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RESEARCH ARTICLE

Eae A gene Detection in *E. coli* from Toddler diarrhea cases in Center of Babylon province

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ABSTRACT:

Background: *E. coli* is considered as one of most important normal flora in animal and human gut. It has many serotypes and it could be isolated from feces of healthy individuals and patients. The aim of current study was the molecular genetics detection of presence of the amplified eae A gene fragment (384bp) to confirm presence of the *E. coli*. **Methods**: A total of 36 feces samples were collected from patients with diarrhea at Marjan Medical city from February 2016 till April 2016. Samples were cultured, identified and followed by DNA extraction. Using PCR amplification for diagnosis of *E. coli* bacteria in feces of toddlers with diarrhea by notification of eae A gene fragment (384bp). **Results**: This study showed that eae A gene was found in 26 out of total cases with a rate of infection reaching 81.3%. **Conclusion:** eae A gene is responsible for the virulence in *E. coli* pathogenic bacteria.

KEYWORDS: eae A gene, *E. coli*, Toddler diarrhea, PCR, test sensitivity.

INTRODUCTION:

E. coli is considered as one of most important normal flora in animal and human gut. It has many serotypes and it could be isolated from feces of healthy individuals and patients. Most of them are non-pathogenic, but might be pathogenic due to acquiring some virulent genes[1,2]. Enterobacteriaceae include many bacterial genus with common characters, some of them are Gram-negative bacilli, aerobic or facilitative non-aerobic and animal or human intestine represent their natural habitat [1-3]. Enterobacteriaceae bacteria contain many virulence factors that had the main role in infection mechanism such as hemolysin, urease also as bacteriocins (normal proteins with antimicrobial effect or antagonistic for the growth of other related bacterial types with produced bacteria)[4,5].

Most of bacteria that belong to this family genus had the ability to form biofilms because it contains many factors for this ability like motility, cellulose forming that had the main role in adhesion to non-living surfaces and interaction between this biofilms with these cells[4,5], bacterial colonies bind with surfaces, they present inside extracellular polymer molds showing various phenotyping in growth, genetic expression and protein production; therefore it might cause medical and economic consequences[6].

Most of studies showed the appearance of Enterobacteriaceae resistance strains for more than one antibiotic. This problem is important from the medical view due to the difficulties in controlling this pathogenicity because of inaccurate selection of antibiotic for curing and randomly increased antibiotics use[2,7]. Many researchers pointed out in previous publications, that focused on treatment of bacterial inflammation, that using antibiotics blending process considered important to get synergistic effect to increase antibiotics activity, reduce toxicity, speed-up treatment, block antibiotics resistance and provide wider spectrum than if they were used alone[10,11,13,15]. The latter studies described isolates by using PCR technique and traditional phenotypic diagnosis. Other molecular

techniques like *E .coli* O157:H method which depends on molecular diagnosis of genes that encoded for the virulence factors. Using the eae A gene as an indicator for presence of this bacteria led to credence of this method for detection of pathogenicity and epidemiology of such infections[1,12]. Therefore, the aim of current study was the molecular genetics detection of presence of the amplified eae A gene fragment (384bp) to confirm presence of the *E. coli*.

MATERIALS AND METHODS:

Plastics objects: 10cm petri dishes, 1.5 Eppendroff's tubes, polyethylene tubes, tips, PCR tubes, cotton swabs. Instruments: Eppendroff's centrifuge, freezer, GFL water bath, Memmert incubator, Memmert oven, Mupid agarose gel electrophoresis system, Atta gel documentation system, applied biosystems Thermocycler, Eppendroff's micropipette, Sartorius water Deionizer.

Chemical materials: Brain Heart broth, Nutrient Agar, Macconkey agar. Ethidium Bromide, TBE Buffer, Ethanol, Isopropanol, Genomic DNA Extraction Wiz. of (Promega), Master Mix (Promega), Deionized Distal Water.

Study included 6 stages:

1 Specimen samples taken from infected toddlers with diarrhea.
2 Culturing of these samples on Nutrient agar, Nutrient Agar, MacConkey agar.
3 Transfer bacterial growth from agar to Brain Heart broth.
4 DNA isolation.
5 Amplification by PCR using eae A gene primers.
6 Agarose gel electrophoresis for PCR product of this gene fragments.

All patient samples were collected then cultured and then harvested for DNA isolation as in Promega Protocol Leaflets.

The primers were prepared according to previous publications referred to this gene eae A sequence with a length of 384bp after PCR amplification[8].

PCR conditions were 35 total cycles as follows:

Initial denaturation 95°C for 1min, Denaturation 95°C for 1min, Annealing 65°C for 2min, Extension 72°C 1.5min and Agarose 1% 70V for 60min.

In current study, a total of 36 feces samples were collected from patent with diarrhea at Mirjan Medical city from February 2016 till April 2016. Samples were cultured, identified and followed by DNA extraction. Using PCR amplification for diagnosis of *E. coli* bacteria in feces of toddlers with diarrhea by notification of eae A gene fragment (384bp). The sensitivity of this test was 90%, therefore it was considered reliable, fast, accurate and precise in epidemiological studies for identification of this bacteria. It might be used for bacterial detection directly without need to specific culture media or any biochemical tests.

RESULTS AND DISCUSSION:

The molecular genetics detection for presence of the amplified eae A gene fragment (384bp) to confirm presence of the *E. coli*[5,8,12]. Results of electrophoresis with agarose gel showed the presence of 384 bp to confirm presence of target gene in bacterial DNA; therefore, the results will be considered as *E. coli* (26 positive samples out of a total of 32 samples used in this study).

The primers used in this study were especially prepared depending on previous publications[1,2] that mainly used in detection for *E. coli* that contain the gene eae A which differentiates it from other type of *E. coli* by the capability of virulence factor production. PCR is rapid and reasonable detection for the exotoxin produced by this type of pathogenic *E. coli*[7,10,12]. Primers were specialty and accurately measured by occurring of amplification process for the eae A (384bp) fragment or not. Most of other *E. coli* bacteria do not give a positive result in this test[1,2,5,7,12]. Presence of target eae A gene, that encoded for intimin, was selected by choosing PCR. All samples for this study were taken from suspicious samples[22]. The pathogenic *E. coli* strains ordinarily cause pathogenicity for people that exposed for food contamination[12,23,27], which is the main cause for diarrhea cases in infants in developing countries[24-27].

All of these study results were similar to[5] that eae A gene is responsible for the virulence in *E. coli* pathogenic bacteria.

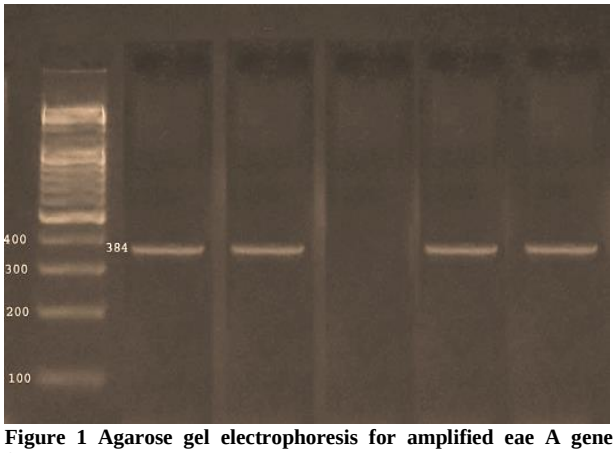

Figure 1 Agarose gel electrophoresis for amplified eae A gene fragment 384bp. Lane 1: ladder. Lane 3: negative result. Lanes 1, 2, 4 and 5 are positive result.

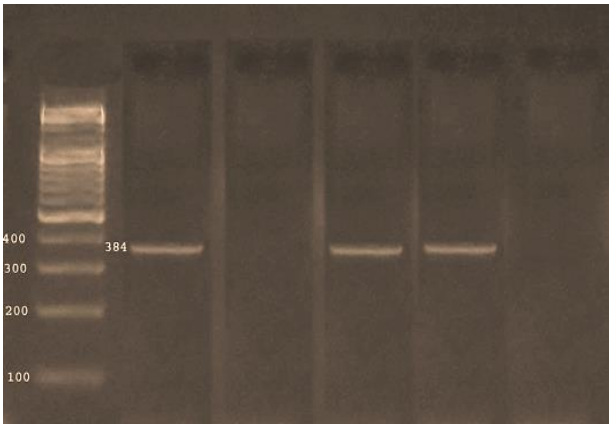

Figure 2 Agarose gel electrophoresis for amplified eae A gene fragment 384bp. Lane 1: ladder. Lanes 2 and 6 are negative results. Lanes 1,4 and 5are positive results.

ETHICAL CLEARANCE:

The research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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