

Prevalence and characterization of antibiotic resistance food borne pathogens isolated from locally produced chicken raw meat and their handlers

Abstract

The present study was undertaken to determine the prevalence and the potential public health significance of *Salmonella* (*S.*) serovars, *Escherichia coli* (*E. Coli*), and *Staphylococcus aureus* (*S. Aureus*) in raw chicken meat which is available in domestic retail shops as well as their handlers in Mansoura City, Dakahlia Governorate, Egypt. Samples of retail raw chicken meat (n=200) as well as equal sized samples of hand swabs and stool specimens (n=50) from retail handlers were bacteriologically tested *E. Coli*, *Staph. Aureus* and *Salmonella* spp., were recovered from the raw chicken meat at the following percentages: (35, 22 and 5%, respectively) using conventional biochemical identification methods. Serotyping of the obtained *Salmonella* spp., revealed that *Salmonella Kentucky* presented at the highest rate of isolation followed by *Salmonella Enteritidis*, *Salmonella Infantis* and *Salmonella Typhimurium*. High frequency of *S. aureus* were found to colonize the skin (40%) and the stool specimens (30%) of chicken meat handlers; whereas four out of 50 stool samples (8%) and one out of 50 hand swabs (2%) from handlers were found to be contaminated with *Salmonella* spp. *E. Coli* was also detected in 40% of the stool samples and in 24% of handlers hand swabs. Serological identification of *E. Coli* isolates revealed the presence of *E. coli* (O26: H11, O103:H2, O128:H2, O111:H2 and O78) in the examined raw meat, O26: H11, O2:H4 and O128:H2 in stool samples and O26: H11, O103:H2 and O125:H21 in hand swabs. All recovered isolates showed various degree of antibiotic resistance. It becomes apparent that retail chicken raw meat sold at the respective shops at Mansoura city is highly contaminated with food-borne pathogens which is considered a potential vehicle for transmitting food-borne diseases. Hence there is an urgent need for increased implementation of consumer food safety education efforts.

Keywords: chicken meat, *E. Coli*, *Staph. Aureus*, *Salmonella* spp, antibiotic resistance

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Introduction

There has been growing awareness of the major public health impact of zoonotic food borne pathogens from foods of animal origin. Several epidemiological reports have implicated foods of animal origin as the major vehicles associated with illnesses caused by food-borne pathogens. Contaminated raw or undercooked poultry and red meat remains the most important source of human infection with the most commonly reported food borne pathogens.^{1,2} Food borne pathogens is the main etiological agents of illness and death in developing countries.³ In the United States, food borne illness causes 37.2million cases per year resulting in 2.612 deaths.⁴ *Salmonella*, *Escherichia coli*, and *Staphylococcus aureus* are the main predominant species in most food poisoning cases associated with contaminated raw meat.⁵⁻⁷ Moreover, foods contaminated with antibiotic resistance bacteria are a major public health problem in many countries due to continues circulation of the resistant bacterial strains in the environment. Antimicrobial agents are extensively used in the poultry industry for disease prevention or as growth promoters.⁸ Wide spread of antibiotic-resistant food borne pathogens threaten the successful treatment of infectious diseases. The presences of multi drug resistant (MDR) pathogens in poultry meat warranted

concern because they are responsible for more serious disease than susceptible bacteria. The vast majority of the population in Egypt's consumed raw chicken carcasses that slaughtered and butchered in small retailers shops. Meat are available in open-air without adequate temperature control also the hygiene is always questionable. The present study is undertaken to determine the prevalence of food borne pathogens with special reference to *Salmonella sp.*, *Escherichia coli*, and *Staphylococcus aureus* in raw chicken meat available in open markets in Mansoura City as well as their handlers and to evaluate the antimicrobial resistance and sensitivity pattern of the obtained isolate.

Materials and methods

Samples collection

A total of 200 raw chicken meat samples were randomly purchased from various local open markets in Mansoura City, Dakahlia Governorates, Egypt in the period between December 2014 to May 2015. Two equal sized samples (n=50) of handler's hand swabs and stool specimens were also collected. Stool Specimens were collected in clean, dry and sterile container. All samples were aseptically collected and transferred into individual sterile bags then transported to the laboratory in insulated coolers containing cold packs and were

analyzed immediately. All the collected samples were bacteriologically tested for the presence of *Salmonella* species, *E. Coli* and *S. Aureus*. All procedures and practices were performed in accordance with the principles and specific guidelines presented in the Guidelines for the Care and Use of Agricultural Animals in Research and Teaching, 3rd ed. (<http://www.fass.org/>), and with those of Mansoura University Animal Care and approved by its Ethical Committee.

Sample preparation

Twenty five grams of the collected samples were transferred to 225mL of buffered peptone water (BPW) and mixed for 10min at 120r/min, the mixture sample were incubated at 37°C for 16-18h.^{9,10}

Isolation of *Staph. Aureus*

0.1ml from the pre-incubated samples in BPW were spread on the surface of Baird-Parker agar based medium (CM0275, Oxoid) supplemented with Egg Yolk Tellurite Emulsion (SR0054, Oxoid) then incubated at 37°C for 24-48h. Five presumptive black colonies surrounded by opaque halo were picked from each selective agar plate. These colonies were purified using Tryptone soya agar (CM131, oxoid) and subjected to biochemical identification.

Isolation of *Salmonella* species

Preparation of meat samples and detection of *Salmonella* were done according to techniques recommended by the International Organization for Standardization.¹¹ Briefly 0.1ml of pre-enriched cultured was transferred to 9.9ml Rappaport Vassilidis (RV) broth (CM0669, Oxoid) and incubated at 41°C for 18 to 24hrs, loop full from the selective enrichment broth was inoculated onto Xylose Lysine Deoxycholate (XLD) (CM0469, Oxoid) agar and incubated at 37°C for (18 to 24)hrs. *The suspected isolates were stored on nutrient agar (CM0309, Oxoid) slant and kept at 4°C for further identification with the aid of Gram's staining and other biochemical tests.*

Isolation of *Escherichia Coli*

0.1ml from the pre-incubated samples in BPW was streaked onto MacConkey agar (CM0007, Oxoid) plates and incubated at 37°C for 24h. Following incubation, lactose-positive colonies (3-5) were streaked onto Eosin-methylene blue (CM0069B, Oxoid) agar plates. Typical *E. Coli* colonies on eosin-methylene blue agar (green and shiny or with dark or purple centers) were sub cultured in nutrient agar slant and incubated for 24h at 37°C then kept at 4°C for further study.

For human samples, hand swabs and one gram of the stool specimens were directly inserted into sterile 9ml BPW tubes under aseptic condition and incubated at 37°C for 18hrs, then subjected to the same laboratory diagnostic procedures as done for the meat samples.¹² Pure cultures of the obtained microorganisms were identified using culture characteristic on selective media, gram-staining and biochemical reactions, according to Bergeys Manual of systematic Bacteriology.¹³ Biochemically tested *Salmonella* isolates and 48 randomly selected *E. Coli* isolates 12 from each of the examined samples were serologically identified at the Center of Food Analysis, Faculty of Veterinary Medicine, Benha University, Egypt.

Determination of antimicrobial susceptibility

Following the identification of different colonies, the confirmed isolates were spread on Mueller-Hinton Agar (oxoid) and the antibiotic discs were placed over the plate and incubated at 37°C for 18-24h

according to Clinical and Laboratory Standard Institute.¹⁴ The criterion for the antibiotic chosen was based on their use in both food production and human therapy. The antibiotics used in this study were Ampicillin (10µg), Nalidixic acid (30µg); Cefoxitin (Ce30µg); Chloramphenicol (30µg); Kanamycin (30µg), Gentamicin (10µg); Ciprofloxacin 5µg; Tetracycline (30µg) and Sulphamethoxazole/Trimethoprim (1.25/23.75µg) (Oxoid UK). The clear zone around each antibiotic disc was measured in millimeter. Strains were evaluated as susceptible, intermediate or resistant.

Results and discussion

In the present study, we evaluated the presence of the most common zoonotic pathogens in retail raw chicken carcasses sold in Mansoura city. Our results showed that 22% out of the examined raw chicken meat samples were contaminated with *S. Aureus* (Table 1). Our findings were in harmony with that previously reported by other researchers.¹⁵⁻¹⁸ In contrast, higher level of *S. aureus* contamination to chicken meat was previously obtained by several researchers¹⁹⁻²³ at the following levels: 46.15 %, 43.3%, 52.04 %, 47.2 % and 92% respectively. On the other side, lower contamination rate was previously reported from Egypt by Osman et al.,²⁴ who detected *S. Aureus* with the percentage of 15% from the examined chicken meat. According to the Centers for Disease Control and Prevention (CDC) this organism is mainly originate from handlers whereas up to 25% of healthy people carry *S. Aureus* on their skin or in their nostrils. High contamination of the examined poultry meat with *S. Aureus* is considered as a reliable indicator for improper personal hygiene of the employees during handling and processing, inadequate sanitation and lack temperature control.

E. Coli was the most prevalent isolates in this study. It was recovered at a percentage of 35% of the examined chicken meat (Table 1). Nearly similar findings were previously reported by other researchers,^{1,25} Worldwide, many reports have documented the isolation of *E. Coli* from chicken meat using conventional methods.^{26,27} In this regard, various prevalence percentages were observed elsewhere: in Morocco 48.4%²⁸ and 98% in India.²⁹ Much higher prevalence rate 100% was also previously reported.^{18,30,31} The presence of *E. Coli* in the examined raw meat is a good indicator of inadequate slaughtering process and could be related to intestinal leakage during the evisceration process because *E. Coli* considered as a normal inhabitant of the intestinal tract of the live bird. The high prevalence of *E. Coli* in the examined meat could be a reason for inferior quality resulting in economic losses and a significant public health hazard.³²

In this study, *E. Coli* isolates from the raw meat were serotyped into five different serogroups (O26: H11, O103: H2, O128:H2, O111:H2 and O78) (Table 3). High number of Shiga toxin-producing *E. Coli* e. g: (O26: H11, O111: H2, O103: H2, O128 and O145:H28) have been associated with food borne illnesses and their importance is increasing worldwide.³³

Despite global improvements in public health facilities, *Salmonella* species remains the main cause of food borne bacterial illness in both developed and developing countries and could be a public health concern worldwide.³⁴ In the present study, only five percentages of the examined samples were found to be contaminated with *Salmonella* spp., this finding was in contrast with previous report worldwide. Chicken meat was extensively contaminated with *Salmonella* spp. at 26.3% in UK,³⁵ 36% in Belgium,³⁶ 39% in the US,¹ 36% in Spain,³⁷ 25% in England,³⁸ 60% in Portugal,³⁹ 56% in Egypt⁴⁰ and 22.6% in

Egypt.¹⁸ Interestingly, some researchers failed to detect Salmonella from 30 chicken quarters collected from different localities in Assiut city, Egypt.⁴¹ The reason behind the different contamination rates could be attributed to the methods of collecting sampling and the procedures of bacterial isolation and identification which can affect the detected prevalence of Salmonella spp.

Serotyping of the obtained Salmonella spp revealed that Salmonella Kentucky was the major isolates obtained 5 out of 10 isolates (50%), followed by Salmonella Enteritidis (20%), Salmonella

Infantis (20%) and Salmonella Typhimurium (10%) (Table 2). Several authors identified S. Kentucky from the commercial broilers production facilities.⁴²⁻⁴⁴ S. Enteritides were previously isolated from broiler chicken and chicken meat from Egypt.⁴⁵ Chicken and its products played an important role in salmonella contamination and considered to be the main cause of salmonella infection that causes enteritis in human beings. Moreover, the most important source for Salmonella infection in human is handling poultry carcasses as well as consumption undercooked poultry meat.⁴⁶

Table 1 Prevalence of *Staph aureus*, *Salmonella* spp. and *E. coli* from the examined samples

Isolated microorganisms	Raw chicken meat (n=200)		Stool specimens from meat seller (n=50)		Hand swabs from meat seller (n=50)	
	N	%	N	%	N	%
<i>E.coli</i>	70	35	20	40	12	24
<i>Salmonella Sp.</i>	10	5	4	8	1	2
<i>Staph aureus</i>	44	22	15	30	20	40

Table 2 Serotyping of *Salmonella Sp.* from the examined samples

Examined samples	Number of <i>Salmonella</i> isolates	<i>Salmonella</i> serotype
Raw chicken meat (n=200)	10(5%)	5 (50%) <i>S. Kentucky</i>
		2 (20%) <i>S. Infantis</i>
		2 (20%) <i>S. Enteritidis</i>
		1 (10%) <i>S. Typhimurium</i>
Stool specimens from meat seller (n=50)	4(8%)	3 (75%) <i>S. Enteritidis</i>
		1 (25%) <i>S. Typhimurium</i>
Hand swabs (n=50)	1 (2%)	<i>S. Kentucky</i>

Table 3 Serogrouping of the isolated *E. Coli*

Examined samples	Sero grouping	Strain characteristic
Raw chicken meat (n=12)	5 (41.7%) O26:H11	EHEC
	3 (25%) O103:H2	EHEC
	2 (16.7%) O128:H2	EPEC
	1 (8.3%) O111:H2	EPEC
	1(8.3%) O78	ETEC
Stool specimens from meat handlers (n=12)	4 (33.3%) O26:H11	EHEC
	7 (58.3%) O2:H4	Cause bacteremia
	1 (8.3%) O128:H2	ETEC
Hand swabs (n=12)	2 (16.7%) O26:H11	EHEC
	6 (50%) O103:H2	EHEC
	4 (33.3%) O125:H21	EPEC

Various microorganisms already present on the skin, feathers or in the alimentary tract of the live bird, in the traditional poultry retailers after slaughtering poultry carcasses, usually scalded in scaling tank

which might be serve as an enrichment media from which pathogens are spread widely to all birds entering the tank. Therefore, microbial contamination can occur at any stage of the production chain, from feather plucking, evisceration, and washing. As well as cross contamination either from other birds, instruments, machines and the operators⁷.

There are limited data regarding the contamination rates of workers who handle the raw meat. In that regard, high frequency of S. Aureus was found to colonize the skins (40%) and the stool specimens (30%) of meat handlers. Nearly similar finding was reported by Jordá et al.,⁴⁷ who identified S. Aureus from 37.5 % of food handlers in Argentina. Higher prevalence of coagulase-positive Staphylococci (50%) was detected in food handlers in Brazil, 28.6% of the isolates were methicillin-resistant S. Aureus (MRSA).⁴⁸ Meanwhile, Awadallah⁴⁹ detected S. Aureus from 20% among the examined hand swabs of meat handlers in Egypt. High prevalence of S. Aureus among meat handlers impose a potential hazard to consumers specially in case of “toxin-mediated virulence, invasiveness, and antibiotic resistance.”

Four stool samples out of 50 representing 8% plus one hand swabs from meat handlers out of 50 (2%) were found to be contaminated with Salmonella spp. These findings were in agreement with that obtained by Abd-Allah⁵⁰ who isolated Salmonella spp. at the rate of 3.1% from hand swabs. Higher isolation rate (8.88%) was previously mentioned by Ibrahim.⁴⁴ It is worthy to mention that eight percentage of the examined humans stool samples from the apparently healthy meat handlers were contaminated with S. Enteritidis and S. Typhimurium. In the present study, Salmonella serovars S. Kentucky, S. Enteritidis, S. Infantis, and S. Typhimurium were isolated from chicken raw meat, S. Enteritidis and S. Typhimurium were isolated from the stool samples of meat handlers and S. Kentucky was isolated from hand swabs of the examined individuals, this provided evidence that direct contact with raw chicken meat might pose great health hazards to humans especially whom their occupation necessitate their contact with poultry and its raw products, because Salmonella are usually transmitted to humans by the fecal-oral route.

E.Coli was detected in 40% of the stool samples of chicken meat handlers and were serologically identified as O26:H11 (4 strains, 33.3%), O2:H4 (7 strains, 58.3%) and O128:H2 (1 strain, 8.3%). In

comparison to our study higher prevalence rate 51.5% was isolated by Behiry et al.,⁵¹ from diarrheic children in Egypt. However, lower prevalence (6% and 20%) was previously recorded by Awadallah et al.,⁴⁹ Bodhidatta et al.⁵²

E.Coli was also detected in 24% of the hand swabs and were serotyped as O26: H11 (2 strains, 16.7%), O103:H2 (6 strains, 50%) and O125:H21 (4 strains 33.3%) (Table 3). Different isolation rates were previously recorded from hand swabs of food handlers in Egypt: 7.5, 32 and 15% by Awadallah et al.,⁴⁹ Samaha et al.,⁵³ Mohamed et al.⁵⁴ respectively. In general, the differences in isolation rates from study to another might be attributed to the number of collected samples, health and hygienic status in addition to the type of handled food.

E.Coli O26: H11 and E.Coli O103:H2 were isolated from (raw chicken meat, stool and hand swabs) and (raw poultry meat and hand swabs), respectively, and were categorized as EHEC. Meanwhile, O128: H2 was isolated from raw poultry meat and stool specimens of handles and categorized as EIEC. Enterohemorrhagic E. Coli (EHEC)

is a well-known cause of severe disease, such as hemorrhagic colitis and hemolytic-uremic syndrome (HUS).⁵⁵ E.Coli O26 is the most frequently isolated non-O157 Shiga-toxicogenic E. Coli (STEC) associated with human clinical illness³³ and E. Coli O26:H11 is the clinically most important and epidemiologically most predominant EPEC and EHEC O26 serotype.⁵⁶ Therefore, the presence of different E.Coli serotypes among the examined samples represents great public health risk. Our findings highlighted the cross contamination from the raw meat to the handlers and confirmed the transmission of pathogenic microorganisms from the contaminated carcasses and their handlers to the consumers. The behavior of antimicrobial sensitivity tests of the obtained isolates showed that the percentages of antibiotic resistance were quite common among E. Coli isolates (n=102). Most of the isolates were resistance to Cefoxitin, Tetracycline and Ampicilline, while different percentages of the resistance to the other used antibiotics were recorded in Table 4. Several reports have shown that enteric bacteria develop resistance to the common antibiotics used in human and veterinary medicine such as Tetracycline, Gentamycin, Kanamycin, and Streptomycin.⁵⁷

Table 4 prevalence of drug resistance food borne pathogens from the examined sample

Antibiotic used (µg)	Resistance % of the isolated pathogens from the examined samples								
	E. Coli			Salmonella spp.			S. Aureus		
	Raw meat n= 70	Stool n=20	Hand swab n=12	Raw meat n= 10	stool n= 4	Hand swab n=1	Raw meat n= 44	Stool n=15	Hand swab n=20
Ampicilline (10µg)	100	89	90	100	85	100	45	50	49
Nalidixic acid (30µg)	45	40	50	30	25	0	100	95	93
Cefoxitin (30µg)	100	99	98	97	96	100	25	30	20
Chloramphenicol (30µg)	18	20	17	20	22	0	35	33	30
Kanamycine (30µg)	50	49	53	66	59	100	20	19	23
Gentamycin (10µg)	45	48	44	25	20	0	15	13	19
Ciprofloxacin (5µg)	20	18	13	30	31	0	22	18	14
Tetracycline (30µg)	100	99	97	100	94	100	100	100	100
Sulphamethoxazole/ Trimethoprim (1.25/23.75µg)	55	60	56	35	38	0	30	31	28

High percentages of resistant were recorded out of all the recovered Salmonella isolates to Tetracyclin, cefoxitin, Ampicillin and Kanamycine (Table 4). In general, various researchers in many countries showed that Salmonella isolates in retail meats was commonly resistant to Tetracycline, Ampecilline, Sulfonamides, and Streptomycin.⁵⁸⁻⁶⁰ As previously reported by Helmuth,⁶¹ the intensive use of antibiotics arouse the prevalence of resistant salmonella strains between 60% and 90% and these bacterial strains are of considerable as a potential clinical importance to human health.

All Staph. aureus isolates were found to be resistance to Tetracycline. High resistance against Nalidixic acid was also found; while various degree of resistance was presented to the examined antibiotics (Table 4). Resistance to Tetracycline was similar to that obtained by Otalu OJ et al.⁶² Nearly similar resistance to Chloramphenicol was recorded by Yurdakul et al.⁶³ On the contrary, Osman et al.²⁴ showed 100% of S. Aureus was resistance against sulfamethoxazole/trimethoprim. S. Aureus has been reported frequently to show multiple antimicrobial

resistance patterns.^{64,65} Our finding revealed that 25% of the isolates were resistance to Cefoxitin and was considered as MRSA²³ identified 44% of the isolated S. Aureus from whole chicken carcass were contaminated with MRSA. MRSA infected birds considered as the main source of MRSA in their meat, consequently cross-contamination may occur to the handlers and their tools which serve as vehicles for further transmission.⁶⁶

Antibiotics have been extensively used in poultry for therapy or as growth promotion, continuous use of antibiotics in poultry feed disrupt the gut flora and thought to be the major cause of drug resistance in food borne pathogens.⁶² Raw chicken meat is usually consumed in Egypt, therefore, the presence of antibiotic resistant strains in chicken meat are considered as an alarming risk factor in the food chain and leading to continuous circulation of resistant strains of the bacteria in the environment and the possible contamination of water and food.

In developing countries involving Egypt, antimicrobial drugs are usually available to consumers with or without prescription from a

medical practitioner this lack of stringent controls on antimicrobial usage in human health and particularly in animal production systems led to misuse of antimicrobial drugs and increases the risk of food-borne microbes harboring an array of resistance genes. In our study multi resistance were observed in most isolates from meat handlers, there may be posse's great health problems especially if these multi-resistance determinants can be transferred to new bacterial hosts. The development of antimicrobial resistance in zoonotic bacteria (e.g. Salmonella, E.Coli and S. Aureus) constitutes a public health risk, as it may potentially affect the efficacy of drug treatment in humans.

Conclusion

The results herein alarming that there is ongoing lack of the a adequate hygienic measures, poor meat handling and bad sales practices in the retail shops which could results in obtaining inferior quality meat and a potential public health hazard. The risk factors for human infection with Salmonella spp., E.Coli, and S. Aureus can occur not only by the consumption of contaminated meat, but also from the handling of contaminated raw meat. Antibiotics should be used with great caution due to the emergence of antibiotic resistance pathogens which provide a significant potential public health hazard. Therefore, surveillance programs concerning the prevalence of potential contaminant pathogens in different kinds of meat are crucial to safeguard the public health. Education of the meat retailers' about the importance of hygienic and sanitary measurements provides wholesome and safe meat to the consumers.

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Conflict of interest

Author declares that there is no conflict of interest.

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