Characterization of antimicrobial resistance of *Salmonella* Newport isolated from animals, the environment, and animal food products in Canada

Cornelius Poppe, Laura Martin, Anne Muckle, Marie Archambault, Scott McEwen, Emily Weir

Abstract

Multi-drug-resistant (MDR) *Salmonella enterica* serovar Newport strains are increasingly isolated from animals and food products of animal origin and have caused septicemic illness in animals and humans. The purpose of this study was to determine the occurrence and the epidemiologic, phenotypic, and genotypic characteristics of *S.* Newport of animal origin that may infect humans, either via the food chain or directly. During the 1993–2002 period, the Office International des Épizooties Reference Laboratory for Salmonellosis in Guelph, Ontario, received 36 841 *Salmonella* strains for serotyping that had been isolated from animals, environmental sources, and food of animal origin in Canada. Of these, 119 (0.3%) were *S.* Newport. Before 2000, none of 49 *S.* Newport strains was resistant to more than 3 antimicrobials. In contrast, between January 2000 and December 2002, 35 of 70 isolates, primarily of bovine origin, were resistant to at least 11 antimicrobials, including the extended-spectrum cephalosporins. The bla_{CMY-2}, flo_{st}, *strA*, *strB*, *sulII*, and *tetA* resistance genes were located on plasmids of 80 to 90 MDa that were self-transmissible in 25% of the strains. Conserved segments of the integron 1 gene were found on the large MDR-encoding plasmids in 3 of 35 strains additionally resistant to gentamicin and spectinomycin or to spectinomycin, sulfamethoxazole–trimethoprim, and trimethoprim. Resistance to kanamycin and neomycin was encoded by the *aphA-1* gene, located on small plasmids (2.3 to 6 MDa). The increase in bovine-associated MDR *S.* Newport infections is cause for concern since it indicates an increased risk of human acquisition of the infection via the food chain.

Résumé

Des souches multi-résistantes de Salmonella enterica *serovar Newport sont de plus en plus isolées d'échantillons provenant d'animaux et de leurs produits; ces souches ont causé des septicémies chez les animaux et les humains. Le but de cette étude était de déterminer l'occurrence et les caractéristiques épidémiologiques, phénotypiques, et génotypiques des* S. *Newport d'origine animale pouvant infecter l'humain, via la chaîne alimentaire ou directement. Durant la période de 1993 à 2002, le Laboratoire de Référence des Salmonelles de l'Office International des Épizooties localisé à Guelph, Ontario, a reçu 36 841 souches de* Salmonella *pour le sérotypage. Ces souches ont été isolées d'animaux, de sources environnementales, et de viandes d'origine animale au Canada. De ces souches, 119 (0,3 %) étaient du* S. *Newport. Avant 2000, aucune des 49 souches de* S. *Newport n'était résistante à plus de 3 antimicrobiens. Par contre, entre janvier 2000 et décembre 2002, 35 des 70 isolats, majoritairement d'origine bovine, étaient résistants à au moins 11 antimicrobiens, incluant les céphalosporines à spectre étendu. Les gènes de résistance* bla_{CMY-2}, flo_{st}, strA, strB, sulII, *et* tetA *étaient localisés sur des plasmides de 80 à 90 MDa, et ceux-ci étaient transférables chez 25 % des souches. Des segments conservés du gène de l'intégron de classe 1 ont été retrouvés sur de larges plasmides de multi-résistance chez 3 des 35 souches également résistantes à la gentamicine et à la spectinomycine ou à la spectinomycine, le sulfaméthoxazole–triméthoprime, et le triméthoprime. Les résistances à la kanamycine et à la néomycine étaient codées par le gène* aphA-1, *localisé sur de petits plasmides (2,3 à 6 MDa). L'augmentation des infections bovines associées aux souches de* S. *Newport multi-résistantes est un problème indiquant un plus grand risque dans l'acquisition de l'infection chez les humains via la chaîne alimentaire.*

(Traduit par Docteur Serge Messier)

Introduction

Salmonella Newport infections increased as a proportion of reported infections by all *Salmonella* serovars in humans in the United States from 5% to 10% during the period 1996–2001 (1) and in Canada from 2.2% to 3.6% during the years 2002 and 2003 (National Laboratory for Enteric Pathogens, Winnipeg, Manitoba, 2003: unpublished data). The percentages of *S.* Newport isolates in particular but also of other *Salmonella* serovars and *Escherichia coli* isolated from humans, animals, and foods of animal origin that are resistant to

Office International des Épizooties Reference Laboratory for Salmonellosis, Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, 110 Stone Road West, Guelph, Ontario N1G 3W4 (Poppe, Martin, Muckle, Weir); Département de Pathologie et Microbiologie, Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec J2S 7C6 (Archambault); Department of Population Medicine, University of Guelph, Guelph, Ontario N1G 2W1 (McEwen).

Address all correspondence and reprint requests to Dr. Cornelius Poppe; telephone: (519) 822-3300; fax: (519) 822-2280; e-mail: Cornelius_Poppe@phac-aspc.gc.ca

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Table I. Epidemiologic summary of *Salmonella* Newport submissions for serotyping during 1993–2002 in Canada

Year	Province (number of strains)	Submissions (number)	Proportion of strains (number/ total)	Source of strain (n = 119)									
				Bovine[a]	Beef products[b]	Chicken[c]	Turkey[c]	Equine	Human	Other animals	Animal feed	Water	Other
1993	Ontario (4)	3	0.2% (4/2 424)			1		1		1	1		
1994	Ontario (3)	3	0.1% (3/3 251)						1	1	1		
1995	Ontario (1)	1	< 0.1% (1/1 860)							1			
1996	Alberta (1)	1	< 0.1% (1/2 015)			1							
1997	Ontario (4), New Brunswick (1)	5	0.2% (5/2 696)	2		1	1			1			
1998	Ontario (2)	2	0.1% (2/3 448)	1				1					
1999	Ontario (31), Quebec (2)	13	0.6% (33/5 093)	1	25	1	4		1		1		
2000	Ontario (17), Alberta (4)	15	0.5% (21/4 523)	5	10	1	1					3	1
2001	Ontario (26), New Brunswick (1), Quebec (1)	25	0.5% (28/5 766)	7	5	2	8	1		1	4		
2002	Ontario (19), Alberta (1), New Brunswick (1)	18	0.4% (21/5 765)	5	2	5	9						
Total	119	86	0.3% (119/36 841)	21	42	12	23	3	2	5	7	3	1

[a] Samples from intestines, feces, and lungs

[b] Samples from fresh and frozen beef, a milk filter, hamburger, and hamburger patties for dogs

[c] Samples from feces, intestines, eggs, carcass rinse, fluff, and environmental swabs

the extended-spectrum cephalosporins (ESCs) are increasing (1–4). Human infections with ESC-resistant *S.* Newport are often foodborne (1), may result from direct exposure to infected dairy cows and calves (1), and have occurred in people handling contaminated pet treats (5). Multi-drug-resistant (MDR) *S.* Newport, *S.* Typhimurium, and other *Salmonella* serovars and phage types have been associated with increased morbidity and mortality in humans (1,6,7) and animals (1,2). Little is known about the occurrence and the phenotypic and genotypic characteristics of the antimicrobial resistance (AMR) of *S.* Newport isolates from animals and food products of animal origin in Canada. Therefore, the purpose of this study was to determine the occurrence of *S.* Newport among food-producing and other animals, food of animal origin, and environmental sources in Canada, to note the clinical signs and pathological findings associated with *S.* Newport infection of animals, and to characterize the strains by examining the phenotypic and genotypic aspects of AMR and the transferability of drug-resistance determinants.

Materials and methods

Bacterial strains

This study was based on passive laboratory-based surveillance. We examined *S.* Newport strains isolated and submitted for serotyping during 1993–2002 in Canada (Table I) as follows.

Antimicrobial susceptibility testing

We determined the antimicrobial susceptibility of *S.* Newport strains and their transformants and transconjugants at resistant and, for certain antimicrobials, at intermediate and susceptible breakpoint levels, as specified by standards M31-A (8) and M100-S12 (9) of the National Committee for Clinical Laboratory Standards, with the agar dilution test, using Mueller–Hinton (MH) agar (Table II). To determine resistance to florfenicol, Aquaflor (Schering Plough Animal Health, Pointe Claire, Quebec), containing 50% florfenicol, was dissolved in dimethylformamide (11).

Table II. Antimicrobials and concentrations (8,9) used to test the susceptibility of the *S.* Newport isolates

Antimicrobial	Abbreviation	Resistance breakpoint level (μg/mL)[a]
Amikacin	Amk	64
Ampicillin	Amp	32
Amoxicillin/clavulanic acid	Amc	32/8[b]
Apramycin	Apr	32[c]
Carbadox	Crb	30[d]
Cephalothin	Cef	32
Ceftiofur	Ctf	8
Ceftriaxone	Cro	64[e]
Cefoxitin	Fox	32
Chloramphenicol	Chl	32
Ciprofloxacin	Cip	4[f]
Florfenicol	Fen	16[g]
Gentamicin	Gen	16
Kanamycin	Kan	64
Nalidixic acid	Nal	32
Neomycin	Neo	16[c]
Nitrofurantoin	Nit	64[h]
Spectinomycin	Spt	64[c]
Streptomycin	Str	32[c]
Sulfisoxazole	Sul	512
Sulfamethoxazole–trimethoprim	Sxt	76/4
Tetracycline	Tet	16
Tobramycin	Tob	8
Trimethoprim	Tmp	16

[a] Susceptible breakpoint levels were also examined for amikacin at 16 μg/mL, ceftriaxone at 8 μg/mL, and ciprofloxacin at 0.125 and 1 μg/mL

[b] Strains were considered resistant when growing on agar plates with this concentration

[c] There are no interpretive standards from the National Committee for Clinical Laboratory Standards for these antimicrobials; strains were considered resistant at these concentrations

[d] Strains were considered resistant to this concentration of this veterinary growth promoter for pigs

[e] Strains that grew on Mueller-Hinton agar with 8 μg/mL were also tested at intermediate-resistance levels of 16 and 32 μg/mL

[f] A 0.125 μg/mL concentration determines reduced susceptibility (10)

[g] Strains were considered resistant at this concentration (11)

[h] Strains were considered resistant at this concentration; however, isolates from the human urinary tract are considered resistant at 128 μg/mL (9)

Plasmids

Plasmid DNA was prepared by the alkaline lysis method (12) and then underwent electrophoresis, staining, and visualization as described previously (13). We used the following plasmids as molecular mass standards: pDT285, 96 MDa; pDT369, 23 MDa (13); pSA971028, an *S.* Typhimurium virulence-associated 62-MDa plasmid (11); and the 8 plasmids of *E. coli* V517 with molecular masses of 1.4 to 35.8 MDa (14).

Strain conjugation

To determine if plasmids were self-transmissible and transferred resistance genes, we conjugated strains as described by Provence and Curtiss (15) with i) *E. coli* C600, ii) a lactose-fermenting, antimicrobial-susceptible *S.* Newport, iii) *S.* Typhimurium LB5000, and iv) *S.* Typhimurium LB5010 (16), strains that had been made resistant to nalidixic acid. To eliminate restriction proficiency, the recipient bacteria were grown for 15 to 20 min at 45°C before mating (15).

Strain transformation

The prepared plasmid DNA was used to transform *E. coli* DH10B (Gibco BRL, Burlington, Ontario) by electroporation (17) to determine if plasmids that could not be transferred by conjugation, and were therefore likely to not be self-transmissible, encoded drug-resistance determinants. Standard conditions (2.5 kV, 200 Ω, and 25 μF) were used (18). The transformants were plated on selective agar to identify isolates that contained plasmids encoding AMR. Plasmid profiles and antimicrobial susceptibility profiles of the transformants were determined.

Amplification, sequencing, and hybridization of gene sequences

Table III shows the primers, amplification product sizes, and GenBank accession numbers of the AMR genes used to examine the parent *S.* Newport strains, their transformants, and their transconjugants. Other genes that were examined by polymerase chain reaction (PCR) but did not yield a product included *aac*(3)-IV, bla_{PSE-1}, *sulIII*, *tetB*, *tetC*, *tetD*, and *tetY*, which encode resistance to apramycin and other aminoglycosides, penicillins and carbenicillin, sulfonamides, and tetracyclines. All primers had previously been shown to produce amplicons in other antimicrobial-resistant isolates of *Salmonella* and other gram-negative pathogens and commensals and were surmised to function similarly in the *S.* Newport isolates. The AMR genes, resistances encoded, and mechanisms of resistance (19–28) are summarized in Table III. Primers for the 5′ and 3′ conserved segments of class 1 integrons were used to identify the integrons and the associated AMR genes (25). A touchdown thermal cycling program was used, with an annealing temperature of 60°C initially; for 15 cycles, the temperature was decreased 1°C per cycle to 46°C, and then there were 40 standard PCR cycles, of 95°C for 15 s, 51°C for 30 s, 72°C for 2 min, and final extension at 72°C for 7 min. Plasmid DNA preparations of parents, transconjugants, and transformants underwent electrophoresis and Southern blotting. Digoxigenin (DIG)-labelled probes were generated by means of the PCR DIG Probe Synthesis Kit (Roche), and hybridizations were performed as described previously (29). The PCR reaction mixtures were cleaned prior to DNA sequencing with the MinElute PCR Purification Kit (Qiagen, Mississauga, Ontario). The DNA sequences were determined with the DYEnamic ET terminator cycle sequencing kit and a MegaBACE 500 sequencing system (Amersham Pharmacia, Piscataway, New Jersey, USA).

Molecular analysis of genetic relatedness

To determine the genetic relatedness of the isolates, we analyzed the patterns produced by pulsed-field gel electrophoresis

Table III. Primers, GenBank accession numbers, and mechanisms of resistance of antimicrobial-resistance (AMR) genes used to examine the parent *S.* Newport strains

Gene	5′ and 3′ primer sequences	Accession number (and position)	Mechanism of resistance	Encodes resistance to
ant(3″)-Ia (*aadA*)	F: GTGGATGGCGGCCTGAAGCC R: ATTGCCCAGTCGGCAGCG	X02340 (514–1040)	Aminoglycoside adenyltransferase (19)	Spt, Str
aph(3″)-Ib (*strA*)	F: TGACTGGTTGCCTGTCAGAG R: CGGTAAGAAGTCGGGATTGA	NC_001740 (101–506)	Aminoglycoside phosphoryltransferase (19)	Str
aph(6)-Id (*strB*)	F: ATCGCTTTGCAGCTTTGTTT R: CGTTGCTCCTCTTCTCCATC	NC_001740 (1248–1650)	Aminoglycoside phosphoryltransferase (19)	Str
ant(2″)-Ia (*aadB*)	F: TCCAGAACCTTGACCGAAC R: GCAAGACCTCAACCTTTTCC	X04555 (1098–1797)	Aminoglycoside adenyltransferase (20)	Gen, Kan, Tob
aphA-1	F: TTATGCCTCTTCCGACCATC R: GAGAAAACTCACCGAGGCAG	U63147 (417–905)	Aminoglycoside phosphoryltransferase (20)	Kan, Neo
bla_{CMY-2}	F: ATAACCACCCAGTCACGC R: CAGTAGCGAGACTGCGCA	U77414 (209–839)	Extended-spectrum β-lactamase (cephamycinase) (21)	Oxyimino-cephalosporins (ceftazidime, cefotaxime, Cro), 7-α-methoxy-cephalosporins (Fox), and β-lactamase inhibitors (clavulanate)
bla_{TEM-1}	F: CAGCGGTAAGATCCTTGAGA R: ACTCCCCGTCGTGTAGATAA	AF309824 (267–910)	Beta-lactamase (22)	Amp, Cef
dhfrI	F: TGGCTGTTGGTTGGACGC R: TCACCTTCCGGCTCGATGTC	X17477 (710–966)	Dihydrofolate reductase inhibitor (23)	Tmp
flo_{st}	F: ACCCGCCCTCTGGATCAAGTCAAG R: CAAATCACGGGCCACGCTGTATC	AF097407 (577–1124)	Efflux (24)	Chl, Fen
int1	F: GGCATCCAAGCAGCAAGC R: AAGCAGACTTGACCTGAT	X12870 (1193–2201) VR[a]	Encoded by genes in integron 1 (25)	Aminoglycosides, Tets, Chl, Fen, Amp, and other drugs if resistance genes inserted into integron 1
qacEΔI-sulI	F: ATCGCAATAGTTGGCGAAGT R: GCAAGGCGGAAACCCGCGCC	X12870 (2278–3075)	Encoded by genes in integron 1 (25)	Quaternary ammonium compounds, sulfonamides
sulI	F: GCGCGGCGTGGGCTACCT R: GATTTCCGCGACACCGAGACCAA	U49101 (2113–2469)	Dihydropteroate synthase inhibitor (26)	Sulfonamides
sulII	F: CGGCATCGTCAACATAACC R: GTGTGCGGATGAAGTCAG	M36657 (338–1059)	Dihydropteroate synthase inhibitor (27)	Sulfonamides
tetA	F: GTGAAACCCAACATACCCC R: GAAGGCAAGCAGGATGTAG	X00006 (1328–2215)	Efflux (28)	Tets
tetG	F: GCTCGGTGGTATCTCTGCTC R: AGCAACAGAATCGGGAACAC	Y19117 (1–468)	Efflux (28)	Tets

[a] VR — variable region, the size of which depends on the number and size(s) of inserted gene cassette(s)

(PFGE) after digestion with *Xba*I and *Bln*I. Whole-cell DNA was prepared, digested, and separated as described previously by the Centers for Disease Control and Prevention (30). Whole-cell DNA of *S.* Newport am01144 restricted with *Xba*I was used as a molecular size marker. The PFGE patterns were determined as described by Liebisch and Schwarz (31). We analyzed the results with BioNumerics (Applied Maths, Austin, Texas), using the Dice similarity coefficient (optimization 1.0%, position tolerance 1.0%).

Results

Epidemiologic observations (Table I)

During the 1993–2002 period, 36 841 *Salmonella* strains, isolated from animals, the environment, food of animal origin, and other sources in Canada, were submitted to our Office International des Épizooties (OIE) Reference Laboratory for Salmonellosis in Guelph, Ontario, for serotyping. Of the strains, 119 (0.3%) were *S.* Newport

Table IV. Genetic and plasmid profiles of the 35 *S.* Newport strains that were resistant to at least 11 antimicrobials, including the extended-spectrum cephalosporins

Strain number(s)	AMRs of parent strains	AMR genes identified in transformants or transconjugants or both	Plasmids (MDa)
00-3183 B	AAcCfCrCtFxCFStSuT	*bla*$_{CMY-2}$, *flo*$_{st}$, *strA*, *strB*, *sulII*, *tetA*	90
00-4272 B	AAcCfCrCtFxCFStSuT	*bla*$_{CMY-2}$, *flo*$_{st}$, *strA*, *strB*, *sulII*, *tetA*	80, 2.3
00-3051 B,[a] 00-3800 B, 00-4056 B, 00-4235 B,[a] 00-4263 B,[a] 00-4264 B, 00-4265 B, 00-4266 B, 01-0606 B, 01-1809 B, 01-4636 B, 01-6249 B	AAcCfCrCtFxCFStSuT	*bla*$_{CMY-2}$, *flo*$_{st}$, *strA*, *strB*, *sulII*, *tetA*	90, 2.3
00-3050 B, 00-3798 B	AAcCfCrCtFxCFStSuT	*bla*$_{CMY-2}$, *flo*$_{st}$, *strA*, *strB*, *sulII*, *tetA*	90, 40, 2.3
01-0991 B,[b] 01-3221 B,[b] 01-3233 B, 02-5850 C,[b] 02-5851 C	AAcCfCrCtFxCFStSuT	*bla*$_{CMY-2}$, *flo*$_{st}$, *strA*, *strB*, *sulII*, *tetA*	90, 72, 2.3
00-4273 B	AAcCfCrCtFxCFStSuT	*bla*$_{CMY-2}$, *flo*$_{st}$, *strA*, *strB*, *sulI*, *sulII*, *tetA*	90, 2.3
01-5180 B, 02-5483 B, 02-5758 B, 02-5858 B	AAcCfCrCtFxCFStSuT + KN	*bla*$_{CMY-2}$, *flo*$_{st}$, *strA*, *strB*, *sulII*, *tetA*, *aphA-1*	90, 3.7
01-3783 B, 01-3784 B	AAcCfCrCtFxCFStSuT + KN	*bla*$_{CMY-2}$, *flo*$_{st}$, *strA*, *strB*, *sulII*, *tetA*, *aphA-1*	80, 6, 2.3
01-4980 B	AAcCfCrCtFxCFStSuT + KN	*bla*$_{CMY-2}$, *flo*$_{st}$, *strA*, *strB*, *sulII*, *tetA*, *aphA-1*	90, 2.3
00-2987 B, 01-3987 T	AAcCfCrCtFxCFStSuT + KN	*bla*$_{CMY-2}$, *flo*$_{st}$, *strA*, *strB*, *sulII*, *tetA*, *aphA-1*	90, 5
02-3908 B	AAcCfCrCtFxCFStSuT + KN	*bla*$_{CMY-2}$, *flo*$_{st}$, *strA*, *strB*, *sulI*, *sulII*, *tetA*, *tetG*, *aphA-1*	90, 3.7
02-4818 B	AAcCfCrCtFxCFStSuT + SpSxtTm	*bla*$_{CMY-2}$, *flo*$_{st}$, *sulII*, *tetA*, *aadA*, *dhfr1*, *int1*, *qacEΔI-sulI*	90, 3.7, 1.6
00-4923 B	AAcCfCrCtFxCFStSuT + GSp + KN	*bla*$_{CMY-2}$, *flo*$_{st}$, *sulII*, *tetA*, *aadA*, *aadB*, *int1*, *qacEΔI-sulI*, *aphA-1*	90, 5
02-1652 B	AAcCfCrCtFxCFStSuT + GSp + KN	*bla*$_{CMY-2}$, *flo*$_{st}$, *sulII*, *tetA*, *aadA*, *aadB*, *int1*, *qacEΔI-sulI*, *aphA-1*	90, 5, 3.2, 2.8

B — bovine; C — chicken; T— turkey; AAcCfCrCtFxCFStSuT — resistant to Amp, Amc, Cef, Cro, Ctf, Fox, Chl, Fen, Str, Sul, and Tet, respectively; Cro — reduced susceptibility to ceftriaxone at 8 μg/mL; K — Kan; N — Neo; Sp — Spt; Tm — Tmp; G — Gen

[a] The transconjugants, obtained by conjugation at 28°C, contained the 90-MDa plasmid with its associated AMRs

[b] The transconjugants, obtained by conjugation at 37°C, contained 90- and 72-MDa plasmids, the larger plasmid encoding the associated resistances

isolates, from 86 submissions; the proportion increased from the 1993–1998 period (< 0.1% to 0.2%) to the 1999–2002 period (0.4% to 0.6%). In 1993–1998, there were 1 to 5 *S.* Newport submissions annually, each from a different location. In 1999–2002, the number of submissions was 13 to 25 per annum, from 40 locations. Reisolation of *S.* Newport strains from the same premises occurred on several occasions. Recrudescence of suspected septicemic salmonellosis occurred particularly in veal calves at veal calf operations and in calves and cows at dairy farms. In 1993–2002, 107 *S.* Newport strains were isolated from premises in Ontario, 6 from Alberta, 3 from New Brunswick, and 3 from Quebec. Two of the Ontario strains were isolated from veal calves within days of importation from New York State. In 2000–2002, 90% of the *S.* Newport strains but only 44% to 51% of all *Salmonella* isolates were from Ontario sources. Of all 119 *S.* Newport isolates in 1993–2002, 63 were of bovine origin, and 56 were from turkeys (n = 23), chickens (n = 12), horses (n = 3), and other sources (n = 18). Among the 63 bovine isolates, 21 were clinical isolates from feces, the intestines, and other tissues, and the other 42 were nonclinical isolates: 27 from frozen beef, 9 from fresh beef, 5 from hamburger, and 1 from a milk filter.

Clinical and pathological observations

Clinical signs and pathological findings in animals from which *S.* Newport was isolated were often severe, but transient colonization (bovine) was also identified in association with outbreaks of MDR *S.* Newport in Ontario. In Holstein cows, fever, diarrhea (watery, bloody, or both), dehydration, and diminished milk production were seen. Fever, diarrhea, dehydration, enteritis, enlarged mesenteric lymph nodes, fibrinous pneumonia, and emaciation occurred in red and white veal calves. In turkey poults, diarrhea, yolk sacculitis,

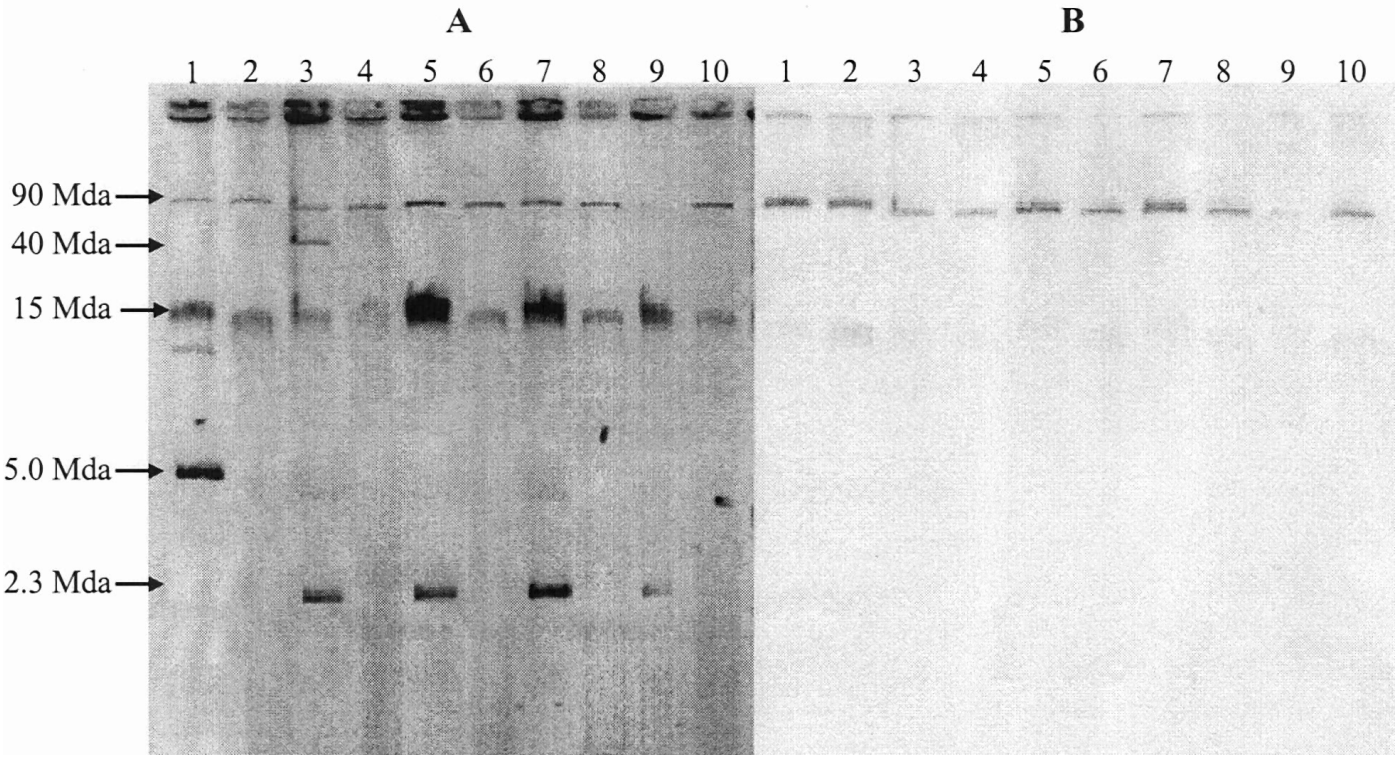

Figure 1. Plasmid profiles (A) and Southern blot hybridization (B) with a digoxigenin-labelled *bla*$_{CMY-2}$ probe of parent and transformant strains of *Salmonella* Newport. Lane 1, SA00-2987; lane 2, SA00-2987T (*Escherichia coli* DH10B transformed with plasmid DNA of the parent strain); lane 3, SA00-3050; lane 4, SA00-3050T; lane 5, SA00-4056; lane 6, SA00-4056T; lane 7, SA01-4636; lane 8, SA01-4636T; lane 9, SA00-3800; lane 10, SA00-3800T.

fibrinous enteritis, fibrinous pericarditis, splenomegaly, and enlarged ceca were seen, and the mortality rate was increased. Fever, diarrhea, ulcerative enteritis, colic, and pneumonia were observed in horses.

Antimicrobial susceptibility

Of the 49 *S.* Newport strains isolated before 2000, 32 were susceptible to all 24 antimicrobials in the testing panel, 14 were resistant only to sulfisoxazole, and 3 showed resistance to 3 or fewer antimicrobials (Amp and Spt; Spt, Str, and Sul; and Kan, Neo, and Tet). Among the 70 strains isolated during the January 2000 to December 2002 period, 32 of 33 from a bovine source, 1 of 18 from turkeys, and 2 of 10 from chickens were resistant to β-lactams, including the 1st-generation cephalosporin cephalothin, showed reduced susceptibility to the ESC ceftriaxone, and were resistant to the ESCs ceftiofur and cefoxitin (a cephamycin), to the amoxicillin/clavulanic acid combination, and to Chl, Fen, Str, Sul, and Tet (Table IV). Some of the ESC-resistant strains were additionally resistant to Kan and Neo (n = 10), Gen, Kan, Neo, and Spt (n = 2), or Spt, Sxt, and Tmp (n = 1). On 2 Ontario farms, veal calves from which resistant *S.* Newport was isolated had been treated with ceftiofur and florfenicol, respectively.

Plasmid profiles

All 35 strains isolated in 2000–2002 that were resistant to Amp, Amc, Ctf, Cro, Cef, Fox, Chl, Fen, Str, Sul, and Tet, including those additionally resistant to Gen and Spt or to Spt, Sxt, and Tmp, harbored a large (80 to 90 MDa) plasmid (Table IV). In contrast, such large plasmids were found in only 3 of 84 strains (4%) that were pansusceptible or were resistant to only 1 to 3 antimicrobials.

Conjugation

Conjugation with each recipient strain was attempted with 24 *S.* Newport strains that were derived from different sources, had different plasmid profiles, or had different AMRs, or some combination. Plasmids of 6 strains produced transconjugants only with the Nal^R *E. coli* C600 recipient strain. Three strains possessed plasmids of 90 and 2.3 MDa that cotransferred at 28°C. An additional 3 strains possessed plasmids of 90 and 72 MDa that cotransferred at 37°C. The 90-MDa plasmid transferred resistance to Amp, Amc, Ctf, Cro, Cef, Fox, Chl, Fen, Str, Sul, and Tet to the transconjugants (Table IV).

Transformation

Transformation by electroporation of *E. coli* DH10B with plasmid DNA of each of the 35 ESC-resistant strains revealed that the large plasmid of all strains was transferable and conferred resistance to Amp, Amc, Ctf, Cro, Cef, Fox, Chl, Fen, Str, Sul, and Tet (Table IV). When the parent strains were additionally resistant to Gen and Spt or to Spt, Sxt, and Tmp, the transformants were resistant to these antimicrobials as well. Similarly, examination of transformants selected on MH agar containing Kan showed that all strains additionally resistant to Kan and Neo transferred these resistances to *E. coli* DH10B. The recipient strains contained the corresponding plasmid of 6, 5, 3.7, or 2.3 MDa that encoded these resistances.

Amplification, sequencing, and hybridization of gene sequences

The presence of the *bla*$_{CMY-2}$ gene was confirmed by sequencing PCR products from each of the 35 strains that were resistant to Amp,

Amc, Cef, Ctf, and Fox and that showed reduced susceptibility to Cro at 8 μg/mL (Table IV). Similarly, all strains resistant to Chl and Fen harbored the flo_{st} gene, strains resistant to Str but not Spt harbored the *strA* and *strB* genes, strains resistant to Sul contained the *sulII* or the *sulI* and *sulII* genes, and strains resistant to Tet harbored the *tetA* gene or the *tetA* and *tetG* genes. Each of the 35 corresponding transformants and the 6 transconjugants also possessed the same resistance genes. Hybridization of Southern blots of plasmid DNA with the bla_{CMY-2} probe showed that the gene was present in each of the transformants on a large plasmid (Figure 1).

Three of the 35 parent strains and their respective transformants produced an amplicon of 1009 base pairs (bp) with the 5′ and 3′ conserved segment primers of the *int1* integron. Upon sequencing, *int1* was shown to contain the aminoglycoside-resistant gene cassette *aadA,* which confers resistance to Str and Spt (Table IV). The 3 strains also contained the *int1*-associated *qacE*Δ*I-sulI* genes. Southern blot hybridization of plasmid DNA showed that *int1* was located on a large plasmid. Two of these strains (00-4923 and 02-1652) were additionally resistant to Gen, Kan, Neo, and Spt and had been isolated twice over 2 y from veal calves at the same location. Resistance to Gen and Spt was encoded by the *aadB* and *aadA* genes, respectively, on the transferable 90-MDa plasmid along with resistance to Amp, Amc, Ctf, Cro, Cef, Fox, Chl, Fen, Str, Sul, and Tet. Strain 02-4818 and its transformant were additionally resistant to Spt, Sxt, and Tmp; resistance to Tmp was encoded by the *dhfr1* gene, on the same 90-MDa plasmid that also contained bla_{CMY-2}, other resistance genes, and *int1*.

In the 12 strains resistant to Kan and Neo, resistance was encoded by the *aphA*-1 gene, carried on a plasmid of 6, 5, 3.7, or 2.3 MDa.

Genetic relatedness

Analysis by PFGE of the 35 ESC-resistant *S.* Newport isolates digested with *Xba*I resulted in 7 distinct patterns, 21 (60%) of the isolates having the same pattern. Digestion with *Bln*I resulted in 6 patterns, 22 (63%) of the isolates having the same pattern. Analysis of the composite data revealed 8 patterns, 20 (57%) of the isolates displaying the same pattern. The same combination of AMR pattern, plasmid profile, and PFGE pattern was seen in 13 (65%) of 20 isolates from 12 sources. A high degree of relatedness was observed in multiple isolates from some locations on different sampling dates, including locations B (96%), C, D, and E (100%), and F (98%). The PFGE patterns of the isolates from location A were dissimilar (73% relatedness) (Figure 2).

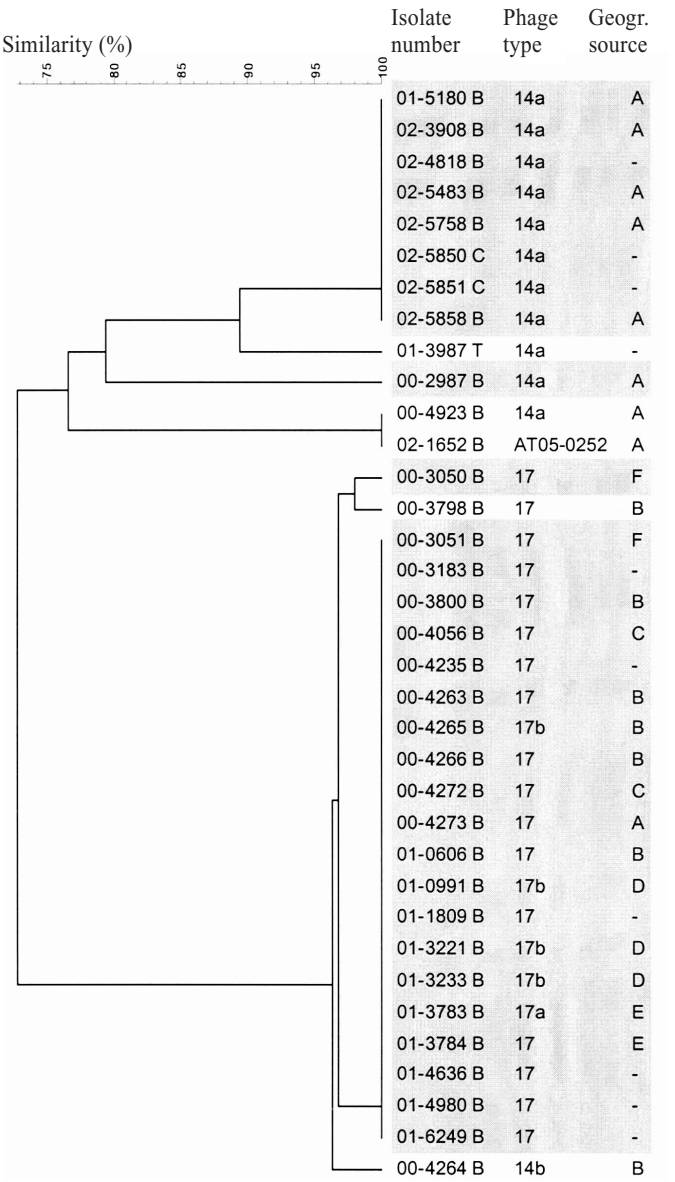

Figure 2. Genetic relatedness of the strains resistant to extended-spectrum cephalosporins, determined from patterns generated by pulsed-gel electrophoresis after digestion with *Xba*I and *Bln*I. The phage types were determined by the use of phages and a preliminary phage typing scheme at the National Microbiology Laboratory in Winnipeg, Manitoba. - — other geographic sources.

Discussion

This study revealed that a high percentage (53%) of the *S.* Newport isolates submitted were derived from dairy cattle or animal food products of bovine origin. The isolation of *S.* Newport from frozen and fresh beef and from an inline milk filter at a dairy farm suggests that humans might acquire *S.* Newport via the food chain (1,6); those consuming unpasteurized milk might be at increased risk of acquiring salmonellosis by this route (32). The clinical and pathological findings in animals infected with *S.* Newport suggest that *S.* Newport causes severe septicemic salmonellosis in susceptible animals and that infection with this pathogen is associated with significant morbidity and mortality in dairy cattle and calves, turkeys, horses, and possibly other animal species. Measures should be taken to prevent human infection with MDR *S.* Newport via the food chain or by direct contact with infected animals. Such measures might include prohibiting the sale of overtly infected calves and cows, isolating and treating infected animals, providing education on the risks of consuming unpasteurized milk, and promoting the prudent use of antimicrobials by veterinarians and farmers.

The epidemiologic observations were based on passive laboratory-based *Salmonella* surveillance. Such a surveillance system has inherent biases. Many factors, including intensity of surveillance,

severity of disease, and association with recognized outbreaks, affect the number of isolates submitted. However, the data do permit broad comparisons and identification of trends.

Of the 70 *S.* Newport strains isolated during 2000–2002, 50% were resistant to amoxicillin/clavulanate, ampicillin, cefoxitin, ceftiofur, ceftriaxone, cephalothin, chloramphenicol, florfenicol, streptomycin, sulfisoxazole, and tetracycline. Resistance to amoxicillin/clavulanate, ampicillin, cefoxitin, ceftiofur, and cephalothin and reduced susceptibility to ceftriaxone are typical of strains producing the AmpC β-lactamase encoded by the bla_{CMY-2} gene. Such strains are resistant to the penicillins, cephalothin, and the 3rd-generation cephalosporins, including the cephamycins. The β-lactamases produced by these strains are not bound by β-lactamase inhibitors such as clavulanic acid and sulbactam (21,29,33). Each of the 35 MDR *S.* Newport strains and their transformants harbored a large plasmid (approximately 80 to 90 MDa, or 120 to 135 Kb) that hybridized with the bla_{CMY-2} DNA probe. The sequence of the amplicon was completely homologous with that of the plasmid-borne bla_{CMY-2} gene in *Klebsiella pneumoniae* (33) and in *S.* Typhimurium DT104, *S.* Bredeney, and *S.* Ohio isolates (29).

Remarkably, all 35 strains carried not only the bla_{CMY-2} gene but also the flo_{st} gene (34) on the large plasmid. Previously, we found the plasmidic flo_{st} gene in only 1 of 90 MDR *S.* Typhimurium DT104 strains, whereas in the other 89 strains, the flo_{st} and other AMR genes (excluding bla_{CMY-2}) were chromosomally located within *int1* (11). In the present study, *int1* was found on a 90-MDa plasmid in 3 of the 35 MDR *S.* Newport strains, with additional resistance to gentamicin and spectinomycin or to spectinomycin, sulfamethoxazole–trimethoprim, and trimethoprim. In the previous study (11), all but 1 of the MDR *S.* Typhimurium DT104 strains produced amplicons of 1009 or 1133 bp (25), or both, with the *int1* primers. In contrast, the 3 *S.* Newport strains in the current study with the additional resistances produced only the 1009-bp amplicon. This suggests that the 3 strains had only 1 plasmidic type-1 integron, containing the *aadA* gene and conferring resistance to spectinomycin and streptomycin, and not another plasmidic integron harboring the bla_{PSE-1} gene (25) and conferring resistance to the penicillins and carbenicillin (22). These findings are reminiscent of those of Rankin and colleagues (2), who identified the *int1* gene, the bla_{CMY-2} gene, and other AMR genes on a large plasmid in 16 of 42 MDR *S.* Newport isolates.

There were 4 AMR patterns and 6 AMR-gene profiles for the 35 resistant strains studied. This suggests that the large plasmids encoding the AMR differ in composition and size. Previously, Carattoli and associates (35) reported that the bla_{CMY-2} gene was encoded on plasmids with 3 different backbone structures (types A, B, and C) in *Salmonella* strains isolated from several sources in the United States, whereas Giles and coworkers (36) found only the type C plasmid in 23 *S.* Newport isolates with 3 AMR patterns.

Additional resistance to kanamycin and neomycin was present in 12 of the 35 MDR *S.* Newport strains and was encoded in all cases by the *aphA-1* gene, located on a small plasmid (2.3 to 6 MDa). These findings are similar to those observed with *S.* Typhimurium DT104 (11): resistance to kanamycin and neomycin was present in 50 of 51 strains and was also encoded by the *aphA-1* gene on a small plasmid (2.0 MDa).

Conjugation is likely an important mechanism by which resistance to ESCs and other antimicrobials spreads among *S.* Newport, other *Salmonella* serovars, and other members of the Enterobacteriaceae. The 90-MDa plasmid was transferred by conjugation to the recipient from 6 of 24 strains (25%); 3 strains possessed 90- and 2.3-MDa plasmids that cotransferred at 28°C, and the other 3 strains possessed 90- and 72-MDa plasmids that cotransferred at 37°C. We have not examined the presence of *tra* and *finO* genes (37) on these large plasmids and do not know if these genes would also reside on the 72-MDa plasmid. Further evidence of the importance of conjugation in transferring AMR, including resistance to the ESCs, was shown in a recent study: a conjugative plasmid containing the bla_{CMY-2} and other resistance genes transferred readily from a commensal *E. coli* to *S.* Newport and other commensal *E. coli* in the intestinal tract of turkey poults (38). These findings support the resistance-gene reservoir hypothesis (39) that bacteria in transit or temporarily present in the ceca and colon acquire or transfer resistance genes by conjugation to the resident intestinal microflora. Therefore, it is tempting to speculate that bacteria such as MDR *S.* Newport may play a role as resistance-gene "traffickers" by exchanging resistance genes with other intestinal bacteria and thereby contributing to the bacterial gene reservoir in the intestines.

Of the 35 MDR *S.* Newport isolates, 20 had the same PFGE profile when digested with *Xba*I and *Bln*I, which indicated genetic relatedness. The same combination of AMR pattern, plasmid profile, and PFGE pattern was seen in 13 of 20 strains that had been isolated from 12 sources. However, differences in PFGE pattern, AMR pattern, and plasmid profile were found among isolates from location A, a veal-calf operation that received multiple entries of calves from a variety of sources (Figure 2). This suggests that methods to prevent the spread of *S.* Newport and other infections on dairy, veal-calf, cow-calf, and other cattle farms would be to limit the entry of other animals into the herd and to segregate new arrivals from the existing herd until they are shown to be free of deleterious infections.

This study showed that 50% of the *S.* Newport strains isolated during the latter part of the review period were resistant to the ESCs and other antimicrobials. Some isolates were additionally resistant to gentamicin and trimethoprim and, because they harbored *int1* genes on the same plasmid as genes encoding drug resistances, they could acquire additional resistance genes through insertion of gene cassettes. The plasmids encoding the drug resistances were self-transmissible in 25% of the strains. Treatment options would be very limited upon infection of the animal or human host with such MDR pathogens capable of causing serious illness. Every effort should be made to prevent the spread of significant zoonotic pathogens such as ESC-resistant *S.* Newport.

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