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A Study on Prevalence and Virulence Determinants of Verotoxic *E. coli* (VTEC) Isolated from Yamuna River Water Around the Mathura Region of India

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Abstract The utility of river water for various purposes is based on different physico-chemical and biological parameters. With the course of time screening of river water for microbiological parameters is mandatory for the implementation of water quality programs. The present study was aimed to find the prevalence and virulence factors of Verotoxic *E. coli* (VTEC) from the surface water of Yamuna river around the Mathura city. The VTEC are considered as the potential risk to human health world wide. A total of 150 water samples were collected aseptically and processed for the isolation of E. coli microorganism. For the identification of VTEC, the *E. coli* isolates were subjected through multiplex PCR to detect the virulent genes (*vt 1*, *vt 2*, *eae*A and *hly* A). The gene carrying isolates were serotyped at Central Research Institute (CRI), Kasauli, Himachal Pradesh. The *E. coli* was 37.33% (56/150) prevalent in Yamuna river water. A total of eleven *E. coli* isolates were revealed with virulent genes through mPCR and categorised as VTEC with a prevalence rate of 7.33% (11/150). The verotoxin genes *vt1*, *vt2* and (*vt1* + *vt2*) were in 27.27% (3/11), 54.54% (6/11) and 18.18% (2/11) *E. coli* isolates, respectively, while the other virulent genes *eae*A and *hly*A were reported as 9.09% (1/11) and 36.36% (4/11). The serotyping of gene carrying isolates were resulted into seven different 'O' serogroups of VTEC in which HUS associated O9 serogroup was also revealed. The presence of VTEC in surface water of Yamuna river are indicating the risk for public health.

Keywords Yamuna river, Verotoxic *E. coli* (VTEC), Verotoxin genes (*vt1* and *vt2*), Intimin gene (*eae*A), Enterohemolysin gene (*hly*A).

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Introduction

Verotoxin–producing *Escherichia coli* (VTEC) is an important zoonotic food and water-borne pathogen causing diarrhoea, hemorrhagiccolitis (HC), and potentially fatal hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TPP) in humans [1]. The VTEC are one of the important group of

E. coli and broadly classified in to two subgroups, *E. coli* O157 and the non-O157 VTEC [2, 3]. The *E. coli* O157 : H7, a serogroup of VTEC is mainly responsible for the outbreaks and severe human illness, world wide. But presently, involvement of the non-O157 VTEC in number of clinical cases and outbreaks changed the direction of work towards this group [4, 5]. The ruminants especially young cattle are the major reservoirs of VTEC, carrying these organisms asymptomatically in their intestine [6, 7]. The ingestion of contaminated food and water may cause the VTEC infections in humans [8].

The major factors affecting the microbiological quality of surface river waters are rapid urbanization, agricultural activities, industrial effluents, sewage and direct defecation and urination of animals [9]. The aquatic ecology of the running water is becoming increasingly affected by anthropogenic discharges that are the sources of water pollution. The data on the prevalence of non-O157 VTEC in aquatic environments is notably scare all over the world [10].

The present work was conducted to assess the prevalence, phenotypic characteristics and virulence determinants of VTEC at different ghats of Mathura city to ensure the Yamunatic water as safe for public health.

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Materials and Methods

Collection, enrichment and phenotypic characterisation

A total of (n = 150) water samples were collected at 10 different ghats (15 samples from each ghat) that located from entry to exit point of the Yamuna river in this region, over a period of six months from January 2012 to June 2012. The samples were collected aseptically in sterilized 1 liter bottles and brought to the laboratory under chilled condition and processed immediately for the better recovery of *E. coli*.

For the isolation of *E. coli*, 1 ml of water samples were inoculated into 10 ml of sterile MacConkeys broth and incubated at 37°C for 24 h as per the method of Edwards and Ewing [11]. The enriched inoculum was streaked on MacConkey agar and the resulting lactose fermenting pink, smooth and round colonies were further streaked on the selective EMB agar (Hi Media, India) and incubated at 37°C for 24 h. The colonies showing green metallic sheen on EMB agar were picked as presumptive *E. coli* and confirmed biochemically by kit (KB010 Hi *E. coli* Identification kit-Hi Media, India). The serogroup of VIEC (*E. coli* 0.57 : H7) was isolated as per the method of Johnson et al. [12] The confirmed *E. coli* isolates were streaked over CT - SMAC (Cefixime Tellurite-Sorbitol MacConkey Agar) and further incubated at 37°C for 24 h.

Virulence detection and serotyping

The presence of virulence genes *vt1*, vt2, *eae*A and *hly*A was verified by multiplex PCR according to method described by Paton and Paton [13]. The template DNA was extracted from single colony of each isolate by kit (Genei, Banglore). The purity and concentration of extracted was detected by nano drop method (Eppendorf, German). The every 25 l PCR reaction mixture of contained 1 × PCR buffer, 1.5 mM $MgCl_2$, each primer at a concentration of 40 nM, 200 µM each of dNTPs, one unit of Taq DNA polymerase and 2.0 µl of template DNA. The PCR reaction was performed in a thermal cycler (Cyber lab, India) using standard cycling conditions: an initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, primer annealing at 59°C for 1 min and extension at 72°C for 1 min and a final extension at 72°C for 6 min and amplified products were separated by electrophoresis in 2% agarose gel and visualized with ethidium bromide. The standard 100 bp DNA ladder (Genei, Bangalore) was used as marker.

All the isolates that turned out to be positive through mPCR method were submitted to the National

Table 1. Gene profile of VTEC serotypes isolated from surface water samples of Yamuna river.

Sl. No.	Serotypes	No. of VTEC isolates	*vt1* gene	*vt2* gene	*vt1* + *vt2* genes	*eae*A gene	*hly*A gene
1	O9	2	–	–	+	–	–
2	O11	2	+	–	–	–	+
3	O27	3	–	+	–	–	–
4	O34	1	–	+	–	–	–
5	O56	1	–	+	–	–	–
6	O81	1	+	–	–	+	–
7	O134	1	–	+	–	–	–
Total	7	11	3	6	2	1	4

Salmonella and *Escherichia* Center, Central Research Institute (CRI), Kasauli, Himachal Pradesh, India for serotyping. Somatig (O) antigen were identified in the isolates by standard methods.

Results and Discussion

The surface water of Yamuna river ghats in the vicinity of Mathura was examined for the presence of zoonotic pathogen VTEC. The city is worls fame as the birth place of lord Krishna and visited by many pilgrims and tourists from different countries.

The TSB enriched samples that produced pink colored smooth colonies on MLA and distinct clear greenish metallic sheen over EMB were considered the presumptive *E. coli*. The isolates showed following biochemical reaction as negative for Voges-Proskauer, hydrogen sulfide, citrate, oxidase and urease production, and positive for indole and methyl red tests were biochemically confirmed as *E. coli*. The *E. coli* were 37.33% (56/150) prevalent in surface water of Yamuna river, the finding of current study had nearly coincided with the findings of other researchers, as 33.33% in U. P. [14], 51.85% in Srinagar [15] while higher value of 70% inkat river of South Africa Nontongana et al. [2] was also reported. The 7.33% of *E. coli* isolates were revealed with Verotoxin and other virulent genes and identified as VTEC through the mPCR. The almost similar prevalence rates 6.67% and 9.2% of VTEC were reported in Yamuna river of India [14] and Lower Fraser river of British Columbia [5], respectively. The lower value of 1% from the kat river of South Africa [2] and the values higher to this study 23.2%, 21.6%, and 19.5% were revealed from the Serpentine, Sumas and Nicomekl Rivers of Canada [5] and 36.4% insurface water of Egypt [8]. The wide variations in prevalence values of *E. coli* and VTEC might be due to difference in geographical and climate conditions of a region, while samples processing in labs are also a critical factor for discrepancies in outcomes of prevalence values [2, 16].

During the course of study isolation rate of *E. coli* O157 : H7 was recorded zero percent. None of the isolates produced the colorless colonies on the selective medium, Cefixim Tellurite Sorbitol-MacConkey (CT-SMAC). Results phenotypically indicated the absence of serotype *E. coli* O157 : H7 in the samples. The scarcity of this serotype of VTEC was also observed in different studies 2.7% [5], 4% by Johnson et al. [10], 6.7% by Jokinen et al. [17] and five-fold higher prevalence of non-O157 isolates than O157 in surface water of California [18]. The observations provide insight that the surface waters can also support diversified non-O157 VTEC populations that have been mainly over looked in assessments of potential risks to human health.

The virulent genes were present either singly or in different combinations in VTEC isolates. The virulence gene *vt1* was detected by multiplex PCR in 27.27%, *vt2* in 54.54%, both *vt1* + *vt2* in 18.18%, *eaeA* in 9.09% and *hly*A in 36.36% of the isolates (Table 1). In the current study gene profile of isolates reflected the higher percentage of *vt2* genes that are frequently reported in clinical isolates [19]. The prevalence of *hly*A gene was also quite high which

is cytolytic to human microvascular end othelial cells [1].

The seven different 'O' serogroups were revealed on serotyping of 11 gene carrying isolates and rest four isolates were identified as untypable (Table 1). The serogroup 'O9' found in surface water of Yamuna river had been associated with clinical cases of HUS in human beings [20].

Conclusion

In the current study, VTEC isolates with variable virulence factors are recovered which historically concerned with human disease. Pollution of surface water of Yamuna river with potentially virulent enteric microorganism poses a public health risk. So the appropriate strategies to control the microbial contamination of surface water by the concerned authorities are desirable.

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