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In silico Pulsed Field Gel Electrophoresis and Molecular characterization of *Escherichia coli*

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ABSTRACT

Sixty-five isolates were analyzed for virulence, antibiotic resistance and adherence genes by *in silico* tools. Thirteen virulence genes were selected to classify *Escherichia coli* isolates. The most prevalent virulence gene was shiga toxin, *stx2* (13.85%). Out of the 65 isolates, 3 isolates were harbouring heat labile enterotoxin (LT1) and isolate NC_017633 *Escherichia coli* ETEC H10407 and NC_017641 *Escherichia coli* UMNK 88 harboured STI and STII genes, respectively, both of which were representative of ETEC. Six isolates were positive for *vt1;* and none of the isolates had the *vt2* gene. The intimin gene, *eaeA* was detected in seven isolates (10.77%). The presence of *Escherichia coli* O157 was confirmed by *rfbE* genes that were present in five isolates (7.69%). Among the 8 *hlyA* positive isolates, 5 were positive for both *hlyA* and *eaeA* genes that classified the isolates into typical EHEC. Two isolates (NC_013941 *Escherichia coli* O55:H7 str. CB9615 and NC_017656 *Escherichia coli* O55:H7 str. RM12579) were classified as atypical EPEC as contained only *eaeA* gene and no *bfpA* gene. Five isolates (7.69%) were positive for *astA* gene and four isolates (6.15%) had the *aggR* gene. So most prevalent verotype was EHEC (11 isolates), followed by ETEC. No Enteroinvasive *E. coli* (EIEC) was found. Eleven isolates (16.92%) had the sulfonamide resistance gene, *sul2*. The curli genes, *csgA* and *crl* were seen in 31 (47.69%) and 40 isolates (61.54%), respectively. Eleven isolates (16.92%) had the type 1 fimbriae and these isolates were likely to form biofilm on abiotic surfaces. The *sfa* gene was detected in 5 isolates and hence these isolates might be able to bind to receptors containing sialic acid residues. None of the isolates had the genes *papC* which are required for colonization on uroepithelial cells. *In silico* pulsed-field gel electrophoresis (PFGE) was able to group isolates into 25 genotypes. Genotype 8 was more virulent and contained only EHEC isolates. Genotype 17 contained all antibiotic resistance genes except *tetC*, *sul3* and *catA1* genes. Genotype 17 also contained three adhesive genes. Virulence profile analyzed in this study helps to compare the genes with previously published human pathogenic strains and verify possible genetic similarities and assess the distribution of these genes based on genotypes. This study helps to select antibiotic for treatment and improve the outcomes with severe bacterial infections based on genotyping.

INTRODUCTION

People in the developing countries suffer from diarrheal disease because of unsafe drinking water, poor sanitation and hygiene practice. Virulence property of *Escherichia coli* may contribute to life-threatening diseases in humans. Heat labile (LT) and heat stable (ST) enterotoxins disrupt the balance of intestinal fluid and cause hypersecretion of fluid and electrolytes (Hughs *et al.*, 1978; Moon 1978). Shiga toxin producing *E. coli* (STEC) produced two toxins (*Stx1* and *Stx2*) which were reported earlier by Jafari *et al.* (2012). Enterohemorrhagic *E coli* (EHEC) strain causing hemorrhagic colitis (HC) was reported by Nataro and Kaper (1998). Aldick *et al.* (2007) published that EHEC hemolysin, *ehx* is cytotoxic to endothelial cells and contribute to the development of HUS (Hemolytic uremic syndrome).

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A new strain named Entero-Aggregative Haemorrhagic *E. coli* (EAHEC) was identified by Wu *et al.* (2011) and Brzuszkiewicz *et al.* (2011) that had the virulence property of EAEC and produced *stx2* but had no LEE pathogenic property. As previously described by Croxen and Finlay (2010) and Johnson and Nolkan (2009), EHEC contains enterocyte effacement locus (LEE) like EPEC and produces attaching and effacing lesions (A/E). Vila *et al.* (1998) demonstrated that EAST-1 is a heat stable enterotoxin encoded by many other pathogens besides EAEC. Mulvey *et al.* (1998) described that UPEC strain is responsible for UTI (Urinary tract infection) infection with the expression of broad-spectrum virulence factors. Adhesin gene helps them to colonize not only to cells but also help them to form biofilms. He *et al.* (2012) reported that biofilms might enhance their resistance to environmental perturbations and help them to survive in harsh conditions. Lopez-Banada *et al.* (2014) described that *papC* gene is responsible for pyelonephritis. Other fimbriae that are associated with adherence are type 1 fimbriae (*fimA*), S fimbriae (*sfa*), curli fimbriae (*csg)* etc. (Mulvey, 2002; Antao *et al.*, 2009; Johnson and Stell, 2000). Anonymous (2004), Danmap (2004), Guerra *et al.* (2003) and Lanz *et al.* (2003) reported that *E. coli* is mostly resistant to tetracyclines, sulfonamides, streptomycin or spectinomycin. Continuous evaluation of antibiotic resistance gene prevents the emergence of multidrug-resistant strains.

These data help to predict virulence, antibiotic resistance and adhesion genes of 65 *E. coli* isolates and identifies genes responsible for *E. coli* infections. Identification of antibiotic resistance genes also help to select effective antibiotic against *E. coli* infections.

MATERIALS AND METHODS

Strains used

Isolates used in this study are summarized in Table 1.

Table 1: Name of the isolates.

Serial number	Isolate name
1	NC_017910 *Escherichia blattae* DSM 4481
2	NC_011601 *Escherichia coli* 0127:H6 E2348/69
3	NC_017626 *Escherichia coli* 042
4	NC_008253 *Escherichia coli* 536
5	NC_011748 *Escherichia coli* 55989
6	NC_017631 *Escherichia coli* ABU 83972
7	NC_008563 *Escherichia coli* APEC O1
8	NC_020163 *Escherichia coli* APEC O78
9	NC_010468 *Escherichia coli* ATCC 8739
10	NC_012967 *Escherichia coli* B str. REL606
11	NC_012892 *Escherichia coli* BL21(DE3)
12	NC_012971 *Escherichia coli* BL21(DE3)
13	NC_012947 *Escherichia coli* BL21-Gold(DE3) pLysS AG
14	NC_012759 *Escherichia coli* BW2952
15	NC_004431 *Escherichia coli* CFT073
16	NC_017638 *Escherichia coli* DH1
17	NC_017625 *Escherichia coli* DH1
18	NC_009801 *Escherichia coli* E24377A
19	NC_011745 *Escherichia coli* ED1a
20	NC_020518 *Escherichia coli* ETEC H10407
21	NC_009800 *Escherichia coli* HS
22	NC_011741 *Escherichia coli* IAI1
23	NC_011750 *Escherichia coli* IAI39
24	NC_017628 *Escherichia coli* IHE3034
25	NC_022648 *Escherichia coli* JJ1886
26	NC_007779 *Escherichia coli* K-12 substr. W3110
27	NC_016902 *Escherichia coli* KO11FL
28	NC_017660 *Escherichia coli* KO11FL
29	NC_011993 *Escherichia coli* LF82
30	NC_022364 *Escherichia coli* LY180
31	NC_017644 *Escherichia coli* NA114
32	NC_013353 *Escherichia coli* O103:H2 str. 12009
33	NC_018650 *Escherichia coli* O104:H4 str. 2009EL-2050
34	NC_018661 *Escherichia coli* O104:H4 str. 2009EL-2071
35	NC_018658 *Escherichia coli* O104:H4 str. 2011C-3493
36	NC_013364 *Escherichia coli* O111:H- str. 11128
37	NC_002655 *Escherichia coli* O157:H7 EDL933
38	NC_011353 *Escherichia coli* O157:H7 str. EC4115
39	NC_002695 *Escherichia coli* O157:H7 str. Sakai
40	NC_013008 *Escherichia coli* O157:H7 str. TW14359
41	NC_013361 *Escherichia coli* O26:H11 str. 11368
42	NC_013941 *Escherichia coli* O55:H7 str. CB9615
43	NC_017656 *Escherichia coli* O55:H7 str. RM12579
44	NC_017646 *Escherichia coli* O7:K1 str. CE10
45	NC_017634 *Escherichia coli* O83:H1 str. NRG 857C
46	NC_017663 *Escherichia coli* P12b
47	NC_022370 *Escherichia coli* PMV-1
48	NC_011742 *Escherichia coli* S88
49	NC_011415 *Escherichia coli* SE11
50	NC_013654 *Escherichia coli* SE15
51	NC_010498 *Escherichia coli* SMS-3-5
52	NC_017632 *Escherichia coli* UM146
53	NC_011751 *Escherichia coli* UMN026
54	NC_017641 *Escherichia coli* UMNK88
55	NC_007946 *Escherichia coli* UTI89
56	NC_017635 *Escherichia coli* W
57	NC_017664 *Escherichia coli* W
58	NC_017906 *Escherichia coli* Xuzhou21
59	NC_010473 *Escherichia coli* str. K-12 substr. DH10B
60	NC_020518 *Escherichia coli* str. K-12 substr. MDS42 DNA
61	NC_000913 *Escherichia coli* str. K-12 substr. MG1655
62	NC_000091 *Escherichia coli* str. K-12 substr. W3110
63	NC_017652 *Escherichia coli* str. clone D i14
64	NC_017651 *Escherichia coli* str. clone D i2
65	NC_011740 *Escherichia fergusonii* ATCC 35469

PCR primers

The primers used in the study are summarized in the Tables 2, 3 and 4.

PCR amplification

An online basis software, http://insilico.ehu.eus/PCR/ was designed to perform *in silico* PCR amplification and endonuclease digestion (San Millan *et al.*, 2013; Bikandi *et al.*, 2004). PCR amplification was performed by selection of genome and introduction of a primer; and resulting PCR product is computed automatically with desired band size of a specific gene (Bikandi *et al.*, 2004).

Table 2: Primers used for virulence gene detection.

Virulence factor	Gene	Primer Sequence (5' to 3')	Amplicon size (bp)	Reference
Verotoxin 1	*vt1*	GAA GAG TCC GTG GGA TTA CG AGC GAT GCA GCT ATT AAT AA	130	Pollard *et al.*,1990
Verotoxin 2	*vt2*	ACC GTT TTT CAG ATT TTGACA CAT A TAC ACA GGA GCA GTT TCA GAC AGT	298	Pollard *et al.*, 1990
Intimin	*eaeA*	CAC ACG AAT AAA CTG ACT AA AAT G AAA AAC GCT GAC CCG CAC CTA AAT-	376	Pollard *et al.*,1990
Heat labile toxin 1	LT1	TGGATTCATCATGCACCACAAGG CCATTTCTCTTTTGCCTGCCATC	360	Pass *et al.*, 2000
Heat stable toxin 1	STI	TTTCCCCTCTTTTAGTCAGTCAACTG GGCAGGATTACAACAAAGTTCACAG	160	Pass *et al.*, 2000
Heat stable toxin 2	STII	CCCCCTCTCTTTTGCACTTCTTTCC TGCTCCAGCAGTACCATCTCTAACCC	423	Pass *et al.*, 2000
Cytotoxic necrotizing factor 1	*cnf1*	GGCGACAAATGCAGTATTGCTTGG GACGTTGGTTGCGGTAATTTTGGG	552	Pass *et al.*, 2000
Cytotoxic necrotizing factor 2	*cnf2*	GTGAGGCTCAACGAGATTATGCACTG CCACGCTTCTTCTTCAGTTGTTCCTC	839	Pass *et al.*, 2000
Hemolysin A	*hlyA*	AGCTGCAAGTGCGGGTCTG TACGGGTTATGCCTGCAAGTTCAC	569	Wang *et al.*, 2002
Perosaminesynthetase	*rfbE*	CTACAGGTGAAGGTGGAATGG ATTCCTCTCTTTCCTCTGCGG	327	Wang *et al.*, 2002
Invasion Plasmid Antigen	*ipaH*	TGG AAA AAC TCA GTG CCT CT CCA GTC CGT AAA TTC ATT CT	422	Luscher *et al*.,1994
Enteroaggregative heat-stable enterotoxin	*astA*	CCA TCA ACA CAG TAT ATC CGA GGT CGC GAG TGA CGG CTT TGT	111	Yamamoto and Nakazawa, 1997
Bundle forming pilus	*bfpA*	GCCGCTTTATCCAACCTGGTA GCTGGAAAAACTCAGTGCCT	326	Sohel*et at.*, 1993
Transcriptional activator	*aggR*	GTATACACAAAAGAAGGAAGC ACAGAATCGTCAGCATCAGC	254	Ratchtrachenchai *et al.*, 1997
Shiga toxin 1	*stx1*	CGC TGA ATG TCA TTC GCT CTG C CGT GGT ATA GCT ACT GTC ACC	302	Blanco *et al.*, 2004
Shiga toxin 2	*stx2*	CTT CGG TAT CCT ATT CCC GG CTG CTG TGA CAG TGA CAA AAC GC	516	Blanco *et al.*, 2004

Table 3: Primers used for adhesin gene detection.

Adhesin gene	Gene	Primer Sequence (5' to 3')	Amplicon size (bp)	Reference
Type1 fimbriae	*fimA*	CGA CGC ATC TTC CTC ATT CTT CT ATT GGT TCC GTT ATT CAG GGT TG	721	Nowrouzian *et al*., 2001.
S fimbrial adhesion	*sfa*	CTC CGG AGA ACT GGG TGC ATC TTA C CGG AGG AGT AAT TAC AAA CCT GGC A	410	Le Bouguenec *et al*., 1992
Curlin Subunit Gene	*csgA*	ACT CTG ACT TGA CTA TTA CC AGA TGC AGT CTG GTC AAC	200	Maurer *et al*.,1998
Curli Regulatory Gene	*crl*	TTT CGA TTG TCT GGC TGT AT CTT CAG ATT CAG CGT CG TC	250	Maurer *et al.*, 1998
Pyelonephritis associated pili C	*papC*	GAC GGC TGT ACT GCA GGG TGT GGC G ATA TCC TTT CTG CAG GGA TGC AAT A	328	Le Bouguenec *et al*., 1992

Table 4: Primers used for antibiotic resistance gene detection.

Antibiotic resistance gene	Gene	Primer Sequence (5' to 3')	Amplicon size (bp)	Reference
Streptomycin resistance gene	*strB*	ATCGTCAAGGGATTGAAACC GGATCGTAGAACATATTGGC	509	Madsen *et al.*, 2000
Tetracycline resistance gene	*tetA*	GGCGGTCTTCTTCATCATGC CGGCAGGCAGAGCAAGTAGA	502	Lanz *et al*., 2003
Tetracycline resistance gene	*tetB*	CATTAATAGGCGCATCGCTG TGAAGGTCATCGATAGCAGG	930	Lanz *et al.*, 2003
Tetracycline resistance gene	*tetC*	GCTGTAGGCATAGGCTTGGT GCCGGAAGCGAGAAGAATCA	888	Lanz *et al.*, 2003
Sulfonamide resistance gene	*sulI*	GTGACGGTGTTCGGCATTCT TCCGAGAAGGTGATTGCGCT	779	Lanz *et al.*, 2003
Sulfonamide resistance gene	*sul2*	CGGCATCGTCAACATAACCT TGTGCGGATGAAGTCAGCTC	721	Lanz *et al.*, 2003
Sulfonamide resistance gene	*sul3*	GAGCAAGATTTTTGGAATCG CATCTGCAGCTAACCTAGGGCTTTGGA	880	Perreten *et al.*, 2003
Chloramphenicol Resistance gene	*cmlA*	CCGCCACGGTGTTGTTGTTATC CACCTTGCCTGCCCATCATTAG	699	Van *et al.*, 2008
Chloramphenicol Resistance gene	*catA1*	AGTTGCTCAATGTACCTATAACC TTGTAATTCATTAAGCATTCTGCC	547	Van *et al.*, 2008

PFGE digestion

In silico pulsed-field gel electrophoresis (PFGE) digestion and construction of the dendrogram was done in the website http://insilico.ehu.es/digest/. The enzyme used for the digestion was *Sgr D*land recognition sequence was CG'TCGA_CG (San Millan *et al.*, 2013; Bikandi *et al.*, 2004).

RESULTS AND DISCUSSION

Fragments of different size were generated by *SgrD*I digestion and separated by pulsed-field gel electrophoresis (PFGE). Dendrogram was constructed in the website. Sixty-five *Escherichia* isolates were separated into 25 different groups at 50% similarity coefficient (Fig. 1).

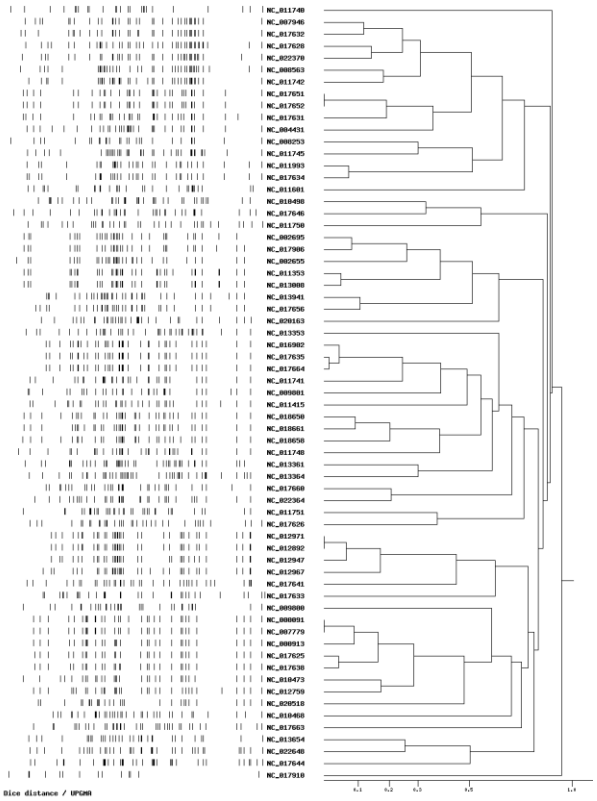

Fig. 1: Phylogenetic diversity of *Escherichia coli* identified by PFGE.

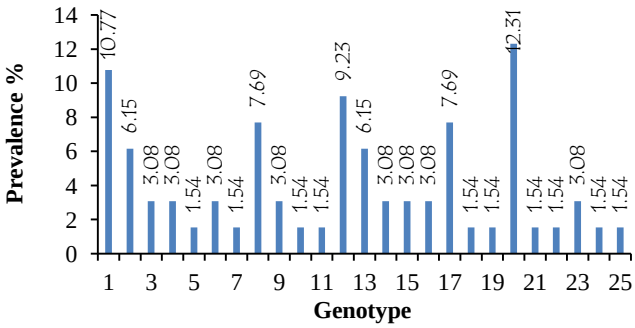

Fig. 2: Prevalence of genotypes.

Genotype 20 was more prevalent (12.31%) followed by genotype 1 (10.77%) and 12 (9.23%). Genotype 5, 7, 10, 11, 18, 19, 21, 22, 24 and 25 was present in low abundance containing 1.54% of the isolates (Fig. 2).

Distribution of virulence genes within genotypes

Lindenthal and Elsinghorst (2001) reported that watery diarrhea of *E. coli* strain was caused by heat labile (LT) or heat stable (ST) enterotoxin. Out of the 65 isolates tested for the LT1, STI, STII, VT1 and VT2 enterotoxins, three isolates (*Escherichia coli* E24377A, *Escherichia coli* ETEC H10407, *Escherichia coli* UMNK88) were found to possess LT1 enterotoxin and the gene product was 360bp., and their percentage of prevalence was 4.62%. The gene product for STI toxin was 160bp while that for STII toxin was 423bp. Isolates, *Escherichia coli* ETEC H10407 and *Escherichia coli* UMNK88, were positive for STI and STII enterotoxin, respectively; and prevalence of STI and STII enterotoxins were 1.54%. These were Enterotoxigenic *E. coli* (ETEC) (Rajkhowa *et al.*, 2009). Palaniappan *et al.* (2006) reported that ETEC causes traveler's and porcine and bovine diarrhea. Isolate *Escherichia coli* ETEC H10407 harboured both LT1 and STI enterotoxin and isolate *Escherichia coli* UMNK88 harboured both LT1 and STII enterotoxin. Verotoxin producing *E.coli*, responsible for foodborne disease in the USA, Canada, Japan and Europe, was reported by Griffin and Tauxe, 1991; Nataro and Kaper, 1998. The 130 bp gene product of *vt1* was found in 6 isolates, but no isolate harboured the *vt2* gene, these might be Enterohemorrhagic *E. coli* (EHEC). Genotypic distribution of this genes found that isolate NC_017633 *Escherichia coli* ETEC H10407 present in genotype 18 harboured both LT1 and STI enterotoxin (100%) (Fig. 3). Low prevalence of LT1 enterotoxin was encountered in genotype 12 (16.67%). The STII enterotoxin was present in genotype 17 and the prevalence was 20%. Genotypic distribution of *vt1* gene found that isolateNC_013353 *Escherichia coli* O103:H2 str. 12009 present in genotype 11and NC_013361 *Escherichia coli* O26:H11 str. 11368 and NC_013364 *Escherichia coli* O111: H- str. 11128 present in genotype 14 harboured the *vt1* gene. The *vt1* gene was also present in genotype 8 but the prevalence was 60%.

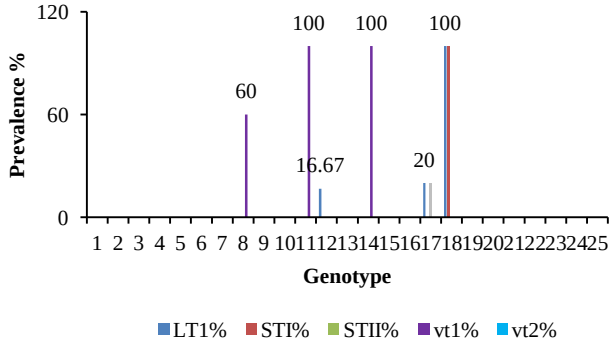

Fig. 3: Genotypic distribution of heat labile, heat stable and verotoxin genes.

Shiga toxin producing *E coli* (STEC) produced two major toxins (*Stx1* and *Stx2*) that causes hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) (Karch *et al.*, 2005; Karmali *et al.*, 2010). The 302 bp gene, *stx1* was found in six

isolates (9.32%) while 516 bp gene, *stx2*was observed in 9 isolates (13.85%). Four isolates had both *stx1* and *stx2* gene. Six isolates were identified to produce302 bp gene product for *stx1* gene by *in silico* PCR amplification which was also found to be positive for *vt1* gene. Osek (2003) previously described that enteroaggregative heat stable enterotoxin 1, (*astA*) was responsible for porcine colibacillosis. Sarantuya *et al.* (2004) reported that two genes, *astA* and *aggR* gene, differentiate Enteroaggregative *E. coli* (EAEC) from other verotypes. The *astA* gene, encoding the toxin EAST1, produce 111bp gene product identified by *in silico* PCR amplification and the prevalence was 7.69%. Out of five *astA* positive isolates, three of them were positive for both *astA* and *lt1* gene. So only isolate *Escherichia coli* 042, *Escherichia coli* P12b were the representative of Enteroaggregative *E coli* (EAEC). Gallegos *et al.* (1993) and Nataro *et al.* (1994) reported that *aggR* gene is the member of AraC/XylS family of bacterial transcriptional activators. Four isolates (their no.) were found to be positive for the *aggR* gene (6.15%). Only *Escherichia coli* 042 contained both *astA* and *aggR* gene. Enteroaggregative *E. coli* (EAEC) causes persistent diarrhea in humans (Palaniappan *et al.,* 2006). Desmarchelier *et al.* (1998) described that *rfbE* gene was used to detect *E coli* O157. The 327 bp gene product of the *rfbE* gene was present in 5 isolates (7.69%). These five isolates were also expressed *eaeA* gene. Figure 4 is representing the genotypic distribution of shiga toxin, enteroaggregative heat stable enterotoxin 1, *rfbE* gene and *aggR* gene. Genotypic distribution shows that all the isolates present in genotype 11 harboured both *stx1* and *stx2* genes (Fig. 4).

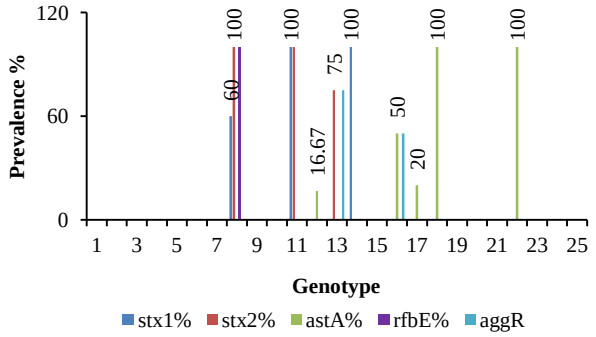

Fig. 4: Genotypic distribution of shiga toxin, *astA, rfbE* and *aggR* genes.

The *stx1* and *stx2* genes were also present in genotype 8 but their prevalence was 60% and 100%, respectively. The *stx1* and *stx2* genes were also present in genotype 14 (100%) and 13 (75%). The *astA* gene was more abundant in genotype 18 and 22 (100%). The *astA* gene was also present in genotype 12,16 and 17 and low prevalence was encountered in genotype 12 (16.67%). All isolates present in genotype 8 contained *rfbE* gene (100%). Seventy five percent isolates present in genotype 13 harboured *aggR* gene. The *aggR* gene was also present in genotype 16 and fifty percent isolates present in genotype 16 harboured *aggR* gene. Other important virulence factors include cytotoxic necrotizing factor (*cnf1* and *cnf2*), hemolysin (*hlyA*), the product of *eaeA* gene, intimin, and *ipaH* gene. Caprioli *et al.* (1983) reported that *cnf* gene was responsible for neonatal enteritis. The cytotoxic necrotizing factors, *cnf1* was found in two isolates (*Escherichia coli* UM146 and *Escherichia coli* UTI89). No isolate was found to be positive for *cnf2*. Ahmed *et al.* (2007) reported that cytotoxic necrotizing factor (CNF)is responsible for urinary tract infection. This is indicating that these were Uropathogenic *E.coli* (UPEC). The *hlyA* gene was found in 8 isolates. The prevalence of *hlyA* gene was 12.3%. The *hlyA* gene was present in both verotoxin and shiga toxin producing *E coli*. So, these were representative of Enterohemorrhagic *E coli* (EHEC). Schmidt *et al.* (1995, 1996) reported that plasmid encoded enterohemolysin causes severe clinical disease in humans. The *eaeA* gene is more commonly associated with Enterohemorrhagic *Escherichia coli* (EHEC) and Enteropathogenic *Escherichia coli* (EPEC). Jerse *et al.* (1990) reported that *eaeA* gene is responsible for the attaching and effacing lesions in human enterocytes. Seven isolates had the *eaeA* gene and the percentage of prevalence was 10.77%. Among the seven isolates, five was positive for both *eaeA* and *rfbE* as mentioned earlier. These five *eaeA* positive isolates were also positive for the *hlyA* gene. It can be concluded that these five isolates were typical Enterohemorrhagic *E. coli* (EHEC) since they contained both *hlyA* and *eaeA* gene (Hegde *et al.,* 2012). Two isolates had the *eaeA* gene only which means that these are Enteropathogenic *E. coli* (EPEC). Palaniappan *et al.* (2006) reported that EPEC is mainly responsible for diarrhea in children and animals. The *bfpA* gene is also used for identification of Enteropathogenic *E coli* (EPEC). No isolates harboured the *bfpA* gene. Hegde *et al.* (2012) reported that *E. coli* harboured only *eaeA* gene and no *bfpA* gene are classified as atypical EPEC. So, these two isolates that harboured only *eaeA* genes were defined as atypical EPEC. Kaper *et al.* (2004) demonstrated that recent studies found more atypical EPEC strains. No invasion plasmid antigen, *ipaH* gene was found which is the common trait of Enteroinvasive *E coli* (EIEC). Palaniappan *et al.* (2006) also published that EIEC and EAEC genes were found only in humans. The cytotoxic necrotizing factor *cnf1* was present in only genotype 1 and the prevalence was 28.57% (Fig. 5).

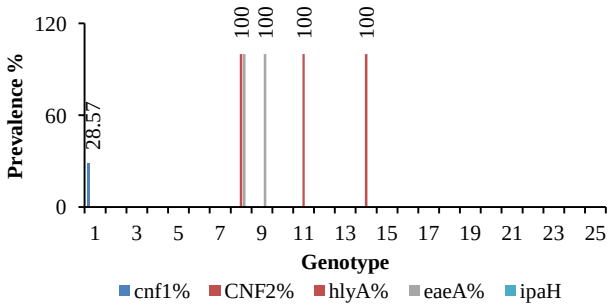

Fig. 5: Genotypic distribution of cytotoxic necrotizing factor, hemolysin, intimin gene.

Genotypic distribution of *hlyA* gene showed that all isolates present in genotype 8, 11 and genotype 14 harboured *hlyA* gene (100%). All the isolates present in genotype 8 and 9 had the *eaeA* gene.

Distribution of antibiotic resistance gene within genotypes

Olowe *et al.* (2013) reported that tetracycline resistance is acquired by energy dependent efflux pump system. Roberts (1996) reported that protein synthesis of tetracycline gene is inhibited by binding of aminoacyl-tRNA with the bacterial ribosome. The tetracycline resistance gene A, *tetA* gene was present in 9 of the 65 isolates and hence the overall prevalence of this gene among the selected isolates was 13.85%. The *tetB* gene was seen in 6 isolates and hence the prevalence is 9.23%. The *tetC* gene was present in only 2 isolates (*Escherichia coli* APEC O1, *Escherichia coli* O83:H1 str. NRG 857C) and the prevalence was 3.08%. Genotypic distribution of *tetA* and *tetB* gene was similar in genotype 13, 14, 16 and 17 and their prevalence was 25%, 50%, 50% and 20%, respectively. The *tetA* gene was also present in genotype 4 and their prevalence in genotype 4 was 50%. The *tetC* gene was present in genotype 1 and 12 and their prevalence was 14.29% and 16.67%, respectively (Fig. 6).

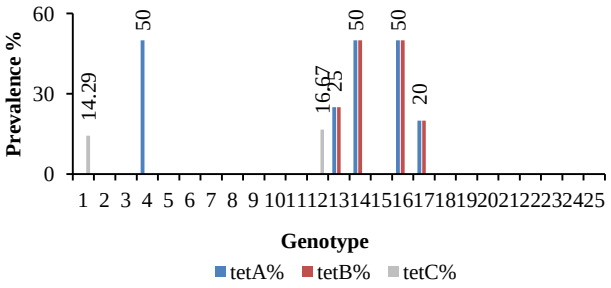

Fig. 6: Genotypic distribution of tetracycline resistance gene.

Ahmed *et al.* (2010) reported that chloramphenicol acetyptransferase, *catA1* gene is responsible for plasmid mediated resistance to chloramphenicol. Dorman and Foster (1982) demonstrated that chloramphenicol resistance is acquired by chloramphenicol acetyltransferase (CAT) enzymes that prevents the binding of chloramphenicol to 50S ribosome. Shaw (1984) also described that none of the *catA* genes has been shown to confer resistance to florfenicol and no homology was observed between CATs and Flo (florfenicol). Among the 65 isolates analyzed, six (9.23%) isolates harboured a 547 bp amplicon for *catA1* gene. Bissonnette *et al.* (1991) reported that chloramphenicol resistance gene, *cmlA* gene is also responsible for chloramphenicol resistance and confers resistance through non-enzymatic efflux pump. Bolton *et al.* (1999) reported that amino acid sequence of *cmlA* and *flo* is 50% similar but *cmlA* resistance to florfenicol is not clear. Only *Escherichia coli* UMNK88 was positive for the 699 bp PCR amplicon for *cmlA* gene. Hence the prevalence was 1.54%. The *strB* gene was present in 9 isolates and produced 509 bp PCR product. So, the prevalence was 13.85%. Genotypic distribution found that *catA1* gene was more prevalent in genotype 16 (100%) (Fig. 7). Fifty percent of isolates present in genotype 4, 14 and 15 harboured *catA1* gene. Genotype 4 and 12 contained both *strB* and *catA1* gene. The *cmlA* gene was present in only genotype 17. Genotype 17 also harboured *strB* gene. Prevalence of *strB* gene in genotype 4, 6 and 9 was 50%. Perrenten and Boerlin (2003) and Skold (2001) reported that resistance to sulfonamide is acquired by *sul1*, *sul2* and *sul3* gene (sulfonamine resistane genes). The *sul1* gene was seen in 5 isolates and hence the prevalence was 7.69%. The *sul2* gene was present in 11 isolates and produced a 250 bp PCR product. So, the prevalence was 16.92%. None of the isolates were positive for *sul3* gene. Figure 8 presented the genotypic distribution of sulfonamide genes. The *sul1* gene was more prevalent in genotype 16 and low prevalence was encountered in genotype 1. All the isolates present in genotype 16 harboured *sul1* gene. The prevalence of *sul2* gene was more abundant in genotype 5. Genotypic distribution of *sul1* and *sul2* gene was similar in genotype 1, 4 and 17 and their prevalence was 14.29%, 50% and 20%, respectively.

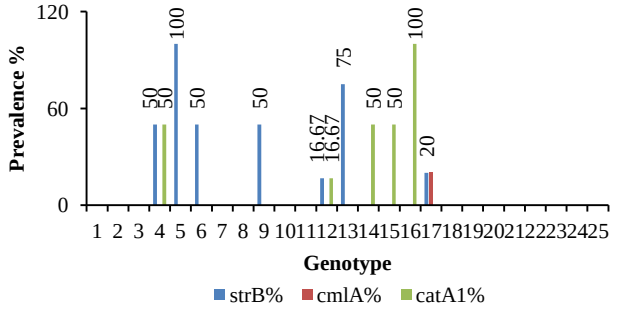

Fig. 7: Genotypic distribution of streptomycin and chloramphenicol resistance genes.

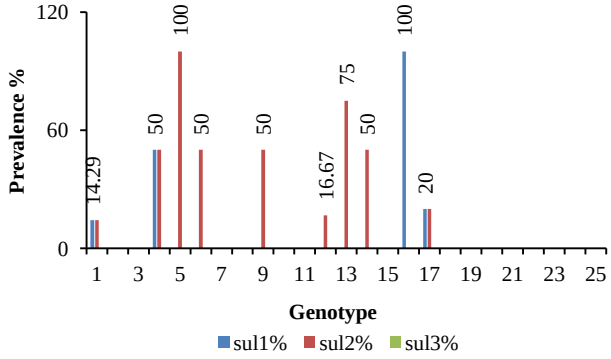

Fig. 8: Genotypic distribution of sulfonamide genes.

Distribution of adhesin gene within genotypes

The curlin subunit gene, *csgA* gene was seen in 31 of the isolates (47.68%) with a 200 bp gene product. The *crl* gene was present in 40 isolates (61.54%). Hence a 250bp PCR product was seen. According to Hammar *et al.* (1996), genes that are involved in curli formation are encoded in *csgBA* operon and *csgDEFG* operon. Chapman *et al.* (2002) reported that without *csgB* gene no curli are assembled and unable to secrete *csgA* from the cell. Arnqyist *et al.* (1992) found that RpoS (RNA polymerase, sigma S) binds with *csgBA* promoter after interaction of *crl* with Rpo Sand therefore *crl* is required in most strains for curli expression.

The curli regulatory gene, *crl* gene has a more regulatory role and *csgA* has a phenotypic role. Nine isolates had the *crl* gene but no *csgA* gene indicating that though has the gene for curli formation didn't exhibit phenotypic properties. All the isolates present in genotype 10, 11, 12, 13, 15, 17, 18, 19, 21 and 22 harboured both *csgA* and *crl* genes (100%) (Fig. 9). Genotype 14 and 20 also contained both *csgA* and *crl* genes. The prevalence of *csgA* and *crl* genes in genotype 14 were 50% and 100%, respectively. The *crl* genes were also present in genotype 8, 9 (100%) and lower abundance was seen in genotype 23 (50%).

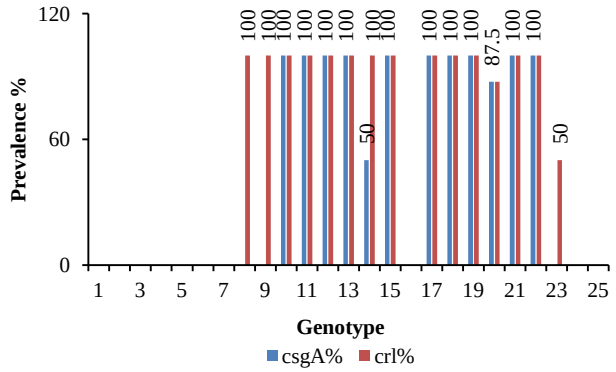

Fig. 9: Genotypic distribution of *csgA* and *crl* genes.

The S fimbrial adhesion, *sfa* gene was present in 5 isolates. The prevalence was 7.69%. Isolates harbouring *sfa* gene were also positive for two UPEC (uropathogenic *Escherichia coli*) strains, *Escherichia coli* UM146 and *Escherichia coli* UTI89. Morschhauser *et al.* (1990) reported that S fimbrial adhesion (*sfa*) enable pathogenic *Escherichia coli* to bind to sialic acid containing eukaryotic receptor molecules. Previous study reported that (Stain et al., 1994) with the help of *sfa* Sadhesin, S fimbriated-*E coli* bind to brain endothelial glycoproteins called NeuAc alpha 2,3-galactose. This binding help the bacteria to penetrate blood brain barrier and develop meningitis. So, this *sfa* containing isolates are more likely to cause sepsis and meningitis. Type 1 fimbriae are encoded by *fim* gene cluster (Iida *et al.*, 2001). Mitra *et al.* (2013) described that it is an importance virulence factor in UTI (Urinary tract infection) infection. The *fimA* gene was present in 11 of the 65 isolates and hence the overall prevalence of this gene among the selected isolates was 16.92%. As previously described by Blumer *et al.* (2005), this gene forms biofilm for cell surface attachment which enables them to persist in the harsh environment. Soto *et al.* (2007) reported that biofilm forming strains demonstrates higher type 1 fimbriae expression. None of the isolates had the *pyelonephritis-associated pili C,papC* gene. Uhlin *et al.* (1985) demonstrated that Pap pili are associated with pyelonephritis and is responsible for binding to digalactoside-containing glycolipids on the uroepithelium. The type 1 fimbriae, *fimA* gene was more prevalent in genotype 17 (Fig. 10). Eighty percent isolates present in genotype 17 harboured *fimA* gene. The prevalence of *fimA* gene in genotype 20 was 62.5%. The *fimA* gene had low abundance in genotype 14 and 16 (50%) and rest of the genotypes harboured no *fimA* gene. The *sfa* gene was present in only genotype 1 (57.14%) and 3 (50%).

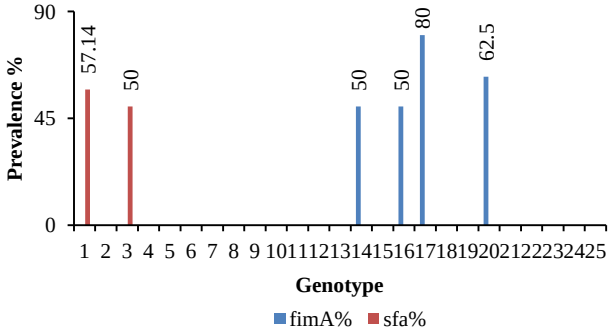

Fig. 10: Genotypic distribution of *fimA* and *sfa* genes.

CONCLUSION

The most prevalent virulence factor was the shiga toxin producing gene, *stx2* (13.85%). Among the 65 isolates analyzed, the most prevalent verotype was EHEC (11 isolates) followed by ETEC (3 isolates). Five isolates were classified as typical EHEC since they harboured both *hlyA* and *eaeA* gene and two isolates were classified as atypical EPEC since they carried only *eaeA* gene. Genotypic distribution showed that genotype 8 was the most virulent where it harbours 6 virulent genes that defined in EHEC. This study showed that the most prevalent antibiotic resistance gene was sulfonamide resistance gene, *sul2* (16.92%). Genotype 17 contained all antibiotic resistance genes, except *tetC*, *sul3* and *catA1* gene. Genotype 4 contained 5 antibiotic resistance genes. The *crl* gene was the most prevalent adhesive gene. Genotype 17 contained three adhesive genes. Adequate hygiene practice should be implemented strictly to control *E coli* contaminations. Adequate heat and temperature should also be maintained for the production of dairy products.

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REFERENCES

Ahmed MO, Clegg PD, Williams NJ, Baptiste KE, Bennett M. Antimicrobial resistance in equine faecal *Escherichia coli* isolates from North West England. Ann Clin Microbiol Antimicrob, 2010; 9(1):12

Ahmed W, Tucker J, Bettelheim KA, Neller R, Katouli M. Detection of virulence genes in *Escherichia coli* of an existing metabolic fingerprint database to predict the sources of pathogenic *E. coli* in surface waters. Water res, 2007; 41(16):3785-3791.

Aldick T, Bielaszewska M, Zhang W, Brockmeyer J, Schmidt H, Friedrich AW, Karch H. Hemolysin from Shiga toxin-negative *Escherichia coli* O26 strains injures microvascular endothelium. Microbes Infect, 2007; 9(3):282-290.

Anonymous. 2004. Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) 2002. Health Canada, Ottawa, Canada.

Antao EM, Wieler LH, Ewers C. Adhesive threads of extraintestinal pathogenic *Escherichia coli*. Gut Pathog, 2009; 1:22.

Arnqvist A, Olsen A, Pfeifer J, Russell DG, Normark S. The Crl protein activates cryptic genes for curli formation and fibronectin binding in *Escherichia coli* HB101. Mol. Microbiol, 1992; 6: 2443–52.

Bikandi J, San Millán R, Rementeria A, Garaizar J.*In silico* analysis of complete bacterial genomes: PCR, AFLP–PCR and endonuclease restriction. Bioinformatics, 2004; 20(5):798-799.

Bissonnette L, Champetier S, Buisson JP, Roy PH. Characterization of the nonenzymatic chloramphenicol resistance (*cmlA*) gene of the In4 integron of Tn1696: similarity of the product to transmembrane transport proteins. J Bacteriol, 1991; 173:4493–4502.

Blanco M, Blanco JE, Mora A, Dahbi G, Alonso MP, Gonza´ lez EA, Berna´rdez MI,Blanco J. Serotypes, virulence genes, and intimin types of Shiga toxin (verotoxin)-producing *Escherichia coli* isolates from cattle in Spain and identification of a new intimin variant gene (eae-j). J Clin Microbiol, 2004; 42:645–651.

Blumer C, Kleefeld A, Lehnen D, Heintz M, Dobrindt U, Nagy G, Wendisch VF. Regulation of type 1 fimbriae synthesis and biofilm formation by the transcriptional regulator LrhA of *Escherichia coli*. Microbiology, 2005; 151(10):3287-3298.

Bolton LF, Kelley LC, Lee MD, Fedorka-Cray PJ, Maurer JJ. Detection of multidrug-resistant *Salmonella enterica* serotype *typhimurium* DT104 based on a gene which confers cross-resistance to florfenicol and chloramphenicol. J Clin Microbiol, 1999; 37:1348–1351.

Brzuszkiewicz E, Thurmer A, Schuldes J, Leimbach A, Liesegang H, Meyer F-D, Boelter J, Petersen H, Gottschalk G, Daniel R. Genome sequence analyses of two isolates from the recent *Escherichia coli* outbreak in Germany reveal the emergence of a new pathotype: Entero-Aggregative-Haemorrhagic *Escherichia coli* (EAHEC). Arch Microbiol, 2011;193:883–891.

Caprioli A, Falbo V, Roda LG, Ruggeri FM, Zona C. Partial purification and characterization of an *Escherichia coli* toxic factor that induces morphological cell alterations. Infect Immun, 1983; 39(3):1300-1306.

Chapman MR, Robinson LS, Pinkner JS, Roth R, Heuser J, Hammar M, Normark S, Hultgren SJ. Role of *Escherichia coli* curli operons in directing amyloid fiber formation. Science,2002; 295:851-5.

Croxen MA, Finlay BB. Molecular mechanisms of *Escherichia coli* pathogenicity. Nat Rev Microbiol, 2010; 8(1):26-38.

Danmap Danmap 2003: Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark. Danish Zoonoses Center, Sørborg, Denmark, 2004.

Desmarchelier PM, Bilge SS, Fegan N, Mills L, Vary JC, Tarr PI. A PCR specific for *Escherichia coli* O157 based on the rfb locus encoding O157 lipopolysaccharide. J Clin Microbiol, 1998; 36(6):1801-1804.

Dorman CJ, Foster TJ. Nonenzymatic chloramphenicol resistance determinants specified by plasmids R26 and R55-1 in *Escherichia coli* K-12 do not confer high-level resistance to fluorinated analogs. Antimicrob Agents Chemother, 1982; 22:912–914.

Gallegos MT, Michan C, Ramos JL. The XylS/AraC family of regulators. Nucleic Acids Res, 1993; 21:807–810

Griffin PM, Tauxe RV. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. Epidemiol Rev, 1991; 13:60-98.

Guerra B, Junker E, Schroeter A, Malorny B, Lehman S, Helmuth R. Phenotypic and genotypic characterization of antimicrobial resistance in German *Escherichia coli* isolates from cattle, swine, and poultry. J Antimicrob Chemother, 2003; 52:489-492.

Hammar M, Bian Z, Normark S. Nucleator-dependent intercellular assembly of adhesive curli organelles in *Escherichia coli*. Proc Natl Acad Sci USA, 1996; 93:6562-6.

He H, Cooper JN, Mishra A, Raskin DM. Stringent response regulation of biofilm formation in *Vibrio cholerae*. J. Bacteriol, 2012; 194(11):2962-2972.

Hegde A, Ballal M, Shenoy S. Detection of diarrheagenic *Escherichia coli* by multiplex PCR. Indian J Med Microbiol, 2012; 30(3):279.

Hughs JM, Murad F, Chang B, Guerrant RL. Role of cyclic GMP in the action of heat-stable enterotoxin of *Escherichia coli*. Nature, 1978; 271**:**755-756.

Iida KI, Mizunoe Y, Wai SN, Yoshida SI. Type 1 fimbriation and its phase switching in diarrheagenic *Escherichia coli* strains. Clin Diagn Lab Immunol, 2001; 8:489–495

Jafari A, Aslani MM, Bouzari S. *Escherichia coli*: A brief review of diarrheagenic pathotypes and their role in diarrheal diseases in Iran. Iran J Microbiol, 2012;4:102–117.

Jerse AE, Yu J, Tall BD, Kaper JP. A genetic locus of enteropathogenic *Escherichia coli* necessary for the production of attaching *anf* effacing lesions on tissue culture cells. Proc Natl Acad Sci USA, 1990; 87(20):7830-7843.

Johnson JR, Stell AL. Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. J Infect Dis, 2000; 181:261–272.

Johnson TJ, Nolan LK. Pathogenomics of the Virulence Plasmids of *Escherichia coli*. Microbiol Mol Biol Rev, 2009; 4:750-774.

Kaper JB, Nataro JP, Mobley HL. Pathogenic *Escherichia coli*. Nat Rev Microbiol, 2004; 2:123-40.

Karch H, Tarr PI, Bielaszewska M. Enterohaemorrhagic *Escherichia coli* in human medicine. IntJ Med Microbiol, 2005; 295**:**405-418.

Karmali MA, Gannon V, Sargeant JM. Verocytotoxin-producing *Escherichia coli* (VTEC). Vet Microbiol, 2010; 140**:**360-370.

Lanz R, Kuhnert P, Boerlin P. Antimicrobial resistance and resistance gene determinants in clinical *Escherichia coli* from different animal species in Switzerland. Vet Microbiol, 2003; 91**:**73-84.

Le Bouguenec C, Archambaud M, Labigne A. Rapid and specific detection of the *pap*, *afa*, and *sfa* adhesin-encoding operons in uropathogenic *Escherichia coli* strains by polymerase chain reaction. J Clin Microbiol, 1992; 30(5):1189-1193.

Lindenthal C, Elsinghorst EA. Enterotoxigenic *Escherichia coli* TibA glycoprotein adheres to human intestine epithelial cells. Infect Immun, 2001; 69(1):52-57.

López-Banda DA, Carrillo-Casas EM, Leyva-Leyva M, Orozco-Hoyuela G, Manjarrez-Hernández ÁH, Arroyo-Escalante S, Hernández-Castro R. Identification of virulence factors genes in *Escherichia coli* isolates from women with urinary tract infection in Mexico. Bio Med Res Int, 2014.

Luscher D, Altwegg M. Detection of *Shigella*, enteroinvasive and enterotoxigenic *Escherichia coli* using the polymerase chain reaction (PCR) in patients returning from tropical countries. Mol Cell Probes,1994;8:285–290.

Madsen L, Aarestrup FM, Olsen JE. Characterization of streptomycin resistance determinants in Danish isolates of *Salmonella typhimurium.* Vet Microbiol, 2000; 75**:**73-82.

Maurer JJ, Brown TP, Steffens WL, Thayer SG. The occurrence of ambient temperature-regulated adhesions, curli, and the temperature-sensitive hemagglutinin Tsh among avian *Escherichia coli*. Avian Dis,1998; 42:106-118.

Mitra A, Palaniyandi S, Herren CD, Zhu X, Mukhopadhyay S. Pleiotropic roles of *uvrY* on biofilm formation, motility and virulence in uropathogenic *Escherichia coli* CFT073. PLoS One, 2013; 8:e55492.

Moon HW. Mechanisms in the pathogenesis of diarrhea: A review. J Am Vet Med Assoc,1978; 172**:**443-448.

Morschhäuser J, Hoschützky H, Jann K, Hacker J. Functional analysis of the sialic acid-binding adhesion SfaS of pathogenic *Escherichia coli* by site-specific mutagenesis. Infect Immun, 1990;58(7):2133-8.

Mulvey MA. Adhesion and entry of uropathogenic *Escherichia coli*. Cell Microbiol, 2002; 4:257–271

Mulvey MA, Lopez-Boado YS, Wilson CL, Roth R, Parks WC, Heuser J, Hultgren SJ. Induction and evasion of host defenses by type 1-

piliated uropathogenic *Escherichia coli*. Science, 1998; 282(5393):1494-1497.

Nataro JP, Kaper JB. Diarrheagenic *Escherichia coli*. Clin Microbiol Rev, 1998; 11:142-201.

Nataro JP, Yikang D, Yingkang D, Walker K. AggR, a transcriptional activator of aggregative adherence fimbria I expression in enteroaggregative *Escherichia coli*. J Bacteriol, 1994; 176:4691–4699

Nowrouzian F, Adlerberth I, Wold AE. P fimbriae, capsule and aerobactin characterize colonic resident *Escherichia coli*. Epidemiol Infect, 2001; 126(01):11-18.

Olowe OA, Idris OJ, Taiwo SS. Prevalence of *tet* genes mediating tetracycline resistance in *Escherichia coli* clinical isolates in Osun State, Nigeria. Eur J Microbiol Immunol, 2013; 3(2):135-140.

Osek J. Detection of the enteroaggregative *Escherichia coli* heat-stable enterotoxin 1 (EAST1) gene and its relationship with fimbrial and enterotoxin markers in *E. coli* isolates from pigs with diarrhoea. Vet Microbiol, 2003; 91(1):65-72.

Palaniappan RU, Zhang Y, Chiu D, Torres A, DebRoy C, Whittam TS, Chang YF. Differentiation of *Escherichia coli* pathotypes by oligonucleotide spotted array. J Clin Microbiol, 2006; 44(4):1495-1501.

Pass MA, Odedra R, Batt RM. Multiplex PCRs for identification of *Escherichia coli* virulence genes. J Clin Microbiol, 2000; 38(5):2001-2004.

Perreten V, Boerlin P. A new sulfonamide resistance gene (*sul3*) in *Escherichia coli* is widely spread in the pig population of Switzerland. Antimicrob Agents Chemother, 2003; 47**:**1169-1172.

Pollard DR, Johnson WM, Lior H, Tyler SD, Rozee KR. Rapid and specific detection of verotoxin genes in *Escherichia coli* by the polymerase chain reaction. J Clin Microbiol, 1990; 28:540–5.

Rajkhowa S, Hussain I, Rajkhowa C. Detection of heat-stable and heat-labile enterotoxin genes of *Escherichia coli* in diarrhoeicfaecal samples of mithun (Bos frontalis) calves by polymerase chain reaction. J Appl Microbiol, 2009; 106(2):455-458.

Ratchtrachenchai OA, Subpasu S, Ito K.Investigation on enteroaggregative *Escherichia coli* infection by multiplex PCR. BullDep Med Sci, 1997; 39**:**211-220

Roberts MC. Tetracycline resistance determinants: mechanisms of action, regulation of expression, genetic mobility, and distribution. FEMS Microbiol Rev, 1996; 19:1-24.

San Millán RM, Martínez-Ballesteros I, Rementeria A, Garaizar J, Bikandi J. Online exercise for the design and simulation of PCR and PCR-RFLP experiments. BMC Res Notes, 2013; 6(1):513.

Sarantuya J, Nishi J, Wakimoto N, Erdene S, Nataro JP, Sheikh J, Miyata K. Typical enteroaggregative *Escherichia coli* is the most prevalent pathotype among *E. coli* strains causing diarrhea in Mongolian children. J Clin Microbiol, 2004; 42(1):133-139.

Schmidt H, Beutin L, Karch H. Molecular analysis of the plasmid-encoded hemolysin of *Escherichia coli* O157:H7 strain EDL 933. Infect Immun, 1995; 63**:**1055-1061.

Schmidt H, Karch H. Enterohemolytic phenotypes and genotypes of Shiga-toxin-producing *Escherichia coli* O111 strains from patients with diarrhea and hemolytic-uremic syndrome. J Clin Microbiol, 1996; 34**:**2364-2367.

Shaw WV. Bacterial resistance to chloramphenicol. Br Med Bull, 1984;40:36–41.

Skold O. Resistance to trimethoprim and sulfonamides. Vet Res, 2001; 32:261-273

Sohel I, Puente JL, Murray WJ, Vuopio-Varkila J, Schoolnik GK. Cloning and characterization of the bundle-forming pilin gene of enteropathogenic *Escherichia coli* and its distribution in *Salmonella* species. Mol Microbiol, 1993; 7:563-575.

Soto SM, Smithson A, Martinez JA, Horcajada JP, Mensa J, Vila J. Biofilm formation in uropathogenic *Escherichia coli* strains: relationship with prostatitis, urovirulence factors and antimicrobial resistance. J Uro, 2007; 177(1):365-368.

Stins MF, Prasadarao NV, Ibric L, Wass CA, Luckett P, Kim KS. Binding characteristics of S fimbriated *Escherichia coli* to isolated brain microvascular endothelial cells. Am J Pathol, 1994; 145(5):1228.

Uhlin BE, Norgren M, Båga M, Normark S. Adhesion to human cells by *Escherichia coli* lacking the major subunit of a digalactoside-specific pilus-adhesin. Proc Nat Aca Sci USA, 1985;82(6):1800–1804.

Van TT, Chin J, Chapman T, Tran LT, Coloe PJ. Safety of raw meat and shellfish in Vietnam: an analysis of *Escherichia coli* isolations for antibiotic resistance and virulence genes. Int J Food Microbiol, 2008; 124:217–223.

Vila J, Gene A, Vargas M, Gascon J, Latorre C, De Anta MJ. A case-control study of diarrhoea in children caused by *Escherichia coli* producing heat-stable enterotoxin (EAST-1). J Med Microbiol, 1998; 47(10):889-891.

Wang G, Clark CG, Rodgers FG. Detection in *Escherichia coli* of the genes encoding the major virulence factors, the genes defining the O157: H7 serotype, and components of the type 2 Shiga toxin family by multiplex PCR. J Clin Microbiol, 2002; 40(10):3613-3619.

Wu CJ, Hsueh PR, Ko WC. A new health threat in Europe: Shiga toxin producing *Escherichia coli* O104:H4 infections. J Microbiol Immunol Infect, 2011;44:390–393.

Yamamoto T. Nakazawa, M. Detection and sequences of the enteroaggregative *Escherichia coli* heat-stable enterotoxin 1 gene in enterotoxigenic *E. coli* strains isolated from piglets and calves with diarrhea. J Clin Microbiol, 1997; 35:223–227

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