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Adherence patterns of diarrheagenic Escherichia coli strains isolated from children with gastrointestinal diarrhea and matched controls in a Hep-2 cell adhesion assay

Zohreh Khodaei[1], Enayatollah Kalantar[2], Mahbobeh Mehrabani[1], Parisa Darabi[3], Afshin Maleki[4]

1 Dietary Supplements and Probiotic Research Center AND Department of Biochemistry, Genetic, and Nutrition, Alborz University of Medical Sciences, Karaj, Iran
2 Department of Microbiology and Immunology, Alborz University of Medical Sciences, Karaj, Iran
3 Dietary Supplements and Probiotic Research Center Alborz University of Medical Sciences, Karaj, Iran
4 Environmental Health Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran

Original Article

Abstract

Escherichia coli is considered as the main cause of epidemic outbreaks and endemic cases of child diarrhea. In the present study, the capacity of a large collection of Escherichia coli (E. coli) strains and matched controls was investigated to determine their adherence pattern on Hep-2 cell lines. A total of 66 pure E. coli strains, which were previously isolated from children aged less than 12 years with diarrhea, were selected for adherence assay. Moreover, 2 E. coli strains, with known adherence patterns, obtained from the Persian Type Culture Collection were used as the control. Hep-2 cell monolayers (50 to 70% confluence) were grown on glass cover slips. Subsequently, 20 µl of bacterial cultures (2 × 10^6 cfu/ml bacteria) was added into each well, and the plates were incubated in a humid atmosphere with 5% CO_2 for 3 hours. Then, culture medium was aspirated from the monolayers, which were washed 4 times with phosphate-buffered saline, fixed in 70% aqueous methanol for 5 minutes, stained with Giemsa stain, and examined using light microscopy. In total, 66 E. coli strains were evaluated for their adherence pattern on Hep-2 cell line. All the isolates adhered to the Hep-2 cells with the exception of 3. The other 63 showed aggregative adherence)AA((34%), localized adherence (LA) (31%), or diffuse adherence (DA) (29%) patterns. The present investigation revealed that this assay is convenient and economical for identification of a large number of diarrheagenic E. coli isolates' adherence to Hep-2 cell lines.

KEYWORDS: Escherichia coli, Adherence Pattern, Cell Line, Children

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Introduction

Today, Escherichia coli is one of the most common causes of diarrhea among children in developing countries. However, precise characterization of diarrheagenic strains can be a difficult task, because strains display great genetic diversity and heterogeneity.[1] As others have reported, Escherichia coli involves an enormous population of bacteria that display a very high degree of both genetic and phenotypic diversity.[2,3] These differences are often demonstrable at the molecular level; however, serotyping and adherence pattern may also be used for classification of Escherichia coli (E. coli) strains.

Adherence has been considered as a preliminary step for the colonization of bacteria on the mucosal surface of host organisms.[4] The adherence pattern of E. coli to Hela or Hep-2 cells is a method for characterization of pathogenic E. coli.

Corresponding Author:
Afshin Maleki
Email: maleki43@yahoo.com

Adherence assays performed with cultured epithelial cells like Hep-2 cell lines show that E. coli strains often express defined patterns like localized adherence (LA), diffuse adherence (DA), aggregative adherence (AA), and etc.; however, non-adherent (NA) E. coli strains have also been reported.[5]

It has also been reported that the Hep-2 cell adherence assay is the gold standard for diagnosing E. coli infection.[6] The pathogenicity of E. coli strains has been demonstrated in childhood diarrhea in developing countries; these strains are currently among the main agents of infectious diarrhea in several countries, including Iran.[7,8] For example, Kalantar et al.[5] in a one year prospective study of 466 children of less than 5 years of age admitted with diarrheal disease to Beassat Hospital, Kurdestan, Iran, demonstrated that enteropathogenic E. coli (EPEC) strains are one of the main groups of enteropathogenic bacteria. Diarrhea remains an important public health problem for children in developing countries. E. coli is an indicator of contaminated water. The present study investigated the capacity of a large collection of E. coli strains and controls, to determine their adherence pattern on Hep-2 cell line.

Materials and Methods

A total of 66 pure E. coli strains, which were previously isolated from children of less than 12 years of age referred to Beasat Hospital, Sanandaj, Iran, with diarrhea, were selected for adherence assay. Bacterial cultures were grown static at 35 °C overnight in Trypticase soy broth. Non-adherent (NA) commensal E. coli obtained from the Persian Type Culture Collection (PTCC 1338) served as a negative control. Moreover, 2 E. coli strains from the bacterial culture collection of Alborz University of Medical Sciences, Iran, with known adherence patterns were used as the controls; (i) LA EPEC strain E2348/69, serotype O127:H6, (ii) AA EAEC strain O42, serotype O44:H18.

Adherence assay

HEp-2 cell monolayers (50 to 70% confluence) were grown on circular 13-mm glass coverslips (Bio Whittaker, Inc., Walkersville, MD, USA). Then, 20 µl of bacterial cultures (2×10^6 cfu/ml bacteria) was added into each well, and the plates were incubated for 3 hours in a humid atmosphere with 5% CO_2. Adherence assay was carried out based on the Haeri et al. procedure.[9] Briefly, culture medium was aspirated from the monolayers, which were washed 4 times with phosphate-buffered saline, fixed in 70% aqueous methanol for 5 minutes, stained with 10% Giemsa stain (Fisher Scientific International, Inc., Pittsburgh, PA, USA), and examined using light microscopy (Zeiss, Germany). Each assay was conducted 3 times in duplicates.

Results and Discussion

Diarrheal infections account for an estimated 12,600 deaths each day among children of less than 5 years of age in Asia, Africa, and Latin America.[10-12]

As others have reported, causes of diarrhea include a wide range of viruses, bacteria, and parasites.[13] However, among the bacterial pathogens, E. coli plays an important role. E. coli strains can be classified according to distinct epidemiological and clinical features, specific virulence factor, and association with certain serotypes and their adherence pattern.[14] The importance of E. coli as an origin of diarrhea and its important role in the prevalence of diarrhea in this area is hardly known. Therefore, the present study was conducted to determine the adherence pattern of different E. coli pathotypes which were isolated from children with diarrhea. This investigation was conducted on children with diarrhea sever enough to require medical attention. In this study, E. coli strains which were isolated from these children were assessed in terms of their ability to adhere to Hep-2 cells.

In total, 66 E. coli strains were evaluated in terms of their adherence pattern on Hep-2 cell line. The isolates which adhered to the

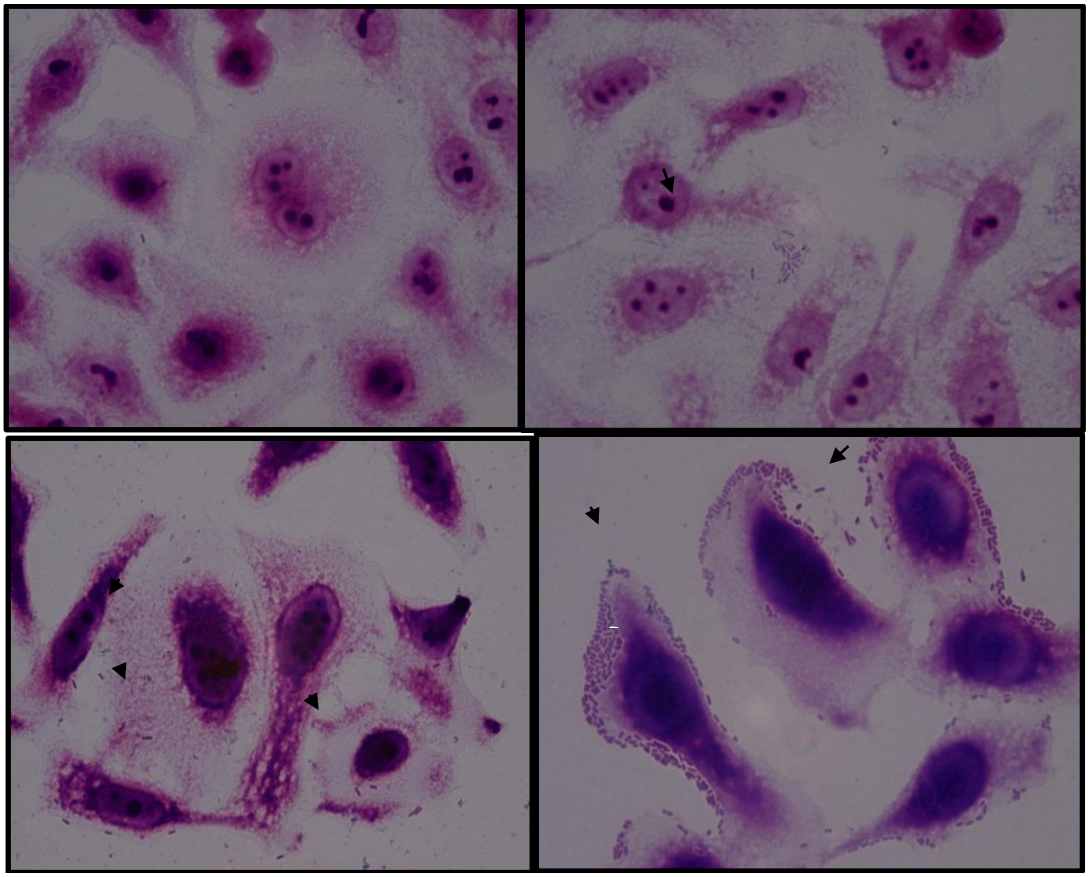

Figure 1. Adherence pattern of four Escherichia coli (E. coli) test strains: A) Non-adherent, B) Localised adherence, C) Diffuse adherence, D) Aggregative adherence, using light microscopy X1000 (arrows show adhered E. coli.)

Hep-2 cells showed different patterns. All the isolates showed at least one kind of adherence on Hep-2 cell line. Figure 1 shows 4 different patterns of adhesion, with A, B, C, and D representing LA, AA, DA, and NA, respectively. Most of the strains showed AA (34%), followed by LA (31%) and DA (29%), adherence patterns (Figure 1).

This finding is almost similar to that of Kalantar et al., who classified E. coli strains based on serological tests.[5] Therefore, the Hep-2 assay may serve as an alternative method of identification of diarrheagenic E. coli strains.

Polotsky et al. compared the Hep-2 cell adherence patterns of E. coli strains isolated from the stools of symptomatic patients with those of five control strains.[15] Isolates displayed 3 adherence patterns including "stacked-brick" AA pattern and LA attaching-and-effacing pattern, which are, respectively, the typical patterns of enteroaggregative E. coli strains and enteropathogenic E. coli strains on Hep-2 cells, and DA pattern. The results of this study are also in agreement with other studies in Iran and neighboring countries.[14,16-18]

Conclusion

The adherence pattern of E. coli to Hep-2 cells is in accordance with serotyping results. On the other hand, this method is a convenient, available, and economical method which is suitable for large collections of bacteria. Isolates from these children displayed different interactive patterns with Hep-2 cells, and thus, further studies on their virulence factors are suggested.

Conflict of Interests

Authors have no conflict of interests.

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