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RESEARCH ARTICLE

Bacterial Phylogenetics

Predominance of phylogenetic group B2 among commensal *Escherichia coli* in humans from Kandy District, Sri Lanka

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Abstract: *Escherichia coli* is a commensal bacterial gut inhabitant in vertebrates, while some strains are pathogens. Analysis of the genetic substructure within *E. coli* has identified nine phylogenetic groups within the species, as group A, B1, B2, C, D, E, F, G and cryptic clade I. These groups vary in attributes such as host characteristics, pathogenicity, antibiotic sensitivity patterns and virulence profiles. The current study was conducted to determine the distribution of phylogenetic groups, commonly encountered sequence types (ST), and group B2 subgroups (SG) of human faecal *E. coli* in a sample population in the Kandy District, Sri Lanka. A total of 158 faecal swabs, collected from healthy individuals, were cultured on MacConkey agar and presumptive *E. coli* isolates were confirmed by a negative reaction on Simmons citrate agar. *E. coli* isolates were characterized according to their phylogenetic group, SG, and ST distribution by a series of PCR protocols. Results revealed a predominance of group B2 (28 %), followed by B1 (23 %), A (17 %), D (14 %), C (5 %), F (4 %), and E (3 %). Within phylogroup B2, SG III was predominant while ST 73, 95, and 131 were detected at low frequencies. ST 69 accounted for 20 % of the group D isolates. Distribution of phylogroups was independent of host gender except group D, which was significantly over-represented by males. As B2 *E. coli* are strongly associated with extraintestinal infections and a high degree of virulence, characterization of these human faecal *E. coli* isolates for antibiotic susceptibility and virulence profiles would be of clinical importance.

Keywords: commensal, *Escherichia coli*, PCR, phylogenetic groups, ST.

INTRODUCTION

Escherichia coli is a well-known member of the bacterial family Enterobacteriaceae. Its primary habitat is the lower gut of vertebrates, where it usually thrives as the predominant aerobic bacterium (Tenaillon *et al*., 2010), whereas water, sediment, and soil can be considered as its secondary habitat (Savageau, 1983). *E. coli* plays a remarkable dual role as a widespread gut commensal and a versatile pathogen.

As a result of phylogenetic studies performed on *E. coli* since the late 20th century, a nine- group phylogeny with groups A, B1, B2, C, D, E, F, G and *Escherichia* clade I has been established (Milkman, 1973; Ochman & Selander, 1984; Walk *et al*., 2009; Chaudhuri & Henderson, 2012; Clermont *et al*., 2013; 2019). Within these phylogenetic groups, clonal complexes (CCs)/ sequence types (STs) are identified based on the genetic sequence similarity between *E. coli* strains that are related and share a common set of characteristics. Numerous studies have shown that the distribution of *E. coli* phylogenetic groups is non-random. For instance, in humans, groups A and B2 have been found to be the most common, while groups B1 and D are less prevalent. In animals, B1 strains are predominant followed by A, B2 and D (Chaudhuri & Henderson, 2012). Analysis of

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human commensal *E. coli* from different climate regions has shown that subjects in tropical areas exhibit a more diverse *E. coli* microbiota than those in temperate areas (Escobar-Páramo *et al*., 2004).

Importantly, human extra-intestinal pathogenic *E. coli* (ExPEC) strains are more likely to belong in group B2, and to a lesser extent, group D, whereas strains belonging to groups A or B1 are less virulent (Boyd & Hartl, 1998; Picard *et al*., 1999; Johnson *et al*., 2001). Further, analyzing large collections of ExPEC has shown a predominance of Sequence Types (STs) 69, 73, 95, and 131 from groups D and B2, leading researchers to investigate the virulence profiles and antibiotic sensitivity patterns of these lineages (Adams-Sapper *et al*., 2013; Banerjee *et al*., 2013). Thus, for instance, being aware of the resistance of the infamous ST 131 of group B2 to antibiotics such as fluoroquinolones and third-generation cephalosporins, clinicians are enabled to plan appropriate therapy for such infections, when the phylogenetic identity of the pathogenic *E. coli* strain is known. Not only is the clinical community interested in phylotyping of *E. coli*, but also a growing number of aquatic microbiologists, the investigators involved in water quality or source of contamination studies (Walk *et al*., 2007; Carlos *et al*., 2010; Stoppe *et al*., 2014), food microbiologists (Bergeron *et al*., 2012), botanists (Méric *et al*., 2013), and scientists in many other disciplines consider that *E. coli* phylotyping is essential in this molecular era of *E. coli* studies.

However, except for a study on avian pathogenic *E. coli* (APEC) strains originating from Sri Lanka by Dissanayake *et al*., (2014), no attempts have been made to assess the phylogroup distribution of human *E. coli* isolates from Sri Lanka. Moreover, to the best of our knowledge, apart from the study on commensal *E. coli* of Pakistani infants by Nowrouzian *et al*., (2009), South Asian human communities/societies have not been screened for the distribution of commensal *E. coli*, though phylogenetic studies on pathogenic *E. coli* have been reported (Chakraborty *et al.*, 2015; Singh *et al*., 2017). This is a major drawback when considering the extent to which *E. coli* phylogenetic studies have developed beyond phylogroup determination and the amount of knowledge generated from studies arising from other regions of the world. Thus, the present study was launched to investigate the distribution of phylogenetic groups, commonly encountered sequence types (ST) and group B2 subgroups (SG) of *E. coli* isolates recovered from human faeces from a sample population residing in Kandy district, Sri Lanka.

MATERIALS AND METHODS

Sample collection

A total of 158 faecal swabs were collected from healthy volunteer Sri Lankan individuals that could be contacted by the investigators (convenience sampling). The subjects were included irrespective of their age or gender, provided that they had not received antibiotic therapy nor had a history of gastrointestinal disease during the previous month. A commercial collection and transport system for bacteria that consisted of a sterile cotton wool swab and a tube of Amies medium without charcoal (BBL™ CultureSwab™ Plus) was provided to each participant, and instructions were given on obtaining the swab sample. The faecal swab samples received were either taken immediately for bacterial culturing or stored in a laboratory refrigerator at 4 °C when immediate culturing was not possible.

Isolation of *E. coli* from faecal samples

Faecal swab samples received were cultured on MacConkey agar plates so as to obtain isolated bacterial colonies and incubated at 37 °C for 18 h. A selected presumptive *E. coli* colony (with a pink/red colour and a non-mucoid appearance) grown from each faecal sample was spot inoculated into a Simmons citrate agar plate and a Luria agar plate and incubated at 37 °C for 18 h. Cultures failing to produce a colour change (citrate negative) on Simmons citrate agar were presumed to be *E. coli*. Cultures on Luria agar were used for short term storage of *E. coli* colonies in a laboratory refrigerator at 4 °C.

Molecular analysis

DNA extraction from bacterial cells (from colonies grown on Luria agar) was performed using the DNAzol® genomic DNA isolation reagent (Molecular Research Center Inc., USA) according to the manufacturer's recommendations with modifications.

Approximately half of each *E. coli* colony on Luria agar was re-inoculated into 100 µL of sterile Luria broth in microfuge tubes and incubated for 18 hours with shaking at 36 °C. Then, 80 µL from each tube were discarded and the tubes with the remaining culture were centrifuged at maximum speed (15,000 r.p.m) for 1 min. After removing the supernatant, the pellet was re-suspended in 25 µL of 10X Sodium chloride-Tris-EDTA (STE) buffer by vortexing. Next, the suspensions

were mixed by inverting the tubes after each addition of 50 µL of DNAzol® followed by 45 µL of 95 % ethanol for 2 min and 1 min, respectively. The tubes were centrifuged for 3 min at maximum speed and the supernatant was discarded. The pellets were then mixed with 250 µL of 75 % ethanol by inverting the tubes 10 times. Tubes were centrifuged at maximum speed for 1 min, the supernatant discarded and drained on paper towels. The pellets were dried at 50 °C for 45 min. Subsequently, 100 µL of a 0.03 M solution of sodium hydroxide in Tris-EDTA (TE) buffer were added to each tube and they were left in the oven at 65 °C for 5 min. After ensuring that the DNA had completely dissolved resulting in a clear solution, the tubes with DNA extracts were stored at -20 °C.

Table 1: Details of primers employed in the various PCR programmes of the current study

PCR programme	Amplification target	Primers	Primer sequences (5′-3′)	PCR product (bp)	Reference
Clermont quadruplex	chuA	chuA.1b	ATGGTACCGGACGAACCAAC	288	Clermont *et al*., 2013
		chuA.2	TGCCGCCAGTACCAAAGACA		
	yjaA	yjaA.1b	CAAACGTGAAGTGTCAGGAG	211	
		yjaA.2b	AATGCGTTCCTCAACCTGTG		
	TspE4.C2	TspE4C2.1b	CACTATTCGTAAGGTCATCC	152	
		TspE4C2.2b	AGTTTATCGCTGCGGGTCGC		
	arpA	AceK.f	AACGCTATTCGCCAGCTTGC	400	
		ArpA1.r	TCTCCCCATACCGTACGCTA		
Group E	arpA	ArpAgpE.f	GATTCCATCTTGTCAAAATATGCC	301	Clermont *et al*., 2013
		ArpAgpE.r	GAAAAGAAAAAGAATTCCCAAGAG		
Group C	trpA	trpAgpC.1	AGTTTTATGCCCAGTGCGAG	219	Clermont *et al*., 2013
		trpAgpC.2	TCTGCGCCGGTCACGCCC		
	trpA	trpBA.f[a]	CGGCGATAAAGACATCTTCAC	489	
		trpBA.r[a]	GCAACGCGGCCTGGCGGAAG		
New primer pool	1, ST73, CFT073 (AE014075)[b]	ST73_for	TGGTTTTACCATTTTGTCGGA	490	Doumith *et al*., 2015
		ST73_rev	GGAAATCGTTGATGTTGGCT		
	19, ST131, NA114 (CP002797)[b]	ST131_for	GACTGCATTTCGTCGCCATA	310	
		ST131_rev	CCGGCGGCATCATAATGAAA		
	9, ST95, UTI89 (CP000243)[b]	ST95_for	ACTAATCAGGATGGCGAGAC	200	
		ST95_rev	ATCACGCCCATTAATCCAGT		
	21, ST69, UMN026 (CU928163)[b]	ST69_for	ATCTGGAGGCAACAAGCATA	104	
		ST69_rev	AGAGAAAGGGCGTTCAGAAT		
Group B2 sub-typing (Panel 1)	pabB II	pabBgpII.f	GAGTCACTGCCAGAAATTGCA	415	Clermont *et al*., 2014
		pabBgpII.r	GGCGAAAGGCTTAAAATTGCACT		
	trpA III	trpAgpIII.f	GACGCGCTGGAATTAGGCTC	255	
		trpAgpIII.r	ATCGGCAACCAGCACCGAAT		
	dinB VI	dinBgpVI.f	CAGCGGTGGAGATGCGCGAT	652	
		dinBgpVI.r	TCGTCAATGCCCTGACTACA		
	icd VII	icdgpVII.f	GCGGTATTCGCTCTCTGAAT	810	
		icdgpVII.r	CAATTAAATCAGCCGCTTCG		
	aes IX	aesgpIX.f	CCTGGCCTGCAACGGGAG	160	
		aesgpIX.r	TCTGGCTGCGGATAAAAGAG		
	chuA Internal control	chuAgene.1	CGATACGGTCGATGCAAAAG	1013	
		chuAgene.2	TTGGACAACATCAGGTCATC		

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PCR programme	Amplification target	Primers	Primer sequences (5′-3′)	PCR product (bp)	Reference
Group B2 sub-typing (Panel 2)	putP I	putPgpI.f	GGTATCGCTTACTTTAACGG	373	Clermont *et al*., 2014
		putPgpI.r	ACCACCGGACCAAACGCC		
	trpA IV	trpAgpIV.f	TGCCAGTGGAAGAGTCCGCT	261	
		trpAgpIV.r	CCGGGGCGGAAATACCAAAG		
	polB V	polBgpV.f	GCCGTTTCGCCGAAGATAAA	530	
		polBgpV.r	TAATGATCTTCAGCGCCTGT		
	aes X	aesgpX.f	GACCGTTGTGAATACTCTTCA	713	
		aesgpX.r	TATAACAGGGCGGCACATTT		
ST 131	gyrB	gyrB47.f	CGCGATAAGCGCGAC	132	Johnson *et al*., 2009
		gyrB47.r	ACCGTCTTTTTCGGTGGAA		
ST 95	Svg	svg.1	TCCGGCTGATTACAAACCAAC		
		svg.2	CTGCACGAGGTTGTAGTCCTG		
ST 69	gyrB	gyrB27.f	GGTGCGTTTCTGGCCA		
		gyrB27.r	GACGCCGATACCATC		

a. The primers trpBA.f and trpBA.r are added in Group E and Group C specific PCR reactions as an internal control.

b. The number designated for the target region of the genome sequence, the sequence type (ST) and the accession number of the relevant *E. coli* genome in GenBank database as cited in the original article by Doumith *et al*. (2015).

Table 2: Concentrations of the primers and PCR conditions used in the current study

PCR programme	Primer	Concentration (µM)	PCR conditions	Electrophoresis conditions
Clermont quadruplex	chuA.1b	0.4	Denaturation for 5 min at 94 °C, 30 cycles of 30 s each at 94 °C, 55 °C and 72 °C and a final extension step of 7 min at 72 °C	1.5 % agarose gel, 100 V for 45 minutes
	chuA.2	0.4		
	yjaA.1b	0.4		
	yjaA.2b	0.4		
	TspE4C2.1b	0.5		
	TspE4C2.2b	0.5		
	AceK.f	0.6		
	ArpA1.r	0.6		
Group E	ArpAgpE.f	0.5	Denaturation for 5 min at 94 °C, 30 cycles of 5 s at 94 °C and 25 s at 57 °C and a final extension step of 5 min at 72 °C	1.5 % agarose gel, 100 V for 45 minutes
	ArpAgpE.r	0.5		
Group C	trpAgpC.1	0.5	Denaturation for 5 min at 94 °C, 30 cycles of 5 s at 94 °C and 25 s at 60 °C and a final extension step of 5 min at 72 °C	1.5 % agarose gel, 100 V for 45 minutes
	trpAgpC.2	0.5		
New primer pool	trpBA.f[a]	0.25	Denaturation for 3 min at 94 °C, 30 cycles of 30 s each at 94 °C, 60 °C and 72 °C and a final extension step of 5 min at 72 °C	1.5 % agarose gel, 90 V for 45 minutes
	trpBA.r[a]	0.25		
	ST73_for	0.4		
	ST73_rev	0.4		
	ST131_for	0.4		
	ST131_rev	0.4		
	ST95_for	0.4		
	ST95_rev	0.4		
	ST69_for	0.6		
	ST69_rev	0.6		

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PCR programme	Primer	Concentration (µM)	PCR conditions	Electrophoresis conditions
Group B2 sub-typing (Panel 1)	pabBgpII.f	0.4	Denaturation for 4 min at 94 °C, 30 cycles for 5 s at 94 °C and 20 s at 63 °C and a final extension step of 4 min at 72 °C.	2 % agarose gel, 85 V for 60 min
	pabBgpII.r	0.4		
	trpAgpIII.f	0.2		
	trpAgpIII.r	0.2		
	dinBgpVI.f	0.4		
	dinBgpVI.r	0.4		
	icdgpVII.f	0.5		
	icdgpVII.r	0.5		
	aesgpIX.f	0.6		
	aesgpIX.r	0.6		
	chuAgene.1	0.4		
	chuAgene.2	0.4		
Group B2 sub-typing (Panel 2)	putPgpI.f	0.4	Denaturation for 4 min at 94 °C, 30 cycles for 5 s at 94 °C and 20 s at 63 °C and a final extension step of 4 min at 72 °C	2 % agarose gel, 85 V for 60 min
	putPgpI.r	0.4		
	trpAgpIV.f	0.4		
	trpAgpIV.r	0.4		
	polBgpV.f	0.4		
	polBgpV.r	0.4		
	aesgpX.f	0.4		
	aesgpX.r	0.4		
ST 131	gyrB47.f	0.4	Denaturation for 10 min at 95 °C, 32 cycles for 30 s each at 94 °C and 65 °C and 30 s, and 2 min at 68 °C	2 % agarose gel, 100 V for 45 min
	gyrB47.r	0.4		
ST 95	svg.1	0.4	Denaturation for 15 min at 95 °C; 30 cycles of 30 s at 94 °C, 90 s at 55 °C, and 90 s at 72 °C and a final extension step of 10 min at 72 °C.	1.5 % agarose gel, 100 V for 45 min
	svg.2	0.4		
ST 69	gyrB27.f	0.4	Denaturation for 10 min at 95 °C, 32 cycles for 30 s each at 94 °C and 60 °C for 1 min at 72 °C and a final extension at 72 °C for 10 min.	1.5 % agarose gel, 100 V for 45 min
	gyrB27.r	0.4		

Polymerase chain reaction (PCR) and gel electrophoresis

All the PCRs (Table 1) were carried out in a TaKaRa PCR Thermal Cycler Dice™ TP600 (Takara Bio Inc, Japan) in 10 µL reaction volumes. The PCR mixture contained 5.0 µL of GoTaq® Green Master Mix, 2X (Promega Corporation, USA; a premix of DNA polymerase, 2X reaction buffer, 400 µM each of dNTPs and 3 mM magnesium chloride), 0.6 µL of DNA extract, relevant primers (concentrations mentioned in Table 2) and nuclease-free water. After each PCR, the products were subjected to horizontal gel electrophoresis (MultiSUB Choice, Cleaver Scientific Ltd.) under conditions mentioned in Table 2. The gels were stained with ethidium bromide (concentration approximately 0.5 µg/mL in the gel) and PCR product sizes were estimated using a molecular marker (HyperLadder™ 50 bp, Bioline). The gel images were captured and saved using a gel documentation system (Bio-Vision -3026).

The Clermont quadruplex PCR was conducted on all isolates to determine their 'quadruplex genotypes' as described in Clermont *et al*., (2013) (Figure 1). Isolates designated 'group D or E' by the quadruplex PCR were then subjected to the group E PCR which differentiated isolates into group D or group E (Figure 2). Similarly, those designated 'group A or C' were differentiated by group C PCR into group A or group C. Next, the isolates designated as groups B2 and D were collectively subjected to new primer pool PCR to identify the major STs (ST 73, 95, 131, and 69) among them (Figure 3).

Group B2 isolates were then again analyzed for their subgroups with group B2 sub-typing PCR (Figure 4). Finally, the STs/subgroups assigned for group B2 ST 95 and 131 isolates and group D ST 69 isolates were confirmed by the corresponding ST PCRs.

Statistical analysis

To determine whether there was a significant association between the *E. coli* phylogenetic group and the gender of the host, Fisher's exact test was performed, as 43% of the cells in the contingency table had expected frequencies below five. The level of statistical significance was defined as 0.05. The statistical analysis was performed using IBM® SPSS® Statistics Version 22.

RESULTS AND DISCUSSION

Commensal populations of *E. coli* show a 'clonal population structure', despite the occurrence of genetic recombination; thus delineation of major phylogenetic groups is possible (Selander & Levin, 1980; Tenaillon *et al*., 2010). Also, it has been shown that for a given host, the predominant isolate (i.e., the faecal *E. coli* isolate that accounts for more than half of the total colonies isolated in a stool culture), and the resident strain, (i.e., the one which persists in a person's gut for a long period) are likely to be the same (Sears *et al*., 1950, Caugant *et al*., 1981). This knowledge forms the basis for assigning *E. coli* phylogenetic groups to individual hosts, by analyzing a single *E. coli* colony from a stool culture (that consists of many *E. coli* colonies).

Out of the 158 faecal swabs collected, 58 were from males and 84 were from females. The gender of the remaining 16 subjects was not provided. On MacConkey agar, 157 swabs yielded bacterial growth. Fourteen of the isolates selected were citrate positive and one isolate failed to produce bands from the phylotyping PCR, resulting in a subtotal of 142 *E. coli* isolates for phylogroup analysis. Representative gel images of the main PCR programmes are shown in Figures 1- 4.

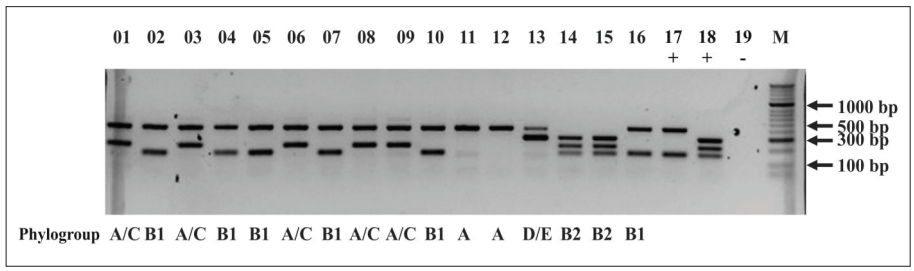

Figure 1: A gel profile of Clermont quadruplex PCR programme. Lanes 1-15, *E. coli* isolates analyzed in this study; lane 17, group B1 positive control; lane 18, group B2 positive control, lane 19, negative control; M- molecular weight marker

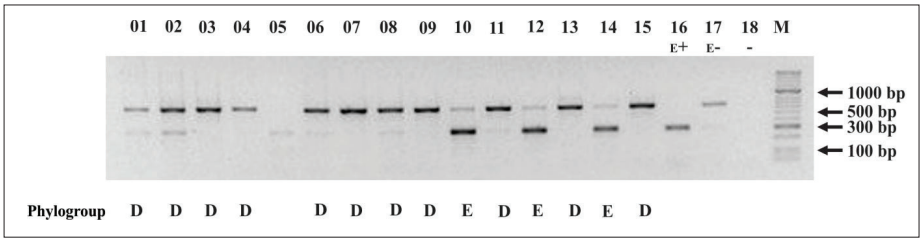

Figure 2: A gel profile of Clermont group E PCR programme. Lanes 1-15, *E. coli* isolates designated as group D or E by Clermont quadruplex PCR; lane 16, group C positive control; lane 17, group C negative control, lane 18, PCR negative control; M- molecular weight marker

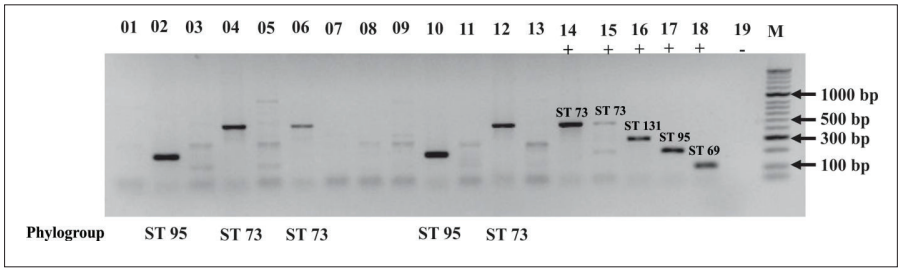

Figure 3: A gel profile of the new primer pool PCR programme. Lanes 1-13, *E. coli* isolates designated as group B2 by Clermont quadruplex PCR; lanes 14-18, positive controls (labeled in the image); lane 19, PCR negative control; M- molecular weight marker

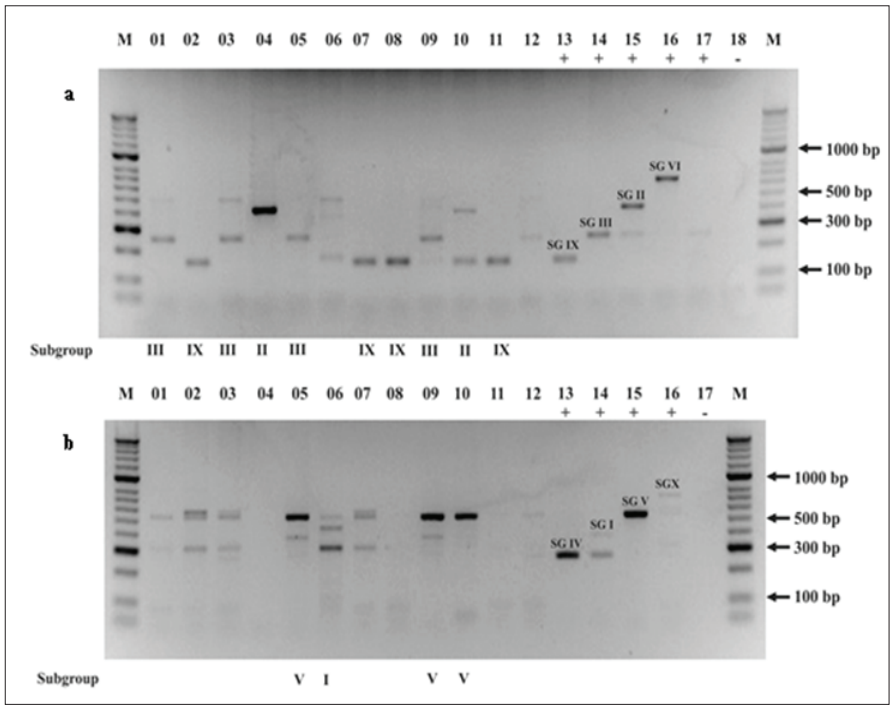

Figure 4: Gel profiles of the panel I/ panel II PCR programme. (a) Panel I. Lanes 1-12, *E. coli* isolates designated as group B2 by Clermont quadruplex PCR; lanes 13-17, positive controls of B2 subgroups (labeled in the image) ; Lane 18, PCR negative control; M- molecular weight marker (b) Panel II. Lanes 1-12, *E. coli* isolates analyzed in this study; lanes 13-16, positive controls of B2 subgroups (labeled in the image); Lane 17, PCR negative control; M- molecular weight marker

The distribution of phylogenetic groups within the study population

Phylogroups B2 and B1 were the most prevalent, followed by phylogroups A and D (Figure 5). Phylogroups C, F, and E each represented ≤5 % of the *E. coli* isolates. The prevalence of *E. coli* phylogenetic groups in the current study population can be arranged as B2 (28 %) > B1 (23 %) > A (17 %) > D (14 %) > C (5 %) > F (4 %) > E (3 %). The dominance of phylogroups A, B1, B2, and D reflects the results of other studies examining *E. coli* phylogroup distribution in humans (Tenaillon *et al*., 2010).

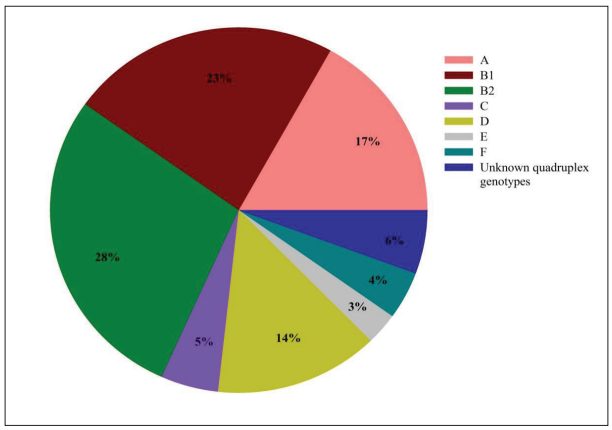

Figure 5: Distribution of the major *E. coli* phylogroups among faecal isolates of healthy individuals from Kandy District, Sri Lanka

Early studies examining the prevalence of *E. coli* phylogroups in samples collected from humans living in different countries, led to the view that phylogroup A strains dominated among isolates taken from people living in the tropics, while phylogroups B2 and D were more prevalent among isolates taken from people in temperate regions (Duriez *et al*., 2001; Escobar-Páramo *et al*., 2004). However, the more recent studies on phylogroup distribution of *E. coli* have established the view that the influence of the environment in terms of socioeconomic factors, (such as the diet and the level of hygiene) in a human population is more important than the climate in which the host lives. For instance, in a study that compared intestinal *E. coli* isolated from subjects who had recently migrated from temperate region (metropolitan France) to tropical region (French Guyana) with that of natives of the two regions, the prevalence of groups A and B2 isolates in the migrant group was intermediate compared to the two native groups, suggesting that the commensal gut flora of humans changes dynamically in response to new environmental conditions (Skurnik *et al*., 2008; Bailey *et al.*, 2010; Tenaillon *et al*., 2010).

In addition, the *E. coli* phylogroup distribution in human hosts has been thought to be influenced by differences in morphology, physiology, and diet of different sexes or ages (Gordon *et al*., 2005). Massot *et al*., (2016) reported that within a single 'locality', phylogroup distribution changes over time. It would have been of interest to look into the findings of Mushtaq *et al*., (2011) and Mukherjee *et al*., (2015) on studies carried out in South Asia on *E. coli* phylogenetic groups. However, since these studies are of pathogenic isolates and not on commensal *E. coli* of human hosts, a comparative analysis on the South Asian region (where Sri Lanka is located) is not possible. In summary, the above reports and the evidence of more recent phylogroup distributions of *E. coli* show that there is a variation by locality, and this variation seems to be due to geographic variation of socio-economic factors rather than the climate.

Predominance of group B2 in the current study population is noteworthy, as isolates belonging to group B2 have been shown to be the most common cause of ExPEC disease such as urinary tract infections (Zhang *et al*., 2002; Matsukawa *et al*., 2019), neonatal bacterial meningitis (Bingen *et al*., 1998; Johnson *et al*., 2002), peritonitis and bacteremia (Hilali *et al*., 2000; Bert *et al*., 2010). From an evolutionary perspective, group B2 is believed to show an early genetic divergence within the species and its extra-intestinal virulence appears to be a 'coincidental by-product of commensalism' (Picard *et al*., 1999). In a study considering only the antibiotic resistant commensal *E. coli* strains and their virulence genes isolated from Indian children, the proportion of antibiotic resistant (extended spectrum beta lactamase producing) commensal *E. coli* carrying a virulence factor associated with urinary tract infections in phylogenetic group B2 has been found to be significantly higher when compared to groups A and D (Chandran *et al*., 2017). Diard *et al*., 2010, stated that traits that enable extra-intestinal infection and long-term colonization of the intestine are more frequent among B2 strains than in other groups. Even though these traits make phylogenetic group B2 more likely to be the most prevalent group in a community, the fact that this phylogroup is not universally predominant indicates the involvement of other factors in shaping phylogroup prevalence. For example, Unno *et al*., (2009) report the absence of group B2 isolates in humans and domesticated animals in a province in the Republic of Korea.

Effect of gender and age

The relative abundance of phylogroups differed between male and female hosts ($p < 0.05$) (Figure 6).

Phylogroups D and C isolates were more prevalent in males, while isolates belonging to phylogroups A, B2, E, and F were more common in female hosts. There was no statistically significant change in phylogroup abundance with respect to host age ($p > 0.1$) (Figure 7).

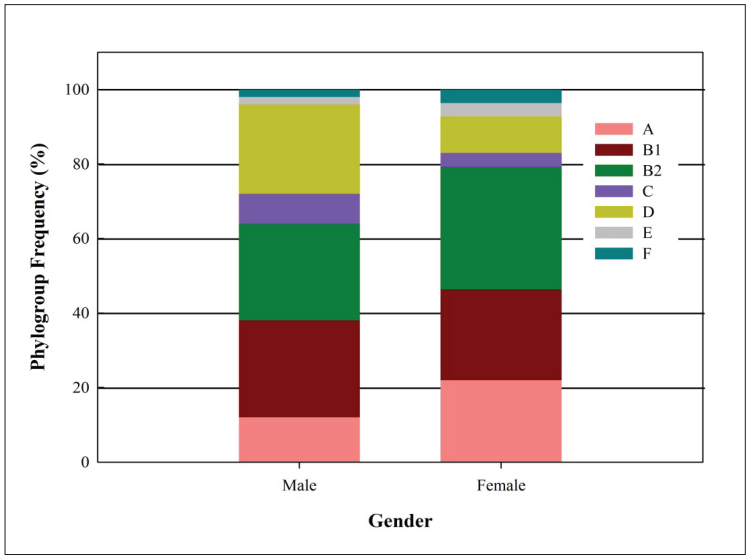

Figure 6: Relative abundance of major phylogenetic groups among male and female participants (n=118 whose gender was provided and phylogenetic group identified)

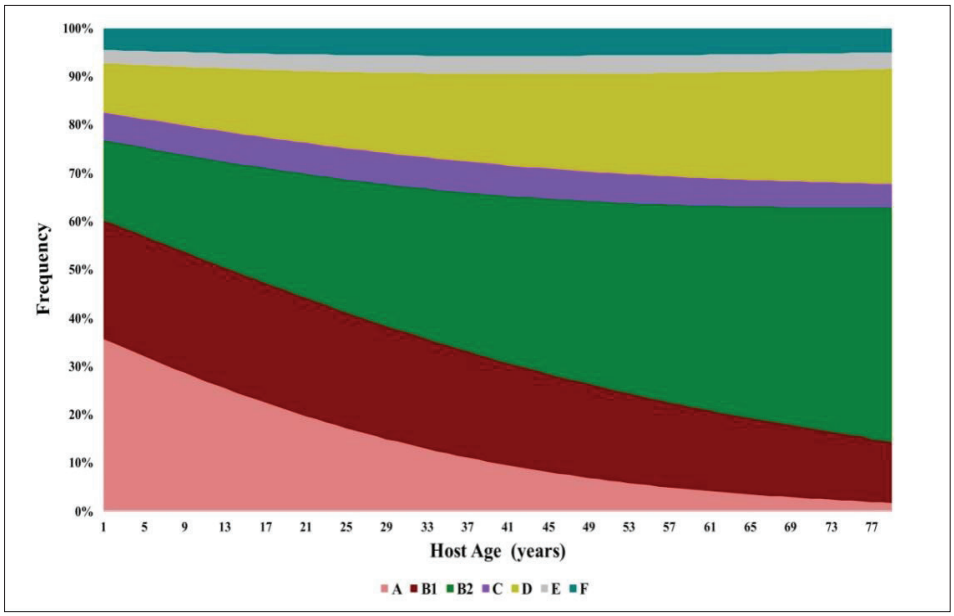

Figure 7: Change in the relative abundance of the commensal *E. coli* phylogenetic groups with respect to the age of host (n = 122 whose age was provided and phylogenetic group identified)

However, there was a tendency for the frequency of phylogroups A and B1 to decline with age, while the frequencies of phylogroups B2 and D increased. A very similar observation is reported in Bok *et al*., (2018), where groups A and B1 have shown a higher prevalence in young children while groups B2 and D prevailed among adults in Poland (only four phylogenetic groups were compared in that study).

Table 3: The distribution of Sequence Types (ST) and group B2 subgroups, as screened by different PCR protocols ($n_{B2} = 40$, $n_{D} = 20$)

ST Detected	B2 subgroup	No. of isolates detected by different PCR protocols (% of D or B2 isolates)		
		Doumith	B2 subtyping	ST Specific
69 [a]	-	4 (20.0)		4 (20.0)
131	I	1 (2.5)	2 (5.0)	0 (0.0)
73	II	4 (10.0)	2 (5.0)	
127	III		15 (37.5)	
141	IV		1 (2.5)	
144	V		8 (20.0)	
12	VI		0 (0.0)	
14	VII		0 (0.0)	
95	IX	8 (20.0)	9 (22.5)	9 (22.5)
372	X	1 (2.5)	0 (0.0)	

a - ST 69 belongs to Group D

Sequence Types and B2 subgroups

The present study investigated the distribution of the four sequence types ST 69 (within group D), ST 73, ST 95, and ST 131 (within group B2) whose involvement in extra-intestinal infections have been shown previously (Mora *et al*., 2009; Riley, 2014). These STs differ in their antibiotic sensitivity profiles. Strains of STs 69, 73, and 95 are still susceptible to antibiotics whereas members of ST131 show increasing resistance to multiple antibiotic classes including fluoroquinolones (Giufrè *et al*., 2012; Banerjee *et al*., 2013; Hu *et al*., 2013; Petty *et al*., 2014). However, the current study detected low levels in all four STs (Table 3). Only two isolates of ST 131 were detected out of the 40 group B2 isolates, despite the high level of antibiotic resistance in *E. coli* that prevail in Sri Lanka (Chandrasiri *et al*., 2013; Jayatilleke *et al*., 2016; Tillekeratne *et al*., 2016; Fernando *et al*., 2017).

Ten (I – X) phylogenetic subgroups within group B2 have been described (Le Gall *et al*., 2007; Clermont *et al*., 2014) and some of these subgroups have been shown to be frequently associated with extra-intestinal pathologies (Bidet *et al*., 2007; Clermont *et al*., 2008; Jaureguy *et al*., 2008). Le Gall *et al*., (2007) have observed all the strains to be virulent in subgroups I, III and IV, both virulent and avirulent strains to be present in subgroups II, V, VI, VII, and IX, and all strains to be avirulent in subgroup VIII. In this study, the rapid and convenient PCR introduced by Clermont *et al*., 2014 to identify group B2 human extra-intestinal pathogenic *E. coli*, was followed in subtyping the group B2 isolates (strains belonging to subgroup VIII are unassignable by this PCR). Group B2 isolates fell into their respective subgroups as shown in Table 3. In North America, Europe, and Australia, ~30% of human hosts sampled harbor an isolate representing STs either 73, 95, or 131 typically (Riley, 2014). By contrast, isolates belonging to these STs represented, at best, only about 10 % (14 of 142 isolates) of all *E. coli* observed in the present study.

CONCLUSIONS

To the best of our knowledge, the current study is the first report from South Asia, on phylogenetic group distribution of human commensal *E. coli* in a human community. Group B2 predominates in the human commensal gut flora of the Sri Lankan study population of the Kandy District, followed by the groups B1, A, D, C, F and E, while the relative abundance of *E. coli* phylogenetic groups varied between male and female hosts. Further, in group B2, SG III is the predominant subgroup while the common ExPEC STs of group B2 (ST 73, 95, and 131) and group D (ST 69) are represented in low frequencies (<25 %). In this study population, the distribution of phylogroups is independent of host gender except in group D, which is significantly over-represented in males.

Future directions

The current study is limited to hosts living in the Kandy district of the country. It would be worthwhile to screen hosts from other areas of the country, to obtain the phylogroup distribution throughout the country. Also, a more precise idea about the effect of gender and/or age of the host on the distribution of *E. coli* phylogroups could have been achieved if sampling method was altered to obtain comparable numbers of individuals from different genders/age groups.

The high levels of antibiotic resistance observed among gram negative clinical bacterial isolates in Sri Lanka make it imperative to investigate antibiotic sensitivity patterns and genetic markers of virulence and pathogenicity of the different *E. coli* phylogenetic groups identified in this study. Even though they are of commensal origin, such knowledge aids in predicting and interpreting pathogenicity/virulence of clinical *E. coli* isolates, understanding the changes in human practices, particularly antibiotic usage, and more importantly, in designing the antibiotic therapy for infections.

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