APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 1991, p. 223–227
0099-2240/91/010223-05$02.00/0

Vol. 57, No. 1

Epidemiology of *Salmonella sofia* in Australia

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Received 20 August 1990/Accepted 31 October 1990

In recent years, the incidence of isolation of *Salmonella sofia* in Australia has risen from 33% of all poultry isolates in 1982 to a peak of 49% of isolates in 1988. A parallel rise has not been seen in *S. sofia* isolated from humans. In Israel, however, *S. sofia* has been commonly isolated from both humans and poultry. We investigated the possibility that the Israeli strains may belong to a different clonal group and express virulence determinants not seen in the Australian isolates, accounting for the apparent differences in the virulence seen within this species. A number of *S. sofia* isolates from Australian chickens and humans, as well as from Israeli humans and chickens, were compared by using sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of outer membrane proteins, plasmid profiles, and restriction fragment length polymorphism analysis. No reproducible differences could be detected by analysis of outer membrane proteins. A small 6.4-kb plasmid, pIMVS2, was detected in all Australian isolates from chickens but not in the Israeli isolates. Restriction fragment length polymorphism studies with cosmid clones as probes provided the most discrimination among isolates, allowing us to divide them into seven groups. This technique revealed that significant differences exist between Australian and Israeli isolates and provided additional insights into the epidemiology of these *Salmonella* isolates.

In common with other subgenus 2 serotypes and in comparison with subgenus 1 serotypes, *Salmonella sofia* may be regarded as having relatively low virulence for humans. Recently there has been a change in the distribution of *S. sofia* in Australia (2). There were no reports of the isolation of *S. sofia* in Australia until 1970 when one isolate from an unidentified source was referred to the Australian Salmonella Reference Laboratory for typing. Over the next 3 years, only two further isolates were referred for typing. These were isolated from a kangaroo in 1972 and from an undisclosed source in 1973.

Strains were next referred in 1977, when 15 isolates from humans and 1 from sewage were typed. Fourteen of the human isolates came from Victoria; the remaining human and sewage isolates were from South Australia. This constituted the highest incidence of *S. sofia* in humans in any single year, and we have no information concerning the clinical significance of the isolates.

In 1980, a dramatic change in the distribution of *S. sofia* in the food chain was noted when the first isolation from chickens was recognized (Fig. 1). The incidence of isolation from chickens rose over the next 2 years and then stabilized. In the years 1982 to 1984, *S. sofia* represented approximately 30% of all *Salmonella* isolations from raw chickens in Australia. Isolation from chickens rose to a peak in 1988; S. sofia accounted for 49% of all isolates. There has been no significant spread to other food animals. Of approximately 1,500 *Salmonella* isolates submitted for typing during 1989 from raw meats other than chicken, including meat of bovine, porcine, and ovine origin, none was *S. sofia*. However, *S. sofia* has been isolated from farmed crocodiles and the meat works where they are processed in the Northern Territory of Australia.

Except for the unexplained large number of isolates referred to the Australian Salmonella Reference Laboratory for typing in 1977, the number of human isolates submitted has not increased in parallel with the number of chicken isolates. The high incidence of *S. sofia* in chickens and the very low incidence in humans have not been reported by other western countries and correlate with the known low virulence of subspecies 2 salmonella for humans. In contrast, *S. sofia* is commonly isolated from both humans and chickens in Israel (11).

We wished to determine why there was a higher incidence of isolation of *S. sofia* from the human population in Israel than from humans living in Australia.

This report details experiments designed to determine, by using plasmid profiles, restriction fragment length polymorphism (RFLP) analysis, and outer membrane protein patterns, if the *S. sofia* populations of Israel and Australia could be differentiated and whether the Israeli strains expressed virulence determinants not seen in the Australian strains. Knowledge of such factors may help to explain the apparent difference in epidemiology of this serotype in the two countries.

MATERIALS AND METHODS

Bacterial Strains. Bacterial isolates were received at the Australian Salmonella Reference Laboratory, Institute of Medical and Veterinary Science, Adelaide, South Australia, from clinical and environmental laboratories within Australia. These isolates were subcultured onto nutrient agar slopes and identified by serotyping by using slide agglutination and biochemical reactions. *S. sofia* isolates were also obtained from I. Sechter, National Salmonella Centre, Jerusalem, Israel. Attempts were made to develop a panel of phages for typing *S. sofia* isolates. However, these phages failed to detect differences in the isolates.

Isolates were also tested for resistance to the antibiotics streptomycin, tetracycline, chloramphenicol, gentamicin, ampicillin, cefoxitin, cephalothin, sulfonamide, and trimethoprim by using an agar dilution method. Derivatives of *Escherichia coli* K-12 used for genetic studies were supplied by the Department of Microbiology and Immunology, Uni-

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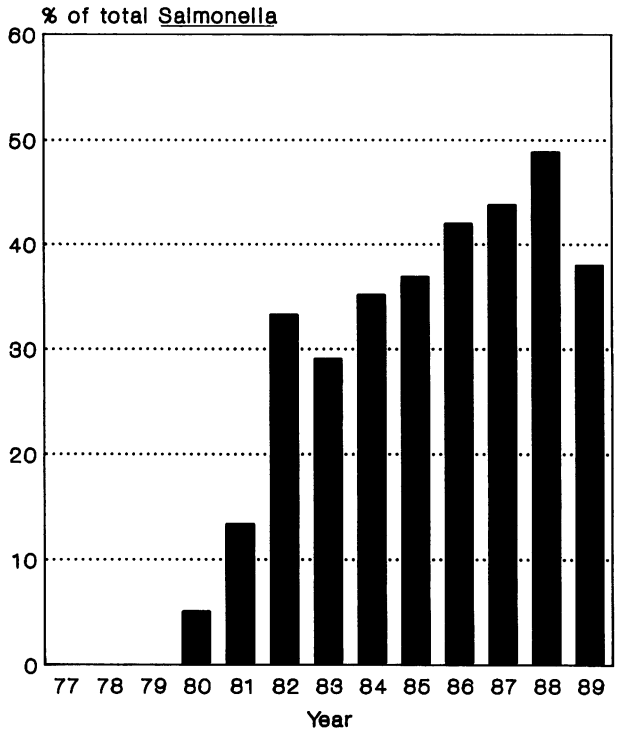

FIG. 1. *S. sofia* isolated from chickens in Australia. The numbers of *S. sofia* isolates are expressed as a percentage of total *Salmonella* spp. from chickens submitted to the Institute of Medical and Veterinary Science, Adelaide, South Australia, for typing.

versity of Adelaide. These were *E. coli* K-12 C600 (F^- *supE tonA1 thr leu*), C600 harboring the F-like R factor R1-19 (Cm^r Km^r Neo^r Ap^r Sm^r Su^r), and DH1 (F^- *gyrA96 recA1 relA1 endA1 thi-1 hsdR17 supE44*).

Plasmid DNA isolation. Bacteria were screened for plasmids by using the method of Birnboim and Doly as modified by Whiley et al. (16). Large-scale plasmid purification was performed by using the three-step alkali lysis method of Garger et al. (4).

Preparation of pili. Crude preparations of pili were prepared by using the procedure of Stirm et al. (13). Cells were grown on solid medium as described by Smyth (12).

Cell envelope and outer membrane proteins. Whole-cell envelopes were prepared by using the small-scale method described by Manning et al. (10). Outer membrane proteins were the Sarkosyl-insoluble fraction of the cell envelope (10).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Electrophoresis in SDS on 12 to 15% polyacrylamide gels was performed by using the modified procedure of Lugtenberg et al. (7) as described by Achtman et al. (1).

Transformation. Transformation of *E. coli* K-12 DH1 by using plasmid DNA was performed as described by Brown et al. (3).

Transposon mutagenesis. Transposon mutagenesis with Tn*3* was used to tag small plasmids in Australian human and chicken isolates. The method used was that detailed previously (16). Plasmid R1-19 harboring the transposon Tn*3* was transferred by filter mating from *E. coli* K-12 C600 (R1-19) to an *S. sofia* strain containing a small plasmid. Only *S. sofia* isolates from Australian humans were used in genetic manipulations. *Salmonella* transconjugants were isolated, and their DNA was extracted and transformed into *E. coli* K-12 DH1. Ampicillin-resistant transformants were selected and purified on nutrient agar containing ampicillin (25 mg/liter). A small Tn*3*-tagged plasmid originally isolated from an Australian human strain (16) was selected for use in these studies.

RFLP analysis. Whole genomic DNA was extracted by using the method of Manning et al. (9), and the DNA was digested to completion with the restriction endonuclease *Hin*dIII (Pharmacia, Uppsala, Sweden) by following the instructions of the manufacturer. Restriction fragments were separated overnight on 0.8% agarose gels by electrophoresis in Tris-acetate buffer (0.04 M Tris-acetate–0.002 M EDTA), stained with ethidium bromide, and viewed under UV irradiation. The fragments were then transferred to nylon filter membranes (8) (Biotrace RP; Gelman Sciences, Inc., Ann Arbor, Mich.) which were then baked at 80°C and stored at room temperature.

Derivation of DNA probe. A genomic bank of *S. sofia* DNA was constructed in cosmid pHC79 by using the method described by Manning et al. (9). Recombinant plasmids were purified from individual clones selected randomly from the bank by using the method of Holmes and Quigley (6). The cosmid clone was labeled with [^{32}P]dCTP by nick translation (8). Several clones were tested empirically to find those which gave optimal differentiation of isolates in Southern hybridizations.

Hybridization. The filters were hybridized overnight at 42°C in hybridization fluid (50% formamide, 7% SDS, 1% skim milk powder, 5× SSPE [1× SSPE is 0.18 M NaCl, 10 mM sodium phosphate, and 1 mM EDTA], 200 μg of salmon sperm DNA per ml, and radiolabeled recombinant probe DNA [5×10^5 cpm/ml]). The next day the filters were washed twice for 15 min at room temperature in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate [pH 7.0])–0.1% SDS and then once at 68°C in 1× SSC–0.1% SDS for 45 min and autoradiographed at −70°C.

RESULTS

Plasmid profile analysis. Plasmid DNA was extracted from a total of 142 isolates of *S. sofia* from a variety of sources in both Australia and Israel. The majority of Australian isolates were from chickens (108), and nine were from humans. A total of 25 Israeli strains were obtained, 14 of which had been isolated from a human source and the remainder of which had been isolated from chickens. After electrophoresis of DNA samples on agarose gels, plasmid profiles were compared. Several different patterns were observed; however, no consistent pattern could be found for any particular group of isolates. Common patterns were found in strains from both chicken and human sources. All Australian chicken strains and six of nine human strains studied contained small plasmids, whereas most (80%) of Israeli isolates did not. A small 6.4-kb plasmid designated pIMVS2 appeared to be common to the majority of Australian chicken and human strains and was analyzed further. Figure 2 shows the restriction map of this plasmid. We examined the distribution of pIMVS2 to determine if it was genetically different from other small plasmids identified in Australian strains and in a small number of Israeli isolates. However, extraction and restriction enzyme digestion of plasmid DNA often proved to be very difficult, and reproducible results were not always obtained.

To enable more precise plasmid characterization, pIMVS2

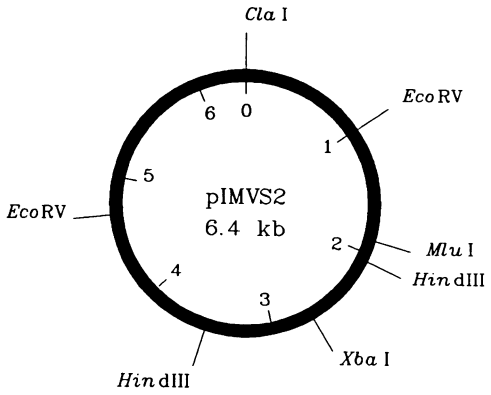

FIG. 2. Restriction map of pIMVS2. A restriction endonuclease cleavage map of pIMVS2 was constructed on the basis of single and double digests in all combinations. The plasmid was not cleaved by *Pst*I.

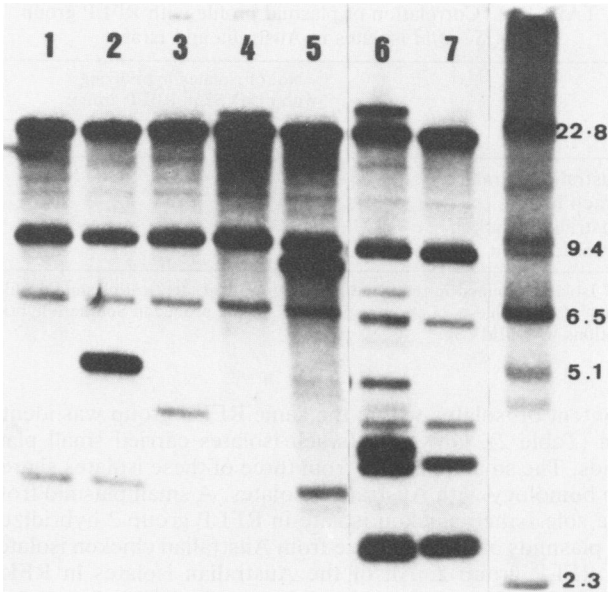

FIG. 3. Differentiation of *S. sofia* into subgroups. Southern transfer demonstrating differentiation of various *S. sofia* strains into seven RFLP groups after probing with cosmid clone A is shown. Lanes: 1 to 7, RFLP groups 1 to 7, respectively; 8, lambda size marker (digested with *Hin*dIII plus *Hin*dIII-*Eco*RI) (BRESATEC, Australia). The sizes of the bands are marked in kilobases.

was tagged with transposon Tn*3* by using the method described previously (16). Radiolabeled plasmid DNA was then used to probe total genomic DNA samples of various strains digested to completion with *Hin*dIII.

The results indicated that the small plasmids found in Australian human and chicken isolates were homologous but were unrelated to those found in the Israeli strains. In addition, the plasmids found in the Israeli chicken isolates were different from those found in isolates from Israeli humans (data not shown).

We sought to determine whether a correlation could be established between the presence of pIMVS2 and the expression of pili or any other surface proteins which may be associated with an attachment or colonization function. SDS-PAGE analysis of crude pili preparations revealed very similar patterns in both Australian and Israeli populations, independent of plasmid content. A putative pilin with a subunit of approximately 22 kDa was observed. However, the production of this protein was not dependent on the presence of pIMVS2. Analysis of pIMVS2 derivatives in *E. coli* K-12 minicells did not allow the identification of any plasmid-associated proteins.

Analysis of cell envelope and outer membrane proteins also did not reveal any differences which could be correlated with plasmid content or source of isolate (data not shown).

Previously, another small plasmid pIMVS1 had been isolated from strains of a *Salmonella typhimurium* phage type 135 outbreak in humans. This plasmid encodes a restriction/modification system (16). Southern hybridization analysis of *S. sofia* with this plasmid did not reveal any homology between pIMVS1 and pIMVS2 (data not shown).

RFLP analysis. Plasmid profiles did not provide a useful typing system for this study because many of the isolates did not contain plasmids. A far more sensitive technique, RFLP analysis, previously developed by Tompkins et al. (15) for use in the identification of *Salmonella* species, was implemented.

A genomic bank of *S. sofia* was constructed by using an isolate from an Australian human, and two clones, A and B, were used to type all 25 Israeli, 9 Australian human, and 23 Australian chicken isolates. The Australian isolates were selected from a variety of Australian states and poultry farms. Three isolates from crocodile meat and three isolates from the meat works where they were processed were also included. Figure 3 shows the patterns obtained when digests of *S. sofia* genomic DNA were probed in southern hybridizations with cosmid A. Seven different patterns (RFLP groups) were observed in isolates from chickens and humans, with most isolates belonging to one of four main patterns (Table 1). All Australian chicken isolates belonged to RFLP group 2. Four of nine Australian human isolates also belonged to this group. In contrast, none of the Israeli human isolates and only one chicken isolate produced this pattern. Most of the Israeli isolates from chickens and humans belonged to RFLP group 3. Only one Australian isolate (from a human) gave a similar pattern. In RFLP group 1, there were three Australian and two Israeli isolates from humans but no isolates from chickens. The RFLP group 4 pattern was commonly seen in isolates from Israeli chickens but not in isolates of any of the other three groups. This group 4 pattern differed from that of RFLP group 1 in that a small band of approximately 3 kb was absent.

When the six isolates of *S. sofia* associated with crocodile meat were analyzed, three were in RFLP group 2 and the other three possessed RFLP patterns which were different from those seen in isolates from either chickens or humans (data not shown).

Although not all strains possessed plasmid, the plasmid

TABLE 1. Differentiation of *S. sofia* isolates in Australia and Israel by RFLP pattern

Source	No. of isolates in RFLP group:						
	1	2	3	4	5	6	7
Australian human	3	4	1		1		
Israeli human	2		11			1	
Australian chicken		23					
Israeli chicken		1	5	4			1

TABLE 2. Correlation of plasmid profile with RFLP group of *S. sofia* isolates in Australia and Israel[a]

Source	No. of isolates hybridizing with pIMVS2 in RFLP group:						
	1	2	3	4	5	6	7
Australian human		4	1		1		
Israeli human			1			1	
Australian chicken		23					
Israeli chicken		1		1		1	

[a] Isolates grouped in the same RFLP group had identical plasmid profiles when *Hin*dIII digests of chromosomal DNA were probed in Southern hybridizations with pIMVS2.

content of isolates within the same RFLP group was identical (Table 2). Only five Israeli isolates carried small plasmids. The small plasmids from three of these isolates shared no homology with Australian isolates. A small plasmid from the sole Israeli chicken isolate in RFLP group 2 hybridized to plasmids of the same size from Australian chicken isolates in RFLP group 2. All of the Australian isolates in RFLP group 2 had the same plasmid type. In RFLP group 3, a plasmid from an Israeli human isolate showed homology with a plasmid detected in an Australian human strain. Overall, the results obtained by grouping according to plasmid content did not disagree with the grouping based on RFLP analysis; however, RFLP analysis was able to provide a mechanism for typing plasmidless strains.

DISCUSSION

Traditional methods such as serotyping, biotyping, and colicin and phage typing are commonly used to characterize *Salmonella* strains (5, 14). The work presented here involved the implementation and evaluation of two molecular typing techniques, i.e., plasmid profile analysis and chromosomal fingerprinting. These techniques were used in an epidemiological study of *S. sofia* isolated in Australia and Israel. Recently, plasmid profile analysis has been used for strain differentiation within serovars and phage types (5). Although this technique enabled us to identify the presence of a small plasmid, pIMVS2, found in all isolates from chickens, it was not sufficiently discriminating to be used as an epidemiological tool. Transposon mutagenesis facilitated tagging of this plasmid, and Southern hybridization analysis demonstrated that pIMVS2 was common to most Australian isolates tested but was unrelated to any of the plasmids in the Israeli isolates.

We previously reported the correlation between the presence of a small plasmid (pIMVS1) encoding a restriction/modification system and an outbreak of salmonellosis. However, we have been unable to associate pIMVS2 with the expression of potential virulence determinants such as antibiotic resistances, pili, or outer membrane proteins.

In contrast to plasmid analysis, RFLP typing was successful in establishing that isolates of *S. sofia* from chickens in Israel were genetically different from the majority of isolates from Australian chickens. All Australian chicken isolates belonged to a single clonal group; however, isolates from Israeli chickens were genetically more heterogeneous. Only one Israeli chicken isolate possessed an RFLP profile identical to the Australian chicken profile, with the majority of Israeli strains belonging to two separate RFLP groups.

S. sofia isolates from humans in both countries gave variable profiles. Approximately half of the Australian isolates from humans were similar to those from chickens (RFLP group 2). The majority of human strains from Israel were also similar to chicken isolates found in that country (RFLP group 3). Therefore, it is feasible that the source of some *S. sofia* infections in humans in Australia and most *S. sofia* infections in Israel is linked to chickens or that they are infected from a common source. However, the strains causing human disease and chicken colonization in Israel are genetically distinct from strains present in Australian chickens. This may explain the difference between the two countries in the epidemiology of *S. sofia*.

In Israel and Australia, the predominant strains of *S. sofia* in chickens do not cause appreciable morbidity in chickens. On the basis of isolation data, the predominant chicken strains in Israeli are more pathogenic for humans than are the strains which colonize Australian chickens. In preliminary studies, we were unable to demonstrate the presence of any virulence determinants in the bacterium which could account for this. The possibility remains that the differences in epidemiology are caused by differing social factors in the two countries.

A proportion of *S. sofia* isolates in both countries possessed an identical profile (RFLP group 1) which was not seen in any isolate from chickens. These infections may originate from other unidentified sources common to both countries.

In conclusion, this study demonstrated that chromosomal fingerprinting or RFLP analysis was a useful epidemiological tool for strain differentiation between Australian and Israeli chicken isolates. Workers wishing to use this technique should be aware that members of the family *Enterobacteriaceae* in particular may possess plasmids which have sequence homologies with the vector used for cloning. Thus, the RFLP types may reflect the plasmid profiles of the isolates. This was not so with the *S. sofia* isolates studied here.

Further research into the potential virulence mechanisms of *Salmonella* spp. and their specificities may provide explanations for the different epidemiological patterns of *S. sofia* worldwide.

ACKNOWLEDGMENT

We thank I. Sechter, National Salmonella Centre, Jerusalem, Israel, and M. Heuzenroeder and T. W. Steele for useful discussions.

This work was supported by Chicken Meat Research Grant IMVS 3C.

REFERENCES

1. **Achtman, M. S., S. Schwuchow, R. Helmuth, G. Morelli, and P. A. Manning.** 1978. Cell-cell interactions in conjugating *Escherichia coli*: Con$^-$ mutants and stabilization of mating aggregates. Mol. Gen. Genet. **164**:171–183.
2. **Australian Salmonella Reference Laboratory, Institute of Medical and Veterinary Science.** 1980. Annual report.
3. **Brown, M. C. M., A. Weston, J. R. Saunders, and G. O. Humphreys.** 1979. Transformation of *Escherichia coli* C600 by plasmid DNA at different phases of growth. FEMS Microbiol. Lett. **5**:219–222.
4. **Garger, S. J., O. M. Griffith, and L. K. Grill.** 1983. Rapid purification of plasmid DNA by a single centrifugation in a two-step caesium chloride-ethidium bromide gradient. Biochem. Biophys. Res. Commun. **117**:835–842.
5. **Holmberg, S. D., I. K. Wachsmuth, F. W. Hickman-Brenner, and M. L. Cohen.** 1981. Comparison of plasmid profile analysis, phage typing, and antimicrobial susceptibility testing in characterizing *Salmonella typhimurium* isolates from outbreaks. J. Clin. Microbiol. **19**:100–104.

6. **Holmes, D. S., and M. Quigley.** 1981. A rapid boiling method for the preparation of bacterial plasmids. Anal. Biochem. **114**:193–197.
7. **Lugtenberg, B. J., R. Meijers, R. Peters, P. van der Hoek, and L. van Alphen.** 1975. Electrophoretic resolution of the major outer membrane protein of *Escherichia coli* K-12 into 4 bands. FEBS Lett. **58**:254–258.
8. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
9. **Manning, P. A., M. W. Heuzenroeder, J. Yeadon, D. I. Leavesley, P. R. Reeves, and D. Rowley.** 1986. Molecular cloning and expression in *Escherichia coli* K-12 of the O antigens of the Inaba and Ogawa serotypes of the *Vibrio cholerae* O1 lipopolysaccharides and their potential for vaccine development. Infect. Immun. **53**:272–277.
10. **Manning, P. A., F. Imbesi, and D. R. Haynes.** 1982. Cell envelope proteins on *Vibrio cholerae*. FEMS Microbiol. Lett. **14**:159–166.
11. **National Salmonella Centre, Israel.** 1986. Summaries of findings.
12. **Smyth, C. J.** 1982. Two mannose-resistant haemagglutinins on enterotoxigenic *Escherichia coli* of serotype O6:H1 or H-isolated from travellers' and infantile diarrhoeae. J. Gen. Microbiol. **128**:2081–2096.
13. **Stirm, S., F. Orskov, I. Orskov, and B. Mansa.** 1967. Episome-carried surface antigen K88 of *Escherichia coli*. II. Isolation and chemical analysis. J. Bacteriol. **93**:731–739.
14. **Threlfall, E. J., and J. A. Frost.** 1990. A review. The identification, typing and fingerprinting of *Salmonella*: laboratory aspects and epidemiological applications. J. Appl. Bacteriol. **68**:5–16.
15. **Tompkins, L. S., N. Troup, A. Labigne-Roussel, and M. L. Cohen.** 1986. Cloned, random sequences as probes to identify *Salmonella* species. J. Infect. Dis. **154**:156–162.
16. **Whiley, S. J., J. A. Lanser, P. A. Manning, C. Murray, and T. W. Steele.** 1988. Plasmid profile analysis of a salmonellosis outbreak and identification of a restriction modification system. Appl. Environ. Microbiol. **54**:1591–1594.