

Original Article

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. Examination of *Salmonella* for *invA* gene was positive for all the strains.

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.

J Infect Dev Ctries 2023; 17(3):388-396. doi:10.3855/jidc.17236

(Received 11 August 2022 – Accepted 08 November 2022)

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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* toxic 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 20^E (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'-tatcgccacgttcgggcaa-3' and reverse 5'-tcgcaccgtcaaaggaacc-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 1. Antimicrobial Agents and the range concentrations tested.

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

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,

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

Antimicrobial	Frequency (%)	
	R (n)	S (n)
Ampicillin	26.25 (21)	73.75 (59)
Cefotaxime	5 (4)	95 (76)
Cefoxitin	1.25 (1)	98.75 (79)
Ceftazidime	2.50 (2)	97.50 (78)
Ceftriaxone	1.25 (1)	98.75 (79)
Chloramphenicol	8.75 (7)	91.25 (73)
Ciprofloxacin	25 (20)	75 (60)
Gentamicin	10 (8)	90 (72)
Ertapenem	1.25 (1)	98.75 (79)
Nalidixic acid	35 (28)	65 (52)
Sulfonamide	45 (36)	55 (44)
Tetracycline	46.25 (37)	53.75 (43)
Trimethoprim	21.25 (17)	78.75 (63)
Trimethoprim-Sulfamethoxazole	16.25 (13)	83.75 (67)

S: Susceptible; R: Resistant.

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,

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

<i>Salmonella</i> Serovar	Food Samples											Total
	Minced Meat	Sausages	Deli Meats	Chickens	MSM*	Land Snails	Breaded Products	Turkeys	Mussels	Sheep Meat	Peppercorns	
Kentucky	7 (33.3%)	5 (23.7%)		2 (9.5%)	1 (4.8%)	1 (4.8%)	1 (4.8%)	2 (9.5%)	1 (4.8%)	1 (4.8%)		21
Muenster	8 (100%)											8
Typhimurium	4 (57.1%)		2 (28.6%)						1 (14.3%)			7
Menston	4 (66.7%)		2 (33.3%)									6
Enteritidis	2 (40%)		1 (20%)	1 (20%)		1 (20%)						5
Montevideo	2 (50%)		1 (25%)	1 (25%)								4
Chester	2 (50%)	1 (25%)						1 (25%)				4
Saintpaul	1 (33.3%)	1 (33.3%)		1 (33.3%)								3
London	3 (100%)											3
Infantis	2 (66.7%)		1 (33.3%)									3
Schwarzengrund	1 (33.3%)				2 (66.7%)							3
Agona		1 (50%)									1 (50%)	2
Virchow	2 (100%)											2
Lindenburg							2 (100%)					2
Hadar		1 (50%)			1 (50%)							2
New Port				1 (100%)								1
Altona	1 (100%)											1
Anatum						1 (100%)						1
Amsterdam	1 (100%)											1
Bloomsbury						1 (100%)						1
Total	40	9	7	6	4	4	3	3	2	1	1	80

*MSM: Mechanically separated meat.

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. menston* (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

Table 4. Antibiotic Susceptibility profile of *Salmonella* strains.

<i>Salmonella</i> Serovars	Antimicrobial resistance profile
Agona	AMP, SMX
Altona	SMX
Chester	AMP, TET
Chester	AMP, CAZ, CHL, SMX, TET, TMP, SXT
Chester (2)	CHL, SMX, TET, TMP, SXT
Enteritidis (2)	NAL
Enteritidis	SMX
Enteritidis	SMX, TET
Enteritidis	TET
Hadar	AMP, CHL, NAL, CIP, SMX, TET, TMP, SXT
Hadar	NAL, CIP, TET
Infantis	NAL
Infantis	NAL, TET
Kentucky (8)	AMP, NAL, CIP, GEN, SMX, TET
Kentucky (2)	AMP, NAL, CIP, SMX, TET
Kentucky	AMP, NAL, CIP, SMX, TET, TMP
Kentucky	AMP, NAL, CIP, SMX, TET, TMP, SXT
Kentucky	AMP, NAL, CIP, TET, TMP
Kentucky	AMP, CTX, FOX, CAZ, CRO, CHL, NAL, CIP, ETP, SMX, TET, TMP, SXT
Kentucky (2)	NAL, CIP, SMX, TET
Kentucky	SMX, TET
Lindenburg	AMP, CHL, NAL, CIP, SMX, TET, TMP, SXT
Lindenburg	TET, TMP
London (2)	CTX, SMX
Muenster	CTX, SMX
Newport	NAL, CIP, SMX, TET, TMP
Saintpaul	NAL
Saintpaul (2)	NAL, SMX, TET, TMP, SXT
Schwarzengrund	CHL, SMX, TET, TMP, SXT
Schwarzengrund	NAL
Typhimurium (2)	AMP, SMX, TET, TMP, SXT
Typhimurium (2)	SMX, TET
Typhimurium	TET, TMP

AMP: Ampicillin; CHL: Chloramphenicol; CRO: Ceftriaxone; CTX: Cefotaxime; CIP: Ciprofloxacin; ETP: Ertapenem; FOX: Cefoxitin; GEN: Gentamicin; SMX: Sulfonamide; CAZ: Ceftazidime; NAL: Nalidixic acid; TET: Tetracycline; TMP: Trimethoprim; SXT: Trimethoprim + Sulfamethoxazole.

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 Ibaguè [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]. Nalidixic 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 nalidixic 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.

Acknowledgements

This work was supported by Regional Analysis Laboratory and Research Casablanca (LRARC-ONSSA) and Institut Pasteur du Maroc - Casablanca (IPM). We would like to express our gratitude to Doctor Fatiha El Mellouli and Professor Abderrahmane Maâroufi, the Directors respectively of the LRARC-ONSSA-Casablanca and IPM, for supporting this work.

References

- World Health Organization (2015) WHO estimates of the global burden of foodborne diseases. Available: https://apps.who.int/iris/bitstream/handle/10665/199350/9789241565165_eng.pdf?sequence=1 Accessed: 19 Dec 2020.
- Krui SL (2011) Phenotypic and genotypic diversity of *Salmonella* isolated in Cambodia from food and human biological samples. University Bordeaux. [Thesis in french]
- Haeghebaert S, Sulem P, Deroudille L, Vanneroy-Adenot E, Bagnis O, Bouvet P, Grimont F, Brisabois A, LeQuerrec F, Hervy C, Espié E, Valk H, Vaillant V (2003) Two outbreaks of *Salmonella enteritidis* phage type 8 linked to the consumption of Cantal cheese made with raw milk, France, 2001. Euro Surveill 8: 151-156.
- ECDC, EFSA, EMA (2017) ECDC/EFSA/EMA second joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in *bacteria* from humans and food-producing animals. EFSA Journal 2017 13: 4006.
- Sanderson KE, Nair S, Barrow P, Methner U (2013) Taxonomy and species concepts in the genus *Salmonella*. *Salmonella* in domestic animals. Oxfordshire, United Kingdom. 1-19 p.
- Amajoud N, Bouchrif B, El-Maadoudi M, Skalli-Senhaji N, Karraouan B, El-Harsal A, El-Abrini J (2017) Prevalence, serotype distribution, and antimicrobial resistance of *Salmonella* isolated from food products in Morocco. J Infect Dev Ctries 11: 136-142. doi: 10.3855/jidc.8026.
- Ziyate N, Karraouan B, Kadiri A, Darkaoui S, Soulaymani A, Bouchrif B (2016) Prevalence and antimicrobial resistance of *Salmonella* isolates in Moroccan laying hens farms. J Appl Poult Res 25: 539-546.
- El-Allaoui A, Rhazi-Filali F, Ameer N, Bouchrif B (2017) Contamination of broiler turkey farms by *Salmonella* spp. in Morocco: prevalence, antimicrobial resistance and associated risk factors. Rev Sci Tech OIE 36: 935-946. [Article in French].
- Cohen N, Ennaji H, Bouchrif B, Hassar M, Karib H (2007) Comparative study of microbiological quality of raw poultry meat at various seasons and for different slaughtering processes in Casablanca (Morocco). J Appl Poult Res 16: 502-508.
- Karraouan B, Fassouane A, Ossmani H-E, Cohen N, Charafeddine O, Bouchrif B (2010) Prevalence and virulence genes of *Salmonella* isolated from raw ground turkey meat in Casablanca, Morocco. Revue Méd Vét 161: 127-132. [Article in French].
- Ben-Moussa A, Chahlaoui A, Rour E, Chahboune M, Aboukacem A, Karraouan B, Bouchrif B (2014) Serotyping and virulence of *Salmonella* isolated in the waters of the River Khoumane region of Meknes, Morocco. J Mater Environ Sci 5: 547-552. [Article in French].
- Faure S (2009) Transfer of a blaCTX-M-9 beta-lactamase resistance gene between *Salmonella* and *Enterobacteria* of the human intestinal flora: influence of antibiotic treatment. University of RENNE. [Thesis in french].
- Cavallo JD, Meyran M (1992) *Salmonella* and its pathology: bacteriological basis for treatment. Med Mal Infect 22: 331-339. [Article in french].
- Bouchrif B, Karraouan B, Ennaji MM, Timinouni M (2008) Emergence of quinolone resistance in *Salmonella* spp. in Casablanca -Morocco. Med Mal Infect 38: 615-616. [Article in French].
- AitMhand R, Soukri A, Moustouai N, Amarouch H, ElMdaghi N, Sirot D, Benbachir M (2002) Plasmid-mediated TEM-3 extended-spectrum β -lactamase production in *Salmonella typhimurium* in Casablanca. J Antimicrob Chemother 49: 169-72.
- Bouchrif B, Karraouan B, Ennaji MM, Timinouni M (2008) Emergence of quinolone resistance in *Salmonella* spp. in Casablanca, Morocco. Med Mal Infect 38: 615-616. [Article in French].
- Nayak R, Stewart T, Wang RF, Lin J, Cerniglia CE, Kenney PB (2004) Genetic diversity and virulence gene determinants of antibiotic-resistant *Salmonella* isolated from preharvest turkey production sources. Int J Food Microbiol 91: 51-62.
- Karraouan B, Aziz F, El-Ossmani H, Cohen N, Charafeddine O, Bouchrif B (2010) Prevalence and virulence genes of *Salmonella* in raw minced meat from turkey in Casablanca, Morocco. Rev Med Vet 161: 127-132.
- Grimont P, Weill F-X (2007) Antigenic Formulae of the *Salmonella* Serovars World Health Organization collaborating Centre for Reference and Research on *Salmonella*, Institut Pasteur of Paris. 166 p.
- James S, Melvin P, April M, Shelley C, Sharon K, Marcelo F, Howard G, Romney M (2018) M100Ed32 | Performance Standards for Antimicrobial Susceptibility Testing, 32nd Edition. Clinical & Laboratory Standards Institute. Available: <https://clsi.org/standards/products/microbiology/documents/m100/>. Accessed: 4th July 2022.
- Berradi T, Karraouan B, Charafeddine O, Bouchrif B, Abouchoaib N (2018) Resistance to fluoroquinolones in *Salmonella kentucky* isolated from food products of animal origin Casablanca, Morocco. Med Mal Infect 48: 488-489. [Article in French].
- Boutaib R, Bouchrif B, Abid M, Karraouan B, Khaddor M (2015) Multidrug resistance of *Salmonella* isolated from shellfish samples, Morocco. BMRJ 8: 663-669.
- Ed-dra A, Filali FR, Karraouan B, El-Allaoui A, Aboukacem A, Bouchrif B (2017) Prevalence, molecular and antimicrobial resistance of *Salmonella* isolated from sausages in Meknes, Morocco. Microb Pathog 105: 340-345.
- Karraouan B, Ziyate N, Ed-dra A, Amajoud N, Boutaib R, Akil A, El-Allaoui A, El-Ossmani H, Zerouali K, Elmdaghri N, Bouchrif B (2017) *Salmonella kentucky*: Antimicrobial resistance and molecular analysis of clinical, animal and environment isolates, Morocco. J Infect Dev Ctries 11: 368-370. doi: 10.3855/jidc.8171.
- Andoh L-A, Dalsgaard A, Obiri-Danso K, Newman MJ, Barco L, Olsen JE (2016) Prevalence and antimicrobial resistance of *Salmonella* serovars isolated from poultry in Ghana. Epidemiol Infect 144: 3288-3299.
- Mansour MN, Yaghi J, El-Khoury A, Felten A, Mistou MY, Atoui A, Radomski N (2020) Prediction of *Salmonella* serovars isolated from clinical and food matrices in Lebanon and

- genomic-based investigation focusing on *Enteritidis* serovar. Int J Food Microbiol 333: 108831.
27. Guerin MT, Martin SW, Darlington G, Rajic A (2005) A temporal study of *Salmonella* serovars in animals in Alberta between 1990 and 2001. Can J Vet Res 69: 88–99.
 28. Cauteren D-Van, Silva N-J, Weill FX, King L, Brisabois A, Delmas G, Vaillant V, Valk H (2009) Outbreak of *Salmonella enterica* serotype Muenster infections associated with goat's cheese, France, March 2008. Euro Surveill 14: 19290.
 29. Erdem B, Ercis S, Hascelik G, Gur D, Aysev A-D (2005) Antimicrobial resistance of *Salmonella enterica* group C strains isolated from humans in Turkey, 2000–2002. Int J Antimicrob Agents 26: 33–37.
 30. Banach JL, Stratakou I, Fels-Klerx H, Den-Besten H, Zwietering M (2016) European alerting and monitoring data as inputs for the risk assessment of microbiological and chemical hazards in spices and herbs. Food Control 69: 237–249.
 31. Centers for Disease Control and Prevention (CDC) (2007) Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food—10 states, 2006. MMWR Morb Mortal Wkly Rep 56: 336–339.
 32. European Food Safety Authority (EFSA), European Centre for Disease Prevention and Control (ECDC) (2016) Multi-country outbreak of *Salmonella enteritidis* phage type 8, MLVA type 2-9-7-3-2 and 2-9-6-3-2 infections. EFSA support publ 13.
 33. Dagnra AY, Akolly K, Gbadoe A, Aho K, David M (2007) Emergence of multidrug-resistant *Salmonella* strains in Lomé (Togo). Med Mal Infect 37: 266–269. [Article in French].
 34. Allaoui AE, Filali FR, Derouich A, Karraoua B, Ameer N, Bouchrif B (2013) Prevalence of *Salmonella* serovars Isolated from Turkey Carcasses and Giblets in Meknès, Morocco. J World's Poult 24: 134-139.
 35. Edris AM, Shaltout F, Salem GH, El-Toukhy EI (2011) Incidence and isolation of *Salmonella* from some meat products. S Afr Med J: 2532-2533
 36. Bouchrif B, Le-Hello S, Pardos M, Karraouan B, Perrier-Gros-Claude J, Ennaji M, Timinouni M, Weill F-X (2009) Ceftazidime-resistant *Salmonella enterica*, Morocco. Emerg Infect Dis 15: 1693b–11695.
 37. Vélez D-C, Rodriguez V, García N-V (2017) Phenotypic and genotypic antibiotic resistance of *Salmonella* from chicken carcasses marketed at Ibagué, Colombia. Rev Bras Cienc Avic 19: 347–354.
 38. Braden CR (2006) *Salmonella enterica* serotype *enteritidis* and eggs: a national epidemic in the United States. Clin Infect Dis 43: 512–517.
 39. Humphrey TJ, Chart H, Baskerville A, Rowe B (1991) The influence of age on the response of SPF hens to infection with *Salmonella enteritidis* PT4. Epidemiol Infect 106: 33–43.
 40. Daube G (2005) Sanitary quality of pork and poultry products: importance of zoonotic agents. university of Liege, Faculty of Veterinary Medicine. [Thesis in french].
 41. Henzler DJ, Ebel E, Sanders J, Kradel D, Mason J (1994) *Salmonella enteritidis* in eggs from commercial chicken layer flocks implicated in human outbreaks. Avian Dis 38: 37–43.
 42. Foley SL, Nayak R, Hanning IB, Johnson TJ, Han J, Ricke SC (2011) Population dynamics of *Salmonella enterica* serotypes in commercial egg and poultry production. Appl Environ Microbiol 77: 4273–4279.
 43. Mohamed K, Najia A, Terta M, Lakranbi M, Senouci S, Ennaji MM (2014) Prevalence and antibiotic-resistance of *Salmonella* isolated from chicken meat marketed in Rabat, Morocco. Int J Innov Appl Stud 6: 2028–9324.
 44. Wegener HC, Hald T, Wong LF, Madsen M, Korsgaard H, Bager F, Gerner-Smidt P, Molbak K (2003) *Salmonella* control programs in Denmark. Emerg Infect Dis 9: 774–780.
 45. Ashraf-A A, Saad S, Nasef-Soad A, Fatma-I E-H, Ahmed S (2018) Study on the effect of some bacterial strains on IB viral infection. Res Vet Sci 11: 98.
 46. Weill F, Le Hello S, Lefevre S, Renaudat C (2016) Annual activity report 2016 National Reference Center for *Escherichia coli*, *Shigella* and *Salmonella*. Available: <https://www.pasteur.fr/fr/sante-publique/CNR/les-cnr/escherichia-coli-shigella-salmonella/rapports-d-activite>. Accessed: 12th December 2020. [Article in French]
 47. Bager F, Helmuth R (2001) Epidemiology of resistance to quinolones in *Salmonella*. Vet Res 32: 285–290.
 48. Fàbrega A, Madurga S, Giralt E, Vila J (2009) Mechanism of action of and resistance to quinolones. Microb Biotechnol 2: 40–61.
 49. Abba H, Somda M-K, Antipas B-B, Barro N, Traore A-S (2017) Prevalence and antibiotic susceptibility of *Salmonella* spp. non-typhoid isolated meat from chickens in Chad. Int J Bio Chem Sci 11: 107. [Article in French].
 50. Omer MM, Abusalab S, Gumaa M, Mulla S, Omer E, Jeddah I, Al-Hassan A, Hussein M, Ahmed A (2010) Outbreak of colibacillosis among broiler and layer flocks in intensive and semi-intensive poultry farms in Kassala State, Eastern Sudan. Asian J Poult Sci 4: 173–181.
 51. Moulin G, Cavalié P, Pellanne I, Chevance A, Laval A, Millemann Y, Colin P, Chauvin C (2008) A comparison of antimicrobial usage in human and veterinary medicine in France from 1999 to 2005. J Antimicrob Chemother 62: 617–625.
 52. Sanders P, Bousquet-Mélou A, Chauvin C, Toutain P (2011) Use of antibiotics in livestock farming and public health issues. INRA Prod Anim 24: 199–204 [Article in french].
 53. Thai TH, Hirai T, Lan N, Yamaguchi R (2012) Antibiotic resistance profiles of *Salmonella* serovars isolated from retail pork and chicken meat in north Vietnam. Int J Food Microbiol 156: 147–151.
 54. Michel-Briand Y, (2007) Resistance to the latest beta-lactams: mechanisms of acquisition and spread of resistance in *Enterobacteriaceae*. Bull Acad Natl Med 191: 35–50.
 55. Yu WL, Chuang YC, Walther-Rasmussen J (2006) Extended-spectrum beta-lactamases in Taiwan: epidemiology, detection, treatment and infection control. J Microbiol Immunol Infect 39: 264–277.
 56. Molbak K, Gerner-Smidt P, Wegener HC (2002) Increasing Quinolone Resistance in *Salmonella enterica* serotype *enteritidis*. Emerg Infect Dis 8: 514–515.
 57. Hwang IS, Song JY, Kim WJ, Jeong H-W, Kim M-S, Cheong H (2013) Prevalence and mechanisms of low-level quinolone resistance among non-typhoidal *Salmonella* isolates from human and poultry livestock in Korea: usefulness of nalidixic acid resistance test. Infect Chemother 42: 230–236.
 58. Herikstad H, Hayes P, Mokhtar M, Fracaro ML, Threlfall E-J, Angulo F-J (1997) Emerging quinolone-resistant *Salmonella* in the United States. Emerg Infect Dis 3: 371–372.
 59. Ed-Dra A, Rhazi-Filali F, Belhaj M, Moumni M, Chahboune M, Bentayeb M, Bouchrif B, Giarratana M (2019) Microbiological and molecular analyzes of non-typhoidal *Salmonella* isolated from sausages, their eradication and bioconservation of meat products by the use of essential oils from aromatic and medicinal plants. University of Moulay Ismail. [Article in french].

60. Yang B, Qu D, Zhang X, Shen J, Cui S, Shi Y, Xi M, Sheng M, Zhi S, Meng J (2010) Prevalence and characterization of *Salmonella* serovars in retail meats of marketplace in Shaanxi, China. *Int J Food Microbiol* 141: 63–72.
61. Carlson SA, Bolton LF, Briggs CE, Hurd HS, Sharma V-K, Fedorka-Cray PJ, Jones BD (1999) Detection of multiresistant *Salmonella typhimurium* DT104 using multiplex and fluorogenic PCR. *Mol Cell Probes* 13: 213–222.

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Conflict of interests: No conflict of interests is declared.