Determination of phylogroup in extended spectrum beta lactamase (ESBL)-*E. coli* from fishes by Clermont's rapid phylotyping method

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Escherichia coli is a normal intestinal inhabitant in majority of the animals including humans. It is a versatile commensal but capable of causing both intestinal and extraintestinal diseases such as diarrhoea, septicaemia, urinary tract infections and neonatal meningitis (Orskov and Orskov, 1992). The strains of *E. coli* are diverse with its genome plasticity and the population structure of this organism remains mostly clonal (Touchon et al., 2009). Despite this, *E. coli* can be delineated into six prominent phylogenetic groups-A, B1, B2, D, E and F. Determining the phylogroup of *E. coli* help to understand the path-ogenicity and also give an insight of how these pathogenic strains acquire virulence genes. Tech-niques like multi locus enzyme electrophoresis and ribotyping are capable of assigning phylogroups, but these are time consuming, complex and need a collection of typed strains (Clermont et al., 2000). In 2000, Clermont et al., (2000) developed a PCR based method to characterize the phylogroup based on three candidate markers –*chuA, yjaA* and *TSPE4.C2*. The primers designed were such that strains could be classified into four groups A, B1, B2 and D. Extensive multi locus sequence data and genome data of *E. coli* lead the Clermont group to further improvise the method and in 2013, characterization of eight phylogroups (A, B1, B2, C, D, E, F and *E. coli* cryptic clade I) of *E. coli* were made possible.

The recent study documented 50 extended spectrum beta lactamase (ESBL) *E. coli* isolated and characterized from fishes collected from Guwahati, Assam through quadraplex PCR phylotyping method of Clermont et al., (2013) (Sivaraman et al., 2020). *arpA* gene was used as an internal control for DNA quality and the *E. coli* and Escherichia clade I strains were expected to give a positive amplicon. Other than the primers used in the quadraplex, two other primers *viz., trpAgpC* and *ArpAgpE* (Lescat et al., 2012) were also used to classify phylogroups C and E, respectively. trp *BA* was used to provide an internal control. The primer sequences and the PCR conditions are listed in Table 1.

***Table1:** Primer sequence, reaction conditions and amplicon size of the gene targets*

Target	Primer Name	Sequence (5'-3')	Length (bases)	Primer concentration (µM)	Annealing temp. (°C)	Amplicon size (bp)
chuA	*chuA.1b* *chuA.2*	ATGGTACCGGACGAACCAAC TGCCGCCAGTACCAAAGACA	20 20	1.0 1.0	59	288
yjaA	*yjaA.1b* *yjaA.2b*	CAAACGTGAAGTGTCAGGAG AATGCGTTCCTCAACCTGTG	20 20	1.0 1.0		211
TspE4.C2	*TspE.C2.1b* *TspE4C2.2b*	CACTATTCGTAAGGTCATCC AGTTTATCGCTGCGGGTCGC	20 20	1.0 1.0		152

arpA	*AceK.f* *ArpA1.r*	AACGCTATTCGCCAGCTTGC TCTCCCCATACCGTACGCTA	20 20	2.0 2.0		400
arpA – *Group E*	*ArpAgpE.f* *ArpAgpE.r*	GATTCCATCTTGTCAAAATATGCC GAAAAGAAAAAGAATTCCCAAGAG	24 24	1.0 1.0	57	301
trpA- Group *C*	*trpAgpC.1* *trpAgpC.2*	AGTTTTATGCCCAGTGCGAG TCTGCGCCGGTCACGCCC	20 18	1.0 1.0	59	219
trpA-Internal *control*	*trpBA.f* *trpBA.r*	CGGCGATAAAGACATCTTCAC GCAACGCGGCCTGGCGGAAG	21 20	0.6 0.6	57/59	489

The results showed that forty percent (*n*=20) of the *E. coli* strains were grouped into phylogroup B1 and 30% (*n*=15) to group A.Other prominent phylogroups amongst the strains were E (12%, 6) and D (8%, 4). Two strains belonged to group F and one to Group C. One isolate could not be typed based on Clermont phylogrouping and was termed 'Unknown' as the PCR results were negative for *arpA/chuA/yjaA/TspE4.C2*. This isolate was positive when screened for *E. coli* specific *uidA* gene. The Fig 1 shows the amplification results of few strains studied. Literature points to the fact that virulent extra-intestinal strains of *E. coli* mainly belong to phylogroup B2 and to a smaller extent to Group D. Most of the commensal strains belong to phylogroup A. Phylogroup E was earlier unassigned to which one of the most important serotype O157:H7 belongs and group F is a sister group of phylogroup B2. Group C is closely related to but distinct from phylogroup B1. These findings emphasizes the phylogenetic diversity of *E. coli* strains isolated from fish source and their ability to disseminate infection. In humans and animals, intra-intestinal infections are caused mostly by phylogroup A/B1 or E and extra-intestinal infections are caused mainly by strains belonging to B2 (Clermont et al., 2011). The findings are in concordance with the reports that *E. coli* strains from animals usually belong to phylogroup B1 with 34-50% incidence. None of the studied strains belonged to pathogenic B2 phylogroup. About 8% of the *E. coli* strains belonged to phylogroup D which is known to be present in extra-intestinal pathogenic strains and calls for greater alert.The study validates Clermont's phylotyping method as a simple, rapid, robust and inexpensive tool for phyogrouping of *E. coli*.

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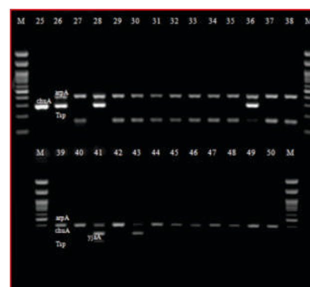

Fig1 : *Quadraplex PCR with the primer combination -arpA/chuA/yjaA/TspE4.C2.*

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