



Detection virulence factors of *E.coli* isolated from diarrhea patients under 2 years

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Abstract:

Diarrhea is one of the most common infectious gastroenteritis diseases in the children under two years old. The present study included 100 diarrhea samples from children patients under two years attending to Al-zahraa teaching hospital for Maternity and Children in Najaf during the period from September 2013 to February 2014. From these 100 samples : 60 samples gave positive stool culture (causes by bacteria) , and 40 samples were negative results (causes by other causative agent such parasitic agent , viral agent , food tolerance and reaction to medicines). Out of 60 samples : 30 isolates of *E.coli* causes diarrhea and 30 other bacteria isolates causes diarrhea.

The study dealt with Identification of *E.coli* from diarrheatic patients by using automated VITEK-2 compact system using Gram Negative-Identification (GN-ID) cards and detect some virulence factors of the *E.coli* such as : hemolysin , proteases, capsule, biofilm production , adhesion. The results explained that all isolates of *E.coli* have polysaccharide capsules , it able to produce hemolysin was 51.6% , also it able to produce proteases enzymes was 35.4% , *E.coli* able to biofilm formation was 74.2% and *E. coli* have able to adhere with epithelial cell was 80.6%.

Key word: diarrhea , *E.coli*, virulence factors of *E.coli*.

Introduction

Diarrhea is abnormal fecal discharge characterized by frequent and /or fluid stool, usually resulting from disease of the small intestine and involving increase fluid and electrolyte loss, leading to the production of unformed or liquid feces [1]. Diarrheal disease are a major cause of childhood morbidity and mortality in the worldwide , especially in developing world including Iraq. It contributes to the death of 4.6- 6 million children annually in Asia, Africa, and America; and 80% of these deaths occur in the first 2 years of life [2].

Deaths of children aged <5 years owing to diarrhea was estimated to be 1.87 million at the global level, which is approximately 19% of total child deaths [3] .

E. coli is gram-negative, facultative anaerobic, rod-shaped, non-spore-forming and belong to the *Enterobacteriaceae* family [4]. *E. coli* strains can cause an impressive variety of different types disease , including septicemia , pneumonia , meningitis , bladder and kidney infections , hemolytic –uremic syndrome (HUS) , diarrhea and dysentery [5]. These *E.coli* are classified by the characteristics of their virulence properties, and each group causes disease by a different mechanism [6]. There are six distinct groups have been defined within gastrointestinal pathogenic *E. coli* commonly associated with intestinal disease: enteroinvasive *E.coli* (EIEC), enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), verotoxigenic *E. coli* (VTEC), enteroaggregative *E. coli* (EAggEC) and Enterohaemorrhagic *E. coli* (EHEC) [7].



Some of the *E. coli* strains have been reported to possess a battery of virulence determinants which enable them to overcome the host's defense mechanisms and produce disease [8]. The production of enterotoxins, haemolysins, colicins, haemagglutinins, proteases, a polysaccharide capsule, colonization factors, cell surface hydrophobicity P-fimbriae, type1 fimbriae and Adhesion are some of the virulence associated factors of *E. coli* [9].

In some strains, the outer membrane of *E. coli* is covered by a polysaccharide capsule composed of K antigens. In other polysaccharides, the M antigens are synthesized under conditions of high osmolarity, low temperature, and low humidity [10]. Bacterial adhesion involves surface interaction between mammalian cell surface (usually carbohydrate), and bacterial surface compound called "ligands" which are protein in nature. The non-specific properties of the bacteria (charge and hydrophobicity) lead to adhesion, and many mechanisms adopted by different bacteria to adhere to mammalian macromolecules or organelles and that needs to overcome repulsion forces between the pathogen and target cell [11]

Materials and Methods:

The present study included 100 diarrhea samples from children patients under two years attending to Al-zahraa teaching hospital in Najaf during the period from September 2013 to February 2014. Out of 100 samples, 60 samples gave positive stool culture (causes by bacteria), and the other 40 samples were considered negative results (causes by other causative agent such as parasitic agent, viral agent, food tolerance and reaction to medicines). 60 Stool samples were collected using plastic containers clean and transported quickly to the department of bacteriology laboratory, and each specimen was inoculated on MacConkey agar for determination bacteria.

E. coli identified with the automated VITEK-2 compact system by using GN-ID cards. This system identifies an organism via a methodology based on the characteristics of the data and knowledge about the organism and reaction being analyzed. This VITEK-2 compact system includes a strip consisting of 47 biochemical tests. Bacteria was suspended in 5 ml normal saline and adjusted to a turbidity of MacFarland No. 0.5. A test tube containing the microorganism suspension was placed into a special rack (cassette) and the identification card is placed in the neighboring slot while inserting the transfer tube into the corresponding suspension tube.

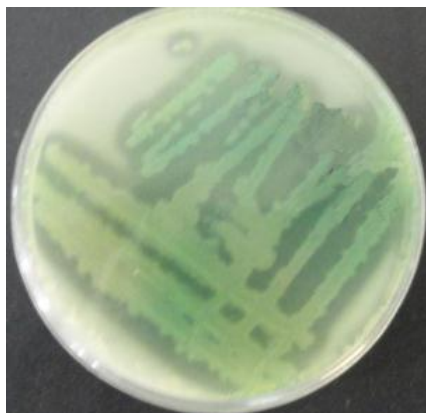
The filled cassette was placed manually into a vacuum chamber station. After the vacuum was applied and air was re-introduced into the station, the organism suspension was forced through the transfer tube into micro-channels that fill all the test wells. Detection of Haemolysin production of *E. coli* according to [12]. The ability of *E. coli* to capsule formation was studied by [13] while Protease production according to [14]. A qualitative assessment of biofilm formation was determined as previously described by [15] and Detection of Adherence activity according to [16].

Results:

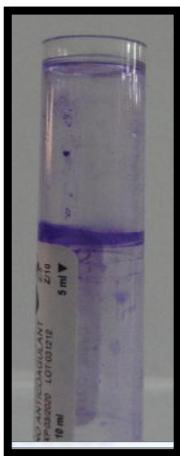
Out of 60 samples: 30 isolates of *E. coli* and 30 isolates of other bacteria such as (*K. pneumoniae*, *Pseudomonas aeruginosa*, *Citrobacter freundii*, *Shigella dysenteriae*, *Salmonella typhimurium*).

38 isolates of *E. coli* diagnosis on MacConkey agar but also diagnosis of isolated *E. coli* were confirmed by Vitek system. 30 isolates gave positive result of *E. coli* by Vitek and 8 isolates gave negative result, some readings gave another species and some isolates did not recognize them by system. Table 1 explained that *E. coli* able to produce hemolysin was 51.6% and 35.4% of *E. coli* able to produce protease enzymes as shown in Figure(1). All isolates had a capsule surrounding

the bacterial cell and study found (74.2 %) of *E.coli* able to biofilm formation as show in figure (2) . The results showed that *E. coli* able to adhere with epithelial cell was 80.6%.



Figure(1): protease formation



Figure(4-15): Biofilm formation

Table (1): The Percentage of Some Virulence Factors of *E. coli*

Virulence Factors	No.(%)
Haemolysin	16(51.6%)
Protease	11(35.4%)
Capsule	31(100%)
Biofilm formation	23 (74.2%)
Adhesion	25 (80.6%)

Discussion

Diarrheal diseases remain the main cause of childhood mortality and morbidity in developing countries. Our results revealed that *E. coli* were the most common causes of diarrhea, therefore we study their virulence factors. The result found 30 isolates gave positive result of *E. coli* by Vitek and 8 isolates negative results, some reading gave another species and some isolates did not recognize them by system, this results similar to other study [17] who diagnostic 70 from 73 isolates of *E. coli* by Vitek 2 system.

The results explained that the capacity of *E. coli* to produce hemolysin on blood agar plate. The hemolysin of these proteins can form pores in biological membranes which occur frequently in gram positive and negative bacteria [18]. Many studies showed the relationship between the bacterial virulence factors and their pathogenicity such as [19] who found that the bacteria which had the ability to produce the hemolysin in some way they were showed increase in its invasion activity and able to resist host immune system. Some studies had reported that hemolysin production is positive among *E. coli* strains were 41% [20]. But another study by [21] who found that out of the total 152 isolates of *E. coli*, only 36(23.7%) were hemolytic on blood agar plate. *E. coli* able to produce many virulence factors this increase their pathogenicity, because of the relationship between the bacterial ability to produce virulence factors and their infectivity. The present study found *E. coli* able to produce protease enzymes was 35.4%. *E. coli* are protecting themselves against the effects of toxic peptides by producing some enzymes such as Extracellular protease plays an important role in the cell survival and cell-cell communication [22]. This result was disagree with [23] who found all *E. coli* able to protease produce. The capsule is considered as one of the most efficient virulence factors of *E. coli* and play role of pathogenic of *E. coli* and it protects this pathogen from the host immunity surveillance (macrophage and serum complement) [24].

In this study, it is shown that 74.2% *E. coli* isolates able to biofilm formation. *E. coli* type 1 fimbriae it has been demonstrated that the FimH adhesin plays a role in biofilm formation by fimbria bacteria on abiotic surfaces [25]. This result disagreed with [26] who found 53% of *E. coli* able to biofilm produce. *E. coli* biofilm initiation and maturation can involve many diverse factors and its ability to form a biofilm depends significantly on environmental circumstances [27]. Biofilm producing bacteria are responsible for many recalcitrant infections and are notoriously



difficult to eradicate [28]. The study has showed that there is 80.6% of *E. coli* able to adherence. This result agreed with [29] who found *E. coli* adhered to epithelial cells were 96% which isolated from the infants with diarrhea. The surface of *E. coli* cells is covered with substances referred to as adhesins which are responsible for the attachment of the cells to the epithelial mucosa [30]. Adhesins are cell-surface components or appendages of bacteria that facilitate [adhesion](#) or [adherence](#) to other cells or to surfaces. Adhesins are a type of [virulence factor](#). It is an essential step in [bacterial pathogenesis](#) or [infection](#), required for [colonizing](#) a new [host](#) [31]. *E. coli* was infecting their hosts by attaching to intestinal epithelial cells (IEC), effacing the epithelial microvilli, and producing pedestal-like structures. The formation of attaching and effacing (A/E) lesions is required for these microbes to cause diarrheal disease [32].

Conclusion: *E. coli* isolates able to produce many virulence factors (biofilm formation and enzymes).

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