ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 2000, p. 1140–1145 Vol. 44, No. 5
0066-4804/00/$04.00+0

Antibiotic Resistance Trends in Enteropathogenic Bacteria Isolated in 1985–1987 and 1995–1998 in Barcelona

GUILLERMO PRATS,* BEATRIZ MIRELIS, TERESA LLOVET, CARMEN MUÑOZ, ELISENDA MIRÓ, AND FERRAN NAVARRO

Departament de Microbiologia, Hospital de la Santa Creu i Sant Pau, Universitat Autònoma 08025 Barcelona, Spain

Received 18 October 1999/Returned for modification 23 December 1999/Accepted 18 January 2000

Trends in resistance to antimicrobial agents used for therapy have been evaluated with 3,797 enteropathogenic bacteria, *Campylobacter*, *Salmonella*, *Shigella*, and *Yersinia*, between 1985–1987 and 1995–1998. The greater increase in the rate of resistance was observed in *Campylobacter jejuni* for quinolones (from 1 to 82%) and tetracycline (from 23 to 72%) and in gastroenteric salmonellae for ampicillin (from 8 to 44%), chloramphenicol (from 1.7 to 26%), and trimethoprim-sulfamethoxazole and nalidixic acid (from less than 0.5 to 11%). Multidrug resistance was detected in several *Salmonella* serotypes. In the 1995–1998 period, 76% of *Shigella* strains were resistant to trimethoprim-sulfamethoxazole, 43% were resistant to ampicillin, and 39% were resistant to chloramphenicol. Seventy-two percent of *Yersinia enterocolitica* O3 strains were resistant to streptomycin, 45% were resistant to sulfonamides, 28% were resistant to trimethoprim-sulfamethoxazole, and 20% were resistant to chloramphenicol.

Campylobacter and *Salmonella* are the bacteria most frequently isolated from patients with sporadic cases of gastroenteritis in our setting, followed, at a smaller proportion, by *Shigella* and *Yersinia* (33). These microorganisms, in particular *Salmonella*, are also responsible for extraintestinal pathologies, such as urinary tract infections, abscesses at diverse locations, and bacteremia (21). Most cases of bacterial gastroenteritis are self-limiting, and it has been suggested that in otherwise healthy patients, administration of antibiotics is not necessary (2); however, for enteritis in infants, elderly people, granulopenic or immunodepressed patients, and also patients with extraintestinal infections, in particular, when bacteremia is suspected, antibiotic therapy is fundamental for illness control (21). The goal of this paper is to review the antimicrobial susceptibilities of *Campylobacter*, *Salmonella*, *Shigella*, and *Yersinia* strains isolated in our hospital from 1995 to 1998 and to compare them with the susceptibilities of those isolated between 1985 and 1987.

MATERIALS AND METHODS

Bacteria: source and identification. The susceptibilities to antibiotics of 1,865 enteropathogenic bacteria belonging to the genera *Campylobacter* (957 strains), *Salmonella* (832 strains), *Shigella* (56 strains), and *Yersinia* (20 strains) isolated from 1995 to 1998 were evaluated. The strains were isolated in the Laboratory of Microbiology of the Hospital de la Santa Creu i Sant Pau, in Barcelona, Catalonia (Spain). Ninety-five percent of the isolates (1,773 strains) were of intestinal origin, and 5% (92 strains) were from extraintestinal sources; a single isolate per patient was included.

For comparison, the susceptibilities of 660 *Campylobacter jejuni*, 1,075 *Salmonella*, 122 *Shigella*, and 75 *Yersinia enterocolitica* strains isolated in our laboratory from 1985 to 1987 were included. Isolation and identification of microorganisms were carried out by standard procedures (25). The serotype and phage type of the *Salmonella* strains were determined in the Servicio de Enterobacterias del Centro Nacional de Microbiología, Instituto Carlos III, Majadahonda, Madrid, Spain.

Antimicrobial susceptibility. Susceptibility was determined by the disk diffusion method, following National Committee for Clinical Laboratory Standards (NCCLS) recommendations (26, 27). Mueller-Hinton agar (Oxoid, Basingstoke, United Kingdom) was used for all strains except the *Campylobacter* strains, for which the agar was enriched with 5% sheep blood. The disks were purchased from Oxoid. Incubation was carried out at 35 to 37°C for 18 h in an aerobic atmosphere for all strains except *Campylobacter* strains, which were incubated under a microaerobic atmosphere (Campy*Gen*; Oxoid) at 35 to 37°C for 24 h. The antibiotics tested varied according to the microorganism and included those used for therapy. In general, ampicillin, amoxicillin-clavulanic acid (co-amoxiclav), cefotaxime, erythromycin, chloramphenicol, tetracycline, streptomycin, kanamycin, neomycin, gentamicin, tobramycin, sulfamethoxazole, trimethoprim, trimethoprim-sulfamethoxazole, nalidixic acid, and ciprofloxacin were included. For selected strains, MICs were determined by a microdilution technique with Sensititre microtrays (Sensititre Ltd., Imberhorne, United Kingdom) or the E test (AB Biodisk NA, Piscataway, N.J.). The breakpoints used were those defined by NCCLS (26, 27) for members of the family *Enterobacteriaceae*. The only exception was for erythromycin, for which the breakpoints for *Staphylococcus* were used.

Escherichia coli ATCC 25922, *E. coli* ATCC 35218, and *Staphylococcus aureus* ATCC 25923 were used as control strains for susceptibility studies.

RESULTS

***Campylobacter*.** During the 1995–1998 period, 909 *Campylobacter jejuni* strains and 48 *Campylobacter coli* strains were studied; among these strains, only 15 *C. jejuni* strains were of extraintestinal origin (12 from blood and 3 from ascitic fluid). These two species are the enteropathogenic bacteria most frequently isolated in our laboratory and the only ones with an increased incidence relative to that in the previous decade (1985–1987).

Table 1 shows the susceptibilities to antimicrobial agents of the *C. jejuni* strains isolated in the two time periods evaluated. Erythromycin, co-amoxiclav, and gentamicin were active against *C. jejuni*, but the rates of resistance to tetracyclines and quinolones increased from 23 to 72% and from 1 to 82%, respectively, and in the later period the rate of resistance to ciprofloxacin reached 81%. Although *C. coli* was less frequently isolated, it was more resistant than *C. jejuni*. Among the 48 *C. coli* strains isolated during 1995–1998, 44 (92%) strains were resistant to erythromycin, 47 (98%) were resistant to tetracycline, 9 (19%) were resistant to gentamicin, and 45 (94%) were resistant to nalidixic acid and ciprofloxacin. However, all 48 strains were susceptible to co-amoxiclav.

***Salmonella*.** During the 1995–1998 period, 832 salmonellae were studied: 756 were isolated from the stools of patients with

* Corresponding author. Mailing address: Departament de Microbiologia, Hospital de la Santa Creu i Sant Pau, Av. Sant Antoni Mª Claret, 167, 08025 Barcelona, Spain. Phone: 34 93 2919071. Fax: 34 93 2919070. E-mail: 2175@hsp.santpau.es.

TABLE 1. Evolution of resistance in *C. jejuni* between 1985–1987 and 1995–1998[a]

Years	No. of strains	No. (%) of resistant strains				
		ERY	TET	GEN	NAL	CIP
1985–1987	660	31 (5)	152 (23)	0 (0)	8 (1)	ND
1995–1998	909	46 (5)	655 (72)	11 (1)	743 (82)	739 (81)

[a] All strains isolated during both time periods were fully susceptible to co-amoxiclav. Abbreviations: CIP, ciprofloxacin; ERY, erythromycin; GEN, gentamicin; NAL, nalidixic acid; TET, tetracycline; ND, not determined.

gastroenteritis, 53 were isolated from blood, 17 were isolated from urine, and 6 were isolated from other sources. The most frequent serotypes were as follows: Enteritidis, 382 (46%) strains; Typhimurium, 275 (33%) strains; Hadar, 44 (5%) strains; Virchow, 15 (2%) strains; and Brandenburg, 13 (1.6%) strains. These five serotypes represented 88% of the *Salmonella* strains isolated; the remaining (12%) belonged to 35 additional serotypes. During the triennium from 1985 to 1987, 1,075 salmonellae were isolated, with Enteritidis (60%), Virchow (8%), Typhimurium (7%), Blockley (3.2%), and Bredeney (2%), being the most prevalent (82%) serotypes.

Table 2 summarizes the resistance of all *Salmonella* isolates and the isolates of the predominant serotypes to nine antimicrobial agents during the two periods of time considered.

During the 1995–1998 period, 44% of the strains were resistant to ampicillin, 42% were resistant to tetracycline, 26% were resistant to chloramphenicol, 11% were resistant to trimethoprim-sulfamethoxazole and nalidixic acid, and 6% showed decreased susceptibility to co-amoxiclav. One nontypeable strain for which the ciprofloxacin MIC was intermediate (2 mg/liter) was isolated from an AIDS patient who had been receiving therapy with norfloxacin for 2 years. Another ciprofloxacin-resistant serovar Enteritidis strain (MIC, 32 mg/liter) was selected in vivo while the patient was receiving fluoroquinolone treatment.

A single strain that belonged to serotype Virchow and that was highly resistant to cefotaxime (MIC, 16 mg/liter) was detected. The strain produced an extended-spectrum beta-lactamase with an isoelectric point of about 8 and had a positive PCR result when two pairs of primers specific for CTX-M-7 were used (the sequence was obtained from GenBank, accession no. AF174129).

Ampicillin resistance was observed in all five prevalent serotypes; discrete incidences of resistance to co-amoxiclav in strains of serotypes Typhimurium (15%) and Hadar (4.5%) and to trimethoprim-sulfamethoxazole in strains of serotypes

TABLE 2. Evolution of antibiotic resistance in *Salmonella* serotypes (1985–1987 and 1995–1998)

Serotype and period	No. of strains	No. (%) of resistant strains[a]								
		AMP	AMC	CTX	CHL	TET	SXT	GEN	NAL	CIP
Enteritidis										
1985–1987	648	56 (9)	3 (0.5)	0 (0)	1 (0.1)	1 (0.1)	0 (0)	1 (0.1)	1/435[e] (0.2)	0 (0)
1995–1998	382	79 (21)	0 (0)	0 (0)	0 (0)	21 (5)	3 (0.8)	6 (1.6)	21 (5)	1 (0.3)
Typhimurium										
1985–1987	79	4 (5)	1 (1.3)	0 (0)	5 (6)	1 (1.3)	0 (0)	0 (0)	0 (0)	0 (0)
1995–1998	275	233 (85)	41 (15)	0 (0)	191 (69)	244 (89)	61 (22)	16 (6)	3 (1.1)	0 (0)
Hadar										
1985–1987	7	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1995–1998	44	24 (55)	2 (4.5)	0 (0)	0 (0)	42 (95)	2 (4.5)	0 (0)	38 (86)	0 (0)
Virchow										
1985–1987	90	8 (9)	0 (0)	0 (0)	6 (7)	0 (0)	1 (1)	6 (7)	0 (0)	0 (0)
1995–1998	15	2 (13)	0 (0)	1[b] (7)	0 (0)	0 (0)	1 (7)	1 (7)	7 (47)	0 (0)
Brandenburg										
1985–1987	13	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1995–1998	13	9 (69)	0 (0)	0 (0)	10 (77)	10 (77)	10 (77)	4 (31)	8 (62)	0 (0)
Other serotypes										
1985–1987[c]	238	16 (7)	1 (0.4)	0 (0)	6 (2.5)	9 (4)	4 (1.7)	0 (0)	0 (0)	0 (0)
1995–1998[d]	103	23 (22)	5 (5)	0 (0)	14 (14)	31 (30)	18 (17)	0 (0)	11 (11)	1 (1)
Total (all serotypes)										
1985–1987	1,075	84 (8)	5 (0.5)	0 (0)	18 (1.7)	11 (1)	5 (0.5)	7 (0.7)	1/684[f] (0.1)	0 (0)
1995–1998	832	370 (44)	48 (6)	1[b] (0.1)	215 (26)	348 (42)	95 (11)	27 (3)	88 (11)	2 (0.2)

[a] Abbreviations AMC, co-amoxiclav; AMP, ampicillin; CHL, chloramphenicol; CTX, cefotaxime; CIP, ciprofloxacin; GEN, gentamicin; NAL, nalidixic acid; TET, tetracycline; SXT, trimethoprim-sulfamethoxazole.

[b] Extended-spectrum beta-lactamase-producing strain.

[c] The number of strains of each serotype was as follows: Blockley, 35; Bredeney, 23; Infantis, 16; Heidelberg, 11; Ohio, 10; Panama, 9; Newport, 8; Goldcoast and London, 7 each; Anatum and Sofia, 4 each; Mikawasima, Montevideo, and Tilburg, 3 each; Saintpaul, Kapemba, and Livingstone, 2 each; Agona, Newbrunswik, Litchfield, Hindmarsh, Newington, Richmond, Goettingen, Muenchen, Derby, Give, and Tournai, 1 each; untypeable or not typed, 78.

[d] The number of strains of each serotype was as follows: Infantis, 9; Muenchen, 6; Ohio, 5; Newport, Heidelberg, Grumpensis, and Derby, 4 each; Bredeney, Mbandaka, and Braenderup, 3 each; Saintpaul, Litchfield, Mikawasima, Richmond, Goldcoast, Give, Bradford, Albany, Oranienburg, Toulon, Washington, Rissen, and Rideau, 2 each; Blockley, London, Agona, Anatum, Kapemba, Goettingen, Tshiongwe, Indiana, Singapore, Nima, Havana, and Tambacounda, 1 each; untypeable or not typed, 20.

[e] Only 435 of the 648 strains were tested for nalidixic acid.

[f] Only 684 of the 1,075 strains were tested for nalidixic acid.

TABLE 3. Antibiotic resistance and phage types of 275 *S. enterica* serotype Typhimurium strains isolated from 1995 to 1998[a]

Phage type	No. of strains	No. (%) of resistant strains												
		AMP	AMC	TET	SUL	TMP	SXT	CHL	STR	KAN	NEO	GEN	TOB	NAL
104	48	48 (100)	3 (6)	46 (96)	46 (96)	3 (6)	2 (4)	45 (94)	46 (96)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
104 b	30	30 (100)	4 (13)	28 (93)	30 (100)	5 (17)	5 (17)	22 (73)	30 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
193	30	18 (60)	4 (13)	26 (87)	24 (80)	16 (53)	16 (53)	6 (20)	25 (83)	5 (17)	5 (17)	0 (0)	0 (0)	1 (3.3)
120	21	21 (100)	5 (24)	21 (100)	20 (95)	4 (19)	4 (19)	13 (62)	20 (95)	4 (19)	3 (14)	0 (0)	0 (0)	0 (0)
195	9	6	1	6	5	5	5	4	4	3	3	1	1	0
10	9	9	1	9	9	9	9	8	9	0	0	9	9	0
U-302	5	4	1	5	5	1	1	5	5	0	0	0	0	0
12	4	2	0	4	3	1	1	1	2	0	0	0	0	0
124	4	3	0	3	4	2	2	3	2	1	1	0	0	1
204	2	0	0	2	0	0	0	0	1	0	0	0	0	0
204 c	1	1	0	1	1	1	1	1	1	0	0	0	0	0
161	2	1	0	1	1	0	0	1	1	0	0	0	0	0
197	1	1	0	1	1	1	1	0	1	1	1	0	0	0
184	1	1	0	1	1	0	0	0	1	0	0	0	0	0
179	1	1	1	1	1	1	0	1	1	1	0	0	0	0
Other[b]	6	0	0	0	0	0	0	0	0	0	0	0	0	0
NSP	19	16 (84)	7 (37)	16 (84)	16 (84)	2 (11)	2 (11)	14 (74)	16 (84)	1 (5)	2 (11)	0	0	1 (5)
NT	82	73 (89)	14 (17)	76 (93)	76 (93)	12 (15)	12 (15)	67 (82)	75 (91)	0	0	6 (7)	6 (7)	0

[a] All strains were susceptible to cefotaxime and ciprofloxacin. Abbreviations: AMC, co-amoxiclav; AMP, ampicillin; CHL, chloramphenicol; GEN, gentamicin; KAN, kanamycin; NAL, nalidixic acid; NEO, neomycin; STR, streptomycin; SUL, sulfonamides; TET, tetracycline; TOB, tobramycin; TMP, trimethoprim; SXT, trimethoprim-sulfamethoxazole; NSP, nonstandard pattern; NT, untypeable.
[b] Other phage types: 22, 23, 29, 52, and 99.

Typhimurium (22%) and Brandenburg (77%) were observed; and a high percentage of resistance to nalidixic acid was observed in strains of serotypes Hadar (86%), Brandenburg (62%), and Virchow (47%). Nalidixic acid resistance was also detected in strains of other serotypes (Enteritidis, $n = 21$ strains; Typhimurium, $n = 3$; Blockley, $n = 1$; Heidelberg, $n = 1$; untypeable strains $n = 3$; and not typed, 4 strains). For most nalidixic acid-resistant strains MICs were >256 mg/liter; the exceptions were one serotype Heidelberg strain (MIC, 64 mg/liter) and all resistant serotype Brandenburg isolates, for which the MICs were in the intermediate range (16 to 32 mg/liter). All but two of these nalidixic acid-resistant strains were susceptible to ciprofloxacin according to the NCCLS criteria. However, the MICs for the strains were 5- to 80-fold those for four susceptible serotype Typhimurium strains included as controls (0.06 to 1 versus 0.008 to 0.012 mg/liter).

Besides, 18 strains of serotypes Typhimurium, Hadar, and Mikawasima had diminished susceptibilities to cefotaxime and ceftazidime (MICs, between 1 and 4 mg/liter). It is obvious from Table 2 that in the last period, serotypes Typhimurium and Brandenburg were the most resistant serotypes but similar multidrug-resistant patterns were found in serotypes Heidelberg (2 strains), Bredeney (1 strain), and Goldcoast (1 strain).

The phage types of the vast majority of serotype Enteritidis and Typhimurium strains isolated since 1996 were determined. A correlation between the phage type and the resistance phenotype was observed. In serotype Enteritidis strains, ampicillin resistance was associated with phage types 6 and 6a (25 of 27 and all 37 strains, respectively) and resistance to nalidixic acid was associated with phage type 1 (15 of 57 strains). Among the serotype Typhimurium strains the predominant pattern of multidrug resistance was ampicillin, chloramphenicol, sulfamethoxazole, tetracycline, and streptomycin resistance. This pattern was found for nearly all phage types. However, certain phage types contained a small number of strains (Table 3).

***Shigella*.** Throughout the 1995–1998 period, 56 *Shigella* strains (30 *S. sonnei*, 19 *S. flexneri*, 3 *S. boydii*, 2 *S. dysenteriae*, and 2 *Shigella* strains) were isolated from patients with enteritis. Twenty-seven were from patients with traveler's diarrhea (11 *S. sonnei*, 9 *S. flexneri*, 3 *S. boydii*, 2 *S. dysenteriae*, and 2 *Shigella* strains), and 29 were autochthonous isolates. When comparing the susceptibilities of the indigenous strains (*S. flexneri* and *S. sonnei*) with those that were imported, no significant differences were observed. The susceptibilities of both species are shown in Table 4; data for the remaining species are not shown. Among all these strains, we did not find resistance to fluoroquinolones or broad-spectrum cephalosporins. However, they showed high rates of resistance—with variations depending on the time period and species—to trimethoprim-sulfamethoxazole (48 to 90%), ampicillin (13 to 89%), and also chloramphenicol (1 to 95%) (Table 4). Only seven *S. flexneri* strains were resistant to co-amoxiclav in the two periods of evaluation (11 and 21%).

***Yersinia*.** All pathogenic *Yersinia enterocolitica* strains isolated in our laboratory belonged to serogroup O3 (biotype 4). From 1985 to 1987, we isolated 75 strains. They were all resistant to ampicillin and cephalothin but were susceptible to co-amoxiclav, cefotaxime, tetracyclines, gentamicin, and nalidixic acid. Seventy-two percent were resistant to streptomycin, 45% were resistant to sulfonamides, 28% were resistant to tri-

TABLE 4. Resistance to antibiotics in *S. sonnei* and *S. flexneri* in the two periods studied[a]

Species[b]	Years	No. of strains	No. (%) of resistant strains					
			AMP	AMC	CHL	GEN	SXT	NAL
S. sonnei	1985–1987	95	48 (51)	0 (0)	1 (1)	0 (0)	54 (57)	ND
	1995–1998	30	4 (13)	0 (0)	1 (3.3)	0 (0)	27 (90)	1 (3)
S. flexneri	1985–1987	27	19 (70)	3 (11)	2 (7)	1 (4)	13 (48)	ND
	1995–1998	19	17 (89)	4 (21)	18 (95)	0 (0)	10 (53)	1 (5)

[a] All strains were susceptible to cefotaxime and ciprofloxacin. Abbreviation: AMC, co-amoxiclav; AMP, ampicillin; CHL, chloramphenicol; GEN, gentamicin; SXT, trimethoprim-sulfamethoxazole; NAL, nalidixic acid; ND, not determined.
[b] The imported strains (traveler's diarrhea) were *S. sonnei* ($n = 11$) and *S. flexneri* ($n = 9$).

methoprim-sulfamethoxazole, and 20% were resistant to chloramphenicol. Only 20 strains were isolated throughout the period from 1995 to 1998. All were resistant to ampicillin and cephalothin but were susceptible to co-amoxiclav, cefotaxime, gentamicin, and ciprofloxacin. The rate of resistance increased up to 90% for streptomycin and sulfonamides, 70% for trimethoprim-sulfamethoxazole, 60% for chloramphenicol, and 5% for nalidixic acid.

DISCUSSION

Campylobacter is the leading cause of bacterial enteritis in Spain and elsewhere (3, 33), and *C. jejuni* accounts for 95% of enteric *Campylobacter* infections. In 1987, *C. jejuni* was generally susceptible to clinically useful antimicrobial agents (Table 1). However, 8 years later most strains became resistant to tetracyclines, nalidixic acid, and the fluoroquinolones. The similar proportions of resistance to nonfluorinated and fluorinated quinolones (82 versus 81%) and the speed at which this level of resistance has been reached (23) are probably related and are due to the fact that a single mutation in either topoisomerase can confer resistance to both nonfluorinated and fluorinated quinolones (11, 13, 37, 50). Similar data have been reported from other locations in Spain (36, 39) and elsewhere (1, 10, 32, 40).

In our laboratory, ampicillin has not been tested regularly against *Campylobacter* because it is not recommended for therapy due to the high incidence of resistance (28), essentially by beta-lactamase production (18, 19). However, in a small number of isolates studied since 1985, an increase in the number of beta-lactamase-producing strains was observed. In 1985, 120 *C. jejuni* strains were tested for beta-lactamase synthesis by using the nitrocefin test and 23% were positive; in 1998, 108 of 130 (83%) strains were positive by the same method. All strains were susceptible to co-amoxiclav; clavulanic acid inhibits some beta-lactamases and has been claimed to have intrinsic antibacterial activity against campylobacter (46). Erythromycin, as well as gentamicin, which has been recommended for use in bacteremic patients (21), remained active (Table 1).

C. coli is only slightly more resistant to quinolones and tetracycline than *C. jejuni* but is resistant to erythromycin (92%) and gentamicin (19%). This is therefore not only a characteristic of strains isolated in developing countries (3) but is also probably a current or potential global concern. Despite differences in the incidence of resistance to macrolides and aminoglycosides in *C. jejuni* and *C. coli*, the mechanisms of resistance to these antibiotics are probably similar in both species (44, 45).

Throughout our 7-year study, we detected in salmonellae an increase in the rates of resistance to ampicillin (from 8 to 44%), tetracycline (from 1 to 42%), chloramphenicol (from 1.7 to 26%), trimethoprim-sulfamethoxazole (from 0.5 to 11%), and nalidixic acid (from 0.1 to 11%) (Table 2).

In regard to the correlation between serotypes and resistance, it is obvious that serotypes Typhimurium and Brandenburg are multidrug resistant but that other serotypes such as Bredeney, Goldcoast, and Heidelberg are also multidrug resistant. Besides, the Hadar, Brandenburg, and Virchow serotypes are associated with nalidixic acid resistance, but this resistance was also found in strains of other serotypes (data not shown).

Currently, serotype Enteritidis is the most prevalent serotype in western Europe (8). In Spain the change from a greater prevalence of serotype Typhimurium to a greater prevalence of serotype Enteritidis observed over the 1970s and the 1980s (6, 34) concerned only fully susceptible strains. At present, the prevalence of resistant serotype Typhimurium strains is increasing, but the frequency of serotype Brandenburg, another resistant serotype, has not increased.

Multidrug resistance in *Salmonella enterica* serotype Typhimurium phage type DT 104 has been the subject of many studies (1, 5, 12, 47). However, numerous phage types of serotype Typhimurium are also multidrug resistant, such as phage types 104 b, 193, 120, 195, U-302, and others (Table 3). Of particular interest is phage type 10, which is not a strictly serotype Typhimurium but which is a monophasic strain, 1,4,5,12:i:−(7). This serotype is resistant to nine antibiotics, including streptomycin, gentamicin, and tobramycin, and its ability to spread in the future remains open.

Relatively elevated co-amoxiclav MICs (16/8 to 32/16 mg/liter) have been found in only 41 serotype Typhimurium strains, 2 serotype Hadar strains, and 5 strains of other serotypes. The presence of several beta-lactamases in these strains, OXA-1, and PSE-1 and TEM-1 hyperproduction (identified by pI, PCR with specific primers, and enzyme kinetics; data not shown) account for this resistance. Some of these strains also display a slight decrease in susceptibility to broad-spectrum cephalosporins (see above). The Virchow strain with a high level of resistance to broad-spectrum cephalosporins produced an extended-spectrum beta-lactamase.

Multidrug resistance in phage type DT 104 in England includes resistance to quinolones (48), but in our study, this phage type remains susceptible to these drugs; however, besides its multidrug resistance, the three first-line treatment antibiotics (trimethoprim-sulfamethoxazole, ciprofloxacin, and cefotaxime) are active against DT 104. Similar results were reported in the United States (12). The clonal character of nalidixic acid resistance can be seen in *S. enterica* serotype Enteritidis phage type 1. This observation differs from what we found in *E. coli* of both human and animal origin, in which resistance to quinolones was not clonal (9).

At present, in developed countries the prevalent *Shigella* species are *S. sonnei* and *S. flexneri* (33). Roughly, about half of the shigellae (27 of 56) isolated in our laboratory over the 1995–1998 period were from travelers, and the susceptibilities of both autochthonous and imported strains were similar. This is not always the case, and various patterns of resistance have been described, depending on the country of origin, but the rates of resistance to tetracycline and trimethoprim-sulfamethoxazole are generally higher in imported strains (43, 49). Our autochthonous shigellae, however, were also highly resistant to the latter antimicrobial agents. Four of 19 (21%) *S. flexneri* strains were resistant to co-amoxiclav. The MIC of co-amoxiclav against these strains ranged from 16/8 to 24/12 mg/liter. Reduced susceptibility or resistance to co-amoxiclav in *E. coli* has been attributed to chromosomal beta-lactamase, TEM-1, TEM-2, or SHV-1 hyperproduction, and production of OXA-1 or an IRT (inhibitor-resistant TEM-derived beta-lactamases) (M. Sabaté, F. Navarro, C. Vergés, E. Miró, and G. Prats, Abstr. 18th Interdisciplinary Meet. Anti-Infectious Chemother., abstr. 252/C24, p. 177, 1998). The seven *S. flexneri* strains with diminished susceptibility to co-amoxiclav produced an OXA-1 beta-lactamase (pI 7.4 and a positive PCR result with specific primers; data not shown).

Although different resistance patterns are observed at different locations, resistance to trimethoprim-sulfamethoxazole, ampicillin, and tetracycline has been observed worldwide (16, 17, 38, 49). It is difficult to ascertain the selective pressures for maintenance of the different resistance patterns. It is worth pointing out that only 2 of 56 (3.5%) strains were resistant to nalidixic acid and none was resistant to ciprofloxacin. This is in contrast to *E. coli*, in which the rate of resistance to fluoroquinolones rose from 0.5 to 21% during the same period.

Perhaps this is due to the fact that *E. coli* undergoes selective pressure by quinolones both in humans and in animals but *Shigella* undergoes selective pressure only in humans. Certain reports, however, show that quinolone resistance in *Shigella* is emerging, and great differences in the rates of resistance have been reported (from 0.3 to higher than 50%) (16).

The worldwide distribution of human pathogenic *Y. enterocolitica* serotypes varies. In the Mediterranean basin, O3 (biotype 4) is the prevalent serogroup (14, 24). The drug of choice for the treatment of infections caused by microorganisms of this serogroup has not been identified, but trimethoprim-sulfamethoxazole, tetracycline or fluoroquinolones, and gentamicin or chloramphenicol have been recommended for use in septicemic patients (4).

In the 1995–1998 period, susceptibility to tetracycline, gentamicin, and ciprofloxacin remained, but in respect to the 1985–1987 period, the rates of resistance to trimethoprim-sulfamethoxazole (70%) and chloramphenicol (60%) rose. Also, 18 of 20 strains (90%) were resistant to both streptomycin and sulfonamides.

Several publications from 1977 to 1995 (15, 22, 30, 31, 42) that have described the results of studies with *Y. enterocolitica* strains of various biotypes and from different locations have reported heterogeneous results for susceptibility to beta-lactam antibiotics, but in all the studies performed *Y. enterocolitica* was found to be susceptible to the non-beta-lactam antibiotics, with only anecdotal resistance to sulfonamides, streptomycin, or chloramphenicol. However, the sulfonamide-streptomycin resistance association was also observed by Soriano and Vega (41) in 17% of 72 O3 strains in 1982 and by Pérez Trallero et al. (29) in 46% of 68 O3 strains in 1986. These data suggest the presence of an integron (20).

Tetracycline resistance is very common in most members of the family *Enterobacteriaceae* and has been considered the antibiotic to which resistance is most extended in nature (35), with the rate of resistance being greater than 70% in *E. coli* in our laboratory. By contrast, *Y. enterocolitica* remains susceptible to tetracycline, and a similar observation can be made for quinolones.

In conclusion, for nearly all enteropathogenic bacteria isolated in our laboratory the rates of resistance to the antibiotics studied increased between the periods studied.

ACKNOWLEDGMENTS

We thank Patrice Courvalin for critical reading of the manuscript and M. A. Usera, A. Echeita, and A. Aladueña from the Servicio de Enterobacterias del Centro Nacional de Microbiología, Instituto Carlos III, Majadahonda, Madrid, Spain, for determination of the serotypes and phage types of the *Salmonella* strains. We thank also R. Solé, M. del Cuerpo, P. Alvarez, and N. Vigil for excellent technical support and C. Vergés and M. Sabaté for preliminary data on resistance mechanisms.

This work was supported by grants 98/1522 and 98/1293 from the Fondo de Investigaciones Sanitarias de la Seguridad Social de España.

REFERENCES

1. **Aubry-Damon, H., and P. Courvalin.** 1999. Bacterial resistance to antimicrobial agents: selected problems in France, 1996 to 1998. Emerg. Infect. Dis. **5:**315–320.
2. **Beers, M. H., and R. Berkow (ed.).** 1999. The Merck manual of diagnosis and therapy, 17th ed. Merck Research Laboratories, Whitehouse Station, N.J.
3. **Blaser, M. J.** 2000. *Campylobacter* and related species, p. 2276–2285. *In* G. L. Mandell, J. E. Bennett, and R. Dolin (ed.), Mandell, Douglas and Bennett's principles and practice of infectious diseases, 5th ed. Churchill Livingstone, Philadelphia, Pa.
4. **Butler, T.** 2000. *Yersinia* species, including plague, p. 2406–2414. *In* G. L. Mandell, J. E. Bennett, and R. Dolin (ed.), Mandell, Douglas and Bennett's principles and practice of infectious diseases. 5th ed. Churchill Livingstone, Philadelphia, Pa.
5. **Centers for Disease Control and Prevention.** 1997. Multidrug-resistant *Salmonella* serotype Typhimurium—United States, 1996. Morbid. Mortal. Weekly Rep. **46:**308–310.
6. **Echeita, M. A., and M. A. Usera.** 1989. Prevalence of *Salmonella* serotypes isolated in Spain from human and non human sources (1983–1987). Microbiologia SEM **5:**95–103.
7. **Echeita, M. A., and M. A. Usera.** 1998. Rapid identification of *Salmonella* spp. Phase 2 antigens of the H1 antigenic complex using "multiplex PCR". Res. Microbiol. **149:**757–761.
8. **Fisher, I. S. T., on Behalf of the Salm-Net Participants.** 1997. *Salmonella enteritidis* and *Salmonella typhimurium* in Western Europe for 1993–1995, a surveillance report from Salm-Net. Eurosurveillance **2:**4–6.
9. **Garau, J., M. Xercavins, M. Rodríguez-Carballeira, J. R. Gómez-Vera, I. Coll, D. Vidal, T. Llovet, and A. Ruiz-Bremon.** 1999. Emergence and dissemination of quinolone-resistant *Escherichia coli* in the community. Antimicrob. Agents Chemother. **43:**2736–2741.
10. **Gaudreau, C., and H. Gilbert.** 1998. Antimicrobial resistance of clinical strains of *Campylobacter jejuni* subsp. *jejuni* isolated from 1985 to 1997 in Quebec, Canada. Antimicrob. Agents Chemother. **42:**2106–2108.
11. **Gibreel, A., E. Sjögren, B. Kaijser, B. Wretlind, and O. Sköld.** 1998. Rapid emergence of high-level resistance to quinolones in *Campylobacter jejuni* associated with mutational changes in *gyrA* and *parC*. Antimicrob. Agents Chemother. **42:**3276–3278.
12. **Glynn, M. K., C. Bopp, W. Dewitt, P. Dabney, M. Mokhtar, and F. J. Angulo.** 1998. Emergence of multidrug-resistant *Salmonella enterica* serotype Typhimurium DT 104 infections in the United States. N. Engl. J. Med. **338:** 1333–1338.
13. **Gootz, T. D., and B. A. Martin.** 1991. Characterization of high-level quinolone resistance in *Campylobacter jejuni*. Antimicrob. Agents Chemother. **35:** 840–845.
14. **Gurguí, M., B. Mirelis, P. Coll, and G. Prats.** 1987. *Yersinia enterocolitica* infections and pork. Lancet **ii:**234.
15. **Hornstein, M. J., A. M. Jupeau, M. R. Scavizzi, A. M. Philippon, and P. A. D. Grimont.** 1985. In vitro susceptibilities of 126 clinical isolates of *Yersinia enterocolitica* to 21 β-lactam antibiotics. Antimicrob. Agents Chemother. **27:** 806–811.
16. **Kelly, P., and M. J. G. Farthing.** 1997. Infections of the gastro-intestinal tract, p. 708–720. *In* F. O'Grady, H. P. Lambert, R. G. Finch, and D. Greenwood (ed.), Antibiotics and chemotherapy, 7th ed. Churchill Livingstone, New York, N.Y.
17. **Keusch, G. T. (ed.).** 1991. Workshop on invasive diarrheas, shigellosis and dysentery. Rev. Infect. Dis. **13**(Suppl. 4)**:**S219–S365.
18. **Kucers, A., S. M. Crowe, M. L. Grayson, and J. F. Hoy (ed.).** 1997. The use of antibiotics, 5th ed. Butterworth Heinemann, Oxford, United Kingdom.
19. **Lachance, N., C. Gaudreau, F. Lamothe, and F. Turgeon.** 1993. Susceptibilities of beta-lactamase positive and negative strains of *Campylobacter coli* to beta-lactam agents. Antimicrob. Agents Chemother. **37:**1174–1176.
20. **Lévesque, C., L. Piché, C. Larose, and P. H. Roy.** 1995. PCR mapping of integrons reveals several novel combinations of resistance genes. Antimicrob. Agents Chemother. **39:**185–191.
21. **Mandell, G. L., J. E. Bennett, and R. Dolin (ed.).** 2000. Mandell, Douglas and Bennett's principles and practice of infectious diseases, 5th ed. Churchill Livingstone, Philadelphia, Pa.
22. **Matthew, M., G. Cornelis, and G. Wauters.** 1977. Correlation of serological and biochemical groupings of *Y. enterocolitica* with the β-lactamases of the strains. J. Gen. Microbiol. **102:**55–59.
23. **Mirelis, B., E. Miró, F. Navarro, C. A. Ogalla, J. Bonal, and G. Prats.** 1993. Increased resistance to quinolone in Catalonia, Spain. Diagn. Microbiol. Infect. Dis. **16:**137–139.
24. **Mollaret, H. H., H. Bercovier, and J. M. Alonso.** 1979. Summary of the data received at the WHO Reference Center for *Yersinia enterocolitica*. Contrib. Microbiol. Immunol. **5:**174–184.
25. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1999. Manual of clinical microbiology, 7th ed. ASM Press, Washington, D.C.
26. **National Committee for Clinical Laboratory Standards.** 1984. Performance standards for antimicrobial disk susceptibility tests, 3th ed. Approved standard. NCCLS Document M2-A3. National Committee for Clinical Laboratory Standards, Wayne, Pa.
27. **National Committee for Clinical Laboratory Standards.** 1995. Performance standards for antimicrobial susceptibility testing; sixth informational supplement. NCCLS Document M100-S6. National Committee for Clinical Laboratory Standards, Wayne, Pa.
28. **Navarro, F., E. Miró, B. Mirelis, and G. Prats.** 1993. *Campylobacter* spp. antibiotic susceptibility. J. Antimicrob. Chemother. **32:**906–907.
29. **Pérez Trallero, E., C. Zigorraga, G. Cilla, P. Idigoras, C. López Lopategui, and L. Solaun.** 1988. Animal origin of the antibiotic resistance of human pathogenic *Yersinia enterocolitica*. Scand. J. Infect. Dis. **20:**573.
30. **Pham, J. N., S. M. Bell, M. J. Hardy, L. Martin, A. Guiyoule, and E. Carniel.** 1995. Susceptibility to β-lactam agents of *Yersinia enterocolitica* biotype 4, serotype O3 isolated in various parts of the world. J. Med. Microbiol. **43:** 9–13.

31. **Pham, J. N., S. M. Bell, and J. Y. M. Lanzarone.** 1991. A study of the β-lactamases of 100 clinical isolates of *Yersinia enterocolitica*. J. Antimicrob. Chemother. **28:**19–24.
32. **Piddock, L. J. V.** 1999. Quinolone resistance and *Campylobacter*. Clin. Microbiol. Infect. **5:**239–243.
33. **Prats, G., T. Llovet, C. Muñoz, R. Solé, B. Mirelis, C. Izquierdo, P. Rodriguez, M. E. Sabanés, N. Rabella, R. Pericas, F. Sanchez, N. Margall, F. Navarro, and P. Coll.** 1997. Etiología de las enteritis en un hospital general universitario en Barcelona (1992–1995). Enferm. Infecc. Microbiol. Clin. **15:** 349–356.
34. **Pumarola, A., G. Prats, A. Rodriguez-Torres, and M. Beltrán.** 1973. Consideraciones sobre el aislamiento e identificación de diversos serotipos del género "salmonella" en coprocultivos. Rev. Diagn. Biol. **XXII:**299–301.
35. **Quintiliani, R., D. F. Sham, and P. Courvalin.** 1999. Mechanisms of resistance to antimicrobial agents, p. 1505–1525. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 7th ed. ASM Press, Washington, D.C.
36. **Reina, J., M. J. Ros, and A. Serra.** 1994. Susceptibilities to 10 antimicrobial agents of 1220 *Campylobacter* strains isolated from 1987 to 1993 from feces of pediatric patients. Antimicrob. Agents Chemother. **38:**2917–2920.
37. **Ruiz, J., P. Goñi, F. Marco, F. Gallardo, B. Mirelis, M. T. Jimenez de Anta, and J. Vila.** 1998. Increased resistance to quinolones in *Campylobacter jejuni*: a genetic analysis of *gyrA* gene mutations in quinolone-resistant clinical isolates. Microbiol. Immunol. **42:**223–226.
38. **Sack, R. B., M. Rahman, M. Yunus, and E. H. Khan.** 1997. Antimicrobial resistance in organisms causing diarrheal disease. Clin. Infect. Dis. **24**(Suppl. 1)**:**S102–S105.
39. **Sánchez, R., V. Fernández Baca, M. D. Díaz, P. Muñoz, M. Rodríguez Créixems, and E. Bouza.** 1994. Evolution of susceptibilities of *Campylobacter* spp. to quinolones and macrolides. Antimicrob. Agents Chemother. **38:** 1879–1882.
40. **Smith, K. E., J. M. Besser, C. W. Hedberg, F. T. Leano, J. B. Bender, J. H. Wicklund, B. P. Johnson, K. A. Moore, M. T. Osterholm, and The Investigation Team.** 1999. Quinolone-resistant *Campylobacter jejuni* infections in Minnesota, 1992–1998. N. Engl. J. Med. **340:**1525–1532.
41. **Soriano, F., and J. Vega.** 1982. The susceptibility of *Yersinia* to eleven antimicrobials. J. Antimicrob. Chemother. **10:**543–547.
42. **Stock, I., and B. Wiedemann.** 1999. An in-vitro study of the antimicrobial susceptibilities of *Yersinia enterocolitica* and the definition of a database. J. Antimicrob. Chemother. **43:**37–45.
43. **Tauxe, R. V., N. D. Puhr, J. G. Well, N. Hargrett-Bean, and P. A. Blake.** 1990. Antimicrobial resistance of *Shigella* isolates in the USA: the importance of international travelers. J. Infect. Dis. **162:**1107–1111.
44. **Taylor, D. E.** 1992. Antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* to tetracycline, chloramphenicol and erythromycin, p. 4–86. *In* I. Nachamkin, M. J. Blaser, and L. S. Tompkins (ed.). *Campylobacter jejuni*. Current status and future trends. ASM Press, Washington, D.C.
45. **Taylor, D. E., and P. Courvalin.** 1988. Mechanisms of antibiotic resistance in *Campylobacter* species. Antimicrob. Agents Chemother. **32:**1107–1112.
46. **Tenover, F. C., C. N. Baker, C. L. Fennell, and C. A. Ryan.** 1992. Antimicrobial resistance in *Campylobacter* species, p. 66–73. *In* I. Nachamkin, M. J. Blaser, and L. S. Tompkins (ed.), *Campylobacter jejuni*. Current status and future trends. ASM Press, Washington, D.C.
47. **Threlfall, E. J., J. A. Frost, L. R. Ward, and B. Rowe.** 1996. Increasing spectrum of resistance in multiresistant *Salmonella typhimurium*. Lancet **347:** 1052–1053.
48. **Threlfall, E. J., L. R. Ward, and B. Rowe.** 1999. Resistance to ciprofloxacin in nontyphoidal salmonellas from humans in England and Wales—the current situation. Clin. Microbiol. Infect. **5:**130–134.
49. **Vila, J., J. Gascón, S. Abdalla, J. Gómez, F. Marco, A. Moreno, M. Corachan, and M. T. Jimenez de Anta.** 1994. Antimicrobial resistance of *Shigella* isolates causing traveler's diarrhea. Antimicrob. Agents Chemother. **38:** 2668–2670.
50. **Wang, Y., W. M. Huang, and D. E. Taylor.** 1993. Cloning and nucleotide sequence of the *Campylobacter jejuni gyrA* gene and characterization of quinolone resistance mutations. Antimicrob. Agents Chemother. **37:**457–463.