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Research Note

Resistance to Antimicrobial Agents among *Salmonella* Isolates Recovered from Layer Farms and Eggs in the Caribbean Region

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ABSTRACT

This investigation determined the frequency of resistance of 84 isolates of *Salmonella* comprising 14 serotypes recovered from layer farms in three Caribbean countries (Trinidad and Tobago, Grenada, and St. Lucia) to eight antimicrobial agents, using the disc diffusion method. Resistance among isolates of *Salmonella* was related to the country of recovery, type of sample, size of layer farms, and isolate serotype. Overall, all (100.0%) of the isolates exhibited resistance to one or more of seven antimicrobial agents tested, and all were susceptible to chloramphenicol. The resistance detected ranged from 11.9% to sulphamethoxazole-trimethoprim (SXT) to 100.0% to erythromycin. The difference was, however, not statistically significant ($P = 0.23$). Across countries, for types of samples that yielded *Salmonella,* significant differences in frequency of resistance were detected only to SXT ($P = 0.002$) in Trinidad and Tobago and to gentamycin ($P = 0.027$) in St. Lucia. For the three countries, the frequency of resistance to antimicrobial agents was significantly different for ampicillin ($P = 0.001$) and SXT ($P = 0.032$). A total of 83 (98.8%) of the 84 isolates exhibited 39 multidrug resistance patterns. Farm size significantly ($P = 0.032$) affected the frequency of resistance to kanamycin across the countries. Overall, among the 14 serotypes of *Salmonella* tested, significant ($P < 0.05$) differences in frequency of resistance were detected to kanamycin, ampicillin, and SXT. Results suggest that the relatively high frequency of resistance to six of the antimicrobial agents (erythromycin, streptomycin, gentamycin, kanamycin, ampicillin, and tetracycline) tested and the multidrug resistance detected may pose prophylactic and therapeutic concerns for chicken layer farms in the three countries studied.

Salmonellosis is a well-established disease of poultry (layers and broilers) worldwide, causing morbidity and mortality and, thus, economic losses *(5, 21)*. Complicating control of salmonellosis in the poultry industry is the fact that, in addition to horizontal transmission on farms, vertical transmission from parent stock to offspring is also common; this makes control a challenge *(9, 17)* and poses a food safety hazard to consumers of contaminated eggs and egg products *(3, 26)*.

Although vaccination to prevent salmonellosis has been used successfully on layer farms in several countries *(9, 21, 36)*, the existence of several pathogenic non-host-adapted *Salmonella* serovars makes successful vaccination difficult. This is particularly a problem in most developing countries, where vaccines are not routinely available or are not used by poultry farmers and where prevention and treatment of salmonellosis have relied predominantly on good sanitary practices and the prophylactic and therapeutic administration of antimicrobial agents in feeds and water *(5, 29, 36)*. The improper use of antimicrobial agents as a result of failed preventive measures has led to the development of resistance to antimicrobial agents, with therapeutic consequences *(8, 29)*.

In addition to the recovery of *Salmonella* isolates from several livestock species, including laying birds and their environments *(1, 35)*, there are reports of increased resistance to antimicrobial agents among isolates *(7, 12, 20)*. *Salmonella* isolates from the shells and contents of table eggs have been reported to exhibit resistance to antimicrobial agents *(10, 16)*. Uncontrolled therapeutic use of antimicrobial agents in the livestock industry *(15, 20)* and the use of antimicrobial agents as additives in animal feeds to promote growth *(11)* may pose food safety, public health, and therapeutic problems in animal and human diseases *(12, 31)*.

There are reports from various countries of variable antimicrobial resistance of *Salmonella* isolates from layers and farm environments *(19, 24, 30, 33)*. It has been documented that antimicrobial resistance among *Salmonella*

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serovars is affected by various factors, such as the type of sample (cloacae, fecal, or feed) taken from poultry farms *(11, 31)* and the serotypes *(35)*.

In the Caribbean region, the only report to document the antimicrobial resistance of *Salmonella* isolates recovered from poultry or table eggs was a 2005 study conducted on table eggs in Trinidad and Tobago *(3)*. To date, there is a dearth of information on the antibiograms of *Salmonella* recovered from layer farms in the region. One objective of this study, therefore, was to determine the antibiograms of *Salmonella* recovered from layer farms in three Caribbean countries (Trinidad and Tobago, Grenada, and St. Lucia), and another was to relate the prevalence of resistance to country of origin, size of farms, type of sample, and serotypes of the isolates.

MATERIALS AND METHODS

Countries where study was conducted. The study was conducted in three Caribbean countries, namely, Trinidad and Tobago, Grenada, and St. Lucia, during the periods 7 September to 3 October 2011, 17 to 21 October 2011, and 14 to 18 November 2011, respectively.

Sample type and collection. Layer farms (small, <5,000 birds; medium, 5,000 to 10,000 birds; and large, >10,000 birds) were visited across the three countries, and the following types of samples were collected: cloacal swabs, environmental swabs of deep litters, freshly laid eggs, and poultry feeds. The procedures used for sample collection and transportation to the laboratory were described previously *(4)*.

Processing of samples. Standard methods were used to process the samples collected and to isolate *Salmonella (4, 6, 23)*. Briefly, samples were preenriched in buffered peptone water (Oxoid, Ltd., Hampshire, England) for 18 to 24 h at 37°C and then were enriched in tetrathionate broth (Oxoid) and in Rappaport-Vassiliadis broth (Oxoid) incubated for 18 to 24 h at 37 and 42°C, respectively.

Isolation and identification of *Salmonella* serovars. The procedures used to isolate *Salmonella* serovars from the samples were described earlier *(4)*. Briefly, the selective broths (tetrathionate and Rappaport-Vassiliadis) were subcultured onto xylose-lysine deoxycholate agar and brilliant green agar (Oxoid) and were incubated aerobically at 37°C for 24 h. All colonies suggestive of *Salmonella* serovars on the selective agar were purified on blood agar plates (Oxoid) at 37°C for 24 h and then were subjected to biochemical tests, including triple sugar iron agar, urea, citrate, methyl red, sulfide-indole-motility medium, *o*-nitrophenyl-*p*-D-galactopyranoside, and lysine iron agar (Oxoid) *(6, 23)*. Initially, serological typing of all isolates biochemically identified as *Salmonella* serovars was done using a commercial slide agglutination test kit *Salmonella* polyvalent antiserum (A-I & Vi, Difco, Detroit, MI). Complete confirmation and serotyping of *Salmonella* isolates were done at the Caribbean Epidemiology Center, Port of Spain, Trinidad and Tobago, which serves as the regional laboratory for the serotyping of *Salmonella*.

Determination of resistance to antimicrobial agents. The antimicrobial resistance of 84 isolates of *Salmonella* recovered from 35 layer farms in the three countries (Table 1) was determined using the disc diffusion method according to the National Committee for Clinical Laboratory Standards (NCCLS) *(27)* guidelines. For the study, the following antimicrobial agents and concentrations were used (all from Difco): erythromycin (E; 15 μg), streptomycin (S; 10 μg), gentamycin (CN; 10 μg), kanamycin (K; 30 μg), ampicillin (AMP; 10 μg), tetracycline (TE; 15 μg), sulphamethoxazole-trimethoprim (SXT; 23.75/1.25 μg), and chloramphenicol (C; 30 μg). The tests were performed on Mueller Hinton agar (Difco) followed by aerobic incubation at 35°C for 24 h. The zones of inhibition were interpreted as recommended by the disc manufacturer and NCCLS *(27)*.

TABLE 1. *Farm sources of* Salmonella *isolates*

Country	Total no. of poultry farms	No. (%) of farms sampled[a]	No. of *Salmonella* isolates tested
Trinidad and Tobago	23	10 (43.5)[b]	43
Grenada	21	10 (47.6)[c]	9
St. Lucia	37	15 (40.5)[d]	32
Total	81	35 (43.2)	84

[a] Types of samples collected: cloacal swabs, environmental samples, eggs, and feeds.
[b] Five large, two medium, and three small farms.
[c] One medium and nine small farms.
[d] Four large and 11 small farms.

Statistical analyses. Data were analyzed using the Statistical Package for Social Sciences (version 22, IBM Corp., Somers, NY). Chi-square analysis was conducted to determine whether there were statistically significant differences in the frequency of resistance to antimicrobial agents among *Salmonella* isolates recovered among and within the three countries and among sources, irrespective of the country of origin. The differences in the frequency of resistance among the various serotypes of *Salmonella* and the predominant resistance patterns were also subjected to analysis. The level of significance was determined at an alpha level of 0.05.

RESULTS AND DISCUSSION

Frequency of resistance of Salmonella isolates to antimicrobial agents. It was of therapeutic significance that all 84 *Salmonella* isolates recovered from three Caribbean countries (Trinidad and Tobago, Grenada, and St. Lucia) exhibited resistance to one or more of seven antimicrobial agents used and that all were sensitive to chloramphenicol, an antimicrobial agent not commonly used in the poultry industry in the region (Table 2). A similarly high prevalence of resistance (98.4%) was reported for isolates of *Salmonella* recovered from poultry houses in Chad *(12)*. The use, or overuse, of antimicrobial agents in the poultry industry and on livestock farms has been shown to result in increased resistance to antimicrobial agents *(8, 29)*, with the resultant therapeutic failures causing economic losses. Equally of therapeutic significance is that, of the six antimicrobial agents used in the poultry industry in the Caribbean region, SXT was the most effective against the *Salmonella* isolates tested from the three countries, with only 11.9% exhibiting resistance. Makaya et al. *(24)* in a study in Zimbabwe reported a 0.0% prevalence of resistance to SXT. In the only published report on the antibiograms of *Salmonella* isolates from eggs (shells and contents) in the

TABLE 2. *Frequency of resistance of* Salmonella *isolate by type of samples from three countries*[a]

Country	Source of sample	No. of isolates tested	No. (%) of resistant isolates[b]	No. (%) resistant to[c]: E	S	*P* value	CN	*P* value	K	*P* value	AMP	*P* value	TE	*P* value	SXT	*P* value
Trinidad and Tobago[d]	Feed	6	6 (100.0)	6 (100.0)	4 (66.7)	0.131	5 (83.3)	0.162	1 (16.7)	0.114	2 (33.3)	0.569	1 (16.7)	0.88	0 (0.0)	0.002
	Egg shell surface	9	9 (100.0)	9 (100.0)	9 (100.0)		7 (77.8)		7 (77.8)		6 (66.7)		2 (22.2)		6 (66.7)	
	Environment	25	25 (100.0)	25 (100.0)	23 (92.0)		11 (44.0)		11 (44.0)		12 (48.0)		4 (16.0)		3 (12.0)	
	Layers	3	3 (100.0)	3 (100.0)	2 (66.7)		2 (66.7)		1 (33.3)		1 (33.3)		0 (0.0)		0 (0.0)	
	Total	43	43 (100.0)	43 (100.0)	38 (88.4)		25 (58.1)		20 (46.5)		21 (48.8)		7 (16.3)		9 (20.9)	
Grenada	Environment	3	3 (100.0)	3 (100.0)	2 (66.7)	0.134	2 (66.7)	0.571	2 (66.7)	0.571	2 (66.7)	1.000	1 (33.3)	0.134	0 (0.0)	NA
	Layers	6	6 (100.0)	6 (100.0)	6 (100.0)		5 (83.3)		5 (83.3)		4 (66.7)		0 (0.0)		0 (0.0)	
	Total	9	9 (100.0)	9 (100.0)	8 (88.9)		7 (77.8)		7 (77.8)		6 (66.7)		1 (11.1)		0 (0.0)	
St. Lucia	Environment	11	11 (100.0)	11 (100.0)	11 (100.0)	NA	3 (27.3)	0.017	2 (18.2)	0.035	1 (9.1)	0.673	9 (81.8)	0.102	0 (0.0)	0.462
	Layers	21	21 (100.0)	21 (100.0)	21 (100.0)		15 (71.4)		12 (57.1)		3 (14.3)		11 (52.4)		1 (4.8)	
	Total	32	32 (100.0)	32 (100.0)	32 (100.0)		18 (56.3)		14 (43.8)		4 (12.5)		20 (62.5)		1 (3.1)	
All countries	Feed	6	6 (100.0)	6 (100.0)	4 (66.7)	0.056	5 (83.3)	0.014	1 (16.7)	0.034	2 (33.3)	0.183	1 (16.7)	0.389	0 (0.0)	0.000
	Egg shell surface	9	9 (100.0)	9 (100.0)	9 (100.0)		7 (77.8)		7 (77.8)		6 (66.7)		2 (22.2)		6 (66.7)	
	Environment	39	39 (100.0)	39 (100.0)	36 (92.3)		16 (41.0)		15 (38.5)		15 (38.5)		14 (35.9)		3 (7.7)	
	Layers	30	30 (100.00	30 (100.0)	29 (96.7)		22 (73.3)		18 (60.0)		8 (26.7)		11 (36.7)		1 (3.3)	
All sources		84	84 (100.0)	84 (100.0)	78 (92.9)	0.137	50 (59.5)	0.491	41 (48.8)	0.179	31 (36.9)	0.001	28 (33.3)	0.00	10 (11.9)	0.032

[a] All isolates were sensitive to chloramphenicol (30 μg).

[b] Resistant to one or more antimicrobial agents.

[c] E, erythromycin (5 μg); S, streptomycin (10 μg); CN, gentamycin (10 μg); K, kanamycin (30 μg); AMP, ampicillin (10 μg); TE, tetracycline (15 μg); SXT, sulphamethoxazole-trimethoprim (23.75/1.25 μg).

[d] Of all the feed and freshly laid eggs sampled on farms from the three countries, *Salmonella* isolates were recovered only from Trinidad and Tobago samples.

TABLE 3. *Antimicrobial resistance patterns among* Salmonella *isolates from three Caribbean countries*

Country	Resistance patterns detected[a]	No. (%) of *Salmonella* isolates[b]
Trinidad and Tobago	S-E	4 (9.3)
	S-E-CN	4 (9.3)
	S-AMP-E	3 (7.0)
	S-E-K-CN	3 (7.0)
	S-AMP-E-CN	3 (7.0)
	S-AMP-E-K-CN	3 (7.0)
	SXT-S-AMP-E-K-CN	3 (7.0)
	SXT-S-E	2 (4.7)
	TE-S-E-CN	2 (4.7)
	TE-S-AMP-E	2 (4.7)
	S-AMP-E-K	2 (4.7)
	Others[c]	12 (27.9)
Grenada	S-AMP-E-K-CN	4 (44.4)
	S-E-K-CN	2 (22.2)
	Others[d]	3 (33.3)
St. Lucia	TE-S-E	9 (28.1)
	S-E-K-CN	8 (25.0)
	TE-S-E-K	3 (9.4)
	TE-S-E-CN	3 (9.4)
	S-E-CN	2 (6.3)
	TE-S-E-K-CN	2 (6.3)
	Others[e]	5 (15.6)

[a] S, streptomycin; E, erythromycin; CN, gentamycin; AMP, ampicillin; K, kanamycin; SXT, sulphamethoxazole-trimethoprim; TE, tetracycline.

[b] Of a total of 43 isolates tested in Trinidad and Tobago, 9 isolates tested in Grenada, and 32 isolates tested in St. Lucia.

[c] Other patterns (one isolate each) include the following: TE-S-AMP-E-K, AMP-E, AMP-E-K-CN, SXT-S-E-K-CN, SXT-TE-S-AMP-E-K-CN, SXT-S-E-CN, TE-S-AMP-E-K-CN, E-CN, E-K, S-E-K, and SXT-S-E-K.

[d] Other patterns (one isolate each) include: S-AMP-E, TE-S-AMP-E-K-CN, and E.

[e] Other patterns (one isolate each) include: SXT-S-E-CN, S-AMP-E, TE-S-AMP-E-CN, TE-S-AMP-E-K-CN, and TE-S-E-K.

region, Adesiyun et al. *(2)* documented that of the 74 isolates of *Salmonella* tested, 17 (22.9%) exhibited resistance to one or more antimicrobial agents (S, TE, K, nalidixic acid, enrofloxacin, CN, and SXT) and, more importantly, that all were sensitive to SXT (compared with 79.1% of the isolates sensitive to the antimicrobial agent in the current study). There is, therefore, a possibility that the increased use of SXT is contributing to the higher frequency of resistance detected, although it is considerably lower than that detected for other antimicrobial agents tested.

Not unexpectedly, across and within countries a statistically significant frequency of resistance was detected to some of the antimicrobial agents, depending on the type of samples from which the *Salmonella* isolates were recovered. Statistically significant differences were detected in St. Lucia for CN ($P = 0.027$, from farm environment and cloacal swabs of layers). Across the countries and within Trinidad and Tobago, resistance was significantly higher to AMP and SXT ($P < 0.05$) primarily due to *Salmonella* isolates from egg shells.

Other significant findings were that the frequency of resistance to a number of antimicrobial agents was relatively high, with a range of 48.8% (K) to 100.0% (E), and that, across the three countries studied, the frequency of antimicrobial resistance differed significantly for the individual antimicrobial agents (the lowest frequency of 11.9% to SXT; highest resistance to E). These findings suggest differences in the use of antimicrobial agents in the three countries.

The finding that 100.0% of the isolates tested in the current study were resistant to erythromycin agrees with the report of Akter et al. *(5)*, who also reported that all isolates of *Salmonella* tested were resistant to the antibiotic. It is, however, pertinent to mention that erythromycin is not routinely used in clinical settings and animal husbandry to prevent or treat salmonellosis but was used in the current study to characterize the isolates.

The frequency of resistance to streptomycin detected in the current study (92.9%) is considerably higher than the 21.9 to 52.9% reported for poultry farms in other countries *(13, 34)*. Of note also is the finding that the prevalence of resistance to CN found in the present study (59.5%) is much higher than that reported by others for isolates recovered from poultry farms (0.0 to 17%) *(12, 25, 33)*.

For kanamycin, 48.8% of the isolates were resistant to the antimicrobial agent, which is comparable to the 50.0% prevalence of resistance reported for *Salmonella* isolates from layer farms in Bangladesh *(18)*.

Of the *Salmonella* isolates from all sources in the three countries studied, 36.9% were resistant to AMP, which is very high compared with the prevalence of <3 and 3% reported by others *(28, 34)*.

In the current study, 33.3% of the isolates of *Salmonella* were resistant to TE, a finding considerably lower than the prevalence reported for isolates from similar sources in other countries (93 to 100.0%) *(14, 18, 22)*.

Frequency of resistance patterns of *Salmonella* isolates to antimicrobial agents by country of origin. Table 3 shows the frequency of resistance patterns displayed, by country of origin. Overall, for the 84 *Salmonella* isolates tested from the three countries, 83 (98.8%) exhibited multidrug resistance. For the 43 *Salmonella* isolates tested from Trinidad and Tobago, 23 multiresistance patterns were determined, compared with 5 patterns for 9 isolates from Grenada and 11 patterns detected among 32 isolates from St. Lucia. The predominant patterns detected were S-E and S-E-CN (9.3% each), S-AMP-E-K-CN (44.4%), and TE-S-E (28.1%) for isolates from Trinidad and Tobago, Grenada, and St. Lucia, respectively. The differences were, however, not statistically significant ($P = 0.23$).

The occurrence of a high frequency of multidrug resistance (98.8%) detected, in addition to the detection of a total of 39 multiresistance patterns in the current study, is further evidence of a potential for therapeutic failure that may be associated with the use of the seven antimicrobial agents across the three countries. Our findings are in agreement with the report on *Salmonella* isolates from poultry houses in Pakistan *(25)*; from country to country,

TABLE 4. *Occurrence of resistance among* Salmonella *isolates by size of farm*[a]

Country	Size of layer farm[b]	No. of *Salmonella* isolates tested	No. (%) resistant to[c]:											
			E	S	*P* value	CN	*P* value	K	*P* value	AMP	*P* value	TE	*P* value	SXT	*P* value
Trinidad and Tobago	Large	36	36 (100.0)	32 (88.9)	0.811	23 (63.9)	0.083	14 (38.9)	0.023	18 (50.0)	0.729	7 (19.4)	0.202	8 (22.2)	0.637
	Small	7	7 (100.0)	6 (85.7)		2 (28.6)		6 (85.7)		3 (42.9)		0 (0.0)		1 (14.3)	
Grenada	Medium	3	3 (100.0)	3 (100.0)	0.453	3 (100.0)	0.257	3 (100.0)	0.257	2 (66.7)	1.000	0 (0.0)	0.453	0 (0.0)	NA
	Small	6	6 (100.0)	5 (83.3)		4 (66.7)		4 (66.7)		4 (66.7)		1 (16.7)		0 (0.0)	
St. Lucia	Large	27	27 (100.0)	27 (100.0)	NA	17 (63.0)	0.075	12 (44.4)	0.854	3 (11.1)	0.581	16 (59.3)	0.379	1 (3.7)	0.662
	Small	5	5 (100.0)	5 (100.0)		1 (20.0)		2 (40.0)		1 (20.0)		4 (80.0)		0 (0.0)	
Total	Large	63	63 (100.0)	59 (93.7)	0.698	40 (63.5)	0.06	26 (41.3)	0.032	21 (33.3)	0.382	22 (34.9)	0.361	9 (14.3)	0.487
	Medium	3	3 (100.0)	3 (100.0)		3 (100.0)		3 (100.0)		2 (66.7)		0 (0.0)		0 (0.0)	
	Small	18	18 (100.0)	16 (88.9)		7 (38.9)		12 (66.7)		8 (44.4)		5 (27.8)		1 (5.6)	

[a] All isolates were sensitive to chloramphenicol (30 μg).
[b] Isolates of *Salmonella* were recovered from large and small farms in Trinidad and Tobago, medium and small farms in Grenada, and large and small farms in St. Lucia.
[c] E, erythromycin (5 μg); S, streptomycin (10 μg); CN, gentamycin (10 μg); K, kanamycin (30 μg); AMP, ampicillin (10 μg); TE, tetracycline (15 μg); SXT, sulphamethoxazole-trimethoprim (27.75/1.25 μg); NA, not available.

multiresistance patterns are known to vary and to reflect the use of antimicrobial agents in the respective poultry industries *(15, 19, 24, 25)*.

Occurrence of resistance among *Salmonella* isolates by size of farm. For the three countries combined, differences in the frequency of resistance for the three sizes of farm were detected to be statistically significantly different ($P = 0.032$) for only kanamycin (41.3, 100.0, and 66.7% for large, medium, and small farms, respectively; Table 4).

Within countries, only in Trinidad and Tobago were statistically significant ($P = 0.023$) differences detected; for kanamycin, resistance was higher for small farms (85.7%) compared with large farms (38.9%). Among countries, regardless of the source of sample, significant differences in the frequency of resistance were detected only for AMP, TE, and SXT. For AMP, the frequency of resistance differed significantly ($P = 0.001$) among countries, 21 (48.8%) of 43, 6 (66.7%) of 9, and 4 (12.5%) of 32 in Trinidad and Tobago, Grenada, and St. Lucia, respectively. For TE, the frequency of resistance was statistically significantly different ($P = 0.000$) across countries, 7 (16.3%) of 43, 1 (11.1%) of 9, and 20 (62.5%) of 32 in Trinidad and Tobago, Grenada, and St. Lucia, respectively. Finally, for SXT, the frequency of resistance was 9 (20.9%) of 43 in Trinidad and Tobago, 0 (0.0%) of 9 in Grenada, and 1 (3.1%) of 32 in St. Lucia; the differences were statistically significant ($P = 0.032$).

In the current study, size of farm was found to have a significant effect only on the frequency of resistance to kanamycin across the three countries and within Trinidad and Tobago; this may reflect differences in management systems and the use of antimicrobial agents, as reported elsewhere *(4, 32)*.

Frequency of resistance of *Salmonella* isolates to antimicrobial agents by serotype. The frequency of resistance to antimicrobial agents among the serotypes of *Salmonella* is shown in Table 5. Of the 14 *Salmonella* serovars tested against the eight antimicrobial agents, the frequency of resistance was statistically significant ($P < 0.05$) only to kanamycin, AMP, and SXT. Among the predominant serovars recovered from the three countries, *Salmonella* Anatum, *Salmonella* group C, and *Salmonella* Kentucky, the frequency of resistance to antimicrobial agents was statistically significantly different ($P < 0.05$) within each country. In Trinidad and Tobago, of the 11 isolates of *Salmonella* Anatum tested, the range of frequency of resistance was from 9.1% (to SXT) to 100.0% (to E); whereas, in Grenada, the lowest frequency of resistance found amongst 8 isolates of *Salmonella* group C was 0.0% (to SXT) and the highest was 100.0% (to E and S). For St. Lucia, among a total of 27 isolates of *Salmonella* Kentucky tested, the frequency of resistance ranged from 3.7% (to SXT) to 100.0% (to both E and S). The significant effect of the serotypes of *Salmonella* isolates on the frequency of resistance to kanamycin, AMP, and SXT is in agreement with published reports *(22, 35)*.

TABLE 5. *Frequency of resistance among* Salmonella *isolates by serovar*[a]

Serotype	No. of *Salmonella* isolates tested	No. (%) of resistant isolates[b]	No. (%) resistant to[c,d]: E	S	CN	K	AMP	TE	SXT
Salmonella group D[e]	1	1 (100.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Salmonella group C[e]	8	8 (100.0)	8 (100.0)	8 (100.0)	7 (87.5)	7 (87.5)	6 (75.0)	1 (12.5)	0 (0.0)
Salmonella Kentucky *(8)*,20:i:z_6	27	27 (100.0)	27 (100.0)	27 (100.0)	15 (55.6)	12 (44.4)	4 (14.8)	17 (63.0)	1 (3.7)
Salmonella Uganda 3,10:l,z_{13}:1,5	10	10 (100.0)	10 (100.0)	8 (80.0)	7 (70.0)	2 (20.0)	4 (40.0)	1 (10.0)	0 (0.0)
Salmonella Anatum 3,10:e,h:1,6	11	11 (100.0)	11 (100.0)	10 (90.9)	7 (63.6)	4 (36.4)	5 (45.5)	2 (18.2)	1 (9.1)
Salmonella Montevideo 6,7:g,m,s:	2	2 (100.0)	2 (100.0)	2 (100.0)	0 (0.0)	1 (50.0)	1 (50.0)	0 (0.0)	1 (50.0)
Salmonella Mbandaka 6,7:z_{10}:e,n,z_{15}	4	4 (100.0)	4 (100.0)	4 (100.0)	4 (100.0)	3 (75.0)	3 (75.0)	1 (25.0)	3 (75.0)
Salmonella Caracas 6,14:(25):g,m,s	6	6 (100.0)	6 (100.0)	5 (83.3)	2 (33.3)	5 (83.3)	3 (50.0)	0 (0.0)	0 (0.0)
Salmonella Muenster 3,10:e,h:1,5	1	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Salmonella Enteritidis 1,9,12:g,m:	1	1 (100.0)	1 (100.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)
Polyvalent A-negative	4	4 (100.0)	4 (100.0)	4 (100.0)	3 (75.0)	4 (100.0)	2 (50.0)	1 (25.0)	3 (75.0)
Salmonella groups I-O[e]	2	2 (100.0)	2 (100.0)	2 (100.0)	1 (50.0)	0 (0.0)	1 (50.0)	1 (50.0)	0 (0.0)
Salmonella group E[e]	2	2 (100.0)	2 (100.0)	1 (50.0)	0 (0.0)	0 (0.0)	2 (100.0)	1 (50.0)	0 (0.0)
Salmonella group B[e]	5	5 (100.0)	5 (100.0)	5 (100.0)	3 (60.0)	2 (40.0)	0 (0.0)	3 (60.0)	0 (0.0)
Total	84	84 (100.0)	84 (100.0)	78 (92.9)	50 (59.5)	41 (48.8)	31 (36.9)	28 (33.3)	10 (11.9)

[a] All isolates were sensitive to chloramphenicol (30 μg).
[b] Resistant to one or more antimicrobial agents.
[c] E, erythromycin (5 μg); S, streptomycin (10 μg); CN, gentamycin (10 μg); K, kanamycin (30 μg); AMP, ampicillin (10 μg); TE, tetracycline (15 μg); SXT, sulphamethoxazole-trimethoprim (27.75/1.25 μg).
[d] *P* values: S, 0.058; CN, 0.129; K, 0.009; AMP, 0.021; TE, 0.246; SXT, 0.000.
[e] Classified at the level of serogroups because the regional typing center did not have the antisera for all the serotypes in the serogroups.

In conclusion, based on the relatively high frequency of resistance to most of the antimicrobial agents tested in the current study, coupled with the equally high occurrence of multidrug resistance among isolates of *Salmonella* recovered from layer birds, farm environments, and feeds, there is a risk of therapeutic failures, which could result in economic losses. There is, therefore, a need for a more judicious use of antimicrobial agents in the poultry industry in the three countries studied.

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