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.

Keywords: *Escherichia coli*, phylogroups, sepsis, *tcpC*, virulence factor

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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-threating 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/interleukin 1 receptor (TIR)-containing proteins [3,4]. Virulent TIR domain-containing proteins (Tcps) have been identified in various bacteria [5]. Bacterial Tcps 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]. Tcps 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 Vardhman 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

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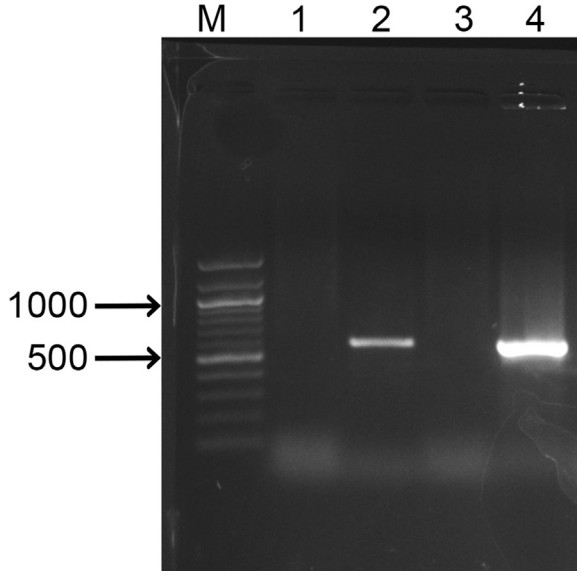

FIG. 1. Presence of *tcpC* gene in *Escherichia coli* isolates from sepsis patients. Lane M, DNA ladder; lane 1, negative control; lane 2, blood *E. coli* isolates; lane 3, faecal *E. coli* isolates; lane 4, positive control.

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′-GCAGTGCCATTTTATC 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

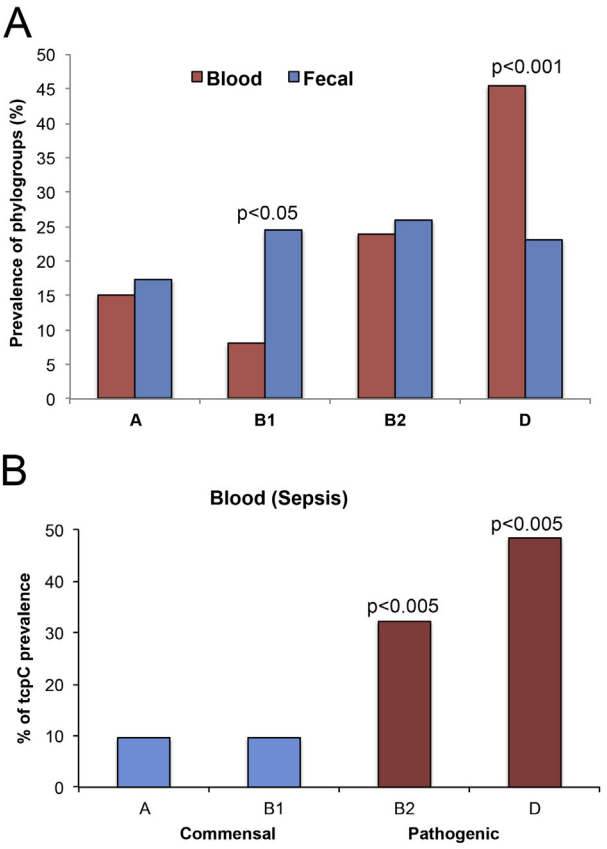

FIG. 2. Comparative prevalence of phylogroups between blood ($n = 78$) and faecal ($n = 83$) *Escherichia coli* isolates (A) by triplex PCR. Correlation between *tcpC* and the phylogroups in *E. coli* isolates by gene-specific PCR method (B). p value calculated by Fisher exact test and indicates significance between sepsis and nonsepsis patients.

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 Tcps 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-κB [12]. *tcpC* from *E. coli* and *TcpB* in a range of *Brucella* species (*B. melitensis, B. ovis* and *B. abortus*) inhibit NF-κB 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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