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Antimicrobial Resistance of *Salmonella* Isolates in a Spanish Hospital

PATRICIA MUÑOZ,* M. DOLORES DÍAZ, MARTA RODRÍGUEZ-CRÉIXEMS, EMILIA CERCENADO, TERESA PELÁEZ, AND EMILIO BOUZA

Servicio de Microbiología Clínica, Hospital General Gregorio Marañón, 28007 Madrid, Spain

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We studied 961 clinical *Salmonella* isolates (one per patient) seen in one Spanish hospital from 1988 to 1991. The incidence of non-*Salmonella typhi Salmonella* infections per 100,000 admissions increased from 3.93 to 5.98. Overall rates of resistance to ampicillin, chloramphenicol, and co-trimoxazole were 32, 11, and 2%, respectively. Resistance to chloramphenicol increased from 9 to 16% during the study period, while resistance to each of the other drugs remained stable. Variations related to serogroups were observed.

Salmonella infections constitute an important problem around the world and may require treatment with antibiotics able to reach high intracellular concentrations (7). Unfortunately, multiresistant *Salmonella* strains are increasingly common and have become an issue of worldwide concern with variable geographical incidence (9, 19).

Salmonellae resistant to antimicrobial agents are common in Spain, a country with many foreign visitors each year. In this article, we report the evolution of the antimicrobial resistance of the *Salmonella* strains isolated at our institution during the last 4 years.

(This work was presented in part at the 31st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, Ill., October 1991 [18].)

Ours is a 2,300-bed teaching institution of the city of Madrid, Spain, serving a predominantly urban population with a low-medium socioeconomic level. All clinical *Salmonella* isolates obtained at our institution from 1988 to 1991 were included in this study.

Identification was made according to standard microbiological procedures (14) by means of the automated MicroScan Autoscan 4 method (Baxter Laboratories, West Sacramento, Calif.). The identification was confirmed by serogrouping with commercial antisera (Difco Laboratories, Detroit, Mich.). In addition, a total of 218 (23%) of 965 strains were sent to a reference national laboratory for further serotyping (Centro Nacional de Microbiología, Virología e Inmunología Sanitarias).

Antimicrobial susceptibility testing was performed according to the criteria of the National Committee for Clinical Laboratory Standards (Villanova, Pa.) by using a broth microdilution automated method (Microscan Autoscan 4 panels). In order to detect salmonellae resistant to extended-spectrum cephalosporins, we determined the MICs of cephazolin, cefotaxime, ceftazidime, and amoxicillin-clavulanate by an agar dilution technique (National Committee for Clinical Laboratory Standards) using concentrations of these antimicrobial agents ranging from 0.06 to 128 μg/ml (20, 21).

Statistical significance of quantitative variables was analyzed by means of the chi-square test, considering P values of <0.05 (two-tailed) significant (25).

During the study, we isolated 1,671 salmonellae, of which 52 strains were *Salmonella typhi*, with all of them being susceptible to the antimicrobial agents tested. Hereafter, we will refer only to the remaining 1,619 (961 patients) nontyphoid salmonellae, considering a single isolate per patient (961 isolates from 961 patients). Table 1 summarizes the increasing number of cases and the distribution of serogroups per year. Origins of isolates were stool (82.6%), blood (14%), urine (2%), and others (1.4%).

Isolates belonging to serogroup $B_{4,5}$ have showed a significant increase, rising from 20 (10%) in 1988 to 66 (24%) in 1991 ($P < 0.001$), while the rate of isolation for serogroup D_9 decreased from 84 to 67% ($P < 0.001$). Further serotyping of strains demonstrated that serogroup D_9 (159 strains studied) corresponded to *Salmonella enteritidis*, serogroup $B_{4,5}$ (46 strains studied) corresponded to *Salmonella typhimurium* (74%), *Salmonella bredeney* (24%), and *Salmonella brandenburg* (2%), and serogroup C_7 (19 strains studied) corresponded to *Salmonella virchow* (89%) and *Salmonella ohio* (11%).

Overall antimicrobial resistance and its evolution are summarized in Table 2; 32% of our strains were resistant to ampicillin, 11% were resistant to chloramphenicol, and 2% were resistant to trimethoprim-sulfamethoxazole. Resistance to chloramphenicol increased from 9% in 1988 to 16% in 1991 ($P < 0.05$), and resistance to tetracycline increased from 8% in 1988 to 19% in 1991. When we studied chloramphenicol resistance monthly, no evidence of an epidemic outbreak was observed (data not shown). Rates of resistance to other drugs have remained stable.

Although resistance to amoxicillin-clavulanic acid is low (4%), 20% of our sensitive strains exhibited an intermediate MIC of 16/8 μg/ml (16 μg of amoxicillin per ml plus 8 μg of clavulanic acid per ml). Resistance to cefazolin and aminoglycosides was uncommon (data not shown). The most frequent pattern of multiresistance was ampicillin plus chloramphenicol, which has increased from 4% in 1988 to 15% in 1991 ($P < 0.05$). None of our strains were resistant to extended-spectrum cephalosporins or to fluoroquinolones.

In our media, serogroups $B_{4,5}$ and C_7 are significantly more resistant than serogroup D_9 ($P < 0.001$) (Table 3). It is noteworthy that 38% of our serogroup $B_{4,5}$ salmonellae were simultaneously resistant to ampicillin and chloramphenicol and 3% were additionally resistant to trimethoprim-sulfamethoxazole. During the years covered by the study,

* Corresponding author.

TABLE 1. Serogroups of non-*S. typhi Salmonella* isolates[a]

Serogroup(s)	No. (%) of isolates[b]				
	1988 [191, 3.93]	1989 [226, 4.72]	1990 [270, 5.81]	1991 [274, 5.98]	Total [961]
D_9	161 (84)	153 (68)	203 (76)	184 (67)	701 (73)[c]
$B_{4,5}$	20 (10)	54 (24)	52 (19)	66 (24)	192 (20)[c]
C_7	7 (4)	19 (8)	12 (4)	17 (6)	55 (6)
Others	3 (2)	0 (0)	3 (1)	7 (2)	13 (1)

[a] One isolate per patient.
[b] The first value in brackets is the total number of patients from whom isolates were obtained in the corresponding year; the second value (if given) is the number of such patients per 100,000 admissions.
[c] $P < 0.001$ (1988 versus 1991).

serogroups $B_{4,5}$ and C_7 have become more resistant, while serogroup D_9 isolates became more susceptible.

An increase in *Salmonella* infections has been reported in recent years for many geographic areas, including countries with very high public health and hygienic standards (23). The knowledge of the resistance patterns for different areas of the world can be very useful in our era of frequent overseas travelling.

Our results show that although gastrointestinal infections remain the most common, 18% of our patients had a variety of extraintestinal infections, as reported elsewhere (8).

The relative proportion of *Salmonella* serogroups and serotypes isolated at a single geographic area or hospital is very variable (5, 16, 27). Our study, as well as other studies, proves that different serogroups have different patterns of resistance, with serogroups $B_{4,5}$ and C_7 usually being more resistant than serogroup D_9 (6).

Our study shows an overall percent resistance to ampicillin of 32%; this rate has reached up to 45.8% in other areas of Spain (2, 22). In other countries, ampicillin resistance has ranged from 1.5% in New Zealand to 85.4% in Iran (10, 12, 17).

Chloramphenicol resistance was present in 11% of our strains, and it is also a problem in North Africa, South America, and Asia, where it reached 10% (7, 28).

Co-trimoxazole resistance was only 2% in our study, as in other Spanish institutions (2, 22), and represents a good oral alternative against ampicillin-resistant strains. However, higher resistance has been reported in Asia and South America (24).

More important among our data is the high percent combined resistance to ampicillin and chloramphenicol, which increased from 4 to 15%. This situation, occasionally described for other geographic areas, may be sporadic or epidemic (15). In our study, we could not demonstrate evidence of an epidemic outbreak to explain this situation.

In the presence of a severe infection caused by a multiresistant strain, the main therapeutic alternatives are new quinolones and extended-spectrum cephalosporins (3). Both have demonstrated excellent bactericidal effects in vivo and in vitro, even against organisms resistant to multiple drugs (1, 4, 26). Although occasionally described, resistance of salmonellae to these agents remains exceptional (11, 13). In our experience, none of the strains was resistant to extended-spectrum cephalosporins or to ciprofloxacin.

In conclusion, in our media, resistance of salmonellae to antimicrobial agents is high, and in the case of chloramphenicol resistance, it is significantly increasing. A continuous surveillance of susceptibility of salmonellae to antimicrobial agents and a more prudent and restricted use of them, in both humans and animals, are desirable.

TABLE 2. Overall resistance of non-*S. typhi Salmonella* isolates to antimicrobial agents[a]

Antimicrobial agent(s)[b]	No. (%) of resistant isolates				
	1988	1989	1990	1991	Total
AMP	56 (30)	76 (34)	86 (32)	83 (30)	301 (32)
CHL	18 (9)	23 (10)	24 (9)	43 (16)	108 (11)[c]
TMP-SMZ	3 (2)	6 (3)	6 (2)	7 (3)	22 (2)
Amoxicillin-clavulanate	11 (6)	12 (5)	8 (3)	10 (4)	41 (4)
Tetracycline	15 (8)	38 (17)	45 (17)	53 (19)	151 (15)
Cefazolin	1 (0.5)	3 (1)	1 (0.4)	0	5 (0.5)
AMP + CHL	7 (4)	16 (7)	20 (7)	40 (15)	83 (9)[c]
AMP + CHL + TMP-SMZ	2 (1)	2 (1)	3 (1)	1 (0.4)	8 (1)

[a] One isolate per patient.
[b] AMP, ampicillin; CHL, chloramphenicol; TMP-SMZ, trimethoprim-sulfamethoxazole.
[c] $P < 0.05$ (1988 versus 1991).

TABLE 3. Evolution of resistance to different antimicrobial agents by serogroup[a]

Antimicrobial agent(s)[b]	Serogroup(s)	No. (%) of resistant isolates				
		1988	1989	1990	1991	Total
AMP	D_9	47 (29)	41 (27)	45 (22)	29 (16)	162 (23)[c]
	$B_{4,5}$	6 (30)	27 (50)	35 (67)	43 (65)	111 (58)[c]
	C_7	3 (43)	8 (42)	3 (25)	10 (59)	24 (44)
	Others	0	0	3 (100)	1 (14)	4 (31)
CHL	D_9	9 (6)	1 (0.6)	1 (0.5)	2 (1)	13 (2)[c]
	$B_{4,5}$	2 (10)	20 (37)	22 (42)	37 (56)	81 (42)[c]
	C_7	5 (71)	2 (10)	1 (8)	4 (24)	12 (22)[c]
	Others	2 (66)	0	0	0	2 (15)
TMP-SMZ	D_9	2 (0.01)	2 (1)	4 (2)	3 (2)	11 (2)
	$B_{4,5}$	0	3 (5)	1 (2)	4 (6)	8 (4)
	C_7	1 (14)	1 (5)	1 (8)	0	3 (5)
AMP + CHL	D_9	1 (0.6)	0	1 (0.5)	1 (0.5)	3 (0.4)
	$B_{4,5}$	4 (20)	14 (26)	20 (38)	35 (53)	73 (38)[c]
	C_7	2 (28)	2 (10)	0	3 (17)	7 (13)[c]
AMP + CHL + TMP-SMZ	D_9	0	0	0	1 (0.5)	1 (0.14)
	$B_{4,5}$	0	3 (5)	3 (6)	0	6 (3)
	C_7	0	1 (5)	0	0	1 (2)

[a] One isolate per patient.
[b] AMP, ampicillin; CHL, chloramphenicol; TMP-SMZ, trimethoprim-sulfamethoxazole.
[c] $P < 0.05$ (1988 versus 1991).

REFERENCES

1. **Ahmad, F., G. Bray, R. W. G. Prescott, S. Aquilla, and N. F. Lightfoot.** 1991. Use of ciprofloxacin to control a salmonella outbreak in a long-stay psychiatric hospital. J. Hosp. Infect. **17**:171–178.
2. **Alos, J. I., R. Gonzalez-Palacios, M. P. Sanchez-Moreno, and P. Calderon.** 1990. Alta frecuencia de elevada resistencia a ampicilina en *Salmonella* spp. no *typhi*. Med. Clin. (Barcelona) **95**:175–177.
3. **Bryan, J. P., H. Rocha, and W. M. Scheld.** 1986. Problems in salmonellosis: rationale for clinical trials with newer β-lactam agents and quinolones. Rev. Infect. Dis. **8**:189–207.
4. **Campos, L. C., E. M. dos Reis, and J. C. Ribeiro Dias.** 1990. In

vitro susceptibility of *Salmonella* to ciprofloxacin and perfloxacin compared with three other antibiotics. Chemotherapy (Basel) **36**:29–32.
5. **Centers for Disease Control.** 1988. *Salmonella* surveillance summary for 1986. Centers for Disease Control, Atlanta.
6. **Cherubin, C. E.** 1981. Antibiotic resistance of *Salmonella* in Europe and the United States. Rev. Infect. Dis. **3**:1105–1126.
7. **Cherubin, C. E.** 1990. Susceptibility testing, antimicrobial resistance and clinical response in *Salmonella* infections. Infect. Dis. Newsl. **9**:1–4.
8. **Cohen, J. I., J. A. Bartlett, and G. R. Corey.** 1987. Extraintestinal manifestations of *Salmonella* infections. Medicine (Baltimore) **66**:349–388.
9. **Cohen, M. L., and R. V. Tauxe.** 1986. Drug-resistant *Salmonella* in the United States: an epidemiologic perspective. Science **234**:964–969.
10. **Farhoudi-Moghaddam, A. A., M. Katouli, A. Jafari, M. A. Bahavar, M. Parsi, and F. Malekzadeh.** 1990. Antimicrobial drug resistance and resistance factor transfer among clinical isolates of salmonellae in Iran. Scand. J. Infect. Dis. **22**:197–203.
11. **Garbarg-Chenon, A., H. Vu Thien, R. Labia, H. Ben-Yaghlane, V. Godard, P. Deny, F. Bricout, and J. C. Nicolas.** 1989. Characterization of a plasmid coding for resistance to broad-spectrum cephalosporins in *Salmonella typhimurium*. Drugs Exp. Clin. Res. **15**:145–150.
12. **Heffernan, H. M.** 1991. Antibiotic resistance among *Salmonella* from human and other sources in New Zealand. Epidemiol. Infect. **106**:17–23.
13. **Hof, H., I. Ehrhard, and H. Tschäpe.** 1991. Presence of quinolone resistance in a strain of *Salmonella typhimurium*. Eur. J. Clin. Microbiol. Infect. Dis. **10**:747–749.
14. **Kelly, M. T., D. J. Brenner, and J. J. Farmer III.** 1985. *Enterobacteriaceae*, p. 263–277. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
15. **Lamb, V. A., C. G. Mayhall, A. C. Spadora, S. M. Markowitz, J. J. Farmer III, and H. P. Dalton.** 1984. Outbreak of *Salmonella typhimurium* gastroenteritis due to an imported strain resistant to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole in a nursery. J. Clin. Microbiol. **20**:1076–1079.
16. **Lester, A., N. H. R. Eriksen, H. Nielsen, P. B. Nielsen, A. Friis-Moller, B. Bruun, J. Scheibel, K. Gaarslev, and H. J. Kolmos.** 1991. Non-typhoid *Salmonella* bacteremia in Greater Copenhagen, 1984 to 1988. Eur. J. Clin. Microbiol. Infect. Dis. **10**:486–490.
17. **Lorian, V.** 1986. *Salmonella* susceptibility patterns in hospitals from 1975 through 1984. J. Clin. Microbiol. **23**:826–827.
18. **Muñoz, P., M. Díaz, M. Rodriguez-Creixéms, J. Baraia, J. Bernaldo de Quirós, P. Nieto, and C. Fron.** 1991. Antimicrobial resistance of clinical isolates of non-typhoid *Salmonella*. Program Abstr. 31st Intersci. Conf. Antimicrob. Agents Chemother., abstr. 110.
19. **Murray, B. E.** 1986. Resistance of *Shigella*, *Salmonella*, and other selected enteric pathogens to antimicrobial agents. Rev. Infect. Dis. **8**:S172–S181.
20. **National Committee for Clinical Laboratory Standards.** 1985. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. M7-A. National Committee for Clinical Laboratory Standards, Villanova, Pa.
21. **National Committee for Clinical Laboratory Standards.** 1991. Performance standards for antimicrobial susceptibility testing. M100-S3. National Committee for Clinical Laboratory Standards, Villanova, Pa.
22. **Pérez de León, A., C. Pérez, D. Ferrer, M. Jordán, and M. Gobernado.** 1990. *Salmonella*: resistencias frente a 3 antibióticos de elección. Enferm. Infec. Microbiol. Clin. **8**:446–448.
23. **Rodrigue, D. C., R. V. Tauxe, and B. K. Rowe.** 1990. International increase in *Salmonella enteritidis*; a new pandemic? Epidemiol. Infect. **105**:21–27.
24. **Rowe, B., J. A. Frost, E. J. Trelfall, and L. R. Ward.** 1980. Spread of a multi-resistant clone of *Salmonella typhimurium* phage type 66/122 in South-East Asia and Middle East. Lancet **i**:1070–1071.
25. **Sackett, D. L., R. B. Haymes, and L. Tugwell.** 1985. Clinical epidemiology: a basic science for clinical medicine. Little, Brown & Co., Boston.
26. **Soe, G. B., and G. D. Overturf.** 1987. Treatment of typhoid fever and other systemic salmonelloses with cefotaxime, ceftriaxone, cefoperazone and other newer cephalosporins. Rev. Infect. Dis. **97**:19–36.
27. **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.
28. **Williams, J. D., F. Moosdeen, C. H. Teoh-Chan, V. K. Lim, and P. Jayanetra.** 1989. Surveillance of antibiotic resistance in South East Asia. Eur. J. Epidemiol. **5**:207–213.