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GENOTYPING OF ENTEROBACTERIACEAE THROUGH ERIC-PCR FROM FISHES OF BHOPAL LAKES

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ABSTRACT

The present investigation was aimed at distribution patterns of enterobacteriaceae in freshwater fish species viz. *Catla catla*, *Labeo rohita*, *Tilapia tilapia* and *Cirrhinus mrigala* collected from 5 water bodies of different regions of Bhopal, Madhya Pradesh, India. Out of all species of enterobacteriaceae observed, occurrence of *E. coli* and *Klebsiella pneumonie* was highest. The molecular diversity analysis clearly indicated considerable genetic diversity among the isolates. The results of present study depict the impact of enterobacteriaceae on the freshwater fauna.

KEYWORDS: Diversity, Enterobacteria, Fish, Genotyping.

INTRODUCTION

Enterobacteriaceae is one of the commonly occurring bacteria infesting fishes and many animal species. Its pathogenesis nature has been reported in 1950 (Austin, 2011). In just six decades, fish production has increased 11–fold in India i.e. from 0.75 million tonnes in 1950-51 to 9.6 million tonnes during 2012–13 resulting in 4.5% annual growth over the years pushedthe country in global fish production, only after China (FAO 2016-2018). Since aqua industry is the backbone of earnings in many developing countries hence fish mortality due to bacterial (infectious and non-infectious) diseases need to be taken into consideration (Ponnerassery, 2012). Any types of environmental stress or injury to fish leads to serious outbreaks of disease with significant mortalities (Sekar *et al*., 2008, Pal and Gupta, 1992).

Sewage pollution is significantly contributed by the enterobacteraceae in fish. Indication about environmental fecal pollution depicts the possibility of isolation of probable pathogenic organisms as *Klebsiella* spp. *Citrobacter* spp. *Salmonella* spp. *E. coli* and *Proteus* spp. from fish (Rajasekaran, 2008).

Identification and characterization of Enterobacteriaceae has been carried out from different fishes by many workers (Diana and Manjulatha, 2012; Bhat *et al*., 2013; Elsherief *et al*., 2014).

ERIC-PCR is based on DNA sequence amplification with primer sets complementary to each end of sequences, representing the short repetitive sequence present in the genomes of Enterobacteriaceae (Versalovic *et al*., 1991). Adding up, shorter sequences as well as longer sequences produced by internal deletions and insertions (about-70 bp) respectively at specific internal sites have also been described (Sharp and Leach, 1996; Cromie *et al*., 1997; Sharp, 1997). Evidently, ERIC sequences have been reported only in Enterobacteriaceae and vibrionaceae but many authors have used these sequences for other bacteria also. ERIC sequences are longer and due to this they deliver more information (Lindsay and Sharp, 2006). Various authors suggested ERIC-PCR may be the useful tool for the genotyping of Enterobacteriaceae members (Wang *et al*., 2011; Bhaumik *et al*., 2012 Griffin *et al*., 2013; Ramazanzadeh *et al*., 2013).

The focus of this study was to evaluate genetic diversity among Enterobacteriaceae population in some water bodies of Bhopal using repetitive sequence based PCR technique (ERIC-PCR). The isolates were recovered from *Catla catla*, *Labeo rohita*, *Tilapia tilapia* and *Cirrhinus mrigala* from different lakes of Bhopal.

MATERIALS AND METHODS

Study sites & sample collection

Sampling sites were Upper Lake, Lower Lake, Shahpura Lake, Sarangpani Lake and Kolar Lake of Bhopal. The

diseased fishes showing symptoms like abnormal swimming, external lesions and changes in colour were collected and examined (Noga, 2010). Four enterobacteriaceae genus and species which are *Escherichia coli, Klebsiella pneumonie, Proteus mirabilis* and *Citrobacter fraundii* were made available from Barkatullah University, Bhopal. The cultures were then subjected to biochemical characterization using the standardized procedures (Aneja (2003).

Genomic DNA extraction

Genomic DNA was extracted using phenol: chlorophorm:isoamyl (25:24:1) with some modifications (Janarthanan and Vincent, 2007).

Enterobacterial Repetitive Intergenic Consensus (ERIC-PCR)

ERIC-PCR was carried out for 19 samples according to established protocols protocol (Versalovic *et al*., 1991; Versalovic *et al*., 1994). Primers ERIC I ATGTAAGCTCCTGGGGATTCAC and ERIC II AAGTAAGTGACTGGGGTGAGCG (Table-1) were used.

Agarose gel electrophoresis

Amplification products of ERIC PCR were electrophorsed through 1.5% agarose (W/V). Band sizes determined by comparison with DNA standard (100bp DNA ladder, BR Biochem).

RESULTS

Biochemical characterization of isolates

Bacterial characterization through conventional method revealed the presence of four genera and four species as *Escherichia coli, Klebsiella pneumonie, Proteus mirabilis* and *Citrobacter fraundii* screened through Gram staining, Indole, H_2S, MR-VP, Catalase, Oxidase and Motility were the confirmatory tests. All the bacterial species belong to the Enterobacteriaceae family (Results not shown).

Enterobacterial Repetitive Intergenic Consensus (ERIC-PCR)

ERIC-PCR fingerprinting showed reproducible pattern of bands and their molecular weights found from 104 to 2431bp (Figure 1). To ensure better results the samples were processed through ERIC-PCR for 3 times individually. The result of all three ERIC-PCR runs was almost similar & indicates the reproducibility of the technique. All tested 19 samples were demonstrated different pattern of ERIC. However, the maximum band intensity was concentrated in the range of 400-500bp. The results indicate that there were clearly distinguished 64 bands ofeEnterobacteriaceae strains which were grouped into 6 distinct clusters showing heterogeneity in enterobacteriaceae (fig. 2).

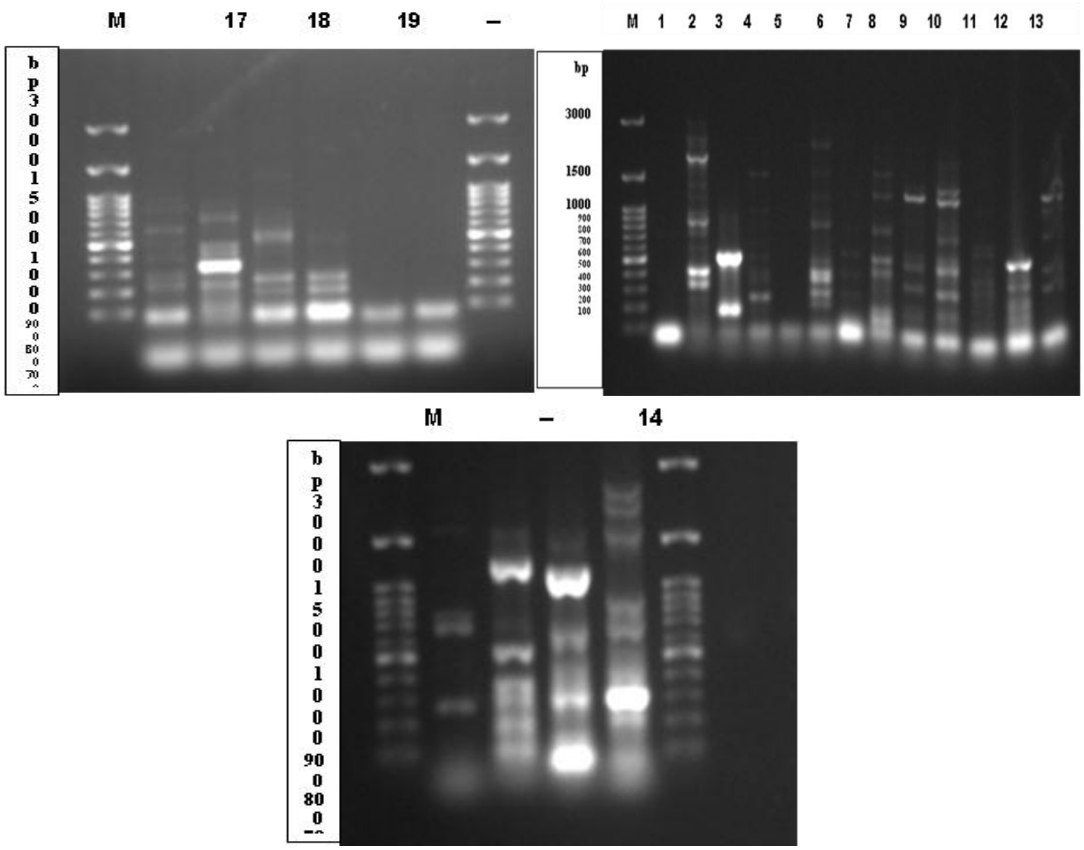

Figure 1: ERIC-DNA fingerprints analyzed through gel electrophoresis using 1.5% Agarose gel obtained from various isolates of Enterobacteriaceae recovered from intestine and liver of sampled fishes. The molecular sizes (in base pair) are indicated on the left. A) M= DNA Standard, 1=Escherichia *coli*, 2=*E. coli*, 3=*E. coli*,

4=*Klebsiella. pneumoniae*, 5=*K. pneumoniae*,, 6=*Citrobacter freundii*, 7=*Proteus mirabilis*, 8=*Proteus mirabilis*, 9=*K. pneumoniae*, 10=*K. pneumoniae*, 11=*K. pneumoniae*, 12=*K. pneumoniae*, 13=*Proteus mirabilis*. B) M= DNA Standard, 14= *Citrobacter freundii* 15=*E.coli*, 16=*E. coli*. C) M= DNA Standard, 17=*K. pneumoniae*, 18=*E. coli*, 19=*E. coli*.

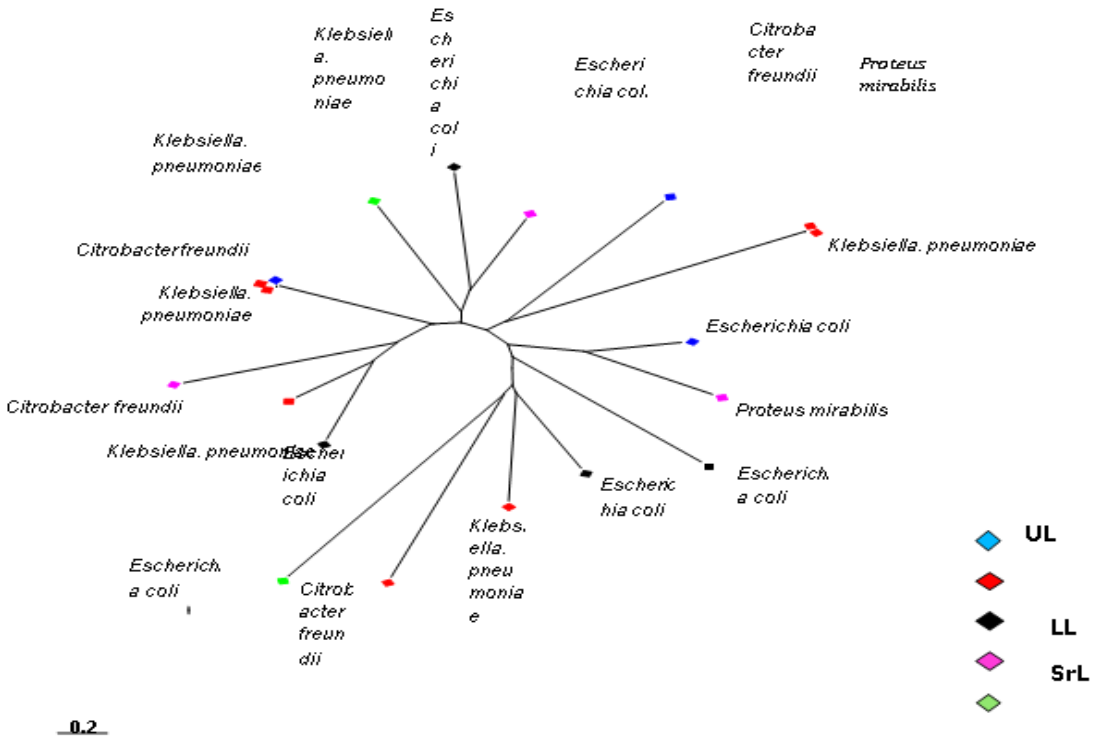

Figure 2: Neighbour joining Tree (Radial Sub Branching Pattern) for showing genetic distance based on ERIC-DNA fingerprints analysis obtained from various isolates of Enterobacteriaceae recovered from Intestine and Liver of sampled fishes.

Table 1: Primers used for repetitive sequence mediated PCR.

Primer	Sequence (5'-3')	Reference	Source
ERIC I	ATGTAAGCTCCTGGGGATTCAC	Versalovic et al., 1991	3D BlackBio Biotech India Ltd
ERIC II	AAGTAAGTGACTGGGGTGAGCG	Versalovic et al., 1991	3D BlackBio Biotech India Ltd

DISCUSSION

Despite of vast distribution and the genetic heterogeneity of the enterobacteriaceae, attention seem to be lowest at population genetics counterpart. Hence, our focus was to illustrate the distribution patterns of genetic diversity in enterobacteriaceae population in variety of fish specimens from different water bodies and to make use of these statistics to assess their genetic reliability.

The results of present study show compatibility with Zhang *et al.* (2018) who characterized *K. pneumonie* isolates by ERIC-PCR and found that isolates from same geographic area have close relationship between them. Likewise, in this study also, it was noticed that isolates from Lower Lake, Sarangpani Lake and Shahpura Lake formed a cluster showing close relationship between them.

Taking into consideration increasing sewage and fecal pollution in the Bhopal area, molecular study of enterobacteriaceae was proceed further through ERIC-PCR as the method proved to be suitable for the evaluation of diversity of enterobacterial species based on conserved repetitive sequences. The result of present study probably helpful to improve the aquatic system, in particular, pisciculture by assessment of change in microflora over the years through molecular assessment of disease causing microflora.

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Conflict of interest

The authors have no conflict of interest to declare.

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