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PREVALENCE OF ESCHERICHIA COLI O157:H7 STRAINS IN BUTCHER SHOPS IN KAFR EL-SHEIKH CITY

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ABSTRACT

This study aims to assess the occurrence of Escherichia coli O157 and O157:H7 in butcher shops in Kafr El-sheikh city. A total of 336 samples (137 ground beef, 152 grinding-machines and 47 manipulator hands) were collected from 20 butcher shops during a period of 8 months from beginning January to end of august 2008 in the Kafr El-sheikh city.The samples were processed according to standard methodsand examined bacteriologically. The resulting E.coli colonies were subjected to agglutination test using specific antisera to E.coli O157 and H7 .

The incidence of E.coli serotypes were observed, only one ground beef sample and one grinding machine was positive for E. coli O157:H7, having a prevalence of 0.73 %.and 0.66% respectively Four samples were found positive for E. coli O157 serotype, having a prevalence of two (1.5%) in ground beef and two (1.3%) from grinding machine . The results of this Study reveal that the level of E. coli O157;H7 was significantly lower as compared to other studies in Egypt, but it is similar to studies performed in many countries around the world. The six E.coli serotypes were screened for antibiotic resistance. High levels of resistance against several antimicrobial agents were detected; those most commonly observed were to tetracycline, Streptomycin, Chloramphenecol and trimethoprim. Such high levels of antimicrobial resistance highlight the need for a more rational use of these agents in cattle.

INTRODUCTION

E. coli is one of the bacteria that exist in the normal micro flora of the intestinal tract of humans and warm-blooded animals. Most strains of *E. coli* are nonpathogenic ***(Stender et al .2001)*** however some strains

differ from commensals in that they express virulence factors molecules directly involved in pathogenesis thereby causing disease ***(Schroeder, et al.2004).***

E.coli O157:H7 strains are associated with a broad spectrum of human illnesses throughout the world, ranging from mild diarrhea to hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) ***(Griffin and Tauxe;1991 and Nataro et al;1998).*** The dominant *STEC* serotype is O157:H7, which is also the most commonly involved in large outbreaks in the United States, Canada, and the United Kingdom. However, strains belonging to more than 100 different O:H types have been associated with human disease. Common *STEC* serogroups associated with pathogenicity include O26, O91, O103, and O111 ***(Caprioli et al;2005).*** *E.coliO157:H7* in undercooked ground beef contaminated by fecal material and unpasteurized dairy products have been linked to several human infections ***(Griffin et al;1991)***. In addition, most cases are not linked to undercooked ground meat, but the means of transmission are often unknown .During processing fecal contamination of the carcass or transfer of bacteria found on the animal hide to the carcass could facilitate transmission of STEC to food . ***(Caprioli et al;2005)& Elder, et al; 2000)*** Cattle have been regarded as a natural reservoir of VTEC organisms for infections ***(Mead and Griffin, 1998) and Meng and Doyle, 1998)*** Most human infections with *E. coli* O157:H7 have been primarily associated with the consumption of contaminated and improperly cooked ground beef and unpasteurised cow's milk ***(Armstrong, et al.1996 and Hancock, et al.1994)*** and many food borne outbreaks of *E. coli* O157:H7 have been reported in different countries (***Christie, 1996).***

Concerning seasonal ,occurrence of enterohaemorrhagic (EHEC) isolates and sporadic or outbreaks caused by these pathogens revealed the increased recovery from various materials were mostly detected in warm seasons such as summer. ***(Kudva, 1997).***

Antimicrobial agents for therapy or prophylaxis aimed at animal growth promotion, have favored propagation of resistant bacteria. ***(White et al.2002)***. Intestinal resistant bacteria due to fecal contamination during slaughtering may be transferred to meat products ***(Elder et al.2000)***. If subsequently transmitted to human food ***(White et al.2002)*** they become reason for concern.

This study is aimed to estimate: (1) the prevalence of E.Coli O157:H7 in cold and warm months in butcher shops with a region of Kafr El-sheikh city; (2)The sensitivity of the isolated bacteria to antimicrobial drug.

MATERIAL AND METHODS

Sampling:

A total of 336 samples(137 ground beef, 152 swabs from grinding-machines and 47 swabs from manipulator hands) were collected randomly from different 20 butcher shops at Kafr El-sheikh city from the beginning January to end of August Samples placed in a sterile bag. and stored in a cool box for transportation to the laboratory and analyzed within 1 h.

Materials:

Preparation of samples:

Ground beef samples were processed according to the *Compendium of Methods for the Microbiological Examination of Foods* with some modifications. Briefly, 25g of each sample was homogenized in 225 mL

of buffered Peptone water (BPW, 1%).Swabs from machines or hands of operators were manually mixed with 10 ml of 1% peptone water. incubated for 24 hours at 35ºC, streaked on MacConkey Sorbitol and incubated for 18-24 hours at 35ºC.

Isolation of *E.coli*:

Five to ten sorbitol positive and negative colonies from each sample were inoculated into (plated on) Eosin Methylene Blue (EMB-Difco) agar and incubated for 24h at 35°C. At least five colonies were taken from the EMB plates for further identification according to ***Farmer, (1999).***

Agglutination test:

All *E.coli* isolates were then subjected to the agglutination test to determine the serotype of the bacteria using specific antisera to E. coli O157 (Oxoid,20007,UK) and Dry spot *E.coli* O157 latex agglutination test (Oxoid, UK) for E. coli O157 carried out in parallel.

The isolates identified as *E.coli* O157 were tested with antisera H7 (Oxoid,211057,UK)

1- A loopful of the suspected colony was emulsified in one drop of sterile physiological saline on glass slide.

2- one drop of *E. coli* H7 antisera was added to the suspension with standard loop and thoroughly mixed.

3- Positive agglutination result was occurred within one minute. As described by the Manufacturer.

Antimicrobial testing:

Antibiotic resistance patterns of *E.coli* O157:H7 and *E.coli* O157 were determined by the disk diffusion method using Mueller Hinton Agar ***(Bauer et al.1966)***. Zone interpretations were based on the recom-

mendations of the National Committee on Clinical Laboratory Standards (NCCL). The antibiotic discs used were: ampicillin (10 µg). streptomycin (10 µg), .tetracycline (30 µg), cefoperazone (30 µg), chloramphenicol (30 µg), ciprofloxacin (10 µg), gentamicin (10 µg), oxytetracycline (30 µg). norfloxacin (10 µg). enrofloxacin (5 µg), trimethoprim (5 µg). trimethoprim-sulfamethoxazole (25 µg).

RESULTS

Table (1): detection of *E.coli* O157:H7 and O157 in different samples.

Type of samples	Noof examined sample/ Noof positive samples	E.coli serotypes	
		O157	O157:H7
Ground beef	137/7 (5.1%)	2 (1.5%)	1(0.73)
Grinding machine	152/10 (6.6%)	2 (1.3%)	1(0.66%)
Manipulator hands	47/2 (4.2%)	0 (0%)	0 (0%)

The incidence of serotypes was calculated according the total No of samples.

Table (2): Antibiotics resistance of E.coli O157:H7 and E.coli O157 serotypes isolated from butcher shops.

Isolates	Resistance pattern
O157:H7	C,S,TE,W
O157:H7	C,S,Ot,W
O157	C,S,TE
O157	C,S,W,nalid
O157	C,S,OT,W
O157	C,S,OT,TE,W

C(Chloramphenicol,30ug);S(Streptomycin,10ug);W(Trimethoprim,5ug);TE(tetracycline,30ug);OT(Oxytetracycline,30ug) ,nild (nalidixic acid,30ug).

DISCUSSION

Outbreaks and sporadic cases of infections due to *E.coli* O157:H7 strains are mainly associated with the consumption of contaminated food of animal origin, especially ground beef ***Mora et al; (2005).*** Isolation of E.coli O157:H7 from ground beef and other foods were described in several countries.

Hussein and Bollinger (2005), analyzing packaged and unpackaged meat from supermarkets and showed that Shiga toxin producing Escherichia coli prevalence ranged from 0.01 to 54.2%.

In our study, 336 samples collected from the regions of Kafr El-sheikh city were contaminated with 2 (0.69%) E.coli O157:H7.Only one (0.73%) was isolated from 137 samples of ground beef and one (0.65%) from 152 samples of grinding machine table (1).

Previously *E.coli* O157:H7 in beef and ground beef samples were noted as a 3.7% of the 164 beef, 29.4% of the 17 beef samples in USA ***Doyle and Schoeni, (1987)*** and 6 % of the 50 ground beef samples in Egypt ***Abdul-Raouf et al., (1996)***. The presence of serotype E.coli O157: H7 isolates in low incidence (0.73%) in ground beef is nearly agree with ***Irino et al;(2005)*** .They isolated *E.coli* 0157:H7 with percentage (0.6%) in Brazilian cattle and with (0.79%) from ground beef. The prevalence similar to those reported by ***Vernozy-Rozand et al;(2002)***, who *E.coli* O157:H7 in minced beef 0.12% (four of 3450). ***Chinen et al. (2001)***. detected *E. coli* O157:H7 from the 6 (3.75%) of 160 ground beef samples in Argentina.

In contrast, the prevalence of O157 was approximately equal to the number of the *E. coli* O157:H7 serotypes found by ***Chinen et al. (2001)***;

Cebiroglu and Nazli,(1999); ***Noveir et al., (2000)***, they isolated *E.coli* O157 in raw meat and raw meat based products With incidence 2% and 2.58% in hamburgers.

Our result showed 2(1.5%) of *E.coli* O157 isolated from minced meats and 2(1.3%) from grinding machine ,this result higher than that detected by ***Heuvelink et al., (1997),*** who detected positive *E.coli* O157 from the 1 % of the 571 minced beef in Netherlands.Other studies in Turkey,conducted to detect *E.coli* O157 and/or*E. coli* O157:H7 revealed that *E. coli* O157 have been isolated from meat and meat products with an occurrence varying from 0 to 5% in meat and meat derived products such as hamburgers and meat balls.***(Akkus,(1996) and Sarimehmetoglu, et al. 1998).*** Few studies,however on the isolation of *E. coli* O157:H7 from ground beef and beef products in Turkey have revealed negative results ***Akkus ,(1996).***

This survey revealed that E.coli O157:H7 serotypes were not detected between January and March. It has been previously noted that the occurrence of *E. coli* O157:H7 in cattle feces is often seasonal, with the warmer and increased moist conditions of summer season contributing the highest incidence ***(Kudva et al., 1997; Johnsen et al.,(2001).***

The antimicrobial susceptibility testing (Table 2) of both *E.coli* O157 and *E.coli* O157:H7 serotypes were resistant to Streptomycin, tetracycline, Chloramphenicol, Nalidixic acid and trimethoprim. These findings agree with ***Sáenz et al;(2001) and Schroeder et al;(2003).*** It has also been supported by recent findings in northern Palestine ***(Adwan et al., 2002; Adwan and Adwan, 2004)*** where 49% of human *Shiga toxin producing Escherichia coli* isolates and 55% of *Shiga toxin producing Escherichia coli* isolates from raw beef were found resistant to three or

more antibiotics. Resistance towards at least one or more antibiotics such as ampicillin, chloramphenicol, and tetracycline has been reported for O157 and H7 serotypes by previous workers from several materials ***(Radu et al., 2001 & Mora et al;(2005)***. The use of antibiotics for the treatment of *Shiga toxin producing Escherichia coli* infections may be contraindicated because certain antibiotics induce the release and dispersion of Shiga-toxin encoding bacteriophages ***(Zhang et al. 2000)***.

Moreover transmission of resistant *Shiga toxin producing Escherichia coli* strains to people, or at least of a transmission of multiresistance encoding determinants from coli form bacteria of animal origin to human strains, may take place ***(Oppegaard et al., 2001).***

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مدى انتشار الميكروب القولوني O157:H7 في محلات الجزارة في مدينة كفر الشيخ

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E.coli O157:H7 هذه الدراسة تهدف إلى معرفة مدى تواجد الميكروب القولوني في محلات الجزارة في مدينة كفر الشيخ في الشهور الباردة والشهور الحارة ،فقد تم تجميع 336 عينة (137 عينة من اللحوم المفرومة ،152 مسحة من ما كينة الفرم ،47 مسحة من ايدي المتعاملين مع اللحوم) على مدى 8 شهور من بداية يناير حتى نهاية أغسطس .جميع العينات تم تجهيزها وفحصها بكتريلوجيا. لعزل الميكروب القولوني ،جميع العترات المعزولة تم E.oli 157 فحصها بواسة اختبارالتلزن البطيء. وقد أثبتت الدراسة ان نسبة الميكروب القولوني في اللحوم المفرومة 0.73% ومن ماكينة الفرم 0.66%.بينما كانت العينات الموجبة للميكروب القولوني أربعة عينات إثنان من اللحوم المفرومة واثنان من ماكينة الفرم بنسبة 1.3%. وقد تبين ان نسبة الميكروب المعزول اقل بالمقارنة بالدراسات المصرية بينما تشبه إلى حد كبير النسبة الموجودة بالبلاد الأخرى تم إجراء اختبار الحساسية على ستة عترات من الميكروب القولوني اثنان منها coli O157. وأربعة من E.coli O157:H7 فوجد أنها عالية المقاومة لثلاثة او اكثر من المضادات الحيوية مثل الكلورام فينيكول، الإستربتو ميسين، التتراسيكلين، الاؤ كسى تتراسيكلين، النالدكسك اسد.