Colonization factors in enterotoxigenic Escherichia coli (ETEC) strains in travelers to Mexico, Guatemala and India compared with children in Houston, Texas

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Introduction

- Enterotoxigenic E. coli (ETEC) is the most common bacterial enteropathogen that causes diarrhea in both children <5 years and among adults from developing countries visiting in these regions ("travelers' diarrhea")[1]
- An estimated 200 million diarrheal episodes and 380,000 deaths are attributed to ETEC in the developing world[2,3].
- Travelers' diarrhea is the most common illness reported among people from industrialized regions visiting developing countries occurring in 15% to 40% depending on region ETEC being the most important etiologic agent[4].
- ETEC virulence factors include enterotoxins, both heat-stable (ST) and heat-labile (LT) toxins and colonization factor (CF)[5].
- Antibodies against CFs are thought to be protective against ETEC infection and thus determining the most prevalent CFs in developing countries may help in developing vaccine candidates that are more effective for children and traveler's to these regions[6].
- A challenge to vaccine development is the temporal, regional and population specific variability in ETEC CFs expressed[7].
- The distribution of enterotoxins and CFs from recently identified ETEC strains from diverse regions of the world should be characterized as we develop effective multivalent vaccines to address all prevalent pathotypes in target populations of interest.

Objective

The objective of this study was to describe the distribution of enterotoxins and CF profiles of ETEC isolates collected from stool samples of adult travelers acquiring diarrhea in Mexico, Guatemala and Goa, India. While not directly comparable, we also included a group of children with acute diarrhea in Houston, Texas. All studies took place between 2007 and 2012

Methods

- We collected stool samples from adult travelers to Mexico, Guatemala and India participating in a series of clinical trials carried between 2007 and 2012. To expand our studies of geographic diversity we also include in the study inpatient children with acute watery diarrhea seen at Texas Children Hospital in Houston, Texas.
- Stools from all subjects were cultured on MacConkey agar with identification of ETEC, ST, LT and ST/LT producing strains by published methods[8].
- To reconfirm the LT/ST toxin type and to determine the colonization factor (CF) type of the ETEC isolates, we used multiplex polymerase chain reaction (PCR) to detect structural genes for LT, ST and 10 CFs using the protocol developed by Rodas C. et al[9]
- We calculated two tailed Fisher's exact test for analysis of independence among different strains expressing LT/ST toxins and CFs.

Results

- Among the 252 isolates, 219 (87%) were from adult travelers developing diarrhea in Guatemala, Mexico or India while 33 (13%) isolates were from children admitted with acute watery diarrhea in Houston.
- Among the 252 ETEC isolates, 37 (15%) were LT-only, 145 (58%) produced ST-only enterotoxin and 70 (28%) producing both LT and ST type enterotoxin
- The most prevalent CF produced was CS21 which was expressed in 165 (65%) of the isolates. The other frequently expressed CF was CS6 which was expressed in 63 (25%) of isolates and CS3 which was expressed in 44 (17%) of the ETEC strains tested.

Table 1: Toxin Type and Colonization Factor (CF) Profiles of 252 ETEC Isolates Obtained from Individual Cases of Travelers' Diarrhea of Adults in Developing Regions and Pediatric Cases of Diarrhea in Houston, 2007-2012

Location ETEC n (%)	LT-only	ST-only	LT/ST	CS1	CS2	CS3	CS4	CS5	CS6	CS7	CS17/19	CS21	CFA1
Guatemala & Mexico, n= 146 (57)	21 (15)	81 (55)	43 (29)	20 (14)	19 (13)	20 (14)	0 (0)	1 (0.7)	47 (32)	0 (0)	1 (0.7)	67 (46)	3 (2)
India, n= 73 (29)	11 (15)	41 (56)	21 (29)	5 (7)	3 (4)	24 (33)	3 (4)	0 (0)	14 (19)	0 (0)	0 (0)	70 (96)	0 (0)
USA, n= 33 (13)	4 (12)	23 (70)	6 (18)	0 (0)	0 (0)	0 (0)	1 (3)	0 (0)	2 (6)	0 (0)	0 (0)	28 (85)	0 (0)
Total, n= 252	37 (15)	145 (58)	70 (28)	25 (10)	22 (9)	44 (17)	4 (2)	1 (0.3)	63 (25)	0 (0)	1 (0.3)	165 (65)	3 (1)

Table 2: Distribution of colonization factors and toxin types seen in 252 ETEC isolates obtained from individual cases of travelers' diarrhea in adults and in pediatric cases of diarrhea in Houston, 2007-2012 (CFs found in only 1-3 ETEC strains removed)

Colonization factor (n)	LT-only n = 37 (%)	ST-only n = 145 (%)	LT/ST n = 70 (%)	p- value
CS1 (25)	2 (5)	7 (5)	16 (23)	0.0003
CS2 (22)	1 (3)	7 (5)	12 (20)	0.0009
CS3 (44)	6 (16)	9 (6)	29 (41)	0.0000
CS4 (4)	0 (0)	3 (2)	1 (1)	0.0028
CS6 (63)	7 (19)	31 (21)	25 (36)	0.0554
CS21 (165)	21 (57)	106 (73)	38 (54)	0.0122

Conclusions

- In the present study we identified ST as the most frequent toxin type expressed among the study ETEC isolates from four regions of the world. This is important because ST is poorly antigenic or non-antigenic, shares epitope homology with the human endogenous peptides of the guanylin family, and has yet to be incorporated into a vaccine construct.
- CS21 was the most frequent colonization factor identified among adult travelers (63%) and pediatric cases (85%).
- CS21 has been shown to be important in endemic pediatric diarrhea[10], identified in half of the strains identified. Limited data are available for CS21 and its role in ETEC virulence.
- Apart from CS21, the other two CFs that were more frequently expressed was CS6 (25%) and CS3 (17%).
- In conclusion, findings of common expression of CS21 and CS6 among recent isolates of ETEC causing travelers' diarrhea over a wide geographic distribution supports the need to perform additional studies of CFs and ETEC diarrhea. If our findings are supported by other studies highly conserved CS21 and CS6 should be included in future multivalent ETEC vaccines.

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