Original Article

Clinico-microbiological Profile of Urinary Tract Infection with Special Reference to Uropathogenic *E coli* : Antibiotic susceptibility Pattern, Phylogenetic Background and Virulent Factor Distribution from West Bengal, India

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Background and Objectives : Routine surveillance and monitoring studies pose a constant need to update clinicians on prevalent pathogens and rational and empirical treatment in Urinary Tract Infection (UTI). Escherichia coli (*E coli*) is the most commonly isolated uropathogen globally. Extended-Spectrum β-Lactamase (ESBL) production and β-Lactamase Inhibitor Resistance (BLIR) among these pathogens together with their uro-virulence determinants further complicate treatment approaches. This study investigated the clinico-microbiological pattern of UTI and determined the antibiotic sensitivity pattern, the phylogenetic background, and virulence determinants of *E coli*, the most commonly isolated uropathogen.

Methods : Uropathogens isolated by urine culture from community and hospitalized patients were biochemically speciated. Antibiotic susceptibility was tested by Kirby-bauer disk diffusion method. Phylogenetic background and virulence determinants of *E coli* isolates were identified by PCR. SPSS 16.0 was used for statistical interpretation.

Results : 45% of the urine samples showed growth positivity. 44% amongst them were *E coli*. All isolates were multidrug-resistant. 50% and 40% were ESBL producers and BLIR respectively. Former showed highest resistance to quinolone, fluoroquinolones, cotrimoxazole, and latter were resistant against all drugs tested except nitrofurantoin. Significant correlation existed between the β-lactams, quinolone, fluoroquinolones, cotrimoxazole ($p \leq 0.05$) resistance pattern. BLIR and ESBL E coli recorded highest prevalence of pathogenic phylogroup B2 and D respectively. Varied prevalence of fimbrial (fimH, papC, papEF, papG, GII) and toxin genes (iroN, hlyA, cnfI, i ucD, cdtBU) in ESBL, BLIR and non-ESBL isolates were observed. Their distribution was statistically significant ($p=0.05$).

Interpretation and Conclusions : Nitrofurantoin is the drug of choice in empirical treatment of uncomplicated UTI. Aggressive and consistent investigation and health education are highly recommended for effective clinical management in UTI.

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***Key words* : Antibiotic resistance, Phylogenetic background, Virulence factor, Uropathogenic E coli , Urinary Tract Infection.**

Urinary Tract Infections (UTIs) pose a major public health issue globally. In everyday practice, at least for the first 72 hours, treating UTI is empirical. The known risk factors associated with UTI primarily constitute recent hospitalization, history of urinary instrumentation or Urological surgery, Neurogenic infections, Prostate cancer, benign hyperplasia of the prostate and bladder. Additionally, other potential dangers include diabetes, HIV, and immuno-suppressive therapies.

Escherichia coli, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis*, and *Staphylococcus saprophyticus* were frequently identified micro organisms in UTI[1]. 80-90% of all UTIs were caused by *E coli* designated as uropathogenic *E coli* (UPEC) to differentiate the pathogenic species from their commensal types in the intestinal flora. UPEC strains cause 75-95% of uncomplicated and 40-50% of

Editor's Comment :

- ESBL producing *E coli* being the most frequent isolate from complicated and uncomplicated UTI, correct choice of antibiotics in empirical treatment is very important.
- Nitrofurantoin is a good option for treating uncomplicated UTI and BL-BLI for complicated cases. Further decision could be taken after getting the urine CS (Culture & Sensitivity) report in hand.

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complicated UTIs[2].

Earlier, extended-spectrum cephalosporins (ceftriaxone, ceftazidime, cefotaxime,) contributed to the UTI treatment arsenal[3]. Unfortunately, the advent of Extended-Spectrum Lactamases (ESBLs) in *E coli* rendered resistance to these antibiotics to complicate treatment options[4,5].The genes encoding ESBLs were frequently harbored on plasmids with genes encoding resistance to aminoglycosides and sulphonamides. Moreover rampant use of quinolones, fluoroquinolones, aminoglycosides and sulphonamides in UTI also resulted in the selection and dissemination of multidrug-resistant bacteria[6].Many *Enterobacteriaceae spp.* reported mutations that exhibited high quinolone and fluoroquinolone resistance. Therefore advent of multidrug resistance complicated UTI therapy and posed a serious health risk[7].

UPEC exhibits varied Virulence Factors (VFs) to establish UTI. Essential VFs were broadly classified into two categories; cell surface and secretory types. Adhesins encoded by type I fimbriae and *pap* operon assisted in adhesion to host cell surface, an important event in the establishment of infection. Toxins and siderophores supported bacterial colonization and helped in persistence against host defense mechanism. Reports revealed that Horizontal Gene Transfer (HGT) enabled the evolution of UPEC from non-pathogenic strains by the acquisition of different VFs[8]. Moreover, studies on the phylogenetic background of pathogenic UPEC isolates indicated an incidence of phylotypes that belonged to commensal *E coli* strains[9].

Appropriate clinical management in UTI demands information on UTI incidence concerning Gender, Age, Socio-economic status, Clinical symptoms, associated risk factors in the propagation of the illness, and characteristics of causative microorganisms and antibiotic exposures.This study was undertaken to investigate the clinico-microbiological aspects of UTI from Kolkata City in Eastern India with an insight into the antibiotic susceptibility pattern and uro-virulence determinants among *E coli*, the most commonly isolated uropathogen to administer appropriate therapeutics according to the severity of infection.

Materials and Methods

Sample Collection and Bacteriology :

A total of 100 urine samples were collected from outdoor patients of the School of Tropical Medicine Kolkata and patients admitted to Carmichael Hospital of Tropical Disease, Kolkata, with clinical features of UTI. The case history and the clinical investigation data were collected from the hospital records. Urine culture positivity was tested for 24-48 hours. Samples that yielded significant monomicrobial growth (>10^5cfu/ml) were subjected to gram staining and biochemical analysis[10]. The study protocol was approved by the Institutional Ethics Committee.

Antibiotic Susceptibility and Extended-Spectrum β-Lactamase Production :

Kirby-bauer disk diffusion assay was conducted using antibiotics: Ampicillin(AMP; 10mcg), Cefelexin (CN;30mcg), Ceftriaxone(CTR;30mcg), Ceftazidime (CAZ;30mcg), Cefotaxime(CTX;30mcg), Cefoxitin (CX;30mcg), Nalidixic acid(NA;5mcg), Ciprofloxacin (CIP;5mcg), Levofloxacin(LE;5mcg), Amikacin (AK;10mcg), Gentamicin(GEN;10mcg), Tobramicin (TOB;10mcg), Chloramphenicol (C;30mcg), Cotrimoxazole (COT;25mcg), Nitrofurantoin (NIF;300mcg), Meropenem (MR;10mcg). Isolates resistant to CAZ, CTR and/or CTX were subjected to ESBL confirmatory test[11].

Bacterial total DNA isolation, Phylogenetic Background and Virulence gene determination :

Total DNA was released from whole bacterial cells using the boiling method. The phylogenetic background and virulence factors (*fimH, papC, papEF, papGI, GII, GII, iroN, hlyA, cnfI, iucD, cdtBU*) were identified by PCR using gene-specific primers on the total DNA as template[12].

Statistical Analysis :

Data were analyzed by One-way ANOVA, the Bonferroni test and Pearson's correlation coefficient for two-tailed bivariate correlation using SPSS 16.0 software[13]. A $p<0.05$ was considered to be statistically significant.

Results

45 out of 100 urine samples showed significant microbial growth after 24 hours of culture. The remaining samples were culture-negative even after 48-hour incubation. Samples collected from females (60%) revealed higher culture positivity rate than males (40%). Moreover highest (51.1%) culture positivity was reported in community-acquired uncomplicated UTI cases. The occurrence of UTI was mostly reported in the elderly population (>60 years, 28.88%), followed by age group; 41-50 years(26.66%) and 51-60 years (20%). 62.22% of the culture-positive patients belonged to a low socio-economic class with 64.44% without any addiction history towards alcohol or tobacco, 86.66% reported fever, 55.55% dysuria, 51.11% urinary frequency, 31.11% burning micturition, 26.67% suprapubic/flank pain and 6.66% reported urgency. 62.22% had a normal total WBC count (4000-11000), followed by 24.45% having raised WBC count (>10000). A very few patients (13.33%) reported a raised serum urea (>40mg/dl) and serum creatinine

level(>1.2mg/dl). 33.33%, 8.89%, and 51.11% patients reported albuminuria, glycosuria, and pyuria respectively. 86.6% had associated one or more risk factors including diabetes mellitus (73.33%), history of recent hospitalization (26.66%), antibiotic intake (20%), urinary catheterization (13.33%), benign prostatic hyperplasia (11.1%), immunocompromised state (6.66%), renal stone (4.44.%), and history of past UTI(4.44%).

The biochemically speciated pathogens were; *Escherichia coli* (20, 44.4%), *Pseudomonas aeruginosa* (11;24.4%), *Enterobacter spp.*(6;13.3%), *Klebsiella pneumoniae* (5;11.1%), *Staphylococcus aureus* (1;2.2%), *Staphylococcus epidermidis* (1;2.2%) and *Enterococcus spp.*(1;2.2%). The highest prevalence of *E coli* (31%) in community-acquired uncomplicated UTI cases and *P aeruginosa*(11%) and *K pneumoniae* (8.8%) in hospital-acquired uncomplicated UTI cases were observed. Moreover comparable incidence of *E coli* (4.4%, 2.2%*), P aeruginosa* (4.4%, 2.2%) and *Enterobacter spp.* (4.4%, 4.4%) were reported from the community and hospital-acquired complicated UTI cases respectively. *E coli* isolates were further characterized for antibiotic sensitivity and the occurrence of virulence determinants.

E coli isolates revealed high resistance to β-lactams; AMP (95%), CN (80%), CX (90%), CAZ (90%), CTR (85%), CTX (85%), quinolone; NA (85%), fluoroquinolones; CIP (80%), LE(80%) and trimethoprim-sulfamethoxazole; COT (80%). The highest sensitivity was observed against NIF(75%) followed by AK (65%), TOB (65%), GEN (60%), C(50%), and MR(55%) respectively (Fig 1). Overall 14 discrete patterns of resistance were observed among the 20 isolates against the 16 antibiotics tested (Table 1). Furthermore, a significant correlation in the antibiotic resistance pattern was observed amongst the different antibiotics for CTR, NIF and MR respectively (Table 2).

Two out of the 20 *E coli* isolates were sensitive to all three third-generation cephalosporins (3-GC; CTR, CAZ, CTX) however the remaining 18 isolates were resistant to either one/all 3-GCs. ESBL confirmatory test revealed that 10 out of 18 were ESBL producers and 8 were BLIR. A discrete difference in the antibiotic resistance pattern was observed amongst the ESBL and BLIR isolates to the 16 antibiotics tested (Figs 2A,B).

The overall predominance of the pathogenic phylogroup D followed by pathogenic phylogroup B2 and non-pathogenic or commensal phylogroup B1 was observed among ESBL isolates. However, the BLIR isolates primarily belonged tophylogroup B2 followed by B1 and D. Moreover the 2 non-ESBL isolates belonged to phylogroup B2 and B1 respectively (Fig 3).

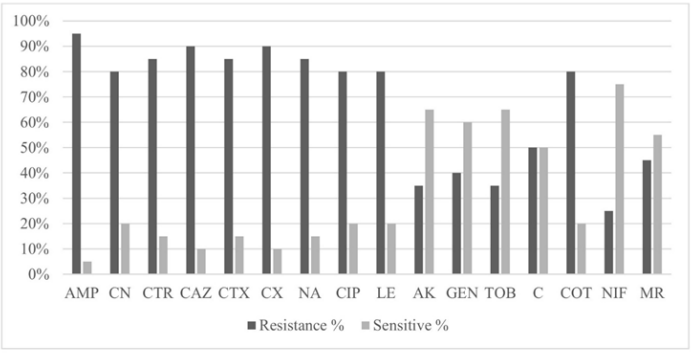

Fig 1 — Drug resistance among the uropathogenic *E coli* isolates (n=20) against various antibiotics. AMP; ampicillin (10μg), CN; cefelexin (30μg), CTR; ceftriaxone (30μg), CAZ; Ceftazidime (30μg), CTX; Cefotaxime (30 μg), CX; Cefoxitin (30μg), NA; Nalidixic acid (30μg), CIP; Ciprofloxacin (5μg), LE; Levofloxacin (5μg), AK; Amikacin (10μg), GEN; Gentamicin (10μg), TOB; Tobramycin (10μg), COT; Cotrimoxazole (25μg), NIF; Nitrofurantoin (300ìg), MR; Meropenem (10μg). All assays were done in triplicate with each pathogenic isolate.

Distribution of virulence gene *fimH* (adhesin gene) from the type I fimbriae operon, *papGI, papGIII* from pap-operon and toxin gene *cdtBU* were higher in BLIR isolates compared to their incidence in ESBL producers. Furthermore, the distribution of other pap-operon genes*papC*, *papEF*, and *papGII* was higher in ESBL isolates in comparison to BLIR isolates. However, the distribution of the toxin genes, *iroN*, *cnf1*, *iucD* were comparable. Additionally, it was observed that the pap-operon genes *papC*, *papEF*, *papGII,* and toxin genes *iroN*, *hylA*, *cdtBU* were absent among non-ESBL producers. (Fig 4). The overall gene frequency of the virulence genes was statistically significant (p=0.05) among the non-ESBL, BLIR, ESBL isolates (Fig 4).

Table 1 — *Resistance pattern of the E. coli isolates (n=20)*

Resistance Pattern	No of isolates
AMP, CAZ, CTX, NA, CIP, COT	1
AMP, CN, CTR, CAZ, CTX, CX, NA, CIP, COT	1
AMP, CN, CTR, CAZ, CTX, CX,	1
AMP, CN, CTR, CAZ, CTX, CX, NA, CIP, LE, AK, GEN, TOB, C, COT, MR	5
AMP, CN, CTR, CAZ, CTX, CX, NA, CIP, LE, C, COT, NIF	2
AMP, CX	1
AMP, CN, CTR, CAZ, CTX, CX, NA, CIP, LE, C, COT, NIF, MR	1
AMP, CN, CTR, CAZ, CTX, CX, NA, CIP, LE, AK, GEN, TOB, COT, NIF, MR	1
AMP, CN, CTR, CAZ, CTX, CX, NA, CIP, LE	1
AMP, CN, CTR, CAZ, CX, NA, CIP, LE, COT, NIF, MR	1
AMP, CN, CTR, CAZ, CTX, CX, NA, CIP, LE, C, COT	1
AMP, CTR, CAZ, CTX, CX, NA, CIP, LE, GEN, C, COT	1
AMP, CN, CTR, CAZ, CTX, CX, NA, CIP, LE, AK, GEN, TOB, COT, MR	1
AMP, CN, CTR, CAZ, CTX, CX, NA, CIP, LE, COT	1

Table 2 — *Correlation of antibiotic resistance pattern in the uropathogenicE. coli isolates (n=20)*

		AMP	CN	CTR	CAZ	CTX	CX	NA	CIP	L	AK	GEN	TOB	C	COT	NIF	MR
CTR	Pearson correlation	.546*	.840**	1	.793**	.608**	.793**	.608**	.608**	.840**	.308	.343	.308	.420	.490*	.243	.343
	Sig. (2-tailed)	.013	.000	-	.000	.004	.000	.004	.004	.000	.186	.139	.186	.065	.028	.303	.139
	Sum of squares & cross products	.850	2.400	2.550	1.700	1.550	1.700	1.550	1.550	2.400	1.050	1.200	1.050	1.500	1.400	.750	1.200
	Covariance	.045	.126	.134	.089	.082	.089	.082	.082	.126	.055	.063	.055	.079	.074	.039	.063
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
NIF	Pearson correlation	.132	.289	.243	.192	-.081	.192	.243	.243	.289	-.182	-.236	-.182	.115	.289	1	.000
	Sig. (2-tailed)	.578	.217	.303	.416	.735	.416	.303	.303	.217	.444	.317	.444	.628	.217	-	1.000
	Sum of squares & cross products	.250	1.000	.750	.500	-.250	.500	.750	.750	1.00	-.750	-1.00	-.750	.500	1.000	3.750	.000
	Covariance	.013	.053	.039	.026	-.013	.026	.039	.039	.053	-.039	-.053	-.039	.026	.053	.197	.000
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
MR	Pearson correlation	.187	.408	.343	.272	.057	.272	.343	.343	.408	.685**	.583**	.685**	.408	.408	.000	1
	Sig. (2-tailed)	.429	.074	.139	.246	.811	.246	.139	.139	.074	.001	.007	.007	.074	.074	1.000	
	Sum of squares & cross products	.400	1.600	1.200	.800	.200	.800	1.200	1.200	1.600	3.200	2.800	2.800	2.000	1.600	.000	4.800
	Covariance	.021	.084	.063	.042	.011	.042	.063	.063	.084	.168	.147	.147	.105	.084	.000	.253
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20

*Correlation is significant at the 0.05 level (2-tailed) ; **Correlation is significant at the 0.01 level (2-tailed)

Discussion

Community-or healthcare-acquired UTIs is clinically divided into complicated or uncomplicated and this classification determines the selection of antibiotics for treatment. Empirical therapy accentuated the emergence of antibiotic resistance, especially in uncomplicated UTI. This study documented information on the various aspects associated with UTI from Kolkata, an Eastern region in India.

Studies from different parts of India revealed a varied urine culture positivity rate ranging from 9.7-53.82%[14-21]. Our study reported an overall high culture positivity rate(51.1%) among community-acquired uncomplicated UTI cases in hospitalized patients with a higher prevalence among females(60%) than males (40%). The latter observation was consistent with findings from other studies[15,18,20]. Highest occurrence of UTI was reported among the elderly population[14,15,17] which was very similar to the observation stated in this study which consisted of a mean age group of 51.95 years that reflected the association between age and complicacy in UTI.

Fever and dysuria were the most common clinical manifestations among the urine culture-positive patients in our study in concurrence to observations from other studies[14,22]. Moreover several studies reported increased frequency to urinate as the commonest symptom in acute uncomplicated UTI patients[14,23]. A similar symptomatology was also observed in our study although the symptoms were not always strong enough to conclude about the predictability of UTI.

Diabetes Mellitus (DM) affects the genitourinary system and frequently causes diabetic nephropathy. It also affects the immune system and enhances the chances of acquiring infection of the urinary tract. In our study, DM posed the most common risk factor associated with UTI (73.33%), very similar to the findings registered in another study[14]. Our study also indicated that clinical manifestations and associated risk factors may not always rightly predict the occurrence of UTI, reaffirming the fact that urine culture is the only confirmatory test to diagnose complicated and uncomplicated UTI.

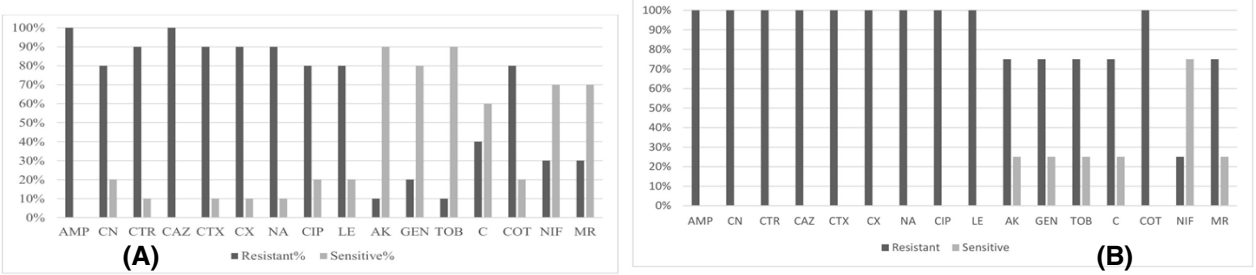

Fig 2 – Antibiogram pattern amongst (A) ESBL and (B) BLIR *E coli* isolates

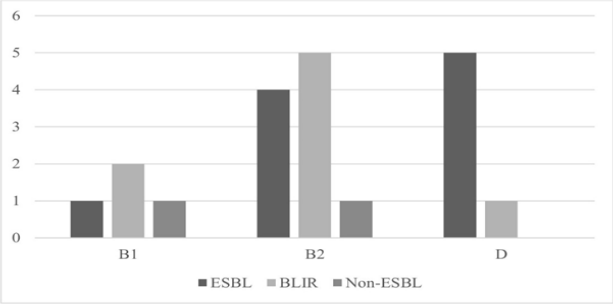

Fig 3 — Phylogenetic background of the ESBL, BLIR, non-ESBL *E coli* isolates

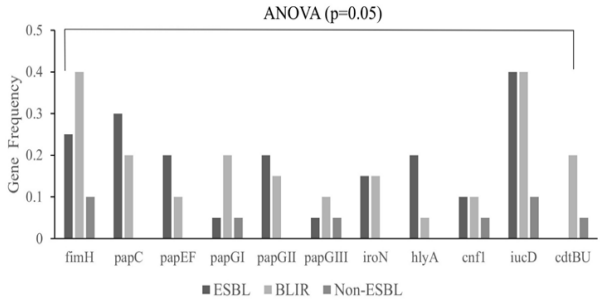

Fig 4 — Distribution of virulence genes among the ESBL, BLIR, non-ESBL *E coli* isolates

Gram-negative bacilli constituted 93.4% of the uropathogens isolated in this study. Our study also demonstrated that *E coli* was the leading uropathogen being responsible for UTI (44.4%) in Kolkata an Eastern region in India which was in concurrence with reports from other studies[15,17,18,20]. However on the contrary several reports also revealed *S aureus*, *P aeruginosa* and *Klebsiella spp.* to be the most predominant uropathogen detected respectively[19,24,25].

The choice of antimicrobials for the treatment of UTI is often based on local resistance profiles of the uropathogen.The increasing pattern of resistance is emerging as a universal threat. In this study, the antibiotic susceptibility was conducted on the most frequent isolate *E coli*. The highest resistance was documented against the 3-GC (CTR, CAZ, CTX) as well as the second generation of fluoroquinolones (CIP, LE) and COT. The highest sensitivity was seen against NIF as well as the aminoglycosides (AK, GEN, TOB). This result was very similar to previous studies conducted in the different States of India[15,17,18].

A significant correlation in the resistance pattern against CTR and CAZ, CTX, CX, CIP, LE, COT and MR and AK, GEN, TOB respectively further delineated the fact that resistance to the cephalosporin (CTR, CX, CAZ, CTX) group of the drug was accompanied by the fluoroquinolone (CIP, LE) group and COT and resistance against aminoglycosides (AK, GEN, TOB) was accompanied by MR respectively. Therefore our study suggested that NIF should be an ideal choice for this population. However orally acceptable NIF formulation has drawbacks and was often not advised for severe upper UTI or individuals with systemic involvement and in such cases, aminoglycosides were the best choice. Therefore routine monitoring and intricate analysis of drug sensitivity patterns among uropathogens pose an absolute necessity to develop proper prescription policies. Our study also successfully detected the ESBL producers (50%) among *E coli* isolates resistant to third-generation cephalosporins (CTR, CAZ, CTX) which was much higher than that reported from other studies[14]. Incidence of BLIR was also observed in 40% of the *E coli* isolates. The ESBL and the BLIR isolates were highly resistant towards the fluoroquinolones in consistent with an Indian study[26]. Our study also indicated that the BLIR isolates were more difficult to treat as they revealed resistance to all drugs tested except NIF. In this study AK,GEN,TOB was found to be effective against the ESBL producers together with NIF, MR and C Siddaramappa, *et al*[26] in his study stated that at least 70% of UTI-associated ESBL-producing *E coli* isolates could be sensitive to chloramphenicol and it may be an appropriate choice to treat the ESBL producers.

UPEC strains that routinely cause infections have been shown to belong to pathogenic phylogroups B2 and D[27]. In our study, 50% of the ESBL producers belonged to phylogroup D followed by B2 (40%) which was in agreement with another previous Indian study[28]. Moreover, for the first time, our study also showed that 62.5% of the BLIR isolates belonged to phylogenetic group B2 followed by B1 (25%) which was distinctly different from the phylogenetic background of the ESBL producers isolated in this study. Furthermore, our study also revealed a varied prevalence of virulence genes (*fimH*, *papC*, *papEF*, *papGII, hlyA, iroN, cnf1, iucD, cdtBU*) across the ESBL and BLIR isolates with the absence of some other virulence genes; *papC*, *papEF*, *papGII, iroN*, *hylA*, *cdtBU* among the non-ESBL isolates very similar to another study that showed a high incidence of multiple virulence genes among the multidrug-resistant ESBL *E. coli*[28]. An earlier study reported that the presence of *papC*, *cnf1* and *hlyA* in UPEC isolates played an important role in recurrent infections of the urinary tract[27]. Therefore the incidence of *papC*(50%), *cnf1*(25%) and *hlyA*(25%) in *E coli* isolated in this study indicated their possibility to cause recurrent infection.

Our study had some limitations. This work relied on very few UPEC isolates from a single hospital. Studies using many isolates from hospitals from different parts of West Bengal could provide broader

insights into the clinico-microbiological aspects of UTI from this Region in India. Future studies of such kind may be very useful to design appropriate clinical management strategies.

Conclusion

Therefore in the present study, we wanted to highlight the importance of investigating the clinico-microbial aspects of UTI together with intricate analysis on the antibiotic sensitivity pattern and distribution of urovirulence determinants among the most frequent uropathogen *E coli.* Moreover increasing resistance against 3GCs together with the presence of β-lactamase inhibitor resistance indicated that Cephalosporins, as well as their inhibitor combination drugs, must not be recommended in treating UTI. However, nitrofurantoin can be started as an empiric antibiotic, which can later be altered according to the drug susceptibility pattern of the uropathogen.

Conflict of interest : The authors declare that they have no conflict of interest.

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