

Fluoroquinolones susceptibility of *Escherichia coli* Strains isolated from frozen chickens meat

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Abstract

Escherichia coli (*E. coli*) is one of the most important food-borne pathogens, and especially in frozen chickens meat. Consequently, this study was conducted to isolate and identify (by using cultural and biochemical tests) *E. coli* from frozen chickens meat samples were available at local markets (retail stores) in different regions of Maisan governorate. Antimicrobial susceptibility testing was determined by using the disc diffusion method. A total of two hundred and fifty-six (256) *E. coli* isolated and identified. The main objective of this study was to investigate the susceptibility of the *E. coli* isolated to fluoroquinolones (Norfloxacin, Nalidixic acid, Enrofloxacin, Ciprofloxacin and Ofloxacin). Results from this study showed that these isolates were found to be highly resistant to the average percentages of resistance for all types of Fluoroquinolones. These results give significantly ($p < 0.05$) high levels of *E. coli* resistance in the frozen chickens meat. However, there was a trend towards higher resistance frequency to the average percentages of Norfloxacin, Nalidixic acid, Enrofloxacin, Ciprofloxacin and Ofloxacin which are reached (58.23, 49.27, 47.90, 17.22 and 11.42%) respectively. In conclusion, The antimicrobial usage is considered the most important factor promoting the emergence, selection and dissemination of antimicrobial-resistant microorganisms in chicken meat .

Keywords: Poultry meat; *Escherichia coli*; Fluoroquinolones; Resistance bacteria



المعزولة من لحوم الدجاج المجمدة لمضادات الحياة *E. coli* حساسية بكتريا

الفلوروكوينولونات

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الخلاصة

تعد بكتريا *E. coli* واحدة من اهم مسببات الأمراض التي تنقلها الأغذية وبشكل خاص لحوم الدجاج المجمدة . لذلك اجريت الدراسة الحالية لعزل وتشخيص (فحوصات مزرعية وبيوكيميائية) بكتريا *E. coli* من عينات لحوم الدجاج المجمد والمتاحة في الأسواق المحلية (متاجر التجزئة) في مناطق مختلفة من محافظة ميسان واختبار حساسية العزلات المشخصة للمضادات الميكروبية الفلوروكوينولون باستخدام طريقة انتشار الأقراص (disc diffusion method) . عزلت 256 عزلة من بكتريا *E. coli* اظهرت جميعها مقاومة عالية المعنوية ($p < 0.05$) لخمس أنواع من المضادات الحياتية الفلوروكوينولون والتي شملت (Norfloxacin, Nalidixic acid, Enrofloxacin, Ciprofloxacin and Ofloxacin) حيث كانت النسب المئوية لأعداد البكتريا المقاومة (58.23, 49.27, 47.90, 17.22 and 11.42%) على التوالي . وعليه يعتبر استخدام مضادات الميكروبات العامل الأكثر أهمية في ظهور، واختيار ونشر الميكروبات المقاومة للمضادات الميكروبية في لحوم الدجاج .

Keywords: لحوم الدجاج ، البكتريا القولونية ، الفلوروكوينولونات ، البكتريا المقاومة .

Introduction

Antibiotics are widely used in human and veterinary medicine to treat and prevent diseases and as growth promoters in animal intensive industries. The consequences are severe. Infections caused by resistant microbes fail to respond to treatment, resulting in prolonged disease and greater risk of death. Bacterial resistance to antibiotics poses a serious challenge to the prospect of chemotherapy. Rational use of antibiotics is most desirable but it cannot provide a permanent solution to the problem. The increasing incidence of resistance to a wide range of antibiotics by microorganisms is a major concern



facing modern medicine (Sengupta and Chattopadhyay, 2012; kareem and Al-Khayyat, 2014).

Antibiotics such as fluoroquinolones (Quinine family) are synthesis derived from Nalidixic acid. Nalidixic acid was first introduced in 1962 and in 1970 the first generation of quinine family was introduced. In the 1980s new potent of drugs were discovered from Nalidixic acid (Poole, 2000; Emmerson and Jones, 2003). This drug has a fluorine atom on six carbon and piperazine ring at seven carbons. It is called Fluoroquinolones. There are different types of Fluoroquinolones drug such as ciprofloxacin, norfloxacin and levofloxacin. They are used for treating many foodborne infections such as *Campylobacter*, invasive *Salmonella*, *Shigella*. and *E. coli*. The most widely used fluoroquinolone antibiotic in poultry production is enrofloxacin, sold under the trade name Baytril. Enrofloxacin is closely related to ciprofloxacin, a fluoroquinolone used in human medicine.

The term 'poultry' principally concerns chickens, but can also include other domestic fowls such as ducks, geese, and turkeys. The poultry industry has undergone considerable expansion over the last 40 years and now accounts for 27% of the worldwide meat consumption (Barbara et al., 2000). The biggest importer of poultry in the world is Russia with 10.6% of the total, followed by the European Union (9.2%) and Japan (8.8%). The Middle East region, particularly countries like Saudi Arabia and United Arab Emirates have increased the imports of poultry in the last years. Asian countries also represent the new markets for the sale of poultry with a considerable growth in its imports from 2005 to 2010, for example Hong Kong, China and Iraq (17.1, 64.4 and 128%) respectively (Winck et al., 2012).

The microbiological safety and quality of poultry meat are equally important to producers, retailers and consumers, and both involve microbial contaminants



on the processed product. Spoilage of poultry meat results mainly from 'off' odour development, and product shelf life is determined both by the number of spoilage organisms present initially and the temperature history of the product at all stages of production and subsequent storage and handling (Mead, 2004; Adeyanju and Ishola, 2014).

Contamination of poultry meat by bacteria can lead to public health problems. In most developing countries, the frequency of infections due to *E. coli* has been increasing steadily and the bacterium is now considered as a main cause of bacterial gastroenteritis. *E. coli* can easily contaminate food products during animal evisceration at slaughter or during food manipulation (Slama et al., 2010). According to the World Health Organization the resistant of *E. coli* can spread from farm animals to humans through the food chain (WHO, 2011).

At slaughter, resistant strains from the gut may contaminate poultry body and as a result poultry meats are often associated with multi-resistant *E. coli* (Chaslus-Dancla and Lafont, 1985; Turtura et al., 1990). The development of resistance to older agents such as ampicillin and trimethoprim-sulfamethoxazole, as well as the emerging problem of fluoroquinolone resistance, may substantially limit our antibiotic choices (Karlowsky et al., 2002; kareem and Al-Khayyat, 2014). As far as can be ascertained, therefore, the current study aimed to comprehensively investigate, for the first time, to isolate, identify and examine the antimicrobial resistance (Fluoroquinolones) of *E. coli* from frozen chicken meat available in the local market in Misan governorate. The aim of this project was achieved by carrying out the following tasks:

- Isolation and identification of *E. coli* from frozen chicken meat obtained from local markets (retail stores) in different regions of Maisan governorate.



- Examining the susceptibility of isolated E coli to fluoroquinolones (Norfloxacin, Nalidixic acid, Enrofloxacin, Ciprofloxacin and Ofloxacin).

Materials & methods

This study was carried out in Maisan governorate. All samples were randomly collected from the retail markets under sterilized conditions during period from December 2010 to February 2011.

E. coli isolation

25 g of meat sample was blended for 2 minutes and a gram of the sample was weighed out and homogenized in 9 ml buffered peptone water in a test-tube to give a dilution of 1:10. Serial six-fold dilution in peptone water was prepared (10^{-1} to 10^{-6}) for test samples. 0.1 ml of dilutions 10^{-6} and 10^{-5} for every sample was respectively plated on McConkey agar (MCA) and spread out using glass spreader. Plates were incubated overnight (18–24 hours) at 37°C (USDA, 2011). Pinkish colonies (coliform) obtained from incubated plates were sub-cultivated unto the fresh MCA plates and incubated overnight (18–24 hours) at 37°C. Isolates were transferred into nutrient slants, labeled and incubated overnight (18-24 hours) at 37°C. On removal from the incubator, slants were stored in a refrigerator at 4°C, ready for biochemical analysis.

Biochemical tests

Catalase test, sugar fermentation, Kovac's test and Gram staining were employed for E. coli isolates.

Antibiotic susceptibility test for isolates

The antimicrobial susceptibility of E. coli isolates were determined by disk diffusion method as described by Bower and Daeschel, (1999). The disk diffusion method recommended by the National Committee for Clinical Laboratory Standards. Disk diffusion antibiotic sensitivity test was used in determining susceptibility of the E. coli Gram-negative bacteria isolates



obtained to various antibiotics at different micrograms. The antibiotic disks used in this study were Norfloxacin (5 µg), Nalidixic acid (30 µg), Enrofloxacin (5 µg), Ciprofloxacin (5 µg) and Ofloxacin (5 µg).

Statistical Analysis

The antimicrobial susceptibility data are expressed as percentages. A one-way analysis of variance (ANOVA) was performed, using SPSS version 21 to estimate overall difference between the percentages. In all cases, $p < 0.05$ was regarded as statistically significant.

Results

A total of 256 isolates of *E. coli* were isolated and identified from frozen chickens meat samples collected from local markets in different regions of Maisan governorate are outlined in Table 1.

Table 1. Number of *E. coli* isolates from frozen chickens meat samples collected from different regions in Maisan governorate

Region ID	Regions	No. isolates
1	AL- Amarah	53
2	AL-Majar Al-Kabeer	35
3	Kalaat saleh	35
4	Ali AL-Garbi	40
5	Kumate	55
6	AL-Maimona	38
Total		256

Antimicrobial susceptibility

A total of 256 isolates of *E. coli* were analyzed. The percentages of isolates susceptible, intermediate and resistant to each antimicrobial agent are represented in Tables (2, 3, 4, 5, and 6). It is clear from these Tables that the average percentages of resistance for all types of Fluoroquinolones values give significantly ($p < 0.05$) high levels of *E. coli* resistance in the frozen chickens meat at retail stores in Maysan governorate. However, there was a trend towards higher resistance frequency to the average percentages of Norfloxacin, Nalidixic acid, Enrofloxacin, Ciprofloxacin and Ofloxacin which reached (58.23, 49.27, 47.90, 17.22 and 11.42%) respectively. Overall, the average percentage of Norfloxacin is the most potent of the currently available fluoroquinolones against Gram-negative bacteria, while the Ofloxacin was the lowest.

Table 2. Percentages of *E. coli* isolates from frozen chickens meat samples susceptible (S), intermediate (I) and resistant (R) to Norfloxacin (5 µg) by disc diffusion methods.

Region ID	Susceptible strains %		
	S*	I**	R***
1	35.3	12.4	52.3
2	18.6	20.2	61.2
3	24.8	17.5	57.7
4	34.4	14.7	50.9
5	22.3	12.3	65.4
6	18.1	20.0	61.9
Average	25.58	16.18	58.23

*sensitive (Inhibition zone diameter 17 mm or more)

**intermediate (Inhibition zone diameter 14-16 mm)

***resistance (Inhibition zone diameter 13 mm or less)

Table 3. Percentages of E. coli isolates from frozen chickens meat samples susceptible (S), intermediate (I) and resistant (R) to Nalidixic acid by disc diffusion methods.

Region ID	Susceptible strains %		
	S	I	R
1	37.8	18.8	43.4
2	22.9	17.7	60.0
3	28.6	14.3	57.1
4	37.5	20.0	42.5
5	27.3	32.7	40.0
6	26.3	21.2	52.6
Average	30.06	20.94	49.27

Table 4. Percentages of E. coli isolates from frozen chickens meat samples susceptible (S), intermediate (I) and resistant (R) to Enrofloxacin (5 µg) by disc diffusion methods.

Region ID	Susceptible strains %		
	S	I	R
1	25.5	26.4	48.1
2	21.2	22.6	56.2
3	23.1	31.2	45.7
4	29.1	20.7	50.2
5	29.1	25.5	45.4
6	26.6	31.7	41.7
Average	25.77	26.35	47.88

Table 5. Percentages of E. coli isolates from frozen chickens meat samples susceptible (S), intermediate (I) and resistant (R) to ciprofloxacin (5 µg) by disc diffusion methods.

Region ID	Susceptible strains %		
	S	I	R
1	48.3	33.5	18.2
2	41.0	38.3	20.7
3	54.9	29.9	15.2
4	53.0	29.4	17.6
5	51.4	32.1	15.9
6	53.9	30.4	15.7
Average	50.42	32.27	17.22

Table 6. Percentages of E. coli isolates from frozen chickens meat samples susceptible (S), intermediate (I) and resistant (R) to Ofloxacin (5 µg) by disc diffusion methods.

Region ID	Susceptible strains %		
	S	I	R
1	82.3	5.9	11.8
2	75.3	11.2	13.5
3	81.4	7.5	11.1
4	81.1	9.1	9.8
5	80.7	7.1	12.2
6	87.1	2.8	10.1
Average	81.32	7.27	11.42

Discussion

Food is a main vehicle to transmit enteric pathogens, most notably E. coli. The most of the recent data suggest the potential role of foods, particularly

retail chickens, as vehicles to transmit fecal pathogens) capable of causing intestinal infections (Salehi and Bonab, 2006). There is also concern that antimicrobial use in food animals can lead to the selection of antimicrobial resistant zoonotic enteric pathogens which may then be transferred to people by the consumption of contaminated food or by direct animal contact. At slaughter, resistant strains from the gut readily soil poultry bodies and as a result poultry meats are often contaminated with multi-resistant *E. coli* (Caudry and Stanisich, 1979; Turtura et al., 1990).

In this study Fluoroquinolones resistance was observed in all of the examined strains similar to the findings of previous studies (Bazile et al., 1996; Blanco et al., 1997; Bass et al., 1999; Guerra et al., 2003; Saenz et al., 2003; Zahraei, 2005; Miles et al., 2006; Miranda et al., 2008; Slama et al., 2010; kareem and Al-Khayyat, 2014; Adib et al., 2014). This is probably because of persistence of previous resistances or illegal use of these agents (Salehi and Bonab, 2006). Furthermore, the total Iraqi poultry imports are increasing steadily in response to population growth and rising incomes due to increased economic activity. These imported poultry are kept in freezers but power supply is not constant resulting in the rise and broadcasting resistant *E. coli*.

Consequently, the results of this study highlighted the importance of in impacting *E. coli* stability and its susceptibility to fluoroquinolones on frozen chickens meat obtained from retail stores in different regions of Misan governorate. These findings have important implications for both the consumers and retail stores of frozen chicken meat, for instance, in terms of how this product may be stored at retail stores, and how they should be labelled as and when health claims associated with frozen chickens meat are to be made.

The indiscriminate use of antibiotics should be cautioned because antibiotics will soon completely lose their effectiveness against microorganisms especially



as *E. coli* acquires antimicrobial resistance faster than other conventional bacteria due to increase resistance. Clearly, more research is needed to further understand the roles of *E. coli* and its resistance in frozen chickens meat.

Conclusion

1. This study is believed to be the first of its kind, which examines the antimicrobial resistance (Fluoroquinolones) of *E. coli* from frozen chicken meat available in the local market in Miasan governorate.

2. This study has shown that imported poultry meat in Maisan markets are contaminated with *E. coli* at high levels.

3. *E. coli* is gradually gaining more resistant to antibiotics.

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