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Phylogrouping of *Escherichia coli* isolated from cases of neonatal calf diarrhoea and detection of their fimbrial genes

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Abstract
Neonatal calf diarrhoea (NCD) is a major problem faced by the livestock farmers world-wide and calves below one month of age are especially prone to this condition. The condition can be caused by infectious agents such as bacteria, viruses and protozoa. A study was conducted to phylogroup Escherichia coli isolates obtained from cases of NCD and to detect the presence of fimbrial genes in them. For this, a total of 120 faecal samples were collected from diarrhoeic calves below 3 months of age. E. coli was isolated from 101 (84.17%) samples. The isolates were phylogrouped on the basis of results of quadruplex polymerase chain reaction (PCR). All the phylogroups except B2 were detected during the analysis. The maximum number of E. coli isolates (27.72%) belonged to the B1 phylogroup which normally contained strains of low virulence. Isolates belonging to Group A (18.81%), Group C (4.95%), Group D(14.85%), Group E(11.88%), Group F(0.9%) and Clade I (0.9%) were also obtained. When the presence of fimbrial genes F5/F41/F17 was tested by PCR, it was observed that 10 out of 62 isolates (16.13%) were positive for F17 gene. None of the samples were positive for F5 or F41 genes. All the isolates in which F17 genes were detected were from animals above one week of age. The results of the study give valuable information about the type of *E. coli* and fimbria associated with NCD.

Keywords: Escherichia coli, neonatal calf diarrhoea, phylogroup, F17 fimbria

Introduction
Diarrhoea caused by *Escherichia coli* is the cause of huge economic losses for farmers. Neonates are mostly affected and calves are no exception. Pathogenic *E. coli* can be divided into six pathogroups based on virulence traits and symptoms they cause in the host: enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), Shiga toxin-producing *E. coli* (STEC), enterohemorrhagic *E. coli* (EHEC), enteroaggressive *E. coli* (EAEC), and EIEC enteroinvasive *E. coli* (Kaper *et al.*, 2004) [15]. ETEC is the cause of most cases of newborn diarrhoea (Nataro and Kaper, 1998; Osman *et al.*, 2012) [18, 20]. ETEC use their enterotoxins and fimbrial adhesins to infect the host animal. Fimbrial adhesions are extracellular proteins that aid in the attachment of bacteria to the small intestinal epithelial cells. The two types of toxins it creates after attachment are heat labile (LT) and heat stable (STa and STb) toxins. These toxins induce the intestinal epithelial cells to discharge fluids and electrolytes, which ultimately results in diarrhoea (Mercado *et al.*, 2003) [17]. ETEC primarily produces the fimbrial adhesions F4 (K88), F5 (K99), F6 (987P), F42, F41, F165, F18, and F17 (Deb Roy and Maddox, 2001) [9]. Among these F5 were mostly found in diarrhoeal calves, along with F41 and F17. Various types of fimbriae appear to be connected with ETEC induced diarrhoea at various ages (Vu-Khac *et al.*, 2004) [29]. F5 and F41 are primarily found in neonatal calves that are around a week old, and as the calves' age, their chance of being found declines. F17 is found in animals that have septicemia or diarrhoea and its prevalence is more in older calves (Ryu *et al.*, 2020) [23]. It has a stronger relationship with the invasion of *E. coli* inside the intestinal villi of calves than F5 and F41. The heterogenicity of F17 fimbriae is determined by receptor specificities. Based on antigenic specificities it can be classified into several subtypes such as F17a, F17b, F17c and F17d (Van Bost *et al.*, 2001) [28].
Organisms can be grouped into different phylogroups based on differences in their phenotypic and genotypic features, ecological niche, life history and susceptibility to transmit disease (Tenaillon *et al.*, 2010; Alm *et al.*, 2011) [27, 2]. The source from which the *E. coli* isolates are obtained is important in the phylogrouping process since there is a close relationship between

the phylogroups and the source. Although multilocus sequence typing (MLST) is helpful for classifying *E. coli*, it was difficult to divide *E. coli* into phylogroups using this data. Therefore, based on the genes chuA and yjaA and a DNA fragment TspE4.C2, Clermont *et al*. (2000) [6] devised a triplex PCR method to categorise *E. coli* into distinct phylogroups A, B1, B2, or D. Later, by including the gene arpA, responsible for group E and making use of the MLST information, a quadruplex PCR, was developed by Clermont *et al*. (2013) [7] that divided the bacteria into seven groups A, B1, B2, D, C, E, F, and clade I. The present study was carried out to identify the phylogroups and fimbrial genes of *E. coli* associated with neonatal calf diarrhoea in Kerala.

Materials and Methods

Collection of samples

Diarrhoeic faecal samples were collected from 120 calves of less than three months of age during the period from October 2019 to September 2022. The samples were collected from calves reared in different farms located in Thrissur and Wayanad districts in Kerala. Rectal swabs or freshly voided faecal samples were collected and transported on ice to the Department of Veterinary Microbiology at the College of Veterinary and Animal Sciences, Mannuthy (in Thrissur district) or Pookode (in Wayanad district) and inoculated on suitable culture media for the isolation of *E. coli*.

Isolation and identification of *E. coli*

All samples were initially inoculated on Brain Heart Infusion Agar (BHIA) and incubated aerobically at 37 ℃ overnight. Suspected colonies were stained by Gram's method and colonies of Gram negative bacilli were further inoculated on MacConkey (MAC) agar and Eosin methylene blue (EMB) agar. Lactose fermenting pink colonies with green metallic sheen were further confirmed as *E. coli* by stand biochemical tests as described by Quinn *et al*. (1994) [22]. After confirmation, these colonies were inoculated on nutrient agar slants and stored at 4 ℃ for molecular analysis.

Phylogrouping of *E. coli*

The DNA of *E. coli* was extracted from cultures using phenol-chloroform method as described by Sambrook and Russell (2001) and used as the template for the polymerase chain reaction (PCR). Phylogrouping was done as per the quadruplex PCR phylogrouping method developed by Clermont *et al.* (2013) [7] with slight modifications Table 1. It is a two-step procedure, on which an initial quadruplex PCR was conducted and based on the results of these a second PCR was conducted to differentiate group C and/or group E. The primers are given in Table 2.

Table 1: Quadruplex genotypes and steps required for assigning *E. coli* isolates to phylogroups (Clermont *et al*. 2013) [7]

Quadruplex genotyping				Phylogroup	Next step
arpA (400bp)	chuA (288bp)	yjaA (211bp)	TspE4.C2 (152bp)		
+	-	-	-	A	
+	-	-	+	B1	
-	+	-	-	F	
-	+	+	-	B2	
-	+	+	+	B2	
-	+	-	+	B2	
+	-	+	-	A/C	Screen using C-specific primers. If C+ then C, else A
+	+	-	-	D/E	Screen using E-specific primers. If E+ then E, else D
+	+	-	+	D/E	Screen using E-specific primers. If E+ then E, else D
+	+	+	-	E/clade I	Screen using E-specific primers. If E- then clade I, confirm using cryptic clade primers
-	-	+	-	Clade I or II	Confirm using cryptic clade primers
-	(476 bp)	-	-	Clade III, IV or V	Confirm using cryptic clade primers
-	-	-	+	Unknown	Perform MLST
-	-	+	+	Unknown	Perform MLST
+	-	+	+	Unknown	Perform MLST
+	+	+	+	Unknown	Perform MLST
-	-	-	-	Unknown	Confirm Escherichia identification using uidA orgadA/B, if positive screen using cryptic clade primers and/or perform MLST

Table 2: Details of primers used for phylogrouping of *E. coli*

PCR reaction	Primer name	Primer sequences (5'- 3'direction)	Target gene	Product size (bp)
Quadruplex	chuA.1b	ATGGTACCGGACGAACCAAC	chuA	288
	chuA.2	TGCCGCCAGTACCAAAGACA		
	yjaA.1b	CAAACGTGAAGTGTCAGGAG	yjaA	211
	yjaA.2b	AATGCGTTCCTCAACCTGTG		
	TspE4C2.1b	CACTATTCGTAAGGTCATCC	TspE4.C2	152
	TspE4C2.2b	AGTTTATCGCTGCGGGTCGC		
	AceK.f	AACGCTATTCGCCAGCTTGC	arpA	400
	ArpA1.r	TCTCCCCATACCGTACGCTA		
Group E	ArpAgpE.f	GATTCCATCTTGTCAAAATATGCC	arpA	301
	ArpAgpE.r	GAAAAGAAAAAGAATTCCCAAGAG		
Group C	trpAgpC1	AGTTTTATGCCCAGTGCGAG	trpA	219
	trpAgpC2	TCTGCGCCGGTCACGCCC		
Internal control	trpBA.f	CGGCGATAAAGACATCTTCAC	trpA	489
	trpBA.r	GCAACGCGGCCTGGCGGAAG		

Detection of fimbrial genes

The isolates that were characterized as belonging to the virulent phylogroup (n=62; other than a and the unknown phylogroups) were further used for the detection of fimbrial genes F5, F41 and F17 by PCR (Table 3).

Table 3: Details of primers used for detection of fimbrial genes

Sl. No.	Primer name	Primer sequences (5'- 3'direction)	Target gene	Product size	Reference
1.	F5-F	TAT TAT CTTGGTGG TAT GG	F5	314	Franck *et al*., 1998 [10]
	F5-R	GGT ATC CTT TAG CAG CAG TAT TTC			
2.	F41-F	GCATCAGCGGCAGTATCT	F41	380	Shams *et al*., 2010 [25]
	F41-R	GTCCCTAGCTCAGTATTATCACCT			
3.	F17-F	GGGCTGACAGAGGAGGTGGGGC	F17	411	Nguyen *et al*., 2011 [19]
	F17-R	CCCGGCGACAACTTCATCACCGG			

Results

Prevalence of *E. coli*

Of the 120 diarrhoeic samples tested, 101 (84.17per cent) were found to be positive for *E. coli*. Of these samples, 96 were collected from animals below 30 days of age.

Phylogrouping of *E. coli* isolates

All *E. coli* phylogroups, with the exception of B2, were detected in the samples tested. Twenty eight isolates belonged to group B1, 15 under group D, 12 under Group E and 5 under Group C. One isolate each belonged to group F and Clade I. Nineteen isolates were grouped as commensals (Group A), while 20 samples were classified as unknown (U) (Table 4).

Detection of fimbrial genes

The presence of F17 gene was detected in ten isolates. Of the 10 isolates, five belonged to the B1 phylogroup, three belonged to group C and one each belonged to groups D and E. None of the isolates tested were positive for the fimbrial genes F5 or F41 by PCR (Table 4). All the animals from F17 gene positive isolates were obtained were collected from animals above 1week of age.

Table 4: Results of phylogrouping of *E. coli* isolates and detection of fimbrial genes

Sl. No.	Sample No.	Age	Result of quadruplex PCR				Quadruplex up	Group C (219bp)	Group E (301bp)	Phylogroup	Fimbrial gene detected
			arpA (400bp)	chuA (288bp)	yjaA (211bp)	TspE4.C2 (152bp)					
1.	1	2 months	+	-	+	+	U			U	
2.	5	3 months	+	+	-	+	D/E			D	
3.	6	45 days	+	-	-	+	B1			B1	
4.	7	3 weeks	+	-	+	+	U			U	
5.	9	10 days	+	-	+	+	U			U	
6.	10	5 days	+	-	+	+	U			U	
7.	11	3 days	+	-	+	+	U			U	
8.	12	23 days	+	-	+	-	A/C	+		C	F17
9.	13	1 week	+	-	+	+	U			U	
10.	14	5 days	+	+	-	+	D/E			D	
11.	15	3 days	+	-	+	+	U			U	
12.	16	5 days	+	+	-	+	D/E		+	E	
13.	17	10 days	+	+	+	+	U			U	
14.	18	1 month	+	-	-	+	B1			B1	
15.	19	3 months	+	+	-	+	D/E			D	
16.	20	20 days	+	+	-	+	D/E			D	
17.	21	37 days	+	+	-	-	D/E			D	
18.	22	45 days	+	+	+	+	U			U	
19.	23	1 month	+	+	-	+	D/E		+	E	
20.	25	45 days	+	+	-	+	D/E			D	
21.	26	45 days	+	+	-	+	D/E			D	
22.	28	45 days	+	+	-	+	D/E			D	
23.	29	45 days	+	+	-	-	D/E		+	E	
24.	31	1 week	+	-	+	-	A/C			A	
25.	32	14 days	+	-	+	-	A/C			A	
26.	34	10 days	+	-	+	-	A/C			A	
27.	35	20 days	+	-	+	-	A/C	+		C	F17
28.	36	14 days	+	-	-	+	B1			B1	
29.	37	25 days	+	+	-	+	D/E			D	
30	38	17 days	+	-	+	-	A/C	+		C	
31.	39	3 days	-	+	-	-	F			F	
32.	40	4 days	+	-	-	+	B1			B1	
33.	41	14 days	+	-	-	-	A/C			A	
34.	42	14 days	+	+	-	+	D/E			D	
35.	43	3 days	+	+	+	-	E/clade I		+	E	

36.	44	1 month	+	-	-	+	B1			B1	
37.	49	7 days	+	-	-	-	A			A	
38.	50	22 days	+	-	-	+	B1			B1	
39.	51	10 days	+	-	-	+	B1			B1	
40.	52	5 days	+	+	+	+	U			U	
41.	53	12 days	+	+	+	+	U			U	
42.	54	15 days	+	-	-	-	A			A	
43.	55	20 days	+	-	-	-	A			A	
44.	56	1 month	+	-	+	-	A/C			A	
45.	57	22 days	+	-	-	+	B1			B1	
46.	58	10 days	+	-	+	-	A/C			A	
47.	59	15 days	+	-	-	+	B1			B1	
48.	60	24 days	+	-	+	-	A/C			A	
49.	61	1 month	+	+	+	+	U			U	
50.	62	12 days	+	+	+	-	E/clade1		+	E	
51.	63	10 days	+	+	+	+	U			U	
52.	64	17 days	+	-	-	+	B1			B1	
53.	65	20 days	+	+	+	-	E/clade I		Clade I	Clade I	
54.	66	1 month	+	+	+	+	U			U	
55.	67	1 month	+	-	-	+	B1			B1	
56.	68	20 days	+	-	-	+	B1			B1	
57.	69	35 days	+	-	-	+	B1			B1	
58.	70	27 days	+	-	-	+	B1			B1	
59.	71	32 days	+	-	-	+	B1			B1	
60.	72	40 days	+	+	-	+	D/E			D	
61.	73	25 days	+	+	+	+	U			U	
62.	74	14 days	+	+	-	+	D/E			D	
63.	75	20 days	+	+	-	-	D/E			D	F17
64.	76	7 days	+	+	-	-	D/E			D	
65.	77	15 days	+	-	+	-	A/C	+		C	F17
66.	78	1 month	+	-	-	+	B1			B1	
67.	79	45 days	+	+	-	-	D/E			D	
68.	80	36 days	+	+	+	+	U			U	
69.	81	30 days	+	+	+	+	U			U	
70.	82	44 days	+	+	+	+	U			U	
71.	83	45 days	+	+	+	+	U			U	
72.	84	46 days	+	+	-	+	D/E		+	E	
73.	85	32 days	+	-	+	-	A/C			A	
74.	86	45 days	+	-	-	+	B1			B1	F17
75.	87	1 month	+	-	-	+	B1			B1	F17
76.	88	33 days	+	+	+	+	U			U	
77.	91	10 days	+	-	+	-	A/C			A	
78.	92	3 months	+	-	+	-	A/C			A	
79.	93	75 days	+	-	-	-	A			A	
80.	94	14 days	+	+	-	-	D/E		+	E	
81.	95	22 days	+	+	-	-	D/E		+	E	
82.	96	16 days	+	+	-	-	D/E		+	E	
83.	97	1 month	+	-	-	+	B1			B1	F17
84.	98	24 days	+	-	-	+	B1			B1	
85.	99	15 days	+	-	-	-	A			A	
86.	101	5 days	+	+	-	-	D/E		+	E	
87.	102	12 days	+	+	-	-	D/E		+	E	F17
88.	104	23 days	+	-	-	+	B1			B1	
89.	105	3 days	+	-	+	-	A/C	+		C	
90.	106	16 days	+	-	-	+	B1			B1	
91.	109	14 days	+	-	-	+	B1			B1	F17
92.	110	17 days	+	+	-	-	D/E		+	E	
93.	111	6 days	+	-	-	-	A			A	
94.	112	12 days	+	-	-	+	B1			B1	
95.	113	20 days	+	-	-	+	B1			B1	
96.	114	10 days	+	-	-	+	B1			B1	
97.	116	1 month	+	-	-	-	A			A	
98.	117	16 days	+	-	-	+	B1			B1	F17
99.	118	20 days	+	-	-	-	A			A	
100.	119	7 days	+	-	-	+	B1			B1	
101.	120	17 days	+	-	-	-	A			A	

Discussion

Newborn calves are susceptible to a number of disease conditions. Around 50 per cent of neonatal calf mortality is caused by diarrhoea. Calves are more susceptible to diarrhoea during the first month of life due to their immature immune system, lack of specific antibodies, stress caused by weaning, and laxity in colostrum feeding. Bacteria are the main disease-causing agents, though parasitic and viral agents are also implicated. Diarrhea has become a serious problem even with improved treatment strategies. In order to lower the incidence rate of diarrhoea, substantial research has to be carried out in this area (Hemashenpagam *et al.*, 2009; Wu *et al.*, 2010; Pereira *et al.*, 2011; Abdullah *et al.*, 2013) [12, 31, 21, 1].

Most of the studies conducted earlier have shown that *E. coli* was the major bacterial cause of diarrhoea. In the present study, 84.17 per cent of the diarrhoeic samples examined yielded *E. coli* isolates. A similar prevalence rate (88.57 per cent) was reported by Begum *et al.* (2014) [4] from Assam. However, Srivani *et al.* (2017) [26] reported a lower prevalence (4.51 per cent) of *E. coli* in diarrhoeic calves in the states of Andhra Pradesh and Telangana. Variations in the prevalence rates of *E. coli* in diarrhoeal calves may be caused by geographic variables, different management practices and hygienic measures that affect the susceptibility of calves to infection (Cho and Yoon, 2014) [5]. Also, the infection may be caused by protozoal or viral agents.

Among the pathotypes of *E. coli* that cause diarrhea in calves, the ETEC strains are the most important ones and strains expressing F5 and/or F41 and producing STa toxin are the most common. However, strains with F17 fimbriae have also been isolated. In a study conducted on calf diarrhoea, Wieler *et al.* (1992) [30] detected 19.3 per cent isolates with F17 fimbriae. In their study they could observe that 31.3 per cent of the isolates carried genes for one of the fimbriae tested (F5, F17 and F41). Van Bost *et al.* (2001) [28] reported that 55 (16 per cent) of 345 *E. coli* isolates from diseased calves carried genes for F17 fimbriae. In the present study, ten out of the 62(16.13 per cent) *E. coli* isolates carried genes for F17, while none of them had genes for F5/F41. This may be due to the variation in the colonization of intestinal epithelial cells by ETEC and F5 are primarily detected in calves of 1-7 days of age. Of the 120 samples collected, only 18 samples come under below 7 days in this study and this may be the reason why F5/F41 were not detected. The F17 fimbrial gene was detected in *E. coli* isolated from calves above 7 days of age. The detected of F17 fimbrial gene in older calves has been reported previously (Ryu *et al*., 2020) [23]. Previous research on F17 demonstrated that it could be found in both humans and calves. There is therefore a possibility of cross-infection which highlights the need for proper management practices of the calves and handling of their manure. It has been demonstrated that spreading fresh or slurry manure over a surface can spread pathogenic *E. coli* to neighbouring food crops and surface water, infecting nearby humans (Meng and Schroeder, 2007) [16].

According to Clermont *et al.* (2011), phylogroups A/B1/E are the main cause of intestinal infections, while phylogroup C, which is closely connected to phylogroup B1. Phylogroups A and B1 strains were less virulent than those from phylogroups B2 and D (Johnson *et al*., 2001; Bashir *et al*., 2012) [14, 3]. In the phylogrouping scheme of *E. coli*, commensals are categorised in group A, while extra intestinal *E. coli* are categorised in groups B2 and D. They were found to be more virulent than the other phylogroups. B1 strains were found to be less virulent and intra-intestinal. Group F was found to be sub group within the D group (Bashir *et al*., 2012; Coura *et al*., 2019) [3, 8]. Group A strains are more prevalent in humans (40.5 per cent) than B2 strains (25.5 per cent), whereas B1 and D strains (17 per cent each) are less frequently encountered. A majority of B1 strains (41 per cent) predominate in animals, followed by A (22 per cent), B2 (21 per cent) and, to a lesser extent, D (16 per cent) (Jaureguy *et al*., 2008; Tenaillon *et al*., 2010) [13, 27]. In the present study, B1 strains were found to be the most prevalent in (27.72 per cent) followed by group A (18.81 per cent) and group D strains (14.85 per cent). This is in concurrence with the results obtained by Bashir *et al*. (2012) [3] and Coura *et al*. (2019) [8] where the most predominant group detected was B1.

E. coli strains that cannot be assigned to a phylogroup may also exist because of the phylogroup's extremely low frequency, the result of extensive recombination involving two different phylogroups, or the highly variable genome content of *E. coli* brought on by gene gain and loss. Using the MLST data, this can be marked as unknown and identified as *E. coli* Clermont *et al.* (2013) [7]. Quadruplex PCR and the MLST data can indicate an 80-95 per cent similarity with phylogrouping. Therefore the quadruplex phylogrouping may be beneficial for the study of genetic diversity of *E. coli* (Gordon *et al*., 2008) [11].

Neonatal calf diarrhoea is one of the most important conditions seen in calves. In the present study, *E. coli* was isolated from cases of NCD. When the isolated bacteria were phylogrouped and it was seen that the majority of the isolates were of the B1 phylogroup which are regarded to be of low virulence. However, isolates belonging to phylogroup D, that contains virulent strains, were also detected but to a lesser extent. Among the fimbrial genes F5, F17 and F41, only F17 was detected in calves older than 1 week. The results of the study give valuable insight into the type of *E. coli*, and fimbria associated with NCD.

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References

1. Abdullah M, Akter MR, Lutful Kabir SM, Abu Sayed Khan M, Abdul Aziz MS. Characterization of Bacterial Pathogens Isolated from Calf Diarrhoea in Panchagarh District of Bangladesh. Journal of Agricultural Food Technology. 2013;3(6):8-13.
2. Alm EW, Walk ST, Gordon DM. The niche of Escherichia coli. In Population Genetics of Bacteria. Walk ST, Feng PCH (Eds.). Washington, DC, USA: ASM Press; c2011. p. 107-123.
3. Bashir S, Haque A, Sarwar Y, Ali A, Anwar MI. Virulence profile of different phylogenetic groups of locally isolated community acquired uropathogenic E. coli from Faisalabad region of Pakistan. Annals of Clinical Microbiology and Antimicrobials. 2012;11:23. https://doi.org/10.1186/1476-0711-11-23 Accessed 20 Dec 2022.
4. Begum S, Hazarika GC, Rajkhowa S. Prevalence of Escherichia Coli from pigs and cattle. Journal of Animal Health and Production. 2014;2(3):38-39.

5. Cho YI, Yoon KJ. An overview of calf diarrhoea-Infectious etiology, diagnosis and Intervention. Journal of Veterinary Science. 2014;15:1-17.
6. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of Escherichia coli phylogenetic group. Applied Environmental Microbiology. 2000;66:4555-4558.
7. Clermont O, Christenson JK, Denamur E, Gordon DM. The Clermont Escherichia coli phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. Environmental Microbiology Reports. 2013;5(1):58-65.
8. Coura FM, De Araújo Diniz S, Silva MX, De Oliveira CHS, Mussi JMS, De Oliveira CSF, *et al*. Virulence factors and phylotyping of Escherichia coli isolated from non-diarrheic and diarrheic water buffalo calves. Ciência Rural, 2019, 49(5). https://doi.org/10.1590/0103-8478cr20180998 Accessed 20 Dec 2022.
9. Deb Roy C, Maddox CW. Identification of virulence attributes of gastrointestinal Escherichia coli isolates of veterinary significance. Animal Health Research Reviews. 2001;1:129-140.
10. Franck SM, Bosworth BT, Harley WM. Multiple PCR for enterotoxigenic, attaching and effacing and shiga toxin-producing *Escherichia coli* strains from calves. Journal of Clinical Microbiology. 1998;36:1795-1797.
11. Gordon DM, Clermont O, Tolley H, Denamur E. Assigning Escherichia coli strains to phylogenetic group: multi-locus sequence typing versus the PCR triplex method. Environmental Microbiology. 2008;10:2484-2496.
12. Hemashenpagam N, Kiruthiga B, Selvaraj T, Panneerselvam A. Isolation, Identification and Characterization of Bacterial pathogens causing Calf Diarrhea with special Iowa, Iowa State University Press; c2009. p. 752.
13. Jaureguy F, Landraud L, Passet V, Diancourt L, Frapy E, Guigon G, *et al*. Phylogenetic and genomic diversity of human bacteremic Escherichia coli strains. BMC Genomics. 2008;9:560. https://doi.org/10.1186/1471-2164-9-560Accessed 20 Dec 2022.
14. Johnson JR, Delavari P, Kuskowski M, Stell AL. Phylogenetic distribution of extraintestinal virulence-associated traits in Escherichia coli. Journal of Infectious Diseases. 2001;183:78-88.
15. Kaper JB, Nataro JP, Mobley HL. Pathogenic Escherichia coli. Nature Reviews Microbiology. 2004;2:123-140.
16. Meng J, Schroeder CM. Infectious Disease Series Editor: Vassil St. Georgiev. Foodborne Diseases. Edited by Shabbir Simjee., Escherichia coli. Humana Press Inc., Totowa, NJ, USA; c2007. p. 1-26.
17. Mercado EC, Rodriguez SM, D'Antuono AL, Cipolla AL, Elizondo AM, Rossetti CA, *et al*. Occurrence and Characteristics of CS31A Antigen-Producing *Escherichia coli* in Calves with Diarrhoea and Septicaemia in Argentina. Journal of Veterinary Medicine Series B. 2003;50:8-13.
18. Nataro JP, Kaper JB. Diarrheagenic Escherichia coli. Clinical Microbiology Reviews. 1998;11(1):142-201.
19. Nguyen TD, Vo TT, Vu-Khac H. Virulence factors in Escherichia coli isolated from calves with diarrhea in Vietnam. Journal of Veterinary Science. 2011;12(2):159-164.
20. Osman KM, Mustafa AM, Elhariri M, Abd Elhamed GS. The Distribution of Escherichia coli Serovars, Virulence Genes, Gene Association and Combinations and Virulence Genes Encoding Serotypes in Pathogenic E. coli Recovered from Diarrhoeic Calves, Sheep and Goat. Transboundary Emerging Disease. 2012;60(1):69-78.
21. Pereira RV, Santos TM, Bicalho ML, Caixeta LS, Machado VS. Antimicrobial resistance and prevalence of virulence factor genes in fecal Escherichia coli of Holstein calves fed milk with and without antimicrobials. Journal of Dairy Science. 2011;94:4556-4565.
22. Quinn PJ, Carter ME, Markey B, Carter GR. Clinical Veterinary Microbiology, Mosby-Year Book Europe Limited, England; c1994. p. 327-344.
23. Ryu JH, Kim SH, Park J, Choi KS. Characterization of virulence genes in Escherichia coli strains isolated from pre-weaned calves in the Republic of Korea. Acta Veterinaria Scandinavica. 2020;62:45.
24. Sambrook J, Russell DW. Molecular Cloning: A Laboratory Manual. (3rd Ed.). Cold Spring Harbour Laboratory Press, New York.
25. Shams Z, Tahamtan Y, Pourbakhsh A, Hosseiny MH, Kargar M, Hayati M. Detection of enterotoxigenic K99 (F5) and F41 from fecal sample of calves by molecular and serological methods. Comparative Clinical Pathology. 2010;21(4):475-478.
26. Srivani M, Reddy YN, Subramanyam KV, Reddy MR, Rao TS. Prevalence and antimicrobial resistance pattern of Shiga toxigenic Escherichia coli in diarrheic buffalo calves. Veterinary World. 2017;10(7):774-778.
27. Tenaillon O, Skurnik D, Picard B, Denamur E. The population genetics of commensal Escherichia coli. Nature Review Microbiology. 2010;8(3):207-217.
28. Van Bost S, Bâbe MH, Jacquemin E, Mainil J. Characteristics of necrotoxigenic Escherichia coli isolated from septicemic and diarrheic calves between 1958 and 1970. Veterinary Microbiology. 2001;82:311-320.
29. Vu-Khac H, Holoda1 E, Pilipcinec E. Distribution of Virulence Genes in Escherichia coli Strains Isolated from Diarrhoeic Piglets in the Slovak Republic. Journal of Veterinary Medicine Series B. 2004;51:343-347.
30. Wieler LH, Bauerfeind R, Baljer G. Characterization of shiga-like toxin producing Escherichia coli (SLTEC) isolated from calves with and without diarrhea. Zentralblatt für Bakteriologie. 1992;276:243-453.
31. Wu G, Mafura M, Carter B, Lynch K, Anjum MF. Genes associated with Genes associated with Escherichia coli isolates from calves with diarrhoea and/or septicaemia. Veterinary Record. 2010;166(22):691-692.