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Original Research Article

Presence of *fim*H and *iss* type 1, 2 and 3 genes in uropathogenic *Escherichia coli* isolates recovered from an apex medical institute in North India

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ABSTRACT

Purpose: To detect the presence of *fim*H and *iss* type 1, 2 and 3 genes in uropathogenic *Escherichia coli* (UPEC) isolates recovered from patients coming to the out patient department (OPD) of our hospital.
Methods: E. coli isolates recovered from patients who had symptoms of urinary tract infection (UTI) were processed for the presence of *fim*H and *iss* genes. DNA was extracted using an in house method after which conventional PCR using forward and reverse primers targeting the four genes was carried out. The amplified products were electrophoresed and visualized in a gel documentation imager. Relevant demographic details of the patients were recorded on a pre-designed pro-forma and antimicrobial susceptibility testing of the isolates was done by disc diffusion method.
*Results: fim*H was present in 87.5% of UPEC isolates whereas *iss* type 1 was seen in 7.3%, type 2 in 4.2% and *iss* type 3 in 71.9% isolates. Age of the patients ranged from 3 months to 82 yrs (mean 43.5 SD ± 18.20). UTI was more common in females (60.2%) as compared to males patients (39.8%). Dysuria (66.7%) was the most common symptom in the studied subjects and diabetes mellitus (42.6%) the most common co-morbidity. A total of 56.5% patients gave a history of prior antibiotic intake. The UPEC isolates were resistant to most of the antibiotics tested. However all the isolates were sensitive to polymyxin B and colistin. Fosfomycin resistance was seen in 9.5% of the UPEC isolates harbouring *fim*H gene.
Conclusion: This is the first study that highlights the presence of *iss* type 3 gene in UPEC isolates along with the *fim*H and *iss* type 1 and 2 genes. The results of this study can serve as a stepping stone for future in depth research into the significance of the *iss* genes in causing UTI.

1. Introduction

Infection of the urinary tract is one of the most commonly encountered bacterial infection in humans and is responsible for significant morbidity in them. It accounts for 25–40% of all nosocomial infections with *Escherichia coli* (*E. coli*) implicated in more than 80% of all such infections [1,2]. The infection usually starts in the bladder and at times can ascend to kidneys causing renal failure and even disseminate to blood causing sepsis. Extra intestinal pathogenic *E. coli* (ExPEC) repertoire has various subtypes namely, uropathogenic *E. coli* (UPEC), neonatal meningitis-associated *E. coli* (NMEC), sepsis-associated *E. coli* (SEPEC) and avian pathogenic *E. coli* (APEC) [3].

Ability of UPEC isolates to cause urinary tract infection (UTI) is due to the presence of various virulence factors [4]. Amongst these, molecules that mediate binding to mannose containing receptors present on uroepithelial cells are the most important determinants of pathogenicity [5]. This attachment is predominantly mediated by *fim*H adhesion located on Type 1 fimbriae. *fim*H is mainly found in UPEC strains causing cystitis [6–8]. Some human ExPEC strains also harbour the *iss* (increased serum survival) gene. This gene is found on a large colicin encoding virulence plasmid, ColV, found in APEC strains [9]. The ability of APEC strains to spread to human beings coupled with the potential to harbour virulence genes that can be transferred to UPEC which in turn helps the microorganism in causing infections has been well documented by Rodriguez-Siek et al. [10]. The *iss* gene has been shown to occur in around 60% of *E. coli* causing UTI and strains causing neonatal meningitis [9].

Whereas the *fim*H gene has been studied extensively the presence of *iss* gene has not been probed to a great extent especially in UPEC isolates.

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The current study was thus undertaken to detect the presence of *fim*H and *iss* type 1, 2 and 3 virulence gene in UPEC strains isolated from patients suspected of having UTI attending the out patient department (OPD) of our hospital and to determine the antimicrobial susceptibility patterns of such isolates.

2. Material and methods

2.1. Study design and settings

This prospective study was carried out in the Department of Microbiology SKIMS, for a period of 6 months from Jan 2021 to June 2021. Urine samples submitted by patients with features of acute cystitis were processed for the recovery of microorganisms. Samples positive for the growth of *E. coli* were subjected to further testing. Demographic characteristics like age, gender, presenting complaints, history of prior antibiotic intake and of a similar illness in the past along with co-morbidity (if any) were noted for each patient.

E. coli were identified using standard microbiological procedures in the laboratory [11]. Only pure cultures of *E. coli* having a colony count of $>10^5$ were included in the study. The antimicrobial susceptibility testing (AST) of the isolates was carried out on Muller Hinton agar by Kirby Baurer disc diffusion using the following agents; ampicillin (10 μg), ampicillin + sulbactam (10/10 μg), piperacillin + tazobactam (100/10 μg), ceftriaxone (30 μg), ceftazidime (30 μg), cefazolin (30 μg), cotrimoxazole (1.25/23.75 μg), amikacin (30 μg), gentamicin (10 μg), nitrofurantoin (300 μg), norfloxacin (10 μg), ciprofloxacin (10 μg), levofloxacin (10 μg), fosfomycin (200 μg) imipenem (10 μg), meropenem (10 μg), and aztreonam (30 μg) as per Clinical and Laboratory Standard Institute (CLSI) guidelines [12]. Polymyxin B and colistin susceptibility was carried out using E-test strips. *E. coli* (ATCC 25922) was used as control strain for susceptibility testing. All the media and antibiotics discs and E-test strips used were procured from Hi Media; Mumbai India.

UPEC isolates were stored in BHI-glycerol media and subcultured on nutrient agar. Overnight pure colonies were screened for the presence of *fim*H and *iss* type 1, type 2 and type 3 genes by conventional uniplex polymerase chain reaction (PCR).

2.2. Extraction of nucleic acid

An in-house method of nucleic acid extraction combined with magnetic bead-based extraction and purification was used. One to two colonies were mixed in 150 μl of nuclease-free water and vortexed for 10 s in 0.2 ml PCR tubes. The samples were subjected to heat shock at 100 °C in a dry heat block (Eppendorf Thermomixer C, Chennai, India), for 10 min and followed by snapped chilling in ice for 5 min. A total of 150 μl of chilled supernatant after high-speed centrifugation at 10,000 g at 4 °C for 5 min was transferred for magnetic bead-based automated nucleic acid extraction (Kingfisher 96, ThermoFisher Scientific, Waltham, USA), using a commercial magnetic bead-based extraction kit (Magmax, total nucleic acid isolation kit, ThermoFisher Scientific, Waltham, USA) The procedure was carried out as per manufacturers protocol.

2.3. Polymerase chain reaction

Conventional PCR was performed with the forward and reverse primers for *iss* type 1, 2 and 3 [3] and *fim*H [13]. Table-1 It was carried out in a total reaction volume of 25 μl using 2X PCR master mix (Promega, Madison, USA), including 5 μl of sample template and 1 μl (0.4 μM final concentration) each of reverse and forward primers.

The thermal cycling conditions for *fim*H and *iss* type 1, 2 and 3 were: initial denaturation at 95 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s (for *fimH*) and 58 °C for 30 s for *iss* type 1, 2 and 3 followed by extension at 72 °C for 1 min and final extension at 72 °C for 10 min (included in the last cycle). A no-template control was used as a negative control using 5 μl of nuclease free water. *E. coli* ATCC 25922 served as a positive control for *fim*H and *E. coli* ATCC 23848 for *iss* type 2, type 3 & *fim*H genes. Known *iss* type 1 available in the laboratory was used as control for *iss* type 1 genes. The amplicon sizes of all four genes are mentioned in Table 1.

PCR amplified products (10 μl) were electrophoresed using 2.0% agarose gel in a horizontal electrophoresis system with a power pack (Consort EV 232, Merck, Kenilworth, USA) and visualized in a gel documentation imager (myECL imager, ThermoFisher Scientific, Waltham, USA).

2.4. Statistics

For comparison of the significance of prevalence of the virulence genes in the clinical isolates and their antibiotic susceptibility profile, Fisher's exact test was used and *P*-value of <0.05 was considered significant.

3. Results

A total of 1612 urine samples were received in the laboratory during the study period from the OPD, out of which 241 (14.9%) yielded significant bacterial growth (colony counts of $>10^5$/ml). Amongst these *E. coli* was isolated from 108 (44.8%) samples and were part of the present study. Age of the patients ranged from 3 months to 82 yrs (mean 43.5 SD ± 18.20). Incidence of UTI was more common in females (n = 65, 60.2%) than males patients (n = 43, 39.8%).

Dysuria (n = 72, 66.7%) followed by increased frequency of micturition (n = 21, 19.4%) were most common presenting complaints and 61 (56.5%) patients gave a history of prior antibiotic intake for their symptoms of which the most common were nitrofurantoin (n = 32, 29.6%) and levofloxacin (n = 30, 27.8%). Thirty two (29.6%) patients gave history of having similar symptoms in the past for which they had been evaluated previously. Of these, culture reports were available for 18 patients. In 17 of the 18 patients *E. coli* had been isolated previously whereas in one patient *Pseudomonas aeruginosa* had been recovered. The most common co-morbid condition noted in the patients was diabetes mellitus (n = 46, 42.6%) followed by hypertension (n = 18, 16.7%) whereas chronic kidney disease was seen in 8 (7.4%) patients. In addition 5 (4.6%) patients had benign prostatic hyperplasia.

Table 1
PCR Primers for evaluation of virulence factors in the study.

Gene	Primer sequence	Amplicon length (bp)	References
iss type 1	FP- 5′-CAGCAACCCGAACCACTTGATG-3′ RP- 5′-TTCTGCCGCTCTGGCAATGCT-3′	323	Johnson et al., 2008
iss type 2	FP-5′-GGTAACCCTGCGTCTGTCAGCACA-3′ RP-5′-CCAGCGGAGTATAAATGCCTAAAG-3′	581	
iss type 3	FP-5′-GTCCCCAACTTCCTCCAATAGTCT-3′ RP-5′-CCAGCGGAGTATAAATGCCTAAAG-3′	390	
fimH	FP-5′-TGCAGAACGGATAAGCCGTGG-3′ RP-5′-GCAGTCACCTGCCCTCCGGTA-3′	506	López-Banda et al., 2014

Out of the 108 isolates of UPEC, 96 were subjected to PCR for the identification of *fim*H and *iss* type 1, 2 and 3 genes. *fim*H was present in 84 isolates (87.5%) whereas *iss* type 1 was seen in 7 (7.3%), type 2 in 4 (4.2%) and type 3 in 69 (71.9%) isolates. Fig. 1 depicts the agarose gel picture of the resistance genes. The antimicrobial susceptibility of the *E. coli* isolates is given in Table 2. UPEC isolates in our study had variable susceptibility to the antibiotics tested. None of the isolates was resistant to polymyxin B or colistin whereas fosfomycin resistance was seen in 9.5% of UPEC isolates. Highest resistance among the isolates was seen for ampicillin (90.5%) followed by ampicillin + sulbactam (69%). Resistance to imipenem and meropenem was seen in 37% and 29.8% isolates respectively. The UPEC isolates exhibited high resistance to fluoroquinolones as well, highest being against ciprofloxacin (60.7%).

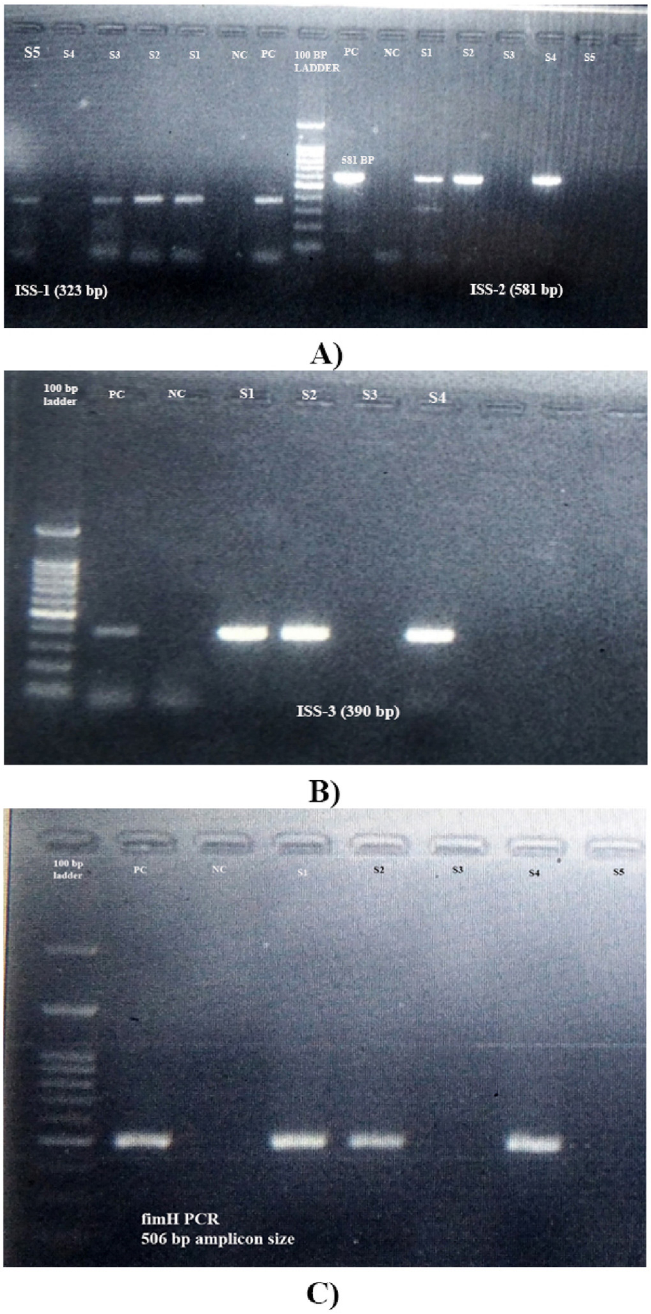

Fig. 1. Agarose gel electrophoresis was performed from the PCR products of conventional uniplex PCR for *iss* type 1, 2, 3 and *fimH* with S1, S2, S3, S4 marked lanes are samples, NC is negative control, PC marked lanes are positive control Fig. 1 (A) displays the gel picture of *iss* type 1 PCR having amplicon size of 323 bp, while *iss* type 2 is shown on the right with amplicon size of 581 bp. Fig (B) & Fig (C) displays the gel picture of *iss* type 3 and *fimH* with amplicon size of 390 bp and 506 bp respectively. A 100 bp DNA ladder was used as size marker.

Table 2
Antibiotic resistance profile of the UPEC isolates.

Antibiotics	*fim*H n = 84	*iss* type 1 n = 7	*iss* type 2 n = 4	*iss* type 3 n = 69
Ampicillin	76 (90.5%)	7 (100%)	4 (100%)	61 (88.4%)
Ampicillin + sulbactam	58 (69%)	7 (100%)	4 (100%)	54 (78.3%)
Piperacillin + tazobactam	43 (51.2%)	1 (14.3%)	–	38 (55.1%)
Amikacin	29 (34.5%)	1 (14.3%)	–	25 (36.2%)
Gentamicin	35 (41.7%)	1 (14.3%)	–	25 (36.2%)
Co-trimoxazole	53 (63.1%)	1 (14.3%)	2 (50%)	41 (59.4%)
Ceftriazone	42 (50%)	2 (28.6%)	–	28 (40.6%)
Ceftazidime	38 (45.2%)	2 (28.6%)	–	28 (40.6%)
Cefotaxime	38 (45.2%)	2 (28.6%)	1 (25%)	28 (40.6%)
Ciprofloxacin	51 (60.7%)	2 (28.6%)	1 (25%)	36 (52.2%)
Norfloxacin	45 (53.6%)	2 (28.6%)	1 (25%)	35 (36.2%)
Levofloxacin	39 (46.4%)	1 (14.3%)	1 (25%)	24 (34.8%)
Nitrofutantoin	18 (21.4%)	–	–	20 (29%)
Fosfomycin	8 (9.5%)	–	–	6 (8.7%)
Imipenem	31 (37%)	2 (28.6%)	–	25 (36.2%)
Meropenem	25 (29.8%)	2 (28.6%)	–	22 (31.9%)
Polymyxin B	–	–	–	–
Colistin	–	–	–	–

Of the studied virulence genes 34 isolates harboured only one such gene. Out of these 22 isolates expressed only *iss* type 3 and 10 had only *fim*H whereas 2 isolates had *iss* type 2 gene. Presence of all the four genes was seen in one isolate only. This isolate belonged to a patient who had undergone cystoscopy 6 months back and was now complaining of symptoms of UTI off and on. His previous culture had grown *P. aeruginosa*. The patient had taken nitrofurantoin, amikacin, fosfomycin and meropenem for his symptoms in the past 3 months without any relief though.

4. Discussion

ExPEC are facultative pathogens and one of the most diverse pathotypes of *E. coli*. They are responsible for causing a multitude of illnesses in human and domestic animals due to the presence of virulence factors like adhesins, toxins, factors that help to evade host defence systems, nutrient acquiring mechanisms etc. [14]. Among the ExPEC, strains of *E. coli* causing community and hospital acquired UTI's, the UPEC; are commonly associated with human disease. Data on the extent to which various virulence factors are present in UPEC isolates are all but non existent from our Union Territory. This study was undertaken to find out the extent to which UPEC isolates recovered from the OPD patients harbour the *fim*H and *iss* genes.

A total of 108 strains of *E. coli* were part of the present study. UTI was more commonly seen in female (60.2%) than male patients (39.8%), a finding consistent with many studies carried out previously [2,15,16]. A short urethra along with proximity to the perineum, vaginal flora, menstrual cycles and pregnancy are known to predispose females to UTI's [17]. Age of the patients in our study ranged from 3 months to 82 yrs (mean 43.5 SD ± 18.20). In a study by Zeng et al. age of 151 female patients ranged from 19 to 94 yrs (median: 62 yrs) [18]. The age range of the patients in a study by Nadagopal et al. was 1 yr–89 yrs with a median of 54.5 yrs [19], whereas it was 18–87 yrs in a study by Shah et al. [15].

In our patient subset, most common symptom of UTI was dysuria (66.7%) and 56.5% patients gave a history of prior antibiotic intake. Over the counter availability of antibiotics especially those taken orally leads to self medication by people. DM was present in 42.6% patients who were a part of our study. Neuropathy involving the urinary tract resulting in dysfunctional voiding and retention of urine, has been postulated as the main reason behind the increased incidence of UTI in diabetics [20]. In a study by Bustos et al. the authors found that 40.5% of UTI patients had DM and 40% of the patients had received prior antibiotic therapy [21]. Likewise Chakraborty et al. in their study found DM (45.5%) and carcinoma to be the most common predisposing factors in patients of UTI [2].

In our study out of 96 UPEC isolates, majority of the isolates harboured the *fim*H gene followed by *iss* type 3. Thirty four isolates harboured only one gene; of which maximum expressed *iss* type 3. A single UPEC isolate harboured all the four genes and belonged to a patient of recurrent UTI who had undergone an invasive procedure 6 months ago. In a study by Hojati et al. *fim*H gene was found in 92.8% UPEC isolates recovered from patients of UTI [8]. Likewise *fim*H was reported in 90% UPEC isolates in a study from South India [2]. Several other studies have demonstrated high prevalence of *fim*H among the UPEC isolates [18]. *fim*H helps UPEC to invade uroepithelial cells and mediates adherence to mannose-containing glycoproteins [22]. Internalization within the uroepithelial cells helps *E. coli* escape the innate immune responses of the host [20].

The presence of all the three different alleles of *iss* especially type 3 has been demonstrated in ExPEC isolates previously however very few researchers have focused on its presence in UPEC isolates. In a study by Derakhshandeh et al. the authors found that the *iss* gene was more commonly found in B2 group of UPEC isolates [1]. The *iss* gene confers resistance against the complement components and its presence is crucial for the development of bacterial sepsis [3] however its role in UTI has not been fully elucidated. In an extensive study on the evolution of the *iss* gene in *E. coli* Johnson et al. described the presence of *iss* on the ColV/BM virulence plasmids as well as within the *E. coli* chromosome. On sequencing the type 3 allele was found to occur in the genomes of all the ExPEC strains. It was also found that the *iss* type 3 gene occurred very frequently in ExPEC isolates, whereas *iss* type 1 was found more commonly among APEC and NMEC isolates but was not that prevalent in UPEC isolates [3].

Isolates harbouring the virulence genes were resistant to most of the antibiotics tested especially ampicillin, ampicillin + sulbactam, various cephalosporins with variable susceptibility to fluoroquinolones. All the isolates of UPEC were susceptible to polymyxin B and colistin.

This is the first study to have screened the UPEC isolates for the presence of *fim*H gene and *iss* type 1, 2 and 3 genes from this part of the country. High antibiotic resistance in UPEC isolates along with the presence of these resistance factors can lead to treatment failures and recurrent UTI. The findings of this study can serve as a stepping stone for researchers who want to study the presence and types of virulence genes in UPEC isolates and to take it a step further and study the clinical implications and relevance of their presence in patients suffering from UTI.

5. Limitations

Our study is limited by the fact that only two virulence genes were targeted. Also an in-depth clinical history and examination of the patients paired together with the presence of the studied genes especially *iss* type 3 in UPEC isolates could have furthered our knowledge regarding its contribution in causing infection in this subset of patients.

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Ethical approval statement

The study was conducted after obtaining clearance from the Institutes ethical committee bearing no: IEC/SKIMS Protocol #RP 063/2022.\

Author contribution

NA gave the concept for the study and wrote the first and the final draft of the study. NA, BB and SR did the literature research. AA collected the samples. TA collected and analyzed the data. QN, SW and RH processed the samples at different stages. All authors read and approved the final submitted version.

Conflict of interest

None.

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