Antibiotic Resistance of *Escherichia coli* O157:H7 Isolated from Chicken Meats

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ABSTRACT

The aim of the study was to estimate the prevalence and antibiotic resistance of *E. coli* 0157:H7 isolated from chicken meats. One hundred chickens from villages (n=50) and farms (n=50) were slaughtered under hygienic conditions. Meat samples were taken from chest area and analysed by conventional, biochemical and microscopic methods. In addition, the antibiotic resistance of the isolates was tested against 12 different antibiotics. Analyses revealed that, probably, four samples contained *E. coli* O157:H7. Confirmation tests were performed with the RapID™ ONE system and the results showed that only four samples contaminated with *E. coli* O157:H7. When the samples origins were examined, the samples that contaminated with *E. coli* O157:H7 were found to be from the villages. The prevalence of *E. coli* O157:H7 was higher in villages than in farms. The probable reason for the high number of *E. coli* O157:H7 in the villages is related to lack of vaccination and hygiene. The antibiotic sensitivity test of *E. coli* O157:H7 showed that isolates were resistance to Amoxicillin, Ampicillin, Chloramphenicol, Ciprofloxacin Doxycycline, Streptomycin and Tetracycline antibiotics.

Tavuk Etlerinden İzole Edilen *Escherichia coli* O157:H7'nin Antibiyotik Dirençi

ÖZET

Bu çalışmanın amacı, tavuk etlerinde E. coli 0157:H7 varlığı ve antibiyotik dirençini araştırmaktır. Köy (n=50) ve çiftliklerden (n=50) toplanan 100 tavuk hijyenik koşullarda kesildikten sonra, göğüs kısmından alınan et örnekleri geleneksel, biyokimyasal ve mikroskobik yöntemlerle analiz edilmiştir. Ayrıca elde edilen E. coli 0157:H7 isolatlarının 12 farklı antibiyotiğe karşı direnç özellikleri test edilmiştir. Geleneksel yöntemlerle yapılan analizlerde 4 örnekte muhtemel E. coli 0157:H7 kolonileri görülmüştür. Doğrulama testleri RapIDTM ONE sistemi ile yürütülmüş ve 4 örneğin E. coli O157:H7 ile kontamine olduğu belirlenmiştir. Bu örneklerin orijinleri incelendiğinde, kontamine olan örnekler köylerden toplanmıştır. Köy tavuklarındaki E. coli O157:H7 varlığı çiftlerinkinden yüksek olmuştur. Bunun muhtemel nedeni, kasabalarında aşı gibi yeterince koruyucu önlemlerin alınmamasından kaynaklanmaktadır. Et örneklerinden izole edilen E. coli O157:H7'ların Amoksillin, Ampisillin, Kloroamfenikol, Kiprofloksin, Doksisiklin, Streptomisin ve Tetrasiklin antibiyotiklerine karşı direnç gösterdiği bulunmuştur.

INTRODUCTION

Food safety covers a wide range of topics from personal hygiene training through contaminant removal. According to Lawley *et al.* (2008), the practices of food safety can be divided into three parts: protection of the food supply from harmful contamination, prevention of the development and spread of harmful contamination, and effective removal of contamination and contaminants. Most food safety procedures fall into one, or more of these categories. For example, good food-hygiene practice is concerned with the protection of food against contamination, effective temperature control is designed to prevent the development and spread of contamination, and pasteurisation is a procedure for removing of contaminants.

Food safety hazard can be any factor that causes harm to consumers, including biological, chemical and physical means and the harm includes illness, injury or both. Biological hazards can be an immediate threat to consumers and are involved in several foodborne out breaks. Bacteria, viruses, parasites and prions all belong in this category. Chemical hazards include chemicals like pesticides, veterinary drugs, plant toxins and environmental contaminants (IUFOST, 2011). The injury caused by chemical hazards is usually less immediate than those caused by biological hazards. Physical hazards that might be involved in food safety problems are objects like metals or stones left in the foods during or after processing. Food allergy is an another important food safety issue, which is growing in importance for the food industry as number of allergy symptoms related incidence increases (Lawley *et al.,* 2008).

Food of animal origin (milk and meat) can become contaminated with bacteria during food processing or slaughtering. Contaminated raw milk and undercooked beef products were implicated in outbreaks of *E. coli* O157 infections and resulting sequelae (Ostroff *et al.,* 1990; Wells *et al.,* 1991; Anonymous, 1993). *E. coli* serotype O157:H7 is one of the most notorious foodborne pathogens with an infectious dose of as low as a few hundred cells (Karmali, 2004). Fresh products such as beef, chicken, dairy products and fruit juices are common foods associated with *E. coli* O157:H7 outbreaks. Hemorrhagic colitis, hemolytic uremic syndrome, nonspecific diarrhea, and other illnesses associated with *E. coli* 0157:H7 were reported with increasing frequency during the past decade (Banatvala *et al.,* 2001; Griffin et al., 1991). The Centers for Disease Control and Prevention reported that Shiga toxin-producing *E. coli* (STEC) is responsible for 265,000 illnesses annually in the United States and *E. coli* O157:H7 causes about 36% of these incidences. Moreover, it estimates that STEC causes 3,600 U.S. hospitalizations and 30 deaths annually (CDC, 2016).

Conventional culture-based methods, involving enrichment, isolation and confirmation steps, are widely used for the detection of *E. coli* O157:H7 (Murakami, 2012). However, it requires four to five days to obtain results. Nucleic acid-based technique can identify target species in a short time. Nevertheless, some molecular technique cannot differentiate viable cells from dead ones (Wang and Levin, 2006). DNA from dead cells can yield false-positive results in conventional PCR, leading to unnecessary product recalls and economic losses. Whereas Real-time PCR can provide a simple and elegant method for determining the amount of a target sequence or gene that is present in a sample. More studies regarding the laboratory diagnosis of *E. coli* O157:H7 were carried out than any other group of food-borne pathogens in recent years, yet *E. coli* O157:H7 remains the most difficult to detect.

The purpose of this study was to establish the prevalence of *E. coli* 0157:H7 in chicken meats by conventional and biochemical methods and determine the antibiotics resistance of isolated strains.

MATERIAL and METHOD

Material

A total of 100 chickens from villages (n=50) and farms (n=50) in Duhok, Arbil and Sulaymaniyah, Iraq were collected during 2013.

Buffered peptone water, MacConkey (MAC) agar, Eosin methylene blue (EMB) agar, Sorbitol MacConkey (SMAC) agar, Hemorrhagic colitis (HC) agar, Mueller Hinton (MH) agar and Brain heart infusion (BHI) broth were provided from LabM, UK. Antibiotic discs were purchased from Bio-analyse, Turkey (Table 1).

Table 1. List of used antibiotics

Antibiotic	Abbreviation	Concentration (μg/ disc)
Amikacin	*AK*	30
Amoxicillin	AMX	25
Ampicillin	AM	10
Cephaloxin	CF	30
Chloramphenicol	C	30
Ciprofloxacin	CIP	25
Doxycycline	DO	30
Gentamicin	GN	10
Norfloxacin	NOR	10
Neomycin	N	30
Streptomycin	S	10
Tetracycline	TE	10

Sampling of chicken meats

Chickens were cut under hygienic conditions in the slaughter house (Duhok), the internal organs were

removed, and the chicken carcasses were placed in sterile sample-collection polyethylene bags, properly. Labelled samples were transferred to the laboratory in a cool box and analysis were performed immediately. Twenty five g meat sample from the breast of each chicken was taken and subjected to the microbial analyses.

Isolation and identification of E. coli

Taken meat samples were homogenized in 225 mL of peptone water using a stomacher (Bagmixer, Interscience) and incubated aerobically at 37°C for 24 h. A volume of 20 µL homogenate was plated on a MAC agar, spread out using glass spreader, and incubated aerobically at 37°C for 24 h for isolation of gram-negative enteric bacteria. After the incubation, up to five suspected pink colour colonies from each agar plate was picked up and transferred to an EMB agar plate and streaked for isolation. EMB Agar plates were incubated aerobically at 37°C for 24 h, and E. coli colony formation (dark centre and usually a green metallic sheen) was examined. When E. coli-like growth was present, one typical, well-isolated colony was streaked onto either a SMAC agar with 5-bromo-4-chloro-3-indolyl-ß-D-glucuronide (BCIG) or a HC agar for the identification and the differentiation of E. coli O157:H7 on the basis of the lack of sorbitol utilization and the absence of ß-D-glucuronidase activity against 4-methylumbelliferyl-ß-D-glucuronide (MUG), respectively. The colony was spread out on a SMAC-BCIG agar using a glass spreader and incubated aerobically at 37°C for 24 h. The non-fermenting sorbitol activity was observed from the colourless colonies on the SMAC-BCIG agars. Similarly, another colony was plated on a HC agar and incubated at 37°C for 24 h and the ß-D-glucuronidase activities were examined under a UV-light in the dark using colonies grown on the HC agars. The colonies showing no fluorescence activity were considered as ß-D-glucuronidase-negative or MUG-negative. ß-D-glucuronidase negative and sorbitol-negative isolates were presumptively identified as E. coli.

The isolates were further tested for biochemical identification using Thermo Fisher Scientific RapID™ ONE System (Remel Inc., KS, USA). Identifications were confirmed with RapID™ ONE kit according to the supplier recommended protocol. Isolates obtained from the EMB agar to produce E. coli O157:H7-like Vero cell cytotoxin(s) were definite as enterohemorrhagic E. coli O157:H7 by inability to ferment sorbitol on the SMAC agar and the absence of ß-D-glucuronidase activity on the HC agar within 24 h at 37°C, as described previously, and by further identification based on the RapID™ ONE System (Remel Inc., KS, USA), by serology with O157 and H7 antisera.

The colonies isolated from the EMB agar were stained by basic Gram staining method. A small sample of the colony was transferred to a clean glass slide. It was decolorized by the alcohol, losing the colour of the primary stain, purple. After decolourization step, a counterstain was used to impart a pink colour to the decolorized gram-negative organisms, and gram negative cells (pink coloured cells) were recorded.

Antibiotic sensitivity test

The standardized disk diffusion test method (NCCLS, 2000), also called as Kirby-Bauer method was used to determine the in vitro antibiotic susceptibility of the identified E. coli isolates to various antibiotics given in Table 1.

Antimicrobial susceptibility screening was conducted using a panel of 12 agents. The concentrations of the antibiotics tested were 10-30 µg/disc. A standardized suspension of the isolated E. coli from EMB agar was prepared by inoculating a colony into 10 mL peptone water and incubated at 37°C for 24 h. It was adjusted to a 0.5 MacFarland turbidity standards and diluted 1:10 ratio. A sterile swab was dipped into the standardized inoculum and used to inoculate evenly the surface of already prepared MH agar. The agar was left for 15 minutes for the surface moisture to dry. A multichannel disc dispenser (Oxoid, Basingstoke, UK) was used to deposit the antibiotics discs onto the surface of the inoculated medium. The plate was then incubated at 37°C for 24 h. The zones of growth inhibition were measured with slipping callipers. The method was replicated three times and the mean zones of inhibition compared with figures provided by the Clinical and Laboratories Standards Institute (CLSI, 2013). E. coli ATCC 25922 was used for quality control for antimicrobial susceptibility tests.

RESULTS

The study was designed to estimate E. coli O157:H7 prevalence in farm and village chicken meat. One hundred chickens from villages and farms were sampled in order to provide a reasonably precise and unbiased estimate of E. coli O157:H7 prevalence.

Bacteriological findings: The distributions of E. coli on the chicken meat samples were detected on the agars (MAC, EMB, SMAC-BCIG and HC) (Table 2). Gram-negative enteric bacteria on the MAC agar was observed as pink colonies. Twenty-two of 100 meat samples were found Gram-negative, 15 of from villages and 7 of which from the farms. Village area sustained the higher prevalence of E. coli among sampled areas. E. coli was detected 15 of 100 meat samples on EMB agars, 5 of which were from the farms and the remaining (10) was from the villages. Sorbitol-negative isolates, as indicated by pale colonies of E. coli O157:H7 on the SMAC-BCIG agar plates were obtained from 4 out of 100 meat samples. All sorbitol-

negative samples were collected from villages. No infection was found in the meat samples from farms. MUG-negative isolates, as indicated as colourless colonies under UV light of *E. coli* O157:H7 on the HC agar plates were obtained from 4 out of 100 meat samples. All MUG-negative samples were collected from villages. The organisms observed under microscope were gram negative, pink coloured with rod shaped appearance and arranged in single or in pair, and suspected as *E. coli.*

Table 2. The distribution of *E. coli* on the chicken meat samples detected on different agar the agars

Area	n	MAC	EMB	SMAC-BCIG	HC
Village	50	15	10	4	4
Farm	50	7	5	0	0
Total	100	22	15	4	4

n: number of sample

Confirmation of E. coli O157:H7: The isolates that were sorbitol-negative and MUG-negative were further characterized and confirmed by using RapID™ ONE system. Four isolates were suspected *E. coli* O157:H7 and subjected to the RapID™ ONE analysis. The change in the colour in the vials was observed after the suspected to contain *E. coli* O157:H7 colonies were incubated.

The suspected *E. coli* O157:H7 colonies gave no colour changes in the vials containing URE, TET, LIP, KSF, β-GLU, β-XYL, NAG, MAL and PYR. 7-digit microcode obtained from the scoring of positive tests before and after the addition of kit reagents, was checked in the electronic RapID™ compendium ERIC® (http://www.remel.com/ERIC/IdentificationSingle.asp x) for the identification of the test organisms. All the test strains showed more than 99.9% similarity with *E. coli.*

Antibiotic susceptibility of E. coli O157:H7: Antibiotic resistance of *E. coli* isolated from chicken meats has been consideration of worldwide. The humans infected with antibiotic resistant bacteria through the food chain lead to difficulty in treatment. Totally 4 *E. coli* O157:H7 isolated from chicken meat was tested for susceptibility with 12 different antibiotics.

The highest resistance was found to AMX, AM, C, DO, S and TE. The highest sensitivity was recorded to GN, NO and N. Only one sample was sensitive to AK and CF, the rest showed intermediate sensitivity to these antibiotics. Only one sample showed intermediate sensitivity to CIP and the rest were resistant to CIP.

DISCUSSION

Escherichia coli O157:H7 has been isolated from dairy cattle, calves, chickens, swine and even sheep and from their meat; although its incidence and prevalence are highly variable (Fu *et al.,* 1995). Chicken and hen eggs are considered as vehicles of transmission of this pathogen, since chicks can be colonized by small populations of *E. coli* O157:H7 and continue to be long-term shedders (Schoeni and Doyle, 1994). Nevertheless, diverse results have been obtained from the isolation of *E. coli* O157:H7 from chickens. Griffin and Tauxe (1991) did not recover this bacterium from raw chicken, contrasting with research carried out by Samedpour and Liston (1994) in Seattle, where 12 of 33 chicken samples were positive. Beery *et al.* (1985) reported the colonization of chicken by this *E. coli* O157:H7strain. The results in this study showed that 22% of the meat samples from chickens were infected with *E. coli* O157:H7. This result is worrying, because Arias *et al.* (2001) has reported that this bacterium in chicken giblets is capable of surviving and multiplying even stored between 0 and 12°C. Since chicken giblets are consumed well cooked, the importance of this finding relies in the potential cross contamination focus they represent during processing, handing and marketing of the product. Human infections of *E. coli* O157 have been mostly attributed or linked to food products from animals (Elder *et al.,* 2000).

In the present study, 4 out of 100 meat samples were found to be positive for *E. coli* O157:H7. The result is comparable with the findings of Abdul-Raouf *et al.* (1996) and Chapman *et al.* (2000). The previous studies have shown that the prevalence of *E. coli* O157:H7 varied from 0.17 to 2.4% (Coia *et al.,* 2001; Kiranmayi *et al.,* 2010; Altae, 2012). On the other hand, Naimi *et al.* (2003) and Tarr *et al.* (1999) did not found any *E. coli* O157:H7 in the chicken meats. The difference in these results may be due to the source of sample contamination, sampling area, and storage conditions. To control and prevent poultry diseases especially colibacillosis, subtherapeutic and therapeutic levels of antimicrobial agents are administered to chickens via food and water. This practice also improves feed efficiency and accelerates weight gain (Bower and Daeschel, 1999). The treatment of whole flocks with antimicrobials for disease prevention and growth promotion became a controversial subject (Witte, 1998; Van dan-Bogaards and Stobberinghs, 1999). However, administration of antimicrobial agents provides a selective pressure which causes detection of resistant bacteria. Therefore, the antibiotic selection pressure for resistance in bacteria in chicken is high and consequently their faecal flora contains a relatively high proportion of resistant bacteria and resistance to existing antimicrobials is widespread and of concern to poultry veterinarians (Cloud, 1985; Hinton *et al.,* 1987; Goren, 1990; Peighambari *et al.,* 1995). 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.

In this study, the representative *E. coli* O157:H7 isolates were found to be sensitive to Gentamicin, Norfloxacin and Neomycin, but resistant to Tetracyclin, Amoxicillin, Doxycyclin, Ciprofloxacin, Chloramphenicol and Ampicillin. This result is consistent with the findings of Salehi and Bonab (2006), and Altaee (2012). On the other hand, Olatoye and Isaac Olufemi (2010) reported different results, probably due to the misuse of antibiotics and the reflection effect in humans, which has generated resistance to such antibiotics.

CONCLUSION

This study was conducted to estimate the prevalence of and to isolate *E. coli* O157:H7 from chicken meats. In this respect, one hundred chickens from villages (n=50) and farms (n=50) were cut in the slaughter house under hygienic conditions and the meat samples from chest area were subjected to the cultural, biochemical and microscopic tests. In addition, the isolates were tested against 12 known antibiotics to measure their antibiotic resistance.

The cultural method for isolation of *E. coli* O157:H7 including pre-enrichment on MAC agar, enrichment in EMB agar and selective enrichment in SMAC-BCIG and HC agars indicated that 4 agar plates were probably suspected with *E. coli* O157:H7. The biochemical confirmation tests performed with the RapID™ ONE test system. The RapID™ ONE test result showed that all 4 suspected cultures contaminated with *E. coli* O157:H7 at the probability of 99.9%.

The tests performed in the study showed that all contaminated cultures (4) with *E.coli* O157:H7 came from the villages. The probable reason for the high number of *E. coli* O157:H7 in the villages is related to lack of vaccination.

The antibiotic sensitivity of *E. coli* O157:H7 was tested against 12 antibiotics using disk diffusion method. The highest resistance of *E. coli* O157:H7 was found to AMX, AM, C, DO, S and TE. The highest sensitivity was recorded to GN, NO and N. Only one sample was sensitive to AK and CF, the rest showed intermediate sensitivity to these antibiotics. Only one sample showed intermediate sensitivity to CIP and the rest were resistant to CIP. These results indicated that *E. coli* O157:H7 contain a multi-drug resistance.

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