





ORIGINAL ARTICLE

Prevalence and molecular characterization of foodborne and human-derived *Salmonella* strains for resistance to critically important antibiotics

Islam Sabeq¹  | Dina Awad¹ | Ahmed Hamad¹ | Mohamed Nabil² |
 Mohamed Aboubakr³ | Mohamed Abaza⁴  | Mohammed Fouad⁵  |
 Amira Hussein⁶ | Sanaa Shama⁷ | Hazem Ramadan⁸  | Shima Edris¹

¹Faculty of Veterinary Medicine, Department of Food Hygiene and Control, Benha University, Takh, Qalyubia, Egypt

²Food Hygiene Department, Animal Health Research Institute (Benha Branch), ARC, Benha, Qalyubia, Egypt

³Faculty of Veterinary Medicine, Department of Pharmacology, Benha University, Takh, Qalyubia, Egypt

⁴Faculty of Veterinary Medicine, Avian and Rabbit Diseases Department, Benha University, Takh, Qalyubia, Egypt

⁵Faculty of Medicine, Microbiology and Immunology Department, Benha University, Benha, Qalyubia, Egypt

⁶Faculty of Medicine, Department of Clinical Pathology, Cairo University, Cairo, Egypt

⁷Laboratory Unit, Benha Fever Hospital, Benha, Qalyubia, Egypt

⁸Faculty of Veterinary Medicine, Hygiene and Zoonoses Department, Mansoura University, Mansoura, Egypt

Correspondence

Islam Sabeq, Faculty of Veterinary Medicine, Department of Food Hygiene and Control, Benha University, Takh, Qalyubia 13736, Egypt.
 Email: islam.sabek@fvtm.bu.edu.eg

Funding information

Benha University, Scientific research Fund, Grant/Award Number: M6/1/5

Abstract

The primary goals of this cross-sectional study were to screen various food/water, and human samples for the presence of *Salmonella* species, and to assess the phenotypic and genetic relationship between resistances found in food and human *Salmonella* isolates to critically important antibiotics. Between November 2019 and May 2021, 501 samples were randomly collected for *Salmonella* isolation and identification using standard culturing methods, biochemical, matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) and PCR techniques. Antimicrobial susceptibility testing was performed on confirmed *Salmonella* species, and PCR was used to investigate the genetic components that confer these resistance traits. *Salmonella enterica subspecies enterica* was confirmed in 35 (6.99%) of the samples (raw food = 23, ready-to-eat food/drink [REF/D] = 5, human = 7). Seventeen of them were antibiotic-resistant to at least one class, and eight were multidrug-resistant (MDR) isolates (raw food = 7, human = 1). All *Salmonella* isolates were susceptible to carbapenems, third- and fourth-generation cephalosporins and monobactam antibiotics. Resistance phenotypes to aminoglycosides (48.57%), β -lactams (20%) and tetracycline (17.14%), as well as associated genes such as *aadA*, *bla*_{TEM}, *bla*_Z and *tetA*, as well as *dfrA* and *sul1*, were prevalent in *Salmonella* isolates. Colistin resistance genotype (*mcr1*) was detected in three (8.57%) isolates recovered from egg, cattle mince and rabbit meat, and the total incidence was 14.29% when two isolates exhibited resistance phenotypes were considered. Furthermore, four (11.43%) MDR isolates shared the *bla*_{TEM} and *bla*_Z genes, and one (2.86%) isolate contained three extended spectrum β -lactams producing genes (ESBL), namely *bla*_{CTX}, *bla*_{TEM} and *bla*_Z. The *gyrA* gene was expressed by one of three foodborne *Salmonella* isolates (8.57%) with ciprofloxacin resistance phenotypes. To the best of our knowledge, this is the first report from Egypt identifying colistin resistance in *Salmonella enterica* recovered from cattle minced meat and rabbit meat. Overall, the highest incidence rate of *Salmonella enterica* was found in cattle-derived products, and it was slightly more prevalent in RTE/D foods than in raw foods. Resistance to critical and clinically important antibiotics, particularly in *Salmonella* from RTE/D food,

suggests that these antibiotics are being abused in the investigated area's veterinary field, and raises the potential of these isolates being transmitted to high-risk humans, which would be a serious problem. Future research using whole-genome sequencing is needed to clarify *Salmonella* resistance mechanisms to critically important antimicrobial agents or those exhibiting multidrug resistance.

KEYWORDS

critical antibiotics, human isolates, MALDI-TOF MS, multi-drug resistance, *Salmonella enterica*, VITEK-2 Compact system

1 | INTRODUCTION

Food safety agencies have considered *Salmonellae* spp. a priority pathogen because of the frequency and severity of illness it causes (IFSAC, 2018). Worldwide, *Salmonella* spp. was estimated as the major causes of diarrheal and invasive foodborne illness and accounted for 93.8 million gastroenteritis cases, 111,000 deaths and around 8.6 million Disability Adjusted Life Years (DALYs) (Havelaar et al., 2015). In the United States, it was estimated that foodborne *Salmonella* spp. was the leading bacterial cause of illness, hospitalizations, and deaths in 2011 (Scallan et al., 2011), and the incidence did not significantly change in 2015 (CDC, 2017). Economically, the total annual quality-adjusted life days (QALDs) loss associated with foodborne *Salmonella* spp. was 1.66 million, while the monetary loss was \$5.49 million which was estimated to be the highest among all other known illnesses, due to the high annual occurrence and related direct costs in the United States (Travis et al., 2015). On the other hand, epidemiological data of *Salmonella* spp. infection from Egypt, Africa, is still incomplete; however, the studies indicated that the *Salmonella* spp. is the major cause of annual illness (1.9 million cases) and deaths in Africa (Uche et al., 2017).

Antimicrobial resistance (AMR) is a major global public health concern and a food safety issue (CAC, 2011). Antibiotics-resistant *Salmonella*, particularly those that are multidrug-resistant (MDR), has been identified as the most serious public health threat resulting from antibiotics misuse in livestock (Mechesso et al., 2020). The spread of AMR amongst food animals in one country can cause human health problems in other countries through food products (WHO, 2007). The unrestricted use of antimicrobial agents in Egypt has contributed to the emergence of ciprofloxacin-resistant *Salmonella* (Ramadan et al., 2018), colistin (Soliman et al., 2021) and carbapenems-resistant *Escherichia coli* (Ramadan et al., 2020) in retail chicken meat, chicken faecal samples, and human and dog samples, respectively. The increased prevalence of *Salmonella* resistance to various antibiotics and higher health-care costs mandates the Centers for Disease Control and Prevention and the World Health Organization to designate antibiotic-resistant *Salmonella* as a serious threat to public health (WHO, 2017b).

Past Egyptian cross-sectional studies compared the presence of *Salmonella* in broiler meat and/or farms, and human samples (Abdelmalek et al., 2019; El-Sharkawy et al., 2017; Hassan et al., 2016). However, little is known or published about the prevalence of this

pathogen in different food types (Ahmed & Shimamoto, 2014), particularly raw cattle products and ready-to-eat/drink food (RTE/D), compared to human cases in the Al-Qalyubia governorate, including assessment of isolated *Salmonella* species for susceptibility to new critical important antibiotics. Furthermore, the growing global threat posed by the emergence of such resistant bacteria prompted us to conduct a cross-sectoral screening study for the presence and distribution of *Salmonella* in food and humans, and the characterization of isolated *Salmonella* for the presence of critical AMR.

2 | MATERIALS AND METHODS

2.1 | Ethical statement

The study was conducted according to the guidelines of the Declaration of Helsinki as well as the rules and regulations of the Egyptian Ministry of Health under full medical supervision. Written consent was obtained from the patients before using the specimens. All human samples were handled and analysed on-site by trained medical personnel under the supervision of a physician. All suspected isolates were anonymized and de-linked from patient information.

2.2 | Study area

The study was conducted in the Al-Qalyubia governorate in Lower Egypt, about 35.26 km north of Cairo in the Nile Delta region (30.41 N; 31.21 E at an altitude of 9.0 m). The Al Qalyubia governorate is the leader in animal production and food packaging and processing in Egypt (CAPMAS, 2021).

2.3 | Sample collection

A total of 501 samples were collected and assessed for the presence of *Salmonella* in foods, water and human patients at Al Qalyubia governorate, Egypt, between November 2019 and May 2021. Raw food categories included cattle and/or beef meat, cattle carcass swabs, mutton, rabbit meat, meat mince, beef and/or cattle liver, turkey meat, duck

meat, milk, dough and egg. While RTE/D categories involved water, Hawawshi, kofta, burger, samosa, beef steak, sausage pizza, sausage, shawarma and Roumy cheese. A total of 145 food handler swabs, as well as blood and stool samples from human patients experiencing gastrointestinal symptoms were collected from hospitals and clinical laboratories in the Al Qalyubia area. Following that, suspected human *Salmonella* isolates were sent for confirmation with those from other food and water sources. The food product samples were bought from slaughterhouses, supermarkets, local markets, retail shops and restaurants. Samples were collected and transported to the laboratory in sterile plastic bags in iceboxes within 1 h for microbiological analysis.

2.4 | Isolation and identification

For the isolation and identification of *Salmonella*, a standard cultivation method recommended by ISO 6579-1: 2017 (ISO 2017) was used with some modifications. In brief, the samples were homogenized at 10% in a buffered peptone solution with the Stomacher 400R (Seward, UK), then incubated overnight at 37°C in a sterile stomacher bag. The pre-enrichment was then inoculated into Rappaport Vassiliadis (RV) broth (Lab M, UK) and incubated at 41°C for 18–24 h. *Salmonella* species were isolated selectively on xylose lysine deoxycholate (XLD) agar plates (BioLife, USA) at 37°C for 18–24 h. Modified semi-solid Rappaport Vassiliadis medium (MSRV), Hektoen enteric (HE) agar and Bismuth Sulfite Agar (Wilson Blair) were also used to confirm atypical colonies. Suspect colonies with typical *Salmonella* morphology were biochemically confirmed using the API 20E® system (BioMerieux, France). Blood samples were enriched using both bile salt broth and streptokinase broth, according to Nagshetty et al. (2010). Then, the enriched blood samples were streaked on specific agar media after turbidity was visible.

2.5 | Identification by MALDI-TOF MS

Presumptive *Salmonella* isolates were confirmed using MALDI-TOF MS (VITEK®MS, database version 3, BioMerieux, France). *E. coli* ATCC 8739 was inoculated on the calibration spots as a calibration and internal identification control. The results were interpreted following the manufacturer's recommendations. The peaks from the spectrum were compared to the typical spectrum for a species, genus or family of microorganism for isolate identification.

2.6 | Antimicrobial susceptibility testing via disk diffusion method

Antimicrobial susceptibility tests were performed using the Kirby-Bauer disk diffusion method. All results were interpreted following the Clinical and Laboratory Standards Institute (CLSI, 2017). *Salmonella* species were initially tested for resistance to eight widely available antibiotics in Egyptian veterinary and medical sectors, includ-

ing trimethoprim (TR, 5 µg), tetracycline (TE, 30 µg) and cefuroxime (CXM, 30 µg), as well as critically important antibiotics such as amoxicillin (AMX, 30 µg), ceftriaxone (CTR, 30 µg), gentamicin (10 µg, GEN), ciprofloxacin (CIP, 5 µg) and norfloxacin (NX, 10 µg). All *Salmonella* isolates susceptible to these antimicrobials were ruled out, whereas resistant isolates to any of the examined antimicrobials were further evaluated using the VITEK® –2 system. *Salmonella* isolates that exhibited resistance to at least three classes of antimicrobial agents evaluated was considered multidrug-resistant (MDR).

2.7 | Antimicrobial susceptibility testing via VITEK® –2 system

Antimicrobial susceptibility testing was also performed using the AST-N222 test card (Ref. No. 413083, BioMerieux, New York, USA). Fresh *Salmonella* colonies were suspended in sterilized physiological saline (aqueous 0.45% NaCl, pH 4.5 to 7.0). The spectrum of antibiotic resistance was assessed for amikacin, minocycline, ceftazidime, ciprofloxacin, imipenem, gentamicin, cefepime, aztreonam, meropenem, piperacillin-tazobactam, piperacillin, tobramycin, ticarcillin, colistin, ticarcillin/clavulanic acid, and trimethoprim/sulfamethoxazole. The multiple antibiotic resistance (MAR) index was estimated by dividing the number of ineffective antibiotics on a specific isolate by the total number of antibiotics tested (Thung et al., 2016).

2.8 | The Blue Carba test

The Blue Carba test (BCT) is a fast, simple and reliable method for detecting carbapenemases producing Gram-negative bacteria directly from the colony without prior extraction (Pires et al., 2013). The BCT is based on the in vitro hydrolysis of imipenem's β-lactam ring by carbapenemases, resulting in the release of an acid product that can be detected by a change in the colour of the PH indicator from blue to yellow. Two independent readers interpreted the test results.

2.9 | Molecular characterization of *Salmonella* isolates

Specific antimicrobial resistance genes in *Salmonella* were detected using PCR. DNA was extracted from a loopful from each colony using the QIAamp DNA Mini Kit (Cat. No. 51304, Qiagen, Hilden, Germany) according to the manufacturer's protocol. All primers and conditions for PCR amplification of *aadA*, *bla*_{CTX}, *bla*_{TEM}, *bla*_Z, *dfra*, *gyrA*, *mcr1*, *Sul1* and *tetA* are listed in Table A1. For PCR, a reaction mixture of 25 µl volume was prepared with 12.5 µl of Emerald Amp GT PCR Master Mix (Cat. No. RR310A, Takara Bio, Shiga, Japan), 1 µl (20 pmol/µl) of each primer (Bio Basic, Ontario, Canada), 6 µl target DNA and the remaining volume needed to reach 25 µl was adjusted with deionized PCR grade water. The reaction was conducted in a T3 Biometra Trio thermal

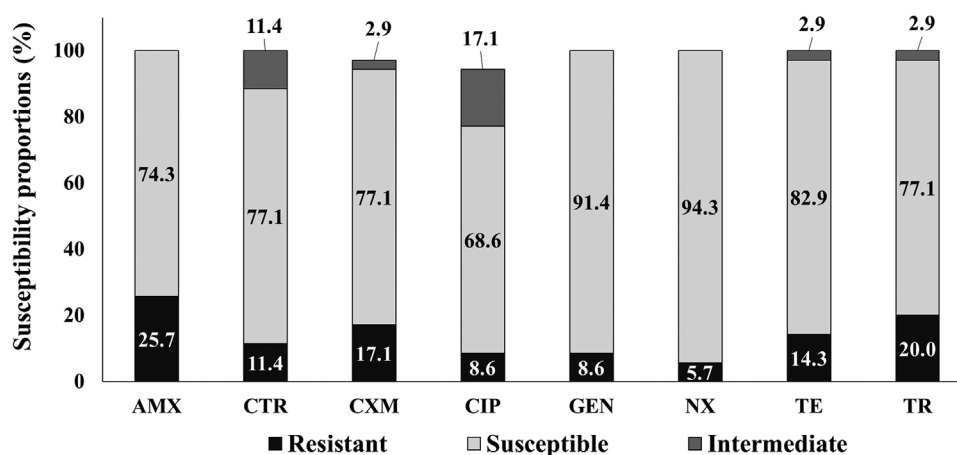
Antimicrobial susceptibility of *Salmonella* isolates (n=35)

FIGURE 1 The antimicrobial susceptibility profile of *Salmonella enterica* subspecies *enterica* isolated from food ($n = 28$) and humans ($n = 7$) via disc diffusion test. Antibiotics assessed: 10 μg gentamicin (GEN), 30 μg amoxicillin (AMX), 30 μg cefuroxime (CXM), 30 μg ceftriaxone (CTR), 10 μg norfloxacin (NX), 5 μg ciprofloxacin (CIP), 5 μg trimethoprim (TR) and 30 μg tetracycline (TE)

cycler (Biometra, Analytik Jena, Jena, Germany). After completing the run, PCR products (6 μl) were subjected to agarose gel electrophoresis (1.5%), stained with ethidium bromide and visualized under UV light in a gel documentation system (Alpha Innotech, Kasendorf, Germany). It should be noted that PCR for *invA* gene amplification was not performed on all isolates, but rather on a random basis on some isolates, particularly those with unclear colony features on selective media (Figure A1).

2.10 | Statistics

Statistical analysis was performed using SPSS Statistics 20 (SPSS Inc., USA). The collected data from various human and food samples and antimicrobial susceptibility test results were computed using descriptive statistics such as frequency, percentage and/or proportion.

3 | RESULT

The MALDI-TOF MS and PCR results revealed that 35 (6.99%, $n = 501$) of the 67 presumptive isolates were confirmed *Salmonella*, and all 35 isolates were classified as *Salmonella enterica* subspecies *enterica* (Tables 1 and 2; Figure A1a and b). The occurrence of *Salmonella* in the food/water group was 7.87% (28/356) and 4.83% (7/145) in human samples.

Using the antimicrobial disc diffusion susceptibility method, 17 (48.57%, $n = 35$) *Salmonella enterica* isolates exhibited resistance phenotypes to at least one class of tested antimicrobials (Figure 1). The susceptibility results using the VITEK® –2 system generated from seventeen *Salmonella* species are summarized in Table 3. Full analysis data including MIC values are provided in Table A2. Four antibi-

otic classes involving carbapenems, third-generation cephalosporins, fourth-generation cephalosporins and monobactam were found to be 100% effective against tested *Salmonella enterica*. Notably, resistance to aminoglycosides was found in seventeen isolates (48.57%, $n = 35$), while resistance to minocycline was found in six *Salmonella* isolates (17.14%, $n = 35$). Resistance to aminoglycosides was observed in both food and human isolated *Salmonella* species, but tetracycline resistance was observed in *Salmonella* species collected from the food/water group (Table 3). Resistance to piperacillin and ticarcillin was also found in seven *Salmonella* isolates recovered from the two screened groups, food/water, and humans (20%, $n = 35$). Using the preceding antibiotics in combination with the β -lactamase inhibitors tazobactam and clavulanic acid reduced the number of resistant *Salmonella* isolates to three (8.57%, $n = 35$) and five (14.29%, $n = 35$), respectively. The class of sulfonamides and dihydrofolate reductase inhibitors was ineffective against four (11.43%, $n = 35$) *Salmonella* isolates, one from a human and three from the food/water group. Colistin and fluoroquinolones were effective against all *Salmonella enterica* isolates except three (8.57%, $n = 35$) and all isolates were derived from raw food samples, particularly minced bovine meat, liver, egg and rabbit (Table 3).

Eight (22.86%, $n = 35$) of the 17 *Salmonella* strains isolated in the current study and assessed using the VITEK® –2 system exhibited MDR (Table 3). One isolate was of human origin (14.29%, $n = 7$), while the remaining seven were derived from food (25%, $n = 28$). In this study, three phenotypic patterns of MDR profiles were identified, with some isolates containing resistance to up to eleven antibiotics and others containing as few as five. Resistance to five antimicrobial classes is at the top of the MDR profiles observed in one eggshell-derived *Salmonella enterica*. Four isolates from minced meat, liver, rabbit and egg exhibited phenotypic resistance to four antimicrobial classes. The last MDR pattern was observed in three isolates, including the human

TABLE 1 The prevalence of *Salmonella enterica* subspecies enterica strains isolated from raw and ready to eat/drink food ($n = 356$) samples

Food samples ($n = 356$)	No. of samples	No. of presumptive <i>Salmonella</i> ^a	No. of MALDI-TOF MS confirmed <i>Salmonella</i> ^b	Incidence %
Raw/fresh food ($n = 300$)				
Cattle meat	25	2	–	0.00
Cattle carcass swabs	10	0	–	0.00
Cattle meat minced	52	6	3	5.77
Mutton meat	13	0	–	0.00
Fat	7	2	1	14.29
Rabbit meat	64	13	4	6.25
Duck	4	0	–	0.00
Cattle liver	36	6	6	16.67
Turkey meat	2	1	–	0.00
Milk	58	4	3	5.17
Egg	27	7	6	22.22
Butter mix	1	0	–	0.00
Dough	1	0	–	0.00
Subtotal ^c	300	41	23	7.67
Ready to eat/drink food ($n = 56$)				
Honey	1	0	–	0.00
Water	6	2	1	16.67
Hawawshi	1	0	–	0.00
Cooked meat	3	0	–	0.00
Kofta	8	1	1	12.50
Burger	3	0	–	0.00
Roumy cheese	1	0	–	0.00
Samosa	7	2	–	0.00
Beef steak	2	0	–	0.00
Sausage pizza	12	2	2	16.67
Sausage	1	0	–	0.00
Shawarma	1	0	–	0.00
Yoghurt	10	1	1	10.00
Subtotal ^c	56	8	5	8.93
Total ^d	356	49	28	7.87

^aSuspected *Salmonella* isolates based on selective media and biochemical test.

^bMALDI-TOF MS, matrix-assisted laser desorption ionization-time of flight mass spectrometry.

^cThe subtotal incidence was calculated by dividing the number of *Salmonella*-positive raw or ready-to-eat/drink foods by the total number of samples evaluated for each category.

^dThe total foodborne incidence was calculated by dividing the total number of *Salmonella*-positive raw and ready-to-eat/drink food samples by the total number of examined food samples.

isolate, and combined three antimicrobial classes. The majority of MDR foodborne *Salmonella* isolates (26.09%, $n = 23$) were associated with raw food samples. While one *Salmonella* isolate was (20%, $n = 5$) obtained from RTE/D food samples, sausage pizza. The MDR human *Salmonella* isolate was identified in stool (Table 3).

The molecular characterization of MDR *Salmonella* for ten antimicrobial resistance genes is shown in Table 3 and Figure A2. In isolated MDR *Salmonella*, the resistance genes *aadA*, *bla*_{TEM}, *bla*_Z and *tetA* pre-

dominated. Notably, none of the recently examined genotypes that contribute to colistin and fluoroquinolone resistance, such as *mcr1* and *gyrA*, were detected in the *Salmonella* isolates that were phenotypically colistin and fluoroquinolone-resistant except for the H19 isolate, which demonstrated colistin resistance in both phenotype and genotype analysis. Nevertheless, some isolates that did not exhibit colistin or fluoroquinolone phenotypes did contain the related genotypes (Table 3).

TABLE 2 The number and prevalence of *Salmonella enterica* subspecies enterica strains isolated from human ($n = 145$) samples

Human samples ($n = 145$)	No. of samples	No. of presumptive <i>Salmonella</i> ^a	No. of MALDI-TOF MS confirmed <i>Salmonella</i> ^b	Incidence %
Blood	47	3	–	–
Stool	58	15	7	12.07
Food handler swabs	40	–	–	–
Total (human) ^c	145	18	7	4.83

^aSuspected *Salmonella* isolates based on selective media and biochemical test.

^bMALDI-TOF MS, matrix-assisted laser desorption ionization-time of flight mass spectrometry.

^cThe total incidence was calculated by dividing the total number of *Salmonella*-positive samples by the total number of examined samples.

TABLE 3 The antibiotic resistance profile patterns and multiple antibiotic resistance (MAR) index of *Salmonella* isolated from food/water and human samples

Isolate			VITEK® –2 system	Genetic resistance	
No.	Code	Origin	Resistance profiles (tested antibiotics = 16)	MAR index ^a	Genes
1	H 19	Egg shell	11 (Amik-Mino-Cipro-Gent-Tobra-Pip/Taz-Pip-Tobra-Ticra-Ticra/Clav-Colistin)	0.69	<i>aadA</i> , <i>bla</i> _{CTX} , <i>bla</i> _{TEM} , <i>bla</i> _Z , <i>gyrA</i> , <i>mcr1</i> , <i>TetA</i>
2	H7	Egg	7 (Amik-Mino-Gent-Pip-Tobra-Ticra-Trime/Sulfa)	0.44	<i>aadA</i> , <i>bla</i> _{TEM} , <i>bla</i> _Z , <i>dfrA</i> , <i>mcr1</i> , <i>TetA</i>
3	N4	Minced meat	9 (Amik-Mino-Cipro-Gent-Pip Pip/Taz-Tobra-Tic-Tic/Clav)	0.56	<i>aadA</i> , <i>bla</i> _{TEM} , <i>bla</i> _Z , <i>TetA</i> , <i>mcr1</i>
4	N 31	Fat	6 (Amik-Mino-Gent-Pip-Tobra-Tic)	0.38	<i>aadA</i> , <i>bla</i> _{TEM} , <i>bla</i> _Z , <i>TetA</i>
5	N7	Liver	8 (Amik-Mino-Cipro-Gent-Pip-Tobra-Ticra-Ticra/Clav)	0.50	<i>aadA</i> , <i>bla</i> _{TEM} , <i>bla</i> _Z , <i>TetA</i>
6	SH15	Human	3 (Pip-Ticra-Ticra/Clav)	0.19	<i>aadA</i> , <i>bla</i> _Z , <i>dfrA</i> , <i>Sul1</i>
7	SH17	Human	8 (Amik-Gent-Pip/Taz-Pip-Tobra-Ticra-Ticra/Clav-Trime/Sulfa)	0.50	<i>bla</i> _Z , <i>dfrA</i> , <i>Sul1</i>
8	N41	Rabbit	6 (Amik-Mino-Gent-Tobra-Colistin-Trime/Sulfa)	0.38	<i>bla</i> _Z , <i>dfrA</i> , <i>TetA</i> , <i>Sul1</i>
9	N42	Rabbit	5 (Amik-Gent-Tobra-Colistin-Trime/Sulfa)	0.31	<i>bla</i> _Z , <i>dfrA</i> , <i>TetA</i> , <i>Sul1</i>
10	DE15	Egg	3 (Amik-Gent-Tobra)	0.19	<i>bla</i> _Z , <i>dfrA</i> , <i>Sul1</i>
11	N6	Liver	3 (Amik-Gent-Tobra)		
12	N36	Sausage pizza	3 (Amik-Gent-Tobra)	0.19	
13	N38	Milk	3 (Amik-Gent-Tobra)	0.19	
14	DE1	Egg	3 (Amik-Gent-Tobra)	0.19	
15	DE3	Egg shell	3 (Amik-Gent-Tobra)	0.19	
16	H23	Egg content	3 (Amik-Gent-Tobra)	0.19	
17	SH73	Human	3 (Amik-Gent-Tobra)	0.19	

^aMAR index = number of ineffective antibiotics/total number of antibiotics assessed.

AMX, amoxicillin; CXM, cefuroxime; CTR, ceftriaxone; NX, norfloxacin; TR, trimethoprim; TE, tetracycline; Amik, amikacin; Mino, minocycline; Cipro, ciprofloxacin; Gent, gentamicin; Pip/Taz, piperacillin-tazobactam; Pip, piperacillin; Tobra, tobramycin; Ticra, ticarcillin; Ticra/Clav, ticarcillin/clavulanic acid; Trime/Sulfa, trimethoprim/sulfamethoxazole.

4 | DISCUSSION

One of the primary concerns of the current investigation was to determine and compare *Salmonella* species prevalence in food/water, and humans in Al-Qalyubia Governorate, Egypt. The confirmatory results of MALDI-TOF MS on the suspected *Salmonella* isolates collected throughout the investigation period between November 2019 and May

2021 revealed a 6.99% overall *Salmonella* prevalence. There is a lack of publications in the literature devoted to this type of research for Egypt. Nevertheless, in other provinces, two cross-sectional studies recorded higher total *Salmonella* occurrence rates of 11.1% (78/700) (Ahmed et al., 2016) and 16% (Abdelmalek et al., 2019).

The *Salmonella* prevalence in the food/water group was assessed to be 7.87% (28/356). In an earlier Egyptian large-scale study that

included four governorates, the overall prevalence of foodborne *Salmonella enterica* from 1600 food samples was 4.3% (69 samples) (Ahmed & Shimamoto, 2014).

In comparison to other raw food types, egg and cattle liver samples had the highest percentages of *Salmonella enterica* at 22.22% and 16.67%, respectively. Unprocessed contaminated eggs and egg-related products are among the most common human salmonellosis vectors (Foley et al., 2011). Moreover, the high occurrence rate in liver samples may be attributed to *Salmonella*'s ability to reach internal organs containing the mononuclear phagocyte system (e.g., the spleen and liver), for surviving and replicating inside macrophages (Nielsen, 2013).

Salmonella enterica was found in 7.69% (10/130) of the cattle-derived raw meat samples evaluated, including meat mince, fat, liver and swabs. The persistent excretion of *Salmonella* from carrier livestock (which can last up to several months) is one of the primary sources of *Salmonella* in farms and slaughterhouses, resulting in the contamination of environments and, of course, associated raw products (Nielsen, 2013). In comparison to the previously recorded *S. enterica* detection rate of 4.9% in Egypt (Ahmed & Shimamoto, 2014) and 5.3% (72,292 cattle samples) across 27 African countries (Thomas et al., 2020), the current finding indicates an increasing trend in the occurrence of *Salmonella enterica* in Egyptian bovine products. This suggestion is supported by the high prevalence rate of *Salmonella enterica*, 5.71%, detected in the seventy dairy products tested in the current study, including milk, butter mix, Roumy cheese and yogurt, compared to the previously recorded 2.0% in dairy products (Ahmed & Shimamoto, 2014). In contrast, *Salmonella enterica* was detected in 1.5% of raw milk samples from the same earlier investigation (Ahmed & Shimamoto, 2014), indicating a decreasing trend compared to the current rate of approximately 1%.

In the current study, rabbit meat was another raw food that had a high *Salmonella* species frequency (6.25%, 4/64). There is little information available about rabbit meat-borne *Salmonella* around the world and in the current research area. However, in the neighbouring province of Sharkia, Egypt, the overall prevalence of *Salmonella* in the examined rabbit farm samples (raw meat, intestinal content, liver and vaginal swabs) was 7.40% (10/135) (Suelam & Reda, 2015).

Salmonella were identified in 8.93% (5/56) of RTE/D foods. This rate is significantly higher than the previously recorded rates of 0.5% and 0% in Egyptian cheese (Ahmed & Shimamoto, 2014). Cross-contamination and/or inadequate cooking of the food are the primary implicated reasons for the survival of this fragile bacteria (Thornton et al., 2009). *Salmonella* species in RTE/D food at such a high rate poses a potential public health risk because these strains are more likely to be transmitted to humans than raw foodborne *Salmonella* species.

Salmonella was detected in 4.83% (7/145) of human samples, which falls between the relatively low (4%) and high (9.3 and 12.6%) percentages reported in previous studies conducted in the Egyptian provinces of Beni-Suef (Hassan et al., 2016), Menoufia and Cairo (Abdelmalek et al., 2019), as well as Al-Qalyubia (Abd El Samie et al., 2018). The most well-known Typhoid disaster in Al-Qalyubia occurred in El Qanater El Khayreya City in 2009, and was caused by sewage mixing with drinking water, resulting in up to 164 Typhoid cases.

The secondary goal of this study was to determine and compare the AMR profile of isolated *Salmonella* from various sources. By the disc diffusion test, only 17 of 35 (48.57%) *Salmonella enterica* isolated from food and human samples showed resistance phenotypes to at least one class of tested antimicrobials in the current study. The total estimated resistance percentages in *Salmonella enterica* originating from food and humans were 50% (14/28) and 42.86% (3/7), respectively. Based on the current VITEK –2 and molecular characterization results, the MDR phenotype was present in 22.86% (8/35) of *Salmonella* isolates. MDR was found in 25% (7/28) of food isolates and 14.29% (1/7) of human isolates, respectively. Previous characterizations of *Salmonella* isolates obtained from broiler meat and human, as well as from retail fish samples in other Egyptian provinces found that 68.1% and 100%, respectively, of isolates were either resistant to at least one antibiotic or carried MDR (Ahmed et al., 2014; Gawish et al., 2021; Hassan et al., 2016).

Polymyxins, carbapenems, third- and higher-generation cephalosporins, aztreonam and quinolones are among the critically important antimicrobial agents identified by WHO as having the highest priority for risk management of severe AMR infections like typhoidal and nontyphoidal fever WHO, (2017a). Besides this, carbapenems and colistin are used as last-line antibiotics and possible alternatives in treating patients infected with serious MDR Gram-positive and Gram-negative bacteria (WHO, 2019). Using VITEK® –2 system and Blue Carba test, the current study results revealed that 100% of all isolated *Salmonella enterica* were susceptible to carbapenems (imipenem and meropenem) and lack the carbapenemases enzyme that confers such resistance (Queenan & Bush, 2007). In addition, the absence of phenotypically resistant *Salmonella* isolates to ceftazidime, cefepime and aztreonam may suggest the absence of ESBLs-producing *Salmonella* because enzymes in such pathogens have the potential to hydrolyse the preceding antibiotics (Winokur et al., 2000). However, genetic analysis of an eggshell-derived isolate revealed the presence of three genes that could contribute to ESBL resistance (Hasman et al., 2005), namely *bla*_{CTX}, *bla*_{TEM} and *bla*_Z. ESBL-bacteria were found to frequently share resistance to quinolones, tetracycline, and aminoglycosides (Petternel et al., 2014), though this pattern was not observed genetically except in one isolate, H19. However, isolates containing other genetic β -lactamases such as *bla*_{TEM} and *bla*_Z were found in four isolates, two of which exhibited resistance phenotypes to quinolones, tetracycline and aminoglycosides.

Four isolates also showed phenotypic resistance to piperacillin/tazobactam (TZP) and ticarcillin/clavulanic Acid. TZP is a critically important antimicrobial with a high priority that was approved to treat serious infections including pneumonia, intra-abdominal infection, sepsis and febrile neutropenia (Barton et al., 2019). The current study looked at only three of the more than 340 β -lactamase genes that have been described as having the ability to mediate resistance in *Salmonella* to β -lactam antibiotics. Fortunately, contrary to previous findings (Lee et al., 2013), TZP resistance phenotypes were not associated with third-generation cephalosporin resistance in the present study.

The high sensitivity of isolated *Salmonella* to carbapenems (Imipenem and Meropenem), third-generation cephalosporins

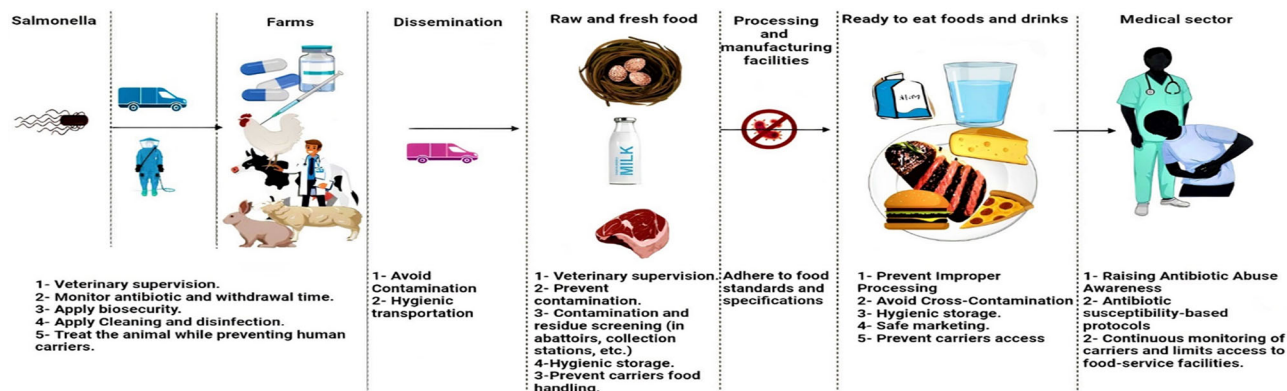


FIGURE 2 Illustration of the various sources, pathways, drivers and factors influencing the spread of antibiotic-resistant *Salmonella* spp. in food, water and humans in the study area, as well as suggested control measures

(ceftazidime), fourth-generation cephalosporins (cefepime) and monobactam (aztreonam) could not be completely attributed to people's awareness of these agents' therapeutic importance. However, this is attributable perhaps to the fact that they are rarely prescribed, except in MDR cases, and their high sale prices may contribute to their infrequent use on farms.

Unfortunately, three *Salmonella* isolates originated from egg and rabbit meat samples (8.75%, $n = 35$) exhibited colistin resistance phenotype. Many mechanisms of bacterial resistance to polymyxin have been described; however, the most dangerous mechanism is plasmid-mediated horizontally transferred resistance (*mcr1*) (Mlynarcik & Kolar, 2019). Here, the *mcr1* gene was found in three *Salmonella* isolates, which could explain the expression of colistin resistance phenotypes in one isolate. Consequently, the overall rate of colistin resistance was 14.29%, whether phenotypic or genotypic. This mechanism has emerged in many countries worldwide, including Egypt (Soliman et al., 2021), reducing colistin therapeutic efficacy against persistent MDR infections (Liu et al., 2016). This resistance phenotype was previously identified in Egypt, where it was detected from *E. coli* isolated from chicken (Soliman et al., 2021) and bovine milk (Tartor et al., 2021). However, the first time detected in *Salmonella enterica* was recovered from cattle minced meat and rabbit meat. The overuse and misuse of colistin in animal production, mainly poultry feed, is the primary cause of the emergence of colistin-resistant pathogens (Bista et al., 2020), which also helps to explain higher levels in egg-derived isolates in the current study.

Here, the presence of a high level of resistance phenotypes to aminoglycosides (48.57%), β -lactams (20%) and tetracycline (17.14%) suggests that these drugs are being misused as growth promoters in animals feed (Bista et al., 2020). Despite the fact that not all suspected genes were investigated, molecular characterization revealed that the attributable resistance genes for these three antimicrobial classes, as well as the trimethoprim/sulfamethoxazole class, were common.

It is also worth mentioning that three *Salmonella* isolates (8.57%, $n = 35$) from cattle liver, minced meat and eggshell were phenotypically resistant to ciprofloxacin, with one of these phenotypes being *gyrA* gene-mediated. Fluoroquinolone antibiotics, like ciprofloxacin,

are usually the first-line antibiotic for *Salmonella* infections, except in children and pregnant women, where treatment is usually limited to β -lactam because fluoroquinolones interfere with cartilage formation (Frye & Jackson, 2013). The co-resistance of these *Salmonella* isolates to TZP, ticarcillin/clavulanic acid, piperacillin and ticarcillin means a serious problem if spread to children and pregnant women (Parry & Threlfall, 2008). Fortunately, these *Salmonella* isolates are still susceptible to cephalosporin β -lactam antibiotics.

It is impossible to conduct a thorough investigation of all genes suspected of contributing to *Salmonella* resistance mechanisms to the critically important antibiotics under investigation in this study. Hence, the presence of an AMR phenotype in isolated *Salmonella*, despite the absence of the resistance gene, could be attributed to the presence of other resistance mechanisms (Ahmed et al., 2016; Chuanchuen et al., 2010). Furthermore, the presence of isolates SH15 and DE15 that are susceptible to trimethoprim/sulfamethoxazole despite the presence of the *dfra* and *sul1* genes support previous findings and assumptions that antibiotic resistance genes in *Salmonella* could be silent (Ahmed et al., 2016; Randall et al., 2004). This is where advanced analysis techniques, such as whole-genome sequencing, come into play to explain possible genetic resistance mechanisms.

The zoonotic nature of *Salmonella*, intracellular pathogenesis and ability to survive medication, generating healthy animal and human carriers, and vertical transmission to the egg from infected poultry are among the potentially implicated sources, pathways, drivers and factors of *Salmonella* spp. spread to food and humans (Kirkwood et al., 2021). Moreover, inadequate processing, sanitary practices, and, of course, cross-contamination from the previously mentioned factors were ranked at the top of the risk factors for the spread of *Salmonella* spp. in cooked foods (Figure 2).

The Egyptian Ministry of Health and Population's surveillance system revealed that among 15 notifiable communicable diseases, typhoid fever had the second highest incidence (12.7 cases/100,000) over 8-year period between 2006 and 2013, with Qalyubia having the fourth highest risk-index score for typhoid (Abdel-Razik et al., 2017). Significant human and economic costs due to morbidity and mortality, as well as lost agricultural productivity, are all key motivators in

characterizing preventive and control measures for *Salmonella* mitigation. Of course, the current findings would aid in assessing *Salmonella* risk in the foods under investigation. However, the most important preventive measure is raising population awareness of the threats associated with antibiotic resistance through the publication of official statistics about human salmonellosis and potential complications caused by increasing MDR *Salmonella* prevalence in food, which is attributed to antibiotic abuse in animals and patients without consulting a medical specialist. Furthermore, to keep such hazards from spreading to humans, the concerned Egyptian food safety authority must implement a more comprehensive national surveillance system that encompasses not only exported food but also locally marketed and produced foods. Recently, Egyptian regulations mandated veterinary supervision on cattle and poultry farms; however, to prevent stakeholder antibiotic abuse and ascertain withdrawal time, medication should be ordered and administered solely by a veterinarian. A biosecurity plan is another critical control point for *Salmonella* that farms must enforce to prevent the introduction and spread through the food chain (WHO & FAO, 2019). Finally, for logical and practical interventions, laboratories in food production facilities, such as slaughterhouses and milk industries, should be outfitted with fast and reliable equipment, or rather guarantee a link with referee laboratories to monitor antibiotic residues in animal-derived food (Figure 2).

5 | CONCLUSIONS

Salmonella enterica were detected at a high rate in cattle-derived food. *Salmonella enterica* was a little more prevalent in RTE/D foods than in raw foods. These findings, along with the detection of resistance to critical and clinically important drugs in foodborne *Salmonella enterica*, particularly from RTE food, imply veterinary use of these antibiotics and the possibility of these isolates being transferred between food and humans, complicating salmonellosis control and posing a serious problem for high-risk groups such as children and pregnant women. To the best of our knowledge, this is the first time colistin resistance was identified in *Salmonella enterica* recovered from cattle minced meat and rabbit meat. In terms of genetic and phenotypic characteristics, there was no complete agreement among MDR *Salmonella* isolates from food and human sources. Finally, additional whole-genome sequencing of isolated *Salmonella* strains from the current study will be required to further explore the range of possible genetic resistance mechanisms involved.

ACKNOWLEDGEMENTS

The authors extend their appreciation to the Scientific Research Fund (SRF), Benha University, Egypt for funding this work through the Research Support Program, Project No. (M6/1/5). The authors acknowledge the effort of Dr. Charlene Jackson, Bacterial Epidemiology and Antimicrobial Resistance Research Unit, USDA-ARS, Athens 30605, GA, USA for language editing of manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORSHIP CONTRIBUTIONS

Islam Sabeq: Conceptualization, data curation, funding acquisition, project administration, writing – review & editing. **Dina Awad:** Formal analysis, methodology, roles/writing – original draft. **Ahmed Hamad:** Conceptualization, formal analysis, writing – review & editing. **Mohamed Nabil:** Methodology, roles/writing – original draft. **Mohamed Aboubakr:** Supervision. **Mohamed Abaza:** Formal analysis, roles/writing – original draft. **Mohamed A. Fouad:** Formal analysis, validation. **Amira Hussein:** Methodology, validation. **Sanaa Shama:** Investigation. **Hazem Ramadan:** Conceptualization, validation, writing – review & editing. **Shimaa Edris:** Formal analysis, roles/writing – original draft.

INSTITUTIONAL REVIEW BOARD STATEMENT

The study was conducted according to the guidelines of the Declaration of Helsinki as well as the rules and regulations of the Egyptian Ministry of Health under full medical supervision. Written consent has been obtained from the patients before using the specimens.

INFORMED CONSENT STATEMENT

Informed consent was obtained from all subjects involved in the study.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received.

DATA AVAILABILITY STATEMENT

All authors agree that the data presented in this study are openly available through publisher platform or others without any restriction.

ORCID

Islam Sabeq  <https://orcid.org/0000-0002-7516-7265>

Mohamed Abaza  <https://orcid.org/0000-0003-4241-3826>

Mohammed Fouad  <https://orcid.org/0000-0002-9656-8041>

Hazem Ramadan  <https://orcid.org/0000-0002-6687-3758>

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SUPPORTING INFORMATION

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How to cite this article: Sabeq, I., Awad, D., Hamad, A., Nabil, M., Aboubakr, M., Abaza, M., Fouad, M., Hussein, A., Shama, S., Ramadan, H., & Edris, S. (2022). Prevalence and molecular characterization of foodborne and human-derived *Salmonella* strains for resistance to critically important antibiotics. *Transboundary and Emerging Diseases*, 69, e2153–e2163. <https://doi.org/10.1111/tbed.14553>