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Detection of Non O-157 Shiga Toxin-Producing *Escherichia coli* from Cases of Diarrhea Children in Iraq

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Abstract: The present work aimed to investigate the non O-175 STEC distribution in Baghdad's hospitals. From different hospitals in Baghdad between November 2022 and November 2024, the samples were taken from children aged 1-5 years. Different selective media were used to retrieve STEC colonies. Results of 21 out of 375 human diarrheal samples came from the pathogenic isolates derived from chromagar. The diagnosis of non-O157 STEC was then confirmed by means of biochemical assays after a single colony from the growth was stained with Gram stain. Targeting the *wzx* (O gene) for serotyping (wzxO26, wzxO45, wzxO103, wzxO111, wzxO121, and wzxO145) using PCR helped to validate the serotypes thereafter. According to PCR findings, fifty seven of 375 (15.20%) samples taken from children had non-O157 STEC. The serotyping revealed O26 presence in ten out of 57 (19%) of children's samples. Serotype O111 was discovered in 6 out of 57 (11%), and O145 in 2 out of 57 (4%) of the juvenile samples. In both scenarios the O45 and O121 came out negative. Among non-O157 STEC, STEC O26 became the most common serotype in children under five years old suffering bloody diarrhea than in those with watery diarrhea.

Keywords: STEC, Wzx, *Stx, Eae* Gene, E. Coli.

Research Paper

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INTRODUCTION

Shiga toxin-producing E. coli (STEC) represent a significant category among the various pathogenic strains of E. coli, classified into two serogroups: O-157 and non-O157 STEC. Examining and analyzing STEC bacteria has been increasingly important in recent years since they are a major factor for food poisoning outbreaks leading to serious infections (Stromberg., 2015). Renal damage is noted, same as hemolytic uremic syndrome, hemorrhagic colitis, and thrombotic thrombocytopenic purpura. The "top six non-O157 STecs are STec O157, O26, O103, O111, O145O, and 121."Globally, non-O157 serotype infections are now more common (EFSA and ECDC, 2014). Recent studies from Iraq show that infections from humans and calves are isolated (Jarad, 2016; Ramadhan, 2017). The reports have been released. Children of both sexes showed the highest observed frequency (Rehman, 2014). The infection happened in the summer and winter, aligning with cattle's higher STEC shedding rate (Dewsbury *et al*., 2015). Especially stx toxin and eae, the degree connected to pathogenic elements is remarkable. The cattle are acknowledged as the primary source for human infections. Small ruminants, birds, and pigs among other species have STEC isolates in their feces. Along with person-to--person and animal-to-- human contacts, STEC transmission occurs by means of contaminated food or water (Conrad *et al*., 2016). One important quality of bacteria is their ability to survive outside the intestines. Additionally, STEC demonstrate tolerance to low acidic conditions. Additionally, both low and high temperatures can lead to the ingestion of a limited number of bacteria, which may result in significant pathogenic effects in humans. STEC strains are often under recognized in laboratory settings, much like other nonpathogenic strains, due to their fermentation of sorbitol. Currently, there is no standardized examination for STEC strains based on gold standards. Molecular techniques—especially traditional PCR and real-time

PCR systems have lately been used to identify the stx1, stx2, eae genes, and other virulence factors.

MATERIAL AND METHODS

Samples Collection

From both sexes housed at Alawi Children Hospital, Ibn AL-Bladi Hospital for Children, Fatima EL-Zahraa Hospital, AL-Sadar Hospital, AL-Imam Ali Hospital, Child Protection Hospital, and Baghdad General Hospital, 375 diarrhea stool samples that were crimson and watery were gathered during 2 years of work. Samples were taken in soy broth using sterile swabs on a transport media and incubated for 18 to 24 hours at 42°C.

Bacterial Isolation and Cultivation

The samples were cultivated on selective media including EMB agar, TC-SMAC agar, TC-RMAC agar, SMAC-BCig, and chromogenic STEC, producing Chrom O175 agar. The samples were biassed in order to find STEC colonies: tests on the chrom O-157 agar including the IVIMIC test, urea, motility Simmons citrate, and colonies displaying mauve hue. found using PCR study.

Molecular Detection of Non-157 Shiga Toxin *E.Coli*

DNA Extraction

Using (PrestoTM Mini g DNA Bacteria Kit Geneaid. USA), genomic DNA of non-O157 STEC isolate was obtained. As stated per the manufacturer's directions.

Table 1: Primers used in this study and their designer

No	Primers Target Gene	Direction	Primer sequences	Size (bases	Reference
1	*WzxO26*	F R	CAATGGGCGGAAATT TTA GA ATAATT TTC TCT GCC GTC GC	55bp	DebRoy *et al.,* *Et al.,* (2011)
2	*WzxO45*	F R	TGCAGT AAC CTGCAC GGGCGAGC AGGCACAACAGC CACTACT	238bp	
3	*WzxO103*	F R	TTGGAGCGTTAACTGGACCT GCTCCC GAGCACGTA TAAAG	321bp	
4	*WzxO111*	F R	TGTTTC TTC GATGTT GCGAG GCAAGGGACATAAGAAGC CA	438bp	
5	*WzxO121*	F R	TCCAACAATTGGTCGTGAAAAGAAG TGT GAA ATG CCC GT	682bp	
6	*Wzx0145*	F R	TTCATTGTTTTGCTTGCTCGGGC AAG CTT TGG AAA TGA AA	750bp	

The amplified DNA products derived from separate microorganisms. Specific-PCR were seen by UV recommendation gel after being electrophoresis on 1% agarose gel stained with 2 µl red safe stain of 20000 u/ml. Depending on the DNA marker a 100–10000 bp DNA ladder.

PCR-Detection of O-Serogroups (WZX)

In order to detect O-serogroups associated with non-O157 STEC, targeting the wzx (O) genes according to DebRoy *et al.* (2011). Under Taq DNA polymerase, primers in table (1), (1 µl of each forwards and reversed), were mixed with 5 µl of master mix, 3 µl of template DNA, 15 µl of nuclease free water to complete the amplification mixture tube to 25µl. relocated to Applied Biosystems/USA's thermocycler. Then commenced the amplification program. Initial denaturation at 95C for 10 min, then 35 cycles of denaturation at 94C for 30 sec, primer annealing at 57C for 30 sec followed by extension at 72C for 1.5 min, and a last extension for 10 min at 72C. Two percent agarose electrophoresed the amplified DNA. Then the product was revealed on 2% Agarose gel and visualized by Gel Documentation system under UV light 336 nm.

RESULTS

After 24 hours at 37˚C, the results of pathogenic isolates from chrom agar were 21 out of 176 human diarrhea samples and produced blue color colonies. Which carried out the biochemical test and compiled for a PCR analysis Tables (5), (6) and pictures (1), (2), (3), (4), (5) revealed the molecular results of *wzx* serogroubs and The Percentage of pathogenic genes (*stx1, stx2, eaeA*) among non-O157 STE serogroup.

Table 2: Detection of *wzx* strain

Strains	Children samples	
	Watery diarrhea	Bloody diarrhea
O26	4	6
O111	2	4
O113	0	
O45	0	
145	1	3
O121	0	

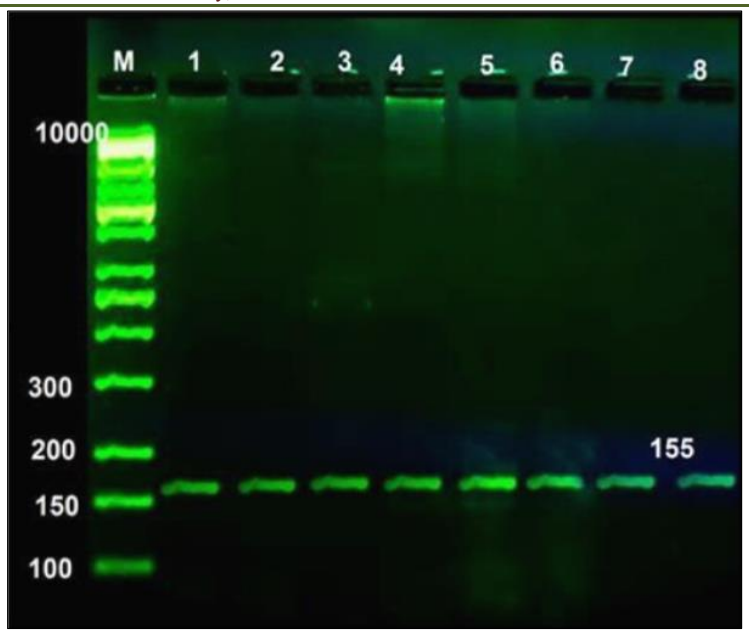

Figure 1: AgarosegelelectrophoresisimageresultsofwzxO26genebandofSTECisolates.Using 2% Agarose stained with red safe dyes and electrophoresed by 5 vol/cm in TBA and lanes (1- 8) showed band size (155bp bp) M: marker (100 bp)

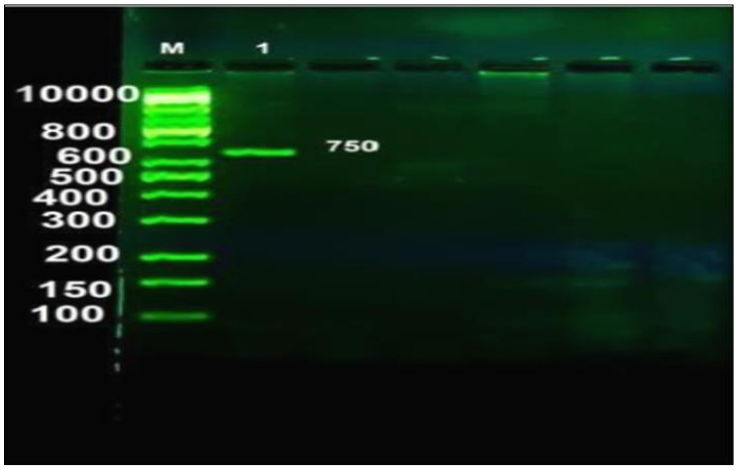

Figure 2: Agarose gel electrophoresis image results ofwzx O145 gene band in STEC isolates. Using2%Agarosestainedwithredsafedyesandelectrophoresed by 5vol/cminTBAM:marker (100bp), lane(1) showed band size (750bp bp)

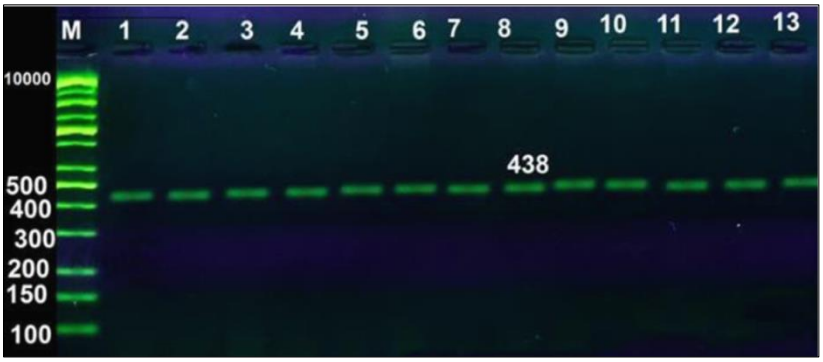

Figure 3: Agarose gel electrophoresis image results of wzx O111 gene band in STEC isolates.Using 2% Agarose stained with red safe dyes and electrophoresed by 5 vol/cm in TBA lane (1-13) showed band size (438bp M: marker (100-b p)

DISCUSSION

Consistent with Verhaegen *et al.,* (2015) and Ramadan (2017), the bacterial isolation shows that Chrom agar STEC showed great sensitivity and efficacy in the identification and separation of the top six STEC strains. As Church *et al*., (2007) have observed, the use of Chrom agar O157 has improved the diagnostic effectiveness of O157 STEC strains. This study offers valuable insights into the prevalence of STEC non-157 serogroups, which are more common in children aged 1-5 experiencing bloody diarrhea compared to those with watery diarrhea, highlighting its significant implications for public health. It was demonstrated that E. coli O26 serotypes were the most prevalent, followed by O111 and O145. Notably, no single sample tested positive for the serotypes O103, O45, and O121 in samples collected from children. This finding aligns with Jared's work from 2016. And EFSA & ECDC, 2014.It has been reported that STEC O26 ranks as the second most prevalent serogroup among EU patients, following O157. Additionally, Ramadhan (2017) identified O111 as the most prevalent serogroup in human diarrheal samples collected from AL-Sader teaching hospital in Missan, Iraq.

CONCLUSION

Children's diarrhea is mostly caused by non-O157 STEC, so *E coli* O26 is the most under observation in Baghdad hospitals with regard to childhood population. With different virulence factors, the isolated strain of Non-O157 STEC displayed most frequency with hemorrhagic diarrhea than watery diarrhea.

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