



Prevalence of Multidrug Resistant *Escherichia coli* O157:H7 Causing Diarrhoea in Children at District Khairpur

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Authors' contributions

This work was carried out in collaboration among all authors. Author SL conceptualized the study and designed the methods. Author SJ collection and assembly of the data. Authors SJ, SL and OP analysis and interpretation of the data. Authors SL, PK, AMA, SAU and Sapna drafting of the article. Authors SL, ZA and MM critical revision of the article for important intellectual content. Authors SL and PK did the statistical expertise. Author SL did the final approval and guarantor of the article.

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ABSTRACT

Aim: Multidrug resistance (MDR) is a major public health problem worldwide. The aim of this study was to assess the prevalence, levels of antimicrobial susceptibility and extended-spectrum beta lactamase (ESBL) production by *Escherichia coli* O157: H7.

Methodology: A cross sectional study was performed and a total of 116 stool samples were collected from children aged ≤ 5 years presenting diarrhoea from hospital located at district

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Khairpur. *E. coli* O157: H7 was isolated on Sorbitol MacConkey agar and isolates were initially characterized by Gram staining. Subsequently biochemical characteristics except oxidase test (which was performed manually) and drug sensitivity test was performed by using Automated MicroScan® walkaway machine. ESBL production by isolates was determined by disc diffusion method as per Clinical & Laboratory Standards Institute (CLSI) guidelines.

Results: Out of 116 diarrheal samples tested, 16 (13.79%) samples were positive for *E. coli* O157:H7. High number of *E. coli* O157: H7 were isolated from children aged up to 1 year followed by 2-3 years aged. Isolates were frequently found in summer season (12 times) followed by winter (3 times) and Autumn (1 time). The isolates were found 100% resistant against Nalidixic acid, Imipenem, Amoxicillin /Clavulanic Acid, Norfloxacin, Chloramphenicol, Vancomycin, Doxycycline, and Ampicillin and 90% resistant to Cifixime. ESBL production was noted in 12 (75%) out of 16 *E. coli* O157:H7 isolates as confirmed by CLSI protocol.

Conclusion: This study revealed that *E. coli* O157: H7 should be considered as major pathogenic bacterium causing diarrhea in children less than three years. The prevalence of ESBL producer MDR *E. coli* O157: H7 is of great concern, which requires monitoring of infection control measures through efficient antimicrobial management to ensure an uncompromised public health.

Keywords: Prevalence; Multidrug resistance; antibiotics; ESBL.

1. INTRODUCTION

Diarrhoeal disease is one of the leading causes of mortality among children of less than five years age [1]. It is accountable for the mortality greater than 1,400 children each day [1]. This disease is particularly prevalent in developing countries where sanitation and hygiene conditions are very poor and safe drinking water is scarce. Bacteria especially *Escherichia coli* is major contributor in causing enteric infection in children. There are various pathogenic strains of *E. coli* linked with diarrhoea, known as diarrhoeagenic *E. coli*. These include Shiga toxin-producing *E. coli* (STEC) which is also known as Verocytotoxin-producing *E. coli* (VTEC) or enterohemorrhagic *E. coli* (EHEC), Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAEC), Enteroinvasive *E. coli* (EIEC) and Diffusely adherent *E. coli* (DAEC) [2]. EHEC serotype *E. coli* O157:H7 is accountable for 20% of epidemic diarrhea worldwide [3]. This serotype is responsible for causing hemolytic uremic syndrome (HUS) in humans. HUS causes hemolytic anaemia, thrombocytopenia and renal failure by causing bloody diarrhoea and extreme abdominal pain, which leads to the formation of clots in capillaries. Rehydration, fever, and pain relief are the only medications available to treat this infection [4-6].

Multidrug-resistant *E. coli* have emerged as a major public health issue around the world [7] and due to the effectiveness of the outer membrane (OM) as a barrier and

multidrug efflux pumps, the *E. coli* O157:H7 strain has much higher intrinsic levels of resistance to different antibiotics. Efflux pumps (EPs) decrease OM permeability, lowering antibiotic absorption and resulting in drug resistance [8]. EPs affects almost all antibiotic groups, including Macrolides, Tetracyclines, and Fluoroquinolones. In addition, the *E. coli* O157:H7 also produce extended-spectrum beta-lactamases (ESBLs), which render them resistant to many antibiotics and these strains have been widely reported in recent years on a global scale [9]. Indeed, Enterobacteriaceae that produce ESBLs are linked to higher rates of morbidity and mortality, longer hospital stays, and higher healthcare costs [10]. According to previous reports ESBL have been related to the development of antibiotic-resistant bacterial strains and due to the spread of these strains, antimicrobials used to treat patients with pathogen bacteria such as *E. coli* are likely to become less successful [11]. Since antimicrobial resistance among enteric bacteria is an increasing global public health concern which limits the treatment options for bacterial infections. Furthermore, antimicrobial resistance also has negative effects on human health and the environment, this study was designed to evaluate prevalence of multidrug resistant *E. coli* O157:H7 causing diarrhoea in children of District Khairpur, Sindh, Pakistan. In addition to this, levels of antimicrobial susceptibility and extended-spectrum beta lactamase (ESBL) production by *E. coli* O157: H7 was also assessed.

2. MATERIALS AND METHODS

2.1 Study Design and Study Area

A cross-sectional study was conducted from December 2019 to December 2020 to evaluate prevalence of multidrug resistant *E. coli* O157:H7 causing diarrhoea in children of District Khairpur, Sindh, Pakistan. Sampling was carried out over a period of 12 months to cover all the four seasons. Children aged ≤ 5 years and presenting severe diarrhoea and admitted to hospital Pir Abdul Qadir Shah Jeelani Institute of Medical Sciences Gambat Khairpur (PAQSJIMS) were included in this research. The patients who had taken antibiotics in last 72 h and critically ill patients were excluded from this study.

2.2 Sample Size and Collection of Fecal Samples

Sample size was determined using the formula reported previously [9]. A total of one hundred sixteen (116) stool samples were collected from both male (58) and female (58) patients equally whose parents gave consent. Rectum stool swab samples were collected and placed in a Cair Blair transport media. Subsequently these were transported to the Microbiology research laboratory of PAQSJIMS at 4°C in polystyrene cool box [12]. A data regarding the age, area, water source in households, feeding stage and antibiotics usage was collected.

2.3 Isolation of *E. coli* O157:H7

Stool swab samples were inoculated onto Sorbitol containing MacConkey agar plates and incubated aerobically at 37°C for 24 hrs [12]. Subsequently light-yellow colored colonies (a characteristic of *E. coli* O157:H7 by fermenting sorbitol, a carbohydrate) were sub-cultured on Eosin Methylene Blue (EMB) agar, and then incubated aerobically at 37°C for 24 hrs. The green metallic sheen colonies appearing on EMB were picked and sub-cultured to nutrient agar for obtaining pure cultures [12,13].

2.4 Characterization and Drug Sensitivity Test of *E. coli* O157:H7

E. coli O157:H7 isolates were initially characterized by Gram staining. Subsequently, biochemical characteristics except oxidase test

(which was performed manually by soaking a filter paper piece with 1% tetramethyl-p-phenylene-diamine dihydrochloride and then inoculating culture with sterilized stick on moist area of filter paper) and drug sensitivity test was performed by using Automated MicroScan® walkaway machine [12-14]. Antibiotics tested were Amikacin (AK), Amoxicillin /Clavulanic Acid (AMC), Ampicillin (AMP), Aztreonam (ATM), Cefepime (FEP), Ceftazidime (CAZ), Cefotaxime (CTX), Ceftazidime (CAZ), Chloramphenicol (C), Cifixime (CFM), Ciprofloxacin (CIP), Doxycycline (DO), Imipenem (IMP), Levofloxacin (LEV), Nalidixic acid (NA), Norfloxacin (NOR), Piperacillin/Tazobactam (TZP), Trimethoprim/Sulfamethoxazole (SXT), Vancomycin (VA). The results for drug sensitivity test were recorded as susceptible, intermediate, and resistant.

2.5 Molecular Identification of *E. coli* O157:H7 Isolates

16SrRNA gene based molecular identification was performed for confirmation of *E. coli* O157:H7 isolates. For this samples were sent to MacroGen Company (Korea) for commercial analysis. After obtaining sequencing results data was analyzed using NCBI Blast available free online.

2.6 Extended Spectrum Beta Lactamase (ESBL) Production by Isolates

Since Microscan machine indicated/assumed the ESBL production by majority of isolates, a phenotypic test as per CLSI guidelines was performed and for this test both cefotaxime (CTX, 30 µg) and ceftazidime (CAZ, 30 µg) disks alone and in combination with clavulanate (CA, 30µg) (Oxoid) were placed on Mueller Hinton Agar plates containing *E. coli* O157:H7 bacterial lawn. The test was interpreted positive when the growth-inhibitory zone around either the CTX with CA or CAZ disk with CA was ≥ 5 mm [15].

2.7 Statistical Analysis

Where required the samples were processed in triplicates and results were declared in average \pm standard deviation. The Chi square test was performed to detect the significant difference in male and female by using XLSTAT365-Freemium.

3. RESULTS

3.1 Prevalence of *E. coli* O157:H7

Out of 116 diarrheal samples tested, 16 (13.79%) samples were positive for *E. coli* O157:H7 as confirmed by cultural characteristics, microscopic characteristics, Biochemical characteristics (Table 1, Fig. 1) and 16 SrRNA sequencing (Fig. 2).

3.2 Demographic Analysis

Out of 116 samples tested (Males: 58, Females 58), 9 (56.25%) *E. coli* O157: H7 strains were isolated from males and 7 (43.75%) from females. (Table 2). The Chi square test did not show significant difference in gender groups (P=0.06).

3.3 Seasonal Distribution of *E. coli* O157:H7

E. coli O157: H7 (Total isolates=16) were isolated more frequently in summer season (12 times) followed by winter (3 times) and Autumn (1 time).

3.4 Antibiotic Resistance Pattern

All the bacterial isolates (N=16) were resistant to most of the antibiotics tested in this study. Phenotypic resistant profile for isolates is shown in Fig. 3.

3.5 ESBL Production

ESBL production was noted in 12 (75%) out of 16 *E. coli* O157:H7 isolates as confirmed by CLSI protocol (Fig. 4).

4. DISCUSSION

Diarrhoeal illness is one of the most important health related problem globally. More than 2 million of mortality happens from diarrhoea among children of below five years age especially in developed countries [16]. *E. coli* is most frequent contributor in causing diarrhea in children. The result of this study revealed high prevalence of *E. coli* O157:H7 in the sample studied, corresponding to children ≤5 years age. These results are almost similar to some studies [17-19] and higher compared to developed countries [20,21].

Table 1. Cultural, microscopic and biochemical characteristics for confirmation of *E. coli* O157:H7

	Characteristics	Result
1. Cultural characteristics	Colonial characteristics on EMB agar	Green metallic sheen colonies
	Colonial characteristics on Sorbitol-MacConkey agar	Colorless colonies (Indicative of Sorbitol negative)
2. Microscopy	Gram staining	Gram Negative, rods
	Motility	Positive
3. Biochemical tests	Catalase	Positive
	Oxidase	Negative
	Indole	Positive
	Methyl red	Positive
	Voges Proskauer	Negative
	Citrate	Negative
	Urease	Negative
	Nitrate reduction	Positive
3.1. Sugar fermentation tests	Glucose	Positive
	Maltose	Positive
	Lactose	Positive
	Sorbitol	Negative

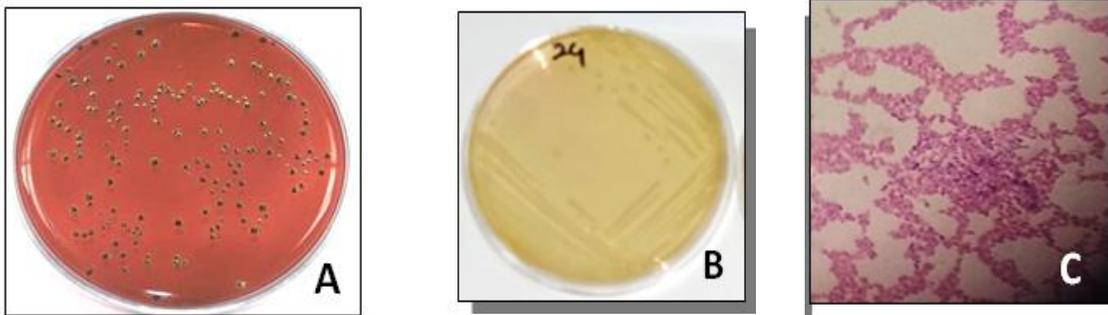


Fig. 1. Cultural and microscopic characteristics of *E. coli* O157:H7: A) Green metallic sheen on EMB agar; B) Colorless colonies on Sorbitol-MacConkey agar; C) Gram negative rods

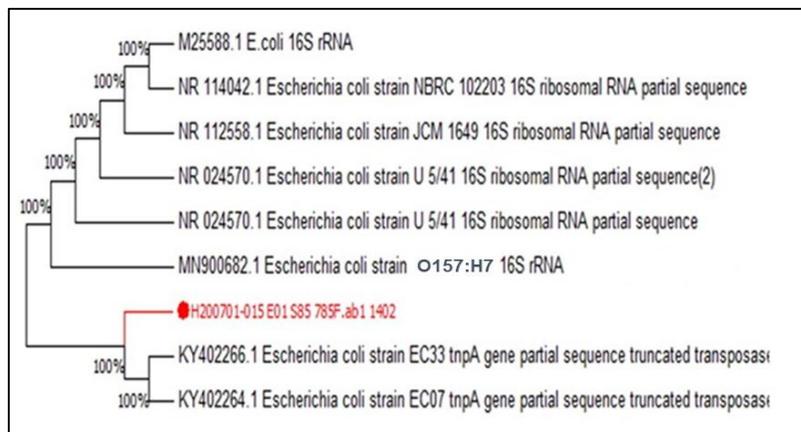


Fig. 2. Phylogenetic tree showing resembling of isolated strain with *E. coli* O157:H7

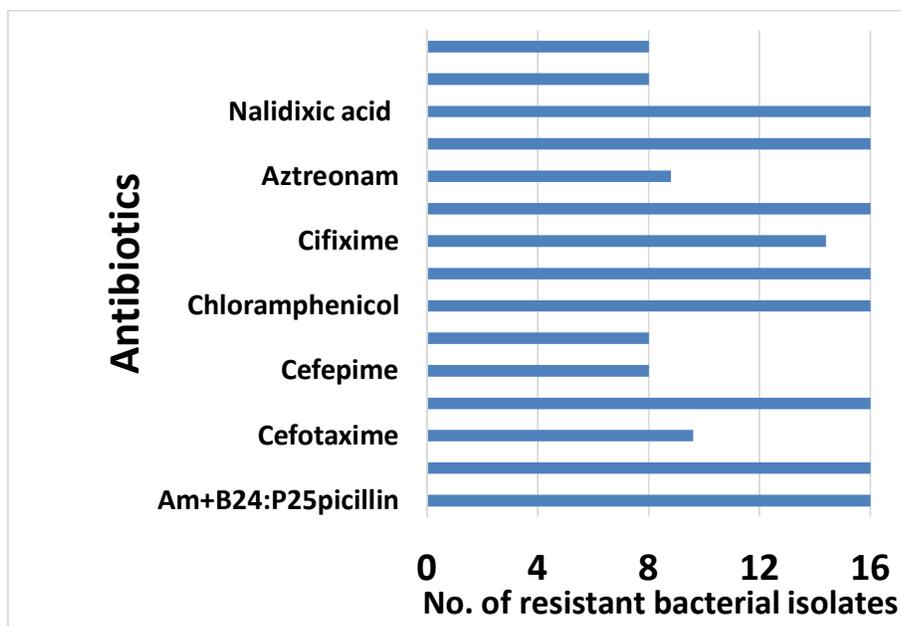


Fig. 3. Antibiotic susceptibility pattern of isolated *E. coli* O157:H7 strains

Table 2. Age and gender wise distribution of *E. coli* O157: H7 isolates

Age group (Years)	Male	Female	Total	(%) Prevalence
0-1	06	04	10	8.62%
2-3	03	03	06	5.17%
4-5	0	0	0	0
Total	09	07	16	13.79%

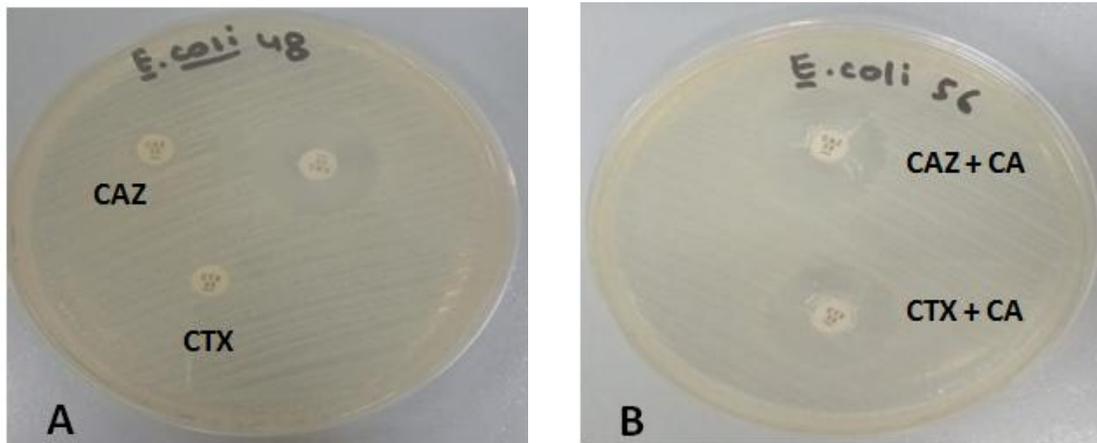


Fig. 4. ESBL confirmatory test: A) CTX and CAZ alone with no zone of inhibition; B) CTX + CA and CAZ + CA with zone of inhibition > 5 mm which confirms the ESBL production

In this study, when age groups were compared, it was noted that children with age < 1 year were more susceptible in getting infection with *E. coli* O157: H7. Increased age decreased the chances of getting infection. The low incidence among elder children must be linked with developed immune system with the increase of age [21]. Previous studies have also reported *E. coli* O157:H7 as the major pathogen causing childhood diarrhoea below two years age in developed countries [22,23]. Although the difference in gender group was observed but it was not significant. This could be because there is no difference in social contact of children and growing children do not have much hormonal difference that could have effect in causing enteric disease.

Season wise distribution of *E. coli* O157:H7 in this study revealed that the infection caused by this organism was more disseminating in summer followed by winter season. A similar pattern has been noted in previous studies [24,25], representing that *E. coli* O157:H7 was associated with ecological factors including heat and moisture.

In this research 100% resistant among *E. coli* O157: H7 was observed against NA, IMP, AMC,

NOR, C, VA, DO, AMP and 90% resistant to CFM.

ESBLs production have been noted in *Enterobacteriaceae* since last few decades. Infections caused by such bacteria often limit the treatment options and thus cause therapeutic failures. Thus, CLSI has suggested that a phenotypic confirmatory test for ESBL development be performed to accurately detect ESBL production. In this study this test was performed to detect the ESBL production by *E. coli* O157: H7 and results revealed the presence of ESBL producer (75%) bacterial isolates. These results support the studies conducted in other parts of world [26,27]. The prevalence of multidrug resistance among bacteria and production of ESBL production is alarming. Physicians in Pakistan should pay attention to these results since *E. coli* is still one of the most common pathogens associated with infectious diarrhea in children.

5. CONCLUSION

The high prevalence of *E. coli* O157: H7 in this study is worrisome, and it emphasizes the fact that this organism is significant cause of infectious diarrhea in children less than three

years in Khairpur region. In addition, high resistance among isolates to commonly used antibiotics for treating diarrhea is alarming which requires routine laboratory identification of pathogen(s) and antibiotic susceptible testing. Active surveillance is also required in devising strategies to counter multidrug resistance in diarrheal cases of this region of Pakistan.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

A written consent from patient attendants has been collected and preserved by the authors.

ETHICAL APPROVAL

Ethical approval from medical superintendent of PAQSJIMS and Research Ethics Committee of our Institute was obtained.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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