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MOLECULAR DETECTION OF *TEM* GENE IN *ESCHERICHIA COLI* O157:H7 ISOLATED FROM CHILDREN AND BUFFALOES IN BASRAH PROVINCE

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Key words: *Escherichia coli* O157:H7, *tem* gene, PCR.

ABSTRACT

During a period of five months (August 2015 to December 2015), a total of 250 samples were collected 125 from hospitalized children suffering from diarrhea, and 125 from buffalo feces samples collected from different regions in Basra Province, (Basra City Center, Abo alkaseeb, alqurna, karmat Ali, A lzobeer). All specimens were screened for the presence of *E. coli* O157H7. A total of 104 (41.6%) of suspected *E. coli* isolates were obtained : 62 from children stool and 42 from buffalo feces, All suspected isolates were tested biochemically. 6 out of 62 from children stool 9.7% and 4 out of 42 from buffalo feces 9.5% were Non-Sorbitol Fermented *E. coli* (NSFEC). All the isolates were found to be resistant to at least 7 antibiotics to which they were subjected. Therefore, all these four isolates were considered to be multidrug resistant. PCR assay for amplification of *tem* gene revealed that 6 of the *E. coli* O157:H7 isolates that isolated from children and buffalo were positive for *tem* gene.

INTRODUCTION

B-lactam are a large group of important antibiotics, because their effectiveness and generally low toxicity [1]. There are four main groups of *B*-lactam antibiotics including penicillins, cephalosprins, carbapenems and monobactams which are arranged according to structure [2]. Bacteria expressing Extended-Spectrum *β*-Lactamases (ESBLs) enzymes hydrolysing penicillins and cephalosporins, may not respond to therapy using some of these antibiotics. ESBLs producing Gram-negative rods have a higher rates of antimicrobial resistance than other areas of hospital [3]. The ESBLs producing organisms are often resistant to several other type of antibiotics, as the plasmids with the gene encoding ESBLs often have

other resistance determinants. The ESBLs are commonly produced by many member of enterobacteriaceae, especially *Escherichia coli* efficiently hydrolyze oxyimino cephalosporins conferring resistance to third generation cephalosporins such as cefotaxime, ceftazidime, ceftriaxone and to monobactums such aztreonam. ESBL-producing enterobacteriaceae might become a major threat to children health as the infection cause by these bacteria have increasingly been reported worldwide in last decade [4].

Escherichia coli is a major cause of food and water-borne illnesses characterized by bloody diarrhea, hemorrhagic colitis (HC) and life-threatening hemolytic uremic syndrome (HUS) in developed nations across the globe [5]. Ruminants are a major source of *E. coli* O157:H7, and transmission principally occurs through consumption of contaminated food but also through direct or indirect contacts with contaminated buffalos or persons [6; 7; 8 and 9]. The aim of the present study is to investigate the antimicrobial susceptibility for *E. coli* O157:H7 isolated from buffalo and children and determine the occurrence of *tem* beta-lactamases genes (extended-spectrum β-lactamase ESBL).

MATERIAL AND METHODS

Collection of Samples

In the present study, isolation of *E. coli* was carried out in stool and faecal samples collected during the period October 2015 through January 2016. Two hundred fifty stool and faecal samples (125 of each group) were collected from children and buffalos, respectively, from outpatients wards at Aben-Kzwan hospital, Basra, Iraq.

Culturing of *E. coli*

Loopful of each samples were inoculated in 5 ml of trypticase Soy broth (TSB-V) supplemented with (1.5 g) bile salt and incubated at 37 °C for 18-24h [10]. A loopfull of bacterial growth was streaked on EMB and MacConkey agar then incubated overnight for *E. coli*. Typical colonies on MacConkey agar and Eosin-Methylene Blue agar (EMB) were streaked on sorbitol MacConkey agar supplemented with cefixime (0.05mg/L) and potassium tellurite (2.5mg/L) and incubated for additional overnight to identify on NSFEC [10].

Identification of *E. coli*

The identification of *E. coli* isolates was performed according to standard method described by [11]. Tentative identification for all the isolates was done by traditional culture characteristics, gram staining, indole test, simmon's citrate test and triple sugar iron test (Hi-Media, India). Definitive identification up to species level was made with the Api 20E System Identification (Pioneer, Germany).

Antimicrobial Sensitivity Testing

The antimicrobial test of all positive isolates were performed according to [12] by modified disc-diffusion method using 10 different antibiotic discs in the following concentrations: amoxicillin (25µg), amikacin (30µg), cephalothin (30µg), cefotaxime (30µg), ciprofloxacin (30µg), gentamicin (10µg), caphalothin (300µg), ceftazidime (5µg), imipenem (20µg) and tetracycline (30 µg), using [12 and 13]. Mueller-Hinton (MH) agar plates (Difco Laboratories, Detroit, USA) were overlaid with each of the *E. coli* strains inoculum (turbidity equivalent to that of a 0.5 McFarland Standard). Inhibition zone diameters were measured after 24 and then 48 hrs incubation [12].

Molecular detection *tem* gene by PCR assay

The DNA was purified and extracted according to the instructions of the company (Kiagen, Germany). PCR amplifications were carried out on a DNA thermal cycler instrument (Tc-312Techne (UK)). The primer design were performed as previously described Table (1). The composition of the reaction mixture was as follows: 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.1% Triton X-100, 1.5 mM $MgCl_2$, each of the four deoxynucleoside triphosphates at a concentration of 0.2 mM, and 1.2 U of *Taq* DNA polymerase in a total volume of 25 µl. A total of 2.5 µl of template DNA was added to the reaction mixture, and the mixture was centrifuged briefly. The PCR program consisted of an initial denaturation step at 95°C for 15 min, followed by 30 cycles of DNA denaturation at 94°C for 30 s, primer annealing at 50°C for 1min, primer extension at 72°C for 2 min, and final extension at 72°C for 10 min. After the last cycle the products were stored at 4°C.

Table (1): Oligonucleotide primer sequences used for PCR amplification of *tem* gene.

Gene	Primer Sequence	Size*	Reference

			(bp)	
tem	**F**	**5'-TCG CCG CAT ACA CTA TTC TCA GAA TGA-3'**	**445**	**[14]**
	R	**5'-ACG CTC ACC GGC TCC AGA TTT AT-3'**		

*: Size: PCR product (bp).

The PCR products (1/10 volume) were analyzed by electrophoresis with 1% agarose gels in TBE buffer (0.04 M Tris-OH, 0.002 M EDTA [pH 8.5]). The gels were stained with ethidium bromide, and the PCR products were visualized with UV light. A single band was observed for *tem* amplified products with a single primer set.

RESULTS

Out of 250 samples 104 isolates were suspected *E. coli* (62 and 42) from children and buffaloes respectively. On the other hand, the prevalence of *E. coli* isolated from children and buffaloes were 33.6% and 49.6% respectively (Table. 2).

The percentage of frequency of NSFEC was 9.6% based on the fermentation of the sorbitol in Sorbitol MacConkey agar (Table 3).

All *E. coli* O157:H7 isolates (100%) were resistance to cephalothin, gentamycin, amoxicillin and ceftazidime. (90%) were resistance to amikacin and cefotaxime and (75%) were resistance to tetracycline, in addition to (50%) were resistant to ceftriaxone. While (100% and 75%) *E. coli* O157:H7 isolates were sensitive to imipenem and ciprofloxacin, respectively (Table 4; Figure 1).

The PCR analysis was applied to DNA extracted from pre-conventional microbiological confirmed of *E. coli* O157:H7 isolates figure (2). Six isolates were produced PCR products corresponding to *tem* gene (445 bp) corresponding for *tem* gene analyzed by PCR assay (Figure 3).

Table (2): Prevalence of *E. coli* isolated from children and buffalos.

Sample source	Total No. of sample	No. of *E. coli* isolate	(%)
children	125	42	(33.6)
buffalo	125	62	(49.6)
Total	250	104	(41.6)

Table (3): Percentage of non-sorbitol fermenting *E coli* (NSFEC).

Sample source	Total No. of sample	Non-sorbitol fermenter (NSF)	(%)
children	62	6	(9.7)
buffalo	42	4	(9.5)
Total	104	10	(9.6)

Table (4): Antimicrobial susceptibility of *E. coli* O157:H7 isolated from children and buffalos.

Type of antibiotic	Sensitive (%)	Intermediate (%)	Resistance (%)
Gentamycin	100	0	0
Cephalothin	0	0	100
Imipenem	100	0	0
Ciprofloxacin	75	25	0
Amoxicillin	0	0	100
Amikacin	10	0	90
Tetracyclin	0	25	75
Ceftazidime	0	0	100
Ceftriaxone	50	0	50
Cefotaxime	0	10	90

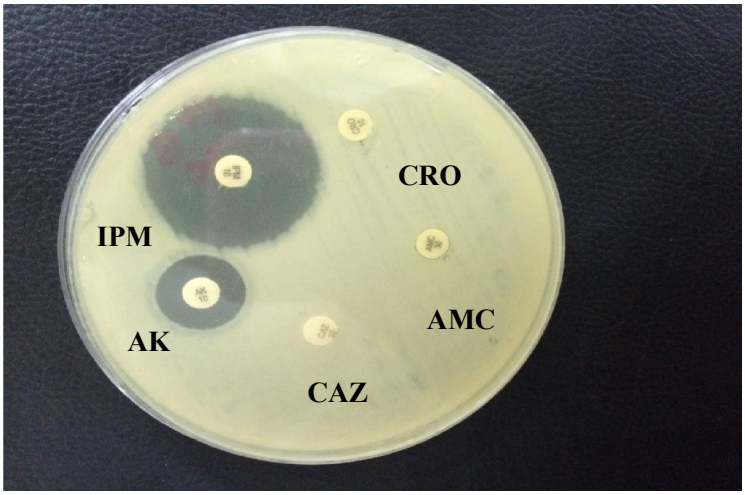

Figure (1): Antimicrobial susceptibility test of *E. coli* O157:H7 isolated from children and buffalos.

IPM: Imipenem; AK: Amikacin; CAZ: Ceftazidime; AMC: Amoxicillin; CRO: Ciprofloxacin.

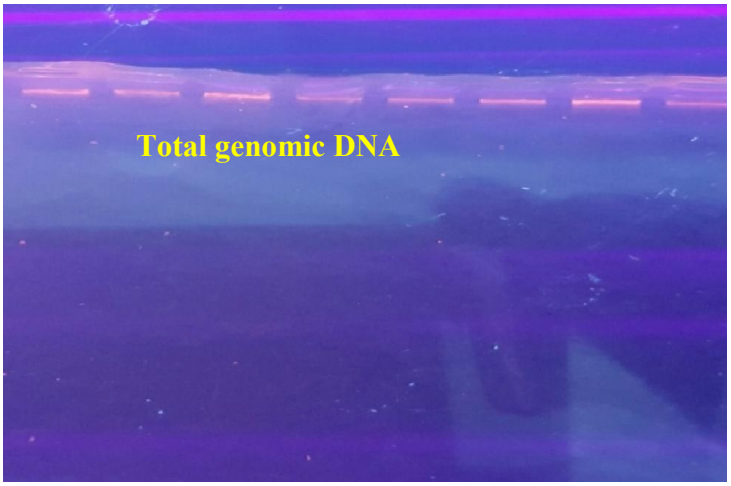

Figure (2): Total genomic DNA extracted from *E. coli* O157:H7 isolates using 1% agarose gel electrophoresis.

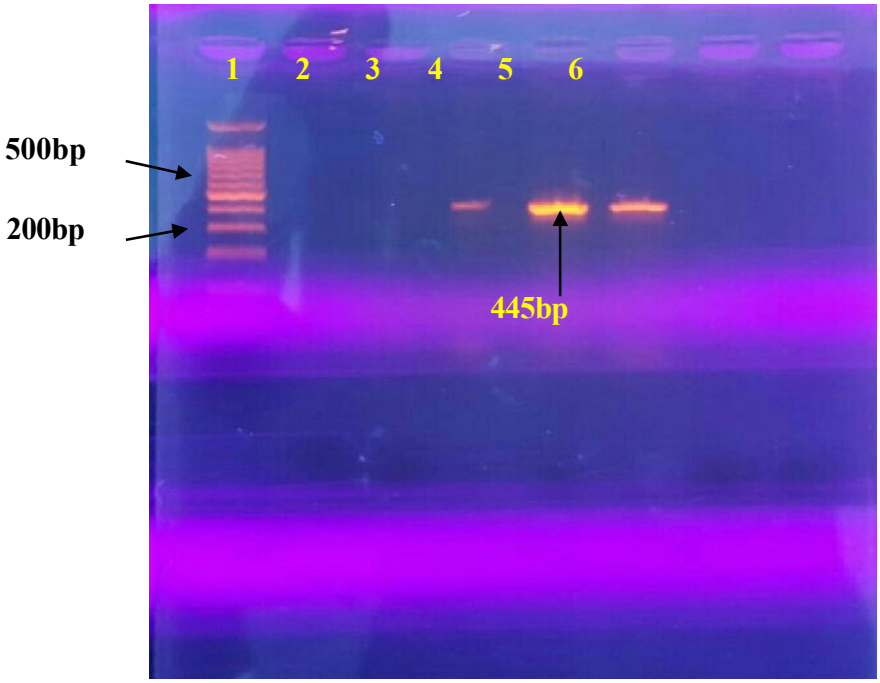

Figure (3): PCR amplification of *tem* gene (445 bp) in *E. coli* O157:H7. Lane 1: molecular size marker; Lanes 4, 5 and 6: *E. coli* O157:H7 *tem* gene isolates.

DISCUSSION

Trypticase Soy broth (TSB) was used as enrichment medium while MacConkey agar and EMB agar were used for selection and isolation purposes. In the present study, 104 *E. coli* isolates out of 250 collected samples were result 62 from children and 42 from buffalo. These results agrees with the results reported by [15 and 16] who found the rates of *E. coli* isolates in stool 38.3% and 38%, respectively. [17] found that the percentage of prevalence of *E. coli* was 59.4%, while [18] found lower rates of *E. coli* isolates in stool 29%.

The occurrence of NSFEC in stool samples which detected by conventional microbiological methods were 9.5% in children. The present occurrence of NSFEC in children stool sample was lower than the result of obtained by [19] who reported 15.6%. It is also lower than the finding of [20 and 21] who detected 57.5% and 73.9% , respectively.

The overall rates of prevalence of NSFEC in all tested samples were found to be 19.2% a factor which accounts for the increased prevalence could be the methodology of isolation employed such as variation in media used for isolation, sampling procedure. In addition, to sows, hooks are not properly cleaned and disinfected.

Multiple antibiotic resistant strains can be transferred from buffalo to children through contaminated food. [22] reported that multiple resistant bacterial strains were transmitted to childrens by raw meat and milk. Cattle feces are a potential source of antibiotic resistant bacteria. If released into the environment, resistant strains may contaminate water and food sources and can be a potential threat to children health [23]. Antibiotic resistance has become a major clinical and public health problem during the lifetime of most people [24]. There are many reasons for this problem, one of which is an over use of antibiotics [25].

The disc diffusion test was used to screening the antimicrobial phenotypes of the four isolates of EHEC O157 : H7. The finding of 100% *E. coli* O157:H7 were resistant to tested

antibiotics cephalothin, amoxicillin and ceftazidime, among the important findings of antimicrobial testing was that 100% of *E. coli* O157:H7 isolates were sensitive to imipenem, gentamycin and 50% was sensitive to ceftriaxone in addition 25% prevalence of intermediate other antimicrobials including ciprofloxacin and tetracycline. The level of resistance was significantly higher than sensitivity. The present result in line with [26] who found *E. coli* O157:H7 that was found to be sensitive to amikacin and ciprofloxacin. On the other hand, it was found resistant to all other antibiotics, ranging from 8.5%-90%. [27] in (1997) study reported multidrug-resistance in *E. coli* with persistent diarrhea in Kenyan children where the isolates were resistant to tetracycline. Another study [28] recorded the highest rates of resistance against amoxicillin, gentamycin, tetracycline and ciprofloxacin (100%) and the most common resistance pattern was for amoxicillin, gentamycin, ciprofloxacin, tetracycline (38.7%) and the least common resistance patterns was for F (3.2%).

The *tem* genes are by far the most widespread with unknown origin [29]. *tem*-1, which is responsible for most of the ampicillin resistance in; 94% of *E. coli* strains isolated in Spain, 89% of *E. coli* strains isolated in Hong Kong, and in 78% of *E. coli* strains isolated in London [30]. However, [31] reported that up to 90% of ampicillin resistance in *E. coli* is due to the production of TEM-1. This enzyme has the ability to hydrolyze penicillins and early cephalosporins such as cephalothin and cephaloridine, TEM -2 β-lactamase is widespread in *E. coli*, although they are much rare than TEM-1.The classical TEM-1 and TEM-2 enzymes have minimal activity against newer cephalosporins [32]. In the past 30 years, however, there have been an increasing emergency of ESBLs, which attack many newer cephems and monobactams as well as third generation cephalosporins and anti-Gram-negative bacterial penicillins (31). Most of these enzymes are mutant of TEM-1 and TEM-2 such as TEM-3, TEM-4, TEM-10, TEM-27, TEM-92 [31]. Although strains that produce ESBL are characteristically resistant to new cephalosporins and/or aztreonam, many strains producing these enzymes appear susceptible or intermediate to some or all of these agents *in vitro*, while expressing clinically significant resistance in infected patients [3].

It was recently reported by [33] in (2011) whom agree with present results which reported presence of *tem* gene in all the 23strains. [34 and 35] founded *tem* β-lactamases genes presence in 50.5% and 57.1 %, respectively.

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الكشف الجزيئي لجينات *tem* المعزولة من جراثيم الاشرشيا القولونية O157:H7 المعزولة من الانسان والحيوان في محافظة البصره

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الخلاصة

خلال فترة خمسة اشهر (اب 2015 - ديسمبر 2015) تم جمع 250عينة ،125 عينة من براز الاطفال الذين يعانون من الاسهال و125 عينة من براز الجاموس من مناطق مختلفة من محافظة البصرة (مركز المحافظة، كرمة علي ، الزبير ، القرنه ، ابو الخصيب). فحصت جميع العينات عن وجود الاشرشيا القولونية و الاشرشيا القولونية النمط المصلي O157:H7. تم الحصول على 104 عزلة (41,6%) من الاشرشيا القولونية المشكوك بها : 42 عزلة من براز الجاموس و 62 عزله من عينات غائط الاطفال. وجد ان 6 من اصل 62 عزلة من براز الجاموس بنسبة 9.5%. و4 عزلة من غائط الاطفال من اصل 42عزلة بنسبة 9.7 % غير مخمرة للسوربيتول. أظهرت جميع العزلات نمط المقاومة المتعددة للمضادات الحيوية. عرضت عزلات الاشرشيا القولونية النمط المصلي O157:H7 المنتجة لإنزيم البيتالاكتاميز لاختبار تفاعل السلسلة المتبلمرة للمورث *tem*. اظهرت النتائج إن 6 عزلات الاشريكيا القولونية معزوله من غائط الاطفال وبراز الجاموس تحتوي على الجين *tem* .

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