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Isolation of *Salmonella* and *Listeria monocytogenes* from different prepared food

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Abstract

The present study aimed to evaluate the prevalence of *Salmonella* and *Listeria monocytogenes* and antimicrobial resistance of *Salmonella enterica* food poisoning in different prepared food in Sharm El Sheikh City in Egypt.

A total of 7 out of 184(3.8%) food samples (chicken meat, red meat and vegetables and cooked food) were found positive for *Salmonella enterica*. However *Listeria monocytogenes* failed to be detected in examined samples. Among the studied food samples, chicken based food showed a lower prevalence of *Salmonella enterica* than red meat based samples with a percentage of (3.6%) and (6.25%), respectively. Vegetable based cooked food samples were found free from both *Salmonella enterica* and *listeria monocytogenes*. Antimicrobial resistance patterns showed that multiple drug resis tance were detected, that all isolated *Salmonella* serotypes are resistant to nalidixic acid. While antibiotic resistance profiles for *Salmonella* Typhimurium were found 100% resistant to Amoxicillin+clavulinic, ciprofloxacin, Nalidixic acid, streptomycin, and Trimethoprim-sulfamethoxazole.

Keywords: *Antimicrobial resistance, Salmonella enteric, Listeria spp., food, Sharm El-sheikh-Egypt.*

Introduction

Salmonella and *Listeria* spp. are among the most common significant pathogens that cause foodborne diseases. These microbes are of great public health concern; especially in developing countries (**Adzitey *et al*., 2012 and Jamali *et al*., 2013**). *Salmonella* is the second most common cause of food poisoning after Campylobacter. It has been found in eggs and raw egg products, meat and poultry. *Salmonella* can survive if food is not cooked properly. Estimating prevalence and antibiotic resistance of food-borne diseases is a common and crucial public health practice. Most infections with antimicrobial-resistant *Salmonella* are acquired by eating contaminated foods of animal origin. Among these, the foods most often incriminated in salmonellosis are contaminated poultry ((**Bokanyi *et al*., 1990 and Boonmar *et al*., 1998**). The use of antimicrobials in animal feed as growth promotion or for control or treatment of animal diseases has been increased. This misuse of antibiotics in animals increases the rates of antimicrobial resistance to several antibiotics (**Kimura *et al.*, 2004; Braden, 2006 and Jamali *et al*., 2013**). Moreover, antimicrobial resistance of the pathogens could complicate the treatment and will represent a serious threat to global public health that requires action across all government sectors and society. (**Engberg *et al*., 2004**). Estimation of prevalence and antibiotic resistance of *Salmonella* and *Listeria* spp. in Sharm el-Sheikh, as one of the most attractive touristic holiday resorts and a significant centre for tourism in Egypt, will serve as an effective guidance and treatment tool against infections caused by food-borne diseases for tourists visiting Egypt.

Materials and Methods

Sample collection

A total of 184 food samples, including chicken meat products (n = 80), meat products (n = 56) and plant origin products (n = 48) were randomly collected from different restaurants located in Sharm El Sheikh Governorate in Egypt. Samples were transferred into sterile plastic bags and transported in an ice box to the Reference laboratory for quality control on poultry production (RLQP) Giza main laboratory in Egypt for further bacterial examination as soon as possible.

Isolation and identification of *Salmonella* species

The standard conventional culture method was used in the isolation of *Salmonella* spp. according to **ISO 6579, 2002**. 25 gm of each sample was placed directly into 225 ml of buffered peptone water (BPW) as pre-enrichment for 18h at 37°C. Further, 1ml of pre-enrichment broth transferred to 10ml Müller-Kaufmann Tetrathionate with novobiocin broth incubated at 37.0°C ±1°C and 0.1ml (100 uL) of the pre-enrichment broth to 10ml Rappaport-Vassiliadis soy peptone (RVS) broth, incubated at 41.5°C±0.5°C overnight (18-24 hours). Loop full from the inoculated and incubated Tetrathionate broth and RVS broth were then spread on XLD and on BGA agar plates and incubated at 37.0°C ± 1°C overnight (18-24 hours). Presumptive *Salmonella* colonies were confirmed by using API 20E (bioMérieux 20100, Marcy L'Etoile, France). The isolates that were identified biochemically as *Salmonella* was subjected to serological identification according to White Kauffman Scheme (**Kauffman, 1974**) **for** determination of somatic (O) and flagellar (H) antigens (Dinka Sieken) (**Cruickshank *et al.*, 1975**).

Isolation and identification of Listeria *monocytogenes*:

Isolation and detection of *Listeria monocytogenes.* were carried out through the **ISO 11290**, **(1996)** method as described by **Becker *et al.* (2006).** Briefly, 25 gm of each sample were added to 225 ml of half Fraser broth (Oxoid, Basingstoke, UK) as the first enrichment culture and incubated for 24 h at 30 ± 1 oC. Then, a loopful of first enriched broth culture was streaked on CHROM agar *Listeria* (CHROM agar, Paris, France) and incubated for another 24 - 48h at 37°C. On the other hand, 0.1ml of half Fraser broth was added to 10 ml of Fraser broth as a second enrichment culture and incubated at 37°C for 48h. Further, a loopful of enriched Fraser broth culture was streaked onto *Listeria* selective agar (Oxford Formulation) (Oxoid, Basingstoke, UK) and PALCAM agar (Oxoid, Basingstoke, UK) and incubated for 24 -48h at 37°C.

Antimicrobial susceptibility test

All identified *Salmonella* isolates were tested for antimicrobial resistance by the disk diffusion method on Mueller-Hinton agar (Oxoid), according to the method recommended by the **National Committee for Clinical Laboratory Standards (NCCLS) 2002.** The following panel of antimicrobial agents and concentrations was applied: amoxicillinclavulanic acid (10-20 µg), chloramphenicol (30 µg), ciprofloxacin (5µg), nalidixic acid (30 µg), nitrofurantoin (300 µg), norfloxacin (10 µg), streptomycin (10 µg), Trimethoprim-sulfamethoxazole (23.75 µg), tetracycline (30 µg), gentamicin (10 µg)

Results

Bacterial identification:

The obtained results showed that 7 out of 184 samples were contaminated with *Salmonella* spp. with a total percentage of (3.8%). *Salmonella* in meat products showed higher prevalence 5/80 than chicken products 2/56 with a percentage of 6.25% and 3.6% respectively. Among meat based products, raw meat samples had the highest incidence followed by a hamburger and cooked meat samples with a percentage of 11.8, 8.7 and 5.9% respectively. The examined beef steak, hot dogs and cooked Chinese beef were not contaminated with *Salmonella.* In chicken based products, *Salmonella* was detected in both chicken panna and raw chicken with a prevalence rate of 12.5 and

7.1% respectively; while fried chicken, cooked chicken and chicken nuggets were free from *Salmonella*. Additionally, *Salmonella* failed to be detected in plant origin food samples. Moreover, *Salmonella Enteritidis* was identified in chicken product samples; whereas *Salmonella Enteritidis* and *Salmonella Typhimurium* were identified in meat product samples. In contrast, all kinds of examined samples, including meat and chicken and plant origin products were not found to be contaminated with *Listeria monocytogenes*. detailed results are summarized in table 1

Table (1). Prevalence of *Salmonella* and *Listeria* spp. In examined food products:

Sample	Sample TYPE	No. of Sample	Salmonella isolation and identification				*Listeria monocytogenes* Isolation	
			Detected		ND*	Serotype	Detected	ND*
Meat Product	Hamburger	23	2	8.7%	21	*S. Enteritidis*	0	23
	Cooked meat	17	1	5.9%	16	*S. Enteritidis*	0	17
	Raw meat	17	2	11.8%	15	*S. Typhimurium*	0	17
	Cooked chinees beef	9	0		9	NA	0	9
	Beef steak	5	0		5	NA	0	5
	Hot dog	9	0		9	NA	0	9
Total %						6.25		
Chicken Product	Cooked chicken	18	0		18	NA	0	18
	Raw chicken	14	1	7.1%	13	*S. Enteritidis*	0	14
	Fried chicken	11	0		11	NA	0	11
	Chicken nuggets	5	0		5	NA	0	5
	Chicken panna	8	1	12.5%	7	*S. Enteritidis*	0	8
Total						3.6		
Plant origin products	Cooked rice	20	0		20	NA	0	20
	Cooked vegetable	10	0		10	NA	0	10
	Pasta	12	0		12	NA	0	12
	Potato	6	0		6	NA	0	6

*ND: Not Detected NA: not applied

Antimicrobial resistance of *Salmonella* Serovars:

Results of antibiotic susceptibility and resistance profiles indicated that *Salmonella Typhimurium* strains showed complete resistance to amoxicillin clavulanic acid (10-20µg), ciprofloxacin (5µg), nalidixic acid (30µg), streptomycin (10µg) and Trimethoprim-sulfamethoxazole (23.75µg). In contrast, *Salmonella Typhimurium* showed good susceptibility to norfloxacin (10µg) (100%) and showed intermediate susceptibility to chloramphenicol (30µg) (100%), gentamycin (10µg) (100%) and nitrofuraontoin (300 µg) (100%).

Salmonella Enteritidis serotypes showed complete resistance to nalidixic acid (30µg) (100%). In contrast, showed good susceptibility to norfloxacin (10µg) and gentamicin with a percentage of 80%, chloramphenicol (30µg) and nitrofurantoin (300µg) with a percentage of 40% for each.

The antibiotic sensitivity and resistance profiles for identified *Salmonella* isolates are shown in table 3 and 4.

Table (2). Result of Antibiotic sensitivity of *Salmonella* Serotypes isolated in food samples from Sharm El sheikh:

Antimicrobial Discs	Disc Potency Mg/disc	Interpretation			Cooked meat		Raw meat		Raw meat		Ham-burger		Ham-burger		Raw chicken		Panna	
		Zone diameter (mm)			S. Ent.		S. Ty-phim.		S. Ty-phim.		S. Ent.		S. Ent.		S. Ent.		S. Ent.	
		Sensi-tive. ≥	Intermedi-ate.	Resis-tant. ≤	Z. D *	Res.	Z. D *	Res.	Z. D *	Res.	Z. D *	Res.	Z. D *	Res.	Z. D *	Res.	Z. D *	Res.
Amoxicillin+clavulinic acid Am+CL	10-20 µg	18	14-17	13	7	R	8	R	8	R	22	S	17	I	22	S	8	R
Chloramphenicol C^{30}	30 µg	18	13-17	12	8	R	15	I	15	I	23	S	7	R	22	S	10	R
Ciprofloxacin CF^{5}	5 µg	21	16-20	15	13	R	14	R	12	R	26	S	18	I	25	S	12	R
Nalidixic acid NA^{30}	30 µg	19	14-18	13	9	R	7	R	7	R	7	R	10	R	11	R	7	R
Nitrofurantoin F300	300 µg	17	15-16	14	10	R	15	I	15	I	23	S	15	I	20	S	16	I
Norfloxacin NX^{10}	10 µg	17	13-16	12	14	I	18	S	19	S	22	S	18	S	19	S	17	S
Streptomycin S^{10}	10 µg	15	12-14	11	6	R	7	R	8	R	18	S	7	R	16	S	15	S
Trimethoprim-sulfamethoxazole SXT	23.75 µg	16	11-15	10	7	R	7	R	6	R	23	S	6	R	20	S	7	R
Tetracyclin T^{30}	30 µg	15	13-15	11	9	R	10	R	25	S	22	S	17	S	20	S	9	R
Gentamycin G^{10}	10 µg	15	13-14	12	13	I	14	I	14	I	18	S	16	S	16	S	19	S

Z.D.: Zone Diameter, Res.: Result, Ham: Hamburger

Table (3). Percentage of antibiotic resistance and susceptibility profiles for different *Salmonella* serotypes isolated in food samples from Sharm El Sheikh:

Antimicrobial Discs	*Salmonella* Enteritidis			*Salmonella* Typhimurium			Both serotypes		
	R	S	I	R	S	I	R	S	I
Amoxicillin+clavulinic acid Am+CL	40.0	40.0	20.0	100.0	0.0	0.0	57.1	28.6	14.3
chloramphenicol C^{30}	60.0	40.0	0.0	0.0	0.0	100.0	42.9	28.6	28.6
ciprofloxacin CF^{5}	40.0	40.0	20.0	100.0	0.0	0.0	57.1	28.6	14.3
Nalidixic acid NA^{30}	100.0	0.0	0.0	100.0	0.0	0.0	100.0	0.0	0.0
nitrofuantoin F300	20.0	40.0	40.0	0.0	0.0	100.0	14.3	28.6	57.1
norofloxacin NX^{10}	0.0	80.0	20.0	0.0	100.0	0.0	0.0	85.7	14.3
streptomycin S^{10}	40.0	60.0	0.0	100.0	0.0	0.0	57.1	42.9	0.0
Trimethoprim-sulfamethoxazole SXT	60.0	40.0	0.0	100.0	0.0	0.0	71.4	28.6	0.0
tetracyclin T^{30}	40.0	60.0	0.0	50.0	50.0	0.0	42.9	57.1	0.0
gentamycin G^{10}	0.0	80.0	20.0	0.0	0.0	100.0	0.0	57.1	42.9

Discussion

It is known that the prevalence of food-borne diseases in developing countries is considerable, in most developing countries, there is limited data through which the prevalence of particular diseases (**Henson, 2003**). The prevalence of *Salmonella* in foods varies greatly from region to region, even within the same country, which is dependent upon the climatic conditions, hygiene and management practices on the farm, handling, processing and storage of raw food. (**Sudhanthiramani *et al.*, 2015**). *Salmonella* present in the chicken meat might originate from poultry farms or contaminate the raw material during slaughter, processing, handling, transport, and storage. Since the cold chain maintenance during transport and consequently the temperature of the product determines its microbiological condition. Additionally, the health hazard from contaminated, raw poultry is mainly one of cross-contamination in the kitchen, where the organism may spread to cooked foods or other ready-to-eat items, such as salad and vegetables. There is also a potential problem with cooked poultry produced commercially. Although, normal cooking destroys *listeria*, recontamination can occur during post-cooking handling at the factory, even with the most rigorous hygiene control.

Lim *et al.*, (1984) mentioned that the total of 774 raw food samples tested, 37 (4.8%) were found to be contaminated with *Salmonella*. Majority of these (30 out of 37 or 81.1%) were from foods of animal origin, namely meats (beef, mutton, pork and chicken), edible offals and processed meats. In a study conducted by **Barrell *et al.*,** (1982) isolation rates of *Salmonella* ranging from 5.2% for raw beef to 36.6% for raw poultry were obtained.

Bucher *et al.*, (**2007**) found that *Salmonella* was found in 9% of the 111 examined samples or lots tested. Feed manufactured from ingredients of both animal and vegetable origin had a higher percentage of *Salmonella*-positive samples (15%) compared with feed manufactured from ingredients of only vegetable origin (8%). The prevalence of *Salmonella* in food in Sharm El-Sheik, Egypt has never been studied; the reported prevalence of *Salmonella* varies from different areas. In this study, overall results showed that 7 out of 184 samples were contaminated with *Salmonella* spp with a total percentage of (3.8%). In this respect **Shanker *et al.*, (2016)** found that *Salmonella* prevalence on meat and poultry ranged from 1% to 10%, depending on the type of meat or produce. In chicken based products, *Salmonella* was detected in both chicken panna and raw chicken with a prevalence rate of 12.5 and 7.1% respectively. In contrast, all kind of examined samples including meat and chicken and plant origin products were not found to be contaminated with *Listeria* spp.

Moreover, the use of antimicrobials in animal's feed has resulted in the development of antimicrobial resistance (**White *et al.*, 2001).** Approaches to preventing and control salmonellosis in the food animal industry by improved biosecurity, vaccination and the introduction of novel immune potentiators with limited success has necessitated the use of antimicrobial chemotherapy in the treatment and control of Salmonellosis (**Zhao *et al.*, 2007**). The use of antimicrobial agents in poultry production for treatment purposes, growth promotion and prophylaxis raise a major concern with regard to antimicrobial resistance and multidrug resistance, which are frequently observed among many *Salmonella* serovars (**Duong *et al.* 2006**).

Antimicrobial resistance patterns showed that multiple drug resistance were detected. In this study, Percentage of antibiotic resistance profiles for *Salmonella* Enteritidis isolated from food samples from Sharm El Sheikh showed that all *Salmonella* serotypes are resistant to nalidixic acid , while the isolates were resistant to chloramphenicol and Trimethoprim-sulfamethoxazole 60% for each and Amoxicillin+clavulinic acid, ciprofloxacin, streptomy-

cin, and tetracyclin were (40%) resistant. While antibiotic resistance profiles for *Salmonella* Typhimurium were found (100%) resistant to Amoxicillin+clavulinic, ciprofloxacin, Nalidixic acid, streptomycin, and Trimethoprim-sulfamethoxazole

In conclusion, the documentation of the food quality will form a basis for control and Prevention of foodborne disease and help with consumer safety, the epidemiological data are needed to report public health authorities about the nature and magnitude of the food diseases.
The Detection of the contamination of food of animal origin with *Salmonella* and *L. monocytogenes* should be part of the compulsory routine microbiological testing of foodstuffs in Egypt. This testing and analysis are important to obtain estimates of the consumer exposure in our country.

Conclusion

The present study revealed the low prevalence or absence of food-borne organisms including *Salmonella enterica* and listeria *spp.* respectively in different examined food samples obtained from Sharm El Sheikh. This reflects the application of good quality in food manufacturing and production practices in that city as well as the application of safety parameters which comply with the international safety standards for the food preparation within that city.
However Continuous monitoring of the prevalence of food-borne pathogens is recommended to insure that international standard of food safety and quality are continuously applied.

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