Molecular analysis of *Salmonella enteritidis* isolates from the Caribbean by pulsed-field gel electrophoresis

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ABSTRACT *Using pulsed-field gel electrophoresis (PFGE), between 1987 and 1996 we analyzed* Salmonella enteritidis *isolates from gastroenteritis cases in four Caribbean countries: Barbados, Saint Kitts and Nevis, Saint Lucia, and Trinidad and Tobago. We also determined the resistance of the isolates to 12 antimicrobial agents. Of the 129 isolates of* S. enteritidis *available for testing, DNA digested by* XbaI *revealed 13 distinctive PFGE patterns. The most prevalent* XbaI *PFGE patterns were group 1 (88 of 129 isolates, 68.2%) and group 2 (26 of 129, 20.2%). The patterns found among* S. enteritidis *isolates correlated with the geographical origin of the isolates. Of the 28 isolates from Barbados, 20 of them (71.4%) belonged to* XbaI *PFGE group 2, and of the 93 isolates from Trinidad and Tobago, 78 of them (83.9%) belonged to group 1.* SpeI *digestion of* S. enteritidis *genome was not as discriminatory as* XbaI.

Overall, of the 129 isolates, 67 of them (51.9%) exhibited resistance to one or more of the 12 antimicrobial agents that we tested. The prevalence of resistance was 53.8% for the S. enteritidis *isolates tested from Trinidad and Tobago, 50.0% for those from Barbados, 28.6% for those from Saint Lucia, and 100.0% for one isolate from the island of Saint Kitts. Resistance was highest to triple sulfur (59 of 129 isolates, 45.7%), followed by furadantoin (10 of 129, 7.8%), ampicillin (7 of 129, 5.4%), and carbamycin (5 of 129, 3.9%).*

Salmonella enteritidis has become an important cause of food poisoning throughout the world (1–3). For a long time in the Caribbean region *S. enteritidis* was rarely associated with human infections (4). In the early 1990s, however, a number of outbreaks and sporadic cases implicating the microorganism were reported (5). There was a subsequent phenomenal increase in the involvement of *S. enteritidis* in reported cases of food poisoning (6).

Various methods have been used for salmonella epidemiological testing, including antibiotic testing, serotyping, and phage typing (7–9). Recently, various molecular techniques have been applied to characterize *S. enteritidis* and to conduct epidemiological investigations. Among these techniques are plasmid profile analysis (10), DNA restriction fragment polymorphisms (11), chromosomal probe fingerprinting (12), and pulsed-field gel electrophoresis (PFGE) (13, 14).

To date, there have been no reports on the characteristics of *S. enteritidis* isolated from the Caribbean, nor have there been reports on the relatedness of isolates implicated in foodborne gastroenteritis.

This study utilized PFGE to investigate the relationship between *S. enteritidis* isolates recovered from gastroenteritis cases in various Caribbean countries and to determine the antibiograms of the isolates.

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MATERIALS AND METHODS

Sources of *S. enteritidis* isolates

The Caribbean Epidemiology Center (CAREC), Port of Spain, Trinidad, is the regional reference laboratory for *Salmonella,* and it served as the source of the *S. enteritidis* isolates we studied. All *Salmonella* clinical isolates from CAREC's 21 Member Countries (mainly English-speaking) in the Caribbean sub-region are sent to CAREC for serological typing. Table 1 shows the number of *S. enteritidis* isolates from gastroenteritis cases (sporadic or outbreak) that we obtained from CAREC and studied. All viable isolates of *S. enteritidis* in the collection of CAREC were selected for study.

Overall, we tested a total of 129 *S. enteritidis* isolates, with 122 originating from human clinical cases and 7 (from Trinidad and Tobago) from animals. It was not possible to determine which of the isolates originated from outbreaks of gastroenteritis since in the Caribbean region there is no routine investigation or reporting of foodborne outbreaks.

Preparation of bacterial DNA, restriction digests, and running of pulsed-field gel electrophoresis

For our testing all the *S. enteritidis* strains were plated for isolation on blood agar. Colonies of pure cultures were then inoculated into 5 mL of brain heart infusion broth and incubated overnight at 37 °C to attain log phase growth. An aliquot (1.3 mL) of cell suspension of each isolate was centrifuged at 12 000 × g for 90 seconds. To prepare bacterial DNA we used a procedure described earlier (15).

Agarose plugs were cut into slices to fit gel wells and equilibrated for 15 minutes at room temperature in tubes containing 200 μL of restriction enzyme buffer (Stratagene, La Jolla, California, United States of America). The buffer was then decanted and replaced with 200 μL of fresh restriction enzyme buffer for a further 15 minutes of equilibration at room temperature. Restriction digests were done as described earlier (15).

A 1.0% SeaKem gel (FMC BioProducts, Rockland, Maine, United States) was prepared in 0.5X TBE buffer 60–90 min prior to completion of the restriction enzyme digestion of bacterial DNA and allowed to solidify at room temperature. DNA digested with either *Xba*I or *Spe*I in gel slices was placed in the wells of SeaKem gel and sealed in place with molten 1% agarose in 0.5X TBE buffer. To wells 1 and 15 of each gel was added standard molecular size markers (lambda ladder). For each run, the sealing agar was allowed to solidify at room temperature and the gel was thereafter transferred into a CHEF-DR III electrophoresis chamber (Bio-Rad Laboratories, Hercules, California, United States) and submerged in chilled 0.5X TBE. Electrophoresis was performed at 200 V for 20 h with the pulse time ramped from 2 seconds to 40 seconds and the buffer temperature maintained at 14 °C. The gel was subsequently stained in ethidium bromide (0.5 μg/mL) solution for 30 min at room temperature and then destained in distilled water for approximately 1 h prior to photography under ultraviolet light.

Determination of PFGE patterns

Polaroid photographs of PFGE patterns were recorded by a desktop scanner (Hewlett-Packard, Boise, Idaho, United States) and subsequently subjected to a computerized artificial neural network analysis described earlier (15). Grouping was based on location and number of bands in the range of 50 kb to 550 kb.

Comparison of *Xba*I and *Spe*I PFGE patterns

Following the identification of the PFGE distinct groups detected by *Xba*I digestion, a representative of each of the 13 groups subjected to *Xba*I digestion was digested with *Spe*I restriction enzyme using fresh plugs, and the pattern was noted.

TABLE 1. Isolates of *S. enteritidis* submitted and studied, by source country and year of isolation, four Caribbean countries, 1987–1996

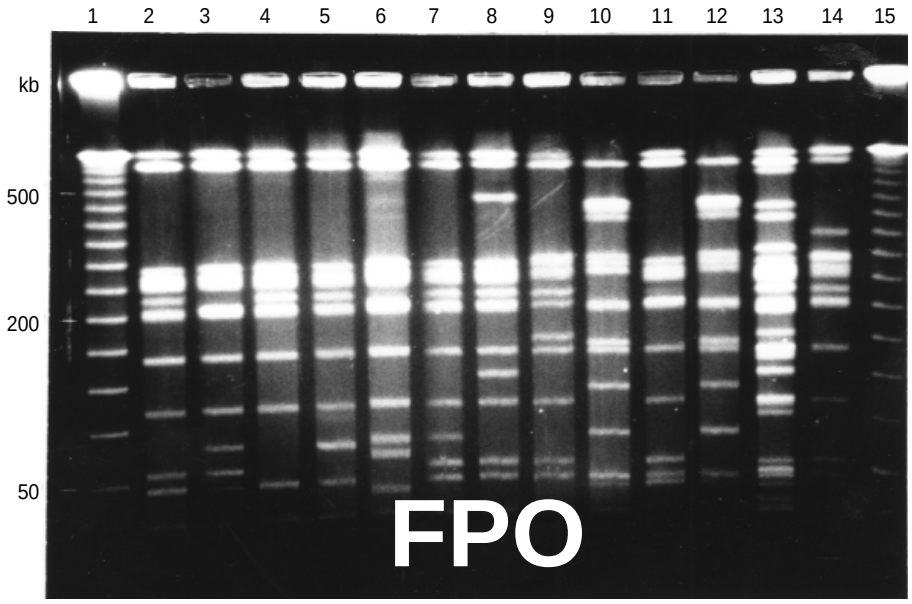
	Trinidad and Tobago			Barbados			Saint Lucia			Saint Kitts and Nevis			All sources		
	Submitted	Studied		Submitted	Studied		Submitted	Studied		Submitted	Studied		Submitted	Studied	
Year	No.	No.	%	No.	No.	%	No.	No.	%	No.	No.	%	No.	No.	%
1987	0	0	0.0	2	1	50.0	0	0	0.0	0	0	0.0	2	0	0.0
1989	0	0	0.0	5	1	20.0	6	1	16.7	0	0	0.0	11	2	18.2
1990	1	0	0.0	10	3	30.0	3	2	66.7	0	0	0.0	14	5	35.7
1991	1	1	100.0	20	3	15.0	0	0	0.0	0	0	0.0	21	4	19.0
1992	1	1	100.0	19	5	26.3	0	0	0.0	0	0	0.0	20	6	30.0
1993	0	0	0.0	22	4	18.2	2	0	0.0	1	1	100.0	25	5	20.0
1994	12	8	66.7	18	5	27.8	4	2	50.0	0	0	0.0	34	15	44.1
1995	50	49	98.0	13	5	38.5	2	2	100.0	0	0	0.0	65	56	86.2
1996	70	34	48.6	4	1	25.0	0	0	0.0	0	0	0.0	74	35	47.3
Total	135	93[a]	68.9	113	28	24.8	17	7	41.2	1	1	100.0	266	129	48.5

[a] Seven of the 93 isolates from Trinidad and Tobago that were studied came from animal sources; the other 86 were all from human gastroenteritis cases.

Determination of antibiograms of isolates

To determine the antibiograms of the *S. enteritidis* isolates we used the agar diffusion method of the National Committee for Clinical Laboratory Standards (16). Twelve antimicrobial agents on disks (Difco Laboratories, Inc., Detroit, Michigan, U.S.A.) were used, and their concentrations were: ampicillin (30 μg), carbamycin (100 μg), chloramphenicol (30 μg), clindamycin (30 μg), furadantoin (300 μg), gentamycin (10 units), kanamycin (30 μg), nalidixic acid (30 μg), streptomycin (10 μg), sulphamethoxazole/trimethoprim (25 μg), triple sulfur (300 μg), and tetracycline (30 μg). We measured the zone sizes and used the criteria provided by the disc manufacturer to determine the resistance or sensitivity of the isolates.

FIGURE 1. Pulsed-field gel electrophoresis patterns of *S. enteritidis* isolates generated by enzyme *Xba*I. Lanes 1 and 15 contained molecular size markers (lambda ladder). Lanes 2–14 show distinct cleavage patterns observed among the 129 *S. enteritidis* isolates tested



RESULTS

Figure 1 shows the fragment patterns of *S. enteritidis* genome digested with *Xba*I; there were 13 distinctive cleavage patterns among the 129 isolates of *S. enteritidis* that we tested.

Digestion with restriction enzyme *Spe*I of the same DNA preparations used for the *Xba*I digestion (shown in Figure 1) is displayed in Figure 2. Only eight distinct fragment patterns were detected (wells 2; 4; 7; 8; 3 and 11; 5, 6, 9, and 10; 12 and 13; and 14).

The sources of the *S. enteritidis* isolates from 1987 through 1996 are shown by country in Table 1; isolates for 1988 were unavailable from CAREC for study. Over the first part of this period, from 1987 through 1993, the four countries submitted a total of 93 *S. enteritidis* isolates: 3 (3.2%) from Trinidad and Tobago, 78 (83.9%) from Barbados, 11 (11.8%) from Saint Lucia, and 1 (1.2%) from the island of Saint Kitts.

The pattern was different in the subsequent period, of 1994 through 1996, with Trinidad and Tobago replacing Barbados as the country from which the largest number of *S. enteritidis* isolates were reported. Of a total of 173 isolates submitted to CAREC over those 3 years, 132 of them (76.3%) were from Trinidad and Tobago, 35 (20.2%) from Barbados, 6 (3.5%) from Saint Lucia, and 0 (0.0%) from Saint Kitts and Nevis.

From 1987 through 1996 we tested a total of 129 isolates of *S. enteritidis*. Of that total, 88 of them (68.2%) belonged to *Xba*I PFGE group 1, and 26 (20.2%) belonged to *Xba*I PFGE group 2 (Table 2). Only 4 (3.1%) belonged to group 3,

FIGURE 2. Pulsed-field gel electrophoresis separation of restriction fragments of *S. enteritidis* genome digested with *Spe*I. Lanes 1 and 15 contained lambda ladder molecular size marker. Lanes 2–14 contained genomes representative of the 13 *Xba*I patterns

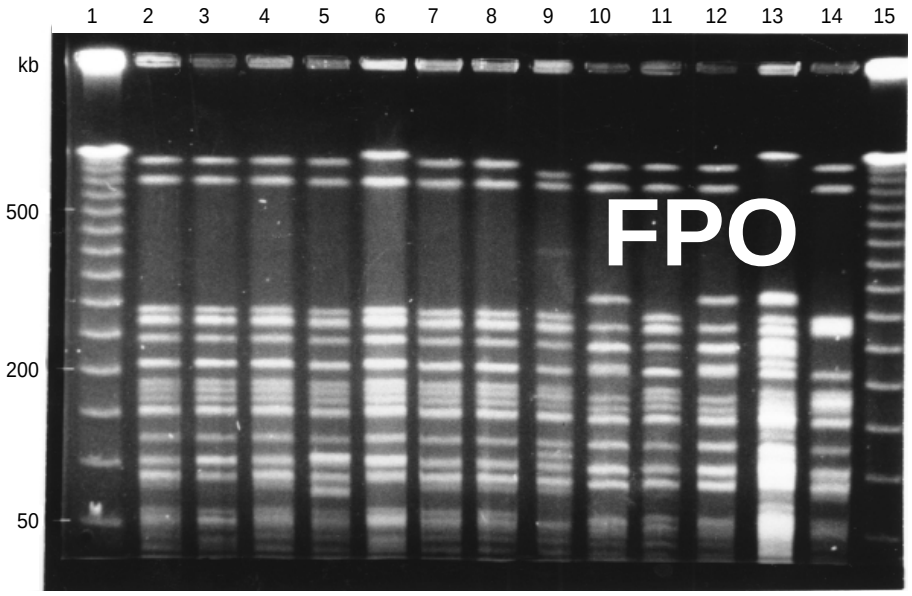


TABLE 2. *Xba*I pulsed-field gel electrophoresis groups of *S. enteritidis* isolates, by year, four Caribbean countries, 1987–1996

Year	No. of isolates tested	Isolates of *S. enteritidis* in PFGE group																							
		1		2		3		4		5		6		7		8		9		10		11		12		13	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1987	1	0	0.0	1	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
1989	2	0	0.0	2	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
1990	5	0	0.0	4	80.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	20.0	0	0.0	0	0.0
1991	4	0	0.0	2	50.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	25.0	1	25.0	0	0.0	0	0.0	0	0.0
1992	6	0	0.0	6	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
1993	5	1	20.0	4	80.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
1994	15	10	66.7	4	26.7	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	6.7	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
1995	56	46	82.1	1	1.18	3	5.4	2	3.6	1	1.8	1	1.8	1	1.8	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	1.8
1996	35	31	88.6	2	5.7	1	2.9	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	2.9	0	0.0
Total	129	88	68.2	26	20.2	4	3.1	2	1.6	1	0.8	1	0.8	1	0.8	1	0.8	1	0.8	1	0.8	1	0.8	1	0.8	1	0.8

2 (1.6%) belonged to group 4, and the other nine groups had 1 isolate each.

In the 1987–1993 period, of the 23 isolates tested, only 1 (4.3%) was classified in group 1, and 19 (82.6%) belonged to group 2. However, for the 1994–1996 period, of a total of 106 isolates tested, 87 of them (82.1%) were in group 1, and only 7 (6.6%) belonged to group 2.

The distribution of *Xba*I PFGE groups among the *S. enteritidis* isolates tested from the four countries is shown in Table 3. In Trinidad and Tobago, group 1 was the most prevalent (78 of 93 isolates tested, or 83.9%). In Barbados, group 2 was the most common (20 of 28, or 71.4%). Saint Lucian isolates of *S. enteritidis* had the same frequency of occurrence, 42.9%, for both group 1 and group 2.

In Trinidad and Tobago, regardless of the year and geographical location of source of *S. enteritidis* isolates within the country, PFGE group 1 isolates were the most prevalent. Five (71.4%) of the 7 *S. enteritidis* of animal origin also belonged to PFGE group 1. In 1994 7 of the 8 (87.5%) isolates that were tested belonged to group 1, in 1995 40 of 49 (81.6%) did so, and in 1996 31 of 34 (91.2%) did so.

Of the 129 isolates of *S. enteritidis* tested, 67 of them (51.9%) exhibited resistance to one or more antimicrobial agents (Table 4). Overall, resistance was highest to triple sulfur (59 of 129 isolates, or 45.7%), a pattern that was true for all four of the countries. The resistance levels for the other antimicrobial agents tested were all noticeably lower, 7.8% or less. The resistance to the various antimicrobial agents was similar in all four of the countries. All of the isolates tested were sensitive to nalidixic acid, chloramphenicol, and sulphamethoxazole/trimethoprim.

A total of 12 resistant patterns were observed, with resistance to triple sulfur alone most frequent (46 isolates). A total of 59 (45.7%) of the 129 isolates, however, were resistant to either triple sulfur alone or triple sulfur in combination with other antimicrobial agents.

DISCUSSION

Of the four countries from which we studied *S. enteritidis* strains, only one, Trinidad and Tobago, recorded a significant increase in the absolute number of *S. enteritidis* isolates from 1994 to 1996. Barbados, on the other hand, had relatively high numbers of *S. enteritidis* from as early as 1990. The situation in Barbados might be explained in part by the country's heavy dependence on tourism. Worldwide, there has been a surge in the involvement of *S. enteritidis* in

TABLE 3. *Xba*I pulsed-field gel electrophoresis groups of *S. enteritidis* isolates, by country of origin, four Caribbean countries, 1987–1996

Country	No. of isolates tested	Isolates of *S. enteritidis* belonging to group																							
		1		2		3		4		5		6		7		8		9		10		11		12		13	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Trinidad and Tobago	93	78	83.9	2	2.2	4	4.3	2	2.2	1	1.1	1	1.1	1	1.1	1	1.1	1	1.1	0	0.0	0	0.0	1	1.1	1	1.1
Barbados	28	7	25.0	20	71.4	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	3.4	0	0.0	0	0.0	0	0.0
Saint Lucia	7	3	42.9	3	42.9	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	14.3	0	0.0	0	0.0
Saint Kitts and Nevis	1	0	0.0	1	100.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Total	129	88	68.2	26	20.2	4	3.1	2	1.6	1	0.8	1	0.8	1	0.8	1	0.8	1	0.8	1	0.8	1	0.8	1	0.8	1	0.8

TABLE 4. Antibiograms of *S. enteritidis* isolates from various sources, using 12 antimicrobial agents, four Caribbean countries, 1987–1996[a]

Country	No. of isolates tested	Isolates resistant to antimicrobials[c,d]		Resistance to specific antimicrobials[b]															
				SSS		FD		AMP		CB		S		CL		GN		K		TE	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Trinidad and Tobago	93	50	53.8	45	48.4	7	7.5	5	5.4	2	2.2	2	2.2	1	1.1	2	2.2	1	1.1	1	1.1
Barbados	28	14	50.0	12	42.9	2	7.1	1	3.6	2	7.1	2	7.1	0	0.0	0	0.0	0	0.0	0	0.0
Saint Lucia	7	2	28.6	1	14.3	1	14.3	1	14.3	0	0.0	0	0.0	1	14.3	0	0.0	0	0.0	0	0.0
Saint Kitts and Nevis	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	0	0.0	0	0.0
Total	129	67	51.9	59	45.7	10	7.8	7	5.4	5	3.9	4	3.1	2	1.6	2	1.6	1	0.8	1	0.8

[a] All 129 isolates of *S. enteritidis* were sensitive to chloramphenicol, nalidixic acid, and sulphamethoxazole/trimethoprim.
[b] SSS = triple sulfur; FD = furadantoin; AMP = ampicillin; CB = carbamycin; S = streptomycin; CL = clindamycin; GN = gentamycin; K = kanamycin; TE = tetracycline.
[c] Number of isolates resistant to one or more of the antimicrobial agents.
[d] A total of 12 resistant patterns were observed, namely, SSS (46 isolates), SSS-FD (7 isolates), AMP (2 isolates), FD (2 isolates), CL-AMP-CB (2 isolates), S-GN-SSS (2 isolates), SSS-AMP (1 isolate), K-SSS (1 isolate), SSS-TE (1 isolate), AMP-CB (1 isolate), S-FD-CB (1 isolate), and S-SSS-AMP-CB (1 isolate). Where only one antimicrobial agent is indicated, it means that resistance was exhibited to that agent only.

human gastroenteritis (2, 17, 18). It is possible that visitors to Barbados brought in the infections from North America or Europe, two of the areas where *S. enteritidis* has been increasingly involved in human gastroenteritis.

It was of epidemiological significance to find that the strains of *S. enteritidis* displayed a distinct geographical distribution. The *Xba*I PFGE group 1 strains were predominantly found in Trinidad and Tobago, while group 2 strains were much more prevalent in Barbados. Although there was a slight overlap of *Xba*I PFGE groups 1 and 2 between the two countries, it was evident that the strains of *S. enteritidis* responsible for gastroenteritis in Trinidad and Tobago and in Barbados differ significantly. The small number of isolates of *S. enteritidis* available for study from Saint Lucia and from Saint Kitts and Nevis made it difficult to draw any inferences on the PFGE patterns of isolates from these countries.

In Trinidad and Tobago, regardless of the source of strains (human versus animals, and geographical location) of *S. enteritidis* and the year of isolation, *Xba*I PFGE group 1 strains predominated, emphasizing the importance of this strain in gastroenteritis in the country. However, it was difficult to associate the various *Xba*I groups with outbreaks of gastroenteritis caused by *S. enteritidis*. That is because outbreaks of foodborne disease are rarely reported or investigated in the Caribbean. Therefore, a high percentage of the *S. enteritidis* isolates sent to CAREC for serotyping may have originated from either sporadic cases or outbreaks. PFGE has been employed in various studies for epidemiological investigations in sporadic and large outbreaks of salmonellosis caused by *S. enteritidis* (3, 14, 19).

If was of zoonotic relevance that in Trinidad and Tobago some of the strains of *S. enteritidis* of animal origin belonged to the same PFGE group as those isolated from human gastroenteritis. *S. enteritidis* infections in humans have been reported to originate frequently from animals, particularly poultry (20–22).

Our finding that *Xba*I restriction enzyme was superior to *Spe*I restriction enzyme in discriminating the strains of *S. enteritidis* was hardly a surprise; other researchers have reported similar findings (14, 19).

Regardless of the country of origin or the year of isolation of *S. enteritidis*, we found that the prevalence of resistance to most of the antimicrobial agents that we tested was low. Resistance to triple sulfur was comparatively high (46%), a finding in agreement with other reports (23, 24). Resistances to furadantoin (7.8%), ampicillin (5.4%), and carbamycin (3.9%) were also similar to what others have reported (21). Outside the Caribbean, however, a considerably higher prevalence of resistance to ampicillin has been reported. In Greece, for example, 30% of *S. enteritidis* isolates were resistant (18). While the antibiograms of *S. enteritidis* strains can be used to determine the relatedness of strains (18), they were not helpful in epidemiological association in this study.

From our research we can conclude that *S. enteritidis* has attained some clinical significance in gastroenteritis in the Caribbean, with a particularly dramatic change in Trinidad and Tobago. In addition, the PFGE we performed clearly demonstrated that in Barbados and in Trinidad and Tobago there are distinctly different strains of *S. enteritidis* involved in gastroenteritis.

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RESUMEN

Análisis molecular de *Salmonella enteritidis* aisladas en el Caribe mediante electroforesis en gel con pulsos eléctricos

Mediante electroforesis en gel con pulsos eléctricos (EGPE), se analizaron las *Salmonella enteritidis* aisladas entre 1987 y 1996 en casos de gastroenteritis de cuatro países caribeños: Barbados, Saint Kitts y Nevis, Santa Lucía y Trinidad y Tabago. También se determinó la resistencia de los aislados a 12 antibióticos. La digestión del ADN con la endonucleasa de restricción *Xba*I reveló 13 patrones distintos de EGPE entre los 129 aislados de *S. enteritidis* analizados; los más prevalentes fueron el grupo 1 (88 de 129; 68,2%) y el grupo 2 (26 de 129; 20,2%). Estos patrones se correlacionaron con el origen geográfico de los aislados. Así, de los 28 aislados de Barbados, 20 (71,4%) pertenecían al grupo 2, y de los 93 aislados de Trinidad y Tabago, 78 (83,9%) pertenecían al grupo 1. La digestión del genoma de *S. enteritidis* con la endonucleasa de restricción *Spe*I no fue tan discriminativa como la digestión con *Xba*I. En general, 67 de los 129 aislados (51,9%) mostraron resistencia a uno o más de los 12 antibióticos probados. La prevalencia de resistencia fue de 51% en los aislados de Trinidad y Tabago, de 50% en los de Barbados, de 28,6% en los de Santa Lucía y de 100% en el único aislado de la isla de Saint Kitts. La mayor resistencia correspondió a la triple sulfamida (sulfamerazina, sulfadiazina y sulfametazina: 59 de 129; 45,7%), seguida de la nitrofurantoína (10 de 129; 7,8%), la ampicilina (7 de 129; 5,4%) y la carbamicina (5 de 129; 3,9%).