A New Primer Set for Detection of fimH Gene in Escherichia coli Isolates

Yun Mei Lai¹, Rina Norgainathai², Myo Thura Zaw¹, Zaw Lin¹*

¹ Department of Pathobiological and Medical Diagnostics, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Malaysia
² Department of Biomedical Sciences and Therapeutics, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Malaysia

* Corresponding author; Email: 56dr.zawlin@gmail.com

Abstract

Background: The FimH protein is the tip fibrillum of the type 1 fimbria which is adhesive organelle of Escherichia coli (E.coli). Nucleotide sequence variations of fimH gene were common among commensal E. coli, intestinal pathogenic strains and extraintestinal pathogens. Because many researchers pointed out FimH adhesin plays an important role in E.coli – mediated urinary tract infections (UTI), the researchers have tried to develop FimH - based anti–urinary tract infection vaccine. There are different prevalence rate of fimH gene in UPEC isolates ranging from 86% to 97.5%.

Aim and Objectives: In this study, a sensitive, specific and reproducible primer sets for Polymerase Chain Reaction (PCR) method to detect fimH gene in uropathogenic Escherichia coli (UPEC) isolates has been designated.

Methodology: To get the fimH whole gene sequence, a set of primer covering whole gene PCR was done on 10 UPEC isolates and DNA sequence of these PCR products were aligned to select conserved areas to design new primer set. Forty UPEC isolates, 12 commensal E.coli isolates, 6 environmental E.coli isolates and 4 Entero-hemorrhagic E.coli (EHEC) isolates were studied for prevalence of fimH gene by using newly designated primer set.

Results: Out of these isolates 37 (92.5%) UPEC isolates, 6 (50%) commensal isolates, 5 (83%) environmental isolates and all the 4 (100%) EHEC isolates were positive for fimH gene.

Conclusion: A new primer set was successfully designed by DNA sequencing for the fimH gene of UPEC isolates and nucleotide alignment analysis. This primer set will be useful for further screening of fimH gene for molecular study of E.coli isolates.

Key words: uropathogenic Escherichia coli, fimH gene, polymerase chain reaction, new primer set

Introduction

E. coli has a number of adhesive organelles which includes P, type1, S, F1C, and long polar fimbriae. Out of these organelles, type 1 fimbriae were present in an average of 95% of all isolates of E. coli. Five parts of type 1 fimbria are FimD (inserted in the outer membrane), FimA (pilus rod), and FimF, FimG,
and FimH (tip fibrillum). The FimH protein is produced as a precursor of 300 amino acids and is processed into a mature form of 279 amino acids. The \textit{fimH} gene is 903bp long and DNA sequence variations were common among commensal \textit{E.coli}, intestinal pathogenic strains and extraintestinal pathogens. However, genetic variations were more common in intestinal pathogens and extraintestinal pathogens because stressful environment make these isolates adaptable by genotypic changes and consequently phenotypic variants were prevalent among these groups. \cite{1,2,3,4}

Mature FimH contains two domains connected by an 8-amino acid linker. These are a mannose-binding lectin domain which expand from residues 1–150 and a pilin domain consisting of residues 159–279. The mannose-binding pocket of FimH has no variation among sequenced UPEC. However, several residues outside the mannose-binding pocket (positions 27, 62, 66, and 163) were found to be mutated under positive selection in clinical UPEC isolates when compared with commensal strains. \cite{4,5,6,7,8,9,10}

Researchers pointed out FimH adhesin as a key player in \textit{E.coli}– mediated urinary tract infections. This information persuaded the researchers to the development of a FimH-based anti–urinary tract infection vaccine. The feasibility of this approach to the vaccine preparation has shown promising results in experimental animal urinary tract models. \cite{11}

The prevalence of \textit{fimH} genes was well studied by various researchers in different countries. The \textit{fimH} gene was 97.5% positive in UPEC in the study of patient with recurrent UTI, attended at the Ambulatory sector of Medical School Hospital of the Campinas State University (UNICAMP), Campinas, São Paulo, Brasil. In the studies done in two clinical institutions of Bucharest, \textit{fimH} had 86% prevalence rate in \textit{E.coli} strains isolated from Romanian adult with UTI. \cite{12,13} Andreu et al. described the presence of \textit{fimH} in \textit{E. coli} strains isolated from patients with pyelonephritis, cystitis, and recurrent UTI as 97%, 97%, and 90%, respectively in the Spanish study. \textit{E.coli} isolates from Mexican women, clinically diagnosed with UTI were studied for virulence genes and \textit{fimH} was 86.1% prevalent, second to \textit{ecp} gene which was 98.1% positive. Therefore, there are different prevalence rate of \textit{fimH} gene in UPEC isolates ranging from 86% to 97.5%. \cite{12,13,14,15}

In this study, we tried to designate the sensitive, specific and reproducible primer sets for Polymerase Chain Reaction method to detect \textit{fimH} gene by sequencing analysis of UPEC isolates. The new primer set PCR was consequently used to screen for UPEC as well as commensal and environmental \textit{E.coli} isolates.

**Methodology**

**Samples**

Forty UPEC isolates, 12 commensal \textit{E.coli} isolates, 6 environmental \textit{E.coli} isolates and 4 Entero-hemorrhagic \textit{E.coli} isolates stocked in microbiology laboratory, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah were included in this study.

**Polymerase Chain Reaction for \textit{fimH} whole gene sequence**

The forward primer including start codon and downstream sequences and reverse primer including stop codon and upstream sequences were designed for \textit{fimH} whole gene sequence PCR. The primer pair is shown in Table 1. The purpose of this PCR is to analyse the genetic variation and single nucleotide polymorphisms (SNPs) of \textit{fimH} ORF region among 10 UPEC isolates. The PCR ingredients were 3 μL of
DNA template, forward and reverse ws primers 0.3 mM each, 1x PCR buffer, dNTPs 0.2 mM and Taq polymerase 1.25U in a total volume of 50μL. The reaction conditions were 94°C for 5 min followed by 30 cycles of denaturation at 94°C for 40 sec, annealing at 55°C for 40 sec and extension at 72°C for 1 min. A final extension of 72°C was run for 5 min before maintained at 4°C. PCR was performed using Applied Biosystems Thermocycler and First base Taq DNA polymerase. The 10 μl of PCR products were run for gel electrophoresis on 1% TAE agarose gel and the gel was visualized with Alpha Imager® HP System after stained with ethidium bromide.

DNA sequencing of ten PCR products

DNA sequencing of PCR products was performed with Applied Biosystems highest capacity-based genetic analyzer using Big Dye® Terminator v3.1 cycle sequencing kit.

Alignment of DNA sequences to find single nucleotide polymorphisms (SNPs)

The resulting DNA sequences were analyzed with A plasmid Editor (ApE) software and nucleotide sequences were aligned with wild type sequences to detect SNPs. Wild type sequence EU124674 was obtained from NCBI website as standard and nucleotide sequence numbers were counted according to wild type sequences.

Designation of Sensitive Primer sets

After DNA alignment was studied, two primer sets were selected from totally conserved nucleotide sequence regions. The GC rich regions and the 5 continuous sequences of G or C or both in 3’ nucleotides of primers were avoided to get proper annealing.

PCR of fimH gene in various E.coli isolates with new two sets of primers

The PCR ingredients were 3 μL of DNA template, the new designated D1-f and D1-r primers 0.3 mM each, 1x PCR buffer, dNTPs 0.2mM and of Taq polymerase 1.25 U in a total volume of 50μL. The reaction conditions were 94°C for 5 min followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec and extension at 72°C for 1 min. A final extension of 72°C was run for 5 min before maintained at 4°C. The 10 μl of PCR products were run for gel electrophoresis on 1.5 % TAE agarose gel and the gel was visualized with Alpha Imager® HP System after stained with ethidium bromide. The same ingredients and PCR conditions were applied for another new primer set D2-f and D2-r.

Results

PCR for fimH whole gene sequence (ws)

The expected PCR product size is 903bp which appeared in the electrophoretic gel. Out of 30 UPEC isolates, only 24 isolates (80%) gave rise to positive results with ws primer set PCR. Gel electrophoresis picture with more of negative isolates was shown in Figure 1.

Alignment of DNA sequences to find SNPs and designation of new primer sets

Excluding primer sequences, there are 30 SNPs in the 10 PCR products sequenced. The conserved regions were analysed in detail following the precautions to design the primers. Two sets of primers (D1
and D2) were selected to get the appropriate PCR product size easy to be handled by agarose gel electrophoresis. The newly designated primers were shown in Table 1 and Figure 2.

**PCR of fimH gene in UPEC isolates with new two sets of primers**

With the new two sets of primers, all the 30 UPEC isolates tested with ws primer set were investigated and D1f-D1r primer set was more sensitive than D2f-D2r. The result of comparison was shown in Figure 3. Because of this result, D1f-D1r primer set was applied for detection of fimH gene in commensal and environmental isolates.

**Detection of E.coli isolates by PCR using new D1f-D1r primer set**

Thirty-seven out of 40 UPEC isolates were positive for fimH gene by D1f-D1r primer set PCR while 6 out of 12 commensal isolates, 5 out of 6 environmental isolates and all the 4 EHEC isolates gave positive reaction with this PCR. As a percentage, 92.5 % of UPEC isolates, 50% of commensal isolates, 83% of environmental isolates and 100% of EHEC isolates were positive with newly designated primer set PCR.

**Discussion**

In the previous studies on virulence factor(VF) profiles in different sources of UPEC isolates, fimH adhesin was one of the most highly prevalent VFs. More than 97% prevalence rate was observed in some studies while the least prevalence rate was 86%. 12-15

One of the causes of this variation may be the selection of primers in non-conserved regions as well as studying in different localities. High percentages of UTI are caused by commensal E.coli having access to urinary tract due to unhygienic habit of the person. Therefore if the commensals have fimH gene in their genome, there will be presence of fimH VF when these organisms cause cystitis and associated ascending clinical conditions.

In this study, we tried to design the sensitive set of primers so that detection rate of fimH gene can be increased. When we aligned the DNA sequences of 10 isolates, the most heterologous nucleotide sequences were observed in ws forward and reverse primers regions. The differences were 1-9 nucleotide difference in forward primer as well as 4-10 in reverse primer. Throughout the whole ORF excluding primers region, there are 30 SNPs with one two continuous nucleotide difference in one isolate. The difference of nucleotides in primer regions will be the cause of negative result in some UPEC isolates.

The newly designed primer sets gave rise to 217bp and 357bp PCR products. Both the primer sets gave positive reactions to three isolates which were negative in ws primers PCR. D1f-D1r PCR (217bp PCR product size) gave positive band in another isolate which was negative in D2f-D2r PCR (357bp PCR product size) indicating that D1f-D1r PCR was more sensitive although both PCRs have appropriate PCR product size to run on 1- 1.5% agarose gel.

In this study with new primer set PCR, UPEC isolates were comparably positive as other previous studies. However, commensal isolates were positive in only 50% while there was as high as 83 % in case of environmental isolates and highest (100%) in EHEC isolates.

As a conclusion, we have succeeded in designing the new primer set by DNA sequencing of fimH gene of UPEC isolates and nucleotide alignment analysis. Because fimH adhesin is interested by molecular
biologist for the study of pathogenesis, molecular epidemiology and prevention of UTI, this primer set will be useful for the identification of \textit{fimH} gene and its mutations.

For the future studies of prevalence rate of the gene which has highly variable nucleotide sequence, the technique laid down in this study will be helpful for the researchers to get the sensitive primer set for amplification of their target gene.

\textbf{Conflict of Interest}

The authors declare no conflict of interest.

\textbf{Authors Contributions}

Conceived and designed the experiment: ZL. Performed the experiments: YML, RN. Analyzed the data: ZL, YML, MTZ. Wrote the paper: ZL, MTZ.

\textbf{Acknowledgement}

We would like to thank Professor Dr. D Kamarudin Mudin, Dean, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah for the continuous support throughout the whole research project. A special thanks goes to Associate Professor Dr. Than Myint, Director, Rural Medicine Research Unit, Faculty of Medicine and Health Sciences for providing me the grant to carry out this research work.

\textbf{References}


**Figure 1:** Gel electrophoresis picture of PCR products of 10 UPEC isolates amplified by using *wsf-wsr* primers. Isolates no.009, 101,104,108,170 were positive for 903bp PCR product while isolates no. 004,005,012,014,105 were negative. 100bp molecular weight markers were run on the left and right sides of the PCR products.

**Figure 2:** Schematic diagram of location of *ws* primer set and newly designed two primer sets in *fimH* gene. *Ws* and *wsr* indicates forward and reverse primers of *fimH* whole gene sequence primer set *ws* designed in this study. *D1f* and *D1r* indicates forward and reverse primers of newly designed primer set *D1* while *D2f* and *D2r* indicates forward and reverse primers of newly designed primer set *D2*. The whole gene is 903bp long to be compared by 100bp scale at the right upper end.
**Table 1:** Nucleotide sequence of oligonucleotide primers used in this study

<table>
<thead>
<tr>
<th>Name of Primer Set</th>
<th>Sequence</th>
<th>Amplicon Size</th>
</tr>
</thead>
</table>
| ws *(fimH whole sequence)* | F: 5’-ATG AAA CGA GTT ATT ACC CT-3’  
R: 5’-TTA TTG ATA AAC AAA AGT CAC G-3’ | 903 bp        |
| D1                 | F: 5’-AAT GTG GGG CAA AAC CTG G-3’  
R: 5’- TAT CCG TTC TCG AAT TAT AAA-3’ | 217 bp        |
| D2                 | F: 5’- ATA ACA GCG ATG ATT TCC AGT-3’  
R: 5’- AAT TTG CCG TTA ATC CCA GA-3’ | 357 bp        |

*Figure 3: Gel Electrophoresis picture of PCR amplification with the two newly designed primer sets D1f-D1r and D2f-D2r which give rise to 217 bp and 357 bp DNA fragment respectively.*

*Isolates number in red colour (004,005,105) were the ones which became positive with the new primer sets. Isolate in yellow colour (021) was the one positive with D1f-D1r and negative with D2f-D2r primer set.*