

Report

Antibiotic resistance of Moroccan strains of *Salmonella enteritidis* isolated between 1996 and 1997

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SUMMARY Antimicrobial resistance is a worldwide problem. The antibiotic resistance of Moroccan strains of *Salmonella enteritidis* was investigated from 1996 to 1997. A total of 51 strains were collected within this period, 31 derived from human sources and 20 from food. Of the 31 human strains, 10 were resistant to antibiotics; 4 were resistant to two or more antibiotics. Of the 20 food strains, 11 were resistant to antibiotics; 6 were resistant to two or more antibiotics. The results are similar to those obtained from strains isolated from other Mediterranean countries.

Introduction

There is a need for worldwide surveillance of antimicrobial-resistant phenotypes of pathogens causing transmissible diseases. Bacterial resistance is a dynamic problem which is increasing in severity in most parts of the world. Misuse and overuse of antibiotics combined with inadequate sanitation and poor hygiene are responsible for the growing level of bacterial resistance.

Antibiotic resistance has long been used as an epidemiological marker for salmonella serovars in many countries [1-3]. *Salmonella enteritidis* was the most frequently isolated serotype in Spain in recent years [4,5], while an increase in the resistance of non-typhi salmonella strains in a Spanish hospital has been observed [6]. The frequency of isolation of *S. enteritidis* and the incidence of antibiotic resistance has recently increased in Greece [7]. In Lithuania, growing resistance of sal-

monella-shigella has also been reported [8]. The correlation between the resistance pattern and the genomic profile has been noted by numerous authors [9-12]. *S. enteritidis* is the serovar responsible for food toxicity in most cases in Europe and Morocco [13-16].

In this study, we investigated the antibiotic resistance of human and food strains of *S. enteritidis* isolated in Morocco between 1996 and 1997.

Materials and methods

A total of 31 human strains of *S. enteritidis* were collected from the stools of patients sent to the Institut National d'Hygiène in Rabat from laboratories belonging to the national network. In addition, 20 food strains of *S. enteritidis* were isolated from different foodstuffs collected by the routine food control system and sent to the Institut National d'Hygiène. The food strains

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were mostly isolated from chicken. All the strains of *S. enteritidis* were collected between January 1996 and December 1997.

The sensitivity of the isolates to antibiotics was determined by the agar disc-diffusion method using Muller-Hinton medium [17]. The antibiotics tested belonged to the families of aminosides, cephalosporins, penicillins, phenicols, sulfamides, tetracyclines and quinolones. The concentrations of antibiotics per disc were as follows: doxycycline (30 µg), oxytetracycline (30 µg), tetracycline (30 µg), gentamicin (10 µg), ampicillin (10 µg) amoxicillin (10 µg), amoxicillin-clavulanic acid [amoxicillin (20 µg), clavulanic acid (10 µg)], nalidixic acid (30 µg), ciprofloxacin (5µg), norfloxacin (10 µg), ofloxacin (5 µg), chloramphenicol (30 µg), trimethoprim-sulfamethoxazole [trimethoprim (1.25 µg), sulfamethoxazole (23.75 µg)], cephalotin (30 µg), ceftazidime (30 µg), cefuroxime (30 µg), cefotaxime (30 µg) and ceftriaxone (30 µg).

Strains resistant to more than one antibiotic were considered multiresistant. The proportion of resistant strains was calculated by dividing the number of resistant strains by the number of strains isolated for the same species in the same period. Similarly, the proportion of multiresistant strains was calculated by dividing the number of multiresistant strains by the number of strains isolated for the same species in the same period. The data were entered into a database using *Epi-Info*.

Results

Of the 31 human strains, 10 were resistant to one or more antibiotics. The proportion of resistant strains was 32.2%: 4 strains were resistant to doxycycline (12.9%); 4 to ampicillin (12.9%); 3 to amoxicillin (9.7%); 3 to amoxicillin-clavulanic acid (9.7%); 2

to gentamicin (6.4%); 1 to nalidixic acid (3.2%); 1 to cephalotin (3.2%); and 1 strain was resistant to ceftriaxone (3.2%). All human strains were sensitive to tetracycline, oxytetracycline, chloramphenicol and quinolones such as ciprofloxacin, norfloxacin and ofloxacin (Figure 1). Of the 31 human strains, 4 were resistant to two or more antibiotics. The proportion of multi-resistant human strains was 12.9%. Of the 10 resistant strains, 4 were resistant to doxycycline alone or doxycycline and another antibiotic.

We found 8 different resistant phenotypes for human strains of *S. enteritidis*: doxycycline, ampicillin, cephalotin, ceftriaxone, gentamicin, doxycycline/nalidixic acid, doxycycline/ampicillin/amoxicillin/amoxicillin-clavulanic acid and ampicillin/amoxicillin/amoxicillin-clavulanic acid (Table 1).

Of the 20 food strains, 11 were resistant to one or more antibiotics. The proportion of resistant food strains was 55.0%: 4 strains were resistant to doxycycline (20.0%); 3 to ampicillin (15.0%); 2 to amoxicillin (10.0%); 1 to amoxicillin-clavulanic acid (5.0%); 1 to gentamicin (5.0%); 2 to nalidixic acid (10.0%); 2 to cephalotin (10.0%); 2 to ceftriaxone (10.0%); 1 to cefotaxime (5.0%); 1 to ceftazidime (5.0%); 1 to cefuroxime (5.0%); and 1 to trimethoprim-sulfamethoxazole (5.0%). All food strains were sensitive to tetracycline, oxytetracycline, chloramphenicol, ciprofloxacin, norfloxacin and ofloxacin (Figure 2). Of the 20 food strains, 6 were resistant to two or more antibiotics. The proportion of multi-resistant food strains was 30%.

A total of 9 different resistant phenotypes found were (Table 2): doxycycline, cephalotin, gentamicin, ampicillin/amoxicillin, doxycycline/nalidixic acid, doxycycline/ampicillin, doxycycline/ampicillin/

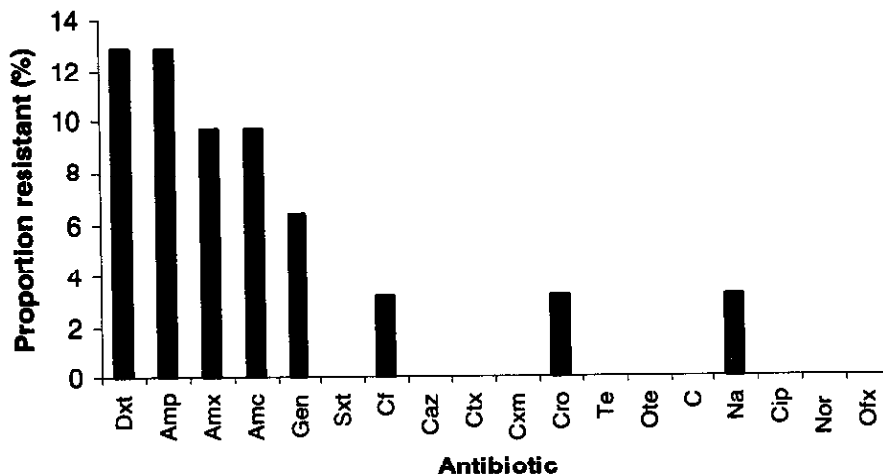


Figure 1 Proportion of resistance to antibiotics in human isolates of *Salmonella enteritidis* (n = 31), Morocco, 1996-97

Dxt: doxycycline, Amp: ampicillin, Amx: amoxicillin, Amc: amoxicillin-clavulanic acid, Gen: gentamicin, Sxt: trimethoprim-sulfamethoxazole, Cf: cephalotin, Caz: ceftazidime, Ctx: cefotaxime, Cxm: cefuroxime, Cro: ceftriaxone, Te: tetracycline, Ote: oxytetracycline, C: chloramphenicol, Na: nalidixic acid, Cip: ciprofloxacin, Nor: norfloxacin, Ofx: ofloxacin

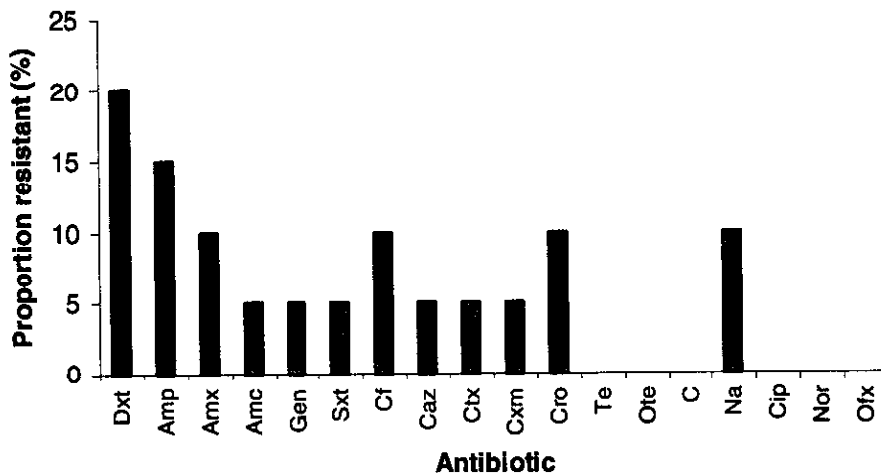


Figure 2 Proportion of resistance to antibiotics in food isolates of *Salmonella enteritidis* (n = 20), Morocco, 1996-97

Dxt: doxycycline, Amp: ampicillin, Amx: amoxicillin, Amc: amoxicillin-clavulanic acid, Gen: gentamicin, Sxt: trimethoprim-sulfamethoxazole, Cf: cephalotin, Caz: ceftazidime, Ctx: cefotaxime, Cxm: cefuroxime, Cro: ceftriaxone, Te: tetracycline, Ote: oxytetracycline, C: chloramphenicol, Na: nalidixic acid, Cip: ciprofloxacin, Nor: norfloxacin, Ofx: ofloxacin

Table 1 Antibiotic resistance phenotypes of *Salmonella enteritidis* in human isolates (n = 31) and their origin, Morocco, 1996-97

Strain	Resistance phenotype	Town	Circumstance
49-97	Dxt	Tetouan	Epidemic
13-96	Amp	Tetouan	Epidemic
4-96	Cf	Agadir	Epidemic
3-96	Cro	Rabat	Epidemic
1-96	Gen	Agadir	Sporadic
8-96	Gen	Al-Hoceima	Sporadic
54-97	DxtNa	Rabat	Sporadic
51-97	DxtAmpAmxAmc	Rabat	Sporadic
35-97	DxtAmpAmxAmc	Rabat	Sporadic
52-97	AmpAmxAmc	Rabat	Sporadic

Dxt: doxycycline, Amp: ampicillin, Cf: cephalotin, Cro: ceftriaxone, Gen: gentamicin, Na: nalidixic acid, Amx: amoxicillin, Amc: amoxicillin-clavulanic acid.

Table 2 Antibiotic resistance phenotypes of *Salmonella enteritidis* in food isolates (n = 20) and their origin, Morocco, 1996-97

Strain	Resistance phenotype	Town	Circumstance
47-97	Dxt	Rabat	Sporadic
46-97	Dxt	Rabat	Sporadic
31-97	Dxt	Rabat	Sporadic
37-97	Cf	Rabat	Epidemic
28-96	Gen	Rabat	Sporadic
36-97	AmpAmx	Rabat	Sporadic
100-97	DxtNa	Rabat	Sporadic
2-96	DxtAmp	Rabat	Sporadic
50-97	DxtAmpAmxAmc	Rabat	Sporadic
27-96	SxtCxmCro	Rabat	Sporadic
32-97	NaCfCroCazCtx	Rabat	Sporadic

Dxt: doxycycline, Cf: cephalotin, Gen: gentamicin, Amp: ampicillin, Amx: amoxicillin, Na: nalidixic acid, Amc: amoxicillin-clavulanic acid, trimethoprim-sulfamethoxazole, Cxm: cefuroxime, Cro: ceftriaxone, Caz: ceftazidime, Ctx: cefotaxime.

amoxicillin/amoxicillin-clavulanic acid, trimethoprim-sulfamethoxazole/cefuroxime/ceftriaxone and nalidixic acid/cephalotin/ceftriaxone/ceftazidime/cefotaxime.

Analysis and discussion

The human strains studied were all sensitive to tetracycline, oxytetracycline, chloramphenicol and quinolones tested. The sensitivity to chloramphenicol was similar to that obtained for Moroccan strains of *S. enteritidis* isolated between 1976 and 1981 [18], for *S. enteritidis* and *S. infantis* serotypes isolated between 1980 and 1991 [19], and for *S. typhi*, *S. typhimurium*, *S. infantis* and *S. virchow* serotypes isolated between 1993 and 1994 [20]. The study conducted between 1980 and 1991 showed that the *S. enteritidis* strains were sensitive to ampicillin, cephalotin, ceftazidime, cefotaxime, gentamicin and nalidixic acid. Our study, conducted 5 years later, shows a change in sensitivity to cephalotin, gentamicin and ampicillin and nalidixic acid, but maintenance of the same sensitivity to ceftazidime and cefotaxime.

The resistance of the human strains to group A penicillins is problematic. Three strains were simultaneously resistant to three different penicillins—ampicillin, amoxicillin and amoxicillin-clavulanic acid. This should be considered in gastroenteritis cases that require antibiotic treatment.

The strains derived from food were all sensitive to chloramphenicol, tetracycline, oxytetracycline and quinolones. One food strain of *S. enteritidis* was resistant to first- and third-generation cephalosporins, while one strain was resistant to second- and third-generation cephalosporins. This phenomenon was specific to food strains of *S. enteritidis*. Resistance to an extended spectrum of cephalosporins has been described in other studies [1].

These patterns of resistance are indicative of the production of a plasmid-encoded beta-lactamase (*dha-1*) that confers a resistance to an extended spectrum of cephalosporins and cephamycins, as has previously been reported in the same serotype of salmonella [1].

We observed that 44.4% of the isolates were from outbreaks and 55.5% from isolated cases. Analysis of geographic origin showed that for both human and food strains, most of the resistant strains originated in Rabat. This is probably not an epidemiological phenomenon but an artefact related to the accessibility to public health facilities. We had no data on age or sex for the human cases.

The incidence of multiresistance did not differ significantly between the food-derived and human strains of *S. enteritidis* (Mantel-Haenszel $\chi^2 = 2.54$, $P = 0.11$). The strains derived from food appeared to be more liable to resistance than the human strains (odds ratio = 1.7, 95% CI: 0.89–3.25). Other studies have given more significant results [7]. Multiresistance was not significantly different between food and human strains ($P = 0.16$).

Three resistant phenotypes were found in both human and food strains: doxycycline, doxycycline/nalidixic acid and doxycycline/ampicillin/amoxicillin/amoxicillin-clavulanic acid. The association of resistance to doxycycline for food and human strains was not significantly different (Mantel-Haenszel $\chi^2 = 2.21$, $P = 0.3$). This is probably due to the small number of strains collected in our study. A strong correlation between antibiotic resistance and doxycycline has been demonstrated for Greek strains of *S. enteritidis* [7].

An increase in resistance to ampicillin in non-typhi strains in a Spanish hospital has also been reported [6]. The association of resistance with doxycycline, the increase in

ampicillin resistance and the resistance to cephalosporins from different generations have all previously been reported in strains isolated from other Mediterranean countries [1,6,7]. These results reveal epidemiological similarities between Mediterranean countries. More precise epidemiological characterization of the strains, for example using ribotyping or rDNA fingerprinting [21,22], is recommended.

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References

- Gaillot O et al. Novel transferable beta lactam resistance with cephalosporinase characteristics in *Salmonella enteritidis*. *Journal of antimicrobial chemotherapy*, 1997, 39(1):85-7.
- McConnell M et al. The value of plasmid studies in the epidemiology of infections due to drug-resistant *Salmonella wien*. *Journal of infectious disease*, 1979, 139:178-90.
- Mered B et al. Epidémie à *Salmonella wien* et *Salmonella typhimurium* dans un service de pédiatrie. Etude bactériologique et épidémiologique. [*Salmonella wien* and *Salmonella typhimurium* epidemic in a pediatric service. Bacteriological and epidemiological study.] *Archives de l'Institut Pasteur Algérie*, 1970, 48:41-52.
- Usera MA et al. Análisis de las cepas de *Salmonella* sp aisladas de muestras clínicas de origen humano en España en el año 1997. [Analysis of *Salmonella* sp. strains of human origin isolated from clinical samples in Spain in 1997.] *Boletín epidemiológico semanal*, 1998, 6(13):129-36.
- Usera MA, Diez R, Echeita A. Análisis de los serotipos de *Salmonella* sp aislados en España en el año 1994. [Analysis of *Salmonella* sp. serotypes isolated in Spain in 1994.] *Boletín epidemiológico semanal*, 1995, 3(15):165-8.
- Campo P et al. Evolution of susceptibility of non-typhi *Salmonella* in a Spanish hospital (1992-1994) and report of a *Salmonella* ser. Typhimurium isolate resistant to quinolones. *European journal of epidemiology*, 1997, 13(2):239-41.
- Tassios PT et al. Molecular epidemiology of antibiotic resistance of *Salmonella enteritidis* during a 7-year period in Greece. *Journal of clinical microbiology*, 1997, 35(6):1316-21.
- Jensen G et al. Antibiotic resistance in *Shigella* and *Salmonella* in a region of Lithuania. *European journal of clinical microbiology and infectious disease*, 1996, 5(11):872-6.
- Esteban E et al. Use of ribotyping for characterization of *Salmonella* serotypes. *Journal of clinical microbiology*, 1993, 31(2):233-7.
- Grimont F, Grimont PAD. Ribosomal ribonucleic acid gene restriction patterns as potential taxonomic tools. *Annales de l'Institut Pasteur. Microbiologie*, 1986, 137B(2):165-75.
- Panigrahi D, Al-Aneziz AH, West PWJ. Plasmid-mediated multidrug resistance in *Salmonella typhi* in Kuwait. *Tropical medicine and international health*, 1996, 1(4):439-42.
- Pignato S et al. Molecular typing of *Salmonella enterica* subsp. *enterica* serovar Wien by rRNA gene restriction patterns. *Research in microbiology*, 1992, 143(7):703-9.
- Benouda A et al. Epidémiologie des salmonelles non typhoïdiques. [Epidemiology of non-typhi salmonella.] *Revue*

- marocaine de biologie-infectiologie*, 1997, III(3):42-6.
14. Doherty L et al. An outbreak of *Salmonella enteritidis* phage type 4 in a rural community in Northern Ireland. *Communicable disease report review*, 1997, 7(5):R73-6.
 15. Holtby I et al. Outbreak of *Salmonella enteritidis* phage type 6 infection associated with food items provided at a buffet meal. *Communicable disease report review*, 1997, 7(6):R87-90.
 16. Majtanova L. Occurrence of *Salmonella enterica* serotype Enteritidis phage types in the Slovak Republic. *European journal of epidemiology*, 1997, 13(2): 243-5.
 17. Bauere AW et al. Antibiotic susceptibility testing by a standardized simple disk method. *American journal of clinical pathology*, 1966, 45:493-6.
 18. Benmansour N, Baaj A, Zouhdi M. Etude bactériologique de 2792 souches de salmonelles isolées au Maroc entre 1976-1985. [Bacteriological study of 2792 strains of salmonella isolated in Morocco between 1976 and 1985.] *Maroc medical*, 1989, XI(4):484-92.
 19. Zouhdi M et al. Sensibilité à 10 antibiotiques des salmonella mineures d'origine humaine isolées au Maroc entre 1980-1991. [Sensitivity to 10 antibiotics of minor salmonella of human origin isolated in Morocco between 1980 and 1991.] *Revue marocaine de biologie-infectiologie*, 1995, I(2):25-30.
 20. Zouhdi M, Benouda A, Alaoui MA. Etat actuel de la resistance des salmonelles aux antibiotiques au CHU Ibn Sina-Rabat. [Current status of salmonella resistance to antibiotics in the central university hospital Ibn Sina, Rabat.] *Revue marocaine de biologie-infectiologie*, 1996, II:24-8.
 21. Nastasi A, Hammina C, Villafrate MR. rDNA fingerprinting as a tool in epidemiological analysis of *Salmonella typhi* infections. *Epidemiology and infection*, 1991, 107:565-76.
 22. Kantama L, Jayanetra F. *Salmonella enteritidis* outbreak in Thailand: study by random amplified polymorphic DNA (RADP) analysis. *Southeast Asian journal of tropical medicine and public health*, 1997, 27(1):119-25.