JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Antibiotic resistance and virulence genes of extraintestinal pathogenic *Escherichia coli* from tropical estuary, south India

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Abstract

Introduction: *Escherichia coli* strains can cause a variety of intestinal and extraintestinal diseases. Extraintestinal pathogenic *E. coli* (ExPEC) strains have the ability to cause severe extraintestinal infections. Multidrug resistance among ExPEC could complicate human infections.

Methodology: *Escherichia coli* strains were isolated during the period of January 2010 to December 2012 from five different stations set at Cochin estuary. Susceptibility testing was determined by the disk-diffusion method using nine different antimicrobial agents. A total of 155 strains of *Escherichia coli* were screened for the presence of virulence factor genes including *papAH*, *papC*, *sfa/focDE*, *iutA*,and *kpsMT* II associated with ExPEC.

Results: Among the 155 *E. coli* isolates, 26 (16.77%), carried two or more virulence genes typical of ExPEC. Furthermore, 19.23% of the ExPEC isolates with multidrug resistance were identified to belong to phylogenetic groups B2 and D. Statistically significant association of *iutA* gene in ExPEC was found with *papC* ($p < 0.001$) and *kpsMT* II ($p < 0.001$) genes. ExPEC isolates were mainly resistant to ampicillin (23.07%), tetracycline (19.23%), co-trimoxazole (15.38%), and cefotaxime (15.38%). The adhesion genes *papAH* and *sfa/focDE* were positively associated with resistance to gentamicin, chloramphenicol, and cefotaxime ($p < 0.05$).

Conclusions: Co-occurrence of virulence factor genes with antibiotic resistance among ExPEC poses considerable threat to those who use this aquatic system for a living and for recreation.

Key words: *Escherichia coli*; ExPEC; antibiotic resistance; phylogenetic groups; virulence factors.

J Infect Dev Ctries 2015; 9(5):496-504. doi:10.3855/jidc.5627

(Received 19 July 2014 – Accepted 26 November 2014)

Introduction

Estuarine waters contaminated by *Escherichia coli* derived from human or animal waste are a growing public health concern. Although most *E. coli* strains are harmless, some strains can cause a variety of intestinal and extraintestinal diseases. According to genetic and clinical criteria, strains of *E. coli* can be classified into three major groups: commensal, intestinal pathogenic, and extraintestinal pathogenic *E. coli* (ExPEC) strains [1]. ExPEC strains have the ability to cause extraintestinal infections such as urinary tract infections, neonatal meningitis, sepsis, and wound infections, which can lead to serious complications and death [2-4]. ExPEC strains possess several virulence traits that facilitate colonization, invasion, and pathogenesis in specific body locations [5].

ExPEC isolates were found to share a characteristic distribution within the widely used *E. coli* phylogenetic classification based on A, B1, B2, and D groups [6]. Most of the virulent extraintestinal pathogenic strains belong to group B2 or, less frequently, to group D, whereas most commensal strains of *E. coli* belong to groups A and B1 [7]. The phylogenetic group B2 strains evolved to become virulent by acquisition of numerous pathogenetic determinants [8]. ExPEC can be characterised by highly effective virulence mechanisms [9] that can be acquired by horizontal gene transfer.

Antibiotic resistance in pathogenic bacteria from environmental sources is recognized as a global problem in public health. Acquired antibiotic resistance is particularly problematic when it occurs in ExPEC, the distinctive *E. coli* strains that possess the specialized virulence factors (VFs) required for extraintestinal disease [10]. Studies have shown an increased prevalence of antibiotic-resistant strains among pathogenic bacteria [11], and over the years, nearly every bacterial pathogen has developed resistance to one or more clinical antibiotics [12]. Antibiotics are major contaminants found in polluted waters [13] and appear to play a significant role in the

natural selection and survival of resistant strains. Antibiotic-resistant bacteria are also reported to be introduced to water bodies through waste discharges from animal husbandry and hospitals [14,15]. Estuarine environments are receptacles for various kinds of wastes draining into the system, which is highly favourable for horizontal gene transfer among pathogenic and non-pathogenic bacterial strains.

Cochin estuary, a part of Vembanad Lake and an important Ramsar site in India, supports a good shellfish and finfish fishery. It is the largest among many extensive estuarine systems along the southwest coast of India and has been identified as one of the most productive estuarine systems along the west coast of India [16]. During the last decade, Cochin City has witnessed a large-scale population explosion. However, insufficient infrastructure to treat the waste has resulted in considerable organic pollution of the estuary, mainly from the satellite townships that are being developed all along its coast. Most of the markets and hospitals situated in this area also discharge partially treated or untreated wastewater into this estuary. Cochin estuary supports excellent fish and shellfish resources apart from being a hub for local and international tourists, who use it for recreation almost year-round. Many people are directly or indirectly making a living from this natural body of water, and the increasing level of organic pollution of the estuary is of concern.

Thus, considering the adverse effects that ExPEC isolates can have on public health, this study focused on assessing the prevalence of antibiotic resistance and distribution of virulence factor genes among different phylogenetic groups of *Escherichia coli* isolated from Cochin estuary, south India.

Methodology

Description of the study area

Water samples were collected from five different stations along the Cochin estuary (Figure 1). The stations were selected based on their closeness to satellite townships and waste inputs. Two of the stations, Chittoor (station 1) and Thevara (station 4), were fixed upstream; two were in the central part of the estuary, Bolgatty (station 2) and Marine Science Jetty (station 3); and one was at the Barmouth (station 5).

Collection of samples

Water samples were collected on a monthly basis from these stations for a period of three years from January 2010 to December 2012. Samples were collected in wide-mouthed sterile plastic bottles (Tarson, Kolkata, India) from one foot below the surface so as to get a better representation of the water column. Water samples were transported to the laboratory in an ice box and subjected to bacteriological examination within four hours of collection [17].

Isolation and identification of E. coli

Samples were analyzed for fecal coliforms by the most probable number (MPN) method using MacConkey broth (Hi-media Labs, Mumbai, India) as a medium. Water samples of 10 mL, 1 mL, and 0.1 mL were inoculated into respective dilution tubes containing inverted Durham's tubes. Inoculated tubes were incubated at 37°C for 24 hours and observed for growth and gas production [18]. For isolation of *E. coli*, one loopful of culture from MacConkey broth tubes showing growth and gas production were streaked onto eosin methylene blue (EMB) (Hi-media) plates and incubated at 37°C for 24 hours. After incubation, plates were observed for typical *E. coli*-like colonies. Whenever present, two colonies per

Figure 1. Cochin estuary map showing sampling location

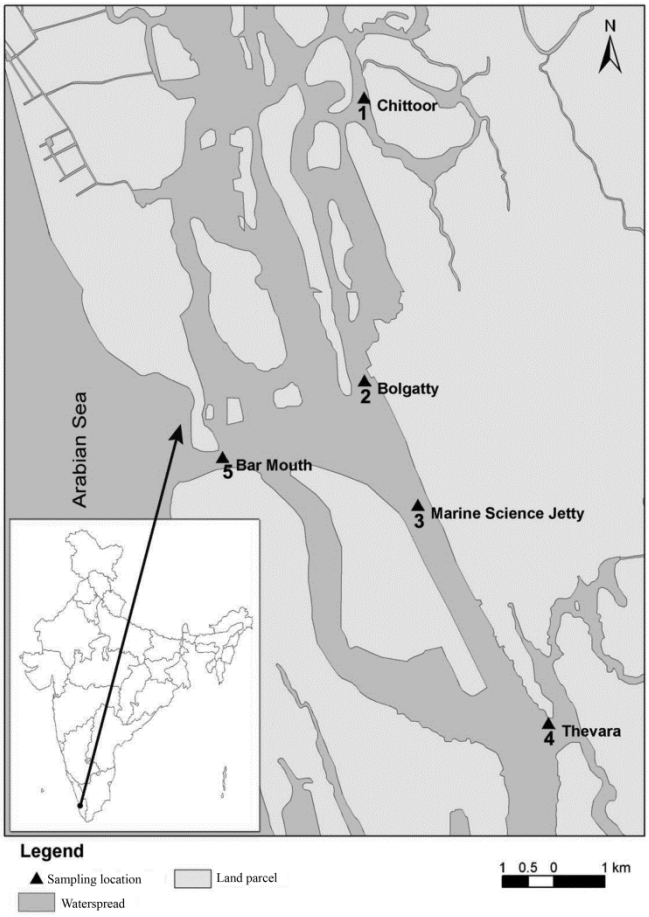


plate were selected, restreaked to ensure purity, and maintained on nutrient agar (Hi-media, Mumbai, India) slants. All isolates were submitted to biochemical screening, which included the indole test, methyl red test, Voges-Proskauer test, and citrate utilization (IMViC) test. Cultures giving + + – – reaction were confirmed as *E. coli* [19]. Confirmed *E. coli* cultures were serotyped at the National *Salmonella* and *Escherichia* Center, Central Research Institute, Kasauli, Himachal Pradesh, India [20].

Isolation of DNA from E. coli

DNA from the bacterial genome was extracted per the standard proteinase K digestion method [21]. Bacterial cultures were inoculated into Luria Bertani broth (HiMedia) and incubated in an orbital shaker incubator (Orbitek, Chennai, India) at 37°C at 110 rpm for 12 hours. After incubation, the cells were harvested by centrifugation at 15,000 g for 10 minutes (Eppendorf, Hamburg, Germany) and then suspended in TEN (Tris-HCl [pH 7.2], 10 mM EDTA, 250 mM NaCl) buffer with 1% sodium dodecyl sulphate (HiMedia, Mumbai, India). Proteinase K (GeNei, Mumbai, India) was then added to a final concentration of 100 µg/mL and mixed gently. The suspension was incubated at 37°C for 60 minutes. DNA obtained by sequential phenol-chloroform and chloroform-isoamyl alcohol extraction was precipitated by adding 2.5 volumes of absolute ethanol. DNA was then suspended in 100 µL of TE buffer (10 mM Tris-HCl, 1 mM EDTA pH 7.5) and checked for purity by agarose gel electrophoresis.

Phylogenetic analysis

The *E. coli* phylogenetic groups were determined by a triplex polymerase chain reaction (PCR) method as described previously [6]. This PCR is based on the amplification of two genes (*chuA* and *yjaA*) and one genomic fragment (TSPE4.C2). The primers used were ChuA.1 (5′-GACGAACCAACGGTCAGGAT-3′) and ChuA.2 (5′-TGCCGCCAGTACCAAAGACA-3′), YjaA.1 (5′-TGAAGTGTCAGGAGACGCTG-3′), and YjaA.2 (5′-ATGGAGAATGCGTTCCTCAAC-3′), and TspE4C2.1 (5′-GAGTAATGTCGGGGCATTCA-3′) and TspE4C2.2 (5′-CGCGCCAACAAAGTATTACG-3′). The optimized protocol was carried out with a PCR mix of 20 µL containing 1.5 mM $MgCl_2$, 2.5 µl of Taq buffer (Tris [pH 9.0] at 25°C, KCl and Triton X-100), 2 mM each of dNTP mixture, 20 pmol each of the primers, 2.5 U of Taq polymerase (GeNei) and 1 µL of the DNA template. The amplification consisted of following steps: initial denaturation at 94°C for 5 minutes, followed by 30 cycles of denaturation (30 seconds at 94°C), annealing (30 seconds at 55°C) and extension (30 seconds at 72°C), and a final extension step of 7 minutes at 72°C. PCR products were then resolved by electrophoresis on 1.5% agarose gel (HiMedia), stained with ethidium bromide (GeNei), and visualized by a gel documentation system (BioRad, Berkeley, USA). The data of the three amplicons resulted in assignment of strains to phylogenetic groups as follows: $ChuA^+$, $YjaA^+/ChuA^+$,$YjaA^+$, $TspE4.C2^+$, group B2; $ChuA^+$, $YjaA^-/ChuA^+$, $TspE4.C2^+$, group D; $ChuA^-$, $TspE4.C2^+/YjaA^+$, $TspE4.C2^+$, group B1; $ChuA^-$, $TspE4.C2^-/ChuA^-$, $YjaA^+$, $TspE4.C2^-$, group A.

Detection of virulence factor genes

All the isolates were screened by PCR for five key virulence factor genes of ExPEC as suggested by Johnson and Stell [7]. Isolates were classified as ExPEC if they were found to be positive for two or more virulence factor genes such as *papAH* (*papA* - P fimbriae major structural subunit; *papH* - P fimbriae minor subunit), *papC* (P fimbrial assembly), *sfa/focDE* (S and F1C fimbriae), *iutA* (aerobactin receptor) and *kpsMT* II (group II capsule), and the remaining ones (without the above genes) were considered as non-ExPEC. *PapA* and *papH* (*papAH*) were coamplified using the same primers set. A universal forward primer for *papAH* (5′-ATGGCAGTGGTGTCTTTTGGTG-3′) was selected from the consensus signal sequence region of *papA*, without regard for peptide structure, whereas reverse primer (5′- CGTCCCACCATACGTGCTCTTC-3′) was from the 5′ end of *papH* [22]. Each reaction mixture consisted of 4 mM MgCl2, 1 µL of 25 pmol of each primer (*papAH*, *papC*, *sfa/focDE*, *iutA*, *kpsMT* II), 2 µL of 2 mM dNTPs and 4 µL of 1X Taq buffer, 1U of Taq DNA polymerase (GeNei) in a total volume of 20 µL, including 1 µL DNA template. The cycling conditions were as follows: 94°C for 5 minutes, followed by 30 cycles of denaturation (94°C, 30 seconds), annealing (64°C, 30 seconds), extension (68°C, 3 minutes), and final extension (72°C, 10 minutes). PCR products were then electrophoresed on 1.5% agarose gel containing ethidium bromide (GeNei), and visualized by a gel documentation system (BioRad).

Antibiotic susceptibility testing

All isolates were subjected to antibiotic sensitivity testing against nine antibiotics using standard methods

[23]. The antibiotics and concentration used were as follows: ampicillin (Amp, 10 mcg), cefotaxime (Ctx, 30 mcg), chloramphenicol (C, 30 mcg), co-trimoxazole (Cot, 25 mcg), gentamicin (Gen, 10 mcg), nalidixic acid (Na, 30 mcg), streptomycin (S, 10 mcg), tetracycline (Te, 30 mcg), and trimethoprim (Tr, 5 mcg). All antibiotic disks used were HiMedia brand. Cultures were enriched in nutrient broth for 6–8 hours. The enriched cultures were then swabbed over Muller-Hinton agar plates using sterile cotton swabs. After 15 minutes of pre-diffusion, the antibiotic disks were placed over the seeded agar plates, sufficiently separated from each other so as to avoid overlapping of antibiotics. After overnight incubation at 37°C, resistance was estimated by measuring the inhibition zone according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [24].

Statistical analysis

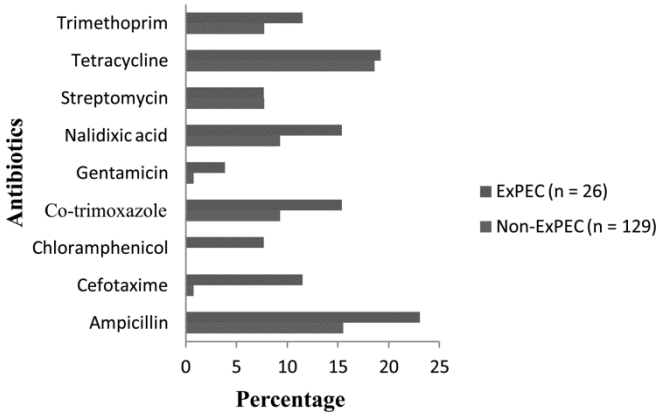
Statistical analysis of the results was carried out with SPSS version 13. Comparisons of associations between extraintestinal pathogenic *E. coli* and antibiotic resistance were performed using Pearson's Chi-square exact test. Statistical significance was set at a p value of < 0.05.

Results

Distribution of virulence factor genes

Results revealed that 29 out of 155 strains carried virulence factor genes, of which 15 (51.72%) belonged to phylogenetic group B2, 9 (31.03%) to group D, 3 (10.34%) to group A, and 2 (6.89%) to group B1. The virulence score used to classify the ExPEC isolates was calculated using the total number of virulence factor genes. The *iutA* (aerobactin acquisition), *papC* (P fimbrial assembly), and *kpsMT* II (group 2 capsule synthesis) genes were frequently encountered, whereas *sfa/focDE* (S and F1C fimbriae) and *papAH* (P fimbriae major and minor structural subunits) were detected rarely. The frequency of distribution of the virulence factor genes varied among the different groups. Interestingly, one isolate belonging to group B2 was found to contain *papAH* + *papC* + *sfa/focDE* I+ *iutA* + *kpsMT* II. Statistical analysis of the result revealed a highly significant association between *iutA* and *kpsMT* II ($p < 0.000$) and *iutA* and *papC* ($p < 0.000$). Furthermore, a significant association was found between *kpsMT* II and *papC* ($p = 0.000$).

Figure 2. Percentage of antibiotic resistance in non-ExPEC and ExPEC isolated from Cochin estuary



Prevalence of antibiotic resistance among ExPEC

All 155 *E. coli* isolates were subjected to antimicrobial susceptibility tests against nine antibiotics. The percentage of resistance to nine antibiotics among ExPEC and non-ExPEC is shown in Figure 2. About 22% of isolates showed resistance to all the antibiotics tested. Results revealed that 23.07% of ExPEC isolates were resistant to at least one antibiotic and that 19.23% were multiresistant. Among the various antibiotics tested, resistance to ampicillin (23.07%), tetracycline (19.23%), co-trimoxazole (15.38%), and cefotaxime (15.38%) was relatively higher among ExPEC. Moreover, 7.69% of the isolates were resistant to chloramphenicol and nalidixic acid, and 3.84% were resistant to gentamicin.

The relationship between antibiotic resistance and carriage of virulence factor genes was statistically examined; p values < 0.05 were used to indicate a significant relationship. The aerobactin receptor gene (*iutA*) was positively associated with resistance to ampicillin ($p = 0.046$), chloramphenicol ($p = 0.025$), and cefotaxime ($p = 0.025$). The S/F1C fimbriae gene, (*sfa/focDE*) on the other hand, showed an association with resistance to gentamicin and chloramphenicol in addition to cefotaxime ($p < 0.05$). Adhesion-related genes such as *papAH* also showed significant association with cefotaxime, chloramphenicol, and gentamicin ($p < 0.05$), whereas *papC* did not show positive association with any antibiotic resistance traits. Group 2 capsule synthesis gene (*kpsMT* II) was positively associated ($p < 0.05$) with chloramphenicol and cefotaxime.

Table 1. Distribution of virulence factors among various phylogenetic group of *E. coli* from Cochin estuary

Virulence factors	% Incidence among all strains (n = 155)	% Incidence among each phylogenetic group			
		A (n = 56)	B1 (n = 37)	B2 (n = 42)	D (n = 20)
papAH	0.64	0	0	2.33	0
papC	8.38	3.57	5.40	19.04	5
sfa/focDE	0.64	0	0	2.38	0
iutA	15.48	3.57	2.70	30.95	40
kpsMT II	14.19	3.57	5.40	23.80	40
papC + *kpsMT* II	1.29	1.78	0	0	5
papC + *iutA*	3.22	1.78	0	9.52	0
iutA + *kpsMT* II	8.38	1.78	0	11.90	0
papC + *iutA* + *kpsMT* II	2.58	0	5.40	4.76	0
papC + *iutA* + *kpsMT* II + *papAH* + *sfa/focDE*	0	0	0	2.38	0

Table 2. Distribution of virulence factors (VF) within resistant and sensitive strains of *E. coli* (n = 155) belonging to different phylogenetic groups

VF	% incidence in Group A (n =56)		% incidence in Group B1 (n =37)		% incidence in Group B2 (n =42)		% incidence in Group D (n =20)	
	S* (n = 43)	R** (n = 13)	S* (n = 28)	R** (n = 9)	S* (n = 34)	R** (n = 8)	S* (n = 15)	R** (n = 5)
papAH	0	0	0	0	0	12.5	0	0
papC	4.65	0	7.14	0	14.70	37.5	0	25
sfa/focDE	0	0	0	0	0	12.5	0	0
iutA	4.65	0	3.57	0	29.41	37.5	40	50
kpsMT II	4.65	0	3.57	0	20.58	25	40	50

*Sensitive; **Resistant

Table 3. Percentage of antibiotic resistance in non-ExPEC and ExPEC and their distribution among four phylogenetic groups

Antibiotics	% resistance in Group A (n = 56)		% resistance in Group B1 (n = 37)		% resistance in Group B2 (n = 42)		% resistance in Group D (n = 20)	
	Non-ExPEC (n = 53)	ExPEC (n = 3)	Non-ExPEC (n = 35)	ExPEC (n = 2)	Non-ExPEC (n = 29)	ExPEC (n = 13)	Non-ExPEC (n = 12)	ExPEC (n = 8)
Ampicillin	9.4	0	22.85	0	13.79	23.07	25	37.5
Cefotaxime	0	0	0	0	0	15.38	8.33	12.5
Chloramphenicol	0	0	0	0	0	3.44	0	12.5
Co-trimoxazole	11.32	0	8.57	0	10.34	15.38	0	25
Gentamicin	0	0	0	0	0	3.44	8.33	0
Nalidixic acid	7.54	0	17.24	0	6.89	15.38	0	25
Streptomycin	13.20	0	2.87	0	6.89	3.44	0	12.5
Tetracycline	24.52	0	22.85	0	10.34	23.07	0	25
Trimethoprim	13.20	0	5.71	0	3.44	15.38	8.33	12.5

Incidence of antimicrobial resistance and virulence genes in phylogenetic groups A, B1, B2, and D

The combination of PCR products obtained (279, 211, and 152 bp) allowed the inclusion of the *E. coli* isolates in one of the four major phylogenetic groups (A, B1, B2, and D). While 36.12% of the isolates belonged to phylogenetic group A, 27.09% belonged to group B2, 23.87% to group B1, and 12.90% to group D (Figure 3). Distribution percentages of non-ExPEC and ExPEC isolates among the four *E. coli* phylogenetic groups are shown in Figure 4. Triplex PCR analysis of the non-ExPEC isolates (n = 129) revealed that 41% of the isolates belonged to phylogenetic group A, followed by group B1 (27.13%), B2 (22.48%), and D (9.3%). Of the 26 ExPEC isolates detected, 13 (50%) belong to group B2, 8 (30.76%) to group D, 3 (11.53%) to group A, and 2 (7.69%) to group B1.

The distribution of virulence factor genes among the four major phylogenetic groups is presented in Table 1. Of the 26 ExPEC isolates, 5 isolates (19.23%) showed multidrug resistance; these belonged to phylogenetic groups B2 and D. More than 37% of group D ExPEC isolates and 23% of group B2 ExPEC isolates showed antibiotic resistance. Therefore, to assess whether the absence of these virulence genes is also related to antibiotic resistance, the frequency of each virulence factor considered (*papAH*, *papC*, *sfa/focDE*, *iutA*, *kpsMT* II) in susceptible and resistant *E. coli* isolates within each phylogenetic group (A, B1, B2, and D) was evaluated (Table 2). A prevalence of *sfa/focDE* and *papAH* was seen in group B2 resistant (12.5%) only. In susceptible *E. coli* B2 strains, the incidence of *papC*, *iutA*, and *kpsMT* II was 14.70%, 29.41%, and 20.58%, respectively, whereas in resistant B2 isolates, the incidence was 25% for *kpsMT* II and 37.5% for *papC* and *iutA*. The results also indicated that within A and B1 phylogenetic groups, *papC*, *iutA*, and *kpsMT* II were present only in susceptible isolates. In phylogenetic group D, prevalence of virulence factor genes such as *iutA* and *kpsMT* II did not show much variation in susceptible (40%) and resistant (50%) isolates. Of the virulence factor genes, *kpsMT* II and *iutA* were significantly ($p = 0.000$) more frequent in all the phylogenetic groups than was *papC* ($p = 0.035$). *PapAH* and s*fa/focDE* were detected only in strains that belonged to phylogenetic group B2. The percentages of antibiotic resistance in non-ExPEC and ExPEC and their distribution among the four phylogenetic groups are listed in Table 3.

Figure 3. Overall distribution of various phylogenetic groups of *E. coli* (n = 155) from Cochin estuary

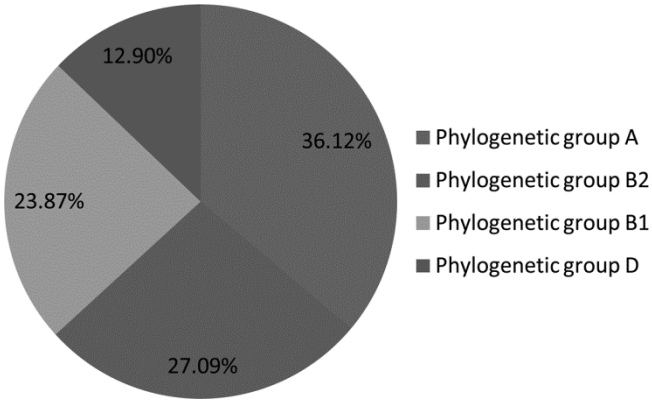
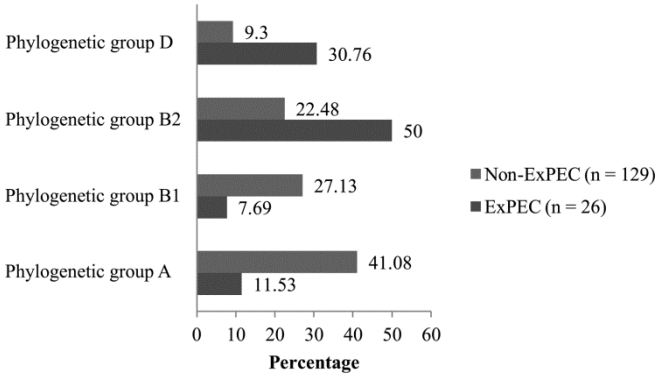


Figure 4. Distribution of different phylogenetic groups among non-ExPEC and ExPEC isolated from Cochin estuary



Discussion

Projection of virulence factor traits onto the phylogenetic background of the isolates revealed, as expected, an association of most virulence factor genes with B2 and D phylogenetic groups and, to a lesser extent, to groups A and B1. This agrees with several studies concerning phylogenetic groups in uropathogenic [25,26] and bacteraemic *E. coli* [27-29]. Our results also confirmed certain findings reported by others [30,31], such as the presence of the *sfa/foc* operon only in phylogenetic group B2. Clinical isolates of ExPEC typically belong to phylogenetic group B2 and, to a lesser extent, group D [32]. In agreement with several authors [33-35], we observed a link between phylogenetic groups and extraintestinal pathogenic strains because the majority of the strains belonged predominantly to phylogenetic group B2 and, to a lesser extent, to group D, whereas they were sparsely represented within groups A and B1. Strains belonging to group B2 harboured a greater number of virulence factors compared to strains from other phylogenetic groups, suggesting a putative association

between virulence factors and pathogenic potential [36].

We found that among all phylogenetic groups, B2 harboured a significantly higher proportion of virulence factors genes. *PapAH* was detected only in phylogenetic group B2. This is in agreement with the report of Nowrouzizn *et al*. [37], who found that most B2 strains carried genes for P-fimbriae. Our result also supported some previous reports indicating a greater association of traditionally recognized uropathogenic virulence factor genes (*e.g*., *pap* and *sfa)* with groups D and B2 as compared with A and B1 [38,39]. Most ExPEC, including those with most robust virulence factor genes and those that are best able to infect non-compromised hosts, are derived from phylogenetic group B2 [40]. High prevalence of B2 in this Cochin estuary is indeed a concern, as the system is used for both fishing and recreation.

Group D contained the second highest number of ExPEC. Extraintestinal pathogenic isolates from this group typically have somewhat fewer virulence factor genes and a different mix of virulence factor genes than do group B2 isolates. *E. coli* strains belonging to groups A and B1 do not frequently cause extraintestinal infection. These strains, which are not highly virulent, generally cause disease only in immunocompromised hosts, and could be pathogenic in healthy hosts only if they were to acquire sufficient extraintestinal factors [40].

In the present study, nearly 77% of the ExPEC isolates were susceptible to all tested antibiotics, while the rest of the isolates demonstrated a wide range of resistance, from resistance to just one antibiotic to resistance to all the antibiotics tested. Overall, this study revealed that resistance to antibiotics is significantly associated with the absence of virulence factor genes. Previous data suggest that among *E. coli* isolates from patients with urosepsis, resistance to antimicrobial agents such as ampicillin, sulphonamides, tetracycline, and streptomycin is negatively associated with virulence [41]. More recent data demonstrate a similar negative association between antibiotic resistance and virulence factors or a B2 phylogenetic background [33,34,42]. This has been interpreted as loss of virulence factors concomitant with mutation to resistance [43]. However, this hypothesis does not account for the phylogenetic shifts (away from group B2) observed among resistant isolates, which suggest that resistant isolates derive primarily from distinct, less virulent bacterial populations [44,45].

Significant associations were observed between sulphonamide resistance and low incidence of *papAH*, *papC*, *sfa/focDE*, *iutA*, and *kpsMT* II. Quinolone-resistant isolates were significantly associated with low incidence of *papAH*, *papC*, *sfa/focDE*, *iutA*, and *kpsMT* II, which supports a previous finding [46]. Soto *et al*. [47] reported that uropathogenic *E. coli* strains exposed to sub-inhibitory concentrations of quinolones showed partial or total loss of PAIs (pathogenicity islands) containing virulence factor genes. Diard *et al*.'s [48] findings that ExPEC PAIs play an important role in intestinal colonization and Moreno *et al*.'s [49] findings that group B2 and D tend to dominate fecal *E. coli* populations prior to extraintestinal infections suggest the presence of shared specific bacterial factors that contribute to fitness within the intestine. A striking finding from the present study is that 37% of group D ExPEC isolates and 23% of group B2 ExPEC isolates exhibited antibiotic resistance, and one ExPEC isolate from group B2 was resistant to all the antibiotics tested. This may be because individual strains have undergone the necessary and appropriate adaptation for survival in the changing antibiotic environment.

In the current study, we found a significant association of virulence factor genes with phylogenetic groups B2 and D. On the other hand, the significant differences in the prevalence of some virulence factor genes, such as *papAH* and *sfa/focDE* in phylogenetic group B2 compared to groups D, A, and B1 may provide evidence of the probable role of phylogenetic background in determining the virulence of a strain.

Conclusions

Our results highlight the presence of multidrug-resistant extraintestinal pathogenic *E. coli* in Cochin backwaters. The high frequency of B2 and D strains carrying virulence factor genes, poses serious questions about the potential risk for humans once they come into contact with contaminated estuarine water. Microbial contamination can limit people's enjoyment of coastal waters for contact recreation or shellfish gathering. Our research on the health status of two popular beaches along the Cochin coast (results unpublished) revealed very high loads of fecal coliforms at Fort Cochin beach, which is frequented by large numbers of both national and international tourists. *E. coli* can make people sick if the bacteria are present in high levels in water used for contact recreation or shellfish gathering. When feeding, shellfish used to filter large volumes of water, resulting in accumulation and concentration of bacteria

in the shellfish flesh. The results of the research call for immediate regulatory control over the classification of bodies of water used for shellfish harvesting; furthermore, the results also call for the monitoring of the health status of beaches and for the implementation of necessary closure when there are high numbers of fecal indicator bacteria and potential pathogens.

Acknowledgements

Divya Sukumaran would like to thank Council of Scientific and Industrial Research (CSIR), Government of India, for the research fellowship to carry out the work. The authors are also thankful to the Head of the Department of Marine Biology, Microbiology and Biochemistry, Cochin University of Science and Technology, for providing the facilities.

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Conflict of interests: No conflict of interests is declared.