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EMERGENCE OF 08 SEROTYPE UROPATHOGENIC *ESCHERICHIA COLI* AS A MULTIDRUG RESISTANT PATHOGEN OF URINARY TRACT INFECTION

[1]Vijayanand Ramakrishnan*, [2]Dr. Mani K Raju, [1]Surendran Lakshmikanthan

*[1]PG and Research Department of Biotechnology, Kongunadu Arts and Science College, GN Mills, Coimbatore, Tamilnadu – 641029, **INDIA***
*[2]Central Research Institute, (Kasauli, Himachal Pradesh, India), Pasteur Institute of India, Coonoor, Tamilnadu – 643103, **INDIA***

Abstract
Urinary tract infection (UTI) has become the most dreadful infectious disease worldwide which is caused most commonly by few serotypes of Uropathogenic *Escherichia coli* (UPEC). Every now and then, one or the other new strain is evolving as a pathogen. Our work is to confirm the emergence of O8 serotype of UPEC as a potential multidrug resistant pathogen by harboring the basic characters like virulence factor for attachment and gene for drug resistant. Out of 100 samples collected from hospitals in and around Coimbatore, Tamilnadu, India, and screened at Bioline laboratories, Coimbatore, 25 were from male and 75 from female. Further analysis of 85 isolates serotyped (at National Salmonella and Escherichia Centre (NSEC), Central Research Institute (CRI), Kasauli, Himachal Pradesh), 16 (19%) were O8 serotype. Mannose sensitive hemagglutination (MSHA) was found in 12 isolates (75%) which indicates the presence of type 1 fimbriae. Isolation of fim H gene confirms the presence of the adhesin fim H subunit, important virulence factor required for the attachment of UPEC to urinary tract. All isolates showed positive for ESBL production and the CTX-M gene was isolated that disclosed the serotype O8 had developed multidrug resistant character. Three isolates produced both fim H and CTX-M genes. Resistance was reported against cephazolin, cefepime, ciprofloxacin, gentamycin, ampicillin and cepalothin and no resistance were developed against ertapenem, imipenem and meropenem. Therefore, the serotype O8 of UPEC can be considered as an emerging potential pathogen of urinary tract infection with multidrug resistant character.

Keywords: Uropathogenic, *Escherichia coli*, Serotype, Fim H, CTX-M.

Corresponding Author:

Vijayanand Ramakrishnan
Research Scholar, Microbiology,
Centre for Research and Development,
Bharathiar University,
*Coimbatore, Tamilnadu, **INDIA***
***E-mail:** vijayanandramakrishnan@yahoo.co.in*
***Phone:** +91-9943447153*

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INTRODUCTION

Each year around 150 million people are affected by Urinary tract infections (UTI) worldwide [1]. In India the principal causative agent is Uropathogenic *Escherichia coli* (UPEC) (68%) followed by other members of Enterobacteriaceae family [2]. Almost 50 to 80% of women will get affected by UTI at least once in their lifetime with a recurrence rate of 20 – 50% [3-4]. Pathogenesis of UPEC are often linked to its O Serotypes [5-6] which seems to be utmost important in epidemiological studies [7-9]. The serotypes O1, O2, O4, O6, O7, O16, O18, O25 and O75 were encountered in 81% of UPEC causing UTI [10-11] and O8 was rarely reported [12]. To avoid elimination by urine flow the UPEC possess a virulence factor, the type 1 fimbriae which can recognize and attach to the receptors in epithelial cells of urinary tract [13] by means of fim H subunit [14]. Presence of type 1 fimbriae indicates cystitis infection since it colonize the lower urinary tract [15-16] and could be detected by mannose sensitive hemagglutination (MSHA) [17-18]. Frequent consumption of antibiotics makes the bacteria to emerge with resistance [19] by mechanism such as production of extended spectrum beta lactamase (ESBL) that can hydrolyse cephalosporins, ceftazidime, cefotaxime and monobactum aztreonam [20]. The most prevalent ESBL enzyme encoding gene in Enterobacteriaceae in the previous decade was found to be CTX-M gene [21-22]. The purpose of this study is to show the emergence of O8 serotype UPEC as a potential uropathogen by screening for MSHA and detecting the expression of fim H gene which is responsible for colonization of UPEC, screening for ESBL and isolating the CTX-M gene which is accountable for the ESBL production and antibiotic resistance.

MATERIALS AND METHODS

SAMPLE COLLECTION: The Bioline laboratory receives samples from various hospitals from in and around Coimbatore. A mid stream urine samples were processed to isolate 100 UPEC cultures from 25 male and 75 female UTI patients. Vitek 2 compact, ID/AST instrument, Biomerieux Diagnostics was used for identification. Further analysis was done in the Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore.

SEROTYPING: For screening O8 serotypes, all the isolates were sub cultured and sent to National Salmonella and Escherichia Center (NSEC), Central Research Institute (CRI), Kasauli, Himachal Pradesh, India.

SCREENING FOR MSHA: A drop of human O group erythrocyte was placed on a Venereal Disease Research Laboratory (VDRL) slide. A drop of phosphate – buffered saline (PBS) with

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and without mannose was added. The culture (100 µl) was added to it and mixed. Hemagglutination in the absence of mannose which is inhibited in its presence are said to be mannose sensitive hemagglutination (MSHA) [23].

ESBL SCREENING: The O8 serotype isolates were screened for the production of ESBL following CLSI guidelines [24]. A zone size of ≤ 22 mm for ceftazidime (30 µg), ≤ 27 mm for cefotaxime (30 µg), and ≤ 25 mm for ceftriaxone (30 µg) were said to be positive for ESBL production. To confirm ESBL production, culture inoculated in 0.5 ml broth and incubated to get turbidity of 0.5 Mc Farland. The broth was swabbed uniformly on to Muller Hinton Agar and the antibiotics ceftazidime (30 µg) and ceftazidime-clavulanic acid (30 µg + 10 µg) and the cefotaxime (30 µg) and cefotaxime clavulanic acid (30 µg + 10 µg) combination disks were placed. The zone of inhibition was noted. A 5 mm zone between single and combination with clavulanic acid confirms the production of ESBL.

ANTIBIOTIC SENSITIVITY TEST: Antibiotic sensitivity test of the O8 serotype UPEC was performed using disc diffusion technique based on CLSI guidelines [24]. Antibiotic discs used for the study were Ampicillin (10 µg), Amikacin (30 µg), Amoxicillin (10 µg), Cephazolin (30 µg), Ciprofloxacin (5 µg), Cepalothin (30 µg), Cefepime (30 µg), Ertapenem (10 µg), Imipenem (10 µg), Meropenem (10 µg), Norfloxacin (10 µg), Tozabactum (10 µg), Tigecycline (4 µg), Co-trimaxazole (25 µg) and Gentamycin (10 µg).

PCR AMPLIFICATION FOR FIM H AND CTX-M: The PCR amplification was performed with DNA from 12 different UPEC isolates which were positive for MSHA. A specific PCR primer set was chosen separately for both fim H and CTX-M gene isolation.

The primers were evaluated on different isolates as primer fim H forward 5`-GATCTTTCGACGCAAATC -3` and reverse 5`-CGAGCAGAAACATCGCAG-3`. Similarly for CTX-M, the specific forward 5`-CGACGCTACCCCTGCTATT-3` and reverse 5`-CCAGCGTCAGATTTTTCAGG-3` primer set were used. The sequences flanked by the primer set were amplified under following conditions: 100ng of genomic DNA 5 µl , PCR master mix 10-12 µl , forward primer 1µl and reverse primer 1µl, and the volume made up to 25µl with Milli-Q water.

RESULT

Among 370 urine samples screened, 100 isolates were identified as UPEC which comprise 25 male and 75 female of ages from 1 to 80 years. The 100 samples screened for serotypes at NSEC,

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CRI, Kasauli, Himachal Pradesh, 15 isolates got contaminated while transport and 8 reported as untypable.

Table 1: Prevalence of urinary tract infection causing O8 serotype uropathogenic Escherichia coli (UPEC) with gender and age group

Age	01 - 10	11 - 20	21 - 30	31 – 40	41 – 50	51 – 60	61 - 70	71 - 80
Female	0	1	4	2	1	1	1	2
Male	0	0	0	0	0	1	0	3

No of females = 13; No of males = 3.

From the remaining 77 isolates, 16 (19%) isolates were found to be of O8 serotype. The O8 serotype UPEC isolates were subjected to further analysis. Upper UTI (Pyelonephritis) was found in 3 individuals and the lower UTI (Cystitis) in 13 patients. The isolates were from 4 males of age above 60 years where 3 of them from the age group 71 to 80 years. Remaining 12 isolates belonged to females of age 11 to 80 years (Table 1). The age group of 21 to 30 years was highly infected. All 16 isolates (O8 serotype) were identified as ESBL producers and 12 (75%) showed positive for MSHA. Highest resistance was found towards cephazolin (94%) followed by cefepime (88%), ciprofloxacin (81%), gentamycin (75%), ampicillin and cepalothin (69%) and co-trimaxazole and amoxicilin (63%). Amikacin, norfloxacin and tozabactum were resistant to less than 25% of isolates and all were sensitive to ertapenem, imipenem and meropenem. (Table 2.)

Table 2: Sensitivity pattern of O8 serotype uropathogenic *Escherichia coli* (UPEC) isolates towards commonly used antibiotics

S. No	Antibiotic	Resistance N = 16 (%)
1	Ampicillin	11 (69)
2	Amikacin	4 (25)
3	Amoxicillin	10 (63)
4	Cephazolin	15 (94)
5	Ciprofloxacin	13 (81)
6	Cepalothin	11 (69)
7	Cefepime	14 (88)
8	Ertapenem	0 (0)
9	Imipenem	0 (0)
10	Meropenem	0 (0)
11	Norfloxacin	4 (25)
12	Tozabactum	3 (19)
13	Tigecycline	0 (0)
14	Co-trimaxazole	10 (63)
15	Gentamycin	12 (75)

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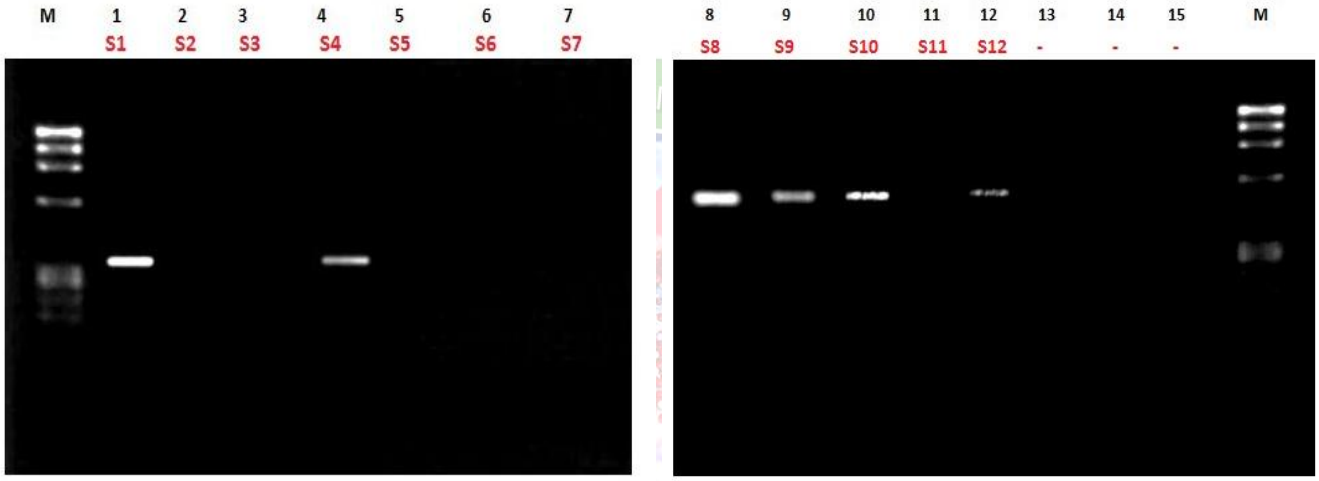
Overall, 2 isolates were resistant to 3 antibiotics, one isolate was resistant to 4 antibiotics, 2 isolates for 6 antibiotics, 5 isolates for 7 antibiotics and 6 isolates for 8 antibiotics. The PCR amplified product of MSHA positive isolates were run on electrophoresis gel and observed for the band formation. The samples S1, S4, S8, S9, S10 and S12 formed band corresponding to the fim H gene marker confirms the presence of the gene in those samples (Figure 1 A and B). Others were negative for band formation which indicates the absence of the gene. Similarly the samples S9, S10 and S12 showed positive for CTX-M gene and others negative (Figure 2).



A **B**

Figure 1: PCR amplification of fim H gene from MSHA positive O8 serotype UPEC samples 1-12

In 1.5% agarose gel, **(A) Lane 2-7:** Sample 1(S1) to Sample 7 (S7). **(B) Lane 1-5:** Sample 8 (S8) to Sample 12 (S12) with DNA marker **M – Lane 1**: 100bp ladder.

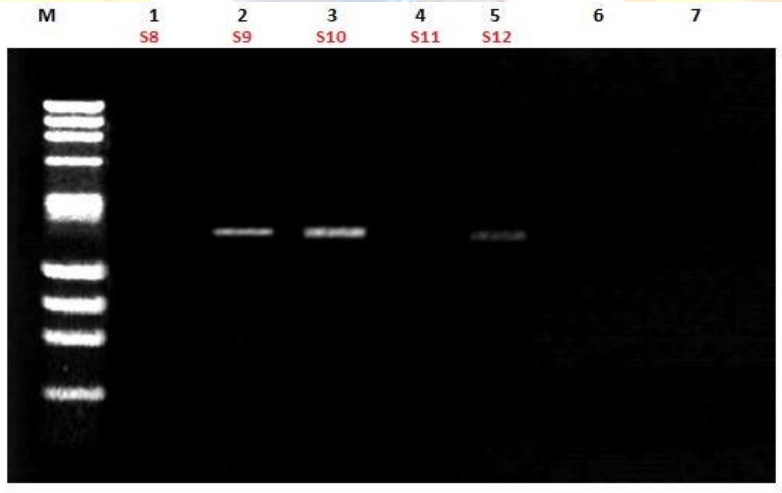


Fig.2: PCR amplification of ESBL producing CTX-M gene from sample 1-12

In 1.5% agarose gel, Lane 2-5: Sample 8(S8) to Sample 12 (S12) with DNA marker M- Lane 1: 100bp ladder. Sample 1 to Sample 7 (S1 to S7) all showed negative (figure not included).

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DISCUSSION

Uropathogenic *E. coli* was responsible for 90% of UTI and 50% of nosocominal infections [25]. The urine samples collected from UTI patients attending hospitals in and around Coimbatore district from both the gender of age 1 to 80 were transported to Bioline laboratories and screened for UPEC. It showed the prevalence of UTI was 3 times more in women when compared to men. Previous studies [26-28] also reported the similar results. Apart from virulence factors, the O serotype of UPEC plays an important role in pathogenecity [29].

The most common serotypes were O2, O4 and O6 [10]. In rare cases we could encounter O8 serotypes in UPEC. Presence of O8 antigen increases the sensitivity towards antibiotics [30]. Since our motive is to check the emergence of O8 serotype as a drug resistant pathogen, we screened and identified 16 isolates. These O8 serotype isolates again showed the prevalence in women of all ages and men above 60 years. Urinary Tract Infection starts from the attachment of UPEC to the epithelial cells of urinary tract by type 1 fimbriae [31] which possess Fim H adhesin protein subunit at its tip. It prevents the pathogens from washing out during urine flow and begins invasion [32]. The MSHA positive isolates (12 samples) which are expected to contain type 1 fimbriae were subjected for gene isolation. Tarchouna et al. [33] said that fim H was most prevalent among all virulence genes by showing 68% of UTI isolates contain the gene. Watts et al. [34] showed 98% fim H in patients with UTI. The PCR products confirms that certain O8 serotype of our isolates (samples S1, S4, S8, S9, S10 and S12) were actively producing fim H gene which helps in attachment and progress of the disease. Yadav et al. [35] reported 79% of UPEC were ESBL producers and were highly resistant. Kandeel et al. [36] demonstrated that location and time of isolation also will influence the development of ESBL. In our study, 100% of the O8 serotype isolates were positive for ESBL production. Studies on ESBL showed that CTX-M was prevalent in about 70% of all the ESBL isolates in Japan, Argentina, United Kingdom, China and Spain [37-41].

In India, CTX-M gene was found in 73% of third generation cephalosporin resistant *E.coli* [42]. We were able to isolate the CTX-M gene from certain isolates (Samples S9, S10 and S12) which indicates the multidrug resistant capability of the O8 serotype UPEC isolates. All the 3 samples were able to produce both fimH and CTX-M genes. The development of ESBL makes the treatment difficult and costlier. Highest resistant of UPEC were seen against ampicillin in Asian countries [43-45]. ESBL strains showed resistance against ciprofloxacin, cotrimoxazole and

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sensitive to amikacin [46]. Yadav et al. [35] reported ESBL cultures that were resistant to cefalexin, norfloxacin, cefrxime, nalidixic acid, ciprofloxacin and ofloxacin. Our O8 serotype isolates showed resistance to cephazolin, cefepime, ciprofloxacin, gentamycin, ampicillin and cepalothin. All O8 serotypes were sensitive to ertapenem, imipenem and meropenem which were similar to the result of Kader et al. [47] who reported sensitivity to imipenem and meropenem.

CONCLUSION

Day by day the emergence of new pathogenic strains and their resistance towards drugs make the treatment of infectious disease very complicated and challenging. This work was designed to identify the emergence of O8 serotype UPEC into a multidrug resistant pathogen of UTI. The rarely occurred O8 serotype has now happened to occur frequently and its sensitivity towards antibiotics has dramatically changed into resistance. We were able to isolate the genes fim H, (encodes an adhesin subunit of type 1 fimbriae) which is meant for attachment to the epithelial cells of urinary tract and CTX-M which are responsible for ESBL production and multidrug resistant. Three isolates were able to produce both the genes which indicates the development of O8 UPEC into a potential pathogen with multidrug resistant. Further molecular studies would help in understanding and treating the pathogen effectively.

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