JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Serotyping, drug resistance, virulence, and antibiotic resistance genes of *Salmonella* isolated from contaminated food

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Abstract

Introduction: Antibiotic resistance (AMR) is a serious problem for veterinary and human health. Its progression is leading to therapeutic failures and risks taking humanity back to the era before the discovery of antimicrobials. The impact of AMR on the economy is considerable. This study was carried out to assess the extent of AMR in *Salmonella* isolated from food products.

Methodology: The European Committee on Antimicrobial Susceptibility Testing (EUCAST) standard method and the recommendations of the Antibiotic Susceptibility Committee of the French Society of Microbiology (CA-SFM) version 2023 V.1.0. 19 were used to test antibiotic susceptibility. A total of 41 antimicrobials were tested on the isolates. Virulence genes *fimA* and *stn*, and the antibiotic resistance gene *CMY-2* were tested by real-time polymerase chain reaction (PCR) on bacterial DNA extracted using the MacheryNagel RNA viral nucleospin extraction kit. The integrated search engine on the National Center for Biotechnology Information (NCBI) website was used to search for the primer sequence of interest for the predefined genes.

Results: All the isolates were resistant to at least one antibiotic, while 90% of isolates were multiresistant (resistant to at least 3 antimicrobial agents). All strains tested positive for the presence of the *stn* and *fimA* virulence genes, with the exception of one isolate. Similarly, with the exception of one strain, all strains tested positive for *CMY-2*.

Conclusions: These results point to the progression of AMR, which is increasingly gaining ground, and to the danger of *Salmonella* virulence as a major agent of food-borne illness in Morocco.

Key words: antibiotic resistance; virulence; foodborne pathogens; *CMY-2*.

J Infect Dev Ctries 2026; 20(3):374-380. doi:10.3855/jidc.21392

(Received 03 February 2025 – Accepted 19 August 2025)

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Introduction

Antibiotic resistance (AMR) is one of the top 10 global health threats which represents an urgent global public health and socio-economic problem [1]. Despite the global nature of AMR, it is difficult to have a clear and shared vision of its epidemiology due to data of widely varying quality and accessibility, particularly in low- and middle-income countries (LMICs) [2]. The knowledge gaps were consolidated into 177 research questions, including 78 (44.1%) specifically relevant to LMICs and 65 (36.7%) targeting vulnerable populations for bacterial priority pathogens, *Mycobacterium tuberculosis*, and selected fungi by the World Health Organization (WHO) [3]. Data collected from across the world have shown an overall decline in the antibiotic pipeline and continually rising resistance to all first-line and some last-resort antibiotics [4].

In 2019, 204 countries and territories reported approximately 4.95 (3.62–6.57) million deaths associated with bacterial AMR. 1.27 million (UI 95%: 0.91–1.71) of these deaths were attributable to AMR in 23 bacterial pathogens; there were 88 pathogen-antibiotic combinations in 204 countries and territories in 2019 [5]. A major issue was reported in the Middle East and North Africa (MENA) region including Morocco where 42 (28.7–59.5) deaths per 100,000 population were associated with bacterial resistance, of which 11.2 (7.5–16.3) deaths per 100,000 population were directly attributable to AMR. This is comparable with Central Europe, Eastern Europe, and Central Asia where the number of deaths associated with AMR in 2019, was 67·7 (45·4–96·6) per 100,000 population and

17·6 (11·7–25·3) attributable deaths. In addition, 1556.6 disability-adjusted life years (1145.2–2300.9) per 100,000 people were lost as a result of bacterial AMR, and 429 (293.7–611.5) disability-adjusted life years were attributable to antibiotic resistance in MENA [5].

Studies suggest that the annual global gross domestic product (GDP) would be most affected in LMICs; a 5–7% loss in LMICs by 2050 compared with a 1% loss in developed countries [6]. The results of the literature review show that the cost of AMR can be classified into 3 different levels. The patient level, in terms of its impact on mortality and morbidity; the healthcare level, in terms of the extra cost of treatment (in the USA, AMR could add $1,400 to the bill); and the economic impact level, in terms of healthcare losses and productivity losses [5]. This has been calculated in the USA and a total cost to society of antibiotic resistance attributable to each outpatient antibiotic prescription is estimated at $13 ($3–95) [7]. The situation in MENA is not clear for humans, but is most likely worse. The AMR situation in the case of animals is analyzed based on data from the USA. The proportion of antimicrobial compounds with resistance greater than 50% (P50) rose from 0.15 to 0.41 in chickens, from 0.13 to 0.34 in pigs, and from 0.12 to 0.23 in cattle, during the period from 2000 to 2018 [8]. In addition, multidrug resistant (MDR) *Salmonella* gradually increased over time in both cecal tonsils and product samples from 2016 to 2019 in USA [9].

A study of factors favoring AMR [10] identified the following as the primary reasons for the rise in AMR: lack of surveillance of resistance development, poor quality of antibiotics available, poor clinical use and easy availability of antibiotics in developing countries, poor level of hospital regulation, and excessive use of antibiotics in food-producing animals in developed countries. However, the data on which these conclusions are based is limited.

Since *Salmonella* is a major foodborne pathogen worldwide, we decided that it should be a top priority to include it in the antimicrobial susceptibility surveillance program [11].

One of the particularities of AMR is that it is transmissible. An important consequence is that the use of antibiotics not only selects for antibiotic resistance in the bacterial populations harbored by the patient or animal treated, but can result in the presence of resistant bacteria at a distance from the site of selection [12]. Horizontal transmission, as well as clonal expansion of successful strains, plays a role in the spread of antibiotic resistance in *Salmonella enterica* serotypes. Resistance genes can move between and are found in resistant plasmids and in the bacterial chromosome [13].

The aim of this study is to serotype *Salmonella* spp strains isolated from contaminated food products in Morocco and to study their resistance to the main antibiotics, using international standards.

Methodology

Salmonella isolates were serotyped according to the 9th edition of antigenic formulae of the *Salmonella* serovars the White-Kauffmann-Le Minor scheme [14] using *Salmonella* serotyping antisera (Bio-Rad, Herculus, California, USA).

The method used for antibiotic susceptibility testing was based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST) standard and the recommendations of the French Society for Microbiology which created an Antibiogram Committee (CA-SFM) version 2023 V.1.0. 19. The antimicrobials were selected from the standard list. Eight antimicrobials were selected from the complementary CA-SFM/EUCAST 2023 list, in addition to 14 other antimicrobials. Table 1 shows the list and characteristics of the 41 antibiotics used and tested for resistance in the present study. A total of 20 strains isolated from food products were tested for sensitivity to these antibiotics.

DNA extraction and virulence gene testing was carried out on 18 *Salmonella* strains, representing the main identified serotype, previously tested for

Table 1. List and characteristics of antibiotics tested.

Code	Antibiotic	Disc content	Higher limit	Lower limit	Code	Antibiotic	Disc content	Higher limit	Lower limit	Code	Antibiotic	Disc content	Higher limit	Lower limit
AK	Amikacin	30 µg	≥ 15	< 15	**CTX**	Cefotaxime	30 µg	≥ 20	< 17	**MA**	Cefamandole	30 µg	≥ 12	< 12
AM	Ampicilline	20 µg	≥ 14	< 14	**CXM**	Cefuroxime	30 µg	≥ 19	< 19	**MEC**	Mecillinam	10 µg	≥ 15	< 15
AMC	Amoxicillin-Clavulanic	20/10 µg	≥ 21	< 14	**CZ**	Cefazoline	30 µg	≥ 12	< 12	**NA**	Nalidixic Acid	30 µg	≥ 20	< 15
ATM	Aztreonam	30 µg	≥ 26	< 21	**DO**	Doxycycline	30 µg	≥ 19	< 17	**NOR**	Norfloxacine	10 µg	≥ 17	< 17
AX	Amoxicilline	25 µg	≥ 21	< 14	**ENR**	Enrofloxacin	10 µg	≥ 19	< 19	**OFX**	Ofloxacin	5 µg	≥ 24	< 22
B	Bacitracin	10 µg	≥ 15	< 14	**F**	Nitrofurantoin	100 µg	≥ 11	< 11	**PRL**	Piperacillin	75 µg	≥ 20	< 20
C	Chloramphenicol	30 µg	≥ 22	< 19	**FEP**	Cefepime	30 µg	≥ 27	< 24	**S**	Streptomycin	500 µg	≥ 15	< 13
CAZ	Ceftazidime	30 µg	≥ 22	< 19	**FF**	Fosfomycin	200 µg	≥ 24	< 24	**SPT**	Spéctinomycine	25 µg	≥ 15	< 13
CFM	Cefixime	5 µg	≥ 17	< 17	**FFC**	Florfénicol	30 µg	≥ 19	< 19	**SXT**	Triméthoprim- sulfaméthoxazol	25 µg	≥ 16	< 10
CIP	Ciprofloxacin	5 µg	≥ 22	< 22	**FLM**	Flumequine	30 µg	≥ 25	< 21	**TE**	Tetracycline	30 µg	≥ 19	< 17
CL*	Cephalexin	30 µg	-	-	**FOX**	Cefoxitin	30 µg	≥ 22	< 15	**TIC**	Ticarcillin	75 µg	≥ 23	< 23
CN	Gentamycine	10 µg	≥ 18	< 16	**IPM**	Imipénème	10 µg	≥ 22	< 19	**TIM**	Ticarcillic-Clavulanic Acid	75/10 µg	≥ 23	< 20
CRO	Ceftriaxone	30 µg	≥ 25	< 22	**K**	Kanamycine	30 µg	≥ 17	< 15	**TOB**	Tobramycin	10 µg	≥ 16	< 16
CT	Colistine	50 µg	≥ 18	< 15	**LEV**	Levofloxacin	5 µg	≥ 23	< 19					

Table 2. Primers selected for the analysis.

Gene	Set	Primer sequence	Tm (°C)	Amplicon size (bp)
FimA	F	TGGCTGTCTCCTCTGCGGAC	58.15	113
	R	CTGCTTCGCCGAGAAGGTCG	57.82	
Stn	F	CAACCCTGGCATGGTGGGC	58.04	101
	R	TAGCAGCAACGTCGACACGC	58.04	
CMY-2	F	TCATGGGTGCATAAAACGGG	58.5	97
	R	TTTTGTTTGCCAGCATCACG	58.1	

antibiotic resistance. Virulence genes *fimA* and *stn*, and the antibiotic resistance gene *CMY-2* were detected by real-time polymerase chain reaction (PCR). The search for the primer sequence of interest for the predefined genes was carried out using the integrated search engine of the US National Center for Biotechnology Information (NCBI), which hosts a gene bank [15]. The choice of primer sets for each gene was based on the analysis of the fluorescence curve profile and the PCR amplification cycle value "Ct". A clear curve and low Ct indicate efficient and specific amplification; consequently, the primer sets (F and R) listed in Table 2 were used.

DNA was extracted using the Machery Nagel "RNA virus nucleospin, (Machery Nagel, Germany) extraction kit, with the addition of proteinase K from 18 *Salmonella* strains previously tested for antibiotic resistance. The reaction mixture consisted of 2.5 µL of *Taq*Man™ Fast Virus 1-Step Master Mix, 1 µL of primer mix, 0.5 µL of EvaGreen dye (20×), and 4 µL of DNA template. The final volume was adjusted to 10 µL using DEPC-treated water. Amplification was performed on the QuantStudio™ 5 Real-Time PCR System, (QuantStudio™, USA), with the thermal cycling conditions set as follows: an initial denaturation step at 95 °C for 20 seconds, followed by 40 cycles of denaturation at 95 °C for 3 seconds, and annealing/elongation at 60 °C for 30 seconds.

Results

Serotypes of Salmonella isolated

A total of 20 strains isolated from food products belonged to the following serotypes: *S. Montevideo* (n = 4), *S. Blockley* (n = 3), *S. Chester* (n = 2), *S. Goldcoast* (n = 2), *S. Anatum* (n = 2), *S. Manhattan* (n = 1), *S. Enteritidis* (n = 1), *S. Kentucky* (n = 1), and 4 non-serotypeable isolates. These isolates were tested for susceptibility to the selected panel of antibiotics. Strains were isolated from the following food products sold at retail or in catering establishments: red meat and meat products (n = 9), poultry products (n = 8), and fish products (n = 3). The food sources from which the strains were isolated are shown in Table 3.

Antimicrobial resistance patterns of isolates

The results showed that all strains were susceptible to the following antimicrobial agents: cefotaxime, imipenem, cefixime, cefamandole, cefazolin, ceftriaxone, and ticarcillic-clavulanic acid combination. The antimicrobial resistance profiles of the *Salmonella* serotypes isolated are summarized in Tables 3 and 4.

Virulence and antimicrobial resistance genes isolation

All strains tested positive for the presence of the virulence genes *stn* and *fimA*, with the exception of 1 isolate. The amplification results are summarized in the Table 5.

Table 3. Antimicrobial resistance profiles of *Salmonella* serotypes isolated from food products.

Strains of *Salmonella*	Food products	Resistance profile
S. Anatum	Red meat	TE-PRL-B-K-AK-SPT-SXT-DO
S. Anatum	Red meat	AX-NA-TE-FLM-LEV-CXM-B-ENR-NOR-OFX-DO
S. Enteritidis	Poultry meat	NA-TE-FLM-FEP-F-B-AK-MEC-NOR-OFX-SXT
S. Goldcoast	Red meat	AX-NA-TE-FOX-FF-B-AM-AK-SPT-FFC-DO-CL
S. Goldcoast	Red meat	B
S. Manhattan	Poultry meat	FLM-FF-B-FFC
S. Montevideo	Poultry meat	AX-NA-CIP-TE-FLM-PRL-FOX-B-AM-SPT-ATM-TIC-SXT-DO-AM-SPT-ATM-TIC-SXT-DO
S. Montevideo	Red meat	CT-CIP-TE-PRL-FEPCAZ-CN-B-S-K-AK-SPT-MEC-ATM-OFX-FFC-DO
S. Montevideo	Red meat	CIP-TE-FLM-PRLF-B-C-SPT-OFX-FFC-DO
S. Montevideo	Red meat	CIP-TE-FLM-B-AM-SPT-DO
S. Blockley	Poultry meat	NA-CIP-TE-FLM-LEV-CN-F-FF-B-MEC-OFX-DO
S. Blockley	Poultry meat	NA-TE-FLM-FEP-CAZ-F-B-NOR-OFX-TOB-DO
S. Blockley	Fish products	NA-TE-FLM-LEV-F-FF-B-OFX-TOB-DO
S. Chester	Red meat	AX-PRL-B-AM-SPT-TIC
S. Chester	Poultry meat	AX-TE-B-AM-SPT-TIC-SXT-DO
S. Kentuckey	Fish products	NA-CIP-TE-FLM-LEV-B-ENR-NOR-OFX-TOB-SXT-DO
Salmonella spp. (OMA-, OMB-, OMC-, Vi-)	Fish products	NA-CIP-TE-FLM-LEV-CAZ-CN-F-FF-B-K-ENR-AK-SPT-MEC-NOR-OFX-TOB-SXT-DO
Salmonella spp. (OMA+, O : 3, 10,15 +, O : 1, 3,19+).	Poultry meat	CN-F-B
Salmonella spp. (OMA+, O3,10,15+)	Red meat	B-SPT
Salmonella spp. (OMA+, O4,5+)	Poultry meat	AX-TE-AMC-FOX-B-AM-SPT-DO-CL

Table 4. Resistance of the isolates to the antibiotics tested.

	Strain/Serotype	AX	CT	NA	CIP	TE	FLM	LEV	PRL	FEP	CAZ	AMC	FOX	CTX	CN	IPM	CXM	MA	F	FF	CFM	B	C	S	AM	K	ENR	AK	SPT	MEC	ATM	CRO	CL*	NOR	CZ	OFX	TIC	TIM	TOB	SXT	FFC	DO
1	*S. Manhattan*	I	I	I	I	S	**R**	I	S	S	I	I	I	S	I	S	S	S	S	**R**	S	**R**	S	S	S	I	S	S	S	S	S	S (19)	S	S	S	S	S	S	**R**	S
2	*S. Montvideo*	R	I	R	R	R	R	I	R	I	S	S	R	S	S	S	I	S	S	S	S	R	S	S	R	I	S	S	R	S	R	S	S (13)	S	S	S	R	S	S	R	S	R
3	*OMA+, O3,10,15+*	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	I	S	S	R	S	S	S	S (19)	S	S	S	S	S	S	S	S	S
4	*S. Montvideo*	I	R	S	R	R	I	S	R	R	R	I	S	S	R	S	S	S	S	S	S	R	S	R	S	R	S	R	R	R	R	S	S (20)	S	S	R	S	S	S	I	R	R
5	*S. Anatum*	S	S	S	S	R	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	R	S	R	R	S	S	S	S (20)	S	S	S	S	S	S	R	S	R
6	*S. Goldcost*	R	I	R	S	R	S	S	S	S	S	I	R	I	I	S	S	S	S	R		R	I	S	R	I	S	R	R	S	S	S	R (6)	S	S	S	S	S	S	S	R	R
7	*S. Anatum*	R	I	R	S	R	R	R	S	S	S	S	I	S	S	S	R	S	S	S	S	R	S	S	S	S	R	S	S	S	S	S	S (21)	R	S	R	S	S	S	I	S	R
8	*OMA+, O4,5+*	R	S	S	S	R	S	S	S	S	S	R	R	S	S	S	S	S	S	S	S	R	S	S	R	I	S	S	R	S	S	S	R (6)	S	S	S	S	S	S	S	S	R
9	*S. Eenteritidis*	S	S	R	S	R	R	S	S	R	S	S	S	S	S	S	S	S	R	S	S	R	S	S	S	S	S	R	I	R	S	S	S (20)	R	S	R	S	S	S	R	S	S
10	*OMA-, OMB-, OMC-, Vi-*	S	I	R	R	R	R	R	S	S	R	I	S	S	R	S	I	S	R	R	S	R	S	S	S	R	R	R	R	R	S	S	S (18)	R	S	R	S	I	R	R	S	R
11	*S. Blockley*	I	S	R	R	R	R	R	S	S	S	I	I	S	R	S	S	S	R	R	S	R	I	S	S	I	S	S	S	R	S	S	S (18)	S	S	R	S	S	S	I	S	R
12	*S. Blockley*	S	I	R	I	R	R	I	S	R	R	S	I	S	S	S	S	S	R	S	S	R	I	S	S	S	S	S	S	S	S	S	S (19)	R	S	R	S	S	R	I	S	R
13	*S. Kentuckey*	S	I	R	R	R	R	R	S	S	S	S	I	S	I	S	S	S	S	I	S	R	S	S	S	S	R	S	S	S	S	S	S (20)	R	S	R	S	S	R	R	S	R
14	*S. Blockley*	S	S	R	I	R	R	R	S	S	S	S	I	S	S	S	S	S	R	R	S	R	I	S	S	I	S	S	S	S	S	S	S (19)	S	S	R	S	S	R	I	S	R
15	*(OMA+, O : 3, 10,15 +, O : 1, 3,19+)*	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	R	S	S	R	S	S	S	S	S	S	S	S	S	S	S (21)	S	S	S	S	S	S	S	S	S
16	*S. Goldcoast*	S	I	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S	S	S	S	R	S	S	S	I	S	S	S	S	S	S	S (20)	S	S	S	S	S	S	S	S	S
17	*S. Montevideo*	S	S	I	R	R	R	S	R	S	S	S	S	S	S	S	S	S	R	S	S	R	R	S	S	S	S	S	R	S	S	S	S (20)	S	S	R	S	S	S	S	R	R
18	*S. Montevideo*	S	S	S	R	R	R	S	S	S	S	S	S	S	I	S	S	S	S	S	S	R	S	S	R	S	S	S	R	S	S	S	S (20)	S	S	S	S	S	S	S	S	R
19	*S. Chester*	R	S	S	S	I	S	S	R	S	S	S	S	S	I	S	S	S	S	S	S	R	S	S	R	S	S	S	R	S	S	S	S (18)	S	S	S	R	I	S	S	S	I
20	*S. Chester*	R	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	R	S	S	S	R	S	S	S	S (22)	S	S	S	R	S	S	R	S	R
	Number of resistant strains	**6**	**1**	**9**	**7**	**15**	**11**	**5**	**5**	**3**	**3**	**1**	**3**	**0**	**4**	**0**	**1**	**0**	**7**	**5**	**0**	**20**	**1**	**1**	**6**	**3**	**3**	**5**	**11**	**4**	**2**	**0**	**2**	**5**	**0**	**9**	**3**	**0**	**4**	**6**	**4**	**14**
	Percentage of resistant (%)	**30**	**5**	**45**	**35**	**75**	**55**	**25**	**25**	**15**	**15**	**5**	**15**	**0**	**20**	**0**	**5**	**0**	**35**	**25**	**0**	**100**	**5**	**5**	**30**	**15**	**15**	**25**	**55**	**20**	**10**	**0**	**10**	**25**	**0**	**45**	**15**	**0**	**20**	**30**	**20**	**70**

*limits are not available on Eucast CA-SFM version 2023 V.1.0. 19.

Nine out of 20 tested strains (45%) were resistant to nalidixic acid. Although the strains showed low resistance to the amoxicillin-clavulanic combination (5%, 1/20), the β-lactamase resistance gene *CMY-2* was detected in 94.44% (17/18) of strains.

Discussion

The strains isolated from food products belonged to the following serotypes: *S. Montevideo* (n = 4, 20%), *S. Blockley* (n = 3, 15%), *S. Chester* (n = 2, 10%), S. Goldcoast (n = 2, 10%), *S. Anatum* (n = 2, 10%), *S. Manhattan* (n = 1, 5%), *S. Enteritidis* (n = 1, 5%), *S. Kentucky* (n = 1, 5%), and 4 non-serotypeable isolates (20%). These isolates were isolated from the following food products sold at retail or in catering establishments: meat products (n = 9), poultry products (n = 8), and fish products (n = 3).

These results differ from those previously reported [14], where the serotypes *S. Kentucky* and *S. Enteritidis* were isolated in different percentages. In addition, most of the serotypes isolated appeared in the distribution list of the 20 most frequently isolated human *Salmonella* serotypes worldwide [16] which include *S. Montevideo*, *S. Anatum*, *S. Enteritidis*, *S. Chester*, and *S. Blockley* [16]. Similarly, certain serotypes among those isolated are among those most isolated in animal-based foods, reported by [17], in particular serotypes *S. Enteritidis*, *S. Anatum*, and *S. Kentucky*. Fonteneau *et al*., [18] reported an increase in cases of salmonellosis in 6 European countries due to *S. Chester* in patients who were reported to have traveled to Morocco between 2014 and 2015. The serotypes *S. Goldcoast* and *S. Manhattan* isolated in this study are not among the most widespread serotypes either in humans or in animal-based foods. Furthermore, the absence of *S. Typhimurium* serotype in this study, given that it is the

Table 5. Presence of the antibiotic resistance (AMR) genes *gyrA* and *CMY-2*, and the virulence genes *fimA* and *stn.*

Strain N°	Isolate/Strain	AMR genes/profiles		Virulence genes	
		Ct *CMY-2*	AMC	*fimA*	*Stn*
1	*S. Manhattan*	27.0	I	17.8	18.7
2	*S. Montevideo*	26.0	S	17.6	18.4
3	*OMA+, O3,10,15+*	29.5	S	19.5	19.9
4	*S. Montevideo*	29.9	I	17.8	19.0
5	*S. Anatum*	29.8	S	18.1	18.6
6	*S. Goldcost*	25.9	I	16.8	17.4
7	*S. Anatum*	28.8	S	16.5	17.2
8	*OMA+, O4,5+*	27.0	R	15.0	16.5
9	*S. Eenteritidis*	n/a	S	17.4	18.2
11	*S. Blockley*	21.9	I	16.2	17.6
12	*S. Blockley*	25.2	S	15.2	15.7
13	*S. Kentuckey*	26.3	S	16.8	17.5
14	*S. Blockley*	26.9	S	15.9	17.0
15	*(OMA+, O : 3, 10,15 +, O : 1, 3,19+)*	19.1	S	17.2	22.4
16	*S. Goldcoast*	16.7	S	n/a	n/a
17	*S. Montevideo*	27.0	S	16.4	17.0
18	*S. Montevideo*	28.0	S	17.4	18.0
19	*S. Chester*	26.5	S	16.2	16.4

R: resistant; I: intermediate; S: sensitive.

most widespread in the world both in humans and in food products, is probably due to the close association of this serotype with pig species and pork meats which are rarely marketed in Morocco

Antibiotic resistance was defined by the "FSIS-NARMS, 2023" as bacterial isolates resistant to one or two drug classes [9]. Multi-drug resistance (MDR) was defined as non-susceptibility to at least one agent in three or more antimicrobial classes [19] and as bacterial isolates resistant to 3 to 7 antimicrobial classes [9]. Here, the definitions for multidrug, extreme (XDR), and pandrug resistance (PDR), as defined by the European Centre for Disease Prevention and Control "ECDC" and the Centers for Disease Control and Prevention "CDC" [19] were used: "MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories; XDR was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories); and PDR was defined as non-susceptibility to all agents in all antimicrobial categories.

AMR reporting of the emergence of resistance is now starting to be implemented in Morocco, but more routine surveillance of foodborne pathogens is needed [8]. All the isolates were resistant to at least one antibiotic by phenotypic antibiogram testing, which is in agreement with other studies [20]; but is higher than some, 65.78%, [21] 80.95% [22], and 27.66% [23]. Also, 90% of isolates presented MDR (resistance to at least 3 antimicrobial agents). This MDR rate, is higher than all those reported previously 62.50% [24], 22.22% [25], 51.3% [23], 39.6% [26], and 44% [20].

The highest rate of resistance was found for the following antibiotics, in descending order: bacitracin (100%), tetracyclines (75%), doxycycline (70%), spectinomycin (55%), flumequine (55%), ofloxacin (45%), and nalidixic acid (45%). The lowest resistance (5%) was observed in the case of colistin, amoxicillin-clavulanic, cefuroxime, chloramphenicol, and streptomycin. A quarter of strains (25%; 5/20) were resistant to fosfomycin.

The strains isolated from fish products showed a very broad resistance profile of 10–20 antimicrobials, while those isolated from red meat products showed resistance to 1–16 antimicrobials. The strains isolated from poultry products showed resistance to 3–14 antimicrobials. Absence of XDR is remarkable compared with the FSI-NARMS 2023 reference list. In fact, antibiotic resistance ranged from 0 to 6 antimicrobial agents among the 12 on the reference list. According to the WHO list of critically important antimicrobials for use in human medicine in the 6th version of the Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR), the results of this study show full susceptibility to the highest priority antimicrobials of 3rd generation cephalosporins such as CFM, CRO and CTX; or low resistance (15%) to CAZ and FEP. In addition, there was low resistance to the antimicrobial polymyxin (colistin) of around 5%. The percentage of quinolone resistance varied according to the antimicrobial agent, from 15% (enrofloxacin) to 55% (flumequine).

In the case of the high-priority antibiotics in the list of critically important antimicrobials for human medicine, monobactams (aztreonam) showed a low percentage of resistance of around 10%, while the percentage of resistance to aminoglycosides varied according to the antimicrobial agent, from 5% (streptomycin) to 55% (spectinomycin). Total sensitivity to carbapenems (imipenem) was noted. Previous studies have used pooled estimates and reported a high level of antibiotic resistance (86%; $p < 0.001$) and MDR (73%; $p = 0.003$) in Africa among the primary bacteria: *Escherichia coli*, *Salmonella*, and *Campylobacter* spp. [27]. Among the *Salmonella* isolates identified from food products in China, 74% were resistant to at least 1 antibiotic; while 42% were resistant to more than 3 antimicrobials [28]. In Poland, 64% of *Salmonella* strains mainly isolated from poultry meat were resistant to one or more antibiotics tested, while phenotypic testing showed that 53.84% of strains were MDR [29]. These results remain lower than those found in this study. Other studies have reported similar results. All strains (n = 26) isolated from minced turkey sold in retail in Oklahoma were MDR and showed resistance to 4 to 10 antibiotics [30]. Similarly, the European Union summary report on antibiotic resistance in zoonotic and indicator bacteria isolated from humans, animals and food in 2020/2021 reported moderate to very high resistance variation for ampicillin, sulfamethoxazole, and tetracyclines [31]. *Salmonella* isolates from broiler chicken and turkey carcasses showed moderate overall resistance to ampicillin of 18.6% and 19.1%, respectively, which is lower than the 37.5% observed in this study. Resistance to sulfamethoxazole was noted in the same report at a very high level for isolates from broiler carcasses and at a moderate level in turkey carcasses of around 53.2% and 14.7% respectively. The same report noted resistance to tetracyclines at a very high level for isolates from broiler carcasses and at a high level in turkey carcasses of 52.6% and 48.1% respectively; this is lower than that identified in this study (75%).

Comparison of the results and occurrence of resistance to selected antibiotics in *Salmonella* spp. reported in the EFSA-ECDC 2023 report shows virtually the same results [31] for chloramphenicol (5/3.9), cefotaxime (0/0.1) and colistin (5/1.5). Higher rates of resistance were found for gentamicin (20/2.3), ampicillin (30/18.6), ceftazidine (15/0.1) and tetracycline (75/52.6). Lower rates of resistance were found for nalidixic acid (45/66.6), ciprofloxacin (35/69.3) and sulfamethoxazole (30/53.1). The overall MDR rate was lower than that found in this study (90/53.6).

The virulence gene *fimA* is associated with the production of fimbriae (or pili) and codes for a subunit of type 1 fimbriae, which are bacterial surface structures that play an important role in host cell adhesion and are key factors in the infectious process. The *fimA* gene is involved in the virulence of certain strains of *Salmonella* spp. [32]. Indeed, it was demonstrated that the presence of an intact *stn* gene contributed significantly to the overall virulence of *S. Typhimurium* [33]. All strains in this study, except for one, tested positive for virulence genes, i.e. 94.44% of strains were carrying these virulence genes. This is clearly higher than that reported by [34] of 77.4% *fimA* and 66% *stn*, who concluded that the presence of one of the three genes (*invA*, *fimA*, and *stn*) can predict the presence of the other, suggesting that the isolates found in food in Morocco have a wide range of virulence genes.

Conclusions

Acquired drug resistance is a public health problem that continues to gain ground in Morocco. Despite a very high rate of MDR to the antimicrobials tested, a limited rate of resistance to certain critically important antibiotics was noted. It is notable however that there was an intermediate rate of resistance to the critically important antibiotics ciprofloxacin and trimethoprim-sulfamethoxazole.

Active surveillance of resistance to antibiotics of critical importance to human and veterinary health is an urgent priority, as evidence-driven action must be taken to limit the increase of AMR. Monitoring the evolution of AMR is key. In order to limit the spread of AMR, we need to step up surveillance, limit the use of antibiotics in both human and veterinary medicine, and above all encourage alternative measures to antibiotics, notably vaccination and biosecurity measures in livestock farming.

Confirmation of the presence of virulence and antibiotic resistance genes highlights the danger of this pathogen as an agent of foodborne disease, and the challenges of limiting antimicrobial treatments to control it. Similarly, the presence of antibiotic resistance genes in foodborne *Salmonella* represents a danger for transmission to humans.

Acknowledgements

The authors are grateful to all the research assistants of the LRARs of Casablanca and Meknes, Poultry unit of IAV Hassan II, veterinary office of Pr. Mouahid Mohamed, and the Pharmacy and Veterinary Input Division (ONSSA).

Special tribute to the memory of Dr. Sami Derkaoui, co-author of this article, who recently passed away during the publication process. May God rest his soul in His holy mercy

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Conflict of interest

No conflict of interest is declared.

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