# **Original Research**

Journal of Advanced Veterinary Research (2023) Volume 13, Issue 9, 1803-1808

# Antimicrobial Resistance of Salmonella Species Isolated from Some Food Products and Human in Alexandria, Egypt

Mohamed A. Nossair<sup>1\*</sup>, Heba M. Habib<sup>1</sup>, Alaa M. Mansour<sup>1</sup>, Sherine A. Shehab<sup>1</sup>, Sahar A.D. El-Lami<sup>2</sup>, Hassan A.M. Samaha<sup>3</sup>

<sup>1</sup>Department of Animal Hygiene and Zoonoses, Faculty of Veterinary Medicine, Alexandria University, Egypt.

<sup>2</sup>Public Health Department, Faculty of Veterinary Medicine, Wasit University, Iraq.

<sup>3</sup>Department of Pharmacognosy and Medicinal Plants, Faculty of Clinical Pharmacy, Al-Baha University, Kingdom of Saudi Arabia.

#### \*Correspondence

Corresponding author: Mohamed A. Nossair E-mail address:mohammadnossair@alexu.edu.eg

#### Abstract

This study was carried out in Alexandria Province for a period of 12 months from November 2021 to October 2022 in the laboratory of Animal Hygiene and Zoonoses Department, Faculty of Veterinary Medicine, Alexandria University for isolation and molecular identification of Salmonella from some food products of animal origin as well as humans. In addition, the antimicrobial susceptibility testing of the identified bacterial strains was assessed. A total of 450 food samples, comprising chicken paneeh, chicken burger, chicken luncheon, minced meat, beef burger, and kariesh cheese (75 each), were randomly gathered from. In addition, a total of 100 human stool samples have been obtained from 60 diarrheal individuals and 40 apparently healthy ones. Isolation of Salmonella from food samples on XLD clarified that the recovery rate was 12, 8, 5.3, 13.3, 9.3 and 5.3 % for Chicken paneeh, Chicken burger, Chicken luncheon, Minced meat, Beef burger and Kariesh cheese, respectively (40 isolates) while biochemical identification showed that the detection rate was 9.3, 5.3, 5.3, 8, 5.3 and 4% from the same samples, respectively (28 isolates only). Moreover, the molecular identification revealed the detection of 11 isolates only. Finally, the serological identification of 11 Salmonella isolates clarified the presence of S. Enteritidis, S. Haifa, S. Inganda, S. Tamale, S. Typhimurium and S. Shangani with various rates. Antimicrobial susceptibility of Salmonella strains (n=11) isolated from food products revealed that Ciprofloxacin was the most effective antibiotic against the tested isolates (90.9 %) followed by Doxycycline (72.73 %) while Cephalexin was the least effective antibiotics as it was noticed that 100% of isolates were resistant. On the other hand, Isolation of Salmonella from the stool samples on XLD (43 isolates) clarified that the recovery rate was 53.3 and 27.5 % for diarrheic and apparently healthy individuals, respectively while biochemical identification tests showed that the detection rate was 25 and 10 % for diarrheic and apparently healthy individuals, respectively (19 isolates only). In addition, the molecular identification of isolates revealed the detection of 16 isolates only. Finally, the serological identification of Salmonella isolates (n=11) recovered from food products clarified the presence of S. Enteritidis, S. Haifa, S. Inganda, S. Typhimurium, S. Montevideo and S. Tsevie with various rates. Finally, antimicrobial susceptibility of Salmonella strains (n=11) isolated from stool samples revealed that Vancomycin and Doxycycline were the most effective antibiotics against the tested isolates (93.75 %) while it was noticed that 100% of isolates were resistant to the remaining antibiotics (Ampicillin, Cefotaxim, Cephalexin, Ciprofloxacin, Penicillin G, Streptomycin and Tetracycline).

KEYWORDS

Antimicrobial Resistance, Salmonella, Food products, Human

# INTRODUCTION

Salmonellosis is one of the most serious issues affecting animal industry, as well as a serious food safety risk. Salmonellosis usually causes self-limiting gastroenteritis with symptoms appear with in 12 to 72 hours after infection including diarrhea, abdominal pain and vomiting which may last for 4-7 days. However, in children, elderly and immunocompromised patients, Salmonellosis may cause severe gastroenteritis which need hospitalization (Foley and Lynne, 2008). Recovered patients may carry Salmonellosis for about 3 to 7 days up to 7 weeks (Jones *et al.*, 2008). People generally acquire the pathogen through food-borne sources, direct contact with infected animals and person to person transmission (Founou *et al.*, 2016). Salmonellosis in animals is often carried asymptomatically and disease occurs usually when animals are placed under stress. Animals which had recovered from infection may continue shedding the bacteria for 2-12 weeks (Hume et al., 2004). Globally, around 86% of salmonellosis was of food borne sources (Hoelzer et al., 2011). Raw or improperly pasteurized milk and their products are considered potential sources of Salmonella infection to humans (Halawa et al., 2016). Feces of infected cattle, contaminated skin, infected udder, milking equipment, air (dust borne infection), feed, animal insects and milkers are main sources of raw milk contamination with Salmonella (Callon et al., 2008). From more than 2600 identified Salmonella serotypes, S. Enteritidis, S. Typhimurium and S. Infantis have a major public health concern (Ezzat et al., 2014). Consumption of animal products like meat, milk, and egg is increased due to rapid human population growth, urbanization, per capita income raise, globalization, and the changes on consumer habits (preference of high-protein diet). This situation results in a high demand of food of animal origin and leads to intensive animal production and processing of products, especially mass production and movement of products globally. During this time, there may be

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defective processing practices at any point of the farm to fork chain which increase the chances of contamination and spread of food-borne pathogens (Dhama et al., 2013). The increased spread of multidrug-resistant Salmonella spp. is attributed to misuse of antibiotics, which has resulted in increased illness severity. The prevalence of multidrug-resistant (MDR) food-borne pathogens is increased after consumption of contaminated food due to the use of drugs for human therapy and animal farming which are responsible for more serious disease than susceptible bacteria (Tsepo et al., 2021). Drug resistance among the pathogens in common and food-borne pathogens in particular is an emerging problem (Swartz, 2022). Prevailing poor food handling and sanitation practices, inadequate food safety laws, weak regulatory systems, lack of financial resources to invest in safer equipment, and lack of education for food handlers are the reasons for common occurrence of food-borne diseases in developing countries including Egypt (Kebede et al., 2014). Antimicrobial resistant bacteria such as Salmonella have evolved as a serious public health concern as a result of extensive abuse of antimicrobial drugs in food animal production as a means of growth. One of the main causes of the rise of multidrug resistance bacteria is the improper use of antibiotics in poultry farms in underdeveloped countries, particularly Egypt (Okeke et al., 2005). The misuse of the antimicrobial agents in veterinary medicine could result in the emerging of multidrug-resistant bacteria (MDR) including Salmonella. The antimicrobial-resistant microorganism and the antimicrobial resistance genes could be transmitted to humans through food derived from animals particularly poultry meat and their products (Sallam et al., 2014). Therefore, the objective of this study was isolation and molecular identification of Salmonella from some food products of animal origin as well as humans. In addition, the antimicrobial susceptibility testing of the identified bacterial strains was assessed.

# **MATERIALS AND METHODS**

#### Sampling

This study was carried out in Alexandria Province for a period of 12 months from November 2021 to October 2022. A total of 450 food samples, comprising chicken paneeh, chicken burger, chicken luncheon, minced meat, beef burger, and kariesh cheese (75 each), were randomly gathered from various shops. Samples were randomly collected from different retail outlets, different shops, and supermarkets in Alexandria City. The samples were obtained as sold to the public and transferred as soon as possible in an insulated icebox at  $4\pm1^{\circ}$ C with a minimum of delay to the laboratory of Animal Hygiene and Zoonoses Department, Faculty of Veterinary Medicine, Alexandria University for conventional bacteriological analysis. In addition, 100 human stool samples have been obtained from 60 diarrheal individuals and 40 apparently healthy ones whose informed consent.

#### Isolation and identification of Salmonella

Salmonella was detected using conventional culture-based methods as reviewed in ISO 6579-1, (2017). Each sample was pre-enriched in buffered peptone water then incubated for 24 hours at 37°C. 0.1 ml of the pre-enriched broth was enriched in 10 ml of Rappaport Vassilliadis (RV) broth then overnight incubation at 42°C A loopful from selective enrichment broth was inoculated on XLD agar (Xylose Lysine Desoxycholate) and incubated for 24 hours at 37°C. Pink to red colonies with or without black center were picked and streaked on nutrient agar slopes and in-

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cubated for 18-24 hours at 37°C for biochemical identification according to MacFaddin (2000) by using indole production test, Simmons citrate test, urease test, triple sugar iron and sugar fermentation test then confirmed with API (Analytical profile index).

#### Molecular Identification of Salmonella targeting InvA gene

Oligonucleotide primers sequence used in PCR for molecular identification of Salmonella targeting InvA gene is 5'GTG AAA TTA TCG CCA CGT TCG GGCA -3' and 3' TCATCG CAC CGT CAAAGG AAC C -5' at 284 bp. Salmonella strains were cultured onto Luria Bartani (LB) broth for 24 hours at 37°C then DNA was extracted according to Sambrook et al. (1989). Amplification process was performed according to Singer et al. (2006). PCR was carried out in 25µl reaction volumes 12.5µl 2x PCR master mix 0.47µl invA1, 0.048µl invA2, invA 0.48µl and 1.7µl NA template. The reaction was completed up to 25µl with distilled water. The PCR system was programmed for denaturation at 94°C, 30 cycles of denaturation at 94°C for 30 seconds. Annealing at 56°C for 30 seconds and extension at 72°C for 2 minutes was performed. Then the reaction was held at 72°C for 7 minutes and stored at 4°C. PCR products were electrophoresed at 2% (wt/vol) agarose and 0.5µg of ethidium bromide (Sigma Aldrich). The samples were electrophoresed at 85 volt for 20-30 minutes. A 300 nm transillumination was used to detect the bands which were then photographed according to Sambrook et al. (1989).

#### Serotyping

Serological identification of *Salmonella* isolates according to Kauffman-White scheme (Grimont and Weill, 2007) were carried out at the Faculty of Veterinary Medicine, Department of Food Hygiene and Control, Benha University, Egypt by the slide agglutination technique of both somatic (O) and flagellar (H) antigens.

#### In-Vitro anti-microbial sensitivity test

On Mueller-Hinton agar, the disc diffusion pattern was used to assess the susceptibility of 16 confirmed strains *Salmonella* to 9 antimicrobial medicines including Ampicillin (5 µg), Cefotaxim (10 µg), Cephalexin (30 µg), Ciprofloxacin (10 µg), Doxycycline (2 µg), Penicillin G (10 IU), Streptomycin (10 µg), Tetracycline (5 µg) and Vancomycin (10 µg). In order to conduct the test, the Muller Hinton agar medium surface was inoculated with bacteria and then streaked with swab sticks. Discs were used to inoculate agar plates, which were subsequently incubated for 24 hours at 37°C. The zone diameters were measured in millimetres using a ruler and the isolators. Isolates were categorized as sensitive or resistant according on the criteria established by CLSI, (2017).

#### Statistical analysis

The Chi-2 test was done on contingency tables to investigate if there were significant differences between isolate sources in terms of isolate's incidence. The significance was recorded when P- value was <0.05. This analysis was done using GraphPad prism software version 8 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com.

## **RESULTS AND DISCUSSION**

Consumption of raw or unsafe food, cross-contamination, improper food storage, poor personal hygiene practices, inadequate cooling and reheating of food items, and a prolonged time lapse between preparing and consuming food items were mentioned as contributing factors to an outbreak of salmonellosis in humans. The bacteria enter the food chain at any point in livestock feed, and in food manufacturing, processing, retailing, catering, and preparation, survive typical catering refrigeration temperatures, and increase in number under conditions of thermal abuse. Salmonellosis is a zoonotic infection that causes substantial economic losses resulting from morbidity, mortality and poor growth with hazard of transmitting food poisoning to human. Antibiotic-resistant Salmonella infections of both humans and animals are universal concerns, particularly in developing countries, where the risk of infection is high because of unhygienic living conditions, close contact and sharing of houses between animals and humans, and the traditions of consumption of raw or undercooked animal-origin food items (Ejo et al., 2016). As shown in Table 1, the recovery rate of Salmonella from food samples on XLD was 12, 8, 5.3, 13.3, 9.3 and 5.3 % for Chicken paneeh, Chicken burger, Chicken luncheon, Minced meat, Beef burger and Kariesh cheese, respectively (40 isolates) while the biochemical identification of the obtained isolates was 9.3, 5.3, 5.3, 8, 5.3 and 4% from the same samples, respectively (28 isolates only). Finally, the molecular identification of 28 Salmonella isolates revealed the detection of 11 isolates only. In addition, data in Table 2 showed the serological identification of 11 Salmonella isolates clarified the presence of S. Enteritidis, S. Haifa, S. Inganda, S. Tamale, S. Typhimurium and S. Shangani with various rates. Isolation and identification of Salmonella from different foods of animal origin were previously performed by Bucher et al. (2007) who recorded that Salmonella strains were isolated from raw, frozen chicken nuggets, chicken nugget/strip meat blend and chicken strips. The identified Salmonella strains were S. Heidelberg, S. Kentucky and S. Enteritidis, Abdellah et al. (2009) who examined raw chicken breast meat purchased from the Moroccan market, and found 9 samples which were positive for Salmonella with percentage of 6.25% and S. Typhimurium was 40.35%, Saad et al. (2019) who examined chilled minced beef, meat block and poul-

#### Table 1. Occurrence of Salmonella in some food products

Food products	No. of samples –	XLD		Biochemical tests		PCR	
		Positive	%	Positive	%	Positive	%
Chicken paneeh	75	9	12	7	9.3	3	42.86
Chicken burger	75	6	8	4	5.3	2	50
Chicken luncheon	75	4	5.3	4	5.3	1	25
Minced meat	75	10	13.3	6	8	2	33.33
Beef burger	75	7	9.3	4	5.3	2	50
Kariesh cheese	75	4	5.3	3	4	1	33.33
Total	450	40	8.9	28	6.2	11	39.29
Chi-square	20.66	p-value	0.00	p-value	0.27	p-value	0.44

Table 2. Serological identification of Salmonella (n=11) recovered from food products.

	No.	07	Group	Antigenic structure		
Salmonella strains		%0		0	Н	
S. Enteritidis	3	27.3	D1	1,9,12	g, m	
S. Haifa	2	18.2	В	1,4,5,12	Z10: 1,2	
S. Inganda	1	9.1	C1	6.7	Z10: 1,5	
S. Tamale	1	9.1	C3	8,20	Z29: e,n,Z15	
S. Typhimurium	3	27.3	В	1,4,5,12	i: 1,2	
S. Shangani	1	9.1	C1	6.7	r: e,n,Z15	
Total	11	100				
Chi-square	1.64		p-value	0.44		

Table 3. Antimicrobial susceptibility of Salmonella (n=11) isolated from food products.

A	Ser	sitive	Resistant		
Antimicrobial agents	No.	%	No.	%	
Ampicillin (AM)	1	9.1	10	90.9	
Cefotaxime (CF)	2	18.18	9	81.82	
Cephalexin (CN)	0	0	11	100	
Ciprofloxacin (CP)	10	90.9	1	9.1	
Doxycycline (DO)	8	72.73	3	27.27	
Penicillin G (P)	1	9.1	10	90.9	
Streptomycin (S)	1	9.1	10	90.9	
Tetracycline (T)	8	72.73	3	27.27	
Vancomycin	5	45.45	6	54.54	
Chi-square	2		p-value	0.69	

try cuts and found that the incidence of Salmonella was 35, 25, 20, 30 and 15%, respectively. In addition, serotyping of Salmonella isolated from meat blocks were S. Enteritidis, S. Typhimurium and S. Virchow while from minced meat were S. Enteritidis, S. Typhimurium, S. Virchow and S. Heidberg, from chicken meat were S. Enteritidis, S. Typhimurium, S. Virchow and S. Kentucky and Shaltout et al. (2019) who examined chicken nuggets and chicken panne and detected S. Typhimurium in 6.7% of panne samples while S. Anatum was detected in 7% of nuggets samples. On the other hand, S. Enteritidis was detected in 6.7% of panne samples. Antimicrobial drugs are crucial in the treatment of infectious illnesses, but their overuse promotes the emergence and spread of antibiotic resistance strains that are linked to serious sickness in human populations. According to Momtaz et al. (2013), there are no restrictions on the use of antibiotics in Egypt, whether they are used to treat ill humans, treat animal diseases, or maybe enhance growth in animals used for food. Inappropriate antibiotic usage has the potential to lead to the formation of antimicrobial-resistant zoonotic bacteria in animal-derived commodities, particularly milk and meat, which are often linked to outbreaks around the globe (Abd El-Ghany et al., 2015). Kareish cheese is one of the most popular cheeses consumed in Egypt due to its high protein, low fat and affordable price. Variable occurrences of Salmonella

in Kareish cheese was reported in many previous literatures. Elafify, et al. (2022) found high Salmonella prevalence rate at 16.67% in the examined kareish cheese samples. In another study, El-Baz et al. (2017) added that the contamination of kareish cheese with Salmonella was high (20%) in their study due to improper sanitation practices during manufacture and handling of cheese and most cheeses were sold uncovered or without container. On the other hand, Omar et al. (2018) recorded a lower prevalence (8%, 2 out of 25) of Salmonella obtained from Kareish cheese than our finding. Antimicrobial susceptibility of Salmonella strains (n=11) isolated from food products was tabulated in Table 3, and revealed that Ciprofloxacin was the most effective antibiotic against the tested isolates (90.9 %) followed by Doxycycline (72.73 %) while Cephalexin was the least effective antibiotics as it was noticed that 100% of isolates were resistant. Antimicrobial susceptibility of Salmonella isolated from different foods of animal origin were previously performed by Donado-Godoy (2015) who found that 98% of Salmonella isolates were reported as multi-drug resistant where Ceftiofur, enrofloxacin, nalidixic acid and tetracycline were the antimicrobials that showed the highest frequency of resistance among Salmonella species and Adel et al. (2021) who investigated the presence of the extended-spectrum β- lactamase (ESBL) and plasmid-mediated quinolone resistance

Table 4. Occurrence of Salmonella in human stool samples in relation to health status.

Stool samples	No. of samples —	XLD		Biochemical tests		PCR	
		Positive	%	Positive	%	Positive	%
Diarrheic individuals	60	32	53.3	15	25	14	93.3
Apparently healthy	40	11	27.5	4	10	2	50
Total	100	43	43	19	16	16	84.2
Chi-square		10.86		p-value	0.01	p-value	0.25

Table 5. Serological identification of Salmonella (n=16) recovered from stool samples.

al II i	No.	07	Group	Antigenic structure	
Salmonella strains		%0		0	Н
S. Enteritidis	5	31.2	D1	1,9,12	g, m
S. Haifa	3	18.8	В	1,4,5,12	Z10: 1,2
S. Inganda	2	12.5	C1	6.7	Z10: 1,5
S. Typhimurium	2	12.5	В	1,4,5,12	i: 1,2
S. Montevideo	2	12.5	C1	6,7,14	g, m, s: 1,2,7
S. Tsevie	2	12.5	В	4,5	i: e, n,z15
Total	16	100			
Chi-square	1.64		p-value	(	).44

Table 6. Antimicrobial susceptibility of Salmonella (n=16) isolated from stool samples.

A	Sen	sitive	Resistant		
Antimicrobial agents -	No.	%	No.	%	
Ampicillin (AM)	0	0	16	100	
Cefotaxime (CF)	0	0	16	100	
Cephalexin (CN)	0	0	16	100	
Ciprofloxacin (CP)	0	0	16	100	
Doxycycline (DO)	15	93.75	1	6.25	
Penicillin G (P)	0	0	16	100	
Streptomycin (S)	0	0	16	100	
Tetracycline (T)	0	0	16	100	
Vancomycin (VA)	15	93.75	1	6.25	
Chi-square	7.41	p-va	alue	0.06	

(PMQR) genes in S. enterica isolated from retail meats and found that 26 out of 34 (82.4%) S. enterica isolates showed MDR phenotypes to at least three classes of antimicrobials where the most prevalent resistance was to ampicillin, streptomycin, oxacillin, and tetracycline. Additionally, 14 (41.2%) of 34 S. enterica isolates showed ESBL-resistant phenotypes. Human salmonellosis represents a public health problem in both developed and developing countries. It is one of the most common causes of foodborne infection in human beings and still the main cause of acute diarrhea (Okareh and Erhahon, 2015; Diab et al., 2019; Geletu et al., 2022; Orabi et al., 2022). The presented data in Table 4, showed that the recovery rate of Salmonella from the stool samples on XLD (43 isolates) was 53.3 and 27.5 % for diarrheic and apparently healthy individuals, respectively while biochemical identification tests showed that the detection rate was 25 and 10 % for diarrheic and apparently healthy individuals, respectively (19 isolates only). The obtained results was higher than results detected by Diab, et al. (2019) who estimated the overall prevalence of Salmonella among human to be 4.4% and Shaaban et al. (2018) who reported 5% prevalence of Salmonella in human. Equale, et al. (2016) explained that contaminated dairy products and contact with dairy cattle represent a common source of Salmonella in humans. Isolation of Salmonella from healthy human clarifies the role of carriers in transmission of infection. A variable findings was obtained by Zhang et al. (2018) who reported 3.75% prevalence of Salmonella (42 out of 1121) from diarrheal patients and 0.31% (1 out of 319) from non-diarrheal patients. Another study was performed by Kadry et al. (2019) in which they found that six strains of Salmonella were isolated from stool samples collected from diarrheal patients with an incidence of 3.75%. A higher occurrence of Salmonella in diarrheal patients was reported by Gong et al. (2022) who examined a total of 255 fecal specimens and found that 20.39% (52 out of 255) were positive for Salmonella. In addition, the molecular identification of isolates revealed the detection of 16 isolates only. Finally, the serological identification of Salmonella isolates (n=11) recovered from food products clarified the presence of S. Enteritidis, S. Haifa, S. Inganda, S. Typhimurium, S. Montevideo and S. Tsevie with various rates (Table, 5). Different findings were recorded by Ketema et al. (2018) who demonstrated that the main serovars in their study were S. Dublin (n=10, 35.7%) and S. Virchow (n=5, 17.9%) followed by S. Braendrerup, S. Haifa and S. Saintpaul which were isolated from 2 samples each (7.1%). Another study was performed by Castañeda-Salazar, et al. (2021) in which the most notable Salmonella serovars isolated were S. Newport (60.87%), S. Typhimurium (17.4%), S. Virchow, S. Bredeney (4.3%) and S. Anatum (4.3%). Finally, the presented data in Table (6) showed the antimicrobial susceptibility of Salmonella strains (n=11) isolated from food products revealed that Vancomycin and Doxycycline were the most effective antibiotics against the tested isolates (93.75 %) while it was noticed that 100% of isolates were resistant to the remaining antibiotics (Ampicillin, Cefotaxime, Cephalexin, Ciprofloxacin, Penicillin G, Streptomycin and Tetracycline). The recorded results were in harmony with Donado-Godoy (2015) who found that 98% of Salmonella isolates were reported as multidrug resistant. Ceftiofur, enrofloxacin, nalidixic acid and tetracycline were the antimicrobials that showed the highest frequency of resistance among Salmonella species and Adel et al. (2021) who investigated the presence of the extended-spectrum β- lactamase (ESBL) and plasmid-mediated quinolone resistance (PMQR) genes in S. enterica isolated from retail meats and slaughterhouses in Egypt by using PCR and DNA sequencing techniques and found that 82.4% of S. enterica isolates showed MDR phenotypes to at least three classes of antimicrobials. The most prevalent resistance was to ampicillin, streptomycin, oxacillin, and tetracycline. Additionally, 14 (41.2%) of 34 *S. enterica* isolates showed ESBL-resistant phenotypes.

## CONCLUSION

From the current study, it was concluded that there was a significant occurrence of resistant *Salmonella* in chicken paneeh, chicken luncheon, minced meat, Kariesh cheese, and beef burger, as well as in humans. Seriously, these resistant bacteria can be transmitted zoonotically to humans which endanger human health through making the treatment of human infections more difficult with routine antibiotics. Finally, it was determined from the data that retail foods in Alexandria pose a risk to human health.

## **CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

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