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Molecular and Epidemiological Study of *Salmonella* Clinical Isolates

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A survey of *Salmonella* infections was carried out over a 1-year period in the rural community covered by the Hospital Reina Sofía (Tudela, Spain). The 255 strains that were collected were studied by serotyping, antimicrobial resistance, and plasmid profile analysis. The predominant serotype was *S. enteritidis* (85.90%), followed by *S. typhimurium* (7.06%) and *S. virchow* (2.36%). Only 7.84% of the strains were resistant to antimicrobial agents. The most common resistance was to beta-lactam antibiotics. This resistance was due to the presence of one of two types of β-lactamases, TEM-1 or TEM-2. Resistance to kanamycin was associated with the synthesis of a 3′-*O*-phosphotransferase. The resistance to streptomycin and chloramphenicol was either not enzymatic or was due to a 3″-*O*-phosphotransferase and a chloramphenicol acetyltransferase, respectively. Analysis of total plasmid DNA content revealed the presence of plasmids in 96.08% of the isolates. According to their plasmid profile, the strains could be classified into different groups. The three main groups, which accounted for 50.19, 20.78, and 4.70% of the isolates, respectively, corresponded to the antimicrobial-susceptible *S. enteritidis* serotype. These results suggested that plasmid profile analysis in conjunction with antimicrobial resistance determination can be useful for subtyping resistant *Salmonella* isolates.

Salmonellas are major, increasingly important, causes of food poisoning throughout the world (1, 7). In Spain, they are the principal etiological agent of gastroenteritis (9). The present understanding of the epidemiology of salmonellosis comes primarily from investigations of outbreaks. Several different methods, including serotyping, biotyping, phage typing, and antimicrobial susceptibility testing, have traditionally been used to trace outbreak strains. In recent years, these epidemiological studies have been improved by the application of molecular techniques, such as plasmid profile analysis (22, 30), DNA restriction fragment polymorphism (34), chromosomal probe fingerprinting (33), and multilocus enzyme electrophoresis (4).

Plasmid pattern determination has proven to be a useful epidemiological tool in outbreaks of salmonellosis caused by different *Salmonella* serotypes (5, 12, 18, 20, 26, 31). In comparative studies of *S. typhimurium*, it was concluded that plasmid profile analysis appears to be as specific as phage typing in identifying related or unrelated isolates from outbreaks (6, 14, 16). Besides, Rodrigue et al. (27a) compared plasmid profiles, phage types, and antimicrobial resistance regarding their usefulness as epidemiological markers for *S. enteritidis* and concluded that the plasmid profile and phage type effectively determined the subtype of this serotype.

Plasmid profiles are most useful when they are combined with other methods for screening or typing (16, 22). Therefore, in this study, the serotype, antimicrobial susceptibility pattern, enzymatic mechanisms of antimicrobial resistance, and plasmid profile were used in combination to investigate the human *Salmonella* infections diagnosed over a 1-year period in the geographic area of the Hospital Reina Sofía (Tudela, Spain), a rural community with 80,000 inhabitants.

(Part of this work was presented at the 16th International Congress of Chemotherapy, Jerusalem, Israel, 1989 [26a].)

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MATERIALS AND METHODS

Bacterial strains and plasmids. A total of 255 strains of *Salmonella*, isolated between 1 February 1988 and 31 January 1989 from patients in the geographic area of the Hospital Reina Sofía, were studied. The strains of *Salmonella* that were isolated more than once from the same patient were eliminated. For each isolate, the date of isolation, the location, and the source were recorded. The standard bacterial strain used for conjugative plasmid transfer experiments was *Escherichia coli* K-12 J62 (F^- Nal *pro his lac*) (2). Reference plasmids for determining the molecular sizes consisted of R144 (93 kb), RP4 (54 kb), N3 (50 kb), and S-a (45 kb) (29).

Phenotypic characterization of isolates. All isolates were serotyped by established procedures for cell wall (O) and flagellar (H) antigen identification (10). The biochemical test reactions and MIC determinations were performed in a Pasco System (Pasco Laboratories, Inc., Wheat Ridge, Colo.). The antibiotics assayed were ampicillin, ticarcillin, mezlocillin, piperacillin, cephalothin, cefoxitin, cefamandole, cefotaxime, gentamicin, tobramycin, amikacin, chloramphenicol, tetracycline, trimethoprim-sulfamethoxazole, and norfloxacin. The strains were further tested for their susceptibilities to antimicrobial agents by using the agar diffusion method of Bauer et al. (3) with commercial disks (Difco Laboratories, Detroit, Mich.). The drugs assayed were carbenicillin, azlocillin, streptomycin, kanamycin, sisomicin, dibekacin, netilmicin, fosfomycin, and nalidixic acid.

Conjugation procedure. Transfer of R plasmids from *Salmonella* isolates to *E. coli* K-12 took place in liquid medium at 37°C (36). Transconjugants were selected on MacConkey agar (Difco) containing the following antibiotics at the indicated concentrations: ampicillin, 50 μg/ml; tetracycline, 10 μg/ml; kanamycin, 25 μg/ml; gentamicin, 10 μg/ml; tobramycin, 10 μg/ml; streptomycin, 10 μg/ml; fosfomycin, 100 μg/ml; and nalidixic acid, 250 μg/ml.

Enzymatic mechanisms of antimicrobial resistance. To as-

say for aminoglycoside-modifying enzymes, crude cell extracts of the aminoglycoside-resistant *Salmonella* strains and *E. coli* K-12 transconjugants were prepared by ultrasonic disruption (11). Enzymatic activity was measured by the cellulose phosphate paper-binding assay (11). The reaction mixture for phosphorylation consisted of 10 μl of phosphotransferase buffer, 10 μl (10 μCi/μmol/ml) of [γ-^{32}P]ATP (Amersham Ibérica S.A., Madrid, Spain), 10 μl of enzyme preparation, and 2 μl of antibiotic (1 mg/ml). Assays were incubated at 30°C for 30 min, and 20-μl samples were counted. For acetylation or adenylylation, 10 μl (8 μCi/μmol/ml) of [1-^{14}C]acetyl coenzyme A (Amersham) or 10 μl (10 μCi/μmol/ml) of [^{14}C]ATP (Amersham), respectively, was used instead of [γ-^{32}P]ATP.

β-Lactamase activity in the crude extracts of beta-lactam-resistant strains was determined by the chromogenic substrate method (25). The rate of hydrolysis of the nitrocefin (Difco) by β-lactamases was monitored with a DU-8 spectrophotometer (Beckman Instruments España S.A., Madrid, Spain). The analytical isoelectric focusing of β-lactamases was performed in a Phast System (Pharmacia Ibérica S.A., Barcelona, Spain).

To determine the mechanism of chloramphenicol resistance, crude cell extracts were tested for the presence of chloramphenicol acetyltransferase (CAT). The radioenzymatic assay for CAT was performed by the method described by Robison et al. (27). The reaction mixture consisted of 10 μl of 1 M Tris hydrochloride (pH 7.8), 65 μl of water, 10 μl of [^{14}C]acetyl coenzyme A (8 μCi/μmol/ml), 10 μl of crude cell extract, and 10 μl of chloramphenicol (16 mg/ml). Assays were incubated at 37°C for 15 min. Direct extraction of ^{14}C-acetylated chloramphenicol from the water phase into the toluene phase was accomplished by the addition of 4 ml of scintillation fluid.

Plasmid profile. The plasmid profiles of the *Salmonella* isolates and *E. coli* transconjugant strains were studied. Plasmid DNA was isolated and purified by the alkaline lysis technique described by Maniatis et al. (21). All plasmid DNAs were electrophoresed on 0.7% horizontal agarose gels in Tris-borate buffer (22). Reference plasmid DNAs and λ DNA *Hin*dIII digest (Pharmacia) were included as molecular weight standards. Gels were stained with ethidium bromide (0.5 μg/ml) and photographed under UV transillumination with Polaroid type 667 film.

Plasmid DNAs from 10 transconjugant strains with identical or similar antibiotic resistance patterns were analyzed by restriction endonuclease digestion, using the enzymes *Eco*RI, *Eco*RV, *Hin*dIII, and *Pst*I, under the conditions recommended by the supplier (Boehringer Mannhein España Biochemicals, Barcelona, Spain). The resulting digests were subjected to electrophoresis on 0.9% horizontal agarose gels.

RESULTS

Table 1 summarizes the serotypes, antimicrobial susceptibilities, and plasmid profiles of all *Salmonella* isolates. Of these strains, 131 isolates (51.37%) corresponded to sporadic cases, and 124 isolates (48.62%) corresponds to outbreak-related cases. There were two *S. enteritidis* outbreaks, with 41 and 14 patients, respectively, associated with wedding receptions and 21 outbreaks, each with no more than five patients, in which the implicated source or exposure was a family reunion. One *S. enteritidis* outbreak, in which 10 patients were involved, was due to a food handler who was an asymptomatic carrier.

Phenotypic characterization and conjugation experiments. *Salmonella* isolates were serologically typed into 11 different serotypes. The predominant serotype was *S. enteritidis* (85.90%), followed by *S. typhimurium* (7.06%) and *S. virchow* (2.36%). Other serotypes included *S. azteca* and *S. muenchen* (0.78%) and *S. bredeney*, *S. agona*, *S. infantis*, *S. gold-coast*, *S. london*, and *S. muenster* (0.39%). The distribution of strains obtained for every serotype is shown in Table 1.

Antimicrobial susceptibility testing showed that only 20 strains (7.84%) were resistant to one or multiple antibiotics. The antimicrobial resistance patterns and the percentage of resistant isolates varied significantly among serotypes (Table 1). A total of 33.33% of *S. typhimurium* isolates were resistant to different antibiotics, whereas only 4.56% of *S. enteritidis* isolates presented this character.

All resistant *Salmonella* strains were mated with *E. coli* K-12 to investigate the linkage of antibiotic resistance determinants to plasmid DNA. Results of these experiments showed that most antimicrobial resistance patterns (70%) were transferred by conjugation (Table 1).

Enzymatic mechanisms of antimicrobial resistance. The phosphocellulose paper-binding assay demonstrated the existence of aminoglycoside-phosphotransferase activity in the crude extracts of three *Salmonella* isolates and on their transconjugant strains, namely, *S. typhimurium* (group VIII) and *S. virchow* (groups III and IV). Resistance to streptomycin in the *S. typhimurium* isolate was due to a 3″-*O*-phosphotransferase, APH(3″). The phosphorylating activity in both *S. virchow* isolates was due to an APH(3′)-I which modifies kanamycin, neomycin, ribostamycin, paromomycin, and lividomycin. No APH(3″) enzyme was detected in these two isolates, suggesting that their mechanisms of streptomycin resistance were not enzymatic. All the other streptomycin-resistant isolates listed in Table 1 were found to be resistant by a nonenzymatic mechanism.

β-Lactamase activity was detected in the crude extracts of all of the beta-lactam-resistant strains except that of the *S. bredeney* isolate. Analytical isoelectric focusing showed that these strains specified two different types of β-lactamases, namely, TEM-1 and TEM-2. The TEM-1 β-lactamases were plasmid-encoded, whereas the TEM-2 β-lactamase corresponded to three *S. typhimurium* isolates (group III) that did not transfer, by conjugation, any of their antimicrobial resistance determinants (Table 1).

Resistance to chloramphenicol was due to the synthesis of CAT in three *Salmonella* isolates, i.e., *S. virchow* (groups III and IV) and *S. bredeney*. This resistance in the two *S. virchow* isolates was transferable by conjugation. However, for the *S. typhimurium* strain (group III) chloramphenicol resistance was neither transferable nor enzymatic (Table 1).

Plasmid profile. Analysis of the total plasmid DNA content revealed the presence of plasmids in 245 *Salmonella* strains (96.08%). The plasmid profiles obtained for every serotype are shown in Table 1. A total of 209 antibiotic-susceptible and 10 antibiotic-resistant *S. enteritidis* isolates were classified into 15 groups according to their plasmid profiles (Fig. 1 and 2). Only eight strains (3.65%) carried no plasmid at all (group XV). The majority (91.32%) of all isolates harbored a 54-kb plasmid that probably coincided with the serotype-specific plasmid described by Helmuth et al. (13).

The 18 *S. typhimurium* isolates could be included in one of the eight plasmid pattern groups. A 90-kb plasmid was the most frequently encountered plasmid in this serotype, because only 2 isolates (11.11%) that did not carry it were detected. This plasmid could correspond to the serotype-

TABLE 1. Plasmid profiles and antimicrobial resistance in 255 *Salmonella* strains

Group	Plasmid profile (kb)	No. of strains	Antimicrobial resistance[a]	Transferred resistance pattern	Conjugative R plasmid (kb)	Enzymatic resistance
S. enteritidis (*n* = 219)						
I	54	128	Susceptible			
II	54, 3.0	53	Susceptible			
III	54, 4.1	12	Susceptible			
IV	54, 32	6	Ap Cb Tic Pip	Ap Cb Tic Pip	32	TEM-1
V	54, 50	2	Ap Cb Tic Pip	Ap Cb Tic Pip	50	TEM-1
VI	54, 5.0, 1.6	2	Susceptible			
VII	54, 41	1	Ap Cb Tic Pip	Ap Cb Tic Pip	41	TEM-1
VIII	78, 54	1	Susceptible			
IX	54, 5.0	1	Susceptible			
X	78, 54, 3.0	1	Susceptible			
XI	104, 54, 3.0	1	Tc	Tc	104, 3.0	
XII	75, 54, 5.0, 1.6	1	Susceptible			
XIII	29, 14.5, 10, 5.8	1	Susceptible			
XIV	85, 54, 36, 29, 23, 5.8, 5.2, 4.1	1	Susceptible			
XV	None	8	Susceptible			
S. typhimurium (*n* = 18)						
I	90	5	Susceptible			
II	90, 0.92, 0.86	5	Susceptible			
III	90, 3.0, 1.6	3	Ap Cb Tic Pip Tc Sm Cm	None	None	TEM-2
IV	90, 4.1	1	Susceptible			
V	102, 90, 6.1	1	Ap Cb Tic Pip Sm	Ap Cb Tic Pip Sm	102	TEM-1
VI	47, 8.4, 3.0	1	Sm Sxt	None	None	None
VII	125, 90, 3.0, 1.6	1	Susceptible			
VIII	114, 47, 30, 6.1	1	Tc Sm	Tc Sm	114	APH(3″)
S. virchow (*n* = 6)						
I	33, 7.1	3	Susceptible			
II	3.0	1	Susceptible			
III	33, 3.0	1	Sm Km Cm Sxt	Sm Km Cm Sxt	None	APH(3′)-I, CAT
IV	120, 33, 3.0	1	Sm Km Cm Sxt	Sm Km Cm Sxt	None	APH(3′)-I, CAT
S. muenchen (*n* = 2)						
I	57, 39, 2.7	1	Susceptible			
II	2.7	1	Susceptible			
S. azteca (*n* = 2)						
I	None	2	Susceptible			
S. bredeney (*n* = 1), I	125, 90, 30, 6.5, 1.95, 1.45, 1.06	1	Ap Cb Tic Pip Tc Sm Cm Sxt	None	None	CAT
S. agona (*n* = 1), I	30, 6.1	1	Susceptible			
S. infantis (*n* = 1), I	120, 111, 7.1	1	Susceptible			
S. gold-coast (*n* = 1), I	5.0	1	Susceptible			
S. muenster (*n* = 1), I	4.8	1	Susceptible			
S. london (*n* = 1), I	0.83, 0.79	1	Susceptible			
Salmonella serogroup D (*n* = 1), I	54	1	Susceptible			
Salmonella serogroup B (*n* = 1), I	33, 7.1	1	Tc Sm	None	None	None

[a] Tc, Tetracycline; Ap, ampicillin; Cb, carbenicillin; Pip, piperacillin; Tic, ticarcillin; Sm, streptomycin; Km, kanamycin; Cm, chloramphenicol; Sxt, trimethoprim-sulfamethoxazole.

specific virulence plasmid of 60 MDa (90 kb) reported by Helmuth et al. (13). Four plasmid pattern groups were obtained for the six *S. virchow* strains isolated in this study. A 33-kb plasmid was the predominant one in this serotype.

The *S. azteca* isolates were plasmid-free. The rest of the isolates, each one of a different serotype, showed plasmid DNAs with heterogeneous sizes.

Table 1 lists both the antimicrobial resistance patterns

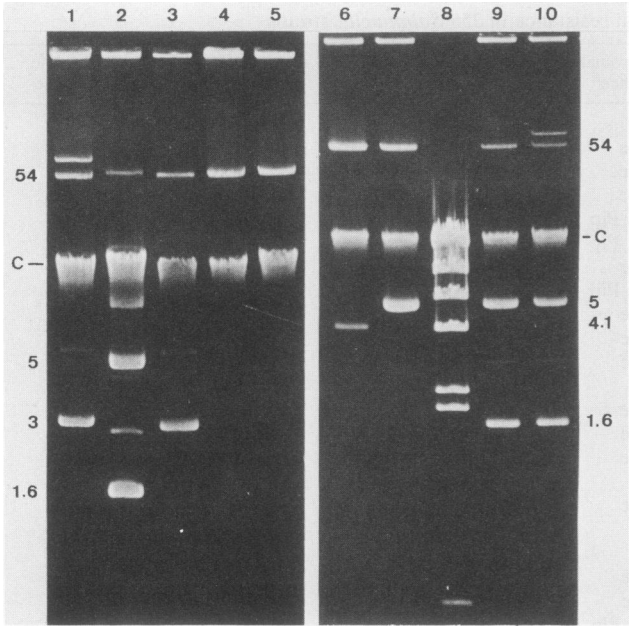

FIG. 1. Plasmid profiles of susceptible *S. enteritidis* strains. Lanes: 1, group X; 2, group VI; 3, group II; 4, RP4; 5, group I; 6, group III; 7, group IX; 8, λ DNA *Hin*dIII digest; 9, group VI; 10, group XII. C, Chromosomal DNA. Numbers next to the gels are in kilobases.

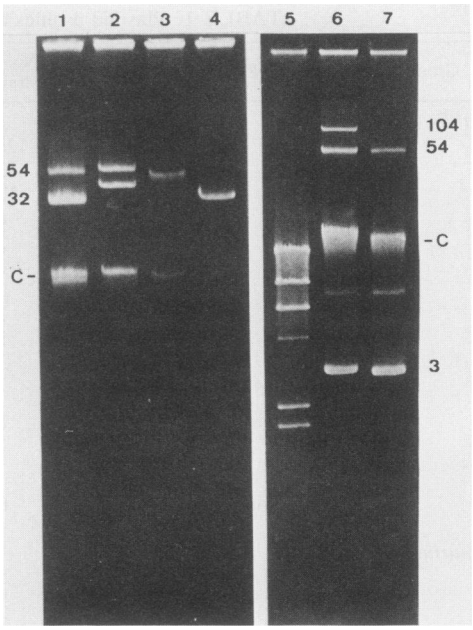

FIG. 2. Plasmid profiles of resistant *S. enteritidis* strains. Lanes: 1, group IV; 2, group VII; 3, N3; 4, S-a; 5, λ DNA *Hin*dIII digest; 6, group XI; 7, group II (susceptible). C, Chromosomal DNA. Numbers next to the gels are in kilobases.

transferred via conjugation to the *E. coli* recipient strain and the conjugative R plasmids. These R plasmids were found in 12 (60%) of the 20 resistant strains.

The *E. coli* transconjugant of *S. enteritidis* strain (group XI) carried two plasmids of 104 and 3.0 kb. Since the 3.0-kb plasmid is too small to be self-transmissible, this finding indicates that the 104-kb plasmid cotransfers the small plasmid.

No plasmid DNAs were detected in any of the two *S. virchow* transconjugant strains (groups III and IV). However, donor strains not only harbored plasmids but also transferred, by conjugation, all of their antibiotic resistance markers at frequencies of transfer of 3×10^{-2} and 2×10^{-4}, respectively. These results suggest the presence of a multiresistant transposon in those strains. Beta-lactam resistance R plasmids of *S. enteritidis* strains (groups IV and V) revealed identical DNA fragment patterns by restriction endonuclease analysis. Moreover, these two banding patterns and the restriction patterns of group VII strains were similar, indicating that all three constituted a subgroup of R plasmids with a common origin, differing only in the number of DNA bands according to the plasmid size (32, 50, and 41 kb). In contrast, very few coincidences were observed in the digestion pattern of the beta-lactam resistance plasmid found in an *S. typhimurium* isolate (group V) and the subgroup mentioned above.

DISCUSSION

The aim of this study was to investigate the epidemiology of human *Salmonella* infections that broke out in our geographic area over a 1-year period. The results presented here provide information on the incidences and distributions of serotypes, antimicrobial resistance, and plasmid profiles among *Salmonella* strains in this geographic area. The most frequently isolated serotype was *S. enteritidis*, which accounted for 85.90% of the collected isolates. Considering that the average percentage of *S. enteritidis* isolates in Spain in 1987 was 63.40% (20), which has grown increasingly since 1983, the results presented above coincide with World Health Organization Salmonella surveillance data for 1979 to 1987 that indicate an international increase of *S. enteritidis* and that it was the most common serotype in eight European countries in 1987 (28). The incidence rate of *S. typhimurium* (7.06%) was very low when compared with the data from other countries where this serotype is more common (15, 23, 35).

Antimicrobial susceptibility analysis revealed that only 7.84% of the strains were antibiotic resistant. However, not all serotypes had similar frequencies of resistance. We draw attention to the high percentage of resistant *S. typhimurium* isolates compared with the percentage of resistant *S. enteritidis* strains and the differences found between the antimicrobial resistance patterns and the resistance mechanisms of these two serotypes. Our results are coincident with other published data which state that the antibiotic resistance frequency for *S. typhimurium* is increasing, while for *S. enteritidis* the incidence remains at low and stable levels (8, 19, 35). On the other hand, antimicrobial resistance studies enabled us to differentiate resistant from susceptible isolates of the same serotype. Moreover, this analysis, in conjunction with plasmid profiles, was useful in subtyping antibiotic-resistant strains, namely, *S. enteritidis* groups IV, V, and VII and *S. virchow* groups III and IV.

Plasmid profile analysis seemed to differentiate the susceptible strains of the serotypes *S. typhimurium*, *S. virchow*, and *S. muenchen*. Furthermore, our data confirm the usefulness of plasmid profiles for identifying epidemiologically related isolates of *S. typhimurium* groups I and II (6, 14) and *S. virchow* group I. With regard to the *S. enteritidis* serotype, this analysis provides a means of grouping the suscep-

tible isolates, showing the distribution of plasmid profiles within our community. The two main groups, i.e., groups I and II, accounted for 50.19 and 20.78% of the reported *Salmonella* infections, respectively. Group I corresponded to 68 sporadic isolates, and 60 isolates originated from 13 foodborne outbreaks. The detection of these isolates in different localities over the study period demonstrated the wide distribution in this geographic area of the plasmid profile mentioned above. Our results agree with those of other authors (13, 20, 24) who reported a high prevalence of susceptible *S. enteritidis* strains carrying a single 54-kb plasmid. Group II corresponded to 8 sporadic isolates and 45 isolates originating from two family reunion outbreaks (4 patients) and isolates originating from a wedding reception outbreak (41 patients). It should be mentioned that the plasmid profile of these isolates was coincident with the profile of an *S. enteritidis* strain isolated from the ovary of a hen belonging to one poultry farm in our geographic area used for egg production (Instituto de Salud Pública Navarra). This result suggests that this particular food source may have been involved in the infections because of the incidence, in our community, of *S. enteritidis* with this plasmid profile (1). Furthermore, this finding coincides with those of investigations in individual countries which suggest that the reason for the global increase in *S. enteritidis* is related to the consumption of eggs and poultry which harbor the organism (28, 32). Group III, which accounted for only 4.70% of the reported *Salmonella* infections, corresponded to six sporadic isolates and six isolates originating from two family reunion outbreaks. These isolates were detected in nearby localities and accounted for all except one sporadic case during the same month.

With regard to the identification of susceptible *S. enteritidis* strains, our results led us to consider the necessity of a further characterization of these isolates. In that respect, we agree with those authors (17, 20, 33) who conclude that much more information of epidemiological value may be obtained when plasmid profile analysis is used as a complement to other typing systems.

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REFERENCES

1. **Archer, D. L., and F. E. Young.** 1988. Contemporary issues: diseases with a food vector. Clin. Microbiol. Rev. **1**:377–398.
2. **Bachmann, B. J.** 1972. Pedigrees of some mutants strains of *Escherichia coli* K-12. Bacteriol. Rev. **36**:525–557.
3. **Bauer, A. W., W. M. M. Kirby, J. C. Sherris, and M. Turck.** 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. **45**:493–496.
4. **Beltran, P., J. M. Musser, R. Helmuth, J. J. Farmer III, W. M. Frerichs, I. K. Wachsmuth, K. Ferris, A. C. McWhorter, J. G. Wells, A. Cravioto, and R. K. Selander.** 1988. Toward a population genetic analysis of *Salmonella*: genetic diversity and relationships among strains of serotypes *S. choleraesuis*, *S. derby*, *S. dublin*, *S. enteritidis*, *S. heidelberg*, *S. infantis*, *S. newport*, and *S. typhimurium*. Proc. Natl. Acad. Sci. USA **85**:7753–7757.
5. **Bezanson, G. S., R. Khakhria, and D. Pagnutti.** 1983. Plasmid profiles of value in differentiating *Salmonella muenster* isolates. J. Clin. Microbiol. **17**:1159–1160.
6. **Brunner, F., A. Margadant, R. Peduzzi, and J. C. Piffaretti.** 1983. The plasmid pattern as an epidemiologic tool for *Salmonella typhimurium* epidemics: comparison with the lysotype. J. Infect. Dis. **148**:7–11.
7. **Chalker, R. B., and M. J. Blaser.** 1988. A review of human salmonellosis. III. Magnitude of *Salmonella* infection in the United States. Rev. Infect. Dis. **10**:111–124.
8. **Cohen, M. L., and R. V. Tauxe.** 1986. Drug-resistant *Salmonella* in the United States: an epidemiologic perspective. Science **234**:964–969.
9. **Echeita, M. A., and M. A. Usera.** 1989. Prevalence of *Salmonella* serotypes isolated in Spain from human and non human sources (1983–1987). Microbiología SEM **5**:95–103.
10. **Ewing, W. H.** 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing, Inc., New York.
11. **Haas, M. J., and J. E. Dowding.** 1975. Aminoglycoside-modifying enzymes. Methods Enzymol. **43**:611–628.
12. **Handfield, T. L., M. H. Monson, and I. K. Wachsmuth.** 1985. An outbreak of antibiotic-resistant *Salmonella enteritidis* in Liberia, West Africa. J. Infect. Dis. **151**:790–795.
13. **Helmuth, R., R. Stephan, C. Bunge, B. Hoog, A. Steinbeck, and E. Bulling.** 1985. Epidemiology of virulence-associated plasmids and outer membrane protein patterns within seven common *Salmonella* serotypes. Infect. Immun. **48**:175–182.
14. **Holmberg, S. D., I. K. Wachsmuth, F. W. Hickman-Brenner, and M. L. Cohen.** 1984. Comparison of plasmid profile analysis, phage typing, and antimicrobial susceptibility testing in characterizing *Salmonella typhimurium* isolates from outbreaks. J. Clin. Microbiol. **19**:100–104.
15. **Kapperud, G., S. Gustavsen, I. Hellesnes, A. H. Hansen, J. Lassen, J. Hirn, M. Jahkola, M. A. Montenegro, and R. Helmuth.** 1990. Outbreak of *Salmonella typhimurium* infection traced to contaminated chocolate and caused by a strain lacking the 60-megadalton virulence plasmid. J. Clin. Microbiol. **28**: 2597–2601.
16. **Kapperud, G., J. Lassen, K. Dommarsnes, B.-E. Kristiansen, D. A. Caugant, E. Ask, and M. Jahkola.** 1989. Comparison of epidemiological marker methods for identification of *Salmonella typhimurium* isolates from an outbreak caused by contaminated chocolate. J. Clin. Microbiol. **27**:2019–2024.
17. **Le Minor, L.** 1988. Typing of *Salmonella* species. Eur. J. Clin. Microbiol. Infect. Dis. **7**:214–218.
18. **Lin, F.-Y. C., J. G. Morris, Jr., D. Trump, D. Tilghman, P. K. Wood, N. Jackman, E. Israel, and J. P. Libonati.** 1988. Investigation of an outbreak of *Salmonella enteritidis* gastroenteritis associated with consumption of eggs in a restaurant chain in Maryland. Am. J. Epidemiol. **128**:839–844.
19. **Lorian, V.** 1986. *Salmonella* susceptibility patterns in hospitals from 1975 through 1984. J. Clin. Microbiol. **23**:826–827.
20. **Luján, R., A. Echeita, M. A. Usera, J. V. Martínez-Suarez, R. Alonso, and J. A. Sáez-Nieto.** 1990. Plasmid profiles as an epidemiological marker for *Salmonella enterica* serotype enteritidis foodborne outbreaks. Microbiología SEM **6**:45–50.
21. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual, p. 368–369. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. **Mayer, L. W.** 1988. Use of plasmid profiles in epidemiologic surveillance of disease outbreaks and in tracing the transmission of antibiotic resistance. Clin. Microbiol. Rev. **1**:228–243.
23. **McDonough, P. L., J. F. Timoney, R. H. Jacobson, and R. Khakhria.** 1989. Clonal groups of *Salmonella typhimurium* in New York State. J. Clin. Microbiol. **27**:622–627.
24. **Nakamura, M., S. Sato, T. Ohya, S. Suzuki, and S. Ikeda.** 1985. Possible relationship of a 36-megadalton *Salmonella enteritidis* plasmid to virulence in mice. Infect. Immun. **47**:831–833.
25. **O'Callaghan, C. H., A. Morris, S. M. Kirby, and A. H. Shingler.** 1972. Novel method for detection of β-lactamases by using a chromogenic cephalosporin substrate. Antimicrob. Agents Chemother. **1**:283–288.
26. **Riley, L. W., G. T. DiFerdinando, Jr., T. M. DeMelfi, and M. L. Cohen.** 1983. Evaluation of isolated cases of salmonellosis by plasmid profile analysis: introduction and transmission of a bacterial clone by precooked roast beef. J. Infect. Dis. **148**:12–17.

26a. **Rivera, M. J., N. Rivera, J. Castillo, A. Mazón, A. Lanas, and R. Gómez-Lus.** 1989. Abstr. 16th Int. Congr. Chemother., abstr. 1225.
27. **Robison, L. R., R. Seligsohn, and S. A. Lerner.** 1978. Simplified radioenzymatic assay for chloramphenicol. Antimicrob. Agents Chemother. **13**:25–29.
27a. **Rodrigue, D. C., R. Tauxe, A. Stubbs, M. St. Louis, N. Puhr, B. Rowe, and I. K. Wachsmuth.** 1989. Program Abstr. 29th Intersci. Conf. Antimicrobial Agents Chemother., abstr. 1002.
28. **Rodrigue, D. C., R. V. Tauxe, and B. Rowe.** 1990. International increase in *Salmonella enteritidis*: a new pandemic? Epidemiol. Infect. **105**:21–27.
29. **Shapiro, J. A.** 1977. Bacterial plasmids, p. 601–677. *In* A. I. Buckari, J. A. Shapiro, and S. L. Adhya (ed.), DNA insertion elements, plasmids and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
30. **Shlaes, D., and C. A. Currie-McCumber.** 1986. Plasmid analysis in molecular epidemiology: a summary and future directions. Rev. Infect. Dis. **8**:738–746.
31. **Taylor, D. N., I. K. Wachsmuth, Y. Shangkuan, E. V. Schmidt, T. J. Barrett, J. S. Schraeder, C. S. Scherach, H. B. McGee, R. A. Feldman, and D. J. Brenner.** 1982. Salmonellosis associated with marijuana. A multistate outbreak traced by plasmid fingerprinting. N. Engl. J. Med. **306**:1249–1253.
32. **Telzak, E. E., L. D. Budnick, M. S. Greenberg, S. Blum, M. Shayegani, C. E. Benson, and S. Schultz.** 1990. A nosocomial outbreak of *Salmonella enteritidis* infection due to the consumption of raw eggs. N. Engl. J. Med. **323**:394–397.
33. **Tompkins, L. S., N. Troup, A. Labigne-Rousell, and M. L. Cohen.** 1986. Cloned, random chromosomal sequences as probes to identify *Salmonella* species. J. Infect. Dis. **154**:156–162.
34. **Wachsmuth, K.** 1986. Molecular epidemiology of bacterial infections: examples of methodology and of investigations of outbreaks. Rev. Infect. Dis. **8**:682–692.
35. **Ward, L. R., E. J. Threlfall, and B. Rowe.** 1990. Multiple drug resistance in salmonellae in England and Wales: a comparison between 1981 and 1988. J. Clin. Pathol. **43**:563–566.
36. **Watanabe, T.** 1964. Selected methods of genetic study of episome-mediated drug resistance in bacteria. Methods Med. Res. **10**:202–220.