

Original Article

Prevalence, serotype distribution, and antimicrobial resistance of *Salmonella* isolated from food products in Morocco

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Abstract

Introduction: Salmonellosis is one of the most common foodborne diseases worldwide. The irrational use of antibiotics in medicine and in animal feed has greatly promoted the emergence and spread of resistant strains of non-typhoidal *Salmonella*.

Methodology: A total of 464 food products were collected in Tetouan from January 2010 to December 2012. The isolation and identification of *Salmonella* were performed according to Moroccan standard 08.0.116. All isolates were serotyped and were then tested for antibiotic resistance using the disk diffusion method.

Results: The microbiological analysis showed that 10.3% of food samples were contaminated with *Salmonella*. Eleven serotypes were identified: Kentucky 22.9% (11/48), Agona 16.7% (8/48), Reading 12.5% (6/48), Corvallis 8.3% (4/48), Saintpaul 8.3% (4/48), Typhimurium 6.2% (3/48), Montevideo 6.2% (3/48), Enteritidis 4.2% (2/48), and 2% (1/48) for each of Israel, Hadar, and Branderup.

Drug susceptibility testing showed that 39.6% of *Salmonella* were resistant to at least one antibiotic and 60.4% were susceptible to all tested antibiotics. The highest percentage of resistance was found to the following antimicrobial agents: nalidixic acid (27.1%), sulfonamides (25%), amoxicillin (12.5%), amoxicillin/clavulanic acid 12.5%, trimethoprim (10.4%), cephalothin (4.2%), and chloramphenicol (2.1%).

Conclusions: This study revealed a relatively high prevalence of *Salmonella* in food products in Tetouan and a large percentage of drug-resistant strains. Hygienic measures should be rigorously implemented, and monitoring resistance of *Salmonella* is required to reduce the risks related to the emergence of multi-resistant bacteria.

Key words: *Salmonella* spp.; serovars; prevalence; antibiotic resistance; Morocco

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Introduction

Salmonella is a Gram-negative, rod-shaped bacteria belonging to the *Enterobacteriaceae* family [1]. Up to now, over 2,600 serovars have been identified in this genus [2].

Non-typhoidal *Salmonella* is an important foodborne pathogen that poses a serious risk to human health throughout the world [3-5]. Although various pathogens have been implicated in produce-related outbreaks, *Salmonella* has been the dominant pathogen [6]. It was most often detected in fresh broiler chicken and turkey meat [7] and was also isolated from seafood, fruits, and vegetables [8,9]. Recently, an increasing number of human salmonellosis cases have been linked to the ingestion of contaminated vegetables [10], juices [11], and low-moisture foods [12].

Most of the non-typhoidal *Salmonella* infections are self-limited, and do not require any antimicrobial therapy [3,4,13]. However, antibiotics are required in invasive or severe cases that may occur in some patients, particularly in vulnerable groups such as young children, the elderly, and immunocompromised persons [14]. In the case of severe infection such as bacteremia or meningitis, the use of antimicrobials is mandatory [15].

Moreover, antibiotics are widely used in animal husbandry for several purposes, including therapeutics, prophylaxis, metaphylaxis, and growth promotion. However, the extensive use and misuse of antimicrobial agents has greatly promoted the emergence and spread of resistant bacteria in both developed and developing countries. This fact constitutes a threat to human health

and presents a major financial burden [4,9,16]. The risk of antimicrobial resistance occurs when bacteria become resistant to the most important antibiotics used to treat human diseases and limits the choice of treatments.

Due to the increased resistance of *Salmonella* species, severe infections are often difficult to treat [17]. Overall, frequency and antimicrobial patterns of resistance may vary greatly between countries [5].

In Morocco, few studies have outlined the prevalence and antibiotic resistance of *Salmonella* in food products. The lack of data on the prevalence of *Salmonella* in the city of Tetouan led us to undertake this study. Thus, we evaluated the prevalence of *Salmonella* spp. in different food products between January 2010 and December 2012. *Salmonella* isolates were serotyped and tested for their susceptibility to antibiotics.

Methodology

Sample collection

A total of 464 samples of dairy products (n = 203), meat products (n = 158), and poultry (n = 103) were collected from different outlets in the city of Tetouan (northwest of Morocco) between January 2010 and December 2012. Food samples were transferred under cold conditions to the laboratory and were directly subjected to microbiological analysis.

Microbiological analysis

Twenty five grams of food samples were homogenized for 2 minutes with 225 mL of buffered peptone water (Biokar Diagnostics, Beauvais, France) using a Stomacher (Mayo Homogenius HG 400V Stomacher, Milan, Italy) and were then pre-enriched at 37°C for 24 hours. Isolation and identification of *Salmonella* were performed according to the International Organization for Standardization (ISO) 6579 [18]. Suspected *Salmonella* colonies were

identified by biochemical tests using the API 20 E gallery (BioMérieux, Marcy l'Étoile, France).

Serotyping

All *Salmonella* isolates were serotyped using direct active slide agglutination. Somatic (O) and flagella (H) antigens were characterized with hyperimmune sera (Bio-Rad, Marne la Coquette, France). The results were interpreted based on the Kauffmann-White scheme [19].

Antimicrobial susceptibility

All serotyped isolates were tested for their antimicrobial susceptibility using the disk diffusion method. The antimicrobial agents used in this study were purchased from Oxoid (Basingstoke, UK) and included nalidixic acid (30 µg), tobramycin (10 µg), amoxicillin (25 µg), amoxicillin/clavulanic acid (20/10 µg), cefoxitin (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg), kanamycin (30 µg), cephalothin (30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), gentamicin (30 µg), sulfamethoxazole/trimethoprim (1.25/23.75 µg), trimethoprim (5 µg), erythromycin (15 µg), and sulfonamide (200 µg). The results were interpreted based on the recommendations of the Antibiogram Committee of the French Society of Microbiology [20].

Results

Salmonella prevalence and serovars

A total of 48 *Salmonella* isolates were recovered from the 464 analyzed samples (Table 1). Eleven serotypes were identified, with a prevalence of 22.9% for Kentucky, 16.7% for Agona, 12.5% for Reading, 8.3% for Saintpaul and Corvallis, 6.2% for Typhimurium and Montevideo, 4.2% for Enteritidis, and 2.1% for Hadar, Israel, and Braenderup (Table 2). Four strains were unidentified.

Table 1. Prevalence of *Salmonella* in the analyzed food samples.

Origin	Number of samples analyzed	Number of positive samples ^a
Traditional cheese	51	3 (5.9%)
Milk and other derivatives	152	0 (00.0%)
Minced meat	138	17 (12.3%)
Sausage	20	1 (5.0%)
Chicken	86	18 (20.9%)
Turkey	17	9 (52.9%)
Total of samples	464	48 (10.3%)

^a Values in parenthesis indicate the percentage of positive samples per type of sample.

Antimicrobial resistance

The results of antimicrobial resistance showed that 27.1% of *Salmonella* strains were resistant to nalidixic acid, 25% to sulfonamide, 22.9% to amoxicillin, 12.5% to ampicillin, 10.4% to trimethoprim and sulfamethoxazole/trimethoprim, 4.2% to cephalothin, and 2.1% to chloramphenicol. In total, 60.4% of the strains were susceptible to all tested antibiotics, and no resistance was found to tobramycin, cefoxitin, ceftriaxone, cefotaxime, kanamycin, ceftazidime, ciprofloxacin, gentamicin, and erythromycin. It was also noted that 39.6% of *Salmonella* isolates (19/48) were resistant to at least one antibiotic and 22.9% were multi-resistant to at least three antibiotics. A resistance

to multiple antimicrobial agents was predominantly seen in Kentucky, Saintpaul, Typhimurium, and Israel serotypes (Table 3), while Agona, Montevideo, and Braenderup showed no resistance to the antimicrobial agents tested in this study.

Discussion

In this study, *Salmonella* was found in 10.3% of the analyzed food products. This high value was mainly associated with the poultry products. In fact, the prevalence of *Salmonella* in turkeys (52.9%) and chickens (20.9%) was higher than that observed in the other products. Indeed, it well known that poultry is the main reservoir of *Salmonella*.

Table 2. Distribution of serovars in the analyzed food products.

Serovars	Food samples					Total
	Traditional cheese ^b	Minced meat ^b	Sausage ^b	Chicken ^b	Turkey ^b	
Kentucky	2 (18.2%)	1 (9.1%)		6 (54.5%)	2 (18.2%)	11
Agona			1 (12.5%)	3 (37.5%)	4 (50.0%)	8
Reading		5 (83.3%)			1 (16.6%)	6
Saintpaul				2 (50.0%)	2 (50.0%)	4
Corvallis		4 (100.0%)				4
Typhimurium				3 (100.0%)		3
Montevideo		3 (100.0%)				3
Enteritidis				2 (100.0%)		2
Israel		1 (100.0%)				1
Hadar		1 (100.0%)				1
Braenderup		1 (100.0%)				1
ND	1 (25.0%)	1 (25.0%)		2 (50.0%)		4

^b Values in parenthesis indicate the percentage of each *Salmonella* serovar relating to the total of the same serovar; ND: not determined.

Table 3. Origin and antibiotic resistance of *Salmonella* serovars.

Serovars	Origin	Resistance
Enteritidis	Chicken	NA
Kentucky	Chicken	NA
Kentucky	Chicken	NA
Kentucky	Turkey	AMX, NA, SSS
Kentucky	Turkey	AMC, AMC, NA, SSS
Kentucky	Chicken	AMC, AMC, NA, SSS
Kentucky	Chicken	AMC, AMC, NA
Kentucky	Chicken	AMX, NA
Kentucky	Meat products	AMX, AMC, NA, SSS
Kentucky	traditional cheese	AMX, AMC, NA, SSS
Saintpaul	Turkey	NA
Saintpaul	Chicken	TMP, SSS, SXT
Saintpaul	Chicken	TMP, SSS, SXT
Typhimurium	Chicken	AMX, CEP, C, TMP, SSS, SXT
Typhimurium	Chicken	AMP, CEP, TMP, SSS, SXT
Reading	Turkey	SSS
Corvallis	Meat products	AMX
Israel	Meat products	TMP, SSS, SXT
Hadar	Meat products	NA

NA: nalidixic acid; AMX: amoxicillin; AMC: amoxicillin/clavulanic acid; CEP: cephalothin; C: chloramphenicol; SXT: sulfamethoxazol/trimethoprim; TMP: trimethoprim; SSS: sulfonamide.

In the dairy products, *Salmonella* was only isolated from cheese (5.9%). The absence of this pathogen in the other dairy products could be attributed to their high level of acidity that can inhibit the survival of *Salmonella*. To our knowledge, only one study in Morocco reported the presence of *Salmonella* in dairy products [21].

Overall, the prevalence found in other studies was lower [22], higher [23], or close to [24] our findings. This discrepancy may be due to the type of analyzed food products and the hygienic conditions in each region.

The distribution of *Salmonella* serovars identified in the present study was particularly heterogeneous (Table 2). Kentucky was the predominant serovar and was mainly isolated from chickens. This result is in agreement with previous studies carried out in Morocco [25-27]. The emergence of resistant strains of Kentucky in Egypt and their expansion to African countries [28] can explain its predominance in Morocco. Le Hello *et al.* suggested that the north and east of Africa could be the origin of infections by Kentucky in Europe [29].

Agona was the second principal serovar found in our study and was frequently isolated from poultry products. This serovar was previously found as the second most frequent serovar in turkey products in Morocco [30]. In Turkey, Agona was isolated by Erol *et al.* as the predominant serovar from turkeys [31]. In the United States, Agona emerged as a public health problem in the seventies. In 1972, Agona became the most frequently isolated serovar [32].

Reading was the third major serovar isolated and was mainly found in meat products. Since the serovar Reading has been frequently reported in both small ruminants and dogs [33], the contamination of meat products with this serovar might occur during the slaughter process.

In this study, the prevalence of Typhimurium and Enteritidis was lower compared to that of the other serovars. However, they were mentioned in several studies as the most frequent serotypes both in humans and animals [16,34] and were considered the most important cause of non-typhoidal salmonellosis [15]. In Morocco, Enteritidis was isolated from samples of table eggs and droppings of laying hens during 1996–1997 [35]. Recently, this serovar was recovered from chickens [26]. The lower percentage of *S. Enteritidis* might be due to the adoption of vaccination and other measures in the production and distribution of chicken meat and eggs as suggested by Lane *et al.* [36].

As for Typhimurium, several authors have reported its presence in food products in Morocco [22,25,26,30], and it was the most isolated serovar from chickens [37].

In this study, 11 different serotypes have been identified, suggesting that the source of contamination was heterogeneous. It should be noted that 9 serotypes were isolated from minced meat, 6 from chickens, and 4 from turkeys. The presence of 9 different serovars in beef meat could be related to the elevated number of farms that supply meat to Tetouan city markets. However, the number of poultry farms that supply the city is restricted, especially those that produce turkeys. Moreover, cross-contamination may occur at any stage, from breeding until consumption.

In Morocco, information regarding the antimicrobial resistance of *Salmonella* isolated from food was not sufficient. In the present study, the antimicrobial resistance of the strains isolated was tested against some classes of drugs recommended to treat *Salmonella* infections. The highest resistance was observed against nalidixic acid, which was reported in previous studies [4,38]. Some investigators linked resistance to nalidixic acid with the isolates from chickens [39]. However, in our study, the resistance to this antibiotic was shown even in isolates from cheese and red meat. In Morocco, enrofloxacin (a fluoroquinolone) is often used for the treatment of colibacillosis caused by *E. coli*. A high rate (76%) of resistance in *E. coli* isolated from chickens in Morocco was observed [40]. Putting these data together suggests that the high resistance of *Salmonella* to nalidixic acid (a quinolone) could be the consequence of the use of enrofloxacin, since nalidixic acid is one of the first generations of quinolones that targets DNA gyrase, so bacteria can rapidly develop resistance against it [34]. Hence, it has been demonstrated that *Salmonella* resistant to quinolones has emerged during the last years in several countries around the world [38], including Morocco [41]. The high level of nalidixic acid resistance may lead to therapeutic failure and can reduce clinical effectiveness of fluoroquinolones [4,9,39], which are the gold standard for the treatment of invasive and systemic salmonellosis [42]. In this study, we noted the lack of resistance to ciprofloxacin (a fluoroquinolone). However, it was reported that the emergence of resistance to this antibiotic has recently increased [7,9,34].

Where treatment with fluoroquinolones is not recommended (*e.g.*, in children and growing adolescents), third-generation cephalosporins are the drugs of choice for treatment of invasive *Salmonella* infections [4]. In the current study, no resistance to

second- and third-generation cephalosporins was found, but a low resistance to cephalothin (first generation) was recorded. Several studies described the reduction of susceptibility to cephalosporins in *Salmonella* strains from food products and veterinary and human sources [7,23,39].

In Morocco, resistance to ceftazidime was detected in 2008 in *S. Typhimurium* that caused the poisoning of 45 people [43]. In some European countries, high resistance to cefotaxime was observed in *Salmonella* isolated from turkeys [7].

The rate of *Salmonella*'s resistance to sulfonamide found in this study was 25%, which is different from the rates reported by other investigators; Khallaf *et al.* reported 64% in Morocco [25], and Proroga *et al.* observed 69% in Italy [44]. This could be due by the wide use of this antibiotic for a long time as an anti-infective [45]. On the other hand, resistance to trimethoprim/sulfamethoxazole in this study was approximately 10.4%, showing the positive effect of trimethoprim in association with sulfonamides.

A low level of resistance was noted to chloramphenicol (2.1%). In fact, the use of this antibiotic is forbidden in veterinary practice in Morocco.

In this study, the resistance to multiple antimicrobial agents was predominantly seen in Kentucky, Saintpaul, Typhimurium, and Israel serotypes. The resistance profile of these serotypes was different from each other. It is noteworthy that our results did not show any correlation between the resistance profile and the origin of the isolate.

On the other hand, the high rate of resistance observed in our study could be explained by the predominance of the serovar Kentucky, which is characterized by a high level of resistance. Indeed, a lower level of resistance was reported in a study where no Kentucky was found [22]. However, the percentages of resistance obtained in other studies in which Kentucky was predominant were higher than those obtained in the present study [25-27]. The multidrug-resistant strains of Kentucky showing resistance to ciprofloxacin have been reported in African countries and emerged in Europe in 2002 [46]. In Morocco, *S. Kentucky* resistant to ciprofloxacin was isolated for the first time from turkeys in 2006, in the same year another strain showing the same resistance profile was isolated from a patient [39].

The results of multidrug resistance for Typhimurium isolated from chickens matched with the results obtained from other studies that isolated serotype Typhimurium from different food products

[22,34,47]. This serotype was considered a new pandemic strain of *Salmonella* in Europe, possessing a high resistance profile ASulTE in humans [7]. According to a study conducted in United States, the frequency of the multidrug-resistant serovar Typhimurium was reportedly increasing [48]. Besides the direct risk for human health of antimicrobial-resistant *Salmonella*, the serovar Typhimurium represents an indirect risk in transferring resistance genes to other pathogenic bacteria through humans [13].

In the current study, *Salmonella* isolated from chickens showed higher levels of multi-resistance than those isolated from other products, since the majority of the isolated strains from chickens were Kentucky, Typhimurium, and Saintpaul, which demonstrated multidrug resistance. This finding was in agreement with that reported in a previous study carried out in Vietnam [38].

To our knowledge, there is no data about serovar distribution and the resistance of *Salmonella* isolated from food products or humans from the city of Tetouan. Therefore, the results of this study could be the starting point for monitoring *Salmonella* serovars and their emerging resistance profiles in animal-derived food in Tetouan. In addition, the results obtained in this study showing high resistance to quinolones, the most-used antibiotics in veterinary practices, would contribute to therapeutic failure. However, it is imperative to continue this research to better differentiate between the strains and to determine the genes of resistance in these resistant bacteria.

Conclusions

Our study is the first report of the prevalence of *Salmonella* in Tetouan city. A high prevalence of *Salmonella* in the screened meat and poultry products was found. A wide diversity was observed in the isolated serotypes, with Kentucky and Agona being the predominant serotypes. High resistance to nalidixic acid and sulfonamides was detected, and different serovars were multidrug resistant. No relationship was found between the resistance profile and the origin of the isolates. These results show a need for a stringent hygiene in retail premises during slaughter and transportation. Furthermore, the multidrug resistance recorded indicates the imperative requirement to monitor the *Salmonella* resistance in food products in order to take the necessary safety measures.

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