Drug Resistance and Biochemical Characteristics of *Salmonella* from Turkeys

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ABSTRACT

A study was conducted to determine the antibiotic resistance and biochemical characteristics of 2690 *Salmonella* strains belonging to 52 serovars and isolated from environmental and feed samples from 270 turkey flocks in Canada. Resistance of the *Salmonella* strains to the aminoglycoside antibiotics varied widely; none of the strains were resistant to amikacin, 14.2% were resistant to neomycin, 25.8% were resistant to gentamicin, and 27.7% of the strains were resistant to kanamycin. Most strains (97.6%) were resistant to the aminocyclitol, spectinomycin. Regarding resistance to the β-lactam antibiotics, 14.3% and 14.4% of the strains were resistant to ampicillin and carbenicillin, respectively, whereas only 5 (0.2%) of the strains were resistant to cephalothin. None of the strains were resistant to the fluoroquinolone ciprofloxacin or to polymyxin B. Resistance to chloramphenicol and nitrofurantoin was found in 2.4% and 7% of the strains, respectively. Only 1.7% of the strains were resistant to the trimethoprim-sulfamethoxazole combination, whereas 58.1% were resistant to sulfisoxazole. Thirty-eight percent of the strains were resistant to tetracycline. *Salmonella* serovars differed markedly in their drug resistance profiles. Biochemical characterization of the *Salmonella* showed that the *S. anatum*, *S. saintpaul* and *S. reading* serovars could be divided into distinct biotypes.

RÉSUMÉ

Le patron de résistance aux antibiotiques et les caractéristiques biochimiques de 2,690 isolats de *Salmonella* appartenant à 52 sérovars provenant d'échantillons de nourriture et de l'environnement chez 270 troupeaux canadiens de dinde ont été déterminés. La résistance des isolats aux aminoglycosides était très variable; aucun des isolats n'était résistant à l'amikacine, 14,2 % étaient résistants à la néomycine, 25,8 % étaient résistants à la gentamicine et 27,7 % résistants la kanamycine. La résistance à la spectinomycine était élevée avec 97,6 % des isolats résistants. En ce qui concerne la résistance aux antibiotiques de la famille des β-lactamines, 14,3 % et 14,4 % des isolats étaient respectivement résistants à l'ampicilline et la carbénicilline, alors que seulement cinq (0,2 %) des isolats étaient résistants à la céphalotine. Aucune résistance au ciprofloxacin et à la polymyxine B n'a été observée. Pour le chloramphénicol 2,4 %, et pour le nitrofurantoin 7 % des isolats étaient résistants. Seulement 1,7 % des isolats étaient résistants à la combinaison trimethoprime-sulfaméthoxazole, alors que 58,1 % des isolats étaient résistants au sulfisoxazole. Pour la tétracycline, 38 % des isolats étaient résistants. Les sérovars de *Salmonella* différaient grandement dans leur patron de résistance aux antibiotiques. La caractérisation biochimique des isolats a permis de démontrer que les sérovars *S. anatum*, *S. saintpaul* et *S. reading* pouvaient être séparés en biotypes distincts.

(*Traduit par Docteur Serge Messier*)

INTRODUCTION

The emergence of drug-resistant pathogens is often the result of the use of antimicrobial agents in animals and humans (1,2,3). The occurrence and proliferation of antibiotic-resistant *Salmonella* in environmental samples, poultry, and other animals and humans may be due to the use of medicated feeds (1,4,5), the practice of dipping hatching eggs in solutions containing antimicrobial agents (6,7,8), routine inoculation of day-old poults with antibiotics (6,7,8), and treatment of other animals (9) and humans (1) with antibiotics. Such practices often lead to the excretion of (4,5), and sometimes illness (1) due to, drug-resistant *Salmonella* in animals and humans. In animals, studies on the prevalence of drug-resistant *Salmonella* are carried out to monitor and improve management and husbandry practices, to promote and ensure the production of feeds, foods, and milk, free of antibiotics and drug-resistant *Salmonella* (1,9), to decrease threats to the health of the consumer, and to further international trade. In human patients ill with *Salmonella*, isolation of the organism, determination of its serovar and drug resistance pattern, and clinical assessment is often carried out to decide whether treatment for salmonellosis is appropriate (10,11) and which drug should be administered (12,13). The severity and prognosis of clinical salmonellosis may depend on the *Salmonella* serovar (14,15), whether or not the *Salmonella* is a host-specific serovar (14,16,17), the phagetype of the *Salmonella* serovar (18,19), host factors (20), and in some cases prior antimicrobial exposure (21).

Antibiotic resistance in *Salmonella*, *Escherichia coli*, *Shigella*, and other genera of the *Enterobacteriaceae* is often mediated by plasmids, some of which are self-transmissible (4,5,7,22, 23,24), whereas others may be cotransferred by conjugative plasmids (23,24,25). Bacteria of the genus

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TABLE I. Number and percentage (%) of *Salmonella* strains of each serovar examined for drug resistance and biotype

Serovar	Total	%	Serovar	Total	%
Anatum	379	14.09	Rough O: b: x[a]	10	0.37
Hadar	344	12.97	Montevideo	8	0.30
Agona	341	12.68	Tennessee	8	0.30
Heidelberg	291	10.82	Worthington	7	0.26
Saintpaul	248	9.22	Brandenburg	6	0.22
Muenster	130	4.83	Kottbus	6	0.22
Schwarzengrund	95	3.53	Ohio	6	0.22
Senftenberg	95	3.53	Haardt	5	0.19
Bredeney	89	3.31	Sandiego	5	0.19
Anatum var. 15+	87	3.23	Rough O: e,h: 6	4	0.15
Reading	74	2.75	Kiambu	3	0.11
Indiana	68	2.53	Muenchen	3	0.11
Kentucky	65	2.42	Orion var. 15+,34+	3	0.11
Typhimurium	59	2.19	Rough O: z_{10}: x	3	0.11
Mbandaka	36	1.34	Group E_1	2	0.07
Broughton	32	1.19	Johannesburg	2	0.07
Albany	25	0.93	Litchfield	2	0.07
Infantis	19	0.71	Rough O: e,h: 2	2	0.07
Typhimurium var. Cop.	17	0.63	Oranienburg	2	0.07
Give	16	0.59	Bareilly	1	0.04
London	16	0.59	Borreze	1	0.04
Meleagridis	16	0.59	Derby	1	0.04
Braenderup	15	0.56	Give var. 15+	1	0.04
Havana	15	0.56	Livingstone	1	0.04
Berta	12	0.45	Subspp. IIIa	1	0.04
Rough O: i: z_6	12	0.45	Taksony	1	0.04

[a] The rough O strains lacked the somatic antigen-determining distal O-specific side-chain of the lipopolysaccharide (LPS)

Salmonella are among those most often found to carry plasmids that encode drug resistance (R plasmids). Use of a single antibiotic may cause an increase in the frequency of bacteria resistant to other antibiotics because the R plasmid may encode resistance to additional antibiotics (6).

Determination of antibiotic resistance, serovar, plasmid profile, phagetype, and/or biotype of *Salmonella* isolates from the environment, feeds, poultry (24,26), horses (25,27), cattle (28,29), pigs (30), other animals, foods (1,31), humans (23,32), and other sources may assist in tracing the resistant bacteria to the source of acquisition of the antibiotic resistance genes (1,3,8). The usefulness of biotyping in studying epidemiological aspects of prevalence of *Salmonella* and salmonellosis has previously been shown for *S. typhimurium* (33,34,35), *S. enteritidis* (35), *S. paratyphi* B, and other *Salmonella* serovars (36).

Turkeys are a common source of *Salmonella* infection for the consumer. The isolation rates of *Salmonella* from turkeys and their environment are often higher than those from chickens and broilers (37,38,39,40). In addition, improper food handling and preparation practices (perhaps related to the larger size of turkeys and insufficient cooking of the turkeys before consumption)(41), may increase the risk of human infection.

During 1990 and 1991, a large number of *Salmonella* strains were isolated in a survey to estimate the prevalence of *Salmonella* among Canadian commercial turkey flocks. Data with respect to the prevalence of *Salmonella* among turkey flocks and the isolation rates of *Salmonella* from environmental samples were reported (40). The most prevalent serovars were *S. anatum*, *S. hadar*, *S. agona*, *S. heidelberg* and *S. saintpaul* which were isolated from 19.6%, 18.1%, 18.1%, 15.6%, and 12.6% of the flock premises, respectively (40). The purpose of the present study was to determine the frequency and patterns of antibiotic resistance and the biochemical characteristics of these *Salmonella* isolates.

MATERIALS AND METHODS

BACTERIAL STRAINS

The 2690 *Salmonella* strains examined for antibiotic resistance and biotype belonged to 52 serovars and were isolated from litter, dust, and feed samples of 270 turkey flocks during a national survey to estimate the prevalence of *Salmonella* among Canadian turkey flocks (40). The total number and percentage of each *Salmonella* serovar examined for drug resistance and biotype are listed in Table I.

SEROTYPING

For serotyping, the somatic (O) antigens of *Salmonella* isolates were determined with slide agglutination tests as described by Ewing (42), whereas the flagellar (H) antigens were identified by a microtechnique utilizing microtiter plates (43). The antigenic formulae of *Salmonella* serovars as listed by Le Minor and Popoff (44) were used to name the serovars.

RESISTANCE TO ANTIMICROBIAL AGENTS

Antibiotic-susceptibility tests were determined with the Cathra Replicator (45) (Automed, Shoreview, Minnesota) using agar plates (Automed) containing the following drugs: amikacin (Amk) and kanamycin (Kan) both at 16, 32, and 64 μg/mL; ampicillin (Amp) at 2, 16, and 64 μg/mL; carbenicillin (Car) at 32, 128, and 256 μg/mL; cephalothin (Clt) at 8, 16, and 256 μg/mL; chloramphenicol (Chl) at 8, 16, and 64 μg/mL; ciprofloxacin (Cip) at 1 and 2 μg/mL; cotrimoxazole (trimethoprim/sulfamethoxazole) (Cot) at 0.5/9.5, 2/38, and 8/152 μg/mL; gentamicin (Gen), and neomycin (Neo) at 4, 8, and 16 μg/mL; nitrofurantoin (Nit) at 64 μg/mL; polymyxin B (Pol) at 2, 8, and 16 μg/mL; spectinomycin (Spc) at 16 μg/mL; sulfisoxazole (Sul) at 256 μg/mL; and tetracycline (Tet) at 2, 8, and 128 μg/mL. The concentrations of antimicrobial agents and interpretation of sensitivity, intermediate sensitivity, and resistance of the strains were those suggested by the National Committee for Clinical Laboratory Standards (NCCLS) (46) and discussed by Prescott and Baggot (47).

BIOTYPING

Thirty biochemical reactions were performed on each isolate by using Gram-Negative Identification (GNI) cards and the automated microbial identification system of bioMérieux-Vitek, Hazelwood, MO.

RESULTS

RESISTANCE TO ANTIMICROBIAL AGENTS

The numbers of the 2690 *Salmonella* strains that were sensitive, intermediately sensitive, or resistant to the antimicrobial agents employed, are shown in Table II. All strains were sensitive to the antimicrobial effects of amikacin, ciprofloxacin, and polymyxin B. Only 5 strains (0.2%) were resistant to the antimicrobial effect of the cephalosporin cephalothin. A high percentage of the 2960 *Salmonella* strains (97.6% or 2626 strains) grew on agar plates containing 16 μg/mL spectinomycin.

Resistance of strains of the individual *Salmonella* serovars to antimicrobial agents other than amikacin, ciprofloxacin, polymyxin B, and spectinomycin are shown in Table III. Only resistance to the highest levels of drugs employed are shown. Three-hundred-and-eighty-six (14.3%) and 388 (14.4%) of the 2690 strains were resistant to the β-lactam antibiotics ampicillin and carbenicillin, respectively. As expected, because both antibiotics are derivatives of 6-amino penicillanic acid, there was almost total agreement in resistances of *Salmonella* serovars to the effect of ampicillin and carbenicillin. *Salmonella reading* and *S. heidelberg* strains were most often resistant to ampicillin and carbenicillin, with resistance of 58.1% and 53.6% of the strains, respectively.

Sixty-six (2.4%) of the *Salmonella* strains were resistant to the antimicrobial effects of chloramphenicol; the most common resistant serovars were *S. kentucky* (41.5%), and *S. havana* (40.0%). Examination of records showed that 60 of the 66 chloramphenicol resistant isolates were recovered from only 8 flocks. All 15 *S. reading* isolates from 1 flock were resistant to chloramphenicol. Only 46 (1.7%) of the 2690 *Salmonella* strains were resistant to the antimicrobial effect of cotrimoxazole. Strains of *S. hadar* were the most often resistant, with 7.0% of these strains growing on agar plates containing 8/152 μg/mL of trimethoprim/sulfamethoxazole (cotrimoxazole).

Comparison of amikacin with the other aminoglycosides neomycin, gentamicin, and kanamycin showed that amikacin more markedly inhibited growth of the *Salmonella* strains. None of the strains were resistant to amikacin as compared to 383 (14.2%) strains interpreted as resistant to neomycin, 694 (25.8%) resistant to gentamicin and 746 of 2690 (27.7%) strains resistant to kanamycin (Table II). Of the 25 most prevalent serovars, *S. give* isolates were the most often resistant to the antimicrobial effects of gentamicin (100%) followed by *S. bredeney* (88.8%), and *S. saintpaul* (66.9%) (Table III). *Salmonella saintpaul* strains were the most commonly resistant to the antimicrobial effects of kanamycin (73.0%), followed by *S. bredeney* (71.9%), *S. indiana* (63.2%) and *S. mbandaka* (55.6%). The most commonly neomycin-resistant serovar was *S. mbandaka* (55.6%) followed by *S. indiana* (42.6%).

Resistance to the antimicrobial effect of high concentrations of nitrofurantoin was uncommon (Table II). Isolates of *S. indiana* were the most frequently resistant (26.5%), followed by *S. hadar* (17.2%) and *S. kentucky* (16.9%) (Table III). A high percentage of isolates from many serovars were resistant to sulfisoxazole. Among the 25 most frequently occurring serovars, all strains of *S. give*, *S. london* and *S. meleagridis* were resistant to sulfisoxazole. Other serovars with a high frequency of resistance were *S. infantis* (89.5%), *S. bredeney* (85.4%), *S. anatum* (85.2%), *S. broughton* (84.4%), *S. saintpaul* (75.8%), *S. havana* (73.3%), *S. heidelberg* (72.5%), and *S. indiana* (72.1%). *Salmonella london*, *S. heidelberg*, and *S. saintpaul* strains were the most commonly resistant to tetracycline, since 93.8%, 80.8%, and 77.0% of the strains were resistant, respectively.

Resistance to sulfisoxazole and tetracycline was the most common combination of drug resistances found in *S. anatum* strains (74 strains; 19.5%) (Table IV). Resistance to ampicillin and carbenicillin together,

TABLE II. Number of *Salmonella* strains sensitive, intermediately sensitive, and/or resistant to antimicrobial agents

Antim.[a]	S/I/R[b]	Conc.[c] (μg/mL)	Number[d]	%[e]	Antim.	S/I/R	Conc. (μg/mL)	Number	%
	S	≤16	2690	100.0		S	≤4	1949	72.5
Amk	I	32	0	0.0	Gen	I	8	47	1.7
	R	≥64	0	0.0		R	≥16	694	25.8
	S	≤2	2298	85.4		S	≤16	1888	70.2
Amp	I	16	6	0.2	Kan	I	32	56	2.1
	R	≥64	386	14.3		R	≥64	746	27.7
	S	≤32	2298	85.4		S	≤4	2292	85.2
Car	I	128	4	0.1	Neo	I	8	15	0.6
	R	≥256	388	14.4		R	≥16	383	14.2
	S	≤8	2561	95.2	Nit	R	≥64	189	7.0
Clt	I	16	124	4.6					
	R	≥256	5	0.2		S	≤2	2689	100.0
					Pol	I	8	1	0.0
	S	≤1	2690	100.0		R	≥16	0	0.0
Cip	I	1	0	0.0					
	R	≥2	0	0.0	Spc	I/R	≥16	2626	97.6
	S	≤8	2622	97.5	Sul	I/R	≥256	1563	58.1
Chl	I	16	2	0.1					
	R	≥64	66	2.4		S	≤2	1109	41.2
					Tet	I	8	557	20.7
	S	≤0.5/9.5	2642	98.2		R	≥128	1024	38.1
Cot	I	2/38	2	0.1					
	R	≥8/152	46	1.7					

[a] Antimicrobial agent: Amk = Amikacin; Amp = Ampicillin; Car = Carbenicillin; Chl = Cephalothin; Cip = Ciprofloxacin; Chl = Chloramphenicol; Cot = Cotrimoxazole; Gen = Gentamicin; Kan = Kanamycin; Neo = Neomycin; Nit = Nitrofurantoin; Pol = PolymyxinB; Spc = Spectinomycin; Sul = Sulfisoxazole; Tet = Tetracycline
[b] S/I/R = Sensitive/Intermediate sensitive/Resistant
[c] Conc. = Concentration
[d] Number of 2690 strains that were sensitive, intermediate sensitive, or resistant at the concentration of antibiotic employed
[e] % = Percentage

Table III. Resistance of *Salmonella* isolates to selected antimicrobial agents

		Antimicrobial agents (≥µg/mL)										
		Amp	Car	Clt	Chl	Cot	Gen	Kan	Neo	Nit	Sul	Tet
Serovar	Total[a]	64	256	256	64	160	16	64	16	64	256	128
Anatum	379	57[b]	57	1	0	2	55	53	33	40	323	170
Hadar	344	1	1	1	0	24	15	126	125	59	75	20
Agona	341	64	65	0	1	8	104	50	21	0	220	92
Heidelberg	291	156	156	1	2	8	71	81	40	3	211	235
Saintpaul	248	10	10	1	0	2	166	181	37	32	188	191
Muenster	130	0	0	0	0	1	13	12	12	0	29	19
Schwarzengrund	95	0	1	0	11	0	14	26	26	8	44	45
Senftenberg	95	3	3	0	1	0	17	19	15	2	31	18
Bredeney	89	0	0	0	0	1	79	64	0	0	76	2
Anatum var15+	87	4	4	0	0	0	18	10	8	8	56	4
Reading	74	43	43	0	15	0	15	0	0	0	34	48
Indiana	68	23	23	0	0	0	23	43	29	18	49	42
Kentucky	65	3	4	0	27	0	25	21	2	11	32	37
Typhimurium	59	0	1	0	0	0	2	1	0	7	14	14
Mbandaka	36	10	9	1	0	0	0	20	20	0	9	22
Broughton	32	0	0	0	0	0	3	2	3	0	27	3
Albany	25	0	0	0	0	0	0	0	0	0	0	1
Infantis	19	0	0	0	0	0	5	0	0	0	17	3
Typhim var Cop	17	0	0	0	0	0	0	0	0	0	0	0
Give	16	0	0	0	0	0	16	5	0	0	16	5
London	16	1	0	0	0	0	0	1	1	0	16	15
Meleagridis	16	0	0	0	0	0	2	0	0	0	16	0
Braenderup	15	0	0	0	0	0	6	0	0	0	6	2
Havana	15	0	0	0	6	0	4	0	0	0	11	7
Berta	12	4	4	0	0	0	4	0	0	0	7	3
Rough O: i: z_6	12	1	1	0	0	0	9	7	0	0	1	7
Rough O: b: x	10	1	1	0	0	0	0	0	1	0	10	1
Montevideo	8	0	0	0	0	0	5	5	0	0	5	5
Tennessee	8	0	0	0	0	0	0	0	0	0	0	0
Worthington	7	2	2	0	0	0	5	3	1	0	7	0
Brandenburg	6	0	0	0	0	0	0	0	0	0	2	0
Kottbus	6	0	0	0	0	0	1	1	0	0	1	0
Ohio	6	0	0	0	0	0	1	0	0	0	2	1
Haardt	5	0	0	0	0	0	0	0	0	0	0	0
Sandiego	5	0	0	0	0	0	5	5	3	0	5	0
Rough O: eh: 6	4	0	0	0	0	0	0	0	0	0	4	4
Kiambu	3	0	0	0	0	0	3	0	0	0	3	0
Muenchen	3	0	0	0	0	0	0	0	0	0	3	0
Orion var15+34+	3	0	0	0	3	0	0	0	0	0	3	3
Rough O: z_{10}: x	3	0	0	0	0	0	0	1	1	0	0	0
Group E_1	2	0	0	0	0	0	2	2	2	0	2	2
Johannesburg	2	2	2	0	0	0	2	2	0	0	2	0
Litchfield	2	0	0	0	0	0	0	1	1	0	1	0
Oranienburg	2	0	0	0	0	0	0	0	0	0	1	0
Rough O: eh: 2	2	0	0	0	0	0	2	2	0	0	2	2
Bareilly	1	0	0	0	0	0	0	0	0	0	0	0
Borreze	1	1	1	0	0	0	1	1	1	0	1	1
Derby	1	0	0	0	0	0	0	0	0	0	1	0
Give var15+	1	0	0	0	0	0	0	0	0	0	0	0
Livingstone	1	0	0	0	0	0	1	1	1	0	0	0
Subspecies IIIa	1	0	0	0	0	0	0	0	0	0	0	0
Taksony	1	0	0	0	0	0	0	0	0	1	0	0
Total	2690	386	388	5	66	46	694	746	383	189	1563	1024

[a] Total number of strains of each serovar
[b] Number of strains that were resistant

or in conjunction with resistance to other antibiotics, was found in 56 (14.8%) of *S. anatum* strains; 37 (9.8%) of these combinations consisted of resistance to ampicillin, carbenicillin, sulfisoxazole, and tetracycline. Sixteen (4.2%) *S. anatum* strains were resistant to the aminoglycosides gentamicin, kanamycin, and neomycin, mostly in conjunction with resistance to sulfisoxazole and/or tetracycline. Resistance to kanamycin and neomycin together or in combination with other drug resistances was found in 43 (11.3%) *S. anatum* strains.

The percentage of *S. hadar* strains resistant to ampicillin, carbenicillin and tetracycline was low when compared with other frequently isolated serovars such as *S. anatum*, *S. agona*, *S. heidelberg* and *S. saintpaul* (Table III). The most common combination of drug resistance in *S. hadar* was to kanamycin and neomycin, either together (76 strains; 22.1%), or in combination with other drug resistances (48 strains;

TABLE IV. Combinations of resistances found in the 15 most frequently occurring *Salmonella* serovars

S. anatum		*S. hadar*		*S. agona*		*S. heidelberg*		*S. saintpaul*	
AC[a]	5[b]	CltCotKNNit	1	AC	10	AC	2	ACCotKNST	1
ACCotT	2	CotS	23	ACGKNS	1	ACCotKNST	1	ACGKNitS	1
ACGKS	9	GKNNitS	1	ACGKNST	11	ACGKNS	1	ACKNS	3
ACS	3	GKNS	2	ACGKS	6	ACGKNST	2	ACST	1
ACST	37	GKS	2	ACGS	2	ACGKST	1	ACKST	4
AST	1	GNitS	1	ACKNST	9	ACGKS	2	GK	1
CltKNitS	1	GS	9	ACS	16	ACGST	10	GKNitST	10
CKNST	1	KN	76	ACST	9	ACKNST	18	GKS	7
GKNNitS	4	KNNit	33	CGKS	1	ACKST	2	GKST	104
GKNNitST	1	KNNitS	1	CotS	4	ACNitS	1	GKT	19
GKNS	6	KNS	7	CotT	4	ACNitST	1	GNST	1
GKNST	4	KNT	3	GKS	15	ACST	92	GNitST	1
GKNT	1	ST	8	GKST	2	ACS	14	GS	1
GKS	8			GS	50	ACT	6	GST	13
GNitS	1			GST	16	CGKNST	1	GT	7
GS	10			KT	5	CST	2	KN	8
GST	8			ST	23	ChlKST	1	KNS	15
GT	1					ChlKT	1	KNST	1
KN	1					CotGKNS	2	KNT	4
KNNitS	7					CotGKNST	2	KST	2
KNNitST	3					CotGKST	1	NitS	9
KNS	2					CotGS	1	NitST	1
KNST	12					GK	1	ST	5
NitS	9					GKNST	9		
NitST	14					GKS	25		
ST	74					GKST	8		
						GS	4		
						GST	4		
						KNT	1		
						KS	1		
						NitT	1		
						ST	6		

S. muenster		*S. schwarzengrund*		*S. senftenberg*		*S. bredeney*		*S. anatum* O15+	
GKNST	12	CKNS	1	ACKNS	1	GK	2	AC	1
GS	1	ChlNitST	4	ACGKNST	1	GKS	61	ACS	3
		ChlST	7	ACK	1	GKST	1	GKNS	8
		GKNST	10	GKNS	3	GS	13	GKST	1
		GST	4	GKNST	9	GST	1	GS	8
		KNS	14	GKST	1			GST	1
		KNST	1	GS	1			NitS	5
		NitT	1	KNS	1				
				ST	6				

S. reading		*S. indiana*		*S. kentucky*		*S. typhimurium*		*S. mbandaka*	
AC	1	ACGKNNitS	1	ACGK	3	CGS	1	ACKNST	8
ACChlST	15	ACGKNNitST	1	CGKN	1	GS	1	ACST	1
ACGST	11	ACGKNS	9	ChlNitST	11	ST	2	AClt	1
ACST	1	ACGKNST	4	ChlST	14			KNT	12
ACT	15	ACGKNitST	3	GK	11				
GS	1	ACGKST	2	GKN	1				
GST	3	ACGNNitST	1	GKT	2				
ST	1	ACKNNitST	1	GS	1				
		GKNST	1	GST	4				
		GNKNitST	1	GT	2				
		KN	3	ST	2				
		KNS	3						
		KNST	3						
		KST	10						
		NitST	2						
		NitT	8						
		ST	1						

[a] Antibiotic resistances; A = ampicillin, C = carbenicillin, Clt = cephalothin, Chl = chloramphenicol, Cot = cotrimoxazole, G = gentamicin, K = kanamycin, N = neomycin, Nit = nitrofurantoin, S = sulfisoxazole, T = tetracycline

[b] Number of strains resistant to the antimicrobial agents

TABLE V. Biochemical tests performed to determine biotype

Description of test	Biotype (number of strains) A (1825)	B (395)	C (163)	D (82)	E (32)	F (27)	G (25)
Glucose fermentation in the presence of 2,4,4′-trichloro-2′-hydroxy-diphenylether	−	−	−	−	−	−	−
Aerobic acid production by glucose oxidation	+	+	+	+	+	+	+
Growth in buffered peptone water containing tryptophan	+	+	+	+	+	+	+
Utilization of acetamide	−	−	−	−	−	−	−
Hydrolysis of esculin	−	−	−	−	−	−	−
Enzymatic activity of indoxyl-β-D-glucoside	−	−	−	−	−	−	−
Production of urease	−	−	−	−	−	−	−
Utilization of citrate as sole carbon source	+	+	+	+	+	+	+
Utilization of malonate as carbon source	−	−	−	−	−	−	−
Production of tryptophan deaminase	−	−	−	−	+	+	+
Growth in the presence of polymyxin B	−	−	−	−	−	−	−
Lactose oxidation	−	−	−	−	−	−	−
Maltose oxidation	+	+	+	+	+	+	+
Mannitol oxidation	+	+	+	+	+	+	+
Xylose oxidation	+	−	+	+	+	−	+
Fermentation of raffinose	−	−	−	−	−	−	−
Fermentation of sorbitol	+	+	+	+	+	+	+
Fermentation of sucrose	−	−	−	−	−	−	−
Fermentation of inositol	−	−	+	−	+	−	−
Fermentation of adonitol	−	−	−	−	−	−	−
Fermentation of glucose in the presence of p-coumaric acid	+	+	+	+	+	+	+
Production of hydrogen sulfide from thiosulfate	+	+	+	−	+	+	+
Hydrolysis of o-nitrophenyl-β-D-galactopyranoside	−	−	−	−	−	−	−
Fermentation of rhamnose	+	+	+	+	+	+	+
Fermentation of L-arabinose	+	+	+	+	+	+	+
Fermentation of glucose	+	+	+	+	+	+	+
Production of arginine dihydrolase	−	−	−	−	−	−	−
Production of lysine decarboxylase	+	+	+	+	+	+	+
Production of ornithine decarboxylase	+	+	+	+	+	+	+
Production of oxidase	−	−	−	−	−	−	−

14.0%) (Table III and IV). *Salmonella agona* strains were commonly resistant to both gentamicin and sulfisoxazole (14.7% of the strains), and to ampicillin and carbenicillin both together and in combination with resistance to other antibiotics (18.8%). *Salmonella heidelberg* strains were resistant to a wide variety of combinations of antibiotics; resistance to ampicillin, carbenicillin, sulfisoxazole, and tetracycline occurred in 92 strains (31.6%). *Salmonella saintpaul* strains were commonly resistant to the antibiotics gentamicin, kanamycin, sulfisoxazole, and tetracycline (41.9%). Other frequently resistant serovars were *S. bredeney*, of which 68.5% of the strains were resistant to gentamicin, kanamycin, and sulfisoxazole, and *S. indiana*, of which 92.6% of strains were resistant to a wide variety of combinations of 2 to 8 antimicrobial agents (Tables III, IV).

BIOTYPING

Results of biochemical reactions enabled grouping of the *Salmonella* strains into numerous biotypes. The 7 most common biotypes (A–G), representing 2549 of the total of 2690 strains, are presented in Table V in order of frequency, with biotype A being the most prevalent. A total of 1825 strains belonged to biotype A, 395 to biotype B, 163 to biotype C, 82 to biotype D, 32 to biotype E, 27 to biotype F and 25 strains belonged to biotype G. Differences in biotype could in some cases be related to the serovar to which the strains belonged. A notable example is *S. anatum*, of which 303 of 379 strains (79.9%) belonged to biotype B. Also, 89.7% of *S. anatum* var 15+ strains belonged to biotype B. The *S. saintpaul* strains could be subdivided into 2 large groups: 53.6% belonged to biotype C (inositol fermenters), whereas 41.5% belonged to biotype A. *Salmonella reading* could also be grouped into 2 major groups: 75.7% were of biotype A, and 24.3% of biotype D (no production of hydrogen sulfide). No associations between biotype and resistance to antimicrobial agents were observed.

DISCUSSION

This study determined the frequency and type of resistance to antimicrobial agents, and the biochemical properties of 2690 *Salmonella* strains isolated from turkeys and their environment. A large number of strains were resistant to 1 or more antimicrobial agents. Drug resistance occurred at high frequency against spectinomycin, sulfisoxazole, tetracycline, the aminoglycosides gentamicin, kanamycin, and neomycin, and the β-lactams ampicillin and carbenicillin. The different *Salmonella* serovars varied widely in number of resistant strains of a particular serovar and in type of drug resistance.

The large number of gentamicin-resistant *Salmonella* strains is likely the result of practices such as dipping hatching eggs into gentamicin sulfate by turkey breeders to prevent mycoplasmosis (6,7,8,29) caused by *Mycoplasma synoviae*, *M. gallinarum*, and *M. iowae* (48), and the injecting of day-old poults with gentamicin, spectinomycin, and norfloxacin as preventative measures against *E. coli* and other infections. Resistance of *Salmonella* to gentamicin in this study (25.8%) was slightly lower than the percentage of gentamicin-resistant *Salmonella* isolates (28.0%) from turkeys reported by Blackburn et al. (29) in the U.S. in 1984. Apparently enrofloxacin, one of the fluoroquinolones, has largely replaced gentamicin in egg dipping practices over the past 4–5 y. In 1990–1991, however, when the isolates were obtained, some operations may still have been using gentamicin. Resistance to one of the aminoglycosides may result in cross-resistance to other aminoglycosides because the R plasmid-specified enzymes, broadly classified as phosphotransferases, acetyltransferases, and adenyltransferases, often affect more than 1 of the aminoglycosides (47). This may explain the common occurrence of combined resistance against 2 or 3 of the drugs in this family.

Despite the likely use of enrofloxacin for dipping eggs, or for injecting day-old poults, resistance of *Salmonella* to ciprofloxacin, another fluoroquinolone, was not found in this study. This observation is of importance to public health because ciprofloxacin is used increasingly in human patients for treatment of acute or extra-intestinal tract salmonellosis, for elimination of the excretion of *Salmonella* in the feces, and for treatment of infections caused by *Escherichia coli*, other *Enterobacteriaceae*, and other pathogens including *Mycobacterium* spp., and *Pseudomonas aeruginosa*. Treatment of human *Salmonella* infections using ciprofloxacin has met with considerable success (12,49). However, resistance of *Salmonella* to the fluorquinolones, and the inability of enrofloxacin or ciprofloxacin to completely curtail excretion of *Salmonella*, has been reported in poultry as well as in humans (13,50,51).

Widespread natural resistance against spectinomycin has been reported (47) and may explain the high percentage of spectinomycin resistant strains observed in the present study. Furazolidone, sulfonamides, β-lactamases, tetracyclines, neomycin, and spectinomycin have all been used in the treatment of poults and turkeys infected with *S. arizonae*, in acute outbreaks of paratyphoid and other infections, and for the prevention of such infections in turkeys, any of which may account for the drug resistances noted in the present study. Although the use of chloramphenicol in food-producing animals has been prohibited for more than 10 y in Canada, there was a small number of flocks from which high numbers of chloramphenicol-resistant *Salmonella* were isolated.

Biotyping of the *Salmonella* isolates showed that tests with 2 substrates, xylose and inositol, which provided the most discrimination of strains of *S. typhimurium* in the scheme of Duguid et al. (33,36), also distinguished *S. anatum* and *S. anatum* var 15+ strains (which were 80–90% xylose oxidation negative), and divided the *S. saintpaul* strains into 2 major groups of inositol fermenters and nonfermenters. It is interesting to note that almost 25% of the *S. reading* did not produce hydrogen sulfide. In recent years, we have observed an increasing number of *Salmonella heidelberg* strains isolated from turkeys or their environment that also do not produce hydrogen sulfide.

In summary, the main findings of this study are as follows: i) none of the strains were resistant to the antimicrobial effects of amikacin, ciprofloxacin, or polymyxin B; ii) resistance to the antimicrobial effects of cephalothin, chloramphenicol, cotrimoxazole, and nitrofurantoin was low; iii) a considerable number of strains were resistant to ampicillin, carbenicillin, tetracycline, gentamicin, kanamycin, neomycin, or sulfisoxazole; iv) a high percentage of strains were resistant to spectinomycin; v) *Salmonella* serovars differed markedly in their drug resistance profiles; and vi) biotyping showed that the *S. anatum*, *S. saintpaul*, and *S. reading* serovars could be divided into distinct biotypes.

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