TEM-type ESBLs, TEM-63 and one 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 (http:www.lahey.org/studies/webt.htm).

Three *S. enterica* serotype Muenchen 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 bla_{CTX-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 (cefotaxime 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 ceftazidime (range 198 to >256 μg/ml), cefotaxime (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 a 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 a 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 Muenchen, indistinguishable from one another by PFGE, were found in two different hospitals in the Eastern Cape. The three CMY-2 producers (all different

TABLE 5. Antibiotic susceptibility (MIC values [in micrograms per milliliter]) of the resistant *Salmonella* strains

Antibiotic(s)	MIC (μg/ml) for:								
	S. enterica serotype Isangi producing (no. of isolates):			*S. enterica* serotype Muenchen producing (no. of isolates):		*S. enterica* serotype Typhimurium producing (no. of isolates):			*S. enterica* serotype Schwarzengrund producing (no. of isolates):
	TEM-63 (6)	TEM-131 (1)	TEM-131 and SHV-5 (1)	TEM-63 (3)	TEM-1 and SHV-12 (5)	TEM-131 (2)	TEM-131 and SHV-5 (7)	CMY-2 (2)	CMY-2 (1)
Cefotaxime	2–3	16	32	1.5–3	12–>256	6	16–64	32	32
Cefotaxime and clavulanic acid	0.094–0.38	0.38	0.38	0.94	0.94–0.38	0.125	0.19–0.38	>1	>1
Ceftazidime	>256	>256	>256	>256	198–>256	>256	>256	198–>256	>256
Ceftazidime and clavulanic acid	3–>4	>4	>4	0.75–>4	0.25–1.5	0.5	0.75–4	>4	>4
Cefepime	4–6	8	12	3–8	4–16	4	8–16	0.5	0.38
Cefoxitin	3–12	4	4	2	2	2	2	>256	>256
Imipenem	0.25	0.5	0.38	0.25–0.38	0.25–0.38	0.25–0.38	0.19–0.38	0.38	0.25
Meropenem	0.023–0.047	0.032	0.032	0.032–0.047	0.032–0.047	0.023–0.032	0.023–0.047	0.032–0.047	0.047
Nalidixic acid	>256	3	4	4	3–>256	4	>256	4	6
Ciprofloxacin	0.094–0.125	0.012	0.012	0.012	0.08–0.25	0.008–0.012	0.19–0.25	0.012	0.012

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 bacteremia 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 $bla_{\text{TEM-63}}$ 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). Ceftazidime 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

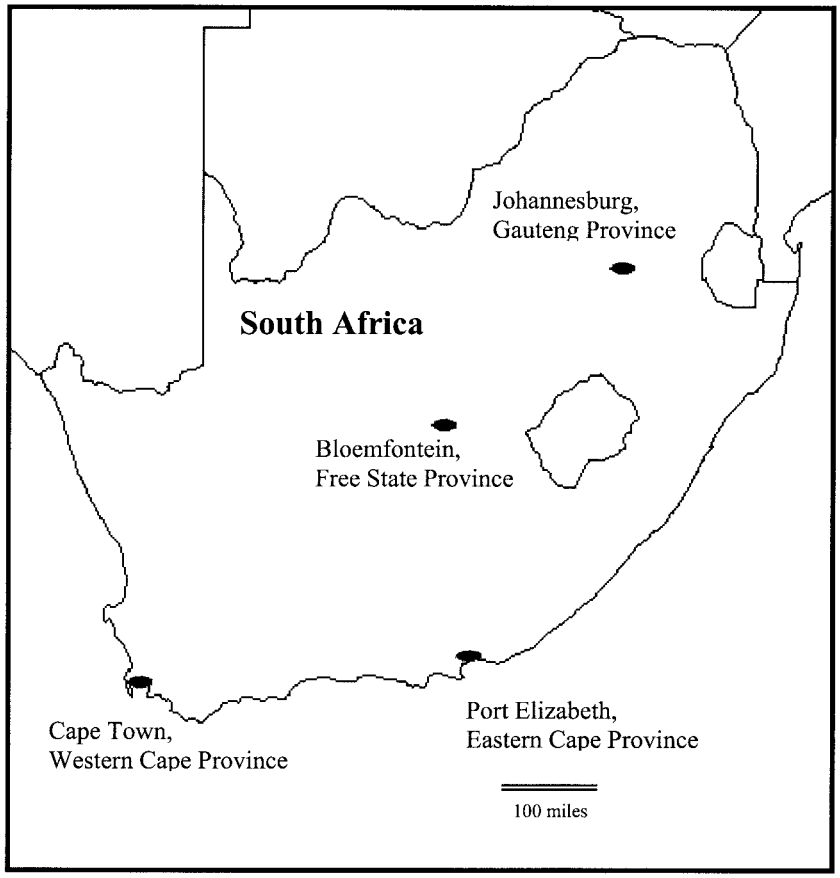

FIG. 1. Locations of the cities in which ESBL- or AmpC-producing salmonellae were obtained.

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,

and others, are presently investigating the clinical impact of ESBL production by isolates that are not resistant to cefotaxime or ceftriaxone by conventional standards (that is, isolates for which drug MICs are less than 64 μg/ml).

ACKNOWLEDGMENTS

We thank Elias Khomane, Lloyd Clarke, and John Kolano for technical assistance.

This work was supported by a grant from the Centers for Disease Control and Prevention (RS1/CCR 322404-02) to David Paterson. The work of Tersia Kruger was supported in part by a grant from the National Health Laboratory Service and the University of Witwatersrand. The Veterans Affairs Department Merit Review Program supported Robert Bonomo.

REFERENCES

1. **Ait Mhand, R., A. Soukri, N. Moustaoui, H. Amarouch, N. El Mdaghri, D. Sirot, and M. Benbachir.** 2002. Plasmid-mediated TEM-3 extended-spectrum beta-lactamase production in *Salmonella typhimurium* in Casablanca. J. Antimicrob. Chemother. **49:**169–172.
2. **Allen, K. J., and C. Poppe.** 2002. Occurrence and characterization of resistance to extended-spectrum cephalosporins mediated by beta-lactamase CMY-2 in *Salmonella* isolated from food-producing animals in Canada. Can. J. Vet. Res. **66:**137–144.
3. **Ambler, R. P., A. F. Coulson, J. M. Frere, J. M. Ghuysen, B. Joris, M. Forsman, R. C. Levesque, G. Tiraby, and S. G. Waley.** 1991. A standard numbering scheme for the class A beta-lactamases. Biochem. J. **276**(Pt. 1)**:**269–270.
4. **Anonymous.** 2002. Outbreak of multidrug-resistant *Salmonella newport*—United States, January-April 2002. Morb. Mortal. Wkly. Rep. **51:**545–548.
5. **Armand-Lefèvre, L., V. Leflon-Guibout, J. Bredin, F. Barguellil, A. Amor, J. M. Pagès, and M.-H. Nicolas-Chanoine.** 2003. Imipenem resistance in *Salmonella enterica* serovar Wien related to porin loss and CMY-4 β-lactamase production. Antimicrob. Agents Chemother. **47:**1165–1168.
6. **Baraniak, A., E. Sadowy, W. Hryniewicz, and M. Gniadkowski.** 2002. Two different extended-spectrum β-lactamases (ESBLs) in one of the first ESBL-producing salmonella isolates in Poland. J. Clin. Microbiol. **40:**1095–1097.
7. **Bell, J. M., J. D. Turnidge, A. C. Gales, M. A. Pfaller, and R. N. Jones.** 2002. Prevalence of extended spectrum beta-lactamase (ESBL)-producing clinical isolates in the Asia-Pacific region and South Africa: regional results from SENTRY Antimicrobial Surveillance Program (1998–99). Diagn. Microbiol. Infect. Dis. **42:**193–198.
8. **Bonnet, R., J. L. Sampaio, R. Labia, C. De Champs, D. Sirot, C. Chanal, and J. Sirot.** 2000. A novel CTX-M β-lactamase (CTX-M-8) in cefotaxime-resistant *Enterobacteriaceae* isolated in Brazil. Antimicrob. Agents Chemother. **44:**1936–1942.
9. **Bradford, P. A.** 2001. Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin. Microbiol. Rev. **14:**933–951.
10. **Bradford, P. A., Y. Yang, D. Sahm, I. Grope, D. Gardovska, and G. Storch.** 1998. CTX-M-5, a novel cefotaxime-hydrolyzing β-lactamase from an outbreak of *Salmonella typhimurium* in Latvia. Antimicrob. Agents Chemother. **42:**1980–1984.
11. **Brown, M., and S. J. Eykyn.** 2000. Non-typhoidal *Salmonella* bacteraemia without gastroenteritis: a marker of underlying immunosuppression. Review of cases at St. Thomas' Hospital 1970–1999. J. Infect. **41:**256–259.
12. **Casin, I., B. Hanau-Berçot, I. Podglajen, H. Vahaboglu, and E. Collatz.** 2003. *Salmonella enterica* serovar Typhimurium bla_{PER-1}-carrying plasmid pSTI1 encodes an extended-spectrum aminoglycoside 6′-*N*-acetyltransferase of type Ib. Antimicrob. Agents Chemother. **47:**697–703.
13. **Crump, J. A., T. J. Barrett, J. T. Nelson, and F. J. Angulo.** 2003. Reevaluating fluoroquinolone breakpoints for *Salmonella enterica* serotype Typhi and for non-Typhi salmonellae. Clin. Infect. Dis. **37:**75–81.
14. **Essack, S. Y., L. M. Hall, D. G. Pillay, M. L. McFadyen, and D. M. Livermore.** 2001. Complexity and diversity of *Klebsiella pneumoniae* strains with extended-spectrum β-lactamases isolated in 1994 and 1996 at a teaching hospital in Durban, South Africa. Antimicrob. Agents Chemother. **45:**88–95.
15. **Fassin, D., and H. Schneider.** 2003. The politics of AIDS in South Africa: beyond the controversies. BMJ **326:**495–497.
16. **Fey, P. D., T. J. Safranek, M. E. Rupp, E. F. Dunne, E. Ribot, P. C. Iwen, P. A. Bradford, F. J. Angulo, and S. H. Hinrichs.** 2000. Ceftriaxone-resistant salmonella infection acquired by a child from cattle. N. Engl. J. Med. **342:** 1242–1249.
17. **Gordon, M. A., H. T. Banda, M. Gondwe, S. B. Gordon, M. J. Boeree, A. L. Walsh, J. E. Corkill, C. A. Hart, C. F. Gilks, and M. E. Molyneux.** 2002. Non-typhoidal salmonella bacteraemia among HIV-infected Malawian adults: high mortality and frequent recrudescence. AIDS **16:**1633–1641.
18. **Gordon, M. A., A. L. Walsh, M. Chaponda, D. Soko, M. Mbvwinji, M. E. Molyneux, and S. B. Gordon.** 2001. Bacteraemia and mortality among adult medical admissions in Malawi—predominance of non-typhi salmonellae and *Streptococcus pneumoniae*. J. Infect. **42:**44–49.
19. **Gruenewald, R., S. Blum, and J. Chan.** 1994. Relationship between human immunodeficiency virus infection and salmonellosis in 20- to 59-year-old residents of New York City. Clin. Infect. Dis. **18:**358–363.
20. **Hammami, A., G. Arlet, S. Ben Redjeb, F. Grimont, A. Ben Hassen, A. Rekik, and A. Philippon.** 1991. Nosocomial outbreak of acute gastroenteritis in a neonatal intensive care unit in Tunisia caused by multiply drug resistant *Salmonella* wien producing SHV-2 beta-lactamase. Eur. J. Clin. Microbiol. Infect. Dis. **10:**641–646.
21. **Hanson, N. D., E. S. Moland, A. Hossain, S. A. Neville, I. B. Gosbell, and K. S. Thomson.** 2002. Unusual *Salmonella enterica* serotype Typhimurium isolate producing CMY-7, SHV-9 and OXA-30 beta-lactamases. J. Antimicrob. Chemother. **49:**1011–1014.
22. **Hanson, N. D., E. Smith Moland, and J. D. Pitout.** 2001. Enzymatic characterization of TEM-63, a TEM-type extended spectrum beta-lactamase expressed in three different genera of *Enterobacteriaceae* from South Africa. Diagn. Microbiol. Infect. Dis. **40:**199–201.
23. **Jarlier, V., M. H. Nicolas, G. Fournier, and A. Philippon.** 1988. Extended broad-spectrum beta-lactamases conferring transferable resistance to newer beta-lactam agents in *Enterobacteriaceae*: hospital prevalence and susceptibility patterns. Rev. Infect. Dis. **10:**867–878.
24. **Kado, C. I., and S. T. Liu.** 1981. Rapid procedure for detection and isolation of large and small plasmids. J. Bacteriol. **145:**1365–1373.
25. **Kauffman, F.** 1972. Serologic diagnosis of *Salmonella* species. Munksgaard, Copenhagen, Denmark.
26. **Koeck, J. L., G. Arlet, A. Philippon, S. Basmaciogullari, H. V. Thien, Y. Buisson, and J. D. Cavallo.** 1997. A plasmid-mediated CMY-2 beta-lactamase from an Algerian clinical isolate of *Salmonella senftenberg*. FEMS Microbiol. Lett. **152:**255–260.
27. **Lee, K., D. Yong, J. H. Yum, H. H. Kim, and Y. Chong.** 2003. Diversity of TEM-52 extended-spectrum beta-lactamase-producing non-typhoidal *Salmonella* isolates in Korea. J. Antimicrob. Chemother. **52:**493–496.
28. **Makanera, A., G. Arlet, V. Gautier, and M. Manai.** 2003. Molecular epidemiology and characterization of plasmid-encoded β-lactamases produced by Tunisian clinical isolates of *Salmonella enterica* serotype Mbandaka resistant to broad-spectrum cephalosporins. J. Clin. Microbiol. **41:**2940–2945.
29. **Miriagou, V., R. Filip, G. Coman, and L. S. Tzouvelekis.** 2002. Expanded-spectrum cephalosporin-resistant salmonella strains in Romania. J. Clin. Microbiol. **40:**4334–4336.
30. **Mulvey, M. R., G. Soule, D. Boyd, W. Demczuk, and R. Ahmed.** 2003. Characterization of the first extended-spectrum beta-lactamase-producing *Salmonella* isolate identified in Canada. J. Clin. Microbiol. **41:**460–462.
31. **Navarro, F., E. Perez-Trallero, J. M. Marimon, R. Aliaga, M. Gomariz, and B. Mirelis.** 2001. CMY-2-producing *Salmonella enterica*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Proteus mirabilis* and *Escherichia coli* strains isolated in Spain (October 1999-December 2000). J. Antimicrob. Chemother. **48:**383–389.
32. **NCCLS.** 1997. Performance standards for antimicrobial susceptibility testing; 10th informational supplement. (M100-S10). NCCLS, Wayne, Pa.
33. **Odeh, R., S. Kelkar, A. M. Hujer, R. A. Bonomo, P. C. Schreckenberger, and J. P. Quinn.** 2002. Broad resistance due to plasmid-mediated AmpC beta-lactamases in clinical isolates of *Escherichia coli*. Clin. Infect. Dis. **35:**140–145.
34. **Paterson, D. L., K. M. Hujer, A. M. Hujer, B. Yeiser, M. D. Bonomo, L. B. Rice, and R. A. Bonomo.** 2003. Extended-spectrum β-lactamases in *Klebsiella pneumoniae* bloodstream isolates from seven countries: dominance and widespread prevalence of SHV- and CTX-M-type β-lactamases. Antimicrob. Agents Chemother. **47:**3554–3560.
35. **Pitout, J. D., M. D. Reisbig, M. Mulvey, L. Chui, M. Louie, L. Crowe, D. L. Church, S. Elsayed, D. Gregson, R. Ahmed, P. Tilley, and N. D. Hanson.** 2003. Association between handling of pet treats and infection with *Salmonella* enterica serotype Newport expressing the AmpC β-lactamase, CMY-2. J. Clin. Microbiol. **41:**4578–4582.
36. **Pitout, J. D., K. S. Thomson, N. D. Hanson, A. F. Ehrhardt, E. S. Moland, and C. C. Sanders.** 1998. 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. **42:**1350–1354.
37. **Popoff, M. Y.** 2001. Antigenic formula of the *Salmonella* serovars, 8th ed. Institut Pasteur, Paris, France.
38. **Rankin, S. C., H. Aceto, J. Cassidy, J. Holt, S. Young, B. Love, D. Tewari, D. S. Munro, and C. E. Benson.** 2002. Molecular characterization of cephalosporin-resistant *Salmonella enterica* serotype Newport isolates from animals in Pennsylvania. J. Clin. Microbiol. **40:**4679–4684.
39. **Revathi, G., K. P. Shannon, P. D. Stapleton, B. K. Jain, and G. L. French.** 1998. An outbreak of extended-spectrum, beta-lactamase-producing *Salmonella senftenberg* in a burns ward. J. Hosp. Infect. **40:**295–302.
40. **Rhimi-Mahjoubi, F., M. Bernier, G. Arlet, Z. B. Jemaa, P. Jouve, A. Hammami, and A. Philippon.** 2002. Identification of plasmid-encoded cephalosporinase ACC-1 among various enterobacteria (*Klebsiella pneumoniae*, *Pro-*

teus mirabilis, *Salmonella*) isolated from a Tunisian hospital. Pathol. Biol. (Paris) **50:**7–11.
41. **Ribot, E. M., R. K. Wierzba, F. J. Angulo, and T. J. Barrett.** 2002. *Salmonella enterica* serotype Typhimurium DT104 isolated from humans, United States, 1985, 1990, and 1995. Emerg. Infect. Dis. **8:**387–391.
42. **Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2233–2239.
43. **Villa, L., C. Mammina, V. Miriagou, L. S. Tzouvelekis, P. T. Tassios, A. Nastasi, and A. Carattoli.** 2002. Multidrug and broad-spectrum cephalosporin resistance among *Salmonella enterica* serotype enteritidis clinical isolates in southern Italy. J. Clin. Microbiol. **40:**2662–2665.
44. **Villa, L., C. Pezzella, F. Tosini, P. Visca, A. Petrucca, and A. Carattoli.** 2000. Multiple-antibiotic resistance mediated by structurally related IncL/M plasmids carrying an extended-spectrum β-lactamase gene and a class 1 integron. Antimicrob. Agents Chemother. **44:**2911–2914.
45. **Winokur, P. L., A. Brueggemann, D. L. DeSalvo, L. Hoffmann, M. D. Apley, E. K. Uhlenhopp, M. A. Pfaller, and G. V. Doern.** 2000. Animal and human multidrug-resistant, cephalosporin-resistant salmonella isolates expressing a plasmid-mediated CMY-2 AmpC β-lactamase. Antimicrob. Agents Chemother. **44:**2777–2783.
46. **Yan, J. J., C. H. Chiu, W. C. Ko, C. L. Chuang, and J. J. Wu.** 2002. Ceftriaxone-resistant *Salmonella enterica* serovar Hadar: evidence for interspecies transfer of blaCMY-2 in a Taiwanese university hospital. J. Formos. Med. Assoc. **101:**665–668.
47. **Yan, J. J., W. C. Ko, C. H. Chiu, S. H. Tsai, H. M. Wu, and J. J. Wu.** 2003. Emergence of ceftriaxone-resistant *Salmonella* isolates and rapid spread of plasmid-encoded CMY-2-like cephalosporinase, Taiwan. Emerg. Infect. Dis. **9:**323–328.