PREVALENCE AND ANTIMICRIBIAL SUSCEPTIBILITY OF ESCHERICHIA COLI O157:H7 ISOLATED FROM HUMAN AND ANIMAL SOURCES IN BASRAH PROVINCE

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ABSTRACT

The present study assessed the prevalence of *Escherichia coli* O157 in diarrhea patients ,beef, and raw milk. A total of 675 samples were inoculated in trypticase soy broth to enhance the growth of *E. coli O157:H7*. Out of total samples 73.5% isolated as *E. coli* then cultured on Sorbitol MacConkey agar ,31.8% non fermenting sorbitol (NSF) *E. coli* colonies were isolated and confirmed by specific biochemical tests . From NSFEC 13.7% were diagnosed as *E.coli O157:H7* by serological test ,the result revealed no significant differences in the level of contamination with *E. coli O157:H7* between beef ,stool and milk .The isolated bacteria were tested for antibiotic susceptibility test which showed resistance 100% to cephalothin ,cefoxitin , cefixime, trimethoprim , amoxicillin, azithromycin, and amoxicillin/clavulanic acid and sensitive 100% to ciprofloxacin ,imepenim ,nitrofurantion gentamycin and amikacin . No major differences in antibiotic susceptibility patterns among the isolates were observed.

INTRODUCTION

Escherichia coli is commonly found in human and animal intestinal tracts and , result of fecal contamination or contamination during food animal slaughter, is often found in soil, water, and foods [1].Diarrheagenic*E. coli* are an important cause of endemic and epidemic diarrhea worldwide .These organisms are currently classified in six categories as follows: enteropathogenic*E. coli* (EPEC), enterotoxogenic*E. coli* (ETEC), enteroinvasive*E. coli* (EIEC),diffusely adhering *E.coli*(DAEC),enteroaggregative*E. coli* (EAEC),and enterohemorrhagic*E. coli* (EHEC) [2].One of the most significant food –borne pathogen that has gained increased attention in recent years is *E. coli* O157:H7 [3] .*E. coli* O157:H7 cause diarrhea , sever abdominal pain , hemorrhagic colitis, hemolytic –uremic syndrome, and thrombotic thrombocytopenic purpura .The pathogenic factors of enterohemorrhagic*E.*

coli include Shiga toxins , the chromosomal LEE locus that carries factors (eaeA,tir) involved in the attaching and effacing process, and a large plasmid carrying the hemolysin genes[4]. *E. coli* O157:H7 serotypes are identified as enterohemorrhagic*E. coli* and categorized as verotoxin-producing *E. coli* [5].Verotoxin-producing *E. coli* (VTEC),including O157:H7,was identified in 1982 as an important human pathogen [6]. Domestic and wild animals are the sources of *E. coli* O157,but ruminants are regarded as the main natural reservoirs. Sporadic cases and outbreaks of human diseases caused by *E. coli* O157:H7 has been linked to ground beef, raw milk, meat and dairy products ,vegetables, unpasteurized fruit juices and water[7].Infections can also be acquired by direct contact with animals and by person-to –person spread [3,8].Recent reports indicate that antimicrobial resistance of *E. coli* O157 is on the rise [9] . Yet the extent to which different antimicrobial use practices have contributed to the increase in antimicrobial resistance is not clear. The usefulness of antimicrobial therapy for *Shiga Toxin E. coli*(STEC) infections is unresolved. Because antimicrobials may lyse bacterial cell walls, thereby liberating Shiga toxins [10],and/ or cause increased expression of *Shiga toxin* genes in *vivo* [11],they are not recommended for treating STEC O157 infections . However ,recent studies suggest that some antimicrobials, if administrated early in the course of infection ,may prevent disease progression to hemorrhagic uremic syndrome (HUS) [12]. Because STEC infections are not aggressively treated with antimicrobial therapy, many isolates may yet be susceptible to numerous antimicrobials.

This study was undertaken to understand the prevalence and antimicrobial resistance pattern in *E. coliO 157: H7* recovered from child's stool, raw milk and beef.

MATERIALS AND METHODS

Sample collection and bacterial isolation.

From July 2011 to January 2012 ,a total of 675 samples , beef (n=225) from the slaughter house , stool from children who were suffering from diarrhea of both sexes under 5 years of old attending Aben – kzwan hospital in Basrah city, and raw milk of different types were collected from different parts in Basra (n=225). All samples were placed in separate sterile plastic containers to prevent spills and cross contamination and were immediately transported to the laboratory in a cooler with ice packs. Twenty –five gram from each sample of beef were homogenized in 225 ml trypticase soy broth TSB (supplemented with vancomycin 4 mg/l and cefixime 0.05 mg/l). 25 ml from each sample of milk added to 225 ml

of TSB-CV , and loop full from each stool sample inoculated with 5 ml of TSB-CV. All enrichment samples incubated at 37°C for 18-24 h.[13]. Loopful from enrichment samples were streaked on to McConkey agar plates (oxoid) and eosin methylen blue and incubated as above.Pure colonies of *E. coli* isolates confirmed by using Api 20 E system. All *E.coli*isolate were screened on sorbitol MacConkey (supplemented with cefixime 0.05 mg/l) agar plates. After incubation at 37°C for 24 h all non-sorbitol fermenter (colorless) colonies were recorded as presumptive aquatic *E. coli* O157 :H7[14]. Additional biochemical tests including cellobiose fermentation and KCN broth turbidity were employed on NSFEC.

Serotyping*;* *E. coli* isolates that gave the following reactions: cellobiose (-),and KCN(-) ,were serotyped by slide agglutination technique use O157,and H7 antisera(Murex Wellcolex,UK)

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was carried out by disk diffusion method according to the recommendation reported by NCCLS [15] .As recommended by the NCCLS Mueller-Hinton agar were used as the culture medium. The antimicrobial agent disks used in this study were: Amoxicillin,Amikacin ,Gentamycin , Cefixime, Cephalothin, Ciprofoxacin,Cefoxitin, Amoxicillin/Clavlanic acid, Naldicxic acid, Nitrofurantoin, Imipenim, Tetracycline , Azithromycin, Trimethoprim. The result was interpreted according to the recommended of the NCCLS(15).

RESULT

Table 1 shows the prevalence of *E. coli* isolated from beef ,stool and milk in Basrah city .The highest prevalence of *E. coli* was found in stool (84.3%) followed by milk (67.2% ,and beef (64.1%). There were no significant differences in the level of contamination with *E.coli* between beef and milk.while there were significant differences (p< o.o5) between stool and both beef with milk.

Table 1. Prevalence of *E*. coli isolates from beef, stool, and milk.

Soures of samples	No. of samples	No. (%)of *E. coli*culture + ve	No.(%)of *E. coli*conrirmed by Api 20 system
Beef	**225**	**67 (29.7)**	**43 (64.1**
Stool	**225**	**134 (59.5)**	**113 (84.3**
Milk	**225**	**55 (24.4)**	**37 (67.2**
Total	**675**	**256 (37.6)**	**193 (73.5**

X^2=19.05	X^2=3.3

From those confirmed as *E. coli* (n=193) there were 29 (31.8%) were identified as non sorbitol fermenter and negative tocellobiose fermentation and KCN broth turbidity. There were no significant differences in frequency of NSFEC between three sources (table 2).

Table 2. Occurrence of NSFEC on sorbitol MacConkey agar among *E. coli* isolates

Samples	*E. coli* isolates	No.(%) Nonsorbitol fermenter (NSF)	% NSFEC isolates with cellobiose and KCN
Beef	**43**	**29 (67.4)**	**12 (41.3)**
Stool	**113**	**44 (38.9)**	**10 (22.7)**
Milk	**37**	**18 (48.6)**	**7 (38.8)**
Total	**193**	**91 (47.1)**	**29 (31.8)**

The distribution ofO157and H7 among isolated NSF *E. coli* were investigated.. Table (3) showed that *E. coli* carrying O157 with a percentage 27.5% and H7 with a percentage 31%. the percentage of NSF *E. coli* carrying both o157 and H7 was 13.7%. There were no significant differences among them regarding source of isolation.

Table 3. Frequency of *E. coli* O157:H7 in NSFEC isolated from beef, stool and milk

Samples	NSFEC	O157	%	H7	%	O157:H7	%
Beef	**12**	**4**	**33.3**	**2**	**16.6**	**2**	**16.6**
Stool	**10**	**1**	**10**	**6**	**60**	**1**	**10**
Milk	**7**	**3**	**42.8**	**1**	**14.2**	**1**	**14.2**
Total	**29**	**8**	**27.5**	**9**	**31**	**4**	**13.7**

Four *E. coli* O157:H7 isolates were tested for antimicrobial susceptibility testing (table 4). The results revealed no antimicrobial resistant to gentamycin , Amikacin , Imepinem, Nitrofurantoin , ciprofloxacin, and Nalidicxic acid .High percentage of antimicrobial resistant

was founded in amoxicillin, cephalothin , trimethoprim, amoxicillin/clvulanic acid, cefixime, cefoxitin , azithromycin, and tetracyclin .

Table 4. Antimicrobial susceptibility tests for bacterial isolates

Type of antibiotic	(%)Sensitive	(%)Intermediate	(%)Resistance
Gentamycin10 µg	100%	0%	0%
Amoxicillin/Clavulanic acid 20/10 µg	0%	0%	100%
Cephalothin30µg	0%	0%	100%
Cefixime30 µg	0%	0%	100%
Cefoxitin10 µg	0%	0%	100%
Imipenem 20 µg	100%	0%	0%
Ciprofloxacin30 µg	75%	25%	0%
Nalidaxic acid30 µg	50%	25%	25%
Amoxicillin25 µg	0%	0%	100%
Trimethoprim5 µg	0%	0%	100%
Azithromycin30 µg	0%	0%	100%
Nitrofurantoin300 µg	75%	25%	0%
Amikacin30 µg	100%	0%	0%
Tetracyclin30 µg	0%	25%	75%

DISCUSSION

The classical screening medium for *E. coli* O157:H7 is sorbitol MacConkey agar. This method exploits the fact that *E. coli O157:H7* ,unlike 90% of *E. coli* isolates did not ferment sorbitol rapidly[16]. Other studies reported that sorbitol MacConkey agar medium is a useful, rapid, and reliable screening aid for the detection *E. coli O157:H7* ,but it is not generally useful of VTEC strains of serotypes other than *E. coli* O157:H7 [17].

Shiga toxin-producing *E. coli* (STEC) is now a major case of food –born disease ,mostly in the United states, Canada, Japan, and Europ [18].In an earlier study STEC O157:H7 was isolated from 3.7% beef and 1.5% of pork samples in United States and Canada[19].Although most sporadic cases and outbreaks have been recorded from developed countries, human infections associated with STEC strains have also been described in Latin American countries[2].It has also been reported from Kenya, Turkey ,and Iraq [20]. Many studies determined the prevalence of *E. coli*O157:H7 on cattle which were from 0.0% to 27% (up to 68%in heifers) [7].

The present study showed that 16.6% of raw beef samples were contaminated with *E. coli* O157:H7 .Our result suggested that cattle could be a reservoir of *E. coli* O157:H7 in Iraq, like many countries [21].The ability of this study to detect serotype O157:H7 in lower rates among non –sorbitol fermenting *E. coli* isolates in beef, stool and milk confirm the results obtained by another author, who reported that this serotype is uncommon and its isolation rates are much lower than those of non O157:H7 serotypes [22].On contrary, Wells *et al*.[23] determined the prevalence of *E. coli* O157:H7 and found that this organism was isolated from 5 of 210 calves (2.3%) .Surveys of United states dairy and beef have found *E. coliO157*:*H7* in 0 to 2.8% of animals.The three isolates of bacteria showed resistant (100%) to cephalothin ,cefoxitin , cefixime, trimethoprim , amoxicillin, azithromycin, and amoxicillin/clavulanic acid followed (80%) resistant to tetracycline and (40%) resistant to nalidixic acid .This result was concordant with Fart *et al*.,[24] .yeon Kim *et al*.,[25] agreement with results that revealed sensitivity *E. coli* O157:H7 to ciprofloxacin ,imepenim ,nitofurantin gentamycin and amikacin .The continued overwhelming sensitivity of *E. coli* O157 in this study to almost all antibiotics tested is astonishing considering the rapid increases in resistance found in other zoonotic bacteria such as *Salmonella* spp., and *Campylobacter*spp. [26].

انتشار و الحساسيةا للمضادات الحيوية في الشرشيا القولونية النمط المصلي O157:H7 المعزولة من الانسان ومصادر حيوانية في محافظة البصرة

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الخلاصة

قمنا بتقييم نسبة انتشار بكتريا الايشريشيا القولونية النمط المصلي O157:H7 في مرضى الإسهال ولحم العجل والحليب الخام . 675 عينة حقنت في الوسط السائل تريبتيكيز سوي لتحسين نمو الايشريشيا القولونية للنمط المصلي O157:H7. 73,5 % من العينات الكلية عزلت كأيشريشيا قولونية وبعد ذلك زرعت على وسط سوربيتولماكونكي أكار،

عزلت 31,8% مستعمرات الايشريشيا القولونية غير المخمرة للسوربيتول واكدت بواسطة أختبارات الكيمياء الحياتية. من الايشريشيا القولونية غير المخمرة للسوربيتول 13,7% شخصت كأيشيريشيا قولونية للنمط المصلي O157:H7 بواسطة الاختبارات المصلية.

أظهرت النتائج بعدم وجود اختلافات معنوية في مستوى التلوث للنمط المصلي O157:H7للايشيريشيا القولونية بين لحم العجل، براز الأطفال والحليب.

فحصت البكتريا المعزولة بواسطة اختبار حساسية المضادات الحياتية والتي أظهرت مقاومة 100% للسيفالوثينوالسيفوكسين،السيفكسيم ،الترايمثبيرين ،الاموكسيلين، الازثرومايسينوالاموكسيلين\كلافولانك اسد وحساسة 100% للسيروفلوكساسين، امبينيمونيتوفورانتينجنتامايسينوالاميكاسين. لوحظ عدم وجود أختلافات كبيرة بين انماط قابلية التحسس للمضادات الحياتية بين العزلات.

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