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Attaching activity pattern of diarrhea genic Escherichia coli strains isolated from children with gastrointestinal diarrhea and matched controls in a HEp-2 cell binding assay

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Running title: Attaching activity pattern of diarrhea genic Escherichia coli

Abstract
Among the bacteria, Escherichia coli, are considered the main cause of epidemic outbreaks and endemic cases of children diarrhea.In this work, we investigated the capacity of a large collection of E. coli strainsand matched controls, to determine their adherence pattern on Hep -2 cell lines. A total of 66 pure E. coli strains which were previously isolated from children of less than 12 years old with diarrhea, selected for adherence assay. Two E. coli strains from the Pasture culture collection, with well-established adherence patterns were used as the standard.[5] Hep-2 cell monolayers (50 to 70% confluence) were grown on circular 13-mm glass cover slips (Bio Whittaker, Walkersville, Md.). Twenty micro liters of bacterial cultures (2* 10
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cfu/ml bacteria
was added into each well, and the plates were incubated for 3 hours in a humid, 5% CO2 atmosphere. Adherence assay was carried out based on the modified Nataro et al. procedure. Briefly, culture medium was aspirated from the monolayers, which were washed four times with phosphate-buffered saline, fixed in 70% aqueous methanol for 5 min, stained with 10% Giemsa stain (Fisher, Pittsburgh, Pa.), and examined by light microscopy. Each assay was conducted in duplicate and repeated in three different occasions.[13] In total, 66 E. coli strains were evaluated for their adherence pattern on Hep-2 cell line. All the isolates adherence to the Hep 2 cells with the exception of three. The other 63 showed aggregative (34%), localized (31%) or diffuse (29%) adherence pattern. This investigation revealed a huge number of E. coli isolates to adhere to Hep – 2 cell lines and they are associated with diarrheal disease.
Key words: E. coli, adherence pattern, Hep- 2 cell line, children

Introduction

Escherichia coli is one of the most common causes of diarrhea in children in developing countries.[2] However, accurate characterization of diarrheagenic groups or strains can be a complex task, because strains display great genetic diversity and heterogeneity

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reported Escherichia coli encompasses an enormous population of bacteria that exhibit a very high degree of both genetic and phenotypic diversity
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.[4] [8] These differences are often detectable at
the molecular level;[4] however, serotyping and adherence pattern may also be used for classification of E. coli strains.
Adherence has been considered as a preliminary step for the colonization of bacteria on the mucosal surface of the host organisms
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. The adherence pattern of E. coli to Hela or HEp 2 cells,
is a method for characterization of pathogenic E. coli. Adherence assays performed with cultured epithelial cells like Hep-2 cell lines show that E. coli strains often express defined patterns like localized adherence, diffuse adherence, aggregative adherence etc. however; non-adherent E. coli strains are also reported
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As others also reported that the HEp-2 cell adherence assay remains the gold standard for diagnosing E. coli infection
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. The pathogenicity of E. coli strains has been demonstrated in
childhood diarrhea in developing countries; as others revealed these strains are currently among the main agents of infectious diarrhea in several countries, including Iran
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. For example,
Kalantar et al
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in one year prospective study of 466 children 5 years olds admitted with
diarrheal disease to Beassat Hospital, Sanandaj, Iran, demonstrated that EPEC strains are a major group of enteropathogenic bacteria.[11] In this work, we investigated the capacity of a large collection of E. coli strainsand controls, to determine their adherence pattern on Hep -2 cell line.
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Materials and methods
A total of 66 pure E. coli strains which were previously isolated from children of less than 12 years old with diarrhea, selected for adherence assay. Bacterial cultures were grown static at 35°C overnight in Trypti case soy broth. Non-adherent commensal E. coli obtained from Persian Type Culture Collection (PTCC 1338), served as a negative control. Two E. coli strains from the Alborz University of Medical Sciences bacterial culture collection, with well-established adherence patterns were used as the standard controls:[5] (i) locally adherent (LA) EPEC strain E2348/69, serotype O127:[5] H6, (ii) aggregatively adherent (AA) EAEC strain O42, serotype O44:H18,
HEp-2 cell adherence assay.[0] [1] [2] [3] ...
HEp-2 cell monolayers (50 to 70% confluence) were grown on circular 13-mm glass coverslips (Bio Whittaker, Walkersville, Md.). Twenty micro liters of bacterial cultures (2* 10
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cfu/ml
bacteria was added into each well, and the plates were incubated for 3hours in a humid, 5% CO2 atmosphere. Adherence assay was carried out based on the modified Nataro et al. procedure
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Briefly, culture medium was aspirated from the monolayers, which were washed four times with phosphate-buffered saline, fixed in 70% aqueous methanol for 5 min, stained with 10% Giemsa stain (Fisher, Pittsburgh, Pa.), and examined by light microscopy. Each assay was conducted in duplicate and repeated in three different occasions.
RESULTS AND DISCUSSION
In total, 66 E. coli strains were evaluated for their adherence pattern on Hep-2 cell line. All the isolates adherence to the Hep 2 cells with the exception of three. Figure 1 showed the morphological appearance of the adherent strains with A, B, C, D representing localized adherence (LA), aggregative adherence AA), diffuse adherence (DA) and non adherent (NA)
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strains respectively.[3] [2] Our results revealed various adhesion patterns like diffusely distributed single E. coli; microcolonies; chains; and some strains clumps. Diarrhea remains as an important public health problem for children in developing areas, Iran with no exception.[0] [1] In addition, diarrheal infections account for an estimated 12,600 deaths each day in children under 5 years of age in Asia, Africa, and Latin America
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As others reported, causes of diarrhea include a wide range of viruses, bacteria, and parasites
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However, among the bacterial pathogens, Escherichia coli play an important role. E. coli strains can be categories on the basis of distinct epidemiological and clinical features, specific virulence determinants, and association with certain serotypes and their adherence pattern
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.[6] [9] [0] The
importance of E.[0] [1] coli as a cause of diarrhea and its attributable fraction to the diarrhea prevalence in this region is hardly known.[0] [1] Therefore, the present study was performed to determine the adherence pattern of different E.[0] [1] coli pathotypes which were isolated from children with diarrhea.[0] [1] This study carried out on E. coli strains which were isolated from children visiting hospital with diarrhea and thus involved children with diarrhea sever enough to require medical attention.[0] [1]
In total, 66 E. coli strains were evaluated for their adherence pattern on Hep-2 cell line. All the isolates showed at least one kind of adherence on Hep 2 cell line which is in agreement with other studies from Iran and neighboring countries
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. Most of the strains showed aggregative
(34%), followed by localized (31%) or diffuse (29%) adherence pattern (Fig 1); which is almost similar to Kalantar et al. study; who classified the E. coli strains based on serological tests
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therefore, the HEp-2 assay may serve as another alternative in identifying diarrheagenic

Escherichia coli strains.

CONCLUSION

Surprisingly, no association was observed between adherence patterns on Hep-2 cells and pathotyping strains which were based on previous study; with the exception of some strains. However, this investigation revealed a huge number of E. coli isolates to adhere to Hep – 2 cell lines.

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