

## Prevalence and antibiotic-resistance of *Salmonella* isolated from chicken meat marketed in Rabat, Morocco

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**ABSTRACT:** Salmonellosis is a one of the major food-borne diseases with a high incidence and severity. In recent years, an increase in the occurrence of antimicrobial drug-resistant *Salmonella* spp. has been observed in several countries including Morocco. This study examines the prevalence of different *Salmonella enterica* (*S. enteric*) serotypes and their susceptibilities to commonly used drugs for treatment of *Salmonella* infections. In total, 300 chicken meat samples collected from 2011 to 2012 were investigated. Isolated *Salmonella* were characterized by serotyping and susceptibilities were determined for 16 antimicrobial drugs using the disc diffusion assay. *Salmonella* was isolated in 12.66% (n=38) specimens. 13 different serotypes have been identified, including *S. Kentucky* (n=9) 23.68% was the most common, followed by *S. Enteritidis* (n=5) 13.15%, *S. Muenster* (n=5) 13.15%, *S. Typhimurium* (n=3) 7.89% *S. Istanbul* (n=3) 7.89% *S. Saintpaul* (n=2) 5.26% and 2.63% for each of the other serotypes. Test results of antibiotic susceptibility showed that 65.78% of the isolates (n=25) were resistant to at least one antibiotic. Resistance to tetracycline and nalidixic acid was the most common (50%), followed by resistance to ampicillin (39.47%), streptomycin (34.21%), the sulfonamide (31.57%), noting that our strains were resistant to ciprofloxacin (26.31%), chloramphenicol (13.15%), amoxicillin + clavulanic acid (18.42%), and trimethoprim sulfamethoxazole (5.25 %). None of the isolates were resistant to cephalosporins of the 3rd generation. Multidrug resistance (>3) was observed in 42.1% of isolates, mainly *S. Kentucky*, *S. Virchow*, *S. Muenster*, *S. Fillmore* and *S. Istanbul*. As these resistant bacteria can be transmitted to humans through food (chicken) they constitute a major public health problem in Morocco and transmission of multiresistant strains mainly *S. Kentucky*.

**KEYWORDS:** *Salmonella*, antibiotic-resistance, chicken meat, Morocco.

### 1 BACKGROUND

The contamination of food products with *Salmonella* generates serious health and economic consequences, which have stimulated numerous studies designed to investigate the survival capacity and the transmission routes of these organisms in different farm animals and the environment [1]. Salmonellosis is one of the most widespread food-borne zoonoses in industrialized as well as developing countries [2], [3], [4], [5]. In most cases, salmonellosis is caused by contaminated food products, particularly those of animal origin such as poultry, eggs [4], [6], [7], [8]. Different studies conducted in Morocco indicated considerable prevalence of *Salmonella* in poultry [1], [9].

Antimicrobial resistance represents a major problem that threatens public health worldwide because it reduces the effectiveness of antibacterial treatment and increases morbidity, mortality, and costs for health-care assistance [10]. The use of antimicrobials in any venue, including disease treatment and growth promotion in domestic livestock, can potentially lead

to widespread dissemination of antimicrobial-resistant bacteria [11]. In recent years, an increase in the occurrence of antimicrobial drug-resistant Salmonella spp. has been observed in several countries including Morocco [1], [9], [12], [13].

Antimicrobial-resistant Salmonella are increasing due to the use of antimicrobial agents in food animals at sub-therapeutic level or prophylactic doses which may promote on-farm selection of antimicrobial resistant strains and markedly increase the human health risks associated with consumption of contaminated meat products. Antimicrobial use in animal production systems has long been suspected to be a cause of the emergence and dissemination of antimicrobial resistant Salmonella [3]. The short and long-term consequences generated by bacterial resistance are severe. Reports have shown that 50-60% of the majority of nosocomial infections in some countries are caused by antibiotic-resistant bacteria [10]. Fatality rates are higher for patients with infections caused by drug-resistant Salmonella spp., and these patients are more likely to require hospitalization and to be hospitalized for longer periods than are patients with infections caused by antimicrobial drug-susceptible Salmonella spp [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 2011-2012 were also evaluated.

## **2 MATERIAL AND METHODS**

### **2.1 FOOD SAMPLES COLLECTION**

During the period from June 2011 to December 2012, a total of 300 chicken meat samples were collected from retail raw chicken meat in Rabat in Morocco. Approximately 50 g of each sample was collected in a sterile plastic pouch and transported to the laboratory refrigerated at 4–10° C not later than 24 hours after collection and samples were immediately analyzed.

### **2.2 MICROBIOLOGICAL ANALYSIS**

The samples were analyzed using standard method the [14]. 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 7-8 hours at 42°C (RV) and at 37°C (SC), respectively. Bacterial isolation was achieved on Hektoen and XLD media Bio-Rad (Marne-la-Coquette- France) at 37°C for 18-24 hours. 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 Le Minor and Popoff [15]. The antigenic formula was determined according to the Kauffman-White scheme [16].

### **2.3 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 16 commercial antibiotic discs Bio-Rad (Marnes-la-Coquette- France): ampicillin (10µg), amoxicillin + clavulanic acid (20+10µg), cefotaxime (30µg), ceftazidim (30µg), Ceftriaxone (30µg), chloramphenicol (30µg), gentamycin (500µg), Kanamycin (30µg), trimethoprim (5µg), Spectinomycin (100µg), streptomycin (10µg), sulfonamides (300µg), Sulphametoxazole (300µg), tetracyclin (30µg), ciprofloxacin (5µg) and nalidixic acid (30µg). In this study, the isolates showing a decrease in susceptibility (intermediate) were considered as resistant. *Escherichia coli* ATCC 25922 was used as a control.

## **3 RESULTS**

### **3.1 PREVALENCE OF SALMONELLA IN CHICKEN MEAT SAMPLES**

A thirty eight out of three hundred (12.66%) chicken meat samples examined at the Food Control laboratory of National Institute of Hygiene during 2010 and 2011 were found positive for Salmonella.

### 3.2 SEROTYPING OF SALMONELLA ISOLATES

A total of fourteen different serotypes were identified among *S. enterica* isolates; the top five included the following serotypes: Kentucky (n=9) 23.68% was the most common, followed by Enteritidis (n=5) 13.15%, Muenster (n=5) 13.15%, Typhimurium (n=3) and Istanbul (n=3) 7.89%. Serotypes Saintpaul (n=2), Djugu (n=1), Fillmore (n=1), Hatford (n=1), Indiana (n=1), Othmarschen (n=1), Tennessee (n=1), Virchow (n=1) and Zerifin (n=1) were also identified. Two 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. Serotype prevalence and distribution in chicken meat samples are reported in Table 1.

**Table 1. Predominant serotypes (%) of *S. enterica* isolated in chicken meat samples**

Serovars	Number	%
Kentucky	9	23,7
Muenster	5	13,16
Enteritidis	5	13,16
Typhimurium	4	10,53
Istanbul	3	7,89
Saintpaul	2	5,26
Salmonella Spp	2	5,26
Fillmore	1	2,63
Djugu	1	2,63
Virchow	1	2,63
Indiana	1	2,63
Othmarschen	1	2,63
Tennessee	1	2,63
Hatford	1	2,63
Zerifin	1	2,63
Total	38	100

### 3.3 RESISTANCE TO ANTIMICROBIALS

Drug susceptibility assay revealed that 34.21% (n=13) of the Salmonella isolates investigated were fully susceptible to all fifteen antimicrobials tested. No resistance to cefotaxime, ceftazidim, ceftriaxone, gentamicine, kanamycin and spectinomycin was observed. Twenty five (65.79%) exhibited resistance to at least one antimicrobial, while twenty (52.63%) were resistant to two or more tested drugs. Resistance to antimicrobials was identified mostly in serotypes Kentucky (9/9), Enteritidis (5/5), Muenster (4/5), Istanbul (3/3), Typhimurium (2/4), Fillmore and Virchow (1/1). However, Salmonella strains serotype Djugu, Hatford, Indiana, Othmarschen, Saintpaul, Tennessee, Zerifin and the nontypeable Salmonella isolates did not show any resistance phenotype. The most common resistance observed was to tetracycline and nalidixic acid, accounting for 50% of isolates, followed by ampicillin 39.47%, streptomycin and the sulfonamide found in 34.21% and 31.57% of isolates respectively. These resistances were variously distributed among different serotypes. Resistance to ciprofloxacin 26.31% and amoxicillin + clavulanic acid 18.42% was found mainly in serovar Kentucky with a rate of 9/10 and 5/7 respectively, while resistance to chloramphenicol 13.15% was found in serovar Muenster (4/5). Resistance to sulfamethoxazole and trimethoprim 5.25% each were observed to a lesser extent and was common in serovar Typhimurium (2/2). It was also evident from the results that 42.1% (n=16) of isolates showed multiple drug resistance (resistant to > 3 drugs) and one strain among the 9 found of serovar Kentucky was Extended-spectrum beta-lactamases (ESBL). The resistance pattern, the origin, and the serotypes of the resistant strains are shown in Table 2.

Table 2. Distribution of antibiotic resistance by serotype identified in *S. enterica* isolates.

antibiotic	Number of resistance	% of resistance
Acide Nalidixique	19	50
Tétracycline	19	50
Ampicilline	15	39,47
Streptomycine	13	34,21
Sulfonamide	12	31,57
Ciprofloxacine	10	26,31
Amoxicilline	7	18,42
Acide Clavulanique	5	18,42
Chloramphénicol	2	13,15
Trimetoprime	2	5,25
Gentamicine	0	0
Spectinomycine	0	0
Ceftriaxone	0	0
Ceftazidime	0	0
Kanamycine	0	0
Cefotaxime	0	0

#### 4 DISCUSSION

Food-borne diseases caused by non-typhoid Salmonella represent a major public health problem worldwide [6]. *S. enterica* is a prevalent foodborne pathogen that can carry multidrug resistance (MDR) and pose a threat to human health [18]. The control of Salmonella and antibiotic resistance are closely related to each other. It means, the control of Salmonella in animal, animals' food, environment and humans is the best possible way for control of antibiotic resistance [2].

The overall prevalence (12.66%) of Salmonella isolated among chicken meat samples in this study was consistently close at that reported from other countries [8], [19]. This result is significantly high to be a potential source of foodborne salmonellosis. The result is higher than a study conducted by Ashraf et al. [20] and Akbar et al. [21] who reported a prevalence of 4.3% and 5.26% respectively and lower than a study conducted by Ta et al. [22] and Wang et al. [23] who reported a prevalence of 43.3% and 48.7% respectively. The difference may be due to different hygienic status of locals where the samples of chicken meats were collected. The analysis of the isolated Salmonella strains in this study showed different serotypes with a strong variability in samples collected in the outlets where hygienic conditions are not satisfactory. The most prevalent Salmonella serovar was Kentucky (23.68% of isolates). This result was consistently close at that reported from other study [9], [24], [25]. Similarly, our study showed a high proportion (52.63%) of Salmonella isolates were resistant to two or more of the antimicrobials that are commonly used in the veterinary and public health set up. This may pose difficulties in the treatment of human clinical cases and other bacterial diseases. This correlates with several studies performed in Morocco [1], [9]. In comparison, Ammari et al. [1] found low level of resistance to nalidixic acid (25%) and the same level of resistance to amoxicillin-clavulanic acid (18.75%) among Salmonella isolated from human and food samples. Bouchrif et al. [9] reported 63% of 30 clinical isolates of *S. Kentucky* obtained in Casablanca between Jan 1, 2003, and Dec 31, 2011 were resistant to ciprofloxacin. In addition, there was one strain among the 9 found of serovar Kentucky Extended-Spectrum Beta-Lactamases (ESBL). The demonstration that meat products are a source of antibiotic resistant Salmonella strains is a serious concern for public health and food safety.

The most important cause of antibiotic resistance is consumption of antibiotic as a growth promoter and treatment for animals and human. Therefore it is very important decrease the consumption of antibiotic in animal and humans [2]. The widespread overuse and misuse of antimicrobial agents are associated with the development of resistance to these drugs that has emerged as a major problem worldwide [26]. Because antimicrobial-resistant bacteria may be transferred to humans through the food chain and the selection of novel antimicrobial resistance mechanisms in Salmonella in animals which specify resistance to antibiotics used in humans, is troubling [11].

The current study indicated the necessity of a further investigation on the prevalence and antimicrobial susceptibility pattern of Salmonella, by considering it as a potential food borne pathogen. Molecular characterization of the isolates with emphasis on resistant strains is also necessary to identify mechanisms of antibiotic resistance. More over judicious and

prudent use of antimicrobials in the veterinary and public health sectors is mandatory since high rate of antimicrobial resistant *Salmonella* isolates were identified.

## 5 CONCLUSION

Three key findings in our study should be noted. First, high proportion (52.63%) of *Salmonella* isolates was resistant to two or more of the antimicrobials. The second notable finding was that the most prevalent *Salmonella* serovar was Kentucky (23.68%) and 9 of 10 of this serovar identified were resistant to ciprofloxacin. Third, the detection of one strain of serovar Kentucky was Extended-spectrum beta-lactamases (ESBL).

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