

Adherence Patterns of Verotoxigenic *Escherichia coli* (VTEC) Non-O157 Strains Isolated from Faecal Samples in Iran

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ABSTRACT

The adherence property of 70 verotoxigenic *E. coli* (VTEC) strains, not belonging to O157: H7 serotype (non-O157) was checked on HeLa cells adherence assay. These strains were isolated from patients with diarrhea and healthy individuals. The results obtained in this study revealed that localised adherence (LA) was manifested by 3 strains of which two belonged to serogroups O111, O127 and the third one was non-enteropathogenic *E. coli*. The LA pattern was not found among strains isolated from healthy persons ($P < 0.001$). Diffuse adherence (DA) was exhibited by six strains equally distributed among both groups and none of them belonged to enteropathogenic strains. However, aggregative adherence (AA) was manifested by 9 strains and was the most frequent among VTEC strains isolated from diarrheal cases ($P < 0.001$) and was comprised of non-enteropathogenic *E. coli* strains. Non-specific adherence (NSA) was observed in 17 strains. Overall, the results obtained revealed that AA and LA adherence were significantly ($P < 0.001$) associated with strains isolated from patients. *Iran. Biomed. J. 3 (3 & 4): 71-75, 1999*

Keywords: *Escherichia coli*, Verotoxin, Non-O157 verotoxigenic, Adherence, Diarrhea

INTRODUCTION

Verotoxigenic *E. coli* (VTEC) has been recognised as an important cause of diarrheal disease. Although, verotoxin (VT) is thought to be important in pathogenesis, other virulence factors are also considered to be critical [1]. The ability to colonise the human intestine is an essential part of the infection [2]. The property of the adherence of *E. coli* strains to HEp-2 or HeLa cells has also been used to identify *E. coli* associated with diarrhea. Cravioto *et al.* [3] reported that most of the enteropathogenic *E. coli* (EPEC) isolated from outbreaks of diarrheal disease adhered to HEp-2 cells whereas non-EPEC strains rarely adhered. They also noted that the EPEC of most classic O:H serotypes associated with infant diarrhea attaches to HEp-2 cells, although this property was rare among isolates from normal flora.

This observation led to the use of tissue culture-adherence assay to identify diarrheogenic *E. coli*. Three patterns of adherence have been described [4], localised adherence (LA) characterised by the formation of bacterial microcolonies, diffuse

adherence (DA) in which bacteria cover the cell surface uniformly and aggregative adherence (AA) in which clumps of bacteria with a characteristic of stacked brick appearance are found on the surface of HEp-2 cells and on glass slides free from cells. Paton *et al.* [5] have found a correlation between the level of *in vitro* adherence of strains and the capacity to cause severe disease. To determine the importance of the adherence among non-O157 VTEC strains isolated from diarrheal and asymptomatic healthy persons, tissue culture adherence assay (HeLa cells) was employed.

MATERIALS AND METHODS

Bacterial strains. The 70 VTEC strains were isolated from faecal samples of diarrheal and asymptomatic healthy persons isolated from Ilam [6] and Mazandaran provinces (unpublished data). The strains were checked for heat-labile (LT) and heat stable (ST) enterotoxins production by VET-RPLA reversed passive agglutination (Denka Seiken, Japan) and infant mice assay [7] respectively due to

*Corresponding Author; E-mail: mmaslani@institute.pasteur.ac.ir. Abbreviations: VTEC, verotoxigenic *Escherichia coli*; LA, localized adherence; DA, diffuse adherence; AA, aggregative adherence

invasive property they were cultured on Congo red plates [8]. These strains were also tested with EPEC (Sanofi Diagnostics Pasteur, France) and O157: H7 (Difco, Detroit, MI, USA) antisera by slide agglutination method. *E. coli* K12 (non-adherent) and *E. coli* strains showing LA (E2348/69), DA and AA were provided by Dr. J.B. Kaper (Centre for Vaccine development, Maryland, USA).

Verotoxin extraction and detection. The MacConkey agar plate (37°C) was used for implementation of the colony sweep polymyxin-B extraction method [6] and Vero cells were employed for VT detection. The strains VT1 and VT2 were used as positive controls and polymyxin-B solution in PBS served as negative controls. Extracted toxin and controls (50 µl) were inoculated in each well (in duplicate) and incubated at 37°C in a 5% CO₂ atmosphere. From VT-positive plates (MacConkey), up to 10 colonies were isolated and used for further analysis. Control strains of VT1 and VT2 producer were provided by Dr. Y. Takeda (Research Institute International Medical Centre of Japan).

HeLa cell adherence assay. Mannose-resistant adhesion to HeLa cells was tested by the method of Scaletsky *et al.* [9] using HeLa cells (National cell Bank of Iran, Pasteur Institute) grown to 50% on coverslips in Leighton tubes in presence of 1% mannose for 3 h at 37°C in 5% CO₂. The slides were washed three times with phosphate -buffered saline (pH 7.2), fixed with 70% methanol, stained with 10% Gimesa and examined for LA, DA, or AA patterns under light microscopy. Three to five individual isolates from each case were assessed by this method (Fig. 1).

Statistical analysis. The χ^2 test and Fisher's exact test were used for statistical analysis.

RESULTS

Of the 70 VTEC isolates, 16 strains (22.9%) were isolated from diarrheal and 54 strains (77.1%) from healthy persons. None of the strains belonged to O157: H7 serotype but 11 strains belonged to O26, O55, O86, O111, O127, O142 serogroups (Table 1). None of 70 strains tested were found to be LT, ST producer or invasive. Out of 16 strains isolated from patients, 2 strains (12.5%) were non-adherent (NA) and 14 strains (87.5%) were adherent. However, AA phenotype was observed in 7 strains and DA and LA

patterns were exhibited by 3 strains each and remaining one strain showed NSA. Among adherence patterns, LA and AA were found to be significantly associated with diarrhea ($P < 0.001$). However of the 54 strains isolates from healthy persons, 21 (38.9%) were adherent and 33 (61.1%) strains were non-adherent. NSA was shown by 16 strains (29.6%) that DA and AA patterns were manifested by 3 (5.6%) and 2 (3.7%) strains respectively (Table 2).

Table 1: Distribution of different patterns of adherence among EPEC and Non-EPEC serogroups

Serogroups	Adherence pattern					Total
	LA	DA	AA	NSA	NA	
026	-	-	-	-	2	2
055	-	-	-	-	1	1
086	-	-	-	-	1	1
0111	1	-	-	2	-	3
0127	1	-	-	2	-	3
0142	-	-	-	-	1	1
NON-EPEC	1	6	9	14	29	59
TOTAL	3	6	9	17	35	70

Table 2: Distribution of patterns of adherence of VTEC strains isolated from healthy and diarrheal cases

Adherence patterns	Symptoms		Total No (%)
	Diarrhoea No (%)	Healthy persons No (%)	
AA	7 (77.7)	2 (22.2)	9 (12.8)
NA	2 (5.7)	33 (94.3)	35 (50)
NSA	1 (5.9)	16 (94.1)	17 (24.3)
LA	3 (100)	0 (0)	3 (4.3)
DA	3 (50)	3 (50)	6 (8.6)
Total	16 (22.9)	54 (77.1)	70 (100)

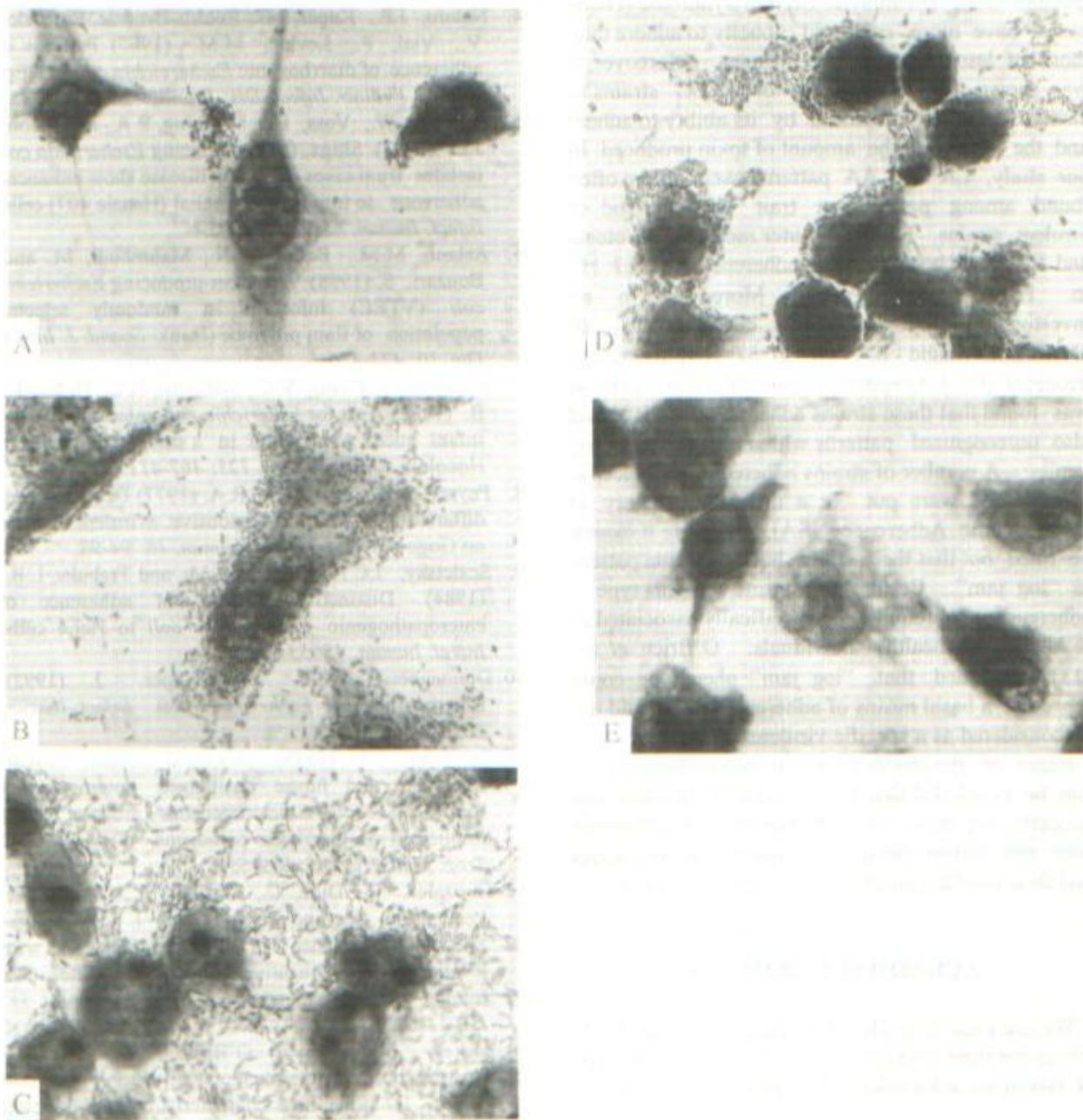


Fig. 1. Different patterns of adherence. **A:** Localised adherence (LA), **B:** Diffuse adherence (DA), **C:** Aggregative adherence (AA), **D:** Non-specific adherence (NSA), **E:** Non-adherence (NA).

DISCUSSION

There are a few studies about adherence of non-O157 VTEC. In the present investigation, the adherence property of non-O157 VTEC strains was examined and the results showed that LA and AA patterns could be of virulence associated factors.

Different studies from various parts of the world

[10-13] also incriminated these patterns as virulence factor. In a study conducted by Paton *et al.* [5], it was concluded that strains producing Verotoxin, isolated from Haemolytic Uremic Syndrome (HUS) cases, have more enhanced capacity to adhere than those of lesser clinical significance. Moreover, it was shown that the capacity of VTEC strains to cause disease is influenced by its ability to adhere

and the type and the amount of toxin produced. In our study, LA and AA patterns were more often found among patients, a trait characteristic of virulent strains. Fimbriae, outer membrane proteins and LPS have been shown in adherence of O157: H7 to HEp-2 cells [14-17]. Moreover, in an investigation on adherence characteristics of enterohemorrhagic *E. coli* O157 [13] to human ileocecal (HCT-8) epithelial cells and HEp-2 cells, it was found that these strains adhered in localised and also unrecognised patterns which they called it "log jam". A number of strains adhered in non-specific manner and were put in a different category of (Non-specific Adherence NSA), therefore it cannot be ruled out that these strains have the same pattern as "log jam". Besides, the strains with this type of adherence were found to be specifically associated to isolates from healthy individuals. O'Brien *et al.* [13] concluded that "log jam" phenotype could represent a basal means of adherence that should not be considered as a specific virulence factor.

Based on the observation obtained in this study, it can be concluded that VTEC strains with adherence property are more virulent than the non-adherence ones and further detail studies on adherence factors and their mechanism of pathogenesis are needed.

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REFERENCES

- O'Brien, A.D. and Holmes, R.K. (1987) Shiga and shiga-like toxin. *Microbiol. Rev.* 51: 206-209.
- Ulshen, H., Rollo, J.L. (1980) Pathogenesis of *Escherichia coli* gastroenteritis in man-another mechanism. *N. Engl. J. Med.* 302: 99-101.
- Cravioto, A., Gross, R.J., Scotland, S. M., Rowe, B. (1979) An adhesive factor found in strains of *Escherichia* belonging to the traditional infantile enteropathogenic serotype. *Curr. Microbiol.* 3: 95-99.
- Nataro, J.P., Kaper, J.B., Robins-Browne, R., Prado, V., Vial, P., Levine, M.M. (1987) Patterns of adherence of diarrhogenic *Escherichia coli* to HEp-2 cells. *Pediatr. Infect. Dis. J.* 6: 829-831.
- Paton, A.W., Voss, E., Manning, P.A., and Paton, J.C. (1997) Shiga toxin producing *Escherichia coli* isolates from cases of human disease show enhanced adherence to intestinal epithelial (Henele 407) cells. *Infect. Immun.* 65: 3799-3805.
- Aslani, M.M., Badami, N., Mahmoudi, M. and Bouzari, S. (1998) Verotoxin-producing *Escherichia coli* (VTEC) infection in randomly selected population of Ilam province (Iran). *Scand. J. Infect. Dis.* 30: 473-476.
- Dean, A.G., Ching, Y.C., Williams, R.G., Hrden, L. B. (1972) Test for *Escherichia coli* enterotoxin using infant mice. Application in a study of diarrhoea in Honolulu. *J. Infect. Dis.* 125: 407-411.
- Payne, S.M., Finkestein, R.A. (1977) Detection and differentiation of iron-responsive avirulent mutants on Gogo red agar. *Infect. Immun.* 18: 94-98.
- Scaletsky, I.C.A., Silva, M.L.M., and Trabulsi, L.R. (1984) Distinctive patterns of adherence of enteropathogenic *Escherichia coli* to HeLa cells. *Infect. Immun.* 45: 534-536.
- Donnenberg, M.S., and Kaper, J. (1992) Enteropathogenic *Escherichia coli*. *Infect. Immun.* 60: 3953-3961.
- Savarino, S.J. (1993) Diarrheal disease: current concepts and future challenges: entero adherent *Escherichia coli*: a heterogeneous group of *E. coli* implicated as diarrhoea pathogens. *Trans. R.Soc. Trop. Med. Hyg.* 87: S49-S53.
- Gonzalez, R., Diaz, C., Marino, M., Cloralt, R., Pequenez, M., and Perez-Schael, I. (1997) Age-Specific Prevalence of *Escherichia coli* with localised and aggregative adherence in Venezuelan infants with acute diarrhoea. *Clin. J. Microbiol.* 35; (5): 1103-1107.
- Mckee, M.L., and O'Brien, A.D. (1995) Investigation of enterohemorrhagic *Escherichia coli* O157: H7 adherence characteristics and invasion potential reveals a new attachment pattern shared by intestinal *E. coli*. *Infect. Immun.* 63: 2070-2074.
- Sherman, P., and Soni, R. (1988) Adherence of Vero cytotoxin-producing *Escherichia coli* of serotype O157: H7 to human epithelial cells in tissue culture: role of outer membranes as bacterial adhesins. *J. Med. Microbiol.* 26: 11-17.
- Zhao, S., Meng, J., Doyle, M.P., Meinersman, R., Wang, G., and Zhao, P. (1996) A low molecular weight outer-membrane protein of *Escherichia coli*. O157: H7 associated with adherence to INT407 cells and chicken caeca. *J. Med. Microbiol.* 45: 90-96.

16. Bilge, S.S., Vary, J.C., Jr. Dowell, S.F., and Tarr, P. I. (1996) Role of the *Escherichia coli* O157: H7 O side chain in adherence and analysis of an rfb locus. *Infect. Immun.* 64: 4795-4801.
17. Cockerill, F., Beebakhee, G., Soni, R., and Sherman, P. (1996) Polysaccharide side chains are not required for attaching and effacing adhesion of *Escherichia coli* O157:H7. *Infect. Immun.* 64: 3196-3200.