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ASYMMETRIC PCR FOR THE CONSTRUCTION OF DNA POOL FOR APTAMER DEVELOPMENT FOR ESBL *E.coli* USING SELEX PROCESS

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***Abstract*— ESBLs are enzymes capable of hydrolysing penicillin, broad spectrum Cephalosporin and monobactam. The enzyme which inhibits the drug activity is present in *Enterobacteriaceae* group of microorganisms like *E.coli*, *Klebseilla pneumonia*, *Staphylococcus aureus, P. Aeruginosa, P. Vulgaris* etc. The treatments of these microorganisms are very difficult because of the presence of Beta-lactamase enzyme in the plasmid DNA. In order to contain the scenario there is a need to use target specific drugs or molecules. Aptamers are single-stranded DNA or RNA that binds to wide variety of target molecules with high affinity and specificity produced by SELEX. The plasmid DNA of 7 kb containing the TEM gene was isolated from ESBL *E.coli*. The PCR amplification was done for the TEM gene of 717 bps. Further, the plasmid DNA containing the TEM gene will be used as a target DNA in Asymmetric PCR for obtaining a pool of ssDNA. The ssDNA and the Beta-lactamase enzyme will be used in SELEX process for obtaining the desired Aptamer.**

Keywords*— *Plasmid DNA, Genomic DNA, PCR

I. INTRODUCTION

The resistance to a multiplicity of common antimicrobials has made the proliferation of multi-drug resistant strains, a serious global health concern that has complicated treatment strategies. One of the most common multi-drug resistant organisms is Extended Spectrum Beta-lactamase (ESBL) *E.coli* that breaks down the Beta-lactam rings of antibiotics. Extended spectrum β-lactamase (ESBL)-producing organisms pose unique challenges to clinical microbiologists, clinicians, infection control professionals and antibacterial-discovery scientists. ESBLs are enzymes capable of hydrolysing Penicillins, broad-spectrum Cephalosporins and monobactams, and are generally derived from TEM and SHV-type enzymes. They are often located on plasmids that are transferable from strain to strain and also between bacterial species. Although the prevalence of ESBLs is not known, it is clearly increasing, and especially in many parts of the world upto 10–40% with the strains of *Escherichia coli.* Currently, Carbapenems are regarded as the drugs of choice for treatment of infections caused by ESBL-producing organisms. Unfortunately, use of Carbapenems has been associated with the emergence of Carbapenem-resistant bacterial species such as *Stenotrophomonas* sp. or *Pseudomonas* sp. Hence the introduction of new higher dosage drugs cannot fully eradicate this pathogen. In order to eradicate it successfully, there is a need to use target specific drugs. Indeed, there is an ongoing need in basic biological research, clinical diagnostics and therapeutics for affinity reagents that can target proteins with high specificity. Monoclonal antibodies continue to be predominantly used for these purposes. However, production of monoclonal antibodies in large quantities is time-consuming and expensive, and there is demand for a high-throughput and low-cost method for generating affinity reagents (Meyer *et al*., 2013). Aptamers have promising advantages compared to antibodies they can be produced easily and inexpensively and are simple to chemically modify and integrate into different analytical schemes. Aptamers can retain their binding and inhibitory behaviour after immobilization on a carrier material or after delivery into animals and can be labelled with various functional groups. SELEX (systematic evolution of ligands by exponential enrichment) is a method to generate DNA or RNA ligands from a combinatorial library. This oligonucleotide library consists of single stranded modified or unmodified RNA or DNA. The ligands that emerge from

SELEX have been called Aptamers (Gou *et al*., 2008). Hence this study deals on the construction of aptamers for the management of ESBL *E.coli.*

II. METHODOLOGY

A. *Selection of Bacteria*

The bacterial isolate obtained from the soil samples from various hospitals in and around Chennai. These soil samples were processed and serial diluted and inoculated on Maconkey agar medium, and in EMB agar medium for the selection of Lactose fermenting colonies. Antibiotic assay was performed for different antibiotics, Amoxicillin, Clavulanic acid with Cefotaxime and then the resistant isolates were, further subjected for the confirmation of ESBL *E.coli* by growing on ESBL agar medium. Gram staining was done to confirm as Gram negative bacteria.

B. ***Plasmid DNA extraction***

Since the ESBL enzyme is expressed by TEM, SHV and CTX genes present in the plasmid DNA of the microorganism the plasmid DNA was extracted by rapid alkaline lysis procedure [13]. The plasmid DNA was resolved by running on 1% agarose gel electrophoresis along with a marker.

C. ***Confirmation of the presence of TEM gene***

The presence of TEM gene in the bacterial sample was confirmed by PCR amplification. Primers used for TEM amplification were 5-CTTCCTGTTTTGCTCACCCA-3 and 5-TACGATACGGGAGGGCTTAC-3. The parameters for PCR amplification were, Intial denaturation at 94°C for 1 minute, Denaturation at 94°C for 30 seconds, Annealing at 63°C for 1 minute, Elongation at 72°C for 1 minute followed by 30 amplification cycles and a final extension of 5 minutes at 72°C. The amplified products were resolved in 1.5% agarose gel.

D. ***Genomic DNA extraction***

Genomic DNA was extracted by phenol choloroform extraction method (Sambrook *et al.,* 2001). The DNA samples were resolved on 0.8% agarose gel. It was examined under UV light to look for the presence of DNA bands of particular size using a marker.

III. **RESULTS**

A. *Confirmation ESBL E.coli*

ESBL *E.coli* was confirmed by streaking the soil supernatant on EMB agar, which imparts metallic sheen that separates *E.coli* from other microrganisms present in the soil. Then it was again inoculated on Hi-chrome ESBL agar and purple color colonies were observed, confirming the presence of ESBL *E.coli*. The Hi-chrome ESBL agar contains the substrate indoxyl-β-glucoside which react with the enzyme β-D-glucosidase, present in ESBL *E.coli* and appeared purple (fig1, 2).

B. ***PCR Analysis***

The plasmid DNA was isolated from ESBL *E.coli* and a product of 7000bp was obtained. The presence of TEM gene in the plasmid DNA was checked by PCR amplification and was found to be of 717bps (fig. 3, 4, and 5).

Fig: 1. EMB agar containing ESBL *E.coli* with metallic sheen appearance.

Fig: 2. Hi-Chrome ESBL agar shows the growth of purple colonies on Hi- Chrome ESBL Agar confirming the presence of ESBL *E. Coli.*

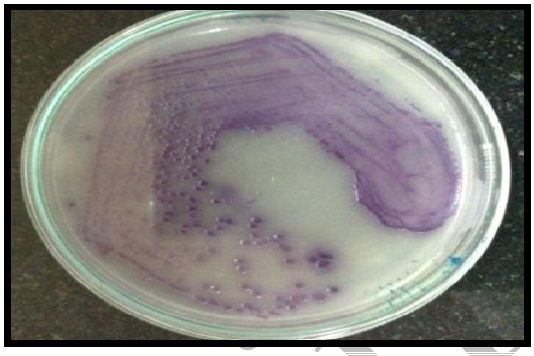
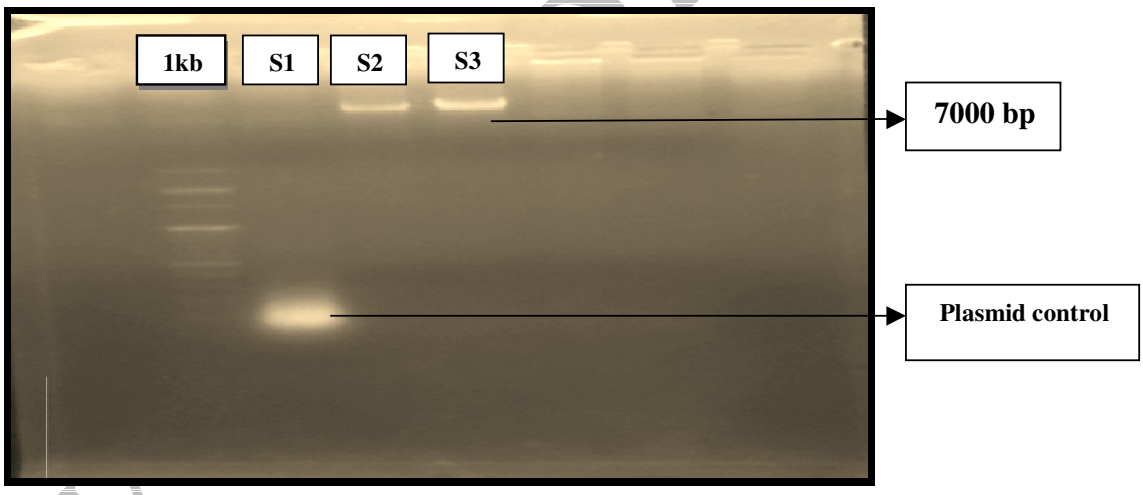
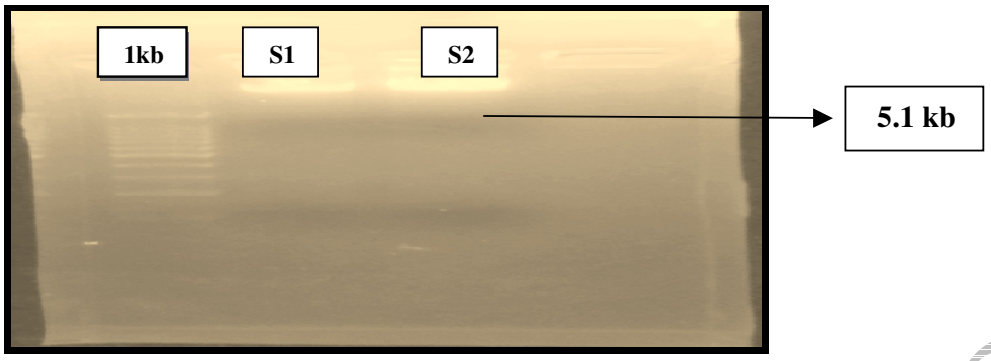


Fig: 3. Plasmid DNA of ESBL producing *E.coli* samples



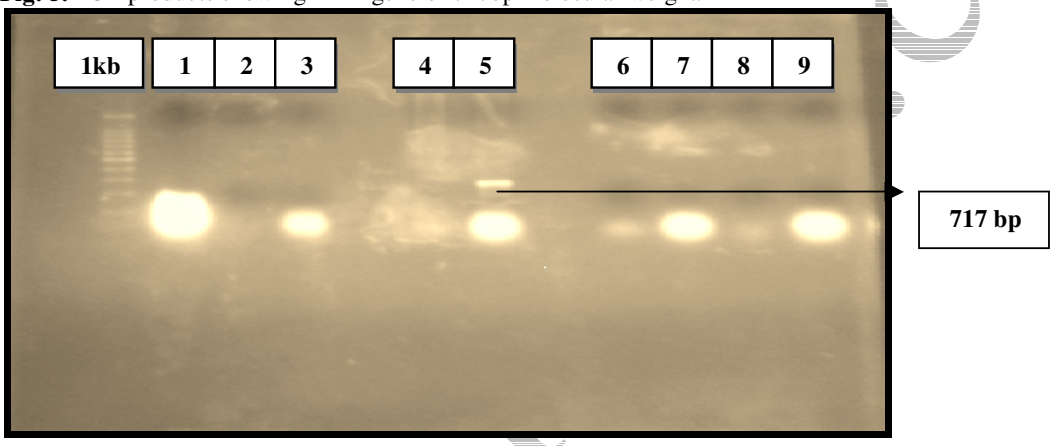
Analysis of the gel: S1- Plasmid control with RNA, S2 and S3: Plasmid DNA of 7000bps.

Fig: 4. Genomic DNA of ESBL producing *E.coli* samples.



Analysis of gel: S1and S2- Genomic DNA of 5.1kb.

Fig: 5. PCR products showing TEM gene of 717bp molecular weight.



Analysis of gel: L1- Plasmid control, L2- SHV control, L3- Indicates the absence of SHV gene in plasmid DNA ,L4- TEM control, L5- Indicates the presence of TEM gene (717 bps), L6- CTX control, L7- Indicates the absence of CTX gene, L8- Asymmetric control, L8- Absence of Asymmetric gene (PCR parameter different for Asymmetric PCR).

IV. DISCUSSION

E.coli is a common pathogen of nosocomial infection and with wide use of antibiotics, multiple drug resistance ESBL producing *E.coli* infection has increased gradually, which is difficult to cure. The prevalence of ESBL-producing strains of *E.coli* varies from country to country and from species to species all around the world. The ESBL enzymes are encoded by genes such as SHV, TEM, and CTX, OXA etc. that are frequently located on mobile genetic elements plasmids, which have the ability to transfer horizontally within and between different bacterial species. Plasmid size ranges from 50 kb to 291 kb. The largest plasmid is of size ~ 242 kb and ~ 194 kb. The molecular analysis demonstrated that the *bla*TEM genes have high frequency compared to *bla*SHV type and *bla*CTX types. Therefore in this study, *bla*TEM gene is used in the construction of DNA pool using Asymmetric PCR for ESBL *E.coli*.

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