DETECTION OF ESCHERICHIA COLI IN CHILDREN STOOL WITH DIARRHEA PATIENTS USING CULTURE AND POLYMERASE CHAIN REACTION METHOD

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ABSTRACT

Introduction : *Diarrheal diseases are a serious issue on health in developing countries and the liquid cause's morbidity and mortality in children that cause the diarrhea pathogenic bacteria including Escherichia coli. This research aims to detect E. coli in stool diarrhea patient of the child with culture method and Polymerase Chain Reaction.* ***Methods :*** *This research is descriptive research design with cross-sectional approach. The sampling was collected from some public health centers namely Pampang, Barabarayya, Antang Perumnas, Tamangapa public health centers. Specimen testing process was conducted at Microbiology laboratory of the Teaching Hospital University of Hasanuddin to identify E. coli bacterium with the culture and to detect eae gene and bfp with PCR technique.* ***Results :*** *The results indicate that from 50 samples, there are 15 samples (30%) of samples positively detection E. coli using culture method and PCR test using eae and bfp primer found 20 (40%) and 1 sample (2%) positive enteropathogen E. coli.* ***Conclusion :*** *PCR methods indicated the result of E. coli bacteria results faster and more accurate that other culture methods.*

Keywords : Diarrhea, Escherichia coli, Polymerase Chain Reaction

INTRODUCTION

Diarrheal diseases are a serious public health problem in developing countries and are a major cause of morbidity and mortality in children [1]. Diarrheal infections caused the deaths of about 3 million people each year in African children's were stricken with diarrhea in seven times each year than in other developing countries experiencing bouts of diarrhea a few times each year [2].

Diarrhea defined as an increased frequency of defecation (three or more times per day or at least 200 g of stool per day), may be accompanied by nausea, vomiting, abdominal cramping, clinically significant systemic symptoms [3]. Diarrhea is a common symptom of gastrointestinal infection caused by a variety of pathogens, including bacteria, viruses, and parasites. Main pathogenic bacteria including *Escherichia coli*, *Vibrio cholera*, *Shigella* spp, *Campylobacter* and *Salmonella* sp [4]. Yousef M. A *et al* (2006) reported the results of his studies; the 1355 stool specimens studied for the presence of EPEC of child diarrhea samples was detected in 140. i.e. Enteropathogens obtained as much as 111 of *E. coli*, 13 of Shiga Toxin-producing *E. coli*, 9 of *Shigella* and 3 of *Salmonella*, 1 of *Aeromonas* [5].

Polymerase Chain Reaction technique (PCR) is one method used to identify infection disease caused by *E. coli* because it has

advantages compared to conventional diagnostic methods [6].

Molecular detection with the use of a specific *primer attaching and effacing eae* and *bulding forming pili* (*bfp) to detect E. coli* enteropathogenic and differentiate with strains of *E. coli*[7]. Bacterial detection on stool specimens with PCR method has a sensitivity level[8]. PCR method it is more specific, sensitive, detection results faster and more accurate[9]. Based on the description above, it will use the method of molecular methods and culture with PCR technique for detection of *E. coli* bacteria in stool child diarrhea patients.

METHODS

Location and Design of the Research

This research was conducted rectal swab specimens in public health centers in Makassar city namely Pampang, Tamangapa, Antang perumnas, and Barabarayya public health centers. Specimen testing process conducted in laboratory of Microbiology teaching hospital, Hasanuddin University. Type of this research is descriptive research with cross sectional design research to detect *E. coli* bacteria in the feces diarrhea children's with PCR and culture methods.

Populations and Samples

Population and sample the study i.e. all patients suffered from diarrhea visiting clinics eligible research namely in accordance with the criteria of inclusion is willing to participate in this study and is willing to sign an informed concept that has been issued by the Committee of ethics of the Faculty of medicine Hasanuddin University. The samples included in this study were 50 samples. This study was conducted over the period from April – July 2016.

Isolation and identification

Rectal swab sample taken to put in Cary-Blair transport medium and was immediately taken to a laboratory to be tested, the next to medium Brain Heart Infusion Broth (BHIB) and incubated at 37^{O}C for 24 hours, Next the inoculum on the plates that contains *MacConkey* Agar medium was streaked out for discrete colonies with a sterile wire, then incubated for at 37^{O}C for 24 hours. Growing bacteria were isolated and identified by studying morphology and biochemical characteristics, test was done including *Triple Sugar Iron Agar* (TSIA), *Sulfite Indol Motility* (SIM), Urea hydrolysis test, citrate test, MR-VP and Carbohydrate test.

PCR Method

DNA Extraction

The process of DNA sample Extraction by the method of Presto™ DNA kit protocol.

PCR Amplification

PCR mix used to PCR amplification, PCR mix master mix containing green each 12,5 ìl, Primer Forward 0,5 ìl, Primer reverse 0,5 ìl, Nuclease-free water 6,5 ìl, DNA product 5,0 ìl, total volume PCR mix 26,0 ìl using two primers there are *bfp* primer: forward 5'-TTC TTG GTG CTT GCG TGT CTT TT 3' reverse 5'-TTT TGT TTG TTG TAT CTT TGT AA-3' and eae primer: forward 5'-TCA ATG CAG TTC CGT TAT CAG TT-3' reverse 5'-GTA AAG TCC GTT ACC CCA ACC TG -3'. The PCR cycles of *bfp* primer consisted of initial denaturation step at 94^{o}C for 10 minutes followed by 36 cycles of denaturation at 94^{o}C for 1 minute, annealing at 60^{o}C for 45 minutes with an extension at 72^{o}C for 1 minutes followed by final extension at 72^{o}C for 10 minutes, The PCR cycles of *eae* primer consisted of the initial denaturation step at 94^{o}C for 2 minutes followed by 30 cycles of denaturation at 94^{o}C for 15 seconds, annealing at 52^{o}C for 8 minutes with an extension at 72^{o}C for 1 minutes followed by final extension at 72^{o}C for 10 minutes. PCR products were visualized after electrophoresis on 2% agarose gel stained with ethidium bromide.

RESULT

Samples stool diarrhea patients brought to the laboratory of Microbiology for culture test and extraction then amplification with a PCR. Total of patients based on this research is 28 male (56%) and female 22 (44%). Table 1 show the total sample most diarrhea patients aged between 12-35 months 29 people (58%) aged 36 – 59 months 12 people (24%) and while the least amount of the age group 0 – 11 months is 9 people (18%).

The observations of *E. coli* bacteria on a Mac Conkey medium that shows the colonies on the medium has the round of the shape, the edges of the flat, smooth surface, and has pink colored. Bacteria colonies that given the

appearance *E. coli* and then biochemical test is done for growing colonies of confirms that is the isolate of *E. coli*, a total of 15 samples (30%) was detected by using culture method.

Detect used PCR method and DNA amplification positive results characterized by the existence of a band formed in accordance with the target band, after electrophoretically in the gel agarose. Identify by Polymerase Chain Reaction method used *E. coli eae* primer with DNA bands 482 bp (figure 1,2 and 3) and 367 bp for *bfp* (figure 4). Positive DNA amplification Results marked with a band formed in accordance with the target band, after electrophoretically resolved on a 2 % agarose.

The results of the test PCR using *eae* primer on children diarrhea sufferers stool samples obtained 20 samples (40%) positive. *bfp* primer to detect any bacterial enteropathogen *E. coli* strains there is one positive sample (2%).

Sensitivity of PCR method in detecting *E. coli* was measured with positive control dilution method starting from the level of dilution 10^{-0} - 10^{-6} (Figure 5). The results of electrophoresis of DNA positive control dilution visible positive results ranging from the level of dilution 10^{-1} - 10^{-3} mark with the formation of the band corresponding to the target band (482 bp), while the level of dilution 10^{-4} – 10^{-6} does not target DNA fragment bands were formed.

DISCUSSION

Diarrhea is a disease endemic in Indonesia and also a potential unusual disease that is often accompanied by death. Diarrhea is the number one cause of death in infants (31.4%) and in children under five of age (25.2%)[10]. Some of the factors that cause the occurrence of diarrhea that is environmental conditions, contamination of food and drinks, beverages, the supply of clean water is lacking, poverty and low education levels. Children aged under five years of age due to diarrhea affected vulnerable at that age have the staying power of the lower body. Additionally, during a toddler, the child was introduced to a variety of foods and began to actively play [11] .

Based on the results of this research the distribution of diarrhea sufferers according to age in table 1, the number of child diarrhea patients is numerous in the age group 0 – 59 months. The most diarrhea sufferers aged between 12-35 months (58%) aged 36 – 59 months 12 people (24%) and while the least amount in the age group 0 – 11 months 9 people (18%) (table 1).

PCR is one of the methods used to identify molecular diseases caused by *E. coli*, because it has many advantages compared to conventional diagnostic methods[6]. Detection of bacteria in stool specimens with PCR method has a sensitivity level[8]. PCR method it is more specific, sensitive, detection results faster and more accurate[9]. PCR results obtained cannot be known directly so that required the presence of an analysis of the PCR product using an electrophoresis method [12].

Based on the results of 50 samples obtained 15 (30%) of samples positive detection *E. coli* using culture method and PCR using primer *E. coli eae* has a long amplified 482 bp (figure 1,2, and 3) and 367 bp *bfp* primer is formed area of the marker gene of *E. coli* bacteria obtained samples 20 (40%) positive detect by *eae* primer. *bfp* primer to detect any bacterial enteropathogen *E. coli* strains obtained one positive sample (2%) (figure 4). The level of sensitivity of PCR method in detecting *E. coli* begins at 10^{-1} dilution – 10^{-3}, So the results of this study indicate that PCR Method indicated the result of *E. coli* bacteria results faster and more accurate that other culture methods (figure 5).

Table 1. Diarrhea patient distribution by age

No	Age (month)	n	Percentage (%)
1	0 – 11	9	18%
2	12 – 35	29	58%
3	36 – 59	12	24%
	Total	50	100%

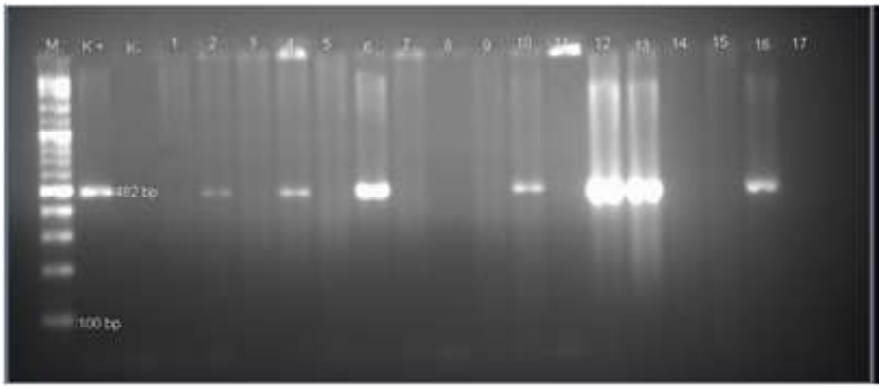

Figure 1. Agorose gel electrophoresis of PCR product sample code of 1-17 amplified with *E. coli* eae gene primer. M = Marker; K+ = positive control K- = negative control

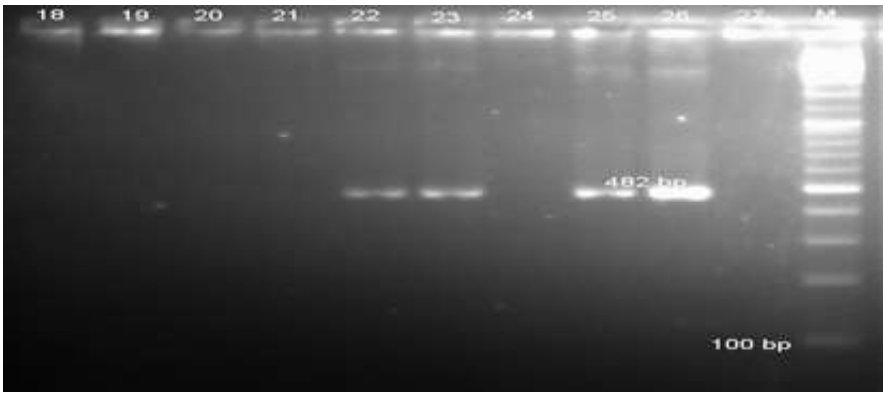

Figure 2. Agorose gel electrophoresis of PCR product sample code 18- 27 amplified with *E. coli* eae gene primer. M = Marker; K+ = positive control K- = negative control

Figure 3. Agorose gel electrophoresis of PCR product sample code of 28- 46 amplified with *E. coli* eae gene primer. M = Marker; K+ = positive control K- = negative control

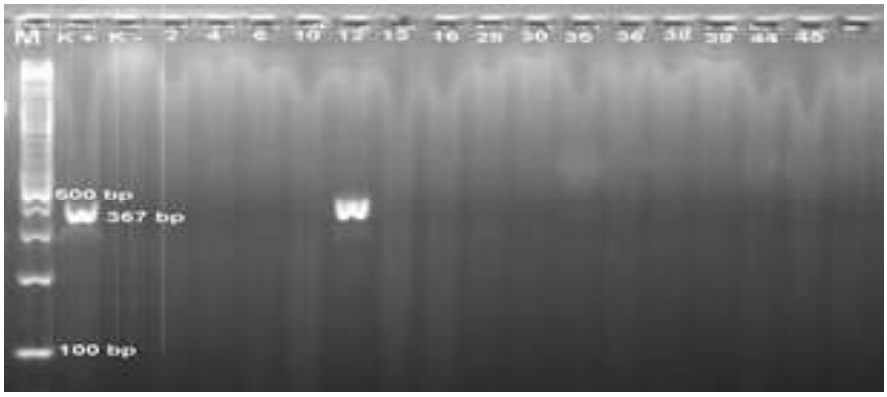

Figure 4. Agorose gel electrophoresis of PCR product sample code of 12 amplified with *E. coli* bfp gene primer. M = Marker; K+ = positive control K- = negative control, Sample; line 12 = Positive sample.

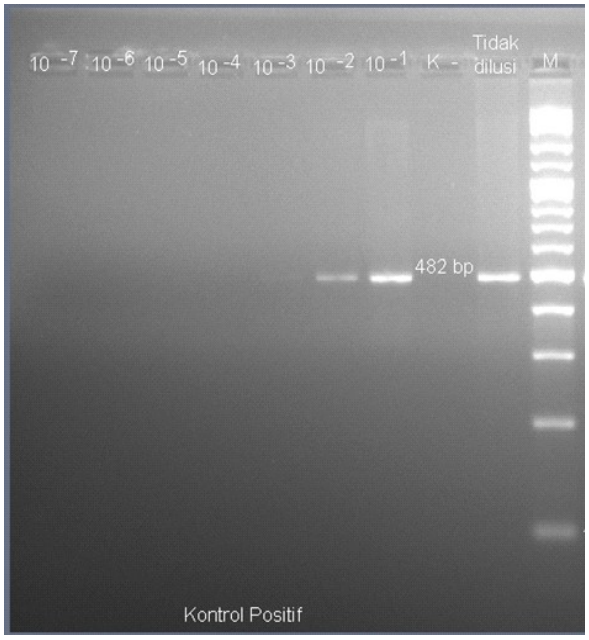

Figure 5. Agorose gel electrophoresis of PCR product amplified positive control *E. coli* on diluted from 10-1, 10-2, 10-3, 10-4,10-5, 10-6

Some of the main pathogenic bacteria cause diarrhea i.e. including E*. coli, Vibrio cholera, Shigella spp, Campylobacter spp.* and *Salmonella sp* [13]. According to Youssef (2006), in this research found some types of bacteria, parasites, and viruses that cause diarrhea in children less than five years in RS Princess Rahma Jordania. Types of bacteria, parasites, and viruses that identification i.e. the following rotavirus (32.5%), enteropathogenic *E. coli* (12.8%), enteroaggregative *E. coli* (10.2), enterotoxigenic *E. coli* (5.7%), *Shigella* spp. (4.9%), *Entamoeba histolytica* (4.9%), *Salmonella* spp. (4.5%), *Campylobacter jejuni*/*coli* (1.5%), *Cryptosporidium* spp. (1.5%), enteroinvasive *E. coli* (1.5%), eae-, Ehly-positive E. coli (0.8%), *Giardia lamblia* (0.8%) and *Yersinia enterocolitic* (0.4%) [5].

Research of Blanco M. *et al.* (2006) at hospital Xeral-Calde from 2015 child diarrhea patients identified 110 enteropathogen strain of *Eschericia coli* that is composed of a enteropathogen strain of *E. coli* (*eae* + *bfp* –) as much as 105 (5.2%) and *E. coli* enteropathogen (*eae* + *bfp* +) as much as 5 (0.2%) [14] from the results of his research i.e. 612 children diarrhea, 412 samples positive *E. coli* culture and biochemical method, the results of the Enteropathogen identification of *E. coli* using *stx*, *eae* and *bfp* primer is not found positive on *stx*, 23 (5.6%) detected positive consists of *bfp* and eae 7 (30.4%) and positive eae (69,6%)[15].

CONCLUSION

From the results it can be concluded that the detection of *E. coli* bacteria in children with diarrhea there are 15 (30%) of samples positively detection *E. coli* using culture method and PCR by using primer *eae* and *bfp* found 20 (40%) and 1 sample (2%) positive enteropathogen *E. coli* by using *bfp* primer. PCR methods indicated the result of *E. coli* bacteria results faster and more accurate that other culture methods. Based on this study it can be suggested that for next research with the number of samples that are more so that can get the maximum results.

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