Original Article

Antimicrobial resistance in *Salmonella enterica* serovar Enteritidis in Morocco

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Abstract

Introduction: *Salmonella enterica* is recognised worldwide as one of the major agents of human gastrointestinal infections. The aim of the present work is to ascertain the antimicrobial susceptibilities of 150 *Salmonella enterica* serovar Enteritidis isolates from humans in Morocco during the period from 2000 to 2008.

Methodology: Antimicrobial resistance determination was performed by disk diffusion method using seven antibiotics. The minimal inhibitory concentration (MIC) of ciprofloxacin was determined for nalidixic acid-resistant (NAR) isolates using E-test strips.

Results: Sixty-one (42%) isolates were resistant to at least one class of antimicrobial agent. The largest numbers of resistant isolates were observed for nalidixic acid with 53 isolates (36%) followed by ampicillin with 7 isolates (5%), tetracycline with 6 isolates (4%), and trimethoprim/sulfamethoxazole with 2 isolates (1%).The resistant isolates were grouped in seven different resistance patterns of which two isolates were resistant to three antibiotics. Among the 53 (36%) NAR isolates, 37 (76%) had a reduced susceptibility to ciprofloxacin.

Conclusion: Resistance rates of *Salmonella enterica* serovar Enteritidis from Morocco are generally low but the resistance to nalidixic acid is worryingly common. Continual surveillance of antibiotic resistance is of primary importance.

Key words: antibiotic resistance; Morocco; *Salmonella enterica* serovar Enteritidis.

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Introduction

Salmonella enterica constitutes a major public health problem. It is recognised worldwide as one of the major causes of human gastrointestinal infections. Baumler *et al*. [1] suggested that the rapid increase of *Salmonella enterica* serovar Enteritidis (*S.* Enteritidis) might be due to the successful campaigns for eradication of *Salmonella* Pullorum and S*almonella* Gallinarum, the causative agents of fowl typhoid and bacillary white diarrhoea, respectively, in chickens.

S. Enteritidis has been the serotype most frequently associated with human salmonellosis in many African countries, such as Morocco [2] and Senegal [3]. *S.* Enteritidis is the only human pathogen routinely found in poultry. Other serovars of *Salmonella enterica* are often found in poultry farm environments and may contaminate eggs when eggshells are cracked [4]. Non-invasive salmonellosis is usually a self-limiting infection and does not require antibiotic treatment. In the event of an invasive disease, effective antibiotic therapy can be lifesaving [5].

In the past, antimicrobial resistance has been unusual in *S.* Enteritidis; however, in the 1990s, the occurrence of resistance to ampicillin and nalidixic acid increased, whereas the resistance to other antibiotics remained sporadic. The widespread use of antimicrobial agents in animal husbandry during food production has contributed to the occurrence of resistant bacteria in animals, including zoonotic pathogens, which can be transmitted to humans via the food chain [6]; therefore, continual surveillance for *Salmonella enterica* antibiotic resistance is of primary importance. The present work aims to ascertain antimicrobial susceptibilities in *S.* Enteritidis in Morocco. This investigation is the third substantial report from Morocco examining antimicrobial susceptibility in this serovar, while the

first work was reported by Rouahi *et al*. [2] and followed by Ammari *et al*. [7].

Methodology

Bacterial strains

A total of 150 isolates of *S.* Enteritidis from humans were identified during the period from 2000 to 2008 at the Laboratory of Medical Bacteriology at the National Institute of Health, Morocco.

Identification and susceptibility testing

All *S.* Enteritidis isolates were biochemically identified using the API 20E system (BioMerieux, Marcy l'Etoile, France) and were serotyped for somatic and flagellar antigen identification according to the Kauffman-White classification scheme [9]. Antimicrobial susceptibility was determined by disk diffusion assay using the Oxoid disks (Dardilly Cedex, France) commercial disks: 10µg of ampicillin (AMP), 30 µg of cefotaxime (CTX), 30 µg of chloramphenicol (CHL), 30 µg of tetracycline (TET), 1.25µg / 23.75µg of trimethoprime /sulfamethoxazole (SXT), 30µg of nalidixic acid (NAL), and 5µg of ciprofloxacin (CIP).

The minimal inhibitory concentration (MIC) of ciprofloxacin was determined for nalidixic acid-resistant (NAR) isolates using E-test strips (AB Biodisk, Solna, Sweden). Decreased ciprofloxacin susceptibility was defined as MIC ≥ 0.125 µg/mL. The tests were done according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [8]. *Escherichia coli* ATCC 25922 was used as a reference strain.

Intermediately resistant isolates were included in the resistant category. Data analysis was performed with WHONET 5 (WHO, Geneva, Switzerland).

Results

The isolates were collected from eight different regions: Agadir (n = 1), Chaouen (n = 10), Alhoceima (n = 5), Khemissat (n = 1), Nador (n = 2), Rabat (n = 76), Settat (n = 4), Tanger (n = 9), and Tetouan (n = 42). A total of 103 isolates were from outbreaks and 47 isolates were from sporadic cases. One hundred and thirty-six clinical isolates were from patients with gastrointestinal infection, eleven isolates were isolated from patients with septicaemia, and three isolates were from patients with a urinary tract infection.The proportion of isolates resistant to each of the seven antimicrobial drugs tested over time on *S.* Enteritidis is presented in Table 1. Overall, 61 (42%) isolates were resistant to at least one class of antimicrobial agent. We noticed that the greatest numbers of resistant isolates were observed for nalidixic acid with 53 isolates (36%), followed by ampicillin with 7 isolates (5%), tetracycline with 6 isolates (4%), and trimethoprim/sulfamethoxazole with 2 isolates (1%). The rate of resistance to all antimicrobials but nalidixic acid (including tetracycline, ampicillin, and trimethoprim/sulfamethoxazole) was low during this period of study and did not exceed 14% in any given year (Table 1). We noted that the resistance to

Table 1. The level of resistance of 150 strains of *Salmonella enterica* serovar Enteritidis isolated from humans in Morocco 2000-2008

Antimic-robial agents	Years, Number of strains (% per year)									
	2000 7 (5)	**2001 19 (12)**	**2002 10 (7)**	**2003 36 (24)**	**2004 9 (6)**	**2005 15 (10)**	**2006 18 (12)**	**2007 24 (16)**	**2008 12 (8)**	**2000- 08 150 (100)**
AMP	0	1 (5)	0	1 (3)	1 (11)	0	0	3 (13)	1 (8)	7 (5)
NAL	6 (86)	2 (10)	3 (30)	3 (8)	4 (44)	2 (13)	5 (28)	17 (71)	11 (92)	53 (36)
TET	0	0	0	2 (6)	1 (11)	2 (13)	0	0	1 (8)	6 (4)
SXT	0	0	0	0	0	0	0	1 (4)	1 (8)	2 (1)
CTX	0	0	0	0	0	0	0	0	0	0
CHL	0	0	0	0	0	0	0	0	0	0
CIP	0	0	0	0	0	0	0	0	0	0
MDR	0	0	0	0	0	0	0	0	0	0

AMP : Ampicillin **; NAL :** Nalidixic acid **; TET :** Tetracycline **; SXT :** Trimethoprim / Sulfamethoxazole **; CTX :** Cefotaxim **; CHL :** Chloramphenicol **; CIP :** Ciprofloxacin **; MDR :** Multi-drug resistance.

Table 2: Origin and resistance patterns of 61 resistant strains of *Salmonella enterica* serovar Enteritidis

Number of strains	Years	Samples	Cities	Cases	Resistance patterns
1	2000	Stools	Rabat	Sporadic	NAL
2	2000	Stools	Tetouan	Sporadic	NAL
3	2000	Stools	Settat	Outbreaks	NAL
4	2000	Stools	Settat	Outbreaks	NAL
5	2000	Stools	Settat	Outbreaks	NAL
6	2000	Stools	Tetouan	Sporadic	NAL
7	2001	Stools	Tetouan	Outbreaks	NAL
8	2001	Stools	Tetouan	Outbreaks	NAL
9	2002	Stools	Agadir	Sporadic	NAL
10	2002	Stools	Chaouen	Sporadic	NAL
11	2002	Stools	Rabat	Sporadic	NAL
12	2003	Stools	Rabat	Outbreaks	NAL
13	2003	Stools	Rabat	Sporadic	AMP NAL
14	2003	Stools	Rabat	Outbreaks	NAL
15	2003	Stools	Rabat	Outbreaks	TET
16	2003	Stools	Rabat	Outbreaks	TET
17	2003	Stools	Rabat	Outbreaks	AMP
18	2004	Stools	Rabat	Sporadic	NAL
19	2004	Stools	Rabat	Sporadic	NAL
20	2004	Stools	Rabat	Sporadic	NAL
21	2004	Stools	Rabat	Sporadic	NAL
22	2004	Stools	Nador	Outbreaks	AMP TET
23	2005	Stools	Rabat	Sporadic	NAL
24	2005	Stools	Rabat	Sporadic	NAL
25	2005	Stools	Tetouan	Outbreaks	TET
26	2005	Stools	Tetouan	Outbreaks	TET
27	2006	Stools	Rabat	Sporadic	NAL
28	2006	Stools	Rabat	Sporadic	NAL
29	2006	Stools	Rabat	Outbreaks	NAL
30	2006	Stools	Rabat	Outbreaks	NAL
31	2006	Stools	Khemissat	Sporadic	NAL
32	2007	Stools	Rabat	Outbreaks	NAL
33	2007	Stools	Rabat	Sporadic	NAL
34	2007	Stools	Tetouan	Outbreaks	NAL
35	2007	Stools	Tetouan	Outbreaks	NAL
36	2007	Stools	Tetouan	Outbreaks	NAL
37	2007	Stools	Tetouan	Outbreaks	NAL
38	2007	Stools	Tetouan	Outbreaks	NAL
39	2007	Stools	Rabat	Outbreaks	NAL
40	2007	Stools	Rabat	Outbreaks	NAL
41	2007	Blood	Rabat	Sporadic	NAL
42	2007	Blood	Rabat	Sporadic	AMP SXT NAL
43	2007	Stools	Tetouan	Outbreaks	NAL
44	2007	Stools	Tetouan	Outbreaks	NAL
45	2007	Stools	Tetouan	Outbreaks	NAL
46	2007	Stools	Tetouan	Outbreaks	NAL
47	2007	Stools	Rabat	Sporadic	NAL
48	2007	Stools	Rabat	Outbreaks	NAL
49	2007	Stools	Tetouan	Outbreaks	AMP
50	2007	Stools	Rabat	Sporadic	AMP
51	2008	Stools	Rabat	Sporadic	NAL
52	2008	Stools	Tanger	Sporadic	NAL
53	2008	Stools	Tanger	Sporadic	NAL
54	2008	Blood	Tanger	Sporadic	NAL
55	2008	Stools	Rabat	Sporadic	TET NAL
56	2008	Stools	Rabat	Sporadic	AMP SXT NAL
57	2008	Stools	Rabat	Sporadic	NAL
58	2008	Urine	Rabat	Sporadic	NAL
59	2008	Blood	Rabat	Sporadic	NAL
60	2008	Blood	Rabat	Sporadic	NAL
61	2008	Urine	Rabat	Sporadic	NAL

AMP: Ampicillin**; NAL:** Nalidixic acid**; TET:** Tetracycline**; SXT:** Trimethoprim / Sulfamethoxazole.

trimethoprim/sulfamethoxazole emerged in 2007 in Rabat. The remaining antimicrobial agents, cefotaxime, chloramphenicol, and ciprofloxacin, showed high efficiency *in vitro* against all isolates throughout the eight years of this study.

Among the 61 resistant isolates of *S.* Enteritidis, seven different resistance patterns were found I included in table 2 all the resistant isolates. (Table 2). Resistance to nalidixic acid was the most common single resistance with 49 (33%) isolates. Thirty one of the (58%) NAR isolates were sporadic cases, 5 (17%) from blood, 24 (77%) from stools, and 2 (6%) from urine, while all the outbreak strains were isolated from stool samples (Table 2). It is worthwhile to note that 5 (4%) of the 150 isolates of *S.* Enteritidis were resistant to two or more antibiotics, among which two were isolated from blood (n = 1) and stool (n = 1) in Rabat were each resistant to three antibiotics (AMP, SXT and NAL).

Among the 53 nalidixic acid resistant isolates, 37 (76%) had a reduced susceptibility to the ciprofloxacin (MIC: 0.125-0.5 μg/mL) with this distribution: 11 (22%) isolates had MIC = 0.125 μg/mL, 21(43%) had MIC = 0.19 μg/mL, and 5 (10%) had MIC = 0.25 μg/mL.

Discussion

S. Enteritidis was the predominant serovar found in our collection, representing 60% of all isolated strains throughout the years studied.Other studies in Morocco have focused on outbreak isolates [7,10]. This study included isolates from outbreaks and sporadic cases due to the ingestion of food products, especially poultry, eggs, meat, or other contaminated food sources.

Our study showed that 89 (59%) isolates of *S.* Enteritidis were susceptible to all antibiotics tested. This prevalence of susceptibility was higher than the reported studies from Spain and Senegal [3,11,12], but similar to the findings of Ammari *et al*. [7] from Northern Morocco and Chiappini *et al*. [12] from Italy; however, susceptibility was lower in reports from the Netherlands and Korea [14,15].

Nalidixic acid is the prototype quinolone. It has been available in many countries since the mid-1960s, but it is now seldom used [16]. Several studies have suggested that the use of fluoroquinolone in veterinary medicine contributes to the emergence and dissemination of nalidixic acid resistance in *Salmonella* among food animals, which may be transmitted to humans [17,18].

NAR is most commonly observed in *S.* Enterica globally [10, 19, 20]. Studies by Hakanen *et al*. [21] and Choi Sang-Ho *et al*. [14] showed that reduced fluoroquinolone susceptibility is becoming more common in many parts of the world. Our study confirmed this for Morocco where 76% of our isolates (NAR) manifested a reduced susceptibility to ciprofloxacin.

A screening test using nalidixic acid disks demonstrated high sensitivity and specificity for detecting *Salmonella enterica* with reduced susceptibility to ciprofloxacin (MIC, ≥ 0.125 μg/mL) [22]. Our study revealed that twelve (24%) of the isolates were nalidixic acid resistant even though their ciprofloxacin minimum inhibitory concentration was less than 0.125 μg/mL. These observations agreed with the results of Crump *et al*. [16], who suggested that this screening test has some limitations and does not identify all NAR *Salmonella* isolates with reduced susceptibility to fluoroquinolones. They also suggested that the current CLSI fluoroquinolone breakpoint for resistance needs to be re-evaluated for *S. enterica* serotypes.

The resistance mechanisms for the isolates in this study were not determined, but reduced fluoroquinolone susceptibility in *Salmonella* is usually associated with a point mutation leading to an amino acid change in their quinolone resistance determining region of the *gyrA* gene [22]. Resistance based on mutations in gyrase genes is selected by the use of antimicrobial agents, either in human medicine or in agriculture. Alternatively, quinolone resistance may be due to decreased permeability or the presence of efflux pump mechanisms [21]. The other antimicrobials agents tested in this study correlated when in comparison with similar data [23, 24]; levels of resistance to ampicillin, trimethoprim/sulfamethoxazole in our isolates were very low, which might be due to the limited use of these classes of antimicrobial agents in Yes, it is the intended meaning animal husbandry during food production ;however, all the isolates from a study in Senegal were resistant to ampicillin [3], in contrast, ampicillin resistance was seen in seven (5%) isolates in the present study.

With the increase in isolates with reduced susceptibility to ciprofloxacin, the third-generation cephalosporins, such as ceftriaxone and cefotaxime, may provide alternative therapy. In our study, all the isolates were sensitive to cefotaxime despite Rouahi *et al*. [2] reporting that 3.2% of their isolates were

resistant to ceftriaxone and 2% to cefotaxime, respectively, elsewhere in Morocco.

Although two isolates were resistant to ampicillin, trimethoprim/sulfamethoxazole, and nalidixic acid, we could not judge that these isolates have multi-drug resistance (MDR). MDR in *Salmonella enterica* is defined as resistance to ampicillin, chloramphenicol, and trimethoprim/sulfamethoxazole [25] combined, and all the isolates in this study were susceptible to chloramphenicol.

Salmonella enterica isolates resistant to tetracycline are also commonly observed and rising rates have been reported from several other studies of human infections [13,20 ,26], perhaps because this class of antimicrobial agent was commonly used in animal production as growth promoters or for therapeutic purposes; however, the percentage of resistance determined in our work was relatively low (4%) in comparison with that found by Rouahi *et al*. [2].

This study revealed that our isolates showed good sensitivity to most antimicrobials agents tested. The increasing prevalence of nalidixic acid resistance associated with reduced susceptibility to ciprofloxacin suggested that ciprofloxacin treatment may not be effective for serious *Salmonella* infection. Thus vigilance and continuous monitoring of this bacterium are essential.

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