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Antimicrobial Resistance Patterns of *Escherichia Coli* O157:H7 Isolated from Stool Sample of Children

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Abstract

Background Enterohemorrhagic *Escherichia coli* is a subset of Shiga toxin-producing *E. coli* that can cause diarrhea or hemorrhagic colitis in humans. Hemorrhagic colitis occasionally progresses to hemolytic uremic syndrome, is a major cause ofacute renal failure in children and morbidity and mortality in adults.

Objective To determine the susceptibility and resistance of the most effective antibiotic to *E.coli* O157:H7 associated with bloody diarrhea.

Methods Two hundred patients with bloody diarrhea were enrolled in this study. *Escherichia coli* were isolated on Sorbitol MacConkey agar with Cefixime and Tellurite and tested by latex agglutination test. The susceptibility and resistance for all bacterial isolates were identified by standard procedures resistance patterns such as disk diffusion test and minimum inhibitory concentration.

Results *E.coli* O157:H7 found in 37 (18.5%) out of two hundred stool samples. The highest rate was found in 18 cases (48.64%) out of 37 infants aged 3-12 months, 12 cases (32.43%) in infants aged 13-24 months, and the lower rates was in children over two years old (18.9%). *E.coli* O157: H7 was completely resistant to gentamicin, ampicillin, nalidixic acid and co-trimoxazole; high rate of resistance to cefotaxime and ceftazidime, moderate-to-low rate of resistance to ciprofloxacin, amikacine, ceftriaxone and Imipenem and no resistances rate to levofloxacin. Out of 37 isolates 29 (78.3%) were β-lactamase producer and 8 (21.6 %) isolates resistant to β-lactamase antibiotic patterns but not produce β-lactamase enzyme.

Conclusion The high-incidence rate of *E. coli* O157:H7 infection in children associated with limited number of drugs effective against *E. coli* O157:H7 with high prevalence of resistance to more than three antibiotics.

Keywords *E.coli* O157:H7, Enterohemorrhagic *Escherichia coli*, disk diffusion test, minimum inhibitory concentration.

List of abbreviation: EHEC = Enterohemorrhagic *Escherichia coli*, Stx = Shiga toxin, HUS = hemolytic uremic syndrome, SMAC-CT = Sorbitol MacConkey Agar with Cefixime and Tellurite, DDT = disk diffusion test, MIC = minimum inhibitory concentration, VTEC = Verotoxin-producing *E. coli*, CLSI = clinical laboratory institute.

Introductions

Enterohemorrhagic *Escherichia coli* (EHEC) is a subset of Shiga toxin (Stx)-producing *E. coli* that can cause diarrhea or hemorrhagic colitis in humans. Hemorrhagic colitis occasionally progresses to hemolytic uremic syndrome (HUS), which is a major cause of acute renal failure in children and morbidity and mortality in adults [(1)]. Humans acquire EHEC O157:H7 by direct contact with animal carriers, their feces, and contaminated soil or water, or via the ingestion of undercooked beef, or other animal products, and contaminated vegetables and fruit. The infectious dose is very low, which increases the risk of disease [(2)]. Verotoxin-producing *E. coli*

(VTEC), including O157:H7 was identified in 1982 as an important human pathogen [3]. Antimicrobial resistance in *Enterobacteriaceae* poses a critical public health threat, especially in the developing countries [4,5] much of the problem has been shown to be due to the presence of transferable plasmids encoding multidrug resistance and their dissemination among different enterobacterial species [6,7]. 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 [8] and/ or cause increased expression of Shiga toxin genes in vivo [9,10].

Methods

Over six months from September 2014 to February 2015 stool samples obtained from children with bloody diarrhea. Two hundred patients whom admitted or outpatients to Central Child hospital, Abu-Ghraib hospital or from private clinic in Baghdad were enrolled in this study; patient's selections were restricted to those who had bloody diarrhea because *E.coli* O157 was mostly associated with this clinical feature. Stool samples were divided into two portions. One portion for the direct stool examinations and the second portion was inoculated in tetrathionate broth for 24 hr at 37^{o}C, and then inoculated in MacConkey agar for isolation and identification of lactose fermenter *E. coli* bacteria. *E. coli* was isolated by sub-cultured on Sorbitol MacConkey Agar with Cefixime and Tellurite (SMAC-CT). This media considered as selective and differential medium for the detection of *Escherichia Coli* serotype O157:H7 which prepared as follows: peptone (20gm), Bile salts (1.5gm), Sodium chloride (5gm), Neutral red (0.03 gm), Crystal violet (0.001 gm), D-Sorbitol (10gm), Cefixime (0.05 mg), Potassium Tellurite (2.5 mg) and Agar (15 gm). These ingredients were suspended in one litter distilled water; PH was adjusted to (7.1) sterilized at 121 ^{o}C for 15 minutes, left to cool before pouring into plates [11,20].

SMAC-CT is modified MacConkey Agar using sorbitol instead of lactose with cefixime and tellurite added. Cefixime inhibits *Proteus* spp. and tellurite inhibits non-O157 *E. coli* and other organisms, thus improving the selectivity of SMAC-CT for *E. coli* O157:H7. Differentiation of enteric microorganisms is achieved by the combination of sorbitol and the neutral red indicator. Colorless or pink to red colonies are produced depending upon the ability of the isolate to ferment the carbohydrate sorbitol, *E. coli* O157:H7 considered as sorbitol negative [11]. For confirmatory identification of *E.coli* O157:H7 Latex agglutinations test for *E.coli* O157:H7 (OXOID-England) were also used in current study. The stranded isolates from central public health laboratory *E.coli* ATCC25922 used as anegative control.

Acidimetric test for β -lactamase production by tube method

β-lactam ring Hydrolysis lead to carboxyl group formations, acidity resulting can be tested in tubes or on filter papers. For the tube method, 2 mL of 0.5% (w/v) aqueous phenol red solution is diluted with 16.6 mL distilled water and 1.2 g of benzyl penicillin is added. The pH is adjusted to 8.5 with 1 M NaOH. The resulting solution, which should be violet in color, can be stored at -20^{o}C. Before use, 100 µl portions are distributed into tubes or microtitre wells and inoculated with bacteria from culture plates (not broth) to produce dense suspensions. A yellow color within 5 min indicates β-lactamase activity. Positive and negative controls must be run in parallel [12].

Antimicrobial susceptibility tests

Resistance patterns of *E.coli*O157:H7 to various selected antibiotics were determined by disk diffusion test (DDT) and minimum inhibitory concentration (MIC). The following antimicrobial discs were tested: cephalexin (30µg), cefotaxime (30µg), ceftriaxone (30µg), amikacine (30µg), gentamycin (10µg), imipenem (10µg), ampicillin (10µg), co-trimoxazole (25µg), ciprofloxacin (5µg),

levofloxacin (10µg), nalidixic acid (30µg), when the incubation was complete, the diameter of the inhibition zone around the disks was measured and compared with the break points of clinical laboratory institute (CLSI) [13]. The MIC was performed by a standard agar dilution method and has been applied for determination the lowest antibiotics concentration that inhibits growth of *E.coli* O157:H7. Stock solutions of each antibiotic at concentrations of 10 mg/ml, 1mg/ml, and 0.1 mg/ml; then two fold dilutions from 512-0.5 µg/ml for all antibiotics were prepared. Muller Hinton agar medium was prepared, sterilized by autoclaving, after cooling, 25 ml were added to each antibiotic container; the content mixed well and poured into petridishes. The inoculum density was adjusted by using 0.5 McFarland standard tubes and then 20 microliters of each inoculum were spotted on the agar surface of Muller Hinton agar medium and incubated at 37°C for 24 hr [12]. After incubation ensure that all of the organisms have grown on the antibiotic-free control plate and observed the growth of bacteria on Muller Hinton agar plate for each antibiotic dilution [12].

Results

Two hundred patients with bloody diarrhea were enrolled in this study, *E. coli* O157:H7 was found in 37 (18.5%) patients, the highest rate were in infants aged (3-12) months, which were 18 cases out of 70 (25.7%) and in (13-24) months, which were 7 cases out of 42(16.7%) and the lowest rates was in children over two years old (13.6%) out of 88 (Table 1).

Table 1. Age distribution among patients with E. coli O157:H7 isolates

Age group (months)	Escherichia coli		
	Positive No. (%)	Negative No. (%)	Total
3-12	18 (25.7)	52 (74.3)	70
12-24	7 (16.7)	35 (83.3)	42
>24	12 (13.6)	76 (86.4)	88
Total	37 (18.5)	163 (81.5)	200

In this study all isolates of *E. coli* O157H7in (SMAC-CT) media gave positive reactions with *E. coli* O157:H7 latex agglutination test.

Antibiotics resistance of *E. coli* O157 H7

Regarding antibiotics resistance by disc-diffusion method, results in current study revealed that *E. coli* O157: H7completely resistance to gentamicin, ampicillin, nalidixic acid and co-trimoxazole, high resistance to cefotaxime and ceftazidime, moderate-to-low resistance to ciprofloxacin, amikacine, ceftriaxone and imipenem. No resistances rate to levofloxacin was observed in this study (Table 2).

Table 2. Number and percentage of resistance *E. coli* O157: H^7 isolates against different antibiotics

Antibiotics	RI	SI	R %
Gentamicin	37	0	100
Ampicillin	37	0	100
Nalidixic acid	37	0	100
Cefotaxime	20	17	54
Amikacine	25	12	67.5
Ceftriaxone	15	22	40.5
Ceftazidime	18	19	48.6
Ciprofloxacin	17	20	45.9
Leafofloxacin	0	37	0
Imipenem	5	32	13.5
Co-trimoxazole	37	0	100

RI = Resistance isolates, SI = Sensitive isolates, R = Resistance

Minimum inhibitory concentration of *E. coli* O157: H7 isolates

The MIC of all antibiotic used in this study were determined by an agar dilution method to measures more exactly the concentration of an antibiotic necessary to inhibit growth of a standardized inoculums under defined conditions [13]. *E. coli* O157: H7was characterized as resistant if the MIC was greater than the breakpoint MIC defined by CLSI while it will be susceptible if it is less than the MIC [13].

MIC results revealed that the resistance pattern of 37 isolates to cefotaxime were as follows: 17 isolates were highly resistant with MIC (512 µg/ml), nine isolates with MIC (256 µg/ml), six isolates with MIC (128 µg/ml), five isolates with MIC (64 µg/ml). MIC of ceftriaxone shows eleven isolates had MIC (256 µg/ml), seven isolates with MIC (128 µg/ml), ten isolates with MIC (64 µg/ml), nine isolates exhibiting high level resistance with MIC (512 µg/ml). MIC of ceftazidime shows three isolates had MIC (256 µg/ml), six isolates (128µg/ml), and twenty isolates had MIC (32 µg/ml).

Imipenem MIC determination shows that two isolates exhibiting highly resistance (512 µg/ml), one isolates had (64 µg/ml), and two isolates (32 µg/ml). MIC respectively determination of MIC to ciprofloxacin reveals that five isolates with MIC (512 µg/ml), seven isolates with MIC (256 µg/ml), two isolates with (128 µg/ml), and three isolates with MIC (16 µg/ml). The results of MIC against Levofloxacin revealed that only one isolates of *E. coli* O157: H7 exhibited less susceptibility with MIC (2 µg/ml), MIC resistant isolates to gentamycin, ampicillin, nalidixic acid and co-trimoxazole reveals completely resistance to this drug. MIC to amikacine showed 25 isolates were highly resistance rate with MIC (512 µg/ml).

Regarding β-lactamase production, current study found that 29 out of 37(78.3%) were β-lactamase producer, and there was 8 (21.6 %) isolates resistance to β-lactamase antibiotic patterns but not produce β-lactamase enzyme which it gave negative results in this test.

Discussion

E. coli O157:H7 recognized as major etiologic agents of two life threatening complication in humans, hemorrhagic colitis and HUS makes *E. coli* O157:H7 infections a public health problem [(14)]. The present study showed that the risk developing of bloody diarrhea caused by *E. coli* O157:H7 was in the first two years of life and the lowest rate was in children over two years of life. Results in the present study are in agreement with previous studies by Baqir *et al* [(15)] and it was in disagreement with finding of Trung *et al* [(16)] who found that *E. coli* O157:H7 was not found in any of the samples of children under two years old. The new medium (TC-SMAC) gave substantial suppression of non-O157 strains and also inhibited non sorbitol fermenting (NSF) bacteria such as proteus species [(17,18)].

Results in current study showed that all isolates on SMAC-CT gave positive results with latex agglutination test, there for the sensitivity of the SMAC-CT media was estimated to be 100% compare with latex agglutination test. This result corresponded with results mentioned by Aseel *et al* [(19)]; Zadik *et al* [(20)]. Result in the current study revealed that out of37 isolates 29 (78.3%) were β-lactamase producer isolates were resistant to β-lactam drugs, high incidence of β-lactamase- producing *E. coli* O157:H7 was previously reported by Israa *et al* [(21)] and Panus *et al* [(22)]. The reminder isolates 8 (21.6 %) resistance to β-lactamase antibiotic patterns but not produce β-lactamase enzyme. The possible explanation for such results is the presence of other mechanisms that make *E. coli* O157:H7 resistant to β-lactamase antibiotic patterns such as efflux pump, alteration of an antibiotic target protein, penicillin-binding protein 2 and reduced antibiotic penetration is also a resistance mechanism for several classes of antibiotics [(23)].

The antimicrobial sensitivity tests showed that *E. coli* O157:H7 isolates were fully resistant to gentamicin, ampicillin, nalidixic acid and co-trimoxazole, a result which quite accord with studies done by Israa *et al* [(21)] and Daniel *et al* [(24)]. The resistance patterns of *E. coli* O157:H7 to several antibiotics were as follows: cefotaxime (54%), amikacine (67.5%), ceftriaxone (40.5%), ceftazidime (48.6%), ciprofloxacin (45.9%). The possible explanation to high level of resistance to this drug may be as a result of it being the most commonly available antibiotic used as a routine therapy among gastrointestinal infections and people readily purchase it across the counter for self-

medication in last years. This could be a reflection of use and misuse of these antibiotics in the society. This finding as a result to fact that outside the hospital environment the general population have easy access to various antibiotics from any pharmacy without prescription from a doctor.

In this study, the majority of *E. coli* O157:H7 isolates showed multidrug resistance to the antibiotics at various percentages. This result is in agreement with the findings by other researchers, who reported multidrug resistance among *E. coli* O157:H7 isolates [25,26].

The development of antimicrobial resistance by the *E. coli* O157:H7 isolates to these drugs poses a major challenge in both human and animal medicine because these drugs are commonly used in the treatment of human patients and in veterinary practice [27].

Results from this study indicate that imipenem and leafofloxacin had high sensitivity since few of the isolates was resistant to them and that might be attributed to the recent introduction of this agent for the treatment of infection in both human and animals in Iraq.

In conclusion, our study made it evident that high incidence rate of *E. coli* O157:H7 infection in children associated with limited number of drugs effective against *E. coli* O157:H7 with high prevalence of resistance to more than three antibiotics, this may provide an evidence for antimicrobial abuse and should be attract more attention about control strategies and used of antibiotic in human and veterinary medicine.

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Conflict of interest

The author declares no competing interests.

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