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Prevalence Of Uropathogenic Specific Protein And Beta Glucuronidase Of UPEC In Relation To Symptomatic UTI And Asymptomatic Bacteriuria.

SM Nachammai*, Karthika Jayakumar, Vinithra Suresh, and M Kousalya

Department of Microbiology, Sri Balaji Vidhyapeeth University, SSSMCRI, Chennai, Tamil Nadu, India.

ABSTRACT

Urinary tract infection is the commonest infection among humans caused majorly by the well known uropathogen Escherichia coli. UPEC possess an array of virulence factors, among the virulence genes the information regarding uropathogenic specific protein coded by usp gene and beta glucuronidase enzyme encoded by uidA gene were less studied. The study was proposed to detect the usp and uidA gene among the UPEC and to analyze is there any significant difference of these genes among UPEC isolated from symptomatic UTI and asymptomatic bacteriuria. DNA was extracted from UPEC once after isolation and PCR amplification was done using specific gene primers. usp gene was found to be statistically significant (p value <0.001) in UPEC from ASB samples (37.5%) whereas in UTI (8.5%). uidA was significant statistically (p value <0.001) in symptomatic UTI samples (88.2%) compared to ASB (3.1%). This study provides added information regarding the infectious potential, housekeeping function of these genes and persistence of UPEC in relation to UTI and ASB. Further animal experimental studies will be helpful in better understanding of the roles of these genes.

Keywords: UTI, ASB, UPEC, usp, uidA

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Corresponding author

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INTRODUCTION

Urinary tract infection are very common among the humans with uncomplicated type in normal structural and functioning of the renal system and with complicated type in case of any abnormalities in the urinary tract. *Escherichia coli* are the well known bacteria among the commensal intestinal flora with mutual relation to the human intestine. As it is the common fecal flora contaminating the perineum and periurethral area, majorly responsible for around 90% of the UTI and 50% of nosocomial infection [1].

UTI are of two types lower and upper UTI classified under symptomatic infection, where urethritis involving the urethral infection, cystitis - bladder infection, pyelonephritis with kidney infection. Colonization and ascension of *E. coli* in any part of the urinary tract as asymptomatic from the source either by fecal *E. coli* which contaminates the periurethral area or the pathogenic *E. coli*. This asymptomatic bacteriuria (ASB) might be transient or causes infection based on the immunity of the host.

The presence of UTI symptoms (frequency, urgency, dysuria, nocturia, lower abdominal pain, fever, flank pain, haematuria etc.,) along with the significant or probable significant culture growth is mostly the characteristic feature of symptomatic UTI. In case of ASB, significant growth of the same bacteria in two consecutive urine samples should be considered. Usually this ASB is not treated as most probably it may be a contamination but still should be notable in case of pregnancy as it leads to severe complications [2].

There are subsets of *E. coli* which possess a range of virulence factors associated with certain serotypes specifically in association with UTI are called as Uropathogenic *Escherichia coli* (UPEC) [3] [4]. Virulence factors like adhesins, toxins, serum resistance factors, enzymes and proteins plays an essential role in UTI pathogenesis against the host interaction. Among the various urovirulence, the prevalence data regarding uropathogenic specific protein and beta glucuronidase enzyme were found less.

Uropathogenic Specific protein:

This protein is coded by the gene usp which encodes for 346 aminoacids and functions like a bacteriocin of nuclease type possessing H-N-H molecule with 38.659 kDa MW. A study in mouse pyelonephritis model done by Zaw MT, mentioned that this usp carries increased infection causing potential in UTI pathogenesis [5], [6].

Bacteriocin is involved in the lysis of cells, in prevention of autolysis the UPEC bacteria co-synthesize an immune protein along with usp bacteriocin and forms a tight complex. These immuno proteins also called as inhibitory protein are predetermined to put off the self killing or suicide of the UPEC itself by expressing bacteriocin and maintains stability similarly even after the release of this bacteriocin [6].

Studies stated that this usp were present in UPEC as well as non-UPEC isolates but predominantly in UPEC and contributes non pathogenic strians to act as infectious causing bacteria [5]. Previous studies states that, UPEC invades the urothelial cells and the intracellular expression of usp gene develops the quiescent state which helps in persistence and survival of the UPEC, thus assigned to be a virulence factor.

Beta-glucuronidase enzyme:

The carbohydrate source were obtained in UPEC through the enzymatic carbohydrate metabolism beta glucuronidase (GUD) coded by the *gene uidA* [7]. In a study by Heninger A 1999 quoted that uidA gene as an identification marker of E. coli [8]. Few studies stated this uidA as one among the urovirulence genes responsible for survival fitness of UPEC and its persistence. The published data's on prevalence of uidA were less among symptomatic UTI and ASB.

This uidA is considered as the 'House Keeping Genes' which is required for maintenance of basic cellular function both in normal condition as well as in pathological conditions [9].

This study has been proposed to note the prevalence of usp and uidA gene in UPEC isolates cultured from symptomatic UTI and ASB urine samples.

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MATERIALS AND METHODS

Bacterial isolates:

A total of 212 UPEC isolated from 924 symptomatic UTI samples and 32 isolates from ASB urine samples from were confirmed by standard biochemical identification.

DNA Extraction:

Boiling lysis method was used to extract the DNA. E. coli isolates were grown in LB broth at 37°C for 18 hours. The broth was then centrifuged and the pellet containing the bacteria were suspended in 200 ml of sterile DNAse free water and incubated for 10 min at 100°C. It was then centrifuged again and the supernatant was used as template DNA and stored at -20°C [10].

PCR amplification:

PCR amplification was carried for usp and uidA gene using specific primers. The amplification of virulence genes was done in a Thermal Cycler (Eppendorf Master) after standardization of PCR conditions as follows:

usp: Initial denaturation: 94°C for 6min 30 cycles of - Denaturation: 94°C for 30s, Anealing: 58°C for 30s, Extension: 73°C for 30s, Final extension: 72°C for 8min, 4°C for 4min.

uidA: Initial denaturation: 95oC for 3min, 35 cycles of - Denaturation: 94°C for 1s, Anealing: 60°C for 50s, Extension: 72°C for 1min, Final extension: 72°C for 5min, 4°C for 4min.

Table: 1 Primer sequences of usp and uidA gene

Gene	Primer sequences (3' to 5')	Base pair size (bp)	Reference
usp	F: GCGTCTGTTGACTGGCAGGTGGTGG R: GTTGCCCGCTTCGAAACCAATGCT	1000 bp	(Aazam, Hassan and Mahbobe, 2012)**44**
uidA	F: ATGCTACTGTTTCCGGGTAGTGTGT R: CATCATGTAGTCGGGGCGTAACAAT	486 bp	(Heninger et al., 1999)45

PCR amplified product were then loaded in agar gel (1%) electrophoresis and the presence specific genes were observed under UV f transilluminator and the base pair size of the amplicons was compared with 100bp DNA ladder added in the same gel.

RESULTS AND DISCUSSION

From the total of 212 E. coli isolates from symptomatic UTI and 32 isolates from ASB, the following are the results of usp and uidA gene.

usp gene were detected in 8.5% and 37.5% of UPEC from UTI and ASB respectively. This gene was found to be statistically significant in UPEC isolated from ASB ($p < 0.001$) compared to UTI.

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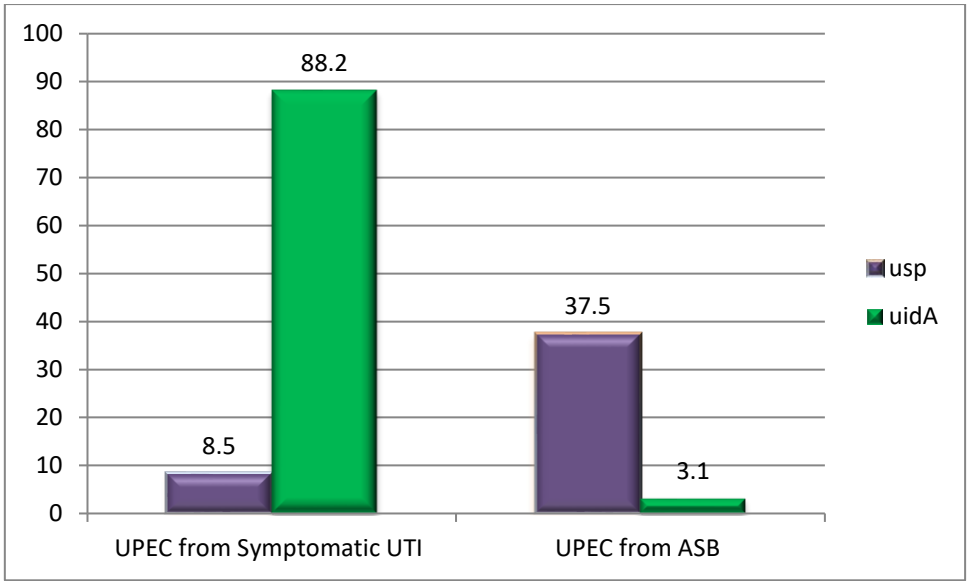

The results from this study suggest that, generally, the uropathogenic specific protein enhances the UPEC persistence in UTI as well as in ASB. The significance of usp gene in UPEC ASB indicated that, this protein converts the bacteria to inactive state and helps in longer survival and adaptation to the urinary tract without causing any symptoms. It might confer pathogenic potential in the host and develops symptomatic infection in future life course of the host under favorable conditions.

uidA gene: 88.2% of UPEC from UTI and 3.1% from ASB possess this uidA gene and the percentage was less in ASB isolates compared to symptomatic UTI.

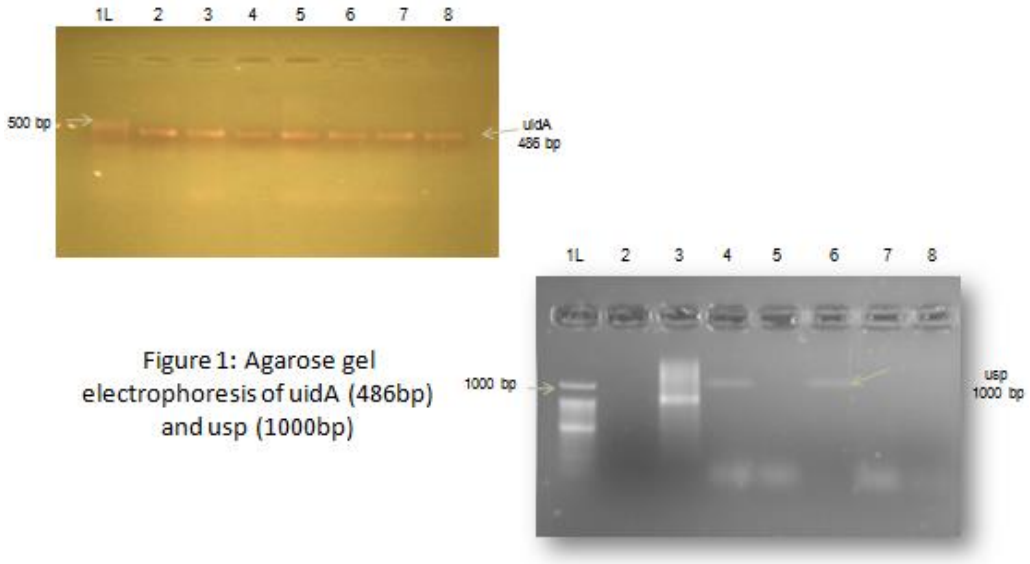

Figure 1: Agarose gel electrophoresis of uidA (486bp) and usp (1000bp)

This gene is statistically significant (p value < 0.001) in UTI representing its central role in bacterial growth, exponential multiplication and infection than ASB. This enzyme focuses mainly to metabolize the carbohydrate and maintains the others genes to be active.

As this uidA is the house keeping gene it may express at constant rates in UTI causing UPEC isolates, where it is found to be higher percent in UTI isolates [12]. It is notable for its stronger enhancement of growth and increased generation to cause infection. Also this uidA apart from metabolic actions keeps the other genes (in UPEC pathogenesis it might be of virulence genes) to be active and under regulation [13].

The decreased percent of uidA in UPEC from ASB points out that, the bacteria just survive with less metabolic actions, in turn persists as quiescent state without causing UTI symptoms. The UPEC with this uidA may turn as a pathogen and those isolates which do not possess uidA may be transient, but further researches are required to understand their property.

CONCLUSION

This study provides added information regarding the prevalence and role of usp and uidA gene from the UPEC and its different ranges in UTI from ASB. Further more reserches and animal experiments help in better understanding the role of uropathogenic specific protein and beta glucuronidase enzyme.

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