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Incidence of Antibiotic Resistant Coliforms in Poultry Meat in Menoufia Governorate, Egypt

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Abstract:
A total of 100 random samples of fresh chicken cuts (50 each of breast and thigh) were collected from different butcher's shops at Menoufia Governorate. The samples were examined for the coliform count, isolation and identification of *coliform strains*, and antibiotic sensitivity. The obtained results indicated that the coliform count varied from $2.7x10^2$ to $9.1x10^4$ CFU/g for chicken thigh and $2.3x10^2$ to $6.3x10^4$ CFU/g for chicken breast respectively. Coliform isolates were identified as *Escherichia coli*, *Citrobacter freundii*, *Citrobacter diversus*, *Enterobacter agglomerans*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Enterobacter hafniae*, *Serratia liquefaciens*, *Klebsiella pneumoniae* and *Klebsiella ozaenae*. Moreover, the serological examination of *E. coli* isolates revealed *E. coli* O86: K6, *E. coli* O55: K59, *E. coli* O125: K70, *E. coli* O128: K67, *E. coli* O26: K60 and *E. coli* O111: K58. Antimicrobial resistance among isolated coliform strains was detected by susceptibility to 8 antibiotics by disc diffusion method. Generally, *E. coli* isolates were resistant to ciprofloxacin, vancomycin, norfloxacin, streptomycin, and kanamycin. *C. freundii* isolates were resistant to ciprofloxacin, vancomycin, erythromycin, cefoxitin, and norfloxacin; *E. agglomerans* isolates were resistant to vancomycin, erythromycin, cefoxitin, streptomycin, and kanamycin. *E. aerogenes* isolates were resistant to vancomycin, cefoxitin, and kanamycin. *E. cloacae* and *K. ozaenae* strains were resistant to ciprofloxacin, norfloxacin, and kanamycin. *S. liquefaciens* strains were resistant to ciprofloxacin, vancomycin, cefoxitin, norfloxacin, and kanamycin. *K. pneumonia* strain was sensitive to all tested antibiotics. These data suggest that antimicrobial-resistant coliform isolates are widely distributed in the meat and processing environment in Egypt, which can play a role in the dissemination of antimicrobial resistance to other pathogenic and commensal bacteria.

Keywords: Antimicrobial resistance, coliforms, chicken meat, Egypt.

INTRODUCTION

Live birds are highly contaminated with different microorganisms on their feathers, skin, and intestinal tract. Accordingly, the contamination of chicken carcasses begins from the time of slaughtering, de-feathering, evisceration, until the final product storage and distribution (Capita *et al.,* 2004). Coliforms, defined as aerobic or facultatively anaerobic, Gram-negative, non-spore-forming rods capable of fermenting lactose with the production of acid and gas at 32–35°C (Davidson *et al.,* 2004), were originally considered to represent only strains from the genera Citrobacter, Enterobacter, Escherichia, and Klebsiella. *Escherichia coli* has emerged as a serious foodborne pathogen associated with numerous outbreaks. *E. coli* strains associated with diarrhea have been classified into six groups based on clinical, epidemiological and molecular criteria (Kalantar, 2013) namely, enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), Enteroinvasive *E. coli* (EIEC) and diffusely adherent *E. coli* (DAEC) (Gomez-Duarte, 2013). The usage of antibiotics is a major factor in the emergence, selection, and dissemination of antibiotic-resistant microorganisms in both veterinary and human (Tollefson and Flynn, 2002). The practice of using antibiotics in poultry is being questioned, owing to increased resistance to antibiotics (Tiwari *et al.,* 2014). Therefore, the present study aimed at the isolation and identification of coliform from poultry meat (breast and thigh), serological identification of *E.coli,* and to determine the antibiotic sensitivity of some isolated coliform strains.

MATERIAL AND METHODS

Collection of samples

A total of 100 random samples of fresh chicken cuts (50 from breast and 50 from thigh) were collected from different butcher's shops at El-monofia Governorate, Egypt. The collected samples were kept in separate plastic bags, transferred directly to the laboratory in an insulated icebox under complete aseptic conditions without any delay.

Determination of Coliform count

The coliform count was done following recommendations by ICMSF (1996), APHA (1992).

Identification of coliforms

Suspected isolates of coliform bacteria were identified following previous studies (MacFaddin, 2000; Iqbal *et al.,* 2016).

Serological typing of *E.coli*

The isolates of *E. coli*, taken, were subjected to serological identification (Varnam and Evans, 1991) using slide agglutination test.

Antibiotic susceptibility testing

Some isolated coliform strains were subjected to the sensitivity test against different antibiotics, using the Kirby-Bauer method on Mueller-Hinton agar (Bauer *et al.,* 1966).

Statistical analysis

Data were tabulated and an appropriate statistical test, either the t-test or ANOVA, was applied using SPSS 16.

RESULTS

The results demonstrated that the mean values of coliform count in the examined samples were varied from $2.7x10^2$ cfu/g to $9.1x10^4$ cfu/g with an average value of $7.95x10^4 \pm 2.66x10^4$ cfu/g for chicken thigh and $2.3x10^2$ cfu/g to $9.3x10^4$ cfu/g with an average value of $6.43x10^3 \pm 1.82x10^4$ cfu/g for chicken

breast (Table 1). The incidence of isolated bacteria was *Citrobacter diversus, Citrobacter freundii, Enterobacter aerogenes, Enterobacter agglomerans, Enterobacter cloacae, Hafnia alvei, Klebsiella penumoniae subsp.ozoenae, Klebsiella penumoniae subsp. penumoniae, E. coli, Serratia liquefaciens* and *Serratia marscens* (4%, 14%, 16%, 4%, 6%, 4%, 8%, 14%, 16%, 10% and 4%) in breast and (8%, 16%, 4%, 8%, 10%, 2%, 4%, 14%, 20%, 8% and 6%) in thigh respectively (Table 2).

The results in table (3) illustrated the incidence of pathogenic *E. coli* serotypes Enteropathogenic *E. coli* (*E. coli* O86:K61, and *E. coli* O55:K59), Enterotoxigenic *E. coli* (*E. coli* O125:K70, and *E. coli* O128:K67) and Enterohemorrhagic *E. coli* (E. coli O26:K60 and *E. coli* O111:K58).

The antimicrobial resistance profile of 8 isolated *coliform* strains was carried out (*E.coli*, *Citrobacter freundii*, *Enterobacter agglomerans*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Klebsiella pneumonia subsp. pneumonia*, *Klebsiella pneumonia subsp. ozaenae* and *Serratia liquefaciens*), by detecting susceptibility to 8 antibiotics (doxycycline, ciprofloxacin, vancomycin, erythromycin, cefoxitin, norfloxacin, streptomycin, and kanamycin) (Table 4). Generally, *K. pneumonia subsp. pneumonia* strain was sensitive to all tested antibiotic disc, also all strain tested were sensitive to doxycycline and resistant to kanamycin except *C. freundii*.

E.coli was resistant to (ciprofloxacin, vancomycin, norfloxacin, streptomycin, and kanamycin), *C. freundii* was resistant to (ciprofloxacin, vancomycin, erythromycin, and kanamycin), *and E. agglomerans* was resistant to (vancomycin, erythromycin, cefoxitin, streptomycin, and kanamycin) antimicrobial agents. While, *E. aerogenes* was resistant to vancomycin, cefoxitin and kanamycin. *E. cloacae* and *Klebsiella pneumonia subsp. Ozaenae* strains were resistant to ciprofloxacin, norfloxacin, and kanamycin however, *S. liquefaciens* strain was resistant to ciprofloxacin, vancomycin, cefoxitin, norfloxacin, and kanamycin.

Table 1. Coliform count (CFU/g) (Mean±SD) in the examined samples of chicken thigh and breast (n=50).

Samples	NO of positive samples	% of positive samples	Minimum	Maximum	Mean±SD
Thigh	45	90	$2.7x10^2$	$9.1x10^4$	$7.95x10^4±2.66x10^4$
Breast	41	82	$2.3x10^2$	$9.3x10^4$	$6.43x10^3±1.82x10^4$

Table 2. Incidence of identified coliforms in the examined samples of chicken meat (N=50).

Isolated coliforms	Samples			
	Breast		Thigh	
	No.	%	No.	%
Citrobacter diversus	2	4	4	8
Citrobacter freundii	7	14	8	16
Enterobacter aerogenes	8	16	2	4
Enterobacter agglomerans	2	4	4	8
Enterobacter cloacae	3	6	5	10
Enterobacter hafniae	2	4	1	2
Klebsiella pneumonia subsp. Ozaenae	4	8	2	4
Klebsiella pneumonia subsp. Pneumonia	7	14	7	14
E.coli	8	16	10	20
Serratia liquefaciens	5	10	4	8
Serratia marcescens	2	4	3	6

Table 3. Incidence of identified *E. coli* serotypes isolated from the examined samples of chicken Breast and Thigh.

Samples / Isolated Bacteria	Breast		Thigh		
	No	%	No	%	
E. coli O86:k61	2	25.0	3	30	EPEC
E. coli O55:k59	1	12.5	2	20	
E. coli O125: K70	1	12.5	1	10	ETEC
E. coli O128:k67	1	12.5	2	20	
E. coli O26:k60	2	25.0	1	10	EHEC
E. coli O111:k58	1	12.5	1	10	
Total	8	100	10	100	

Table 4. Antibiotic sensitivity test of isolated coliform strains.

Strain	Doxycycline (DO30)	Ciprofloxacin (CP5)	Vancomycin (N30)	Erythromycin (E15)	Cefoxitin (Fox30)	Norfloxacin (NOR10)	Streptomycin (S10)	Kanamycin (K30)
Enterobacter agglomerons	19	22	R	R	R	20	R	R
Enterobacter aerogenes	19	23	R	25	R	18	18	R
Enterobacter cloacoe	18	R	19	26	19	R	16	R
Serratia liquefaciens	16	R	R	25	R	R	18	R
E.coli	17	R	R	27	19	R	R	R
Klebsiella ozqence	17	R	23	28	20	R	17	R
Klebsiella pneumonia	20	27	20	24	20	18	16	20
Citrobacter freundii	17	R	R	R	R	R	20	19

DISCUSSION

The contamination with Coliforms may occur during slaughtering, cutting or dressing of carcasses, soiled hands, shopping blocks, or knives for handling and cutting, also contaminated water considered as a source of Coliforms in meat (Yadav *et al.,* 2006). The detection of coliform is used as a general indicator of sanitary conditions in the food-processing environment (Feng *et al.,* 2002). Nearly similar results were reported by (Mohammed *et al.,* 2015) who mentioned that the mean value of total coliform count in chicken meat was $1.7x10^3$cfu/g (Hassanien-Fatin *et al.,* 2016) recorded that the average value of coliform count is $2.61\times10^3\pm0.60\times10^3$ /g for chicken thigh and $2.07\times10^3\pm0.60\times10^3$/g for chicken breast.

Higher coliforms counts were obtained by (Ruban and Fairoze, 2011) who found that the mean value of fecal coliforms of chicken thigh and breast were 1.42 ± 0.15 and 1.34 ± 0.16, respectively. Moreover, higher values of coliforms counts were observed in thigh meat compared to breast meat. These results agreed with those obtained by (El-Khawas and Hendy, 2015) who found the mean value of coliforms counts for thigh samples (1.83 log CFU/g) and breast (1.42 log CFU/g).

The presence of coliforms in greater numbers may be responsible for the inferior quality of chicken meat resulting in economic losses and the possibility of the presence of other enteric pathogens, which constitute at time public health hazard (Chaem *et al.,* 2002). Nearly similar results of isolated coliforms were obtained by Kilonzo et al. (2013) and Olobatoke et al. (2015) who isolated *Enterobacter* (n = 34), *Klebsiella* (n = 13), *Citrobacter* (n = 6), *Serratia* (n = 14), *Hafnia* (n = 9) and *Escherichia* (n = 11). Higher results were observed by Gad (2004), Purabi and Joshi, (2010), Shawish (2011), and Shrestha et al. (2017). Lower results were obtained by Yulistiani (2017) and Arueyingho (2019) who isolated *E. coli* (27.82%), *Enterobacter* sp (4.15%), *Klebsiella* sp (1.84%) and *Citrobacter* sp (3.65%). On the other hand, the results of serotyping E.coli those obtained by Lee et al. (2009) who isolated enterotoxigenic *E. coli* (34.6%) followed by enterohaemorrhagic *E. coli* (35.9%) and finally enteropathogenic *E.coli* (20.5%) and Saif- Marwa (2015) who examined 100 random meat samples of fresh-marketed chicken meat (breast and thigh) and isolated Enteropathogenic *E. coli* (O55: H7 and O78), Enterotoxigenic *E. coli* (O125: H18, O128: H2 and O127: H6), Enterohaemorrhagic *E. coli* (O26 and *E. coli* O111: H4) and Enteroinvasive *E. coli* (O124). Enteropathogenic *E. coli* is a common cause of infantile diarrhea in developing countries. The high prevalence of resistance in poultry meat isolates is alarming given the evidence of possible transmission of antibiotic-resistant foodborne bacteria to consumers and food handlers (Bester and Essack, 2010). The results of antibiotic sensitivity were similar to previous studies. Millman et al. (2013) reported that *E.coli* was susceptible to ciprofloxacin, Amosun et al. (2012) found *E.coli* resistant to erythromycin, streptomycin, and norfloxacin. Arueyingho (2019) found that *Enterobacter* sp was resistant to erythromycin and gentamicin but *E. coli* isolates were resistant to amoxicillin/clavulanate and erythromycin.

Rasool et al. (2003) reported that *K. pneumoniae* was resistant to several antibiotics such as ampicillin, streptomycin, gentamicin, chloramphenicol, tetracycline, and ofloxacin. Another study by Shuhong et al. (2018) documented *K. pneumoniae* was resistant to several antibiotics such as ampicillin, streptomycin, and piperacillin.

CONCLUSION

Results concluded that the chicken thigh had a higher significant result than chicken breast for the total coliform count, due to the processing of carcass into parts and cross-contamination from unclean water, cutting tables and knives. Therefore, good hygienic practices should be followed in every step of processing.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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