tcpC as a prospective new virulence marker in blood *Escherichia coli* isolates from sepsis patients admitted to the intensive care unit

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**Abstract**

The prevalence of the tcpC in the blood *Escherichia coli* isolates collected from the sepsis patients admitted to the intensive care unit was investigated for the first time. The blood and faecal samples were collected from sepsis and nonsepsis patients, respectively. The prevalence of the tcpC and phylogroups was confirmed by gene-specific PCR. The occurrence of the tcpC in the blood *E. coli* isolates from sepsis patients was significantly higher than the faecal isolates. The higher prevalence of blood *E. coli* isolates among the pathogenic groups (B2, D) compared to the commensal groups (A, B1) suggests tcpC as a prospective new virulence marker for sepsis.

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*Escherichia coli* is a highly versatile microorganism that causes a wide range of diseases, including self-limiting diarrhoea and urinary tract infection to life-threatening sepsis [1]. It is one of the leading causes of blood infection and has the ability to trigger a vigorous inflammatory response in the host, leading to sepsis. Bacterial pathogens have virulence factors (VFs) that contribute to their pathogenesis. Bacterial virulence is multifactorial and thus depends on host susceptibility and microbe characteristics [2]. The *E. coli* VFs can be categorized as adhesins, toxins, iron uptake, protectins, pathogenicity-associated islands and Toll-like receptor (TIR)-containing proteins [3,4]. Virulent TIR domain-containing proteins (TcpPs) have been identified in various bacteria [5]. Bacterial TcpPs in Gram-negative/positive bacteria such as *Brucella melitensis* (tcpB), *Paracoccus denitrificans* (PdTIR), *Staphylococcus aureus* and *E. coli* (tcpC) is known; however, the exact mechanism of their effect has not been fully understood [3,5,6]. In addition, there is strong evidence of the involvement of the homologous recombination in the spread of the bacterial tcpC virulence [6].

The Toll-like receptor (TLR) signaling domain is the TIR domain, which is found in the cytosolic face of TLRs as well as their adaptors. Previous studies have revealed that a diverse range of both pathogenic and nonpathogenic microorganisms express proteins containing TIR domains [3,7]. TcpPs may play a role in protein–protein interaction; however, in Gram-negative bacteria, it has been shown to shut down TLR signaling [5]. Among other microbes, TIR domain contain protein (BtpB) is known to contribute to the virulence and control of local inflammatory responses in the establishment of chronic brucellosis [8]. SaTlp1 and SaTlp2 are potential VFs of *S. aureus* that interact with the innate immune signaling machinery of the host cells [7]. Previously, we have shown a role of tcpC in the urinary tract infection pathogenesis [3]; however, its role in pathogenesis of sepsis is unknown.

In order to investigate the prevalence of the tcpC gene in the blood *E. coli* isolates of the sepsis patients under a challenging environment, blood samples (*n* = 78) were collected from patients diagnosed with sepsis and admitted to the intensive care unit (ICU) of Vardhaman Mahavir Medical College (VMCC) and Safdarjung Hospital, Delhi, India, during 2011–2013. The randomly selected faecal samples (*n* = 83) were collected as a control from patients of cardiovascular surgeries and road accidents admitted to the ICU of VMCC and Safdarjung Hospital who were not diagnosed with sepsis. The study was approved by the institutional ethical committee of VMCC and Safdarjung Hospital (S.No-VMMC/SJH/Ethics/SEP-11/29).

The *E. coli* isolates were collected and screened by standard procedures. The isolates were grown as lactose-positive colonies on MacConkey blood agar media and then subcultured in tryptone soy broth by incubating at 37°C for 18 hours. A portion of the broth was pooled, and genomic DNA was...
isolated. PCR was performed for amplification of the tcpC gene of 544 bp (Fig. 1) using bacterial genomic DNA as a template and gene-specific primers (tcpC FP: 5’-GAGTGGAAG-GAGGTTGAGGC-3’ tcpC RP: 5’-GCAGTGCCATTTTTC CGCC3’). All the isolates were tested with positive and negative control in duplicate. E. coli commensal strains belong to groups A and B1, whereas extraintestinal pathogenic strains belong to groups B2 and D. We observed no significant difference in the prevalence of the phylogroups in the faecal E. coli isolates, whereas the phylogroups B2 (24%) and D (45.4%) were found to be more prevalent (p <0.001) among the blood E. coli isolates (Fig. 2A). Individually, we did not find any significant difference in the prevalence of the phylogroups A and B2 in blood and faecal E. coli isolates; however, group B1 was found to be significantly higher in the faecal isolates (p <0.05). Phylogroup D (45.4%) was more prevalent in the blood (p <0.001) compared to the faecal E. coli isolates, thus corroborating earlier observations of extraintestinal pathogenic strains belonging to group D.

In addition to the VFs, the discrete phylogroup (A, B1, B2 and D) of E. coli is indicative of their origins from natural populations that may become commensal or pathogenic [9]. The prevalence of tcpC in the E. coli isolates in various disease conditions and their correlation with the phylogroups has not been elucidated. We were interested in investigating a possible link between the strain phylogeny and the tcpC gene among the blood E. coli isolates of sepsis patients. For this, we performed triplex PCR using primers specific to genes chuA and yjaA and DNA fragment TSPE4.C2 [10]. We found that the prevalence of tcpC in the pathogenic group (B2 and D) was significantly higher (40.3%) than the commensal groups (A and B1) (9.6%) (p <0.005) (Fig. 2B). A high prevalence of the tcpC gene among the blood E. coli isolates compared to the faecal ones suggests a role of tcpC in the development of sepsis.

The TcpPs of Gram-negative bacteria is known to interact with adapter proteins MyD88 and TLR4 TIR domains and demonstrate an inhibitory effect on the TLR signaling. In contrast, P. denitrificans TIR protein (PdTIR) interacts with MyD88 and TLR4 TIR domains without showing an inhibitory function [11]. TlpA from Salmonella enterica inhibits activation of the transcription factor NF-kB [12]. tcpC from E. coli and TcpB in a range of Brucella species (B. melitensis, B. ovis and B. abortus) inhibit NF-kB activation by direct interaction with MyD88 [13]. The exact mechanism of the action of tcpC after its interaction with the host proteins is unknown.

Our data have shown the presence of tcpC in a significantly high amount in the blood E. coli isolates of the sepsis patients,
indicating its possible role in disease pathogenesis [14]. A correlation between the tcpC gene and the bacterial phylogroups has been confirmed by analysing the distribution of E. coli tcpC in different phylogroups. As expected, the prevalence of the E. coli pathogenic group (B2 and D) was significantly higher than the commensal group (A and B1). The high prevalence of the tcpC gene among blood isolates from sepsis patients also indicates its role in the development of infection and suggests that the challenged host environment, such as the presence of catheters or hospital-acquired infections, or even poor hygiene due to infrequent urination of the patient leading to a high vulnerability, may have altered the bacteria’s pathogenicity [15].

In summary, we report for the first time the prevalence of the bacterial virulence gene tcpC in blood E. coli isolates from sepsis patients admitted to the ICU and their correlation with E. coli phylogroups. Further studies are ongoing to understand the mechanism of action of tcpC in an appropriate animal model in order to envision tcpC as a target for creating treatment strategies.

Conflict of interest

None declared.

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