

Antimicrobial resistance in non-typhoidal *Salmonella* from human sources, Spain, 2001–2003

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Objectives: To determine the current state of antimicrobial resistance among non-typhoidal *Salmonella* strains isolated from humans in Spain.

Methods: All strains of *Salmonella* from human sources received in the reference laboratory from 2001 to 2003 were serotyped and phage types were determined in the most common serovars. A systematic sampling procedure was carried out in order to obtain a random sample for susceptibility testing. The selected strains were tested for susceptibility to 12 different antimicrobial agents by a disc diffusion method using Mueller–Hinton agar. Results were scored as susceptible, moderately susceptible or resistant, according to CLSI criteria.

Results: From 2001 to 2003, 5777 strains of *Salmonella* were tested for susceptibility. Fifty per cent of strains of *Salmonella* Enteritidis were resistant to nalidixic acid. This was the most frequent resistance pattern of this serovar and it was characteristic of PT1, the most frequent phage type of *Salmonella* Enteritidis in Spain. Seventy-four per cent of *Salmonella* Typhimurium strains were resistant to four antibiotics or more. Resistance to ampicillin, chloramphenicol, streptomycin, sulphonamide and tetracycline was the most frequent resistance pattern of *Salmonella* Typhimurium and it was characteristic of DT104, the most frequent phage type in Spain. Sixty-nine per cent of *Salmonella* Hadar strains were resistant to at least four antibiotics.

Conclusions: The results of our study showed both a worrying percentage of strains of *Salmonella* Enteritidis resistant to nalidixic acid and of strains of *Salmonella* Typhimurium with a pattern of resistance to four antibiotics or more. Surveillance of antimicrobial resistance should carry on and improve in order to be able to evaluate the control measures carried out for decreasing resistance in *Salmonella*, specifically that addressed to the prudent use of antimicrobial agents by farmers and veterinarians.

Keywords: salmonellosis, zoonoses, drug-resistant

Introduction

Salmonella is the most commonly notified cause of bacterial diarrhoea in Spain, with an average of 7621 cases reported from 2000 to 2004 (data from Spanish Microbiological Information System). Non-typhoidal salmonellosis is usually a self-limiting disease; however, it can give rise to severe disease, especially septicaemia. The annual average number of hospital admissions

due to salmonellosis in Spain from 1999 to 2002 was 6143 and in the majority of these cases effective antimicrobial treatment may have been essential for recovery.

The surveillance of salmonellosis in Spain is carried out by three systems. Two of them are based on the microbiological diagnoses made by the laboratories. The Microbiological Information System is a basic system and its source is the laboratories of clinical microbiology (mainly hospital laboratories).

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The second, the Spanish National Reference Laboratory of *Salmonella* and *Shigella* (LNRSSSE) is complementary and analyses the strains sent voluntarily from the clinical microbiology laboratories. The third surveillance system is the Outbreaks Reporting System, which obtains data on outbreaks of salmonellosis. Surveillance of antimicrobial resistance is only carried out by the LNRSSSE, which uses the same methods as the European countries that participate in the International Surveillance Network for the Enteric Infections *Salmonella* and VTEC O157 (Enter-net). This network monitors, amongst other things, the antimicrobial resistances of the human isolates of *Salmonella*.¹ Spain has participated in Enter-net since its beginning in 1994.

We conducted a retrospective study of antimicrobial resistance of non-typhoidal *Salmonella* strains isolated from human sources from 2001 to 2003 in order to assess the current state of this problem in Spain.

Materials and methods

All the 21526 strains of *Salmonella* from human sources received in the LNRSSSE from 2001 to 2003 were serotyped by standard methods.² Phage types were determined in the most common serovars (*Salmonella enterica* serovar Enteritidis, *Salmonella enterica* serovar Typhimurium and *Salmonella enterica* serovar Hadar).³⁻⁵ A systematic sampling procedure was carried out in order to obtain a random study sample. This involved the selection of one of every three strains received according to order of arrival in the laboratory. Only one strain per person and per outbreak was included in the sample. The selected strains were tested for susceptibility to 12 different antimicrobial agents by a disc diffusion method using Mueller-Hinton agar. The respective quantities ($\mu\text{g}/\text{disc}$) of the active compounds were as follows: ampicillin, 10; chloramphenicol, 30; sulphonamide, 300; streptomycin, 10; tetracycline, 30; gentamicin, 10; kanamycin, 30; nalidixic acid, 30; ciprofloxacin, 5; cefotaxime, 30; cefalotin, 30;

trimethoprim/sulfamethoxazole, 25. Results were scored as susceptible, moderately susceptible or resistant, according to CLSI criteria.⁶ *Escherichia coli* ATCC 25922 was used as a reference strain.

All statistical analyses were performed using the STATA computer software program (version 8.2).

Results

From 2001 to 2003, 5777 strains were tested for susceptibility: 60.4% were *Salmonella* Enteritidis, 21% *Salmonella* Typhimurium, 2.5% *Salmonella* Hadar and the remaining 16.1% were 111 different serovars. The proportion of serovars tested was similar to the proportion of serovars received in the laboratory.

The strains tested came from all but one of the 19 regions of Spain; 51.3% of them were from males and 60.6% from people younger than 14 years (31.5% younger than 4 years). The sample could not be representative of the Spanish population as a whole since the strains are sent to the LNRSSSE voluntarily from the clinical microbiology laboratories.

The frequency of resistance to individual drugs and multiple resistance is shown in Table 1. For *Salmonella* the most common resistances detected were to nalidixic acid, ampicillin, tetracycline and sulphonamide. Of the strains, <5% presented resistance to gentamicin, kanamycin and cefalotin and <1% to ciprofloxacin and cefotaxime. Five strains were moderately susceptible (zone diameter of 15–21 mm) to ciprofloxacin. The distribution of zone diameters for ciprofloxacin (Figure 1) showed two peaks of frequency. The distribution for the strains resistant to nalidixic acid presented only one peak which fitted in with the less susceptible peak of the total distribution. Of the strains, 34.3% were susceptible to all the antimicrobial agents studied and 21.6% were resistant to four antibiotics or more.

A total of 3491 strains of *Salmonella* Enteritidis were tested. Of the strains <5% showed resistance to all the antimicrobial

Table 1. Non-typhoidal *Salmonella* isolates from human sources. Percentage of strains resistant to antimicrobials, Spain, 2001–2003

Antimicrobial agents	Total <i>Salmonella</i> <i>n</i> = 5777	<i>Salmonella</i> Enteritidis <i>n</i> = 3491	<i>Salmonella</i> Typhimurium <i>n</i> = 1211	<i>Salmonella</i> Hadar <i>n</i> = 147
Ampicillin	28.4	11.7	76.0	70.1
Chloramphenicol	14.2	0.5	56.4	0.0
Sulphonamide	22.3	2.7	78.7	1.4
Gentamicin	2.6	1.0	3.1	0.0
Ciprofloxacin	0.0	0.0	0.0	0.0
Kanamycin	1.6	1.1	1.2	1.4
Streptomycin	19.5	1.8	61.4	89.1
Cefalotin	3.2	1.0	1.9	57.1
Nalidixic acid	35.0	49.9	7.5	91.2
Cefotaxime	0.1	0.0	0.2	0.0
Tetracycline	27.3	5.0	80.4	91.8
Co-trimoxazole	6.0	1.7	10.7	0.7
Number of resistances				
None	34.3	37.3	13.5	4.7
One	36.8	55.2	6.2	1.4
Two	4.1	4.4	3.3	0.0
Three	3.2	1.4	3.2	25.2
Four or more	21.6	1.7	73.8	68.7

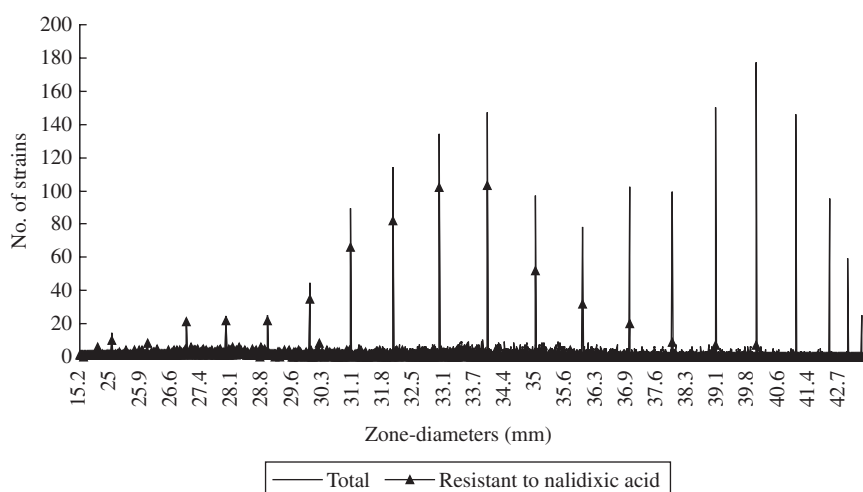


Figure 1. Non-typhoidal *Salmonella* isolates from human sources. Distribution of zone diameters for ciprofloxacin, Spain, 2001–2003.

Table 2. *Salmonella* Enteritidis ($n = 3491$); percentage of strains resistant to antimicrobials according to phage type, Spain, 2001–2003

Antimicrobial agents	Phage type (number of strains)					
	1 (1383)	4 (599)	21 (280)	6A (268)	6 (157)	other (804)
Ampicillin	2.9	0.5	6.4	74.6	45.9	9.5
Chloramphenicol	0.2	0.2	0.0	0.4	0.6	1.2
Sulphonamide	1.4	0.7	1.4	2.6	1.9	7.2
Gentamicin	1.2	0.5	1.8	0.4	0.0	1.2
Ciprofloxacin	0.0	0.0	0.0	0.0	0.0	0.0
Kanamycin	0.1	0.0	0.4	2.6	0.0	3.5
Streptomycin	1.0	0.2	1.1	0.8	0.6	5.2
Cefalotin	1.7	0.2	0.7	0.0	0.0	1.1
Nalidixic acid	89.1	4.5	58.9	24.3	7.6	29.9
Cefotaxime	0.1	0.0	0.0	0.0	0.0	0.0
Tetracycline	1.2	0.7	10.0	6.7	1.9	13.2
Co-trimoxazole	0.7	0.3	1.1	2.2	1.3	4.5

agents studied except for nalidixic acid and ampicillin with 49.9% and 11.7% of strains resistant, respectively; 37.3% of the strains were susceptible to all the antimicrobials and resistance to four antibiotics or more was unusual (1.7% of the strains). The resistances found according to the phage types of *Salmonella* Enteritidis are shown in Table 2. The most common phage types were PT1, PT4, PT21, PT6A and PT6. In general, PT4 isolates were more susceptible to drugs than isolates of the other phage types. PT1 and PT21 isolates showed resistance to nalidixic acid in 89.1% and 58.9% of the strains, respectively, and most PT6 and PT6A isolates (45.9% and 74.6%, respectively) were resistant to ampicillin. The most common pattern was nalidixic acid resistance (45.2% of the strains) and 74.4% of the strains with this pattern showed the phage type PT1.

A total of 1211 strains of *Salmonella* Typhimurium were tested. *Salmonella* Typhimurium is a more resistant serovar than *Salmonella* Enteritidis (13.5% of the strains were susceptible to all the antimicrobial agents) (Table 1), especially to tetracycline,

Table 3. *Salmonella* Typhimurium ($n = 1077$); most frequent resistance patterns detected and the predominant associated phage types, Spain, 2001–2003

Resistance pattern ^a	Number of strains (%)	Phage type most common in the resistance pattern	Number and percentage of strains with that phage type
ACSSuT	403 (37.4)	104	118 (29.3%)
ASSuT	153 (14.2)	193	62 (40.5%)
ACSSuTNx	42 (4.0)	104	17 (40.5%)
T	40 (3.7)	208	17 (42.5%)
ACSSuTSxt	23 (2.1)	104	11 (47.8%)
ASuTSxt	15 (1.4)	193	10 (66.7%)
Other	401 (37.2)		

^aA, ampicillin; C, chloramphenicol; S, streptomycin; Su, sulphonamide; T, tetracycline; Sxt, trimethoprim/sulfamethoxazole; Nx, nalidixic acid.

sulphonamide, ampicillin, streptomycin and chloramphenicol. However, more than 90% of the strains were susceptible to the rest of the antimicrobials studied. Resistance to four antibiotics or more was very frequent (73.8% of the strains) and 47.2% were resistant to five or more drugs. The most frequent phage types were DT104 (180 strains), DTU302 (180 strains), DT104b (140 strains), DT193 (128 strains) and DT208 (33 strains). DT104 was more resistant to nalidixic acid (15.0%) and chloramphenicol (87.8%) in comparison with the other phage types (6% and 30.7%, respectively). DT208 was the most susceptible although it showed high resistance to tetracycline (97.0%) and co-trimoxazole (21.2%). The most frequent resistance pattern was ampicillin/chloramphenicol/streptomycin/sulphonamide/tetracycline ('ACSSuT') and the phage type that presented this pattern with most frequency was DT104 (Table 3).

A total of 147 strains of *Salmonella* Hadar were tested (Table 1). *Salmonella* Hadar is a more resistant serovar than Enteritidis and Typhimurium, especially to ampicillin (70.1%

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of the strains), streptomycin (89.1%), cefalotin (57.1%), nalidixic acid (91.2%) and tetracycline (91.8%); 95.3% of the strains showed resistance to at least one antimicrobial agent and 68.7% to four antibiotics or more. The most frequent resistance pattern was streptomycin/tetracycline/nalidixic acid/cefalotin (49.0% of the strains) and the second most frequent resistance pattern was streptomycin/tetracycline/nalidixic acid (21.5%).

Discussion

Salmonella enterica is one of the most important zoonotic pathogens in which resistance to multiple antimicrobial agents is becoming more and more frequent.⁷ Also, a number of studies have shown increased morbidity and mortality for infections with resistant strains.^{8,9} In our study resistance to four antibiotics or more was found in 21.6% of the total of the studied strains. Ciprofloxacin and cefotaxime are the antimicrobial agents recommended for the treatment of invasive infections due to *Salmonella* and hence the current interest in the emergence of resistance to these antimicrobial agents.^{10,11} In the present study these resistances were encountered in very low percentages, 0.12% for cefotaxime and 0.01% for ciprofloxacin. However, resistance to nalidixic acid was frequent, with 35.0% of the strains presenting it. Although we do not know the MICs of ciprofloxacin, our results suggest, as well as other authors,^{12,13} that the breakpoint for ciprofloxacin is high and this could be distorting the real situation. In comparison with the results published by Enter-net corresponding to data of 10 European countries in 2000,¹⁴ our results showed a low percentage of strains resistant to sulphonamide (22% in Spain versus 30% in Europe) and a higher percentage of resistance to nalidixic acid (36% in Spain versus 14% in Europe). The participants in the European study were mainly northern European countries and perhaps the results would have been different if more southern European countries had participated.

Salmonella Enteritidis is a comparatively susceptible serovar showing a percentage of resistance to at least four antibiotics lower than 2% and resistances lower than 5% for all the antimicrobial agents studied except for ampicillin (11.7%) and nalidixic acid (49.9%). Both results were significantly higher than those found in the European study, which showed percentages of resistance of 6% and 13%, respectively.¹⁴ Resistance to nalidixic acid (nalidixic acid resistance is the most frequent resistance pattern of *Salmonella* Enteritidis) is characteristic of the PT1, which is the most frequent phage type of *Salmonella* Enteritidis in Spain, both in human and food and animal samples.^{15–18} Resistance to nalidixic acid in human samples corresponded with the high percentage found in food and animal samples^{19–22} and it may be associated with the past use in Spain of quinolones, such as enrofloxacin, in cattle, pigs and poultry and difloxacin in poultry.

Salmonella Typhimurium is the serovar that showed the highest percentage of strains with resistance to four antibiotics or more, the most frequent resistance pattern being ACSSuT. The phage type which appeared most frequently with this resistance pattern was DT104. *Salmonella* Typhimurium DT104 R-type ACSSuT has been frequent in industrialized countries since the mid-1980s when it spread internationally.²³ When our results are compared with other European countries¹⁴ significant differences are found for ampicillin, chloramphenicol, streptomycin

and tetracycline, with the Spanish percentages being higher than the corresponding European figures. Also more strains showed resistance to at least four antibiotics in Spain than in the European study (74% versus 51%).

During the 3 years of this study temporal variation was not found in the percentages of resistance for serovar or antimicrobial agent. This stability could be due to the short period of time of analysis (3 years).

The results of our study showed both a worrying percentage of strains of *Salmonella* Enteritidis resistant to nalidixic acid and of strains of *Salmonella* Typhimurium with a resistance pattern ACSSuT. Outbreak investigations in Spain have demonstrated that foods, particularly foods of animal origin, are the most important source of *Salmonella* infections in humans and so the majority of antimicrobial-resistant *Salmonella* infections may be acquired from ingestion of foods contaminated with antimicrobial-resistant *Salmonella*.²⁴ Also, the results of surveillance in Spain of resistance in *Salmonella* in animals,²² although limited as yet, show a high percentage of strains of *Salmonella* Enteritidis resistant to nalidixic acid. Surveillance of *Salmonella* strains for resistance to antimicrobial agents at the animal, food and human levels should carry on and improve in order to identify a potential emergence of resistance patterns and in order to be able to evaluate the control measures carried out for decreasing resistance in *Salmonella*, specifically that addressed to the prudent use of antimicrobial agents by farmers and veterinarians.

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Transparency declarations

None to declare.

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