



Molecular Detection of *Escherichia coli* (*E. coli*) from Diarrheal Stool Samples from Children in Quetta, Balochistan, Pakistan

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Abstract

Diarrhea is one of the major causes of death in children, particularly in developing countries. Rapid detection and treatment is necessary to control disease transmission in the community and thus limiting the huge number of death toll. The major cause of diarrhea in developing countries is *Escherichia coli* (*E. coli*). This study was aimed to isolate *E. coli* from diarrheal stool samples from children aged 05 months to 05 years visited/ hospitalized in Quetta due to acute/persistent diarrhea. Diarrheal stool samples from 200 children were collected from Lady Sandeman Hospital Quetta and cultured on nutrient agar and later transferred to *E. coli* specific growth media for initial detection. For further confirmation the colonies were subjected to polymerase chain reaction (PCR). The PCR results revealed that 44(22%) samples out of 200 samples were positive for *E. coli*. These results indicate a high proportion of *E. coli* infection among children suffering with diarrhea

Keywords: *Escherichia Coli* (*E. Coli*), Polymerase Chain Reaction (PCR), Padiatric Diarrhea

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INTRODUCTION

Diarrhea is the foremost reasons for morbidity and death among children below five years of age in the developing world (1). In these countries every child has suffer an average of three incidences of diarrhea each year. In developing countries, diarrhea is the second most prevalent disease in children following respiratory diseases (2). Diarrhea is caused by a broad variety of pathogens such as bacteria, viruses, and parasites. Among bacteria *Escherichia coli* (*E. coli*) is the main etiologic agent of childhood diarrhea in undeveloped nations. *E. coli* usually reside in the intestines of healthy humans and animals (3). Diarrhea is also caused by salmonella bacteria which affect the intestine and the disease caused is known as Salmonellosis (4). Similarly, shigellosis is an intestinal disease induced by shigella. The sign of shigella infection is bloody diarrhea. It can spread through direct contact with infected stool (5). *Campylobacter* is comma or S shaped Gram negative bacteria and can cause a gastrointestinal infection known as campylobacteriosis. The mode of transmission of this bacteria is the fecal-oral route (6). *Giardia* is a parasite which exist in the intestines of animals and humans. The signs of giardia infection are nausea, abdominal cramps, and watery diarrhea (7). Among viruses, *Rotavirus* is the most frequent diarrheal pathogen in children throughout the world. It is a circular shape virus and causes inflammation in the stomach. It

can spread readily through hand to mouth contact. Rotavirus infection is prevalent in children aged between 3 to 35 months (8). There are two varieties of vaccines prepared for prevention against rotavirus namely Rota Teq and Rotarix (9). Diarrhea is of three types particularly: i) acute diarrhea which lasted less than or equal to 13 days, ii) persistent diarrhea defined as diarrhea with a span of 14 days and above, and iii) dysentery diarrhea associated with bloody stool and abdominal cramps (10). Diarrhea is also associated with antibiotics. This research project was aimed to check the infection of *E. coli* in children with chronic, persistent and acute diarrhea, between 5 months to 5 years of age in Quetta by using PCR.

MATERIALS AND METHODS

The study was conducted in the Center of Advanced Studies in Vaccinology and Biotechnology (CASVAB), Quetta and civil hospital Quetta from May 2019 to October 2019. Subjects were children between 5 months to 5 years of age who visited or admitted in hospital for the treatment of diarrhea.

Collection and transportation of stool samples

Stool samples from the children suffering with diarrhea were obtained and transported by using a sterile plastic containers to the microbiology laboratory for examination.



Culturing

The specimens were cultured on nutrient agar media. After incubation at 37°C overnight the colonies were observed over the surface of plate.

Detection of *E. coli* by PCR

The colonies from the plate were analyzed for the confirmation of *Escherichia coli* (*E. coli*) by using *uidA* gene specific primers (12).

The genomic DNA was extracted by using the boiling method (13). The colonies were boiled at 95 °C for 10 minutes in (500 µl) of 1 X TE buffer. After boiling the solution was subjected to centrifugation. The supernatant containing the genomic DNA was collected and stored while the pellet was discarded. The detailed sequence of *uidA* gene primers set is shown in Table I.

Table I. Primer sequence and predicted length of PCR products of *uidA* gene.

Target gene	Nucleotide sequences 5'-3'	Product size bp	Ref
<i>uidA</i> gene	F.CCAAAAGCCAGACAGAGT R.GCACAGCACATCAAAGAG	623-bp	14

Polymerase chain reaction was carried out by mixing 3µl of template DNA, 10 µl of master mix, 1-1 µl of each forward and reverse primers and 5 µl of molecular grade water. This mixture was prepared in PCR tubes. The initial temperature for Taq polymerase activation was set at 95°C for 5 minutes. Followed by 35 cycles of denaturation temperature at 94°C for 1 minute, annealing at 52°C for 30 seconds, extension at 72°C for 1 min. At the last final extension at 72°C for 7 minutes.

The PCR product was examined by electrophoresis on agarose gel (15) and the product size was checked by molecular maker at gel concentration of 2 % (W/V). The voltage for gel electrophoresis was set at 110 V and electrophoresis for 45 minutes. The PCR products were visualized under UV trans illuminator chamber.

The specimens obtained from children were cultured on EMB media. After incubation at 37 °C overnight the *E. coli* colonies were identified by their greenish colonies on this selectable media (11).

Ethical considerations

A permission from ethical review board of CASVAB and the hospital involved was obtained.

RESULTS AND DISCUSSION

Throughout the study period, 200 children with diarrhea were randomly included in this study. These children were between 5 months to 5 years of age. *E. coli* was detected by polymerase chain reaction(PCR) method in 44 (22%) patients. The stool samples collected from children were cultured on nutrient agar as shown in figure 1. For further confirmation the colonies were picked from nutrient agar plates and grown on EMB agar plates for 24 hours. A high level of aseptic environment is maintained. The *E. coli* presented typical metallic shine on EMB agar as shown in figure 2.



Fig. 1. Colonies of *E.coli* on nutrient agar media.

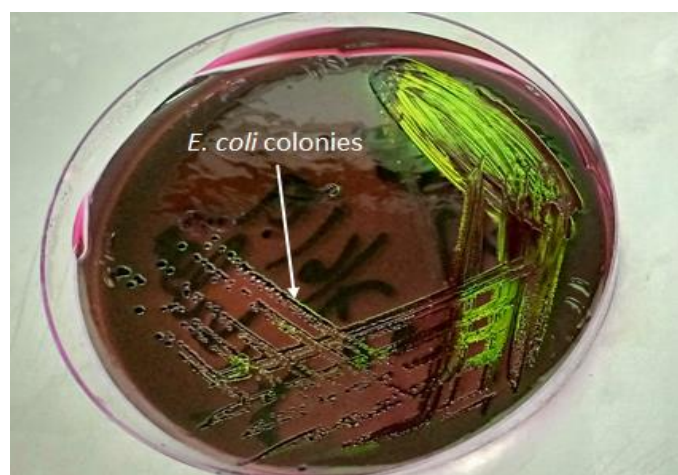


Fig. 2. EMB culture result of *E. coli*.

The Genomic DNA was isolated from each of the sample by means of boiling method Fig. 3 shows the result of DNA extraction from stool samples.

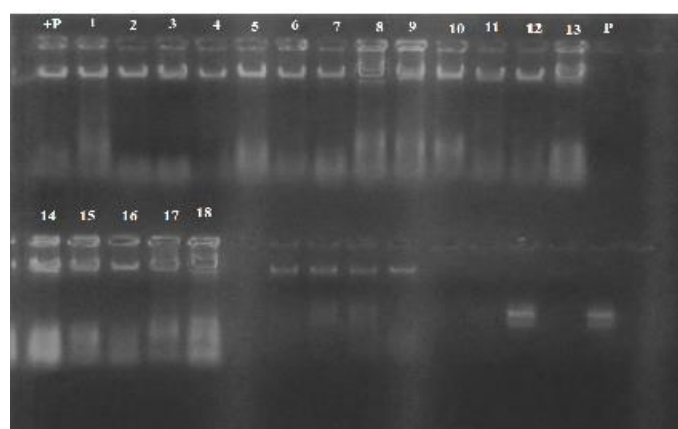


Fig. 3. Isolation of genomic DNA of bacterial growth from stool samples. +P: positive control -P: negative control. 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17 and, 18: positive genomic DNA respectively

All the stool samples were subjected to PCR analysis for final confirmation. The PCR amplification of stool samples indicated that 44 samples were positive for *E. coli*.

All the (n=200) collected stool samples from the patients aged 5 months to 5 years were analysed for the presence *E. coli* through PCR. The representative results of PCR amplification of uidA gene is shown in Fig. 4, 5, 6, and, 7 respectively.

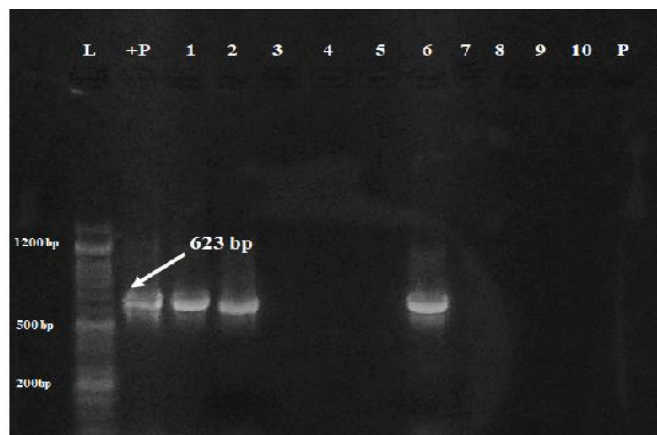


Fig. 4. Detection of *Escherichia coli* (*E. coli*) in stool samples. The 623 bp amplification of targeted gene of *E. coli* is shown in sample numbers 1, 2 and, 6. While sample 3,4,5,7,8,9 and, 10 shows negative results. L denotes the marker while +P and -P represents the positive and negative control respectively.

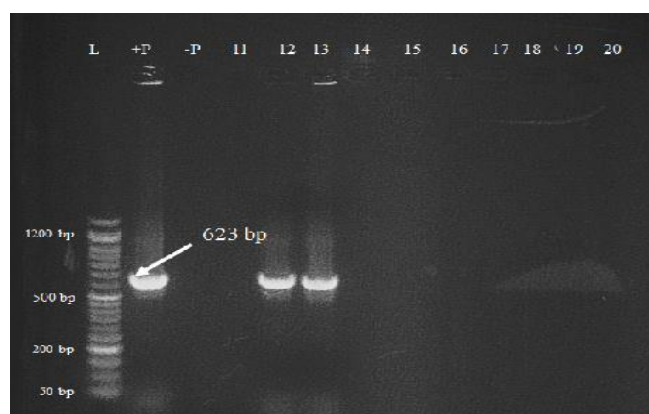


Fig. 5. A PCR based detection of *Escherichia coli*. The 623bp amplification of targeted gene of *E. coli* is shown in sample numbers 12 and,13. They were positively amplified. Lader is denoted as "L" while positive and negative control are represented by the +P and -P respectively. All other samples shown in figure were found negative.

After performing the PCR from all the (n=200) stool samples the PCR products of positive samples were subjected for gel electrophoresis in single gel to present the results in uniformity as shown in fig. 8.

The current study was performed to detect the incidence of *Escherichia coli* (*E. coli*) as a possible etiology of diarrhea in children. The PCR based amplification of uidA gene results showed that 44(22%) out of 200 samples were positive for *E. coli* isolated from diarrheal stools samples. Several studies have reported the incidence of *E. coli* as pathogenic bacteria involved in paediatric diarrhea in Pakistan and around the globe (16, 17, 18, 19). Similarly, *E. coli* has been reported as one of the leading cause of gastrointestinal tract infections (20).

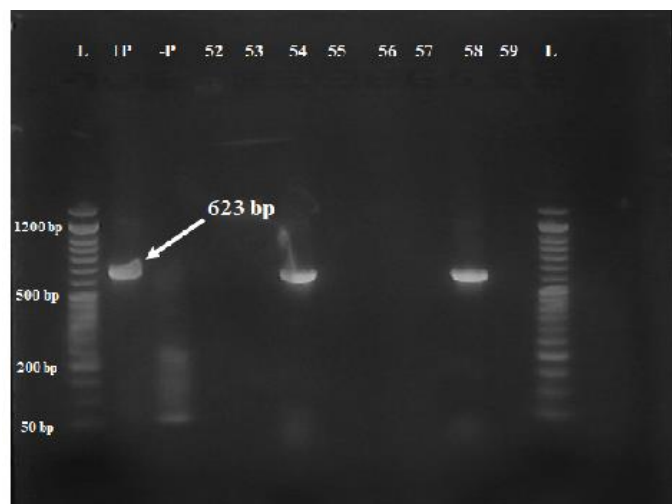


Fig. 6. Diarrheal Stool samples examination by PCR to detect *Escherichia coli* (*E. coli*). Sample 54, and 58 were found positive while the other 06 samples were negative for *E. coli*.

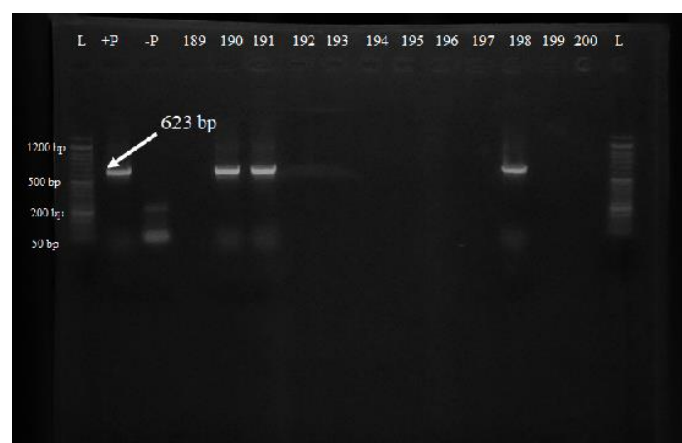


Fig. 7. Sample in 190, 191 and 198 shows positive result for the presence of *Escherichia coli* (*E. coli*). Sample number 189,192,193,194,195,196,197,199, and 200 are negative for *E. coli*. Ladder is represented by "L" while +P and -P represents the positive and negative control respectively.

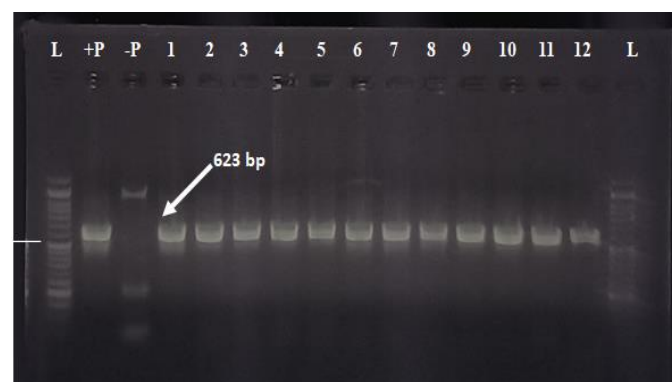


Fig. 8. Detection of *Escherichia coli* (*E. coli*) in stool samples. The 623 bp amplification of targeted gene of *E. coli* is shown in sample numbers 1,2,3,4,5,6,7,8,9,10,11 and, 12. L denotes the marker while +P and -P represents the positive and negative control respectively.

Besides infections in developing world, *E. coli* has been reported to strike even in developed world and its outbreak

have been reported previously (21). Several pathogenic factors/antigens harboured by *E.coli* lead to cause infection in humans. Normally, *E.coli* are recognized on the basis of antigens present on its surface and these antigens are O, H and K and more than 700 serotypes of *E. coli* have been documented to date (22). Most common infections caused by *E. coli* are infections of Urinary tract, meningitis and gastrointestinal tract. The children, elderly and immunocompromised persons are most affected by these infections caused by pathogenic strains.

For effective treatment, rapid diagnosis is of utmost importance. The current developments in molecular biology have eased the path of diagnosis. The co-infection/mixed infection of several pathogens or different strains of same pathogen can be detected with help of single PCR by help of designated primers used for specific species or strains of pathogens (23, 24).

In developing countries like Pakistan, these molecular techniques are not so well adopted. This study was an attempt to present the evidence to detect *E. coli* more efficiently and accurately from stool samples.

Conclusion

This study concludes that *Escherichia coli* (*E. coli*) are one of the main causes of diarrhea in childhood. The results also shows that use of *uidA* gene targeting primer is useful, sensitive and rapid tool for the detection of *Escherichia coli* (*E.coli*) from stool samples

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