

Prevalence and antibiotic-resistance of *Salmonella* isolated from food in Morocco

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Abstract

Background: Salmonellosis remains one of the most frequent food-borne diseases worldwide, especially in developing countries. The emergence of antimicrobial resistance in *Salmonella* isolates from food can potentially compromise the treatment of these infections. This investigation was conducted for the first time in Morocco both to detect the occurrence of *Salmonella* in foods as well as to determine the antibiotic resistance profile of the *Salmonella* isolates.

Methodology: In total, 11,516 food samples collected from 2002 to 2005 were investigated. Isolated *Salmonella* were characterized by serotyping and susceptibilities were determined for 15 antimicrobial drugs using the disc diffusion assay.

Results: The overall percentage of *Salmonella* prevalence ($n=105$) was 0.91% with rates of 71% for slaughterhouses and 9% for seafood. Sixteen different serotypes were identified among 104 *Salmonella enterica* isolates including serotypes Infantis ($n=25$), Bredeney ($n=13$), Blokley ($n=11$), Typhimurium ($n=9$), Mbandaka ($n=8$), Branderup II ($n=7$), and Kiambu ($n=6$); 1 isolate of *Salmonella enterica* belonged to subspecies II salamae. Twenty-nine percent of isolates ($n=30/105$) were resistant to at least one antimicrobial. Resistance to tetracycline was the most common finding (21%), followed by resistance to ampicillin (13%), amoxicillin+clavulanic acid (9%), streptomycin (7%), chloramphenicol (4%) and nalidixic acid (3,8%). None of the isolates was resistant to 3rd-cephalosporin and fluoroquinolones (i.e. ciprofloxacin). Multidrug resistance (MDR) was seen in 9.5% of the isolates, mainly in *S. Typhimurium* DT104 with R-type ACSSuT and *S. Hadar*.

Conclusions: Despite a low frequency of *Salmonella* isolation, *S. Typhimurium* DT104 was identified in the first step of the food chain. The study points out the need control antibiotic resistance in *Salmonella* isolated from food in Morocco to avoid the spread of MDR.

Key Words: *Salmonella*, food, antibiotic-resistance, Morocco

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Introduction

Food-borne diseases represent a serious threat to public health resulting in considerable economic consequences in many parts of the world. Salmonellosis is one of the most frequent foodborne diseases in almost all countries and *Salmonella enterica* Enteritidis followed by Typhimurium represent the most frequently isolated serotypes [1]. In the African continent in 2002 among non-human sources, *S. Anatum* and *S. Enteritidis* constituted the largest proportion of isolates [2].

In most cases, salmonellosis is caused by contaminated food products, particularly those of

animal origin such as poultry, eggs, beef and pork. Fruits and vegetables also have been reported as vehicles in *Salmonella* transmission, and contamination can occur at multiple steps along the food chain [3].

In developed countries *Salmonella* is recognized as a major food-borne human pathogen and is responsible for collective food poisoning with approximately 65% of cases in France [4] and 95% in the United States of America [5]. Although the declaration and recording of (12%) *Salmonella* cases remain underreported, *Salmonella* is the major cause of food poisoning in Morocco [6].

In meat production, the leading source of contamination of carcasses by *Salmonella* is the evisceration step at the slaughterhouse [7]. In Ethiopia, approximately 20% of camel carcasses were found positive for *Salmonella* at slaughtering, and 15% of the meat samples were found positive at the retail level [8]. In the last two decades, the emergence of antibiotic-resistant *Salmonella* has become a serious health hazard worldwide. The widespread use of antimicrobial agents in food animal production and the routine practice of giving antimicrobial agents to domestic livestock as a means of preventing and treating diseases has contributed to the occurrence of *Salmonella* with decreased susceptibility to drugs [9].

The intestines of animals and humans remain the major reservoirs of the contamination of food, and animals play a role in the distribution of the salmonellosis [10,11].

The occurrence of strains resistant to one or several antimicrobials is essentially intensified in the Typhimurium serotype isolated from both humans and animals [12]. The drug resistance spectra of MDR strains of *Salmonella* serovars have also been expanding in recent years, especially for the aminopenicillines, tetracyclines, sulfonamides and chloramphenicol, and often more than 50% of *S. Typhimurium* strains isolated in France are multidrug resistant [13].

The present study was undertaken to evaluate the occurrence of *Salmonella* in foods in Morocco. The identification of circulating serotypes and the antimicrobial resistance profiles of *Salmonella* isolates collected in Morocco during 2002-2005 were also evaluated.

Materials and Methods

Food samples collection

During the period from March 2002 to December 2005, a total of 11,516 different food samples were collected from slaughterhouses located in Rabat city, and from hotels, restaurants, snack bars, and public and private companies in several cities in Morocco (mainly from Casablanca, Tanger, Rabat and Marrakech). The samples were analysed using the AFNOR method (Standard NF VO8-52). Food samples are summarized in Table 1. Samples from slaughterhouses were beef meat samples. Approximately 50 g of each sample (having no prolonged contact with the surroundings) was collected in a sterile plastic pouch and transported to the laboratory refrigerated at 0 –10° C not later than 24 hours after collection. Samples were stored at -20° C until analysis.

Table 1. *Salmonella* spp prevalence in food samples collected in Morocco during 2002-2005.

Samples source	No of samples	
	Analysed	Positive
Cooked meat	2952	2
Sausages	2052	2
Chicken meat	1200	5
Pastry	2232	5
Chopped meat	196	4
Seafood	562	10
Spices	80	1
Water	120	1
Slaughterhouses	2122	75
Total	11.516	105

Microbiological Analysis

Twenty-five g of each sample was blended in 225 ml buffered peptone water (BPW), Bio-Rad (Marne-la-Coquette- France) homogenized in Colworth Stomacher and incubated 16-20 hours at 37° C as pre-enrichment for *Salmonella*. From each pre-enriched sample, 0.1 ml was used to inoculate 10 ml of the Rappaport-Vassiliadis medium (RV) Bio-Rad (Marne-la-Coquette-France) and 1 ml of the pre-enriched sample was used to inoculate 10 ml of the selenite-cystine medium (SC) Bio-Rad (Marne-la-Coquette- France). Samples were incubated for 7 hours at 42° C (RV) and at 37° C (SC), respectively. Bacterial isolation was achieved on EKM and XLD media at 37° C for 24 hours Bio-Rad (Marne-la-Coquette- France). Lactose-negative colonies were kept for further studies.

Salmonella was identified using the API20E system (Sanofi Diagnostics Pasteur). Serotypes were identified by agglutination tests with *Salmonella* specific anti-sera Bio-Rad (Marne-la-Coquette- France) as described by Popoff and Le Minor [14]. The antigenic formula was determined according to the Kauffman-White scheme [15]. Identification of *S. Typhimurium* DT104 was conducted according to previous published procedures by Alvarez *et al.* [16]. *S. Typhimurium* DNA templates were multiplexing amplified by PCR with specific primers for six different genetic targets: a sequence specific for serotype Typhimurium DT104 (band 1, 102 bp); a *Salmonella* genus-specific sequence (band 2, 204 bp); a serotype Enteritidis-specific sequence (band 4, 304 bp); a serotype Typhimurium-specific sequence (band 8, 401 bp); a *Salmonella* C2 serogroup-specific sequence (band 16, 502 bp); and a sequence specific for serotype 4,5,12:i:- (band 32, 705 bp). Every

amplification profile has a code number obtained by the addition of the values corresponding to each amplified band. An *S.Typhimurium* DT104 strain was used as positive control and was identified by code 11 (positive amplification for bands 1, 2, and 8).

Antibiotic sensitivity test

Each of the *Salmonella* isolates was tested for susceptibility to antimicrobials on Muller-Hinton agar following the disc diffusion method according to the recommendation of the Clinical and Laboratory Standards Institute (CLSI) [17] by using commercial antibiotic discs Bio-Rad (Marnes-la-Coquette- France) (µg): ampicillin (10), amoxicillin + clavulanic acid (20/10), ceftazidim (30), cefotaxime (30), chloramphenicol (30), cefazolin (30), cefamandol (30), gentamycin (500), mecillinam, Trimethoprim/Sulphametoxazole (1,25/23,75), trimethoprim (5), streptomycin (10), Sulphametoxazole (300), tetracyclin (30), ciprofloxacin (5) and nalidixic acid (30). In this study, the isolates showing a decrease in susceptibility (intermediate) were considered as resistant. *Escherichia coli* CIP.76-24 was used as a control.

Results

Prevalence of Salmonella in food samples

A hundred five out of 11.516 (0.91%) food samples examined for routine analysis at the Food Control laboratory of Institut Pasteur during 2002 to 2005 were found positive for *Salmonella*. Table 1 shows the prevalence of *Salmonella* isolates from a variety of food products. Among the sources, *Salmonella* strains from slaughterhouses constituted the largest proportion of isolates (75/105; 71%) followed by seafood (10/105; 9.5%).

Serotyping of Salmonella isolates

A hundred-four isolates of *Salmonella enterica* belonged to subspecies I *enterica* and one isolate to subspecies II *salamae*. Serotype prevalence and distribution in food samples are reported in Table 2. A total of fifteen different serotypes were identified among *S. enterica* I isolates; the top five included the following serotypes: Infantis (23.8%), Bredeney (12.4%), Blokley (10.5%), Typhimurium (8.6%) and MBandaka (7.6%). In five *S. Typhimurium* strains we could detect an amplification profile code (code 11) which is specific for *S.Typhimurium* phage type DT104 by using a specific multiplex PCR described above. Serotypes Anatum, Bareilley, Berta, Bovismorbificans, BraenderupII, Enteritidis, Hadar, Kiambu, Labadi, and

Montevideo, were also identified in different percentages ranging from 6.7% to 1%. Three isolates were nontypeable strains. These were positive for *Salmonella* in biochemical tests; however, during grouping, they were found to be positive with polyvalent O antisera but negative with the available monovalent antisera.

Table 2. Predominant serotypes (%) of *Salmonella enterica* by source of isolation.

<i>Salmonella</i> serotypes	No of strains by source										
	Cooked meat	Sausages	Chopped meat	Seafood	Pastry	Chicken meat	Spices	Slaughterhouse	Water	N	(%)
Anatum			1		1			2		4	(3.8)
Bareilley			1							1	(0.95)
Berta			2							2	(1.9)
Blokley		1		5		3		2		11	(10.4)
Bovismorbificans								2		2	(1.9)
BraenderupII								7		7	(6.6)
Bredeney					1			12		13	(12.3)
Enteritidis	1							2		3	(2.8)
Hadar				3		1				4	(3.8)
Infantis					1		1	22	1	25	(23.8)
Kiambu								6		6	(5.7)
Labadi				1				1		2	(1.9)
MBandaka								8		8	(7.6)
Montevideo								4		4	(3.8)
Typhimurium ^a	1	1		1	1			5		9	(8.5)
Other serotype*					1			2		3	(2.8)
<i>Salamae</i> (type II)						1				1	(0.95)
Total No of isolates(%)	2 (1.9)	2 (1.9)	4 (3.8)	10 (9.5)	5 (4.7)	5 (4.7)	1 (0.95)	75 (71.4)	1 (0.95)	105	(100)

* nontypeable isolates of *Salmonella*.

^a Six *S.Typhimurium* strains were DT104 according to specific PCR (15).

Resistance to antimicrobials

Drug susceptibility assay revealed that 71% (75/105) of the *Salmonella* isolates investigated were fully susceptible to all fifteen antimicrobials tested. No resistance to ciprofloxacin, ceftazidim, cefotaxime, cefamandol, gentamycin and mecillinam was observed.

Thirty isolates (29%) exhibited resistance to at least one antimicrobial, while sixteen (15%) were resistant to two or more tested drugs. Resistance to antimicrobials was identified mostly in serotypes Typhimurium (6/9) Blokley (5/11), BranderupII (4/7), Hadar (4/4), Infantis (4/25), and in nontypeable *Salmonella* isolates (3/3). However, *Salmonella* strains serotype Bredeney, Kiambu, Bovismorbificans, Anatum, Berta, Bareilley, Labadi and *S. salamae* did not show any resistance phenotype.

The most common resistance observed was to tetracycline, accounting for 21% of isolates, followed by ampicillin and amoxicillin+clavulanic acid found in 13% and 9% of isolates respectively. These resistances were variously distributed among different serotypes. Resistance to streptomycin was exhibited by 6.7% of isolates, predominantly in Typhimurium and Hadar. Resistance to nalidixic acid was common in serovar Hadar (4/4). Resistance rates to chloramphenicol (4%), kanamycin (3.8%), trimethoprim-sulfamethoxazole (2.8%) and cefazolin (0.9%) were observed to a lesser extent and distributed among serotypes MBandaka, Blokley, Hadar, and Typhimurium.

It was also evident from the results that 9.5% (10/105) of isolates showed multiple drug resistance (resistant to ≥ 3 drugs). These included serotype Typhimurium (n=5), Hadar (n=2) and 3 isolates for which it was not possible to associate to a serotype. The resistance pattern, the origin, and the serotypes of the resistant strains are shown in Table 3.

Table 3. Distribution of antibiotic resistance by source and serotype identified in *Salmonella enterica* isolates.

<i>Salmonella</i> serotype	Source* (n of resistant isolates)	MDR isolates	Antibiotic resistance profile
Blokley	SF(2)		TET
	C(1)/SL(1)		SXT
	C(1)		SXT TET
Branderup II	SL(1)		TET
	SL(3)		K
Enteritidis	CM(3)		AMP TET
Hadar	SF(2)	2	NA TET
	SF(1)		NA AMP STR TET
	C(1)		NA AMP CEF STR TET (AMC)
Infantis	P(1)/SL(2)		TET
	SL(1)		AMP
MBandaka	SL(1)		AMP TET
	SL(1)		TET
Montevideo	SL(1)		K
Typhimurium	S(1)		AMP (AMC)
Typhimurium DT104	SL(5)	5	AMP C STR SU TET (AMC)
other serotype	P(1)/SL(2)	3	AMP TET (AMC)

AMP, ampicillin; AMC, amoxicillin+clavulanic acid; CEF, cefazolin; CHL, chloramphenicol; NA, nalidixic acid; K, kanamycin; SXT, trimethoprim/sulfamethoxazole; SU, sulphamethoxazole; STR, streptomycin; TET, tetracycline. MDR multiresistant

* SF: seafood; S: sausages; SL: slaughterhouse; C: chicken; P:pastry; CM: cooked meat

More than a half of *Salmonella* resistant strains were detected in beef meat samples from

slaughterhouses 18/30 (56.6%), comprising five pentaresistant (ACSSuT) *S. Typhimurium* DT104.

Discussion

Food-borne diseases caused by non-typhoid *Salmonella* represent a major public health problem worldwide. Since these pathogens are transmitted through contaminated food or water, the presence of *Salmonella* strains in food animals and in raw meat products has relevant public health implications. Thus, monitoring food safety is a key point in preventing and controlling the spread of *Salmonella*, as well as in providing healthier food products.

In this survey, it was evident from the bacteriological analysis of the investigated samples that *Salmonella* contaminates a large number of food products. However, bovine meat samples from slaughterhouses, seafood, and chicken meat were the products most often identified in the spreading of this pathogen.

The overall prevalence of *Salmonella* was consistently lower than that reported from other countries [18-20]. This observation can be correlated to better hygiene in Morocco than in the other countries studied; however, other factors including sampling procedures and storage, and bacterial isolation might have affected the lower prevalence.

The analysis of the isolated *Salmonella* strains in Morocco showed different serotypes with a strong variability in samples from slaughterhouses.

The same serotypes appear among the top five non-human sources reported, although in a different order. In Africa, *S. Enteritidis* and *S. Anatum* are the most common *Salmonella* serotypes. In Europe, *S. Infantis* followed by *S. Enteritidis* and *S. Typhimurium* constituted the largest proportion of isolates [2]. In Morocco, the National Institute of Hygiene reported serotypes Gallinarum, Enteritidis, Typhimurium and Infantis as the most isolated from animals in 2003 (WHO Global Salm-Surv). Unfortunately a score for the isolation of *Salmonella* from food for a comparison was not available.

In our study, we found Infantis to be the predominant serotype followed by Bredeney. Stevens *et al.* reported a prevalence of this serotype in slaughterhouse samples in Senegal, which is similar to our findings [18].

S. Enteritidis was only the ninth most common serotype among our isolates; only 3 strains were isolated from cooked meat samples and

slaughterhouses. This finding partially reflects the capacity of *S. Enteritidis* to contaminate eggs in low numbers and the difficulty of isolating it from food or the environment; in our study, few samples from eggs were submitted for routine testing.

Since drug resistance in zoonotic microbes has prompted therapeutic intervention in humans, antimicrobial resistance in food-borne pathogens has become a public health issue [21,22].

Food-borne pathogens can acquire resistance in response to antimicrobial drug use in food animals, contaminate food products at the time of slaughter, and possibly transmit the resistance genes to humans via the food chain [23].

The most frequent antimicrobial resistance among all different *Salmonella* serotypes studied was against tetracycline. This observation was predictable as a consequence of the high use of tetracycline as an antimicrobial in animal husbandry and in human medicine. Next to tetracycline, ampicillin and amoxicillin+clavulanic acid resistance was found in 13% and 9% of isolates respectively regardless of the serotype. Despite the low frequencies, resistance to these antimicrobials in food-borne pathogens may still create problems for disease treatment.

Resistance to nalidixic acid was common in serovar Hadar. However, the four isolates showed susceptibility to ciprofloxacin. Fluoroquinolones are the current drugs of choice for the treatment of invasive *Salmonella* infections in adults, and resistance to NAL may invalidate fluoroquinolone therapy [24].

Five *S. Typhimurium* strains from beef meat samples originating from slaughterhouses were multidrug-resistant phage-type DT104 (according to specific PCR) with R-profile ACSSuT. The occurrence of *S. Typhimurium* DT104 with multidrug resistance was the most important finding with regard to human health. Similarly, other authors also described the strains with this pentaresistant pattern as the most frequent [25].

DT104 represent an important international human pathogen, and it is widespread in Western and Eastern Europe, North America, and the Middle East [26]. Its presence and spread emphasize the importance of having strong surveillance and control programmes in Morocco.

In conclusion, although the overall low prevalence of *Salmonella* isolation is encouraging compared to that reported from other countries, our findings suggest that beef meat is a reservoir of *Salmonella* spp. in Morocco.

The results obtained in this survey can be used in a programme of *Salmonella* monitoring and control in Morocco that can be launched in the near future.

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