Resistance to expanded-spectrum oxyimino-cephalosporins among *Salmonella* strains is mostly due to acquisition of plasmids encoding various class A extended-spectrum β-lactamas (1, 8, 13, 17, 19–21). Production of plasmid-mediated class C β-lactamas by *Salmonella* isolates has also been described previously (4, 22, 23). The emergence of such strains may have serious implications because of the limitation of therapeutic choices for patients with invasive *Salmonella* infections and by facilitation of the spread of *bla* genes in the community. We describe here an imipenem-resistant *Salmonella enterica* serotype Cubana isolate that produces the recently described KPC-2 β-lactamase (E. S. Moland, J. Johnson, J. A. Black, T. J. Lockhart, A. Hossain, V. L. Herrera, N. D. Hanson, and K. S. Thomson, Abstr. 41st Intersci. Conf. Antimicrob. Agents Chemother., abstr. 2226, 2001).

**MATERIALS AND METHODS**

*Salmonella* serotype Cubana was isolated in December 1998 from a stool specimen of a 4-year-old boy with diarrhea in a hospital in Maryland. The patient was chronically ill with Wiskott-Aldrich syndrome. In the 6 months before isolation of the *Salmonella* Cubana isolate, he had been hospitalized three times and had received intravenous antibiotics. During each of those hospitalizations he had received intravenous β-lactams, including ceftiraxone and ceftazidime, but not carbapenems. There was no history of recent travel. The isolate (AM04707) was submitted to the Centers for Disease Control and Prevention as part of the National Antimicrobial Resistance Monitoring System for enteric bacteria (http://www.cdc.gov/NARMS) and was subsequently forwarded to the Hellenic Pasteur Institute.

*Escherichia coli* K-12 strain 14R525 (Nal+) was used as the recipient in conjugation experiments. *E. coli* DH5α (GIBCO-BRL, Carlsbad, Calif.) was used for transformation. Chloramphenicol-resistant plasmid pBCKS(+) (Stratagene, La Jolla, Calif.) was used for cloning and expression of *bla* genes.

The MICs of β-lactams were determined by an agar dilution technique (10). Susceptibilities to other antimicrobial agents were assessed by a disk diffusion method (11) and by a partial-range broth microdilution method (*Sensititre*; Trek Diagnostics, Westlake, Ohio), according to the instructions of the manufacturer.

Conjugation experiments were carried out in mixed broth cultures as described previously (5). Transconjugant clones were selected on Mueller-Hinton agar containing ampicillin (50 μg/ml) plus nalidixic acid (200 μg/ml). Plasmid DNA preparations were obtained by an alkaline lysis technique and resolved in 0.8% (wt/vol) agarose gels. Individual plasmids were excised as discrete bands from low-melting-point agarose (0.8%) and were subjected to partial digestion with various restriction enzymes including HindIII. Digests were ligated into the multicloning site of pBCKS(+). The resulting recombinant plasmids were used to transform *E. coli* DH5α competent cells. β-Lactam-resistant transformants were selected on Luria-Bertani agar containing chloramphenicol (20 μg/ml) and ampicillin (50 μg/ml). The nucleotide sequences of the cloned fragments were determined with an ABI Prism 377 DNA sequencer (Perkin-Elmer, Applied Biosystems Division, Foster City, Calif.). Sequence similarity searches were performed with the BLAST program (available at the website of the National Center for Biotechnology Information).

β-Lactamases were extracted in one of two ways. One method involved ultrasonic treatment of bacterial cells, followed by suspension in phosphate buffer (100 mM; pH 7.0) and clarification by centrifugation (Fig. 1A). A small-scale freeze-thaw method with modifications provided by J. W. Biddle and J. K. Rasheed was used for comparison of KPC-1 and KPC-2 (3) (Fig. 1B). Detection of carbapenemase activity was performed by bioassay as described previously (24). Analytical isoelectric focusing was performed in polyacrylamide gels containing ampholytes (pH range, 3.5 to 9.5; APBiotech, Piscataway, N.J.). β-Lactamase activity was visualized with nitrocefin (Oxoid Ltd., Basingstoke, United Kingdom, or Becton-Dickinson, Lexington, Ky.). In situ inhibition of β-lactamase activity was performed by soaking the gels in solutions containing clavulante (0.5 mM), tazobactam (0.5 mM), or EDTA (5 mM). Maximum hydrolysis rates for various β-lactam substrates were estimated by UV spectrophotometry, and inhibition by clavulanate and tazobactam was assessed by using nitrocefin as the reporter substrate. The respective procedures were as described previously (5).

Nucleotide sequence accession number. The sequence of the 5.2-kb fragment of pST-H1 containing *bla* genes has been submitted to the GenBank database and assigned accession number AF481906.

**RESULTS**

*Salmonella* serotype Cubana 4707 exhibited either resistance or decreased susceptibility to all β-lactams tested including β-lactam–β-lactamase inhibitor combinations, oxyimino-cephalosporins, aztreonam, and carbapenems. In the presence of clavulanic acid, the MICs of ceftazidime and imipenem were decreased by 4 and 2 doubling dilutions, respectively (Table 1). The isolate was also resistant to streptomycin, trimethoprim, and sulfamethoxazole. The isolate was susceptible to nalidixic acid (MIC, ≤4 μg/ml) and ciprofloxacin (MIC, ≤0.015 μg/ml).
Transfer of β-lactam resistance to E. coli by conjugation was successful. Transconjugants exhibited a phenotype of resistance to β-lactams similar to that of Salmonella serotype Cubana 4707 (Table 1). They were also resistant to streptomycin, trimethoprim, and sulfonamides. Analysis of plasmid DNA indicated transfer of a plasmid (pST4707), and an E. coli (pST4707) showed that the rate of imipenem hydrolysis relative to that of penicillin G, which was set at 100, was 45 ± 5. The value for ceftaxime was 58 ± 3.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Serotype Cubana 4707</th>
<th>E. coli(pST4707)</th>
<th>E. coli DH5α(pSTH-1)</th>
<th>E. coli DH5α</th>
</tr>
</thead>
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<tr>
<td>Amoxicillin</td>
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<td>&gt;256</td>
<td>&gt;256</td>
<td>4</td>
</tr>
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<td>Amoxicillin-CLA</td>
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<td>32</td>
<td>8</td>
<td>2</td>
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<td>&gt;128</td>
<td>&gt;128</td>
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<td>&gt;128</td>
<td>&gt;128</td>
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<tr>
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<td>&gt;128</td>
<td>1</td>
</tr>
<tr>
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<td>&gt;128</td>
<td>32</td>
<td>1</td>
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</tr>
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<td>2</td>
<td>2</td>
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<td>8</td>
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</tr>
<tr>
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<td>Meropenem</td>
<td>8</td>
<td>8</td>
<td>2</td>
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</tr>
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</table>

a CLA, clavulanic acid. Penicillin/inhibitor ratio, 2:1.
b The inhibitor concentration was fixed at 2 μg/ml.
c TAZ, tazobactam. The inhibitor concentration was fixed at 4 μg/ml.

Cloning of a 5.2-kb HindIII fragment of pST4707 yielded a pBCSK(+) derivative (pST-H1) which mediated resistance only to β-lactams. E. coli(pST-H1) exhibited resistance or decreased susceptibility to all β-lactam antibiotics tested, including carbapenems (Table 1). Also, it was positive in the carbapenemase bioassay and produced a single carbapenemase bioassay (pST-H1) (lane 1, TEM-12, pI 5.2; TEM-2, pI 5.6; SHV-3, pI 7.0; SHV-5, pI 8.2).

pBCSK(+) derivative (pST-H1) which mediated resistance/H1
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only to/H9252
successful. Transconjugants exhibited a phenotype of resistance to/H9252
increased susceptibility to all/H9252
bla
serotype Cubana 4707, Salmonella
E. coli
4707 and
contained two
shown). Isoelectric focusing demonstrated that the extracts
bioassay performed as described by Yigit et al. (24; data not
respectively (Fig. 1). Both enzymes were inhibited in situ by
presence of which was supported by sequencing of 95% of the
tamase with a pI of 5.4 is consistent with a TEM-1 enzyme, the
24.5 and 42 MDa (data not shown).
conventional gel electrophoresis in a 0.8% gel, was between
indicated transfer of a plasmid (pST4707) that, according to
conventional gel electrophoresis in a 0.8% gel, was between
24.5 and 42 MDa (data not shown).

β-Lactamase extracts from Salmonella serotype Cubana 4707 and E. coli(pST4707) were positive in a carbapenemase bioassay performed as described by Yigit et al. (24; data not shown). Isoelectric focusing demonstrated that the extracts contained two β-lactamases with apparent pIs of 5.4 and 6.7, respectively (Fig. 1). Both enzymes were inhibited in situ by clavulanic acid and tazobactam but not by EDTA. The β-lactamase with a pI of 5.4 is consistent with a TEM-1 enzyme, the presence of which was supported by sequencing of 95% of the blαTEM-1-coding region (data not shown).

Cloning of a 5.2-kb HindIII fragment of pST4707 yielded a pBCSK(+) derivative (pST-H1) which mediated resistance only to β-lactams. E. coli(pST-H1) exhibited resistance or decreased susceptibility to all β-lactam antibiotics tested, including carbapenems (Table 1). Also, it was positive in the carbapenemase bioassay and produced a single β-lactamase with a pI of 6.7 (Fig. 1). Hydrolysis experiments performed with extracts from E. coli(pST-H1) showed that the rate of imipenem hydrolysis relative to that of penicillin G, which was set at 100, was 45 ± 5. The value for ceftaxime was 58 ± 3. Hydrolysis rates for ceftazidime and cefoxitin were too low to obtain reliable values, although the slightly elevated cefoxitin MIC indicates some degree of hydrolysis (Table 1). The hydrolysis rates for nitrocefin and cephalothin were significantly higher than that for penicillin G (2.4- and 3.1-fold, respectively). The 50% inhibitory concentrations of clavulanate and tazobactam were 1.4 and 0.08 μg/ml, respectively. The 5.2-kb HindIII fragment included an 882-bp open reading frame (ORF) that differed by only 1 bp (nucleotide 520) from blαKPC-1 found in Klebsiella pneumoniae (24). An identi-
The high degree of homology between \( \text{bla}_{\text{KPC-1}} \) and \( \text{bla}_{\text{KPC-2}} \) and the identity of their flanking sequences indicate that these variant \( \text{bla} \) genes could be parts of a single structure. However, KPC-2-encoding plasmid pST4707 was self-transferable, while the \( \text{bla}_{\text{KPC-1}} \)-encoding plasmid could not be conjugated into \( E.\ coli \) (24). Also, the \( \text{bla}_{\text{KPC-1}} \)-encoding plasmid, unlike pST4707, neither encoded TEM nor mediated resistance to antibiotics other than \( \beta \)-lactams. Therefore, it can be speculated that the \( \text{bla}_{\text{KPC}} \) variants resulted from insertion of a similar or common mobile element into different plasmid backgrounds. Sequencing data are compatible with this notion, although the existence of a transposable element was not proven.

KPC-1 was found in a \( K.\ pneumoniae \) isolate from a hospital in North Carolina. KPC-producing \( K.\ pneumoniae \) strains have also been found in a Maryland hospital (Moland et al., 41st ICAAC). The isolation of \( Salmonella \) serotype Cuba 4707, also in Maryland, indicates a certain degree of spread of \( \text{bla}_{\text{KPC}} \) genes in the United States. The plasmid locations of these genes and their association with possibly mobile structures may facilitate their spread. Continued surveillance is essential to monitor for the potential spread of \( \text{bla}_{\text{KPC}} \) genes among \( Salmonella \) strains.

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REFERENCES

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