Indian J Microbiol (July–Sept 2011) 51(3):355–358
DOI 10.1007/s12088-011-0175-5

ORIGINAL ARTICLE

Distribution of Pathogenic Genes *aatA*, *aap*, *aggR*, among Uropathogenic *Escherichia coli* (UPEC) and Their Linkage with *StbA* Gene

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Received: 26 July 2010 / Accepted: 21 March 2011 / Published online: 3 April 2011

Abstract Urinary tract infection (UTI) with *E. coli* (UPEC) is one of the most common bacterial infections among human beings. In addition to the host predisposing factors, genes are also proposed to have an important role in the occurrence of UTIs. This study investigated the distribution of three pathogenic genes including *aggR*, *aap* and *aatA* among UPEC infected samples and their linkage with *stbA*, the essential gene for maintaining of pAA plasmid. A total of 244 samples were collected from patients with UTIs through clinical laboratories located in western side of Tehran (Iran) during years 2008–2009. *E. coli* isolation was performed according to standard laboratory methods. DNAs were extracted from samples using Boiling method, and the presence of *aap*, *aggR*, *aatA* and *stbA* genes were investigated by PCR. No pathogenic genes (*aap*, *aggR*, *aatA*) were found in 104 out of 244 UPEC samples, while 14 of them were carrying *stbA* gene. Out of 140 UPEC samples with pathogenic genes, 94 (46.6%) were carrying aap gene, 52 (23%) *aggR* gene, and 80 (35.4%) *aatA* gene. A total of 18 samples were also carrying all pathogenic genes together. Moreover, 44 out of 144 samples were carrying *stbA* gene. The results obtained by this study showed that the *aggR*, *aap* and *aatA* pathogenic genes have different existence patterns in different *E. coli* strains that infect different organs. Our study also showed that these three plasmid genes in EAEC strains are able to transpose in the genome and change their level of linkage with pAA plasmid essential gene *stbA*. Meanwhile, this study confirmed that *aggR*, *aap* and *aatA* genes are not specific to only EAEC strains.

Keywords Urinary tract infections · Uropathogenic *Escherichia coli* · Plasmid pAA

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Introduction

Urinary tract infections (UTIs) are among the most prevalent infections in human beings. Surveys conducted around the world have indicated that intestinal bacteria (family Enterobacteriaceae) are the main causes of UTIs, of which *Escherichia coli* is the most common one. To create an infection, fecal bacteria colonize at the entrance of the urinary tracts where they can enter into the internal organs such as bladder and kidney to make infections. Regarding the origin of intestinal bacteria and their moving out of the digestive system due to change in the conditions and horizontal transfer, they undergo some genetic differences [1, 10, 12]. Enteroaggregative *E. coli* (EAEC) is a pathogenic strain in the digestive tract that causes a severe and stable diarrhea [3].

The EAEC pathogenic genes, such as Fembria, enterotoxin, *aggR*, *aap* and *aatA* are mostly located on the 55,989 bp plasmid or pAA. Estimates show that 50–100% of EAEC bacteria carry pAA plasmid. The *aap* gene produces anti-aggregation protein that forms a bacterial capsule with the capacity to prevent bacterial accumulation and helps bacteria to disperse around.

The *aatA* gene produces a membrane protein that is part of *aat*-PABCE transmitter system and is necessary for translocation of pathogenic proteins such as *aap*. The AggR gene also produces a transcription activator for

several pathogenic genes [2, 4–6, 13]. Since pAA plasmid carries many transposable elements, the presence of these genes is not in direct relationship with the presence of the plasmid.

In contrast, *stbA* (Stable plasmid inheritance protein A) gene, another gene located on pAA, has no role in the pathogenesis [2]. This gene produces a protein that is necessary for the low copy number plasmid to be passed to the daughter cells. Therefore, it is presumed that the transferring of plasmid to the daughter cells is directly dependent to the presence of *stbA* gene.

In this study, we examined the distribution of *aap*, *aggR*, *aatA*, and *stbA* genes and their linkage with pAA plasmid in the Iranian patients with UPEC infection.

Materials and Methods

A total of 244 Uropathogenic *Escherichia coli* were collected after culturing the samples obtained from UTI patients with (colony cunt > 100,000 CFU/ml) the highest level of infection on EMB and Blood agar medium. Sample collection was done through medical laboratories located in west of Tehran, Iran. The *E. coli* strains were determined using IMVIC biochemical tests and API kit (API Rapid 20E, Biomerieux, USA) according to manufacturer's instructions.

The total DNAs were extracted from bacteria by boiling method. The Multiplex PCR method introduced by Cerna et al. [2] was used to detect *aggR*, *aap* and *aatA* pathogenic genes. The StbA gene amplified separately using a set of primers designed by GeneRunner software (GenBank Accession no. CU928159).

A full loop of bacterial colony resuspended into 200 µl of distilled water. The suspension was boiled for 5 min and then centrifuged at 14,000 rpm for 5 min to form cell lysate pellet.

A 5-µl aliquot of the supernatant was used as template for PCR amplification. Multiplex-PCR was carried out using thermal cycler (Eppendorf, Germany) in a total volume of 50 µl containing 10 pmol of each three pairs of primers (Table 1), 25 µmol of deoxynucleoside triphosphates, 5 µl of 10× Taq buffer [50 mM KCl, 10 mM Tris–HCl (pH 8.3)], 2 mM $MgCl_2$ and 2.5 U of *Taq* polymerase (Fermentas, USA).

The Multiplex-PCR condition was set up as follows: 5 min at 95°C as initial denaturation; 45 s at 95°C as denaturation phase, 35 s at 57°C as annealing phase, and 45 s at 72°C as extension phase, for 40 cycles; and 5 min at 72°C as final extension. The *stbA* gene was also amplified in a total volume of 25 µl containing 10 pmol of each primers (Table 1), 25 µmol of deoxynucleoside triphosphates, 3 µl of template, 2.5 µl of 10× Taq buffer [50 mM KCl, 10 mM Tris–HCl (pH 8.3)], 1 mM $MgCl_2$ and 2 U of *Taq* polymerase (Fermentas, USA).

The *stbA* PCR condition was set up as follows: initial denaturation for 5 min at 95°C; 40 cycles of denaturation for 45 s at 95°C, annealing for 35 s at 57°C, and extension for 45 min at 72°C; followed by the final extension for 5 min at 72°C. PCR products were separated by gel electrophoresis on a 2% agarose gel. In order to confirm the accuracy of genes amplified in this study, a PCR product of each gene was sent for sequencing to the Macrogen Company (South Korea) and the result was confirmed by NCBI Blast Tool.

Results

The results obtained by amplification of *aggR*, *aap* and *aatA* genes are shown in Fig. 1. The result obtained by amplification of *stbA* gene is also shown in Fig. 2.

The genotypes of all samples are presented in Table 2. Out of 244 UPEC samples, 104 had no pathogenic genes (*aap*, *aggR*, and *aatA*) while 14 of them were carrying *stbA* gene. Of 140 UPEC samples with pathogenic genes, 94 (46.6%) were carrying *aap* gene, 52 (23%) *aggR* gene, and 80 (35.4%) *aatA* gene, with a total of 18 samples carrying

Table 1 Primers used for PCR

Primer concentration (pmol)	PCR product size (bp)	Primer sequence	Gene	Reference
10	310	**F** CTT GGG TAT CAG CCT GAA TG	*aap*	[2]
		R AAC CCA TTC GGT TAG AGC AC		
15	457	**F** CTA ATT GTA CAA TCG ATG TA	*aggR*	[2]
		R AGA GTC CAT CTC TTT GAT AAG		
20	629	**F** CTG GCG AAA GAC TGT ATC AT	*aatA*	[2]
		R CAATGT ATA GAA ATC CGC TGT T		
10	200	**F** CAAACCTGGCTATTGCT	*stbA*	Current study
		R ATCCACTATTACATCATCGAAC		

F forward, **R** reverse

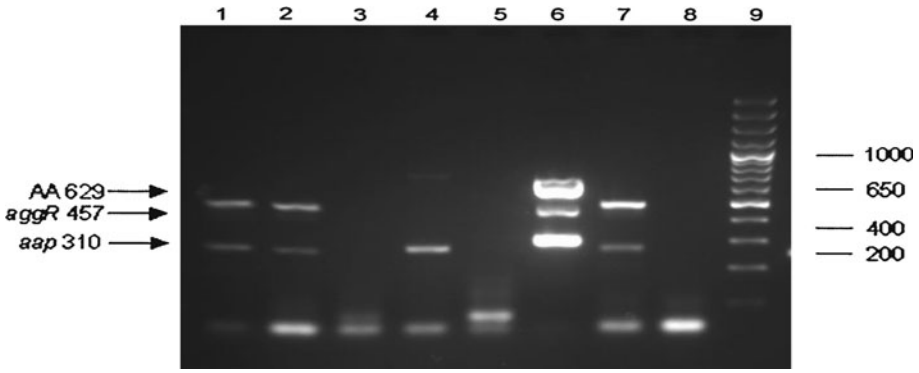

Fig. 1 Gene amplification result of pathogenic genes *aggR*, *aap* and *aatA* run on 2% agarose gel. *Rows 1–7 E. coli* isolated from patients, *row 8* negative control and *row 9* size marker 100 bp

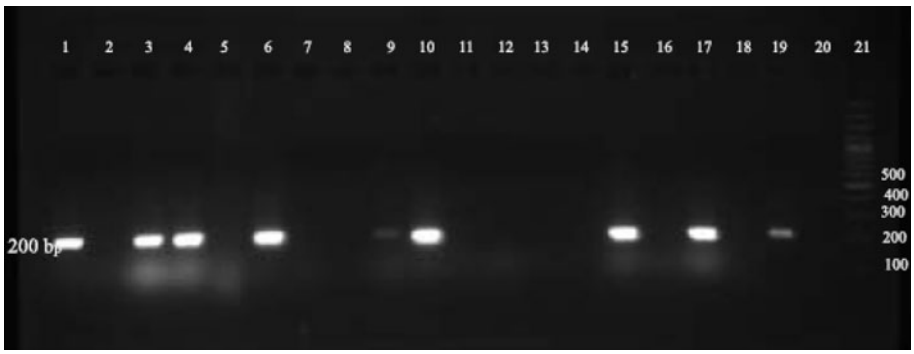

Fig. 2 The *stbA* gene amplification results of UPEC samples on 2% agarose gel. *Rows 1–19* PCR products of some samples, *row 20* negative control and *row 21* 100 bp size marker

Table 2 The results of presence or absence *aggR*, *aap*, *aatA* and *stbA* genes by PCR

UPEC samples $n = 244$	*aatA*	*aap*	*aggR*	*stbA*
104	(−)	(−)	(−)	14/104(+)
26	(−)	(+)	(−)	6/26(+)
18	(−)	(−)	(+)	8/18(+)
28	(+)	(−)	(−)	4/28(+)
16	(−)	(+)	(+)	2/16(+)
34	(+)	(+)	(−)	12/34(+)
18	(+)	(+)	(+)	12/18(+)

all three pathogenic genes together. 44 out of 144 samples were also carrying *stbA* gene (Table 2).

Discussion

Pathogenic Escherichia coli strains including EAEC as the cause of diarrhea and UPEC as the cause of UTI, have the common pathogenic factors including adhesion and Fimbria [1]. In UPEC strains, *kps*, *afa*, *sfa*, *auf*, *pap*, *usp*, *ireA*, *prs*, *aer*, *cnf* and *hly* are the genes which participate in the pathogenicity; while *aap*, *aatA*, *aggc*, *agg3c*, *aggR*, *eae*, *ehly*, *iha*, *irp2*, *lpfA*, *pet*, *pic*, *pils*, *shf*, *astA* genes are those participating in EAEC pathogenicity [5–7, 9]. In the investigation carried out by Abe et al. [1], it was shown that UPEC strain might acquire same properties as EAEC strain, which means some genes can be shared between these two strains. In the study done by Cerna et al. [2] it was determined that 24 (86%) out of 28 EAEC strain were carrying pAA pathogenic genes. 23 samples had all three *aatA*, *aggR*, *aap* plasmid genes, while one sample carried only *aap* gene. Out of 70 genes amplified by PCR, 23 genes (32.8%) were *aggR*, 23 genes (32.8%) were *aatA* (AA), and 24 genes (34.4%) were *aap* [2]. The study done by Gadsden et al. [8] showed that unlike the EAEC strain, UPEC strain has no *aatA*, *aggR*, *aap* genes. Also, Monteiro et al. [11] demonstrated that *aap* gene is not specific for EAEC strain. However, the results obtained by our study showed that 140 (57.4%) out of 244 UPEC samples contained one, two or three *aatA*, *aggR*, *aap* genes. We identified 226 genes by PCR, of which 94 (46.6%), 52 (23%), and 80 (35.4%) were *aap*, *aggR*, and *aatA*, respectively. In our study, the *stbA* stabilizer gene was also amplified by PCR to determine the presence of pAA plasmid. To the best of our knowledge, this study is the first study done in Iranian population.

Interestingly, it seems that the gene existence patterns for *aggR*, *aap* and *aatA* plasmid genes tend to change when the bacterium moves from the intestine to the urinary tract from 86% in the intestine to 57.4% in the urinary tract. In our study, only 44 out of 140 samples with *aggR*, *aap*, and *aatA* genes had *stbA* gene; while one out of 14 samples

with none of *aggR*, *aap* and *aatA* genes had *stbA* gene. These results show that the pathogenic genes can exit from the plasmid and re-locate in another place in the genome, and therefore, greatly decrease their linkage with pAA plasmid. Meanwhile, this study shows that the *aggR*, *aap* and *aatA* pathogenic genes are not specific to the EAEC strain. It seems that despite the numerous transposons on plasmids and bacterial genome and also a decreased level of evolutionary pressure, a logical change occurs in the existence patterns of these genes.

Acknowledgments This study was part of a MSc thesis done in the Islamic Azad University, Tonekabon branch. The authors would like to gratitude all people who helped and participated in sample collection.

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