Frequency of type 1 fimbriae among *E.coli* subtypes isolated from patients with urinary and gastrointestinal tract infection

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Abstract: The gut constitutes an important reservoir of bacteria causing extra intestinal infections such as urinary tract infection (UTI). According to the fecal–vaginal–urethral hypothesis, *E. coli* strains causing UTI usually derive immediately from the host's own fecal and perineal flora. We assessed multiplex PCR assays to detect type 1 fimbriae among *E.coli* subtypes in children with symptom of urinary tract or gastrointestinal infection. **Material and Methods:** We used multiplex PCR assays that detect enteropathogenic *E. coli* (EPEC) isolates, enteroaggregative *E. coli* (EAEC) isolates, enterotoxigenic *E. coli* (ETEC) isolates, enteroinvasive *E. coli* (EIEC) isolates, and enterohemorrhagic *E. coli* (EHEC) isolates. Also the isolates were examined for type 1 fimbriae. The targets selected for each group were *eae* for EPEC isolates, *aggR* for EAEC isolates, and the genes encoding heat-labile and heat-stable toxins for ETEC isolates, *stx*1 and *stx*2 for EHEC isolates, *invE* for EIEC isolates and *fimH* for detection of type 1 fimbriae. **Results:** In this study more than 80% of *E. coli* isolates from the Urine and rectal swab samples of childrens have the genes for type 1 fimbriae. Among 101 rectal swab specimens tested, 48.5% had *fimH* gene, 2% were EHEC, 3% ETEC and 4% EAEC; we also detected mixed infections, 1% with ETEC and EHEC, 4% EHEC with *fimH* gene, 11.9% ETEC with *fimH* gene, 5.9% EAEC with *fimH* gene, 6.9% EHEC with ETEC with *fimH* gene, 1% EHEC and EAEC with *fimH* gene, 2% ETEC and EAEC with *fimH* gene. EIEC and EPEC were not found among the isolates tested. From 101 urine specimens tested 56.4% had *fimH* gene, 2% were EHEC, 2% ETEC, 3% EAEC; we also detected mixed infections, 1% with EAEC and EHEC, 7.9% EHEC with *fimH* gene, 9.9% ETEC with *fimH* gene and 3% EAEC with *fimH* gene. EIEC and EPEC were not found among the isolates tested. **Discussion:** As our understanding of the molecular aspects and detection of more than 80% *fimH* gene in *E. coli* strains it has been possible to design vaccines that target adaptive responses against specific bacterial proteins such as *FimH* tip adhesin of type 1 fimbriae. The antibodies produced can interfere with the function of essential bacterial virulence factors and can prevent the bacteria from adhering to and invading the host.
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Introduction:

The gut constitutes an important reservoir of bacteria causing extra intestinal infections such as urinary tract infection (UTI). The fecal *Escherichia coli* population structure may influence the occurrence and etiology of extra intestinal infection, but is poorly understood [1]. The structure of the fecal *E. coli* population may be important in the pathogenesis of extra intestinal infections [2]. Diarrhea is one of the leading causes of morbidity and mortality among children under five years in the developing world [3]. Among the bacterial causes diarrhea, diarrheagenic *Escherichia coli* (DEC) is the most important etiologic agent of childhood diarrhea and represents a major public health problem in developing countries [4]. Identification of DEC strains requires that these organisms be differentiated from non-pathogenic members that constitute normal intestinal flora. Molecular identification and classification of DEC is based on the presence of different chromosomal or plasmid-encoded virulence genes, which are absent in the commensal *E. coli* [5]. Five categories of *E. coli* have been well associated with diarrhea in several epidemiological studies [5]: enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), and enterohemorrhagic *E. coli* (EHEC). In addition infections caused by pathogenic *E. coli* are often initiated by binding of the bacteria to the host cell surface via specific bacterial adhesins with fimbriae. Fimbriae or fimbrial adhesins are thread-like structures that reach out from the bacterial surface enabling bacteria to adhere to host cells. Type 1 fimbriae were the adhesin first described in *E. coli* [6]. They are the most common adhesins produced by these bacteria and mediate adherence to mannose-containing glycoprotein found on the surfaces of many eukaryotic cells [7].This study attempted to develop a multiplex PCR for identification of EPEC, EIEC, ETEC, EAEC, EHEC

strains and also detection of *fimH* gene in *E. coli* isolates of rectal swab and urine.

Methods:

Rectal swab specimens from children were collected using wide mouthed sterile plastic containers and transported immediately to the microbiology laboratory for analysis within two hours of collection. Also urine samples had been collected by supra pubic aspiration, catheterization, or use of urine bags.

Bacteriological procedures

All bacterial isolates were microbiologically identified in the microbiology laboratory of CMC (Children's Medical Center) using standard biochemical identification methods [8,9].

DNA Extraction:

To extract DNA a sweep of growth on a nutrient agar slant were boiled in 500 µL of sterile distilled water for 10 minutes. Then centrifugation was done at 13000 rpm for 5 minutes to pellet the cell debris. 1.0 µL of the supernatant was used as template for the PCR amplification. Positive and negative controls were used with each PCR set up.

Detection of virulence factors of diarrheagenic and Uropathogenic E. coli.

Detection of specific virulence genes by polymerase chain reaction (PCR) is frequently used because this method gives rapid, reliable results with a high sensitivity and a high specificity [5, 10]. Having confirmed the specificity of each primer set by single PCR, we combined primers sets and tested the control strains in several PCR cycling protocols. PCR products of the expected sizes was for *eae* 454 base pairs (bp), *stx1* 817 bp, *stx2* 474 bp, *InvE* 382 bp, *aggR* 254 bp, *ST* 166 bp, *lT* 130 bp, and *fimH* 506 bp. The targets selected for each category were *fimH* for type 1 fimbriae, *aggR* for EAEC, *eae* for EPEC, *stx* for EHEC, *LT* and *ST* for ETEC, and *InvE* for EIEC. For each of the target genes, different pairs of primers were selected from the literature [11, 12] (Table 1). PCR was carried out with 1.0 µL of the template added to 24 µL mix containing 10 mM Tris-HCl (pH 8.3); 50 mM KCl; 0.1% Triton X-100; 100 mM MgCl2; 2.5 U of *Taq* DNA polymerase; 10 mM deoxynucleoside triphosphate and each of primers. The PCR program was 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, for 30 cycles, and 72°C for 3 min. PCR products were then electrophoresed on a 2.5% agarose gel, stained with ethidium bromide, and visualized by UV transillumination. The buffer in the electrophoresis chamber and in the agarose gel was 0.5X Tris-borate-EDTA.

Results:

Among 101 rectal swab specimens tested, 48.5% had *fimH* gene, 2% were EHEC, 3% ETEC, 4% EAEC; we also detected mixed infections, 1% with ETEC and EHEC, 4% EHEC with *fimH* gene, 11.9% ETEC with *fimH* gene, 5.9% EAEC with *fimH* gene, 6.9% EHEC and ETEC with *fimH* gene, 1% EHEC and EAEC with *fimH* gene, 2% ETEC and EAEC with *fimH* gene. EIEC and EPEC were not found among the strains tested (Table 2).From 101 urine specimens tested 56.4% had *fimH* gene, 2% were EHEC, 2% ETEC, 3% EAEC; we also detected mixed infections, 1% with EAEC and EHEC, 7.9% EHEC with *fimH* gene, 9.9% ETEC with *fimH* gene and 3% EAEC with *fimH* gene. EIEC and EPEC were not found among the strains tested (Table 2).

Discussion:

Type 1 fimbriae, the single most commonly expressed virulence factor, are produced by more than 80% of all Uropathogenic *E. coli* [13]. Type 1 fimbriae are encoded by the fim gene cluster, fimA-H [14, 15]. In our study type 1 pili like other study, has been shown to be the most commonly expressed virulence factors in UPEC and also DEC [16]. This pili is commonly found among UPEC as well as non-UPEC strains [17, 18]. In confirm of other study our results show more than 80% of *E. coli* isolates from the urine and rectal swab samples had *fimH* gene [13]. More than 90% of *E. coli* isolates from the gut of healthy Swedish adults have the genes for type 1 fimbriae [19]. According to the fecal–vaginal–urethral hypothesis, *E. coli* strains causing UTI usually derive immediately from the host's own fecal and perineal flora. This model, first suggested by serological data [20], is now supported by cross-sectional molecular data [20-24]. The fecal-perineal-urethral hypothesis has been widely recognized to explain the ascending UTIs caused by enteric bacteria [25, 26]. To assess the validity of the hypothesis, we examined the distribution of virulence factors (VFs) of *E. coli* isolates from both urine and rectal swab with urinary tract or gastrointestinal symptom. Almost urinary *E. coli* isolates causing UTIs show the same genes as those discovered predominantly in the rectal swab isolates in each subject. Further, demonstrated that the isolates sharing the same VFs in the urine and stool from each subject were identical genetically, indicating that *E. coli* strains residing in stool serve as a reservoir for UTIs [27,28]. However, diarrheagenic *E. coli* strains possess specific fimbrial gene that enhance their intestinal colonizing ability and allow adherence to the small bowel mucosa, a site that is not normally colonized [29, 30].

Table 1. Multiplex PCR primer sets used to identify the recognized virulence markers, and PCR product sizes used for detection of ETEC, EIEC, EHEC, EPEC and EAEC

Target gene	Sequence (5′ to 3′)	Amplification Size (bp)
Stx1	AGTTAATGTGGTGGCGAA GACTCTTCCATCTGCCG	817
Stx2	TTCGGTATCCTATTCCCG TCTCTGGTCATTGTATTA	474
EaeA	AAACAGGTGAAACTGTTGCC CTCTGCAGATTAACCTCTGC	454
InvE	ATATCTCTTATTTCCAATGCGT GATGGCGAGAAATTATATCCCG	382
AggR	GTATACACAAAAGAAGGAAGC ACAGAATCGTCAGCATCAGC	254
ST	CCCTCAGGATGCTAAACCAG TTAATAGCACCCGGTACAAGC	166
LT	AGCAGGTTTCCCACCGGATCACCA GTGCTCAGATTCTGGGTCTC	130
FimH	TCGAGAACGGATAAGCCGTGG GCAGTCACCTGCCCTCCGGTA	506

Table 2. Frequency of *E. coli* subtypes from rectal swab and urine samples

E. coli subtypes	Number from rectal swab	Number from urine
EHEC	2	2
ETEC	3	2
EAEC	4	3
UPEC	49	57
ETEC + EHEC	1	-
EHEC+ EAEC	-	1
EHEC + *fimH* gene	4	8
ETEC + *fimH* gene	12	10
EAEC + *fimH* gene	6	3
EHEC + ETEC + *fimH* gene	7	4
EHEC + EAEC + *fimH* gene	1	-
ETEC + EAEC + *fimH* gene	2	-
Other	10	11
Total	101	101

ETEC strains cause diarrhea through the action of the enterotoxins LT and ST. These strains may express an LT only, an ST only, or both an LT and an ST and also express *fimH*. The mechanisms by which ETEC strains adhere to and colonize the intestinal mucosa have been the subject of intensive investigation [31, 32]. Also we detected EHEC and EAEC with *fimH* gene. Type 1 fimbriae were suggested to be involved in the adherence of some EHEC strains on the basis of inhibition by growth in mannose [33-35].Certain molecular epidemiological data suggest that some of what traditionally have been regarded as virulence factors in Extracellular pathogenic *E. coli*, particularly for UTI, may also promote intestinal colonization [36-38].Type 1 fimbriae are thought to be responsible for increasing the inflammatory response associated with bacterial adhesion and invasion studies have shown specific receptors on mast cells that bind to the FimH tip to initiate an inflammatory response by releasing inflammatory [13]. Adhesins, such as the FimH produced by most *Enterobacteriaceae* (including uropathogenic *E. coli*), are highly conserved proteins [39]. This lack of major variation is most likely due to the requirement that all pathogenic strains recognize invariant host receptors. Although minor changes in the adhesin protein have been observed (2% divergence) and correlate with decreased or increased affinity for binding to sugars [40], antibodies against a single FimH protein cross-react with >90% of *E. coli* strains expressing the FimH adhesin and block adhering and invading the host [41,42] and can interfere with the function of essential bacterial virulence factors [13]. As our understanding of the molecular aspects and detection of *fimH* gene in *E. coli* strains it has been possible to design vaccines that

target adaptive responses against specific bacterial proteins such as *FimH* tip adhesin of type 1 fimbriae. Furthermore, antibodies against Fim H from a single isolate protect against in vivo colonization by >90% of uropathogenic strains in a murine model for cystitis. This high degree of antigenic conservation is another reason why adhesins may serve as ideal vaccines [43]. Blocking the primary stages of infection, namely bacterial attachment to host cell receptors and colonization, may be the most effective strategy to prevent bacterial infections.

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