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# Characterization of the First Extended-Spectrum Beta-Lactamase-Producing *Salmonella* Isolate Identified in Canada

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**A single *Salmonella enterica* serovar Typhimurium isolate with an UT2 phage type producing an extended-spectrum beta-lactamase (ESBL) was identified in Canada in 2000. The isolate harbored two plasmids, one containing a $bla_{TEM-1}$ gene and the other containing a $bla_{SHV-2a}$ gene. The ESBL gene was located on a 70-kb transferable plasmid which also carried tetracycline and trimethoprim resistance elements.**

The emergence of extended-spectrum beta-lactamases (ESBLs) in gram-negative bacteria is an increasing problem worldwide (8, 13, 20). Although reports of ESBLs associated with *Salmonella* 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 (20). In particular, the ESBLs reported for *Salmonella enterica* serovar Typhimurium include PER-1 (26), PER-2 (4), CTX-M-2 (5), CTX-M-4 (12, 24), CTX-M-5 and CTX-M-6 (11), TEM-3 (1), TEM-52 (25), SHV-2 (6), SHV-2a (3), and SHV-9 (14).

*Salmonella* serovar Typhimurium DT104 harboring the antibiotic resistance element SGI1, which confers resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline, is a major concern worldwide (7). In Canada, the Multi-provincial *Salmonella* Typhimurium Case Control Study Group began a detailed study in four provinces (British Columbia, Alberta, Saskatchewan, and Ontario) to examine clonality and antimicrobial resistance patterns in relation to risk factors and burden of illness associated with infection in the year 2000. Of the 1,033 human isolates submitted to the National Laboratory for Enteric Pathogens for characterization, only 30 isolates displayed reduced susceptibility or resistance to ceftriaxone by broth microdilution (17). The distribution of ceftriaxone MICs for the 30 isolates were as follows: ≥2 μg for 4 isolates, 4 μg for 4 isolates, 16 μg for 12 isolates, 32 μg for 7 isolates, and ≥64 μg for 3 isolates. Twenty-three strains were isolated from stool samples, one strain was isolated from a blood sample, one strain was isolated from a urine sample, one strain was isolated from skin, and the source was unknown for four isolates. These isolates were further tested for the production of an ESBL using ceftazidime and cefotaxime with and without clavulanic acid as described by the National Committee for Clinical Laboratory Standards (NCCLS) (16). A single strain displayed potentiation with clavulanic acid and was labeled N00-0114. It was isolated from a blood culture from a patient with bacteremia. A stool sample was not obtained. It was identified by standard biochemical tests (10) and confirmed to belong to serovar Typhimurium by serogrouping and serotyping using the somatic (O) and flagellar (H) antigens described previously (19). The strain was nontypeable by the current standardized phage typing protocol for serovar Typhimurium (2); however, it was subtyped as PT UT2 with experimental phages.

To determine if the ESBL phenotype was transferable, liquid mating experiments were conducted with the rifampin-resistant recipient strain *Escherichia coli* J53-2 (kindly provided by G. Jacoby, Burlington, Mass.). Transconjugants were selected on brain heart infusion agar containing 5 mg of cefotaxime per liter and 200 mg of rifampin per liter. The MICs of various antimicrobial agents were determined for the original isolate and the transconjugant by using broth microdilution according to the NCCLS (17). The results are presented in Table 1. The MICs of the various beta-lactam antibiotics (with the exception of meropenem and cefoxitin) for both *S. enterica* serovar Typhimurium N02-0114 and the transconjugant were higher than those for the rifampin-resistant recipient strain *E. coli* J53-2, and the addition of clavulanic acid reduced the MICs of all beta-lactams tested. Interestingly, the MICs of ceftazidime, aztreonam, and cefepime for the transconjugant were lower than those for the parent N02-0114. Both strains were resistant to tetracycline and trimethoprim-sulfamethoxazole and remained sensitive to chloramphenicol; however, only the parent was resistant to kanamycin and streptomycin.

To determine the number and isoelectric points (pIs) of the beta-lactamase(s) present in *S. enterica* serovar Typhimurium strain N02-0114 and the transconjugant, isoelectric focusing was conducted using precast gels (pH 3 to 10) in a Mini-Protean II apparatus (Bio-Rad) from crude extracts prepared by sonification as described previously (15). Beta-lactamase activity was visualized by a colorimetric assay with nitrocefin. There were two enzyme activities observed in extracts derived from strain N02-0114 with pIs of 5.4 and 7.6, respectively. The

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TABLE 1. MICs of antimicrobial agents for clinical isolate *S. enterica* Typhimurium N02-114, transconjugant *E. coli* N02114M, and *E. coli* J53-2

| Beta-lactam(s)[a] | MIC (μg/ml)[b] | | |
|---|---|---|---|
| | *S. enterica* Typhimurium N02-114 (TEM-1, SHV-2a) | *E. coli* N02114M (SHV-2a) | *E. coli* J53-2 |
| Ampicillin | >32 | >32 | ≤2 |
| Cephalothin | >32 | >32 | 8 |
| Ceftiofur | 16 | 16 | ≤0.5 |
| Amoxicillin + CLA | 8 | 8 | 4 |
| Cefpodoxime | >16 | >16 | ≤0.25 |
| Cefpodoxime + CLA | ≤0.25 | ≤0.25 | ≤0.25 |
| Ceftriaxone | >16 | 16 | ≤0.25 |
| Ceftriaxone + CLA | ≤0.25 | ≤0.25 | ≤0.25 |
| Cefotaxime | **>16** | **16** | ≤0.25 |
| Cefotaxime + CLA | **≤0.25** | **≤0.25** | ≤0.25 |
| Ceftazidime | **>16** | **2** | ≤0.25 |
| Ceftazidime + CLA | **0.5** | **≤0.25** | ≤0.25 |
| Aztreonam | 8 | 0.5 | ≤0.25 |
| Aztreonam + CLA | ≤0.25 | ≤0.25 | ≤0.25 |
| Cefipime | 8 | ≤0.5 | ≤0.25 |
| Cefoxitin | 4 | 4 | 2 |
| Meropenem | ≤0.25 | ≤0.25 | ≤0.25 |
| Amikacin | ≤4 | ≤4 | ≤4 |
| Apramycin | ≤2 | ≤2 | ≤2 |
| Chloramphenicol | ≤4 | 8 | 8 |
| Trimethoprim-sulfamethoxazole | >4/76 | >4/76 | ≤1.2/2.3 |
| Nalidixic acid | ≤4 | <4 | ≤4 |
| Ciprofloxacin | ≤0.015 | ≤0.015 | ≤0.015 |
| Gentamicin | 0.5 | 0.5 | ≤0.25 |
| Kanamycin | >64 | ≤16 | ≤16 |
| Streptomycin | 256 | <32 | <32 |
| Tetracycline | >32 | >32 | 8 |

[a] CLA, clavulanic acid at a fixed concentration of 4 μg/ml.
[b] The NCCLS ESBL-positive breakpoints for *E. coli* and *Klebsiella* spp. are shown in bold type.

transconjugant contained only a single band of pI 7.6, suggesting that the ESBL had a pI of 7.6. This result suggests the genetic element coding for the beta-lactamase with the pI of 5.4 does not reside on the same transferable element that codes for the ESBL of pI 7.4. PCR was used to determine the type of *bla* gene contained in the two strains under study. Total DNA was isolated as previously described (21) and was used in PCRs with universal primer sets to detect $bla_{\mathrm{TEM}}$ (23) and $bla_{\mathrm{SHV}}$ (18) genes. Detection of CTX-M-type genes was conducted using in-house-designed primers CTX-M-U1 (5′-ATG TGCAGYACCAGTAARGTKATGGC) and CTX-M-U2 (5′-TGGGTRAARTARGTSACCAGAAYCAGCGG) (R is a purine; Y is a pyrimidine; S is G or C) in PCRs similar to that of SHV but with annealing at 58°C. Strain N02-0114 produced amplicons corresponding to $bla_{\mathrm{TEM}}$ and $bla_{\mathrm{SHV}}$, whereas the transconjugant produced only an amplicon corresponding to $bla_{\mathrm{SHV}}$. These amplicons were purified using commercially available methods (Amicon) and sequenced by the DNA Core Facility of the National Microbiology Laboratory. The amino acid sequence encoded by $bla_{\mathrm{TEM}}$ was identical to the sequence encoded by $bla_{\mathrm{TEM\text{-}1}}$, and the $bla_{\mathrm{SHV}}$ sequence was 100% identical to the $bla_{\mathrm{SHV\text{-}2a}}$ sequence. These results correlate the isoelectric focusing findings of pIs 5.4 and 7.6 corresponding to $bla_{\mathrm{TEM\text{-}1}}$ and $bla_{\mathrm{SHV\text{-}2a}}$, respectively.

Plasmid DNA was purified in *S. enterica* serovar Typhimurium strain N02-114 and the transconjugant using a plasmid mini kit (Qiagen). N02-0114 appeared to contain two plasmids of 70 and 120 kb, respectively. The transconjugant contained a single 70-kb plasmid which was not present in the recipient J53-2 (data not shown). The 70-kb plasmid was restricted using *Cla*I, blotted onto nylon membranes, and probed with labeled $bla_{\mathrm{SHV\text{-}2a}}$ PCR product following the manufacturer's instructions (ECL kit; Amersham). The SHV-2a coding sequence resided on a 7.5-kb *Cla*I fragment (data not shown). The first SHV-2a in *Salmonella* serovar Typhimurium was recently reported in an isolate from Poland, but the size of the plasmid and additional antimicrobial resistance genes on the element was not reported (3).

To our knowledge, this is the first report of an ESBL in a *Salmonella* in Canada. The multidrug resistance characteristics of this strain in combination with the mobility of the element carrying the ESBL are a major concern. The finding that the tetracycline resistance element resides on the same plasmid as the ESBL genes raises the possibility that the use of oxytetracycline in feedlots or the aquaculture industry in Canada could coselect for this multidrug resistance ESBL phenotype (9, 22). Although standards do not exist for the identification of ESBLs in salmonella, data presented in this report support the breakpoints used for *E. coli* and *Klebsiella* spp. (16, 17). On a positive note, only a single *Salmonella* serovar Typhimurium isolate with the ESBL phenotype was identified out of more than 1,000 isolates examined in the multiprovincial study over a 1-year period. However, the limited scope, with only *Salmonella* serovar Typhimurium studied, raises concerns that ESBLs may be found in other serotypes. Since this appears to be an isolated incident, we do not recommend that front-line laboratories test all salmonella for the presence of ESBLs; however, an enhanced surveillance study should be performed to determine if the problem is increasing and to determine the breadth of the issue in other serotypes in Canada.

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