

Uropathogenic Specific Protein: Epidemiologic Marker of Uropathogenic *Escherichia coli* as well as Non-specific DNase

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Abstract

Background: Uropathogenic *Escherichia coli* (UPEC) are the most common bacteria causing urinary tract infection (UTI). The putative pathogenicity island (PAI) which contains the gene encoding uropathogenic specific protein (*usp*) and three small open reading frames (*orfU1*, *orfU2* and *orfU3*) encoding OrfU1, OrfU2 and OrfU3 proteins respectively is widely distributed in UPEC strains. This pathogenicity island was designated as PAI*usp*.

Objective: To provide an overview of previous studies on uropathogenic specific protein (Usp) and to recommend future studies on Usp.

Methods: We performed the detailed review of the previous reports on Usp with special emphasis on structural diversity and molecular epidemiology of PAI*usp*, role of Usp in urovirulence and molecular activity of Usp.

Results: As *usp* gene is frequently detected in *E. coli* strains isolated from UTI and enhances the infectivity of non-pathogenic host *E. coli* strains in mouse pyelonephritis model, Usp is thought to be an important virulence factor. In addition, Usp is thought to have bacteriocin activity because Usp shares homology with nuclease-type bacteriocins. PAI*usp* can be divided into four subtypes based on its sequence and structural diversity. A recent study has demonstrated that Usp is a non-specific DNase belonging to H-N-H nuclease superfamily.

Conclusion: Although there are many questions regarding Usp, it has been revealed that *usp* gene is widely distributed in UPEC strains and PAI*usp* subtyping is a useful method to characterize *usp*-positive UPEC strains epidemiologically. Moreover, Usp has non-specific DNase activity. Future studies should investigate the expression of Usp in UPEC during UTI.

Keywords: uropathogenic specific protein, uropathogenic *Escherichia coli*, urinary tract infection, epidemiologic marker, DNase

Introduction

Urinary tract infections (UTI) are one of the most common infections in human.¹ Uropathogenic *Escherichia coli* (UPEC) are the most common bacteria causing uncomplicated UTIs.² UPEC possesses a diverse array of virulence and fitness factors. Adherence factors such as type 1, P, S fimbriae and Dr family adhesins help the UPEC to attach to uroepithelium and establish infection.³ The UPEC strains also possess iron uptake systems which enable it to survive under iron limiting host environments.⁴ UPEC also produces toxins such as alpha-hemolysin and cytotoxic necrotizing factor 1 which can inflict direct damage on the urinary tract tissues.^{5,6} This review article focuses on the gene encoding uropathogenic specific protein (*usp*) which is widely distributed in UPEC strains.

The 1038 bp open reading frame (ORF) encoding 346 amino acid protein was discovered in UPEC strain Z42 isolated from a prostatitis patient when searching for homologues of zonula occludens toxin among UPEC strains.⁷ The zonula occludens toxin is produced by *Vibrio cholerae* and was demonstrated to act on zonula occludens, a component of tight junction between small intestinal mucosal cells, thereby increasing the intestinal permeability.⁸ Because this ORF was frequently associated with UPEC strains, it was thought to encode the previously unknown virulence factor of UPEC and designated as *uropathogenic specific protein gene (usp)*.

The *usp* gene is located on previously unknown pathogenicity island of UPEC

The *usp* gene is predicted to code for 346 amino acid protein designated as uropathogenic specific protein (Usp). The predicted molecular weight of Usp is 38.659 kDa. The downstream region of *usp* gene is occupied by 3 small ORFs (*orfU1*, *orfU2*, *orfU3*) putatively encoding 98, 97 and 96 amino acid proteins designated as OrfU1, OrfU2 and OrfU3 respectively.⁷ The *orfU1-3* are transcribed in the same direction as the *usp* gene. Comparison of the DNA fragment containing *usp* gene and *orfU1-3* from UPEC strain Z42 with whole genome sequence of *E. coli* strain K12 showed that the 4167 bp fragment carrying *usp* gene and *orfU1-3* occupies the 270 bp intergenic region between *aroP* and *pdhR* genes in *E. coli* strain K12.⁷ The intergenic region between *aroP* and *usp* contains the sequence characteristic of the Tn3 family transposon and the direct repeats of 4 base pairs are present on each side of this fragment, which is similar to the target size duplication at the insertion sequence insertion site.⁹ In addition, the G+C content of this DNA fragment is different from that of surrounding region. These features suggest that this DNA fragment is a horizontally acquired transposon-like element⁹ and together with the frequent association of the *usp* gene with UPEC strains, imply that this DNA fragment could be the previously unrecognized pathogenicity island (PAI) of UPEC.⁷ This pathogenicity island is distinct from the pathogenicity islands previously reported in UPEC strains and was designated as PAI_{usp}.

Structural and sequence diversity of PAI_{usp}

Nakano *et al.* performed the sequencing analysis of PAI_{usp} in seven representative *usp* gene-

positive *E. coli* strains (one from cystitis case, four from prostatitis case, one from pyelonephritis and one from stool).⁹ It was found that *usp* gene has DNA sequence heterogeneity starting from 230 bp before 3' end. Based on this sequence variation *usp* gene can be classified into 2 types - *uspI* and *uspII*. The sequence variation in 3' end of *uspI* and *uspII* gene results in 26 differences in amino acid sequences (Figure 1). In addition, structural diversity also exists in region downstream of *usp* gene containing *orfUs*. The number of *orfUs* and position of *orfU* in relation to each other differ from strain to strain. Interestingly, PA*usp* in *E. coli* strains isolated from UTI cases has two or three *orfUs* whereas PA*usp* in fecal *E. coli* strain has only one *orfU*.⁹

Prevalence of *usp* gene in extraintestinal pathogenic *E. coli* strains including uropathogenic *E. coli* strains

The *usp* gene is designated as such because it is more frequently associated with UPEC strains than fecal *E. coli* strains.⁷ When 378 UPEC strains and 50 *E. coli* strains from stools of the healthy individuals were examined with colony hybridization test using the *usp* gene-specific DNA probe, most of the UPEC strains (84%) was positive for *usp* gene whereas only 24% of the fecal *E. coli* strains hybridized with the probe. When the prevalence of *usp* gene in the *E. coli* isolates from urine and feces of companion animals (dogs and cats) was examined, the pattern of distribution of *usp* gene in urinary and fecal *E. coli* isolates of these animals is same as that observed in human *E. coli* isolates.¹¹ UPEC strains are associated with limited number of O serotypes such as O1, O2, O4, O6, O16, O18, O22, O25 and O75. The presence of *usp* gene is significantly associated with all common serotypes of UPEC.¹²

As mentioned earlier, *usp* gene can be divided into 2 variants – *uspI* and *uspII* having sequence variation in 3' end. Depending on the *usp* variants and sequential arrangement of *orfUs*, PA*usp* can be classified into 4 subtypes (Figure 2).¹³ When PA*usp* in *usp*-positive UPEC strains isolated in Japan was subtyped using a polymerase chain reaction (PCR) method, type IIa was found to be most common (42.4%) followed by type Ia, type Ib and type IIb (Table 1). It was also revealed that 96.6% of *E. coli* strains belonging to phylogenetic group B2 possess *usp* gene and 94.9% of *usp*-positive *E. coli* strains belong to phylogenetic group B2. However, the prevalence of *usp* gene is comparatively low in *E. coli* strains of other phylogenetic group: 11.4% in phylogenetic group A, 13.8% in phylogenetic group B1 and 24.3% in phylogenetic group D.¹³ Extraintestinal pathogenic *Escherichia coli* (ExPEC) strains including *E. coli* strains isolated from UTI were reported to be frequently associated with phylogenetic group B2.^{14,15,16,17,18}

Recently *E. coli* strains belonging to sequence type 131 (as determined by multilocus sequence typing) has emerged as the globally disseminated cause of multi-drug resistant extraintestinal infections including UTIs. Interestingly, it was found that *usp* gene was almost always detected in *E. coli* strains of sequence type 131.^{19, 20, 21, 22}

Despite the high prevalence of *usp* gene in UPEC strains, the *usp* gene can also be detected in *E. coli* strains isolated from patients with sepsis and bacteremia.^{23, 24} However, the prevalence of *usp* gene is significantly lower in these *E. coli* isolates than in urinary *E. coli* isolates. A study conducted in Sweden monitored 130 healthy infants during the first year of life with regular stool

culture and examined the *E. coli* isolated from stool for the presence of *usp* gene. It was revealed that the carriage of *usp* gene is significantly associated with the *E. coli* strains persisting in intestinal microbiota for more than one year.²⁵ These studies suggest that Usp may also have role in pathogenesis of *E. coli* infection outside urinary tract.

Virulence of *usp* gene in vivo

The virulence of *usp* gene was examined in vivo using mouse pyelonephritis model. The plasmid vector carrying *usp* gene was found to enhance the infectivity of host *E. coli* cells in mouse pyelonephritis model whereas the vector containing *orfU1-3* and the vector lacking both *usp* gene and *orfU1-3* did not exhibit this effect, suggesting that Usp may contribute to the causation of UTI and could be an important virulent determinant of UPEC.¹²

Homology with nuclease-type bacteriocins

Sequence homology analysis has revealed that Usp shares homology with nuclease-type bacteriocins such as colicin E9 and pyocin AP41. In addition, OrfUs proteins encoded by 3 small ORFs downstream of *usp* gene have homology with immunity proteins for these bacteriocins.¹⁰ Based on sequence homology, it is thought that Usp is a nuclease-type bacteriocin and OrfU proteins are immunity proteins for Usp. Bacteriocins are defined as bactericidal peptides or proteins produced by bacteria and are different from traditional antibiotics in that bacteriocins are active against the bacteria closely related to the producing strain.²⁶ Nuclease-type bacteriocins kill the target bacterial cells by degradation of their nucleic acid. Nuclease-type bacteriocins are produced together with immunity proteins, which inhibit the bactericidal activity of bacteriocin preventing the bacteriocin producing cell from killing by its own bacteriocin.^{27, 28} As there are more than one *orfU* downstream of *usp* gene, it was speculated that the *orfU* immediately downstream of *usp* gene protects the Usp producing cell from bacteriocin activity of Usp and additional *orfUs* are responsible for conferring immunity towards other bacteriocins and provide advantage when competing with other strains.¹⁰ As the result, the strains possessing 3 *orfUs* are resistant to more bacteriocins and more prevalent than those possessing 2 *orfUs*. In agreement with this speculation, it has been reported that UPEC strains containing three *orfUs* are more common than those containing two *orfUs*.¹³ Moreover, *orfU1* lies immediately downstream of *uspI* while *orfU2* lies immediately downstream of *uspII*, suggesting that OrfU1 confer immunity towards UspI and OrfU2 is responsible for immunity against UspII.¹⁰ Amino acid sequence variation in 3' end of UspI and UspII determines which *orfU* should be immediately downstream.

Usp is a non-specific nuclease belonging to H-N-H nuclease superfamily

Zaw *et al.* overexpressed Usp in *E. coli*, purified it and characterized its activity. Initial attempt to overexpress Usp in *E. coli* was unsuccessful because the pET vector encoding Usp could not

be maintained in *E. coli* BL21(DE3) due to cellular toxicity of Usp.²⁹ As mentioned earlier, it is thought that Usp is a nuclease-type bacteriocin and OrfU proteins are immunity proteins for Usp based on sequence homology.⁹ When recombinant colicins were overexpressed in *E. coli*, their cognate immunity proteins were expressed together with colicins to protect the host *E. coli* cell from cellular toxicity of overexpressed colicin.^{30,31} Similarly, the pET vector encoding Usp together with OrfU allowed the overexpression of Usp in *E. coli* BL21(DE3), suggesting that OrfU could mask the cellular toxicity of Usp.²⁹ In addition, co-purification of Usp and OrfU1 was possible, indicating that there was tight complex formation between Usp and OrfU1. To purify Usp, Usp complexed with 6xhistidine tagged OrfU1 (OrfU1-His) was bound to Ni²⁺-chelating agarose and Usp was separated from OrfU1-His bound to the agarose using denaturing reagent.²⁹

Purified Usp was found to have DNase activity and the DNase activity of Usp/OrfU1 complex was significantly lower than that of Usp suggesting that OrfU1 could reduce the DNase activity of Usp (Figure 3).²⁹ The C terminal region of Usp contains the sequence homologous with H-N-H motif.¹⁰ The H-N-H motif is the 30-33 amino acids consensus sequence containing two pairs of conserved histidines surrounding a conserved asparagine and found in various nucleases including E-group DNase colicins such as colicin E7³², and E9³³ and homing endonucleases³⁴. Site-directed mutagenesis of the conserved residues in H-N-H motif in Usp showed that H-N-H motif was important for DNase activity of Usp, indicating that Usp is a member of H-N-H nuclease superfamily.²⁹

Future recommendations

Although the *usp* gene was discovered more than ten years ago, there are many questions that need to be answered. Usp shares homology with nuclease-type bacteriocins. However, there has been no report demonstrating the bacteriocin activity of Usp. Zaw *et al.* demonstrated that Usp is a protein having DNase activity whose expression is toxic to *E. coli* host cell and is not possible unless the OrfU proteins are expressed together.²⁹ Future study on *usp* should investigate the expression of Usp in UPEC strains. It can be suggested that the expression of Usp in UPEC strain is tightly regulated and will be elusive to detect. As the *usp* gene is predicted to encode a virulence factor of UPEC, it would be interesting to see if UPEC express Usp during the course of UTI, which could be investigated using experimental animal UTI model. Another way of detecting the expression of Usp in UTI would be to develop an ELISA system based on purified Usp and screen the blood of UTI patients for presence of specific antibodies against Usp. Previous studies have demonstrated that UPEC can multiply or persist in quiescent state after invading the uroepithelial cells,³⁵ so it is interesting whether Usp is expressed while UPEC exist intracellularly.

Zaw *et al.* characterized the activity of UspI from UPEC strain Z42. However, *uspII* gene is more common than *uspI* gene suggesting that strains possessing *uspII* has competitive advantage compared with those possessing *uspI* gene. So the activity of UspII needs to be characterized.

Kurazono *et al.* reported that the amino acid sequence of Usp contains a putative cleavage site for signal peptidase between serine 24 residues and alanine 25 residues and therefore is a

secreted protein.⁷ However, experimental evidence that Usp is secreted into the extracellular medium is lacking.

In nucleotide sequence databases, ORFs of differing lengths from various UPEC strains such as UTI89 and 536 are annotated as *usp* gene. For example, a 1782 bp ORF encoding 593 amino acid protein in complete genome sequence of UPEC strain UTI89 (Accession number CP000243) is annotated as *usp* gene although this ORF starts with the codon coding for amino acid other than methionine. Parret and De Mot also anticipated that Usp may be a 600 amino acid protein containing N terminal extension homologous with hemolysin coregulated pilus produced by *Vibrio cholerae*.¹⁰ Future study should seek to purify native Usp from wild-type UPEC strains to clarify whether Usp is 346 amino acid protein as described by Kurazono *et al.* or 600 amino acid protein as anticipated by Parret and De Mot.

List of abbreviations

UTI: Urinary tract infection; UPEC: Uropathogenic *Escherichia coli*; Usp: Uropathogenic specific protein; PAI: Pathogenicity island; ORF: Open reading frame; PCR: Polymerase chain reaction.

Conflict of Interest: The authors have no conflict of interest to declare.

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Table 1: Prevalence of PAI*usp* subtypes in urinary *E. coli* isolates¹³

Presence or absence of <i>usp</i> genes/ PAI <i>usp</i> subtypes	Number of UPEC isolates (%)
Number of <i>usp</i> -positive UPEC isolates	320 (84.9)
Type Ia	107 (28.4)
Type Ib	37 (9.8)
Type IIa	160 (42.4)
Type IIb	10 (2.7)
Non-typed	6 (1.6)
Number of <i>usp</i> -negative UPEC isolates	57 (15.1)

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uspI  M L L F P S S V L A T Q I W G K W S L S G V L S A T R G S Y I G
uspII . . . . . G . . . . . A . . . . . . . . . . I . . . . . . . . . .
uspI  A L A S A L Y I P S A G E G S A R V P G R D E F W Y E E E L R Q
uspII . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
uspI  K A L A G S T A T T R V R F F W G T D I H G K P Q V Y G V H T G
uspII . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
uspI  E G T P Y E N V R V A N M Q W N E Q T Q R Y E F T P A H D V D G
uspII . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
uspI  P L I T W T P E N P E H G N V P G H T G N D R P P L D Q P T I L
uspII . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
uspI  V T P I P D G T D T Y T T P P F P V P D P K E F N D Y I L V F P
uspII . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
uspI  A G S G I K P I Y V Y L K E D P R K L P G V V T G R G V P L S P
uspII . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
uspI  G T R W L D M S V S N N G N G A P I P A H I A D K L R G R E F K
uspII . . . . . . . . . . I . . . . . . . . . . . . . . . . . . . . . .
uspI  T F D E F R E A L W L E V S K D S V L L A Q F I K S N Q N N V S
uspII . . . . . . . . . . . . . . . . . . . . . . Q . P E . I . . . . S S G . . T R I K
uspI  Q G Y S P Y V P E E G Y Y Y G P N E I V K K F Q I H H V V A I E
uspII . . L T A K A . I D . R H . . . . K D . . . . . . . . . . R . . . .
uspI  H G G G V Y D I D N F R I V T P R L H D E I H Y R R *
uspII Y . . S . . . . . L . . . . . . . . . . . . . . . . . . . . . *

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Figure 1: Amino acid sequence variations in *uspI* and *uspII*. UPEC strain Z42 is the representative UPEC strain possessing *uspI* gene and UPEC P17 is the representative UPEC strain possessing *uspII* gene.

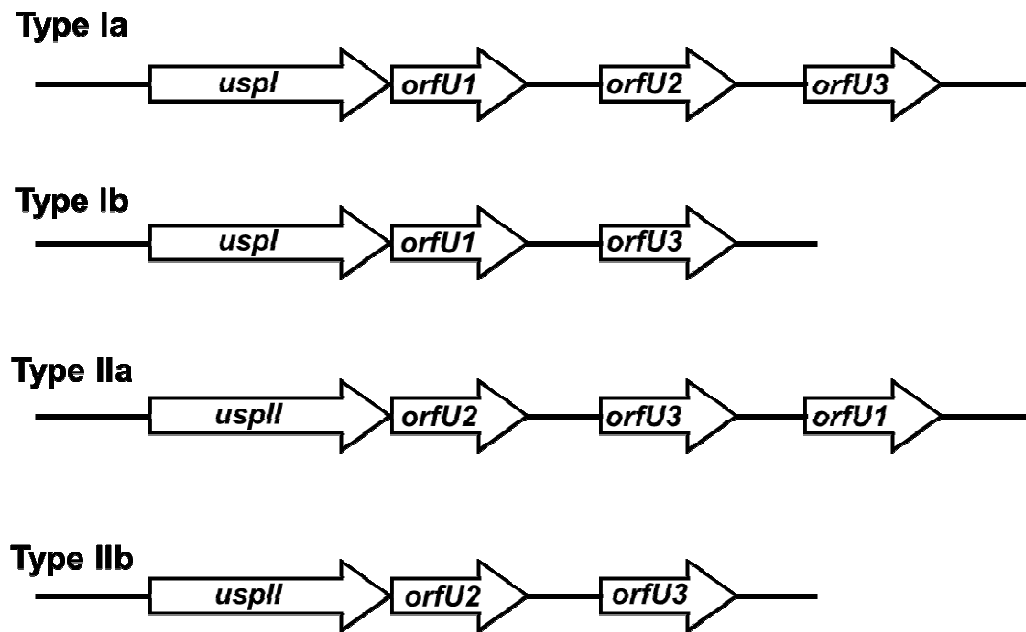


Figure 2: Structures of *usp* gene and *orfUs* in each subtype of PAIusp. The large arrows indicate the length and transcriptional direction of the genes in PAIusp.¹³

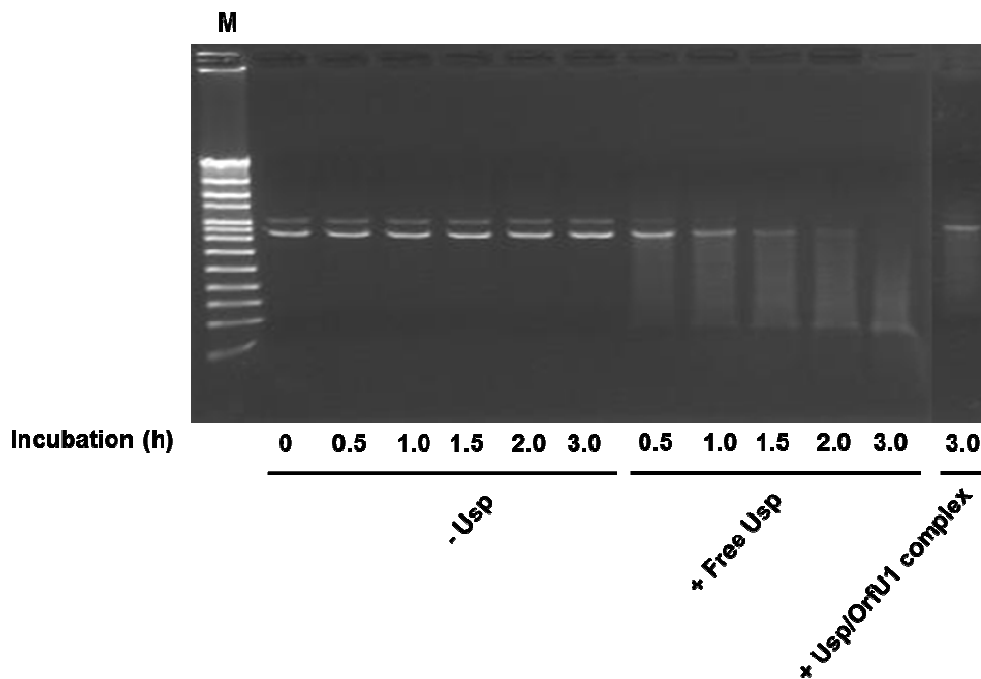


Figure 3: Comparison of DNase activity of Usp and Usp/OrfU1 complex. The DNase activity was examined using linear pUC18 DNA as substrate. DNA degradation was checked by electrophoresis in 1.0% (w/v) agarose gel.²⁹