Prevalence and antimicrobial susceptibility profile of *Salmonella* isolated from food products in the region of Casablanca, Morocco

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Abstract

Introduction: Salmonellosis is a foodborne bacterial disease responsible for food epidemics around the world. The objective of this study is to determine the prevalence and diversity of *Salmonella* serotypes in several food products isolated at the Casablanca Regional Analysis and Research Laboratory and to test their resistance to different antimicrobials.

Methodology: 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. The *Salmonella* isolates were further analyzed by PCR to detect the presence of virulence genes *invA*.

Results: 20 different serotypes were identified from 80 strains isolated from 2015 to 2019, the most common of which are *S. kentucky* (26.3%) followed by *S. muenster* (10%), *S. typhimurium* (8.7%), *S. menston* (7.5%) and *S. enteritidis* (6.3%). Antimicrobial susceptibility testing revealed that 66.25% of isolates were resistant to at least one of the 14 antimicrobial agents tested. Bacterial resistance was most frequently observed for tetracycline with 46.25%, 45% to sulfonamide, 35% to nalidixic acid, 26, 25% to ampicillin, and 25% to ciprofloxacin. *Salmonella* serotypes *S. montevideo*, *S. virchow*, *S. amsterdam*, *S. anatum*, and *S. bloomsbury* were 100% susceptible to all antimicrobials tested.

Conclusions: The results of this study have shown that minced meat has a high level of *Salmonella* contamination, which can be considered one of the main potential sources of human salmonellosis in Morocco.

Key words: *Salmonella*; serovars; prevalence; antimicrobial resistance; Morocco.


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Introduction

Foodborne illness following ingestion of food contaminated with pathogens is an important cause of morbidity and mortality worldwide. The World Health Organization estimates that about 600 million people become ill from contaminated food and cause more than 420,000 deaths each year [1].

*Salmonella* infections is one of the major causes of food diseases worldwide, therefore it is a serious public health problem [2]. These foodborne pathogens are responsible for about 65% of food-borne illness cases in France and 95% in the United States [3], and it is the second bacterial etiology behind *Campylobacter* toxin-collective food infection and the leading cause of hospitalization and mortality in Europe [2], with a reporting rate of 21.2 cases per 100,000 population [4].

Globally, 2600 *Salmonella* serotypes have been identified worldwide [5]. Hence, in Morocco, several previous studies have been carried out to confirm locally the presence and evaluate the prevalence of *Salmonella* in certain food products [6], including, laying hen farms [7], turkeys of the flesh [8], meat and animal derivatives [9,10], and finally, those present in the environment [11].

Antimicrobials are probably the most successful form of bacterial infectious disease treatment in the history of medicine since their discovery in the early part of the 20th century [12]. However, massive and abusive use of antibiotics has contributed to the
development of resistant bacteria, and Salmonella serotypes apart from serovar Typhi have shown antibiotic resistance all over the world [13].

In Morocco, an evolution of bacteria susceptibility profile to antimicrobials showed high rates of resistance to quinolones in Salmonella serovar kentucky [8,14] and to third-generation cephalosporin in S. enterica serotype Typhimurium [15,16].

In this context, few data are available on emergence phenomena in terms of bacterial resistance linked to food consumption.

The primary objective of our study is to determine the prevalence of antigenic diversity in the foodborne Salmonella population isolated from the Regional Laboratory of Analysis and Research of Casablanca (LRARC) between 2015 and 2019 in order to establish a profile of antimicrobial susceptibility.

**Methodology**

**Origins of strains**

The strains analyzed in this study come from the Regional Laboratory of Analysis and Research of Casablanca (LRARC) under the tutorial of the National Office of Health Safety of Food Products (ONSSA), within the framework of compliance checks, self-checking or official controls. The strains that have been isolated are from different foods. The study was conducted over two periods, a first period from 2015 to 2018 that involved a retrospective study in which we used the strains collection, and a second period from January 1 to December 31, 2019, where we searched, isolated, and identified strains of Salmonella.

**Isolation and identification of Salmonella**

The detection of Salmonella strains was carried out according to techniques recommended by the Standard (ISO 6579), following four steps: pre-enrichment, enrichment, isolation, and biochemical identification.

25 grams of the test sample was inoculated into 225 mL of buffered peptone water (Biokar Diagnostics, France) and incubated at 37 °C for 24 hours. Enriching simultaneously on two broths, 0.1 mL of pre-enrichment culture was inoculated into 10 mL of Rappaport-Vassiliadis broth (Biokar Diagnostics, France) and 1 mL was inoculated into 10 mL of Muller Kauffmann Novobiocin Tetrathionate broth (Biokar Diagnostics, France), the culture was incubated at 42 °C and 37 °C for 2 hours, respectively.

The isolation was carried out on Xylose Lysine Deoxycholate agar (Biokar Diagnostics, France), then purified on nutrient agar. To confirm Salmonella, all suspected colonies underwent oxidase and urease tests, followed by biochemical identification using the API 20E (BioMérieux, Marcy l’Étoile, France).

**Molecular identification of Salmonella**

DNA is extracted by the fast method, adapted to small volumes: colonies collected from a fresh culture of 18 to 24 hours on TCS nutrient agar are suspended in 500 μL of water molecular biology, lysed by thermal action in boiling water for 10 minutes, followed by centrifugation at 12000 rpm for 5 minutes. The supernatant was recovered and stored at -20 °C until use. The molecular confirmation of Salmonella strains was performed by amplification of the 275-bp fragment of the invA gene (Accession number M90846.1) using the primer pair: Forward 5'-tattgcaacctggggaat-3' and reverse 5'-tacgacctgcaaaaagac-3'[17], using the conventional polymerase chain reaction (PCR) described by Karraouan et al. [18].

**Salmonella serotyping**

All Salmonella isolates were serotyped by slide agglutination tests, using group serum immunes and specific serum immunes (BioRad, Marnes-La-Coquette, France), directed against somatic “O” and flagellar “H” antigens, of phase 1 and phase 2, if the latter is negative, the process by swarming according to Sven Gard was carried out for the induction of the second flagellar phase. The results were interpreted based on the Kauffmann-White Scheme [19].

**Antimicrobial susceptibility testing**

Antibiotic susceptibility testing was performed using the disc diffusion method on Muller-Hinton agar (Bio-Rad, Marnes-La-Coquette, France) by flooding. A total of 14 antimicrobial agents were used in this study (Table 1).

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>Code</th>
<th>Concentration Disc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>AMP</td>
<td>10 µg</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>CTX</td>
<td>30 µg</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>FOX</td>
<td>30 µg</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>CAZ</td>
<td>30 µg</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>CRO</td>
<td>30 µg</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>CHL</td>
<td>30 µg</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>CIP</td>
<td>5 µg</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>GEN</td>
<td>10 µg</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>ETP</td>
<td>10 µg</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>NAL</td>
<td>30 µg</td>
</tr>
<tr>
<td>Sulfonamide</td>
<td>SMX</td>
<td>200µg</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>TET</td>
<td>30 µg</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>TMP</td>
<td>5 µg</td>
</tr>
<tr>
<td>Trimethoprim/</td>
<td>SXT</td>
<td>1.25 µg/23.75 µg</td>
</tr>
<tr>
<td>Sulphamethoxazole</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Antimicrobial Agents and the range concentrations tested.
The measurements of the diameters of the inhibition zones were carried out, then interpreted according to the recommendations of the Committee of Antibiotics of the French Society of Microbiology (CA-SFM) version 2018 [20]. *Escherichia coli* ATCC 25922 was used as a quality control strain.

**Results**

**Prevalence and distribution of serotypes**

In this study, eighty strains of *Salmonella* were isolated from the different foodstuffs analyzed. Results showed a higher prevalence of *Salmonella* contamination of minced meat at 50% (40/80), followed by sausages at 11.25% (9/80), deli meats at 8.75% (7/80), chickens at 7.5% (6/80), land snails and mechanically separated meat (MSM) at 5% (4/80), breaded products and turkeys at 3.75% (3/80), and with a lower contamination rate of 1.2% (1/80) for peppercorns and sheep meat (Table 2).

Molecular identification has shown that invasion gene *invA* was detected in all *Salmonella* isolates in this study.

The isolated strains were serotyped by a slide agglutination test. Of the strains isolated from *Salmonella*, twenty different serotypes were identified from 2015 to 2019. The most common serovar was *S. kentucky* (21/80, 26.3%). However, other serovars including *S. muenster* (8/80, 10%), *S. typhimurium* (7/80, 8.7%), *S. menston* (6/80, 7.5%), *S. enteritidis* (5/80, 6.3%), *S. montevideo* (4/80, 5%), *S. chester* (4/80, 5%), *S. saintpaul* (3/80, 3.8%), *S. london* (3/80, 3.8%), *S. infantis* (3/80, 3.8%), *S. schwarzengrund* (3/80, 3.8%), *S. agona* (2/80, 2.5%), *S. virchow* (2/80, 2.5%), *S. lindenburg* (2/80, 2.5%), *S. hadar* (2/80, 2.5%), *S. newport* (1/80, 1.2%), *S. altona* (1/80, 1.2%), *S. anatum* (1/80, 1.2%), *S. amsterdam* (1/80, 1.2%), *S. bloomsbury* (1/80, 1.2%) were also identified (Table 2).

**The antimicrobial susceptibility of isolated Salmonella**

The determination of resistance phenotype to 14 antibiotics was obtained by the Muller-Hinton agar diffusion method. The proportions of resistance isolates are shown in Table 3.

Drug susceptibility data showed that 46.25% of the strains has a high level of resistance to tetracycline, followed by 45% to sulfonamide, 35% to nalidixic acid, 25% to ciprofloxacin, 26.25% to ampicillin, 21.25% to trimethoprim, and 16.25% to trimethoprim/sulfamethoxazole. Antibiotic molecules with a low resistance rate of 1.25% are cefoxitin, 2.5%.

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

<table>
<thead>
<tr>
<th>Salmonella Serovar</th>
<th>Minced Meat</th>
<th>Sausages</th>
<th>Deli Meats</th>
<th>Chickens</th>
<th>MSM</th>
<th>Land Snails</th>
<th>Breaded Products</th>
<th>Turkeys</th>
<th>Mussels</th>
<th>Sheep Meat</th>
<th>Peppercorns</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kentucky</td>
<td>7 (33.3%)</td>
<td>5 (23.7%)</td>
<td>2 (9.5%)</td>
<td>1 (4.8%)</td>
<td>1</td>
<td>1</td>
<td>1 (4.8%)</td>
<td>1</td>
<td>2 (9.5%)</td>
<td>1 (4.8%)</td>
<td>1 (4.8%)</td>
<td>21</td>
</tr>
<tr>
<td>Muenster</td>
<td>8 (100%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>4 (57.1%)</td>
<td>2 (28.6%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>Menston</td>
<td>4 (66.7%)</td>
<td>2 (33.3%)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Enteritidis</td>
<td>2 (40%)</td>
<td>1 (20%)</td>
<td>1 (20%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Montevideo</td>
<td>2 (50%)</td>
<td>1 (25%)</td>
<td>1 (25%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Chester</td>
<td>2 (50%)</td>
<td>1 (25%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Saintpaul</td>
<td>1 (33.3%)</td>
<td>1 (33.3%)</td>
<td>1 (33.3%)</td>
<td></td>
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<td></td>
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<td>1</td>
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<td></td>
<td>3</td>
</tr>
<tr>
<td>London</td>
<td>3 (100%)</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>3</td>
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<tr>
<td>Infantis</td>
<td>2 (66.7%)</td>
<td>1 (33.3%)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Schwarzengrund</td>
<td>1 (33.3%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Agona</td>
<td>1 (50%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Virchow</td>
<td>2 (100%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Lindenburg</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Hadar</td>
<td>1 (50%)</td>
<td></td>
<td>1 (50%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>New Port</td>
<td>1 (100%)</td>
<td></td>
<td>1 (100%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Altona</td>
<td>1 (100%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Anatum</td>
<td>1 (100%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Amsterdam</td>
<td>1 (100%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Bloomsbury</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>9</td>
<td>7</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>80</td>
</tr>
</tbody>
</table>

*MSM: Mechanically separated meat.

**Table 3. Antibiotic susceptibility of isolated strains of *Salmonella* spp.**

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R (n)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>26.25 (21)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>5 (4)</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>1.25 (1)</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>2.50 (2)</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>1.25 (1)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>8.75 (7)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>25 (20)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>10 (8)</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>1.25 (1)</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>35 (28)</td>
</tr>
<tr>
<td>Sulfonamide</td>
<td>45 (36)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>46.25 (37)</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>21.25 (17)</td>
</tr>
<tr>
<td>Trimethoprim-</td>
<td></td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>16.25 (13)</td>
</tr>
</tbody>
</table>

S: Susceptible; R: Resistant.
ceftriaxone, and ertapenem. On the other hand, the interpretation of the results to determine susceptible strains led to a determination of several antibiotic susceptibility profiles tested by serotype (Table 3, 4).

S. montevideo, S. virchow, S. amsterdam, S. anatum and S. bloomsbury serotypes were 100% susceptible to all antibiotics tested, whereas the S. kentucky strain (serovar with the highest percentage) showed high resistance to tetracycline 81%, nalidixic acid, sulfonamide and ciprofloxacin with 76.2%.

From this study, a diversity of sensitivity profile toward the different classes of antibiotics was observed. 60% of the strains showed resistance to at least one antimicrobial, a multidrug resistance ranging between 2 and 13 antibiotics was recorded, the resistance to multiple antimicrobial agents was mainly observed in 50% of isolates to at least two antimicrobials, 35% to more than 3 antimicrobial molecules and 33.75% to 4 antibiotics.

**Discussion**

**Prevalence and distribution of serotypes**

In this study, Salmonella was isolated from different foods. The results showed a predominance of contamination in meat and animal derivatives. It appears that the isolation rate of Salmonella in minced meat (50%) is higher than in other products. Meat and animal derivatives are considered to be the main source of Salmonella Typhi contamination. The high frequency of isolated Salmonella is due to cross-contamination, which may be due to inappropriate hygiene practices, either on livestock farms or during slaughtering, processing, transportation, or distribution. In addition, Salmonella contamination rates in seed pepper showed the lowest level (1.2%). To our knowledge, no previous study has been conducted to assess the level of Salmonella contamination in spices in Morocco.

As a result, control and mentoring of Salmonella contamination must be based on rapid and specific screening to prevent any foodborne disease outbreaks.

The distribution of Salmonella serovars identified in the present study was particularly heterogeneous (Table 4). Of the eighty isolated strains of Salmonella, 20 different serotypes were identified. This wide distribution of serotypes is due to the diversity of the raw material, and the inappropriate conditions of preparation, storage, and distribution. Serotypes S. kentucky (26.3%), S. muenster (10%), S. typhimurium (8.7%), S. muenster (7.5%), and S. enteritidis (6.5%) have been the five most frequently isolated serotypes, indicating the predominance of S. kentucky with a high occurrence of isolation from poultry products. This finding is similar to previous studies conducted by Amajoud et al. [6] and Karraouan et al. [10], showing that S. kentucky is the most frequently isolated serotype.

On the other hand, S. kentucky has an alarming emergence of isolation in various food products and as a cause of human pathologies in Morocco and around the world [21–24].

Moreover, S. muenster serotype was previously isolated from ground turkey meats in Morocco [10], from chickens in Ghana [25], from food and human samples in Lebanon [26], and from cattle samples in the Alberta province of Canada [27], present the second serovar primarily detected with a frequency of 10%. It
is important to mention that in 2009, S. muenster serotype cause a epidemic outbreak in France following the consumption of goat cheese [28].

Surprisingly, S. menston, which has rarely been associated with human salmonellosis cases, was among the serotypes frequently isolated in our study. Nevertheless, our result is in line with previous studies conducted on human samples (stool, blood, and urine) in Turkey [29] and from isolates of different foodstuffs in Morocco [21].

Zoonotic serovars of Salmonella enterica present a continuing global threat to the poultry industry and public health in many countries, and S. enteritidis and S. typhimurium are the most associated serotypes with human salmonellosis [30]. They also have been identified by the Centers for Disease Control and Prevention (CDC) as the most reported serotypes by public health laboratories [31]. Our result showed an isolation rate of 8.7% and 6.3% respectively for S. typhimurium and S. enteritidis. Since 2016, S. enteritidis occupied the first place of the serotypes responsible for human salmonellosis followed by S. typhimurium and its monophasic variant and remains actively involved in epidemics European amplitude [32]. A study carried out in Togo on various pathological products showed the predominance of these two serovars [33], also in the 12th report of the Algerian Network of Surveillance of Resistance of Bacteria to Antibiotics, where the dominance of two S. enteritidis and S. typhimurium in human medicine was clear.

In Morocco serotype S. typhimurium has been isolated in previous studies, from food in the city of Tetouan [6], and from turkey carcasses and offal in Meknès [34]. This serotype was also isolated from sausages in studies conducted in Egypt [35] and Morocco [23,36]. A study conducted in Colombia showed the presence of this serotype in chicken carcasses marketed at Ibague [37].

In the United States, S. enteritidis is responsible for 80% of epidemics and about 50,000 to 110,000 cases associated with contaminated eggs each year [38]. It is possible that, after laying, the eggs are contaminated through the shell [39], on the other hand, Salmonella can contaminate the inside of the eggs during its formation in the genital tract of the hen, which makes the egg an important reservoir of Salmonella [40]. Poultry, especially chicken, is the principal reservoir of this serotype [41]. In 2002, except for the Scandinavian countries, in all European countries, between 10 and 15% of chickens were sold in butcher shops infected with S. enteritidis, which was the most common serotype in meat (11.1% of serotypes) and in production units (10.8%) [42]. In Morocco, S. enteritidis has been isolated from laying hens [7] and broiler turkeys [8], and recently this serovar has been discovered from chicken samples [43].

Study of antibiotic susceptibility

The present study highlights the resistance rates of 14 antibiotics (Table 1), in Salmonella associated with contaminated food. The results recorded showed high levels of resistance to tetracycline (46.25%), sulfonamide (45%), nalidixic acid (35%), ciprofloxacin (25%), and ampicillin (26.25%). Antibiotic resistance in bacteria has continued to progress since antibiotics have been misused in human medicine and as a growth promoter in animals [44]. The resistance of Salmonella strains to fluoroquinolones, particularly nalidixic acid and Ciprofloxacin, is linked to the introduction of the latter into veterinary medicine, for prophylactic or therapeutic ends. Although resistance to ciprofloxacin is around 26.25%, which is low compared to previous studies conducted in Egypt (86.4%) [45] and Morocco 30.5% [22], it remains worrisome as this antibiotic is of first choice for severe non-typhoidal Salmonella infection in adults [14].

11% of the drugs prescribed by doctors are fluoroquinolones, they are effective in the treatment of a wide variety of infectious diseases [46]. As in human medicine, veterinary therapy began to prescribe them in Europe early 1990s, and a few years later in the United States [47]. The quinolone family is widely used in poultry for the treatment of juvenile chicks less than one week old.

Indeed, higher rates than those recorded in our study for nalidixic acid (35%) were recorded in other studies [7,10,22]. This antimicrobial belongs to the first generation of quinolones, which inhibit the activities of DNA gyrase and topoisomerase IV, two enzymes essential for the viability of bacteria. The acquisition of quinolone resistance is frequently linked to chromosomal mutations such as those of the genes encoding the A and B subunits of the protein targets (gyrA, gyrB, parC and parE), or to mutations leading to a decrease in the drug accumulation, either by decreased uptake or increased efflux, and plasmid-associated quinolone resistance genes have also been described [48]. The isolated Salmonella strains showed higher resistance to tetracycline. These results are higher than those found in a previous study in laying chicken farms 25% [7], and lower than those found in other studies [8,10,49], in which isolates were resistant to more than
the 46.25% found in our study. The presence of high resistance to tetracycline is due to the long time use of the molecule that is used in the first line, and its use, in recent years, in poultry industries for growth promotion and disease prevention [50]. 80% of tons of antibiotics sold each year in France is represented by four families: Tetracyclines, sulfonamides, Penicillins, and Macrolides, while tetracycline alone accounts for half of total sales [51,52].

Antibiotic resistance of isolated strains for sulfonamide showed a lower rate than the results of Truong Ha et al. in Vietnam [53] and that of Karraouan et al. in Morocco [10], which found respectively 58.1% and 64.1%.

Moreover, the study found that among the strains of *Salmonella* isolated, there was resistance to the beta-lactam and ampicillin family. Resistance of this family is usually due to beta-lactamase expression, through mutations involving the beta-lactamase structure (TEM, SHV, OXA, CTX-M beta-lactamase families) and/or mutations of beta-lactamase synthesis regulators (AmpC beta-lactamases); or the appearance of new enzymes (PER, VEB, CMY, DHA-1, ACC-1, etc.) [54]. Between 1962 and 1964, studies in the United Kingdom identified the first outbreaks of ampicillin resistance. In Morocco, a study by el Allaoui et al. showed a resistance of 33% in broiler turkey farms [8]. Molecular epidemiology studies suggest that the ESBL-encoding genes have been disseminated either by the proliferation of epidemic strains or by the transfer of plasmids carrying the resistance traits [55]. In contrast, we noticed a high percentage of *S. kentucky* resistant to fluoroquinolone (Table 4).

In 2006, *S. kentucky* resistance to ciprofloxacin with a CMI value of 4-16 mg/L was isolated in Morocco from a child hospitalized in the pediatric ward [14]. However, strains with resistance to ciprofloxacin and nalidixic acid have emerged around the world, especially in Europe; in 2002, it has been isolated in a French tourist who suffered from gastroenteritis. Resistance to ciprofloxacin increased in Denmark from 0.8% in 1995 to 8.5% in 2000 in non-typhoidal *Salmonella* isolated from humans [56]. Nalidix acid and ciprofloxacin resistance in human isolates of non-typhoidal *Salmonella* in Finland increased from 3.9% in 1995 to 23.5% in 1999 and this rise was greater (5.6% to 50%) among persons who traveled to Thailand [57]. Studies from the USA have reported resistance to nalidix acid in 0.5% and to ciprofloxacin in 0.02% of strains of non-typhoidal *Salmonella* [58]. Although in early 2006 the EU decided to ban the use of most antibiotics in animal feed, the emergence of multi-resistance continues to increase. In the case of our study, 50% of strains are resistant to more than 2 antibiotics, and 35% are resistant to more than three antibiotics. The highest multi-resistance index was observed in our study in *S. kentucky* isolates (Table 4), resistant to almost all families of antibiotics tested, quinolones, ampicillin, third-generation cephalosporins such as ceftriaxone, ceftazidime, cefoxitin, cefotaxime, sulfonamide, and tetracycline. In Morocco, resistance to several antibiotics has been reported for two strains of *S. typhimurium* [59]. The multi-resistance results of this study are lower than those found in China in meat 53% to at least three antibiotics [60], and those found in Meknes (Morocco), 44.12% to more than three antibiotics [23].

The effectiveness of antimicrobials in infections therapy has decreased and resistance strains have appeared in humans, this is the case of the study carried out on *Salmonella* responsible for human infections in Lomé in Togo where the strains taken from various products pathological showed high levels of resistance [33].

Invasion gene operon, *invA* was detected in all *Salmonella* spp. isolates in our study. This gene is essential for full virulence in *Salmonella* and is considered to trigger the internalization required for invasion of deeper tissue [61]. In line with our study, other studies also reported the detection of this gene in all *Salmonella* spp. isolates [7,22,23].

**Conclusions**

The study of strains isolated from different foods has shown a higher prevalence of *Salmonella* contamination in minced meat. The diversity of serotypes showed the presence of 20 different serotypes of which *S. kentucky* was the most isolated, followed by *S. muenster*, *S. typhimurium*, *S. menston*, *S. enteritidis*. Antimicrobial susceptibility testing results revealed that 66.25% of *Salmonella* isolated were resistant to at least one antibiotic, with 46.25% resistance to tetracycline followed by 45% to sulfonamide, 35% to nalidixic acid, and 25% to ciprofloxacin. This study determined the prevalence of serotypes and evaluated the antibiotic resistance profile of isolated strains of *Salmonella* spp. The recorded data highlight the importance of rigorous surveillance following antibiotic resistance, rationalizing the use of fluoroquinolones in veterinary and medical practice, and strengthening food hygiene measures, in order to limit the emergence of mutants resistant to antibiotics.
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