ORIGINAL ARTICLE

Faecal *Escherichia coli* isolates show potential to cause endogenous infection in patients admitted to the ICU in a tertiary care hospital

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Abstract

Nosocomial infections are acquired during hospital treatment or in a hospital environment. One such infecting agent, *Escherichia coli,* harbours many virulence genes that enable it to become pathogenic, causing damage to the host. The mechanism of the *E. coli* virulence factors provenance to cause infection in host environments is not clearly elucidated. We investigated the virulence and pathogenicity of *E. coli* affected by the host environment. For this, blood (n = 78) and faecal (n = 83) *E. coli* isolates were collected from patients with and without sepsis, respectively, who had been admitted to the intensive care unit. The *E. coli* genomic DNA was isolated; the phylogenetic grouping was conducted by triplex PCR. The occurrence of nine virulence genes among the all the isolates was confirmed by gene-specific PCR. The prevalence of *E. coli* in blood isolates was more in phylogenetic groups B2 and D compared to groups A and B1. However, in faecal isolates, there was no significant difference. The prevalence of adhesin and toxin (*papG, sfa, afa, cnf1, hlyA*) genes was higher in blood compared to faecal *E. coli* isolates. However, the prevalence of *aer, traT* and *PAI* was similar as well as higher among both of these groups. These observations indicate a role of external environment (hospital setting) on host susceptibility (development of infection) in the faecal *E. coli* isolates, thereby making the patient prone to a sepsis condition.
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Introduction

Nosocomial infections are the major cause of death and increased morbidity among hospitalized patients. The incidence of such infections is estimated to be 5–10% of patients admitted to tertiary care hospitals, although this may go up to 28% in the intensive care unit (ICU). Nosocomial infections contribute to 0.7–10% of deaths compared to 0.1–4.4% of all the deaths occurring in hospitals [1]. Alarmingly, 10–30% of patients in the Indian population acquire such infections [1]. Nosocomial infections are most commonly acquired as a result of surgical wounds, urinary tract infections (UTIs) and lower respiratory tract infections and may be cross-infections or endogenous. Modern diagnostic procedures such as biopsies, endoscopic examinations, catheterization, intubation/ventilation and surgical procedures increase the risk of infection by microorganisms like *Escherichia coli* that are normally innocuous but may become pathogenic when the body's immunological defenses are compromised [1].

E. coli is the most common pathogen causing diarrhoea, neonatal septicemia, UTI, bacteraemia and urosepsis [2]. It is responsible for 80% of community-acquired UTIs and 30% of nosocomial infections [3]. *E. coli* is one of the leading causes of bloodstream infections and comprises 17–37% of all bacteria isolated from patients with bloodstream infections [4]. Such

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bloodstream infections with extraintestinal *E. coli* are frequently associated with patients who have undergone major surgeries; who were admitted to hospitals for long durations; or who had a peripheral or urinary catheter [1]. *E. coli* in the bloodstream can trigger vigorous host inflammatory response, leading to sepsis associated with high morbidity and mortality [5].

A phylogenetic analysis indicates discrete origins of a diverse natural populations of the pathogenic *E. coli* [6] that can be classified into major phylogenetic groups A, B1, B2 and D [7]. The commensal strains usually belong to groups A and B1, whereas the extraintestinal pathogenic strains belong to groups B2 and D [8,9]. The intestinal *E. coli* are mixture of all the phylogenetic groups and may act as a reservoir for the pathogenic isolates. The pathogenicity of intestinal or extraintestinal *E. coli* may be attributed to its genetic virulence markers [10]. Strains of groups B2 and D often carry virulence factors (VFs) that are lacking in A and B1 [8,11,12]. The reason for a commensal strain becoming virulent may be attributed to the multiple strategies of genome plasticity wherein the random point mutations were incorporated for adaptive pathogenic environments [13]. The VFs in *E. coli* are required to overcome host defenses, invade host tissues and trigger a local inflammatory response. *E. coli* virulence and phylogeny are intertwined because VFs from both the host and the environment shape its genetic structure [14].

To date, no single virulence factor has been demonstrated as being specific, unique or definitive to cause a particular disease. Virulence is multifactorial because it depends on both the characteristics of microbe and the susceptibility of the host [15]. On the basis of functional groups, the *E. coli* VFs can be categorized as adhesins, such as P fimbriae (*papG*), type 1 fimbriae (*fimH*), S fimbriae (*sfa*) and A fimbriae (*afa*); toxins, such as hemolysin A (*hlyA*) and cytotoxic necrotizing factor 1 (*cnf1*); iron uptake, such as aerobactin (*aer*); protectins, such as serum resistance (*traT*); and others, such as pathogenicity-associated islands (*PAI*s) and Tir-containing protein of *E. coli* (*tcpc*) [16–18]. *PAI*s have previously been investigated in pathogenic bacteria. However, to our knowledge, no study has been conducted for commensal strains [19]. Houdouin *et al.* [20] reported a susceptibility of the *E. coli* isolates from blood of urosepsis patients to diverse antibiotics according to the prevalence of VFs and phylogenetic groups.

The *E. coli* phylogenetic grouping and their correlation with the VFs in disease conditions is well established [11,15,21]; however, a correlation between phylogenetic groups and virulence profile of the blood and faecal *E. coli* isolates is not known. Most of the previous studies compared the virulence properties of *E. coli* isolates from disease condition with those from healthy individuals [11,15,22]; however, the potential of faecal *E. coli* isolates (usually commensals) to cause the disease in a compromised host is unknown. Therefore, in order to understand the host susceptibility, we investigated the blood *E. coli* isolates from the patients diagnosed with sepsis and compared them with the faecal *E. coli* isolates from patients without sepsis admitted to the ICU. We found that the prevalence of the *E. coli* isolates among the pathogenic groups B2 and D was significantly higher than the commensal groups A and B1 in faecal *E. coli* isolates. The overall prevalence of *E. coli* isolates among pathogenic groups was similar to commensal groups faecal *E. coli* isolates, indicating that this group of patients has a higher chance of contracting a severe form of infection. In addition, an overall prevalence of VFs among all the phylogenetic groups was higher in the blood *E. coli* isolates. However, the high prevalence of *aer, traT* and *PAI* among faecal *E. coli* isolates indicated that the host environment may have an important role to play for a differential expression of these virulence genes to induce pathogenicity in the hospital setting. A knowledge of such virulence patterns and their correlation with phylogenetic groups is critical for our understanding of bacterial infection and is indispensable to the development of devising related novel therapeutic strategies.

Methods

Selection of patients and bacterial isolates

Blood samples (n = 78) were collected from patients with sepsis admitted to the ICU of Vardhman Mahavir Medical College (VMCC) and Safdarjung Hospital, New Delhi, India, from February 2011 to August 2013. The blood culture was carried out as a part of compulsory diagnostic testing. The samples were selected from patients who had shown a clinical response arising from a nonspecific insult, which include more than two of the following: multiple positive blood culture results; bacteremia associated with systemic symptoms; temperature >38.5°C; hypotension (systolic blood pressure <90 mm Hg); and leucocytosis (white blood cell count $\geq$13 000/mm^3). In addition, randomly selected faecal samples (n = 83) were collected from patients admitted to the ICU (on day 1) for cardiovascular surgeries and road transport accidents who were not diagnosed with sepsis at VMCC and Safdarjung Hospital during the same period of time. A total of 161 blood and faecal *E. coli* isolates (Table 1) were screened for *E. coli* positivity by standard biochemical procedures. Only one isolate from each patient is included in this study.

Subculturing of bacterial isolates and isolation of DNA

The *E. coli* isolates were grown as lactose-positive colonies on MacConkey blood agar medium in the Department of Microbiology at VMCC and Safdarjung Hospital. Thereafter, the

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TABLE 1. Clinical diagnosis of 161 patients with and without sepsis

Clinical diagnosis	n (%)
Sepsis patients treated in ICU	78 (48.4)
Sepsis	19 (24.3)
UTI	9 (11.5)
Bronchopneumonia	10 (12.8)
Nephritic syndrome	8 (10.2)
NA (blood culture positive for *Escherichia coli*)	32 (41)
Nonsepsis patients treated in ICU	83 (51.6)
Medical device in patient's body after major surgery *or* patients underwent invasive procedure[a]	83 (100)

ICU, intensive care unit.
[a]Patients with cardiovascular surgeries and patients who experienced road transport accidents who were not diagnosed with sepsis.

samples were subcultured on tryptone soy broth by incubating at 37°C for 18 hours. A portion of the broth containing bacterial isolates was pooled and processed for isolation of the bacterial genomic DNA by the standard sodium acetate precipitation method. DNA was quantified using NanoDrop (NanoDrop ND 100).

Triplex PCR

A triplex PCR was performed using a bacterial genomic DNA as template and primers specific to *chuA* and *yjaA* genes and the TSPE4.C2 DNA fragment, as described elsewhere [7] (Table 2). For PCR amplification, a 25 μL reaction containing 1 μL template DNA, 2 mM $MgCl_2$, 0.5 mM dNTPs, 0.5YU of Taq polymerase (Promega) and 0.3 pmol/μL of forward and reverse primers each in 1× PCR buffer was set up. The reaction conditions included one cycle of initial denaturation for 5 minutes at 94°C and thereafter 35 cycles of denaturation for 30 seconds at 94°C, annealing for 30 seconds at 55°C and an extension of 5 minutes at 72°C. A final extension of 10 minutes at 72°C was carried out before termination. Thereafter the PCR products were run on 1.8% agarose gel (Saekem Lonza). All the strains were tested in triplicate with positive and negative controls. The positive control was *E. coli* strain CFTO73 obtained from ATCC (no. 700928), and the negative control was PCR master mix without DNA template.

Phylogenetic grouping

A phylogenetic grouping (A, B1, B2 and D) of *E. coli* isolates from patients with and without sepsis was determined on the basis of triplex PCR data by making a dichotomous decision tree based on an amplification of *chuA* and *yjaA* genes and the TSPE4.C2 DNA fragment, as previously reported [7]. The fragment size of the *chuA* and *yjaA* genes and the TSPE4.C2 DNA fragment was 279, 211 and 152 bp, respectively. Group B2 was designated with a positivity of *chuA* and *yjaA* genes; group D with a positivity of *chuA* gene and a negativity of *yjaA* gene; group B1 with a positivity of TspE4.C2 DNA fragment and a negativity of *chuA* gene; and group A with a negativity of *chuA* gene and TspE4.C2 DNA fragment.

PCR for virulence markers

The presence of virulence genes encoding P fimbriae (*papG*), type 1 fimbriae (*fimH*), S fimbriae (*sfa*), A fimbriae (*afa*), cytotoxic necrotizing factor 1 (*cnf1*), hemolysin (*hlyA*), aerobactin (*aer*), serum resistance (*traT*) and pathogenicity-associated island marker (*PAI*) was evaluated by performing a PCR using gene-specific primers (Table 2). Using a bacterial genomic DNA from patients with and without sepsis as the template, a PCR amplification was performed for each gene in a standard 25 μL reaction.

TABLE 2. Primers for amplification of *Escherichia coli* genes related to phylogenetic grouping and virulence genes

Functional Category	Gene	Oligonucleotide sequence (5′–3′)	Annealing temperature (°C)	Product size (bp)	Reference
		Phylogenetic grouping			
	chuA	f GACGAACCAACGGTCAGGAT r TGCCGCCAGTACCAAAGACA	55	279	Clermont *et al.* 2000 [7]
	YjaA	f TGAAGTGTCAGGAGACGCTG r ATGGAGAATGCGTTCCTCAAC	55	211	
	TspE4.C2	f GAGTAATGTCGGGGCATTCA r CGCGCCAACAAAGTATTACG	55	152	
		Virulence factors			
Adhesins	*papG*	f GCATTTCTACGGTAACC r TCGTCAAATTTCTCAGTCAGA	50	295	Norinder *et al.* 2012 [40]
	Sfa	f CTCCGGAGAACTGGGTGCATCTTAC r CGGAGGAGTAATTACAAACCTGGCA	54	408	Le Bouguenec *et al* 1992 [41]
	Afa	f GCTGGGCAGCAAACTGATAACTCTC r CATCAAGCTGTTTGTTCGTCCGCCG	60	793	Le Bouguenec *et al.* 1992 [41]
	fimH	f TGCAGAACGGATAAGCCGTGG r GCAGTCACCTGCCCTCCGGTA	55	506	Johnson & Stell 2000 [22]
Toxins	*hlyA*	f AACAAGGATAAGCACTGTTCTGGCT r ACCATATAAGCGGTCATTCCCGTCA	55	1177	Yamamoto *et al* 1995 [42]
	cnf1	f AAGATGGAGTTTCCTATGCAGGAG r CATTCAGAGTCCTGCCCTCATTATT	52	498	Kuhar *et al* 1998 [43]
Iron uptake	*aer*	f TACCGGATTGTCATATGCAGACCGT r AATATCTTCCTCCAGTCCGGAGAAG	56	602	Yamammoto *et al* 1995 [44]
Protectins/serum resistance	*traT*	f GGTGTGGTGCGATGAGCACAG r CACGGTTCAGCCATCCCTGAG	57	288	Johnson and Stell 2000 [22]
Pathogen associated island	*PAI*	f GGACATCCTGTTACAGCGCGCA r TCGCCACCAATCACAGCCGAAC	57	922	Johnson and Stell 2000 [22]

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The reaction conditions included one cycle of initial denaturation for 5 minutes at 94°C and thereafter 35 cycles of denaturation for 30 seconds at 94°C, annealing for 30 seconds at specific temperature (Table 2) and an extension of 5 minutes at 72°C. A final extension of 10 minutes at 72°C was carried out before termination. Thereafter the PCR products were run on 1.8% agarose gel (Saekem Lonza). All the strains were tested in triplicate with positive and negative controls. The positive control was *E. coli* strain CFTO73 obtained from ATCC (no. 700928), and the negative control was PCR master mix without DNA template.

Statistical analysis

The *Z* test was used to compare the virulence factor's prevalence among the blood and faecal *E. coli* isolates. A one-way ANOVA nonparametric test was performed to analyse the virulence profile among various *E. coli* phylogenetic groups. A p value of <0.05 was considered significant.

Ethical clearance

The isolates were previously approved by the Institutional Ethical Committee of Vardhman Mahavir Medical College (VMCC) and Safdarjung Hospital, New Delhi, India (S.No-VMMC/SJH/Ethics/SEP-11/29). As per the guidelines, an informed written consent was taken from all the adult subjects included in this study.

Results

Phylogenetic grouping of blood and faecal *E. coli* isolates

E. coli commensal strains belong to groups A and B1, whereas extraintestinal pathogenic strains belong to groups B2 and D [8,11]. We observed no significant difference in the prevalence of phylogenetic groups A (19.2%), B1 (26.5%), B2 (30%) and D (24%) (p 0.47) (Fig. 1, Table 3) in the faecal *E. coli* isolates, whereas phylogenetic groups B2 (25.6%) and D (47.4%) were found to be more prevalent (p 0.0001) among the blood *E. coli* isolates (Fig. 1, Table 3). Individually, we did not find any significant difference in the prevalence of phylogenetic group A in blood (17%) and faecal (19.2%) *E. coli* isolates; however, group B1 was found to be significantly higher in the faecal *E. coli* isolates (p 0.0008) (Fig. 1, Table 3), as previously described [23]. Contrary to expectation, we did not find any significant difference in the prevalence of phylogenetic group B2 from blood and faecal *E. coli* isolates. However, phylogenetic group D (47.4%) was more prevalent in blood (p 0.002) compared to faecal *E. coli* isolates (Fig. 1, Table 3), thus corroborating earlier observations of extraintestinal pathogenic strains belonging to group D [8,24,25].

Virulence profile of blood and faecal *E. coli* isolates in phylogenetic groups

E. coli phylogenetic grouping and its correlation with the VFs in various disease conditions has been previously described [11,21,22]. The combined virulence profile of all the nine VFs (*papG, fimH, sfa, afa, cnf1, hlyA, aer, traT* and *PAI*) among the phylogenetic groups (A, B1, B2 and D) of the blood *E. coli* isolates was found to be significantly higher than the faecal isolates (p 0.002; Fig. 2A, dotted lines). Relatively higher levels of VFs among the faecal *E. coli* isolates suggest the entry of commensal *E. coli* into the bloodstream, thus enhancing its pathogenicity.

We investigated a possible link between strain phylogeny and individual virulence genes among blood and faecal *E. coli* isolates. Our data showed that the adhesin coding genes *PapG*

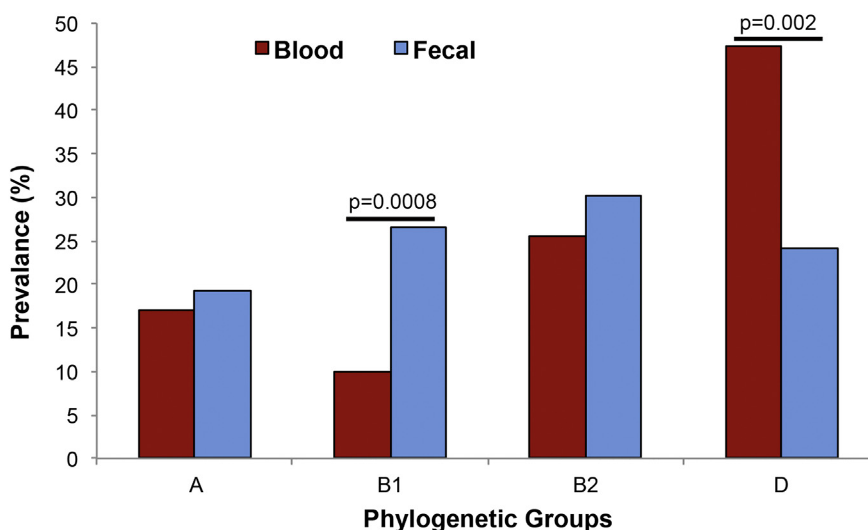

FIG. 1. Triplex PCR–based comparison of prevalence of phylogenetic groups between blood (*n* = 78) and faecal (*n* = 83) *Escherichia coli* isolates. The p value is calculated by *Z* test and indicate significance between blood and faecal *E. coli* isolates.

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TABLE 3. Distribution of phylogenetic groups and virulence genes in blood and faecal *Escherichia coli* isolates from patients with and without sepsis

Category/variable	No. (%) of isolates for: Total (*n* = 161)	Sepsis, blood (*n* = 78)	Nonsepsis, faecal (*n* = 83)	p
Phylogenetic groups				
Group A	29 (18)	13 (17)	16 (19.2)	NS
Group B1	30 (18.6)	8 (10)	22 (26.5)	0.0008
Group B2	45 (27.9)	20 (25.6)	25 (30)	NS
Group D	57 (35.4)	37 (47.4)	20 (24)	0.002
Virulence factors				
Adhesins				
P fimbriae (*papG*)	114 (70.8)	75 (96)	39 (47)	0.0001
Type 1 fimbriae (*fimH*)	150 (93.2)	70 (90)	80 (96)	NS
S fimbriae (*sfa*)	92 (57.14)	65 (83)	27 (32.5)	0.0001
A fimbriae (*afa*)	52 (32.2)	38 (49)	14 (17)	0.0001
Toxins				
Hemolysin A (*hlyA*)	19 (11.8)	14 (18)	5 (6)	0.02
Cytotoxic necrotizing factor (*cnf1*)	46 (28.6)	38 (49)	8 (10)	0.0001
Iron uptake				
Aerobactin (*aer*)	110 (68.3)	53 (68)	57 (69)	NS
Protectins				
Serum resistance (*traT*)	136 (84.5)	63 (81)	73 (88)	NS
Other				
Pathogenicity associated island (*PAI*)	80 (49.7)	36 (46)	44 (53)	NS

NS = nonsignificant.
The p value is calculated by *Z* test and indicates significance between patients with and without sepsis.

and *fimH* were the most prevalent (90–100%) among all the phylogenetic groups in blood *E. coli* isolates, and *fimH* was equally (80–100%) high in the faecal isolates (Fig. 2B). A significant difference (p <0.0001) was observed in the prevalence of *papG* between blood and faecal *E. coli* isolates. The overall prevalence of *sfa* in the blood *E. coli* isolates was higher in groups B1 and B2 compared to groups A and D (p <0.0001). *Afa* in the blood *E. coli* isolates was higher in groups A and D compared to groups B1 and B2, whereas it was varied for the faecal *E. coli* isolates. Of the toxin-coding genes studied, the overall prevalence of *cnf1* in the blood *E. coli* isolates was higher in groups B1 and B2 compared to groups A and D (p <0.0001), whereas *hlyA* was very low in both groups.

A significant difference was found in the prevalence of *aer* in groups B1 and B2 (p <0.01) of blood and faecal *E. coli* isolates; however, the reverse scenario was seen for the group D. The prevalence of *traT* in groups B1, B2 and D was higher in faecal *E. coli* isolates compared to blood isolates (p <0.05). The prevalence of *PAI* in groups A and B1 was found to be higher in the blood *E. coli* isolates (p <0.05); however, the prevalence of group D was significantly higher in faecal *E. coli* isolates (100%) compared to the blood isolates (35%, p <0.002) (Fig. 2B). Interestingly, an unexpected high expression of the virulence genes among the faecal *E. coli* isolates indicates the role of these genes in the development of infection.

Combined virulence profile of blood and faecal *E. coli* isolates

The pathogenic *E. coli* possesses an array of VFs leading to its pathogenesis [26]. The prevalence of the adhesin and toxin categories—that is, *papG, sfa, afa, cnf1* and *hlyA* among blood *E. coli* isolates—was found to be significantly higher than faecal isolates, indicating their significant association to the pathogenic conditions (Fig. 3A, Table 3). However, no significant difference was observed in the prevalence of *fimH* among the blood and faecal *E. coli* isolates, as we expected. Surprisingly, no difference was observed in the prevalence of *aer, traT* and *PAI* among the blood and faecal *E. coli* isolates, indicating a role of the host environment in stimulating the bacteria to acquire these virulence genes (Fig. 3A, Table 3).

The prevalence of the *E. coli* isolates with respect to the *papG, sfa, afa, cnf1* and *hlyA* in the phylogenetic groups (A, B1, B2 and D) belonging to the blood *E. coli* isolates was higher compared to faecal *E. coli* isolates. However, no discrete difference in the prevalence of the *E. coli* isolates with respect to *aer, traT* and *PAI* was observed among various phylogenetic groups (Fig. 3B). Instead, a high prevalence of *fimH, aer, traT* and *PAI* among the faecal *E. coli* isolates suggests an effect of external environment to induce bacterial pathogenicity [27].

Discussion

Characterization of *E. coli* is important for both clinical and epidemiologic implications. A community- or hospital-acquired *E. coli* infection is the primary cause of neonatal meningitis, UTI, urosepsis and sepsis [11,21,24,28], but reports of *E. coli* causing bloodstream infections are limited. The pathogenic *E. coli* strains belong to phylogenetic groups B2 or D, out of which B2 isolates are more prevalent among the intestinal pathogenic strains whereas the commensal one belongs to group A or group B1 [8,16]. Irrespective of the presence or absence of virulence genes or factors, the status of the host can be critical for the development of an infection [29]. Studying bacterial isolates from different diseased hosts like pyelonephritis, cystitis and asymptomatic bacteriuria (ABU) is common, but for sepsis it is not known.

Pathogenic behaviour is predicted both by virulence factor repertoire and by phylogenetic background [8,30]. Genetically, extraintestinal pathogenic *E. coli* harbours a variety of VFs, which gathered into pathogenicity-associated islands and enhance the capacity of *E. coli* to cause systemic infections [31]. However, it is still not clear how strains with apparently low virulence can cause sepsis, not only in compromised but also in noncompromised hosts [32]. It is conceivable that these strains may possess unrecognized VFs or specific VFs that may facilitate bacteraemia [20]. Previous studies described *papC* and *aer* as

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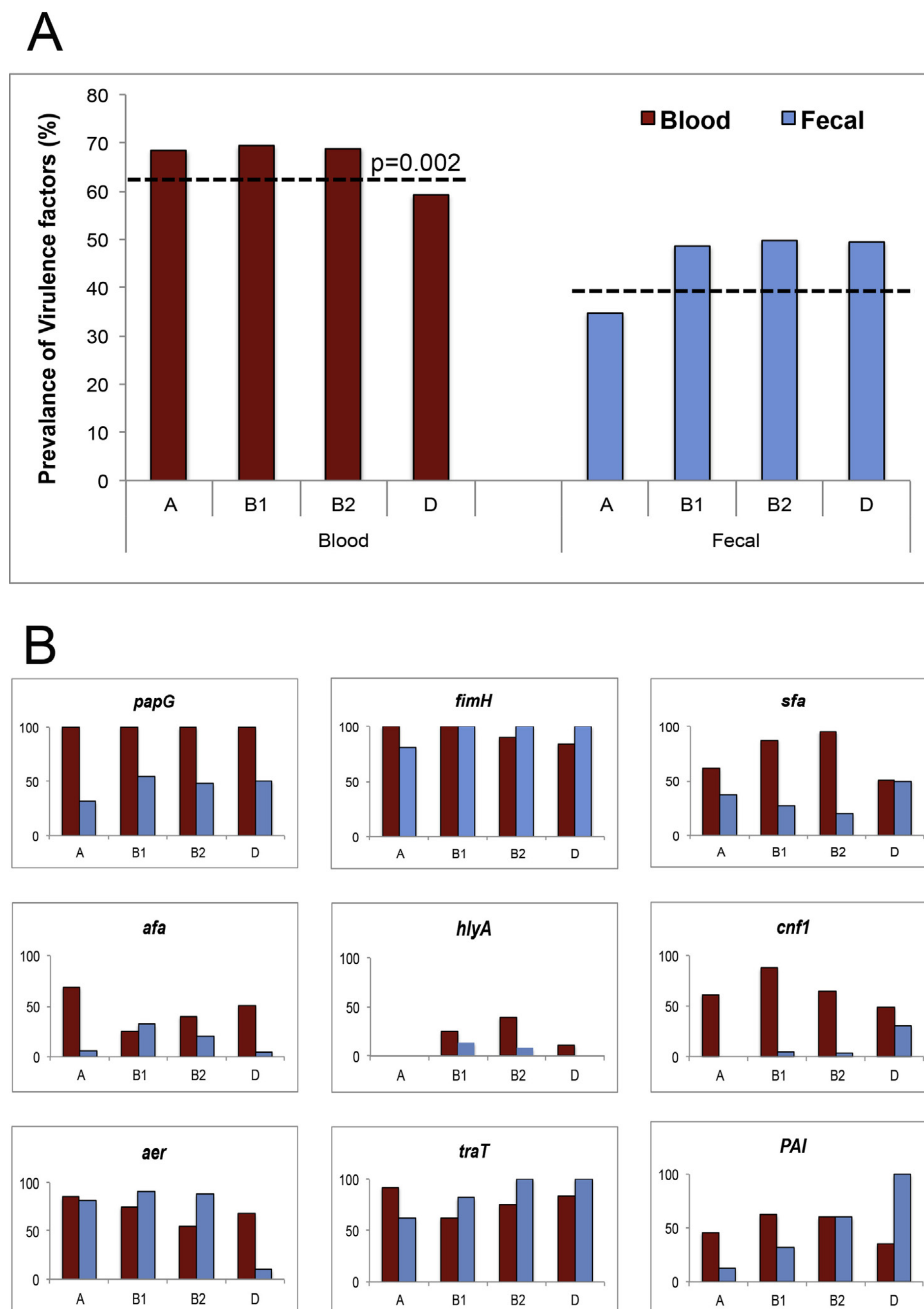

FIG. 2. (A) Combined prevalence of virulence factors among phylogenetic groups of blood and faecal *Escherichia coli* isolates. Dotted lines indicate mean values among blood and faecal *E. coli* isolates. The p value is calculated by *Z* test and indicates significance among both groups. (B) Expression of virulence genes among phylogenetic groups in blood and faecal *E. coli* isolates.

the minimal prerequisite for bacterial passage from kidney infection into the bloodstream [21,33,34]. The role of *E. coli* VFs in the pathogenesis of sepsis in relation to the site of its primary infection is virtually unknown.

Virulence properties of the isolates belonging to phylogenetic groups A and B1 were not analysed. To understand the role of commensal intestinal *E. coli* as a potential source for pathogenic and *E. coli* populations, we investigated the phylogenetic groups and virulence profile of the blood and faecal *E. coli* isolates from patients with and without sepsis, respectively, admitted to the ICU. The faecal isolates were not collected from the same sepsis patients because such patients

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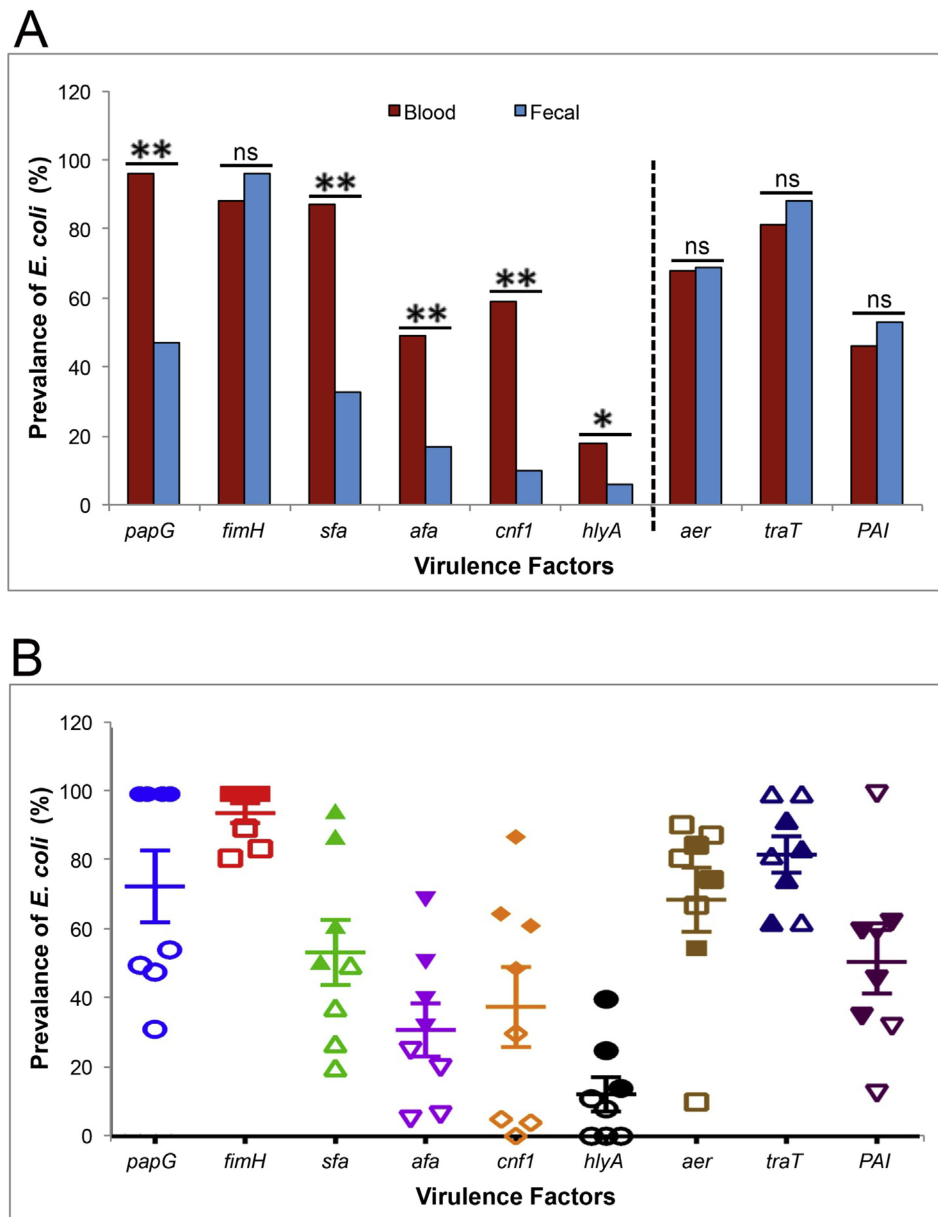

FIG. 3. PCR-based prevalence of virulence genes (*papG, fimH, sfa, afa, cnf1, hlyA, aer, traT, PAI*) between blood (*n* = 78) and faecal (*n* = 83) *Escherichia coli* isolates (A). Solid and empty shapes represent blood and faecal *E. coli* isolates, respectively (B). The p value is calculated by *Z* test and indicates significance among both the groups. *p 0.02; **p 0.0001; ns, nonsignificant.

will definitely have an effect on their gut flora and would not have acted as a suitable control. We established phylogenetic grouping of *E. coli* isolates into four groups: A, B1, B2 and D [7]. No significant difference was observed in the prevalence of the commensal and pathogenic phylogenetic groups in the blood and faecal *E. coli* isolates. However, group B1 was more prevalent in the faecal *E. coli* isolates, suggesting they may have been acquired from the hospital environment by these patients. A high prevalence of *E. coli* in the group D blood isolates corroborates the nature of the *E. coli* as an extraintestinal pathogenic strain [8]. The aberrant observation of high prevalence of groups B2 among the faecal *E. coli* isolates may be attributed to hospital-acquired infections. A collective high prevalence (54%) of groups B2 and D among the faecal *E. coli* isolates may be ascribed to the varied bacterial characteristics, antibiotic usage or genetic factors of the host. The observed difference in distribution of *E. coli* phylogenetic groups between pathogenic and commensal *E. coli* populations was similar to

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those in an earlier study that compared faecal and urine isolates from different host population cohorts [30].

Because faecal flora is considered to be the natural reservoir of pathogenic strains in extraintestinal infections [35], the phylogenetic distribution of commensal *E. coli* isolates from healthy individuals could provide an important comparison of and insight into the spread of the potential pathogenic lineage. Previous reports indicated that group B2 *E. coli* strains are rare in faecal samples [30], which is contrary to our data. This implies that acquiring the group B2 strain in faecal samples is important in the risk of infection. In a similar phylogenetic study among patients with UTIs, group B2 dominated in the uropathogenic strains, while they also accounted for about 50% of the rectal specimen [35]. The high percentage of group B2 and group D in the faecal *E. coli* isolates prompted us to ask how frequently the virulent B2 pathogens that are routinely carried by healthy humans would affect the disease dynamic in this population.

In order to find possible link between strain phylogeny and individual virulence genes, we analysed an overall virulence profile (*papG, fimH, sfa, afa, cnf1, hlyA, aer, traT* and *PAI*) of blood and faecal *E. coli* isolates. The aggregate score of the virulence was found to be higher in blood than in faecal *E. coli* isolates. We observed a variable expression of virulence genes among the commensal (A and B1) and pathogenic (B2 and D) groups of blood and faecal *E. coli* isolates. An unexpected high expression of the virulence genes among the faecal *E. coli* isolates indicated a role of these genes in the development of infection and suggested 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 bacterial pathogenicity [1].

In *E. coli* extraintestinal infections, phylogenetic group B2 was found to be predominant and more virulent than other phylogenetic groups. An ascending route was proposed to be the major pathway of *E. coli* causing UTIs [36]. In addition to the virulence factors, host factors such as obstructions and immune-compromising conditions may favour the development of UTI among such patients [37]. Out of the VFs examined in this study, *papG, sfa, afa, cnf1* and *hlyA* were found to be more prevalent in the blood than the faecal *E. coli* isolates. Our data suggest that the adhesin coding gene *fimH* was the most prevalent among all the phylogenetic groups of blood and faecal *E. coli* isolates. FimH-mediated biofilm formation is known to facilitate bacterial colonization of urinary catheters and other medical implants—an unfortunately common problem for hospitalized individuals [38]. An unexpected high expression of the virulence genes *aer, traT* and *PAI* among the faecal *E. coli* isolates indicates their role in the development of infection. A high prevalence of these virulence genes may have led to the entry of commensal *E. coli* into the bloodstream, leading to sepsis [21,33,34]. *hlyA* and *traT* are known to predict the bacterial pathogenicity [39] among both compromised and non-compromised hosts [22].

The prevalence of adhesin and toxin (*papG, sfa, afa, cnf1* and *hlyA*) genes was significantly higher in the *E. coli* isolates from the blood compared to faecal *E. coli* isolates, indicating a possible association to the pathogenic conditions [22]. The high prevalence of adhesins in blood and faecal *E. coli* isolates than the expression of toxins or other virulence factors is arguably the most important determinant of pathogenicity. Surprisingly, no difference was observed in the prevalence of *fimH, Aer, traT* and *PAI* among the *E. coli* isolates of both these groups, indicating a role of the host environment in stimulating the bacteria to acquire these VFs and thus inducing the bacterial pathogenicity [26]. The analysis of virulence gene expression suggested a variable prevalence of *aer, traT* and *PAI* in the phylogenetic groups among blood and faecal *E. coli* isolates. The unusual high prevalence of *PAI* in the faecal *E. coli* isolates in our study indicates its role in making these patients vulnerable to severe infection. These observations suggest that the faecal *E. coli* isolates may transform a nonsepsis condition into sepsis in a hospital environment. In addition, the similar prevalence of *aer, traT,* and *PAI* in blood and faecal *E. coli* isolates is contrary to the findings of previous studies in *E. coli* strains from urosepsis [22].

In summary, we report for the first time a correlation of phylogenetic groups with important virulence markers in blood and faecal *E. coli* isolates from patients with and without sepsis admitted to the ICU. The high prevalence of all the VFs studied in the blood and faecal *E. coli* isolates and a similar prevalence of *aer, traT* and *PAI* indicate their role in the sustenance and development of infection. The specific association of *hlyA* and *cnf1* in the blood and faecal *E. coli* isolates indicate that the host environment may have an important role to play for a differential expression of virulence genes, thereby causing pathogenicity among these isolates in hospital settings. Therefore, our data suggest that faecal isolates could become pathogenic in the immunocompromised patients under challenging host conditions. Our study also indicates that patients prone to a severe form of infection in the hospital environment may be identified on the basis of their virulence profile. Further investigation is required to determine the interplay of these VFs and concurrently identifying the mechanisms regulating the expression of these traits in sepsis and nonsepsis *E. coli* isolates in order to improve the management of infectious diseases.

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Conflict of Interest

None declared.

Acknowledgements

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