Molecular identification of blaCTX-M and blaTEM genes among multi-drug resistant Enteropathogenic Escherichia coli isolated from children

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Abstract: In this cross sectional study (June 2016 to June 2017), we studied the isolation and molecular characterization of multi-drug resistant Escherichia coli (MDR-E. coli) from children suffering from diarrhea. For this purpose, a total of 100 fecal samples were collected with the consent of the parents/ guardians on a prescribed form. The bacterial isolation was done by employing conventional and standard microbiological procedures. Subsequently, all the isolates were identified on the basis of biochemical tests and were further characterized by amplification of 16S rRNA gene followed by di-deoxy sequencing of the amplified product. Afterwards, the isolates were subjected to antimicrobial susceptibility profiling using Kirby Bauer disc diffusion method. A total of 87 E. coli isolates were identified in the current study and majority of the isolates were found sensitive to all or few antimicrobials. However, 14 E. coli isolates were found resistance to multiple drugs including amoxicillin-clavulanic acid, ciprofloxacin, gentamicin, cefoperazone and ofloxacin, hence termed as MDR-E. coli. All of the 14 isolates were further analyzed for the identification of blaCTX-M and blaTEM genes through PCR using specific primers. This resistant was found to be associated with the presence of plasmid encoded beta lactamases genes including blaCTX-M (13/14 E. coli isolates) and blaTEM (9/14 E. coli isolates). Altogether, it was found that ESBLs harboring E. coli is potential source of diarrhea among pediatric diarrheal patients. Therefore, molecular identification and characterization of bacterial pathogens along with antimicrobial susceptibility are critical to understand MDR-E. coli infections.

Keywords: Enteropathogenic E. coli, children, multi-drug resistant, diarrhea.

INTRODUCTION

The members of the family Enterobacteriaceae have a substantial impact in the microbiology of gastrointestinal tract (GIT) in humans as well as in animals (Moura et al., 2009; Jandhyala et al., 2015). While, Escherichia coli is one of the top ranked bacterium in this family which is responsible for several disorders including diarrhea in infants, children and adults throughout the world (Allard et al., 2017; Gallardo et al., 2017). E. coli is classified into different groups including enterohemorrhagic, enteropathogenic, enteroinvasive, enterotoxigenic, diffusely adherent, enteroaggregative and necrotoxic E. coli on the basis of pathogenicity and the mechanism of virulence as described previously in different studies (Berings et al., 2017).

Among these groups the enteropathogenic E. coli (EPEC) is foremost important and is responsible for acute and chronic diarrhea in infants and children in developing as well as in developed countries. This is also a well-established fact that EPEC associated diarrhea is the second highest cause of mortalities among infants following the rotavirus infections (Lanata et al., 2013).

EPEC do not produce any toxins and exert its pathogenic effects by attachment/effacement (A/E) lesions in the intestines. The attachment is mediated through an outer membrane protein of the bacterium termed as intimin which is encoded by eae gene of the bacterium (Das et al., 2013). The lesions are characterized by bacterial attachment to the intestinal wall of the host and disturbing the cell surface which ultimately lead to thinning and shortening of microvilli of intestine (effacement). EPEC is predominantly transmitted through fecal-oral route by contaminated hands, foods and by fomites (Rhouma et al., 2016; Mourand et al., 2017). EPEC strains are a potential cause of acute to chronic diarrhea with high morbidity and mortality rates in developed countries (Moura et al., 2009).

Similarly, the antimicrobial resistance among different E. coli isolates has been reported in different regions of the world. Few of the studies described that E. coli is responsible for diarrhea in almost 200 million infants and children annually resulting in huge numbers of deaths in developed as well as in developing countries. Antimicrobial resistance or multi-drug resistance is also high particularly in developing countries (Das et al., 2013). The diagnosis of EPEC could be performed either...
on phenotypic or on genotypic basis including 16S rRNA or eae gene amplification followed by sequencing of the amplified PCR product (Tariq et al., 2012; Abriouel et al., 2017). However, the multi drug resistance is detected by molecular identification and characterization based on blaCTX-M or other beta lactamase genes. Experimental animal models could also be used to investigate the type of E. coli strains as described recently (Fayyaz et al., 2018). Therefore, in this study, we have focused on isolation, identification and molecular characterization of multi-drug resistant enteropathogenic E. coli from children suffering with diarrhea along with antibiogram studies of different E. coli isolates.

MATERIALS AND METHODS

Isolation of Enteropathogenic Escherichia coli (EPEC)
In the present study, a total of 100 fecal samples were collected from 1-5 years children for isolation of E. coli during June 2016-June 2017. For this purpose small amount of fecal material was dissolved in 2 ml of normal saline and directly inoculated on MacConkey’s agar (Oxoid™, UK) followed by an incubation at 37°C for 24-48 hours (Ahmed et al., 2009). Initial identification was performed on the basis of standard microbiological and biochemical procedures as described recently (Fayyaz et al., 2018).

Antibiogram studies of EPEC
The antibiogram profiles of all isolates of E. coli was investigated against routinely used antimicrobial agents including amoxicillin-clavulanic acid, ciprofloxacin, imipenem, gentamicin, cefoperazone, vancomycin, amikacin, meropenem, levofloxacin, ofloxacin, teicoplanin, piperacillin-tazobactam and ceftriaxone. The antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion method on Muller Hinton agar (Oxoid™, UK) according to the guidelines of Clinical Laboratory Standards Institute (CLSI-2016) as described (Berings et al., 2017; Fayyaz et al., 2018).

Molecular Identification of multi-drug resistant EPEC, blaCTX-M and blaTEM
For the molecular characterization of EPEC, the bacterial DNA was extracted using commercially available DNA extraction kit (Favorgen® Biotech Corp. Taiwan) according to manufacturer’s instructions. DNA amplification was carried out by targeting the 16S rRNA gene of the bacterium using universal set of primers 27F (5’-AGAGTTTGATCMTGGCTCAG-3’) and 1492R (5’-TACGGYTACCTTGTTACGACTT-3’) as described (Dupont et al., 2016). DNA amplification was also performed targeting the blaCTX-M and blaTEM genes for identification of beta lactamase resistance using CTX-M and TEM forward/reverse primers as described (Ghorbani-Dalini et al., 2015). The amplification was carried out in total volume of 20 µL which was consisted of 1 µL of bacterial DNA template, 1 µL of each forward and reverse primers and Master Mix (12.5 µL). Whereas negative control contained water instead of template DNA, while E. coli genomic DNA was used as positive control. Finally, the amplified product was subjected to electrophoresis using 1.5% agarose gel stained with ethidium bromide and visualized under UV light. After the gel purification the amplified products were dispatched to Macrogen, Korea for di-deoxy Sanger sequencing method. The obtained sequences were analyzed with Chromas Pro® (Technelysium, Technelysium Pty Ltd, Australia) to assess the sequencing quality and contigs were formed and sequences were aligned using BLASTn online program. The aligned sequences were compared with the available bacterial 16S rRNA sequences in NCBI database as described (Dupont et al., 2016).

RESULTS
A total of 87 E. coli were identified out of 100 samples on the basis of the biochemical profiles. The antibiogram data indicated that majority of E. coli were sensitive to several antibacterial agents. However, a total of 14 (16%) E. coli isolates were found resistant to different groups of antibiotics including amoxicillin-clavulanic acid, ciprofloxacin, gentamicin, cefoperazone and ofloxacin, hence termed as MDR E. coli strains as shown in table 1.

Fig. 1: Phylogenetic position of multi drug resistant E. coli AZ1 strain

Fig. 2: Molecular identification of blaCTX-M and blaTEM genes among MDR- E. coli

The genomic amplification of MDR E. coli resulted in 1500 bp product, the sequencing data and homology search showed that isolated bacteria were...
enteropathogenic E. coli. One sequence was submitted to GenBank and named as multidrug resistant E. coli AZ1 strain (GenBank Accession Number: MF185146). The phylogenetic position of this strain was shown in fig. 1. Molecular identification of blaCTX-M and blaTEM was identified among 13/14 (93%) and 9/14 (64%) E. coli isolates, respectively as shown in fig. 2.

DISCUSSION

The impact of enteropathogenic Escherichia coli (EPEC) could not be neglected as it continued as major etiological agent of diarrhea and significant morbidities and mortalities in infants and children throughout the world (Gallardo et al., 2017). The actual duration and progression of EPEC infection is not clearly defined. In the present study, we characterized the enteropathogenic E. coli on the basis of sequence analysis of 16S rRNA gene as already reported in previous studies (Dupont et al., 2016). In previous studies, the molecular characterization and phylogenetic analysis was performed for EPEC strains which were originated from different sources using same gene amplification and sequence analysis (Saeed et al., 2009; Saravia et al., 2017).

In the present study, antimicrobial susceptibility data showed 14 MDR- E. coli (16%) whereas beta lactamase enzyme blaCTX-M gene was identified among 93% (13/14) of MDR-E. coli. One of the recent studies described that extended spectrum beta lactamases are enzymes encoded by different genes on bacterial plasmids and are responsible for degradation of antimicrobials (Mourand et al., 2017). Overall an increased occurrence of MDR- E. coli was found in the current study. Many of the previous studies described E. coli as primary pathogen of gastrointestinal tract causing diarrhea and gastric disturbances by eruption of intestinal epithelium and imbalance of fluids (Fayyaz et al., 2018). An increased occurrence of E. coli was observed in previous studies indicating the significance of antimicrobial resistance as an alarming situation to children health in developing as well as in developed countries.

CONCLUSION

This study showed that the occurrence of diarrhea is contributed to enteropathogenic E. coli which could be multi-drug resistant, therefore we suggested the empirical antimicrobial therapy to combat the EPEC infections particularly among children to control diarrhea. Further, molecular identification and characterization is also critical to confirm the accurate cause of the infection.

REFERENCES


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