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Hygienic status of meat served at hospitals and its improvement after HACCP implementation

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Abstract

Examination of the microbial quality of meat served to patients at hospitals received little attention. Therefore, this study investigated the microbial status of meat served at hospitals in Zagazig city, Egypt. Furthermore, the effects of the implementation of hazard analysis and critical control point (HACCP) on the microbial status of meat were examined. Microbiological examination in this study included general microbial indicators (total aerobic plate count and most probable number of coliforms), isolation and identification of specific food-poisoning microorganisms including *Escherichia coli*, *Salmonella spp.*, and *Staphylococcus aureus*. Due to the lack of the available information about the virulence of the isolated pathogens and their multidrug resistance profile in Egypt, multiplex PCR was used to detect the virulence-associated genes of *Escherichia coli* including shiga toxin, shiga toxin 2 and intimin in addition to invasive and hyper-invasive locus genes of *Salmonella spp*. Furthermore, *Staphylococcus aureus* enterotoxin (SE) coding-genes including SEA, SEB, SEC and SED were also investigated. Finally, antibiograms of the isolated food poisoning organisms were tested. The achieved results revealed inadequate hygienic measures performed at hospital kitchens, in terms of the high microbial load of meat either raw or cooked. Such meat was subjected to contamination by different types of microorganisms. The isolated strains showed variable degrees of virulence and multidrug resistance for the commonly used antibiotics in Egypt, which may therefore cause sever adverse outcomes to patients and stuff if such contaminated meat is served. Implementation of HACCP parameters significantly improved the microbiological quality of meat.

Key Words: Antibiograms, Hygienic status, Food poisoning, HACCP, Hospitals, Meat microbiology

Introduction

Foodborne illnesses become of central significance especially in hospitals worldwide. Improper handling of meals served at hospitals results in their contamination by many biological hazards that naturally found everywhere in the environment. In hospitals, foodborne outbreaks are facilitated by many factors including; bad hygienic measures inside the kitchen, food handling carriers, carelessness and untrained food handlers[40)]. Inside the kitchen, meat can be contaminated by different species of bacteria from the contaminated raw materials, equipment, meat

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contact surfaces and inadequate personal hygiene. Furthermore, patients especially children are more susceptible to food poisoning because of their weak immune system and their high risk of exposure to diseases through cross infection.

In developing countries like Egypt, foodborne diseases occur commonly because of inadequate food safety laws, weak regulatory systems, lack of financial resources and lack of food-handlers education[39]. However, there is lack of information about the microbiological quality of foods especially, that of animal origin, which served at hospitals in Egypt.

Escherichia coli and *Salmonella spp.* are the leading causes of foodborne infection. In addition, *Staphylococcus aureus* enterotoxigenic strains are responsible for foodborne intoxication due to the production of heat-stable enterotoxins. Nevertheless, there is a clear shortage about the available information about the prevalence of such organisms in meat served at hospitals in Egypt.

It is believed that more hygienic measures that focus on kitchen utensils, meat contact surfaces, and hand washing to reduce the contamination of food, water and kitchen environment must be followed in hospitals[18]. Thus, implementation of the microbiological guidelines including hazard analysis and critical control points (HACCP) and good manufacturing practices, recommended by WHO and FDA may help to prevent microbial contamination[17]. HACCP system is approved internationally as a preventive measure for food safety and it is the way of identification of different hazards that affect meat quality and provides corrections to these hazards through the application of the seven HACCP principals at any stage of the food supply chain. HACCP is a food safety management system based on reduction to an acceptable level or elimination of hazards in the food in hospital catering[27], it requires enumeration and identification of critical steps to serve safe food in addition to identification and evaluation of safety measures[5]. However, there is no published reports about the effects of the implementation of HACCP principles in reduction of the microbial load in meat served at hospitals in Egypt. Therefore, this study was conducted to evaluate the microbiological quality of meat (beef and chicken) served at hospitals in Egypt. Additionally, the prevalence of foodborne organisms including *E. coli, Salmonella spp.,* and *Staphylococcus aureus* was investigated. Furthermore, the expressions of virulence attributes in the isolated organisms were investigated using PCR. We further investigated the antibiogram of the isolated organisms. Finally, the effects of the implementation of HACCP principles on the microbial quality of meat served at hospitals were examined.

Materials and methods

This study was conducted according to the guidelines of Zagazig University, Egypt during the period of February to December 2017. These guidelines include approval of the research project form the section of the scientific research and postgraduate affairs in the university prior to the project's startup. Additionally, to follow the ethics of animal use in the experiments.

This study was conducted in five hospitals' kitchens in Zagazig city, Egypt in two phases. At each phase, the number of the collected samples was set to be 100. The first phase included evaluation of the general hygienic conditions inside the kitchens and meat distribution rooms through visual observations and collection of different samples of beef and chicken meat for microbiological examination. The second phase was conducted by implementation of HACCP principles in the same hospitals' kitchens followed by microbiological examination to a newly collected meat samples to evaluate the efficiency of the implemented points.

Collection of samples: One hundred random and equal samples (n = 25) from each of raw beef,

raw chicken, cooked beef and cooked chicken meat were collected equally from five different hospital kitchens in Zagazig city, Egypt. Samples were rapidly transferred in a cooled condition (4°C) to Food Control Laboratory, Faculty of Veterinary medicine, Zagazig University, Egypt for bacterial isolation and identification.

Preparation of samples, enumeration and isolation procedures: Twenty-five grams of each sample were aseptically homogenized in 225 mL of 1% sterile peptone water (Oxoid CM9) to make a dilution of 10^{-1} then were allowed to stand for 5 minutes, then 1 mL was transferred aseptically to a test tube containing 9 mL sterile 0.1% buffered peptone to prepare tenfold decimal serial dilution up to 10^{-7} dilution[4].

For aerobic plate count, one mL of each dilution was pipetted into separate duplicate petri dishes, and then 12–15 mL of nutrient agar (CM003, Oxoid, England) were added and mixed by alternate rotation. After solidification, dishes were incubated at 37°C for 24 h. All colony-forming units (pinpoint size) were counted[16].

For *Staphylococcus aureus (S. aureus)*, isolation was done on Baird Parker agar (Biolife, Italy) supplemented with egg yolk-tellurite emulsion (Himedia, India). After incubation at 37°C for 48 h, up to five typical colonies (black, shiny, convex, 1–1.5 mm in diameter, and surrounded by a clear halo zone) and/or atypical colonies (black with no zones) presumptive colonies were subcultured on blood agar plates (Difco Laboratories, Detroit, MI) and incubated for 24 h at 37°C[17]. Gram's stain, mannitol fermentation, catalase, coagulase and DNAs tests were performed on suspected colonies for identification of *S. aureus*[32].

For *Salmonella spp.*, pre-enrichment in buffered peptone water 1% at 37°C for 24 h then 1 ml of pre-enriched peptone water was enriched in Rappaport Vassiliadis broth with soya broth at 41.5°C. A loopful was streaked on XLD agar, incubated at 37°C for 24 h and red colonies with black center were enumerated[20]. The obtained purified isolates were identified biochemically using Oxidase test, hydrolysis of urea, H_2S production and Utilization of citrate. Serotyping was performed according to Kauffman White scheme with commercial antisera (Difco Laboratories Deteroeit, Mitchigeu, USA) for cell wall (O) and Flagellar (H) antigen identification[24].

For the most probable number (MPN) of coliforms and *E. coli*; one mL of each dilution was inoculated separately into 3 MacConkey broth tubes with inverted Durham's tubes. Then, tubes were incubated at 37°C and examined after 24 and 48 h. Positive tubes showing acid and gas productions in inverted Durham's tubes were recorded as MPN of coliforms. Then a loopful from positive tubes was inoculated into 7 ml *E. coli* broth, incubated at 44.5°C for 24 to 48 h. Positive tubes, showing gas production, were used to calculate MPN of *E. coli*. A loopful from each positive tube was streaked onto Levine's eosin-methylene blue agar (Difco). Then, incubated at 37°C for 24 h. Typical colonies of *E. coli* (greenish metallic with dark purple center) were transferred to nutrient agar slants and incubated at 37°C for 24 h and then stored at 4°C for further identification based on staining and biochemical tests (catalase, oxidase, indol, methyl red, Voges-Proskauer test, citrate utilization, nitrate reduction, urease, H_2S production, gelatin liquefaction and Eijkman test)[3]. Finally, serological identification was done by using rapid diagnostic *E. coli* antisera sets (Difco) for diagnosis of the enteropathogenic types[26].

Genomic DNA extraction and PCR analysis: Genomic DNA extraction was done using QIA amp kit according to the manufacturer's instructions. Primer sequences for identification of Shiga toxins (*stx1 and stx2*) and intimin (*eaeA*) genes of *E. coli*; invasive (*invA*) and hyper-invasive locus (*hilA*) genes of *Salmonella spp., and S. aureus* enterotoxin coding genes (SE A, B, C, and D) were described in Table 1. PCR assays were carried out using the methods described before[35,37]. The formed PCR products were electrophoresed in 2%

Table 1. Sequences and specificities of primers used in the present study

Strains	Target gene	Oligonucleotide sequence (5´ → 3´)	Product size (bp)
E. coli	*stx1*	F-5´ ACACTGGATGATCTCAGTGG ´3	614[11]
		R-5´ CTGAATCCCCCTCCATTATG ´3	
	stx2	F-5´ CCATGACAACGGACAGCAGTT ´3	779[11]
		R-5´ CCTGTCAACTGAGCAGCACTTTG ´3	
	eaeA	F-5´ GTGGCGAATACTGGCGAGACT ´3	890[29]
		R-5´ CCCCATTCTTTTTCACCGTCG ´3	
Salmonella spp.	*invA*	F-5´ GTGAAATTATCGCCACGTTCGGGCA´3	284[36]
		R-5´ TCATCGCACCGTCAAAGGAACC ´3	
	hilA	F-5´ CTGCCGCAGTGTTAAGGATA ´3	497[19]
		R-5´ CTGTCGCCTTAATCGCATGT ´3	
S. aureus	*SEA*	F-5´ TTGGAAACGGTTAAAACGAA´3	120[33]
		R-5´ GAACCTTCCCATCAAAAACA ´3	
	SEB	F-5´ TCGCATCAAACTGACAAACG ´3	478[33]
		R-5´ GCGGTACTCTATAAGTGCC ´3	
	SEC	F-5´ GACATAAAAGCTAGGAATTT ´3	257[33]
		R-5´ AAATCGGATTAACATTATCC ´3	
	SED	F-5´ CTAGTTTGGTAATATCTCCT ´3	317[33]
		R-5´ TAATGCTATATCTTATAGGG ´3	

agarose gel and stained with ethidium bromide[14].

Antibiogram: Antibiotic sensitivity testing of *E. coli*, *Salmonella spp.,* and *S. aureus* was performed by single diffusion assay using 15 commercially prepared antibiotic discs (6 mm) with variable concentrations[30] including amoxicillin-clavulanic acid (AMC) (30 μg), amoxicillin (AML) (10 μg), cefpodoxime (CPD) (10 μg), ampicillin (AMP) (10 μg), chloramphenicol (CL) (30 μg), ciprofloxacin (CIP) (5 μg), erythromycin (E) (15 μg), gentamicin (G) (10 μg), flumequine (UB) (30 μg), cefotaxime (CTX) (30 μg), cefardine (CE) (30 μg), enrofloxacine (EN) (5 μg), sulphamethoxazol-trimethoprim (SXT) (25 μg), streptomycin (S) (10 μg) and penicillin (P) (10 IU).

Multiple drug resistance index (MDR) = resistance isolates/tested antibiotics.

Implementation of HACCP inside the kitchen and food distribution rooms: HACCP systems were implemented in the same kitchen and meat distribution rooms according to the seven principles[21]: conduct a hazard analysis, determine critical control points (CCPs), establish critical limits, establish monitoring procedures, establish corrective action, establish verification procedures, and establish record-keeping and documentation[15]. The critical control points for the examined samples were evaluated and monitored then corrected according to the following steps:

Training of food handlers on the hygienic practices during food preparation

High standards of personal hygiene were maintained by; clean clothes, cutting of nails, no jewelers, and hand washing before and after meat handling, gloves were worn immediately after hand washing with a periodical change, hair covers were worn, smoking was prohibited and food handlers with respiratory and skin diseases were not allowed to prepare meat.

Cleaning process inside the kitchen and distribution rooms

Cleaning with water only is not enough to remove microorganisms while washing using detergent, hot water, and mechanical scrubbing

are effective to decrease cross-contamination. Cleaning to whole kitchen, meat storage rooms, meat distribution rooms and all meat contact surfaces (hands, cutting tables, cutting boards, knives, utensils and transportation vehicles), all obvious materials were removed then flushed with warm water (50°C), then thorough cleaning with water and detergent followed by rinsing with warm water to remove the suspended objects[34]. All trash baskets were closed tightly and quickly disposed. Disinfection was carried out using TH_{4+} (combination of quaternary ammonium compounds and glutaraldehyde). It is the one of the most powerful disinfectant as it has virucidal, bactericidal and fungicidal effects in a dilution of 1 : 200 for one minute[13] and rinsed with warm water to remove any disinfectant residues then utensils were tightly closed and meat instruments were placed inside clean plastic bags till use.

Handling of raw and cooked meat

Raw beef and chicken have been stored in separate deep freezers than vegetables. Thawing was done in clean disinfected sinks with water flowing slowly and replaced frequently. Utensils containing meat and chicken were closed and rapidly cooked without any delay. After cooking, meat was quickly removed from the soup and put in cleaned and closed utensils then kept in boiling water bath to keep internal meat temperature at 63°C or above; then meat was transferred by clean closed vehicles to the distribution rooms in which packaging inside aluminum plates to be distributed to consumers was done.

After HACCP implementation, one hundred samples collected randomly and equally equal from raw beef, raw chicken, cooked beef and cooked chicken meat and examined microbiologically to evaluate the effect of HACCP program.

Statistical analysis: Using SPSS-14, one-way analysis of variance (ANOVA) was performed to compare the samples while differences among individual means were compared by Duncan's multiple range test. In addition, the t-test was used to compare between samples before and after HACCP at 95% level of confidence, ($P < 0.05$) was considered as significant.

Results

Microbiological hazards associated with meat served at hospitals

In this study, total aerobic plate count (APC) was used to assess the hygienic measures inside the kitchen. The achieved results declared that the mean values of APC were 4.9 ± 2.0 and 4.8 ± 1.0-log cfu/g in raw beef and chicken meat, respectively. High levels of contamination by coliform and *E. coli* were detected in raw chicken meat with mean values of 4.0 ± 1.4 and 3.4 ± 0.3 (log MPN/g), respectively; while in raw beef, these values were 3.8 ± 0.9 and 3.2 ± 0.3 (log MPN/g), respectively (Table 2). Furthermore, the prevalence rates of *S. aureus* in the examined raw beef, raw chicken, cooked beef and cooked chicken were 16%, 20%, 8% and 12%, respectively. The mean values of APC, MPN of coliform, MPN of *E. coli,* and *S. aureus* count in the cooked beef samples were 2.8 ± 1.1, 2.9 ± 1.2, 2.5 ± 1.0 and 0.8 ± 0.4-log cfu/g, respectively; while were 3.3 ± 1.7, 3.1 ± 0.2, 2.6 ± 0.7 and 0.7 ± 0.4-log cfu/g in cooked chicken meat samples, respectively (Table 2). *Salmonella spp.* was isolated from raw chicken meat only with a prevalence rate of 24%. Serological identification of the isolated *Salmonella spp.* revealed that *S. Typhimurium, S. Enteritidis, S. Infantis, S. Bargny and S. Tsevie* were the isolated serovars. Four serotypes of *E. coli* were serologically identified in this study namely *O128:H2 (ETEC)* (two strains isolated from raw beef and chicken meat); *O26:H11 (EHEC)* (two strains isolated from raw chicken meat); *O142 (EPEC)* isolated from raw beef only and *O55:H7 (EPEC)* isolated from cooked chicken meat (Table 2).

Table 2. Hygienic status of beef and chicken meat samples collected from hospitals in Zagazig city, Egypt

Samples	Aerobic plate count	Coliform (MPN)	*Escherichia coli* (MPN)	*Escherichia coli serotypes*	*Staphylococcus aureus*		*Salmonella spp*
	Mean ± SD	Mean ± SD	Mean ± SD	Prevalence	Mean ± SD	Prevalence	Prevalence
Raw beef	4.9 ± 2.0^{a}	3.8 ± 0.9^{a}	3.2 ± 0.3^{a}	2 (8%)	2.2 ± 0.3^{a}	4 (16%)	ND
Raw chicken	4.8 ± 1.0^{a}	4.0 ± 1.4^{a}	3.4 ± 0.3^{a}	3 (12%)	3.3 ± 0.8^{a}	5 (20%)	6 (24%)
Cooked beef	2.8 ± 1.1^{c}	2.9 ± 1.2^{b}	2.5 ± 1.0^{b}	0	0.8 ± 0.4^{b}	2 (8%)	ND
Cooked chicken	3.3 ± 1.7^{b}	3.1 ± 0.2^{b}	2.6 ± 0.7^{b}	1 (4%)	0.7 ± 0.4^{b}	3 (12%)	ND

Counts are in log cfu/g; n = 25 each
SD is the standard deviation; ND: *Salmonella spp.* is not detected in all samples except raw chicken
Means within the same column with a different superscript letter $^{(a, b, c)}$ are significantly different at $P < 0.05$

Fig. 1. Expression of enterotoxin-coding genes in the isolated *S. aureus* strains using multiplex PCR. Lane M: 100 bp ladder as a molecular size DNA marker (*SEA* (120 bp), *SEB* (478 bp), *SEC* (257 bp) and *SED* (317 bp)). Lane C+: Control positive for *SEA*, *SEB*, *SEC* and *SED* genes. Lane C−: Control negative. Lane 9: Positive *S. aureus* strain for *SEA* gene. Lanes 5 and 11: Positive *S. aureus* strains for *SEC* gene. Lane 6: Positive *S. aureus* strain for *SEA* and *SED* genes. Lanes 1, 2, 3, 4, 7, 8, 10, 12, 13 and 14: Negative strains for enterotoxins.

Screening of the expression of virulence-associated genes in the isolated bacteria

A multiplex PCR was designed to confirm the expression of toxin and virulence associated genes in the isolated bacteria. Ten out of fourteen (71.4%) of the isolated *S. aureus* strains were non-toxin producing, while 28.6% of the isolated *S. aureus* strains were enterotoxigenic. Two strains harbored SEA and SEC genes, while one stain was positive for SED (Fig. 1). It was found that all isolated *Salmonella* serovars (100%) harbored *invA* gene, while four isolates (66.7%) were positive for Hyper-invasive locus (hilA) (Fig. 2). *E. coli* toxin-associated genes (*stx1*, *stx2* and intimin (*eaeA*) were expressed in the isolated *E. coli* strains (Fig. 3).

Antibiogram of foodborne bacterial isolates

Results obtained from the disc diffusion test revealed that the isolated strains of *S. aureus, Salmonella spp* and *E. coli* showed variable degrees of antibiotic resistance. The average MDR of *S. aureus* strains was 0.5. The resistance profile of the isolated strains for the antibiotics tested in this study were as follows P (100%), CL (100%), S (85.7%), UB (71.4%), CE (71.4%), E (71.4%), AMP (50%), SXT (57.1%), EN (28.6%), AML (28.6%), CTX (14.3%), CPD (14.3%), G (14.3%), CIP (0%) and AMC (0%) (Table 3).

Salmonella spp. had an average MDR of 0.6. *S. Typhimurium* was the highly resistant strain; it was resistant to EN, G, UB, S, E, CTX, CIP, AMP, P, CE, CPD and AML. *S. Tsevie* was resistant to G, S, E, CL, AMP, P, CE, AMC, CPD and AML. *S. Bargny* was resistant to EN, G, UB, S, E, AMP, CIP, P, CPD and AML. *S. Enteritidis* was resistant to EN, S, SXT, E, CTX, AMP, P and AML. Finally, *S. Infantis* was the lowest resistant strain, it was resistant to G, CL, P, CE, CPD and AML (Table 4).

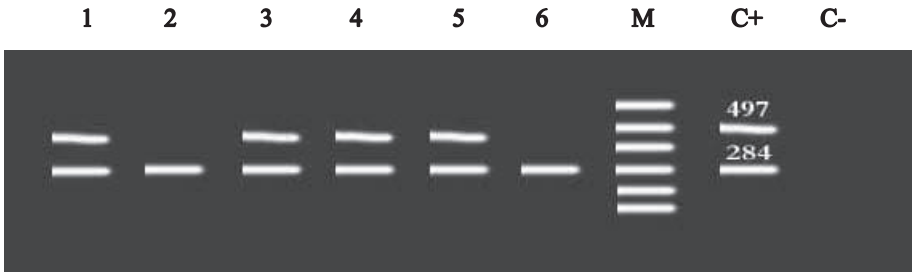

Fig. 2. Expression of virulence-associated genes in *Salmonella* serovars using multiplex PCR. Lane M: 100 bp ladder as a molecular size DNA marker (*invA* (284 bp) and *hilA* (497 bp)). Lane C+: Control positive strain for *invA* and *hilA* genes. Lane C−: Control negative. Lanes 2 (*S. Bargny*) and 6 (*S. Tsevie*): Positive strains for *invA* gene. Lanes 1 (*S. Infantis*), 3 (*S. Enteritidis*) and 4, 5 (*S. Typhimurium*): Positive for *invA* and *hilA* genes.

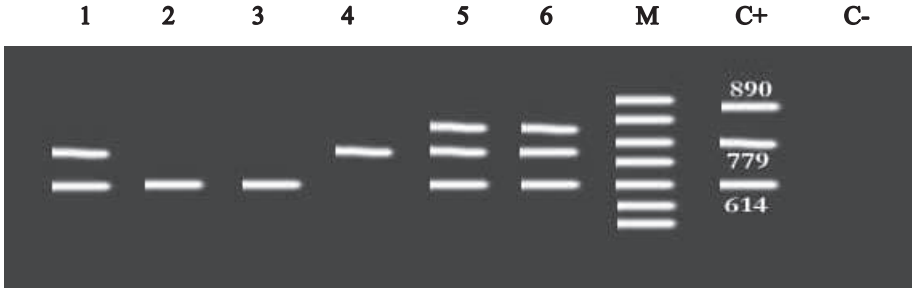

Fig. 3. Expression of shigatoxin-producing genes in the isolated *E. coli* serotypes using multiplex PCR. Lane M: 100 bp ladder as a molecular size DNA marker (*stx1* (614 bp), *stx2* (779 bp) and *eaeA* (890 bp)). Lane C+: Control positive *E. coli* for *stx1*, *stx2* and *eaeA* genes. Lane C−: Control negative. Lane 1 (O142): Positive for *stx1* and *stx2* genes. Lanes 2 and 3 (O128): Positive for *stx1* gene. Lane 4 (O55): Positive for *stx2* gene. Lanes 5 and 6 (O26): Positive for *stx1*, *stx2* and *eaeA* genes.

E. coli isolates had an average MDR of 0.4. *E. coli O26:H11* was resistant AML, AMP, P, SXT, S, E, Cl and CIP. *E. coli O128:H2* was resistant AML, AMP, P, SXT, S and E. *E. coli O142* was resistant AML, AMP and P. Finally, *E. coli O55:H7* was resistant AML, AMP and SXT (Table 5).

Effect of HACCP implementation on the microbiological quality of meat

The high microbial load in beef and chicken meat served to patients indicated unsatisfactory hygienic measures during meat preparation and handling. Therefore, HACCP principles were applied in the kitchen, food handlers were trained for the good practices during meat handling and effective cleaning and disinfection to walls, floors, roofs, cutting tables, cutting boards, knives, utensils and transporting vehicles. As a result, the microbiological quality of meat was significantly improved. APC, coliforms, *E. coli*, *S. aureus* contamination levels were significantly decreased (Table 6).

Discussion

Microbial quality of meat served at hospitals

Contamination of meats by biological hazards has been recognized as a global health concern especially in hospitals where a large number of patients is found. In the present study, microbial examination of meat served at hospital in Zagazig city, Egypt revealed high microbial load indicated by the high APC, MPN of coliforms and MPN of *E. coli*. This may indicate poor hygienic standards inside the hospital kitchens and unsanitary measures during meat preparation. Cooking temperature has a destructive effect on microorganisms and enzymes, consequently

Table 3. Percentages of antimicrobial susceptibility of the isolated *Staphylococcus aureus*

Antimicrobial agent	Sensitive		Intermediate		Resistant	
	No	%	No	%	No	%
P	0	0	0	0	14	100
CL	0	0	0	0	14	100
S	0	0	2	14.3	12	85.7
UB	0	0	4	28.6	10	71.4
CE	0	0	4	28.6	10	71.4
E	3	21.4	1	7.2	10	71.4
AMP	2	14.3	5	35.7	7	50
SXT	4	28.6	2	14.3	8	57.1
EN	7	50	3	21.4	4	28.6
AML	7	50	3	21.4	4	28.6
CTX	6	42.8	6	42.8	2	14.4
CPD	5	35.7	7	50	2	14.3
G	8	57.1	4	28.6	2	14.3
CIP	11	78.6	3	21.4	0	0
AMC	11	78.6	3	21.4	0	0
Multiple Drug Resistance (MDR)	Average 0.5					

Table 4. Virulence attributes and antimicrobial resistance profile of the isolated *Salmonella spp*

Salmonella spp	Virulence attributes	Antimicrobial resistance profile	MDR index
S. Typhimurium[1]	*invA* and *hilA*	EN, G, UB, S, E, CTX, CIP, AMP, P, CE, CPD, AML	0.8
S. Tsevie	*invA*	G, S, E, CL, AMP, P, CE, AMC, CPD, AML	0.7
S. Bargny	*invA*	EN, G, UB, S, E, AMP, CIP, P, CPD, AML	0.7
S. Enteritidis	*invA* and *hilA*	EN, S, SXT, E, CTX, AMP, P, AML	0.5
S. Typhimurium[2]	*invA* and *hilA*	S, CL, AMP, P, CE, CPD, AML	0.5
S. Infantis	*invA* and *hilA*	G, CL, P, CE, CPD, AML	0.4
Average MDR = 0.6			

S. Typhimurium[1,2]: indicates the 2 isolated strains of *S. Typhimurium* in this study, they had different antibiotic susceptibility profiles

cooked beef and chicken meat should be free from most pathogenic microorganisms, however mishandling of cooked meat inside the kitchen and meat distribution rooms resulted in an increase in the microbial contamination of final cooked products.

S. aureus is considered as one of the most important causes of food poisoning worldwide that responsible for food borne intoxication due to the production of heat-stable enterotoxin. *S. aureus* was isolated at different percentages from the examined samples, this reflects unsatisfactory hygiene measures during handling and processing of meat. Food handlers may be responsible for meat contamination by *S. aureus* as a result of cross contamination from their hair, nails and skin. Higher values were recorded in Nigeria as indicated by Nnachi and Ukaegbu[31] who reported higher counts of APC, coliform and *S. aureus*, while lower values were reported in Korea by Kim and Yim[25].

Salmonella spp. is a natural inhabitant in the

Table 5. Virulence attributes and antimicrobial resistance profile of the isolated *Escherichia coli*

E. coli strains	Virulence attributes	Strain Characteristics	Antimicrobial resistance profile	MDR index
O26 : H11	*stx1*, *stx2* and *eaeA*	EHEC	AML, AMP, P, SXT, S, E, Cl and CIP	0.5
O128 : H2	*stx1*	ETEC	AML, AMP, P, SXT, S and E	0.4
O142	*stx1* and *stx2*	EPEC	AML, AMP and P	0.2
O55 : H7	*stx2*	EPEC	AML, AMP and SXT	0.2
Average MDR = 0.4				

Table 6. Effect of HACCP implementation on the microbiological quality (log cfu/g) of meat (n = 25 each)

Samples	Aerobic plate count	Coliform	*Escherichia coli*	*Staphylococcus aureus*
Raw beef	3.3 ± 0.5**	2.5 ± 0.5**	2.2 ± 0.5**	0.5 ± 0.2**
Raw chicken	3.4 ± 0.9**	2.8 ± 1.1**	2.4 ± 0.5**	1.8 ± 0.2**
Cooked beef	1.6 ± 0.4**	1.7 ± 1.5	0.7 ± 0.5	0.3 ± 0.3
Cooked chicken	1.9 ± 0.4**	2.0 ± 0.2**	0.9 ± 0.3**	0.4 ± 0.3*

Values represent means ± SD of the positive samples
t- test significance**: high significant difference between the examined samples before and after HACCP ($P < 0.01$)
t- test significance*: significant difference between the examined samples before and after HACCP ($P < 0.05$).

intestinal tract of live birds and can contaminate chicken carcasses via cross contamination by meat contact surfaces, meat handlers, low hygienic standards, inadequate storage, dust and insects. In our study, *Salmonella* was detected only in raw chicken meat. Isolation of *Salmonella* from chicken meat is an indication of bad hygienic measures during carcass preparation and cross contamination from the intestinal tract as poultry is the main source of *Salmonella spp*. Unlikely, Yousif *et al*.[40] did not isolate *Salmonella spp.* from raw chicken meat collected from a hospital in Egypt.

Presence of *Enteropathogenic E. coli (EPEC)* such as *E. coli O55:H7* in chicken meat samples is an indication of fecal contamination because of inappropriate sanitation, poor handling and post-cooking contamination. The prevalence rate of *E. coli* (12.0%) in this study is higher than the contamination rate of poultry in Korea (4.6%) detected by Lee *et al.*[28], while lower than the contamination rate (16%) recorded by Darwish *et al*.[10] in duck meat and giblets.

Virulence-associated genes and antibiogram of the isolated foodborne pathogens

Some strains of *S. aureus* has the ability to produce one or more enterotoxins resulting in many cases food poisoning symptoms, these toxins are classified according to the antigenic properties into five SEs including SEA, SEB, SEC, SED and SEE, which are heat stable enterotoxins and resistant to proteolysis by enzymes. Consequently, it is very important to detect the level of beef and chicken meat contamination with enterotoxigenic strains of *S. aureus*, which is quite high in this study. The disease caused by SEs has a short incubation period (4.4 hours), nausea, vomiting, abdominal cramps, headache, and diarrhea. Although this disease is usually a self-limiting, death may occur among susceptible peoples like children and the elderly[38]. In agreement with the recorded results in the present study, SEs were detected in meat in Turkey[6] and Egypt[8].

Virulence-associated genes of *Salmonella spp.* are found mainly on its chromosomes, plasmids,

and prophages; they are known as *Salmonella* pathogenicity islands (SPIs) that play vital roles in adhesions, invasions, intracellular survival, systemic infections, antimicrobial resistance, toxin production, and magnesium and iron uptake[6]. Invasive gene is one of the SPIs, which consists of two additional invasion genes and aids in *Salmonella spp.* invasion to phagocytic and non-phagocytic cells. In the current investigation, the isolated strains harbored *invA* and *hilA* genes. In line with this result, Karmi[23] detected these virulent genes in the isolated *Salmonella spp* in Egypt.

Shiga toxin-producing *E. coli (STEC)* is identified as a toxin producing group of *E. coli* and one of the most important foodborne pathogens resulted in many outbreaks all over the world through consumption of contaminated beef and chicken meat. Shiga toxin 1 and 2 are the principle genes of virulence properties and pathogenicity, however, intimin encoded by the *eaeA* gene is another virulent factor which is responsible for adhesion of STEC to the intestinal epithelium[7] as shiga toxin alone is not enough to cause diseases. STEC can result in severe life-threatening diseases, as the hemolytic uremic syndrome (HUS), hemorrhagic colitis (HC) and thrombotic thrombocytopenic purpura (TTP), in addition to watery and bloody diarrhea[22]. In the present study, *stx1* gene was detected in *O26:H11*, *O128:H2* and *O142*, while *stx2* gene was expressed in *O26:H11*, *O55:H7* and *O142*, but *eaeA* gene was detected only in *O26:H11*.

Large numbers of bacteria have become resistant to antibiotics while some are multi-drug resistant (MDR), resistance can be passed from one strain to another by gene transfer because of antibiotics misuse. In the current study, *S. aureus* strains showed variable degrees of resistance to 13 out of 15 tested antibiotics. *Salmonella spp.* had the highest MDR; *S. Typhimurium* was the highest resistant strain, it was resistant to 12 out of 15 tested antibiotics, while *S. Infantis* was the lowest resistant strain, it was resistant to 6 out of 15 tested antibiotics. The low sensitivity of the isolated strains against most of the used antibiotics in Egypt could be attributed to the misuse of these antibiotics in poultry farms in Egypt. *E. coli* strains had the lowest MDR index, *O26:H11* and *O128:H2* were resistant to 8 and 6 out of 15 tested antibiotics, respectively; while both of *O55:H7* and *O124* were resistant to three antibiotics only. This high resistance might be due to the transfer of drug resistance among bacteria, and/or developing drug resistance due to bacterial mutational changes[9]. It was found that AML, AMP, P, SXT, S and E were the most resistant drugs among the isolated strains of *E. coli, Salmonella spp.,* and *S. aureus*. This may be attributed to the frequent use of these drugs in treatment of most of bacterial diseases in large animals and birds in Egypt. The high rate of antimicrobial resistance among the isolated pathogens in the current investigation may lead to severe adverse outcomes, especially among patients at hospitals, with weak immune system, if such contaminated meat is served. Similarly, high rates of resistance were detected in Egypt[2] and England[10].

Implementation of HACCP and its effect on the microbiological quality of meat

In hospitals, food hygiene requires attention to all preventive measures to minimize the hazards of food poisoning.

Effect of HACCP implementation on the microbiological quality of meat

HACCP system significantly improved the microbiological quality of meat served to patients inside the hospital. The achieved results in this study were in accordance with other studies conducted in Egypt[40], Ghana[1] and Greece[27]. The microbiological quality of meat collected after HACCP implementation was significantly improved achieving a clear reduction in APC, and a lower incidence of coliform organisms, *E. coli*, and *S. aureus*; whereas *Salmonella spp.* was not detected in meat samples. The possible explanation to this result is attributed to the

effectiveness of HACCP system that identified the critical points inside the hospital kitchen, which resulted in meat contamination by different biological hazards then correction to these points to provide a wholesome meat.

Conclusion

The results achieved in this study revealed poor hygienic measures adopted during preparation of meat meals at hospitals in Zagazig city, Egypt. Foodborne pathogens were isolated from meat served at hospitals. Implementation of HACCP principles during prepation of meat strongly reduced the bacterial contamination levels. Thus strict legislations should be adopted to ensure safety of meat served at hospitals with restrict observation of HACCP principles.

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