Prevalence and Characterization of Antibiotic Resistance Food Borne Pathogens Isolated from Locally Produced Chicken Raw Meat and their Handlers

Abstract

The purpose of this study was to determine the prevalence and the potential public health significance of Salmonella (S.) serovars, Escherichia coli (E. coli), and Staphylococcus aureus (Staph. 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 Staph. aureus were found to colonize the skin (40%) and the stool specimens (30%) of chicken meat handlers; whereas four out of 50 stool specimens (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 specimens and in 24% of handlers hand swabs. Serological identification of E. coli isolates revealed the presence of different serotypes in the examined samples. Briefly, E. coli serotypes O26: H11, O103:H2, O128:H2, O111:H2 and O78 were found in raw meat; E. coli serotypes O26: H11, O2: H4 and O128:H2 and O26: H11, O103:H2 were detected in the tested stool specimens; while E. coli serotype O125:H21 was only detected in the examined 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 are considered a potential vehicle for transmitting food-borne diseases. Hence implementation of consumer food safety education efforts is urgently needed.

Keywords: Chicken meat; E.coli; Staph. aureus; Salmonella spp; Antibiotic resistance

Introduction

There has been growing awareness of the major public health impact of antibiotic 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 were the main etiological agents of illness and deaths in developing countries [3]. In the United States, food-borne illness causes 37.2 million cases per year resulting in 2.612 deaths [4]. Salmonella (S.), Escherichia coli (E.coli) and Staphylococcus aureus (Staph. 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 continuous 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]. Widespread of antibiotic-resistant food-borne pathogens threaten the successful treatment of infectious diseases. The presences of multidrug resistance (MDR) pathogens in poultry meat warrant concern because they are responsible for more serious disease than susceptible bacteria. In Egypt, the vast majority of the population 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 purpose of this study was to determine the prevalence of food-borne pathogens with special reference to Salmonella spp., E. coli, and Staph. aureus in raw chicken meat available in open markets at Mansoura city as well as their handlers and to evaluate the antimicrobial resistance and sensitivity pattern of the obtained isolates.

Materials and Methods

Samples collection

A total of 200 raw chicken meat samples were randomly purchased from various local open markets at 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 collected. Stool Specimens were also collected in clean, dry and sterile containers. 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 Staph. 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 225 ml of Buffered Peptone Water (BPW) and mixed for 10 min at 120 r/min, the mixture sample were incubated at 37°C for 16-18 h [9,10].

Isolation of Staphylococcus aureus

0.1ml from the pre-incubated samples in BPW were spread on the surface of Baird-Parker agar based medium (CM00275, Oxoid) supplemented with Egg Yolk Tellurite Emulsion (SR0054, Oxoid) then incubated at 37°C for 24-48 h. Five presumptive black colonies surrounded by opaque halo were picked from each selective agar plate. These colonies were purified using Tryptone soy 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 9.9 ml Rappaport Vassiliadis (RV) broth (CM0069, Oxoid) and incubated at 41°C for 18 to 24 h, 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 h. 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 24 h. 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 24 h at 37°C then kept at 4°C for further study.

Hand swabs and the stool specimens were directly inserted into sterile 9 ml BPW tubes under aseptic condition and incubated at 37°C for 18 h, 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 Bergey's 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 (10 μg), Nalidixic acid (30 μg); Cefoxitin (Ce30μ); Chloramphenicol (30 μg); Kanamycin (30 μg). Gentamicin (10 μg); Ciprofloxacin 5 μg; Tetracycline (30 μg) and Sulphamethoxazole/Trimethoprim (1.25/23.75 μg) (Oxoid). The clear zone around each antibiotic disc was measured in millimeter.

Results and Discussion

In the present study, we evaluated the presence of the most common zoonotic pathogens in retail raw chicken carcasses sold at Mansoura city. Our results showed that 22% out of the examined raw chicken meat samples were contaminated with Staph. aureus (Table 1). Our findings were in harmony with that previously reported by other researchers [15-18]. In contrast, higher level of Staph. aureus contamination to chicken meat was previously obtained by several researchers [19-23] 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 Staph. 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 originated from handlers whereas up to 25% of healthy people carry Staph. aureus on their skin or in their nostrils. High contamination of the examined poultry meat with Staph. 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 from 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 that regard, various prevalence rates were observed elsewhere: in Morocco 48.4% [28] and 98% in India [29]. Much higher prevalence rate 100% was also previously reported by several investigators [18,30,31]. The presence of E. coli in the examined raw meat is found to be 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 in ceasing 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 Egypt [40] 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 *Salmonella Kentucky* from the commercial broilers production facilities [42-44]. *Salmonella Enteritidis* were previously isolated from broiler chicken and chicken meat from Egypt [45]. Chicken and its products have 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 of undercooked poultry meat [46].

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 scaled 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 operators.

There are limited data regarding the contamination rates of these workers who handle the raw meat. In this regard, high frequency of *Staph. aureus* was found to colonize the skins (40%) and the stool specimens (30%) of meat handlers. Nearly similar finding was reported by Jordà et al. [47] who identified Staph. 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 *Staph. aureus* (MRSA) [48]. Meanwhile, Awadallah et al. [49] detected *Staph. aureus* from 20% among the examined hand swabs of meat handlers in Egypt. High prevalence of *Staph. 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-Allah [50] who isolated *Salmonella* spp. at the rate of 3.1% from hand swabs. Higher isolation rate (0.88%) was previously mentioned by Ibrahim et al. [44]. It is worthy to mention that eight percentage of the examined humans stool samples from the apparently healthy meat handlers were contaminated with *Salmonella* Enteritidis and *Salmonella Typhimurium*. In the present study, *Salmonella* serovars, *Salmonella Kentucky*, *Salmonella Enteritidis*, *Salmonella Infantis*, and *Salmonella Typhimurium* were isolated from chicken raw meat, *Salmonella* Enteritidis and *Salmonella Typhimurium* were isolated from the stool specimens of meat handlers and *Salmonella Kentucky* was isolated from hand swabs of the examined individuals, this provide 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 was 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

| Isolated Microorganisms | Raw Chicken Meat (n = 200) | Stool Specimens from Meat Handler (n = 50) | Hand Swabs from Meat Handler (n = 50) |
|-------------------------|---------------------------|------------------------------------------|--------------------------------------|
|                         | N  | %  | N  | %  | N  | %  |
| *E.coli*                | 70 | 35 | 20 | 40 | 12 | 24 |
| *Salmonella* Spp.       | 10 | 5  | 4  | 8  | 1  | 2  |
| *Staph. aureus*         | 44 | 22 | 15 | 30 | 20 | 40 |
food handlers in Egypt: 15, 7.5, 32 % 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 Enterohemorrhagic *E. coli* (EHEC). Meanwhile, O128: H2 was isolated from raw poultry meat and stool specimens of handlers and categorized as Enteroinvasive (EIEC). 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-toxigenic *E. coli* (STEC) associated with human clinical illness [33] and *E. coli* O26:H11 is the clinically most important and epidemiologically most predominant Enteropathogenic (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 highlight 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 2: Serotyping of *Salmonella* Spp. from the examined samples.

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

### Table 3: Serogrouping of the isolated *E.coli*.

| Examined Samples                      | Serogrouping | Strain Characteristic |
|---------------------------------------|--------------|-----------------------|
| Raw chicken meat (n = 12)             | 5 (41.7%) O26: H1 1 3 (25%) O103:H2 2 (16.7%) O128:H2 1 (8.3%) O111:H2 1(8.3%) O78 | EHEC EHEC EPEC EPEC ETEC |
| Stool specimens from meat handlers (n = 12) | 4 (33.3%) O26: H1 1 7 (58.9%) O2:H4 1 (8.3%) O128:H2 | EHEC Cause bacteremia ETEC |
| Hand swabs (n = 12)                   | 2 (16.7%) O26: H1 1 6 (50%) O103:H2 4 (33.3%) O125:H21 | EHEC EHEC EPEC |

High percentages of resistant were recorded out of all the recovered *Salmonella* isolates to Tetracyclin, Cefoxitin, Ampicilline and Kanamycine (Table 4). In general, various researchers in many countries showed that *Salmonella* isolates in retail meats was commonly resistant to Tetracycline, Ampicilline, 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.
Table 4: prevalence of drug resistance food borne pathogens from the examined samples.

| Antibiotic used (µg) | 
|----------------------|-----------------|-----------------|-----------------|-----------------|
|                      | **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 |
| Ampicillin (10 µg)   | 100             | 89              | 90             | 100             | 85              | 100             | 45              | 50              | 49             |
| Nalidixic acid (30 µg) | 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             |
| Kanamycin (30 µg)    | 50              | 49              | 53             | 66              | 59              | 100             | 20              | 19              | 23             |
| Gentamycin (10 µg)   | 45              | 48              | 44             | 25              | 20              | 0               | 15              | 13              | 19             |
| Ciprofloxacin (5 µg) | 20              | 18              | 13             | 30              | 31              | 0               | 22              | 18              | 14             |
| Tetracycline (30 µg) | 100             | 99              | 97             | 100             | 94              | 100             | 100             | 100             | 100            |
| Sulphamethoxazole/Trimethoprim (1.25/23.75 µg) | 55            | 60              | 56             | 35              | 38              | 0               | 30              | 31              | 28             |

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 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 *Staph. aureus* was resistance against Sulfamethoxazole/Trimethoprim. *Staph. 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 MRSA. Girtschaft and Duman identified 44% of the isolated *Staph. aureus* from whole chicken carcass were contaminated with MRSA [23]. 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 was observed in most isolates from meat handlers, these may pose 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 *Staph. aureus*) constitutes a public health risk, as it could potentially affect the efficacy of drug treatment in humans.

**Conclusion**

The results herein alarming an ongoing lack of the adequate hygienic measures, poor meat handling and bad sales practices in the retail shops thereby obtaining inferior quality meat and a potential public health hazard. The risk factors for human infection with *Salmonella spp.*, *E.coli*, and *Staph. 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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