

## Predisposition of Blood Group Non-secretors to Urinary Tract Infection with *Escherichia coli* Anti-microbial Resistance and Acute Kidney Injury

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## Abstract

Urinary tract infection (UTI) causes significant renal damage and disease severity is compounded by antimicrobial resistance (AMR) and other comorbidities in the patient. Blood group antigens secreted in body fluids (secretor status) are known to play a role in bacterial adhesion and we studied its influence on AMR in UTI. A total of 2758 patients with UTI were studied with urine culture, qualitative and semiquantitative urine microscopy, serum creatinine and secretor status in saliva samples by adsorption-inhibition method. Of these, AMR from 300 patients with *E. coli* infection were assessed as per CLSI 2019 guidelines and extended-spectrum beta-lactamase (ESBL) genes ( $bla_{TEM}$ ,  $bla_{CTX-M}$ ,  $bla_{SHV}$ ) and *NDM1* genes were studied using TaqMan probes in Real-time polymerase chain reaction. Patients with UTI were followed up for two weeks. Female patients had higher predilection (57%) for *E. coli* infection while patients with diabetes or non-secretors had none. In our study, ESBL producers were seen in 62% of the *E. coli* isolates and fosfomycin had 100% susceptibility. Non-secretors were significantly associated with acute kidney injury (AKI), AMR and ESBL genes. Multidrug-resistant (MDR) was noted in 127/160 (79.4%) ESBL and 17/18 (94%) *NDM1* gene encoding strains. Quantitative urine microscopy scoring predicted AKI both at presentation and at end of follow up. ESBL producers were common in our study population and non-secretors had a significant association with AMR genes. Urine microscopy scoring system may be a useful tool to predict AKI in patients with UTI.

**Keywords:** Antimicrobial resistance, *Escherichia coli*, Blood group secretor, extended-spectrum beta-lactamase, Acute Kidney Injury, Quantitative urine microscopy

## INTRODUCTION

Upper urinary tract infection (UTI) remains the third most frequent infectious disease next to respiratory and gastrointestinal infections.<sup>1</sup> Women are affected commonly and exhibit two incidence peaks during child bearing age and postmenopausal period with more than 50% of them infected with UTI at least once in their life time. UTI has a global prevalence about 150–250 million cases per year.<sup>2</sup>

*E. coli* is the most frequent uropathogen, followed by *Klebsiella* species across the globe.<sup>3-5</sup> Increased AMR in Enterobacteriaceae restrains the therapeutic options and affects clinical outcome. The overuse of common over the counter antimicrobials is responsible for acquired microbial resistance. Development of AMR is a dynamic event and is a rapidly evolving event with regional differences based on local prescription practices and hence, periodic assessment of antibiotic susceptibility patterns is warranted to facilitate the selection of therapy.

MDR is a major public health concern where there is no reserve drug to treat the critically ill patients. MDR is defined as nonsusceptibility to at least one agent of three different classes of commonly used antimicrobial agents<sup>6</sup>. MDR is commonly seen in methicillin resistant

*Staphylococcus aureus* (MRSA), vancomycin resistant *Staphylococcus aureus*, vancomycin resistant Enterococci, ESBL producing Enterobacteriaceae and metallo beta lactamases (MBL) producing bacteria.<sup>6</sup>

Increasing occurrence of AMR for beta-lactam groups of antimicrobials is a major concern. ESBL has the ability to confer bacterial resistance to penicillin; first-, second- and third-generation cephalosporins; and aztreonam (but not the cephamycins or carbapenems) by hydrolysis of these antimicrobials, and which are inhibited by  $\beta$ -lactamase inhibitors such as clavulanic acid.<sup>7-10</sup> ESBL was first identified in 1983. Within a short span of time ESBL has evolved drastically with over 300 variants being detected in different members of the family Enterobacteriaceae and other non-enteric organisms.<sup>7</sup>

New Delhi metallo- $\beta$ -lactamase-1 (*NDM-1*), a newly described MBL has emerged as a major public health hazard as these organisms hydrolyze all  $\beta$ -lactams including carbapenems except monobactam.<sup>11</sup> Following this, screening for presence of NDM mutation was done in various regions of India, Pakistan and UK, highlighting widespread dissemination of *NDM1* gene.<sup>12,13</sup> Similarly, Jamal WY et al., has reported high prevalence of *NDM1* gene, where all the

patients needed hospitalisation and all the isolates reporting *NDM1* were MDR.<sup>14,15</sup> This led to a situation where there were limited options to treat the deadly infection. Twenty-one *NDM1* variants have been identified in different countries in a rapid pace since 2009, all of which are archived by Khan AU et al.<sup>16</sup> The knowledge of local and regional antimicrobial susceptibility pattern is critically needed to prepare reference of the antimicrobials to be prescribed for first line therapy.

Urinary tract infection is more frequent in Diabetes mellitus due to poor metabolic control, defects in immunity and autonomic neuropathy resulting in incomplete bladder emptying, contribute in the pathogenesis of UTI.<sup>17</sup> UTI and its resistance profile of antimicrobials were studied in patients with diabetes mellitus.

Persons with secretion of blood group antigen in body fluids are called secretors. Absence of respective antigen are called non-secretors. Non-secretor has increased inflammatory response to UTI, such as fever and higher levels of acute phase reactants such as C-reactive protein and erythrocyte sedimentation rate.<sup>18</sup> Blood group secretor status (secretor status) predisposing to several diseases involving mucus layers such as inflammatory bowel diseases as well as other multi-system diseases such as Rheumatic fever and rheumatic heart disease.<sup>19,20</sup> Non-secretor synthesize unique glycolipid receptor on their vaginal epithelial cells and results in increased adherence to *E. coli* and increased risk for recurrent UTI.<sup>21</sup> A study from Japan interpreted association of non-secretor with female acute uncomplicated pyelonephritis caused by *E. coli*<sup>22</sup> and studies from Iraq and Sudan proved that non-secretor had increased susceptibility to UTI in women and children with a greater tendency for adverse symptoms.<sup>23,24</sup> These indicate that the expression of Fut2 gene coding for secretors and its various single nucleotide polymorphisms (SNPs), play a pivotal role in protecting the host from infection.<sup>25</sup> There are limited studies on the association of non-secretors which have crucial effect on the adhesion of *E. coli* and thereby contributing to AMR. This study was designed to aid in the understanding of the role of secretor status in UTI.

UTI commonly causes AKI and is a significant cause of morbidity and can lead to progressive renal damage in settings of MDR. Identification of renal tubular damage and AKI is a critical requirement in clinical settings for early intervention to aid in recovery and minimise damage to renal parenchyma.<sup>26,27</sup> Quantitative urine microscopy scoring system proposed by Perazella et al., is a simple investigative tool for early assessment of renal tubular damage and AKI.<sup>28</sup>

The study was designed to find the association of secretor status with anti-microbial resistance in patients with *E. coli* infection and its correlation with other risk factors such as diabetes mellitus and sex of the patient. We also studied the presence and persistence of AKI in these patients on follow up.

## METHODOLOGY

A prospective study was conducted from June 2017 to June, 2019 in the Department of Microbiology in a tertiary care hospital in South India. A total of 2758 patients with clinical manifestations of upper UTI from both outpatients as well as those admitted in Departments of Medicine and Urology were studied.

### Diagnosis

The diagnosis of UTI were suspected in patients with characteristic symptoms. Pathognomonic features of lower UTI are frequency, urgency, dysuria, and suprapubic pain; and while upper UTI was distinguished by presence of costovertebral angle pain/tenderness, fever, and chills, along lower urinary tract symptoms.<sup>1</sup> All the patients underwent abdominal ultrasound and those with shrunken kidney, suggesting chronic kidney disease were excluded from the study.

Urine specimen were collected from 2758 patients with clinical manifestations of upper UTI except antenatal mothers and paediatric population. Clean-catch midstream urine and catheter aspirates were taken with aseptic precautions<sup>29</sup>, centrifuged and examined for significant pyuria (>10 WBC's/HPF). Urine specimens were processed without delay in the Microbiology laboratory by inoculating into MacConkey and blood agar and incubated

aerobically at 37°C for 24 hours for semi-quantitative culture. Colony count of 10<sup>5</sup> colony forming units (CFU)/ml was defined as significant bacteriuria. Isolates with CFU ≥ 10<sup>5</sup> was processed for further study. Bacterial identification was done by standard biochemical test<sup>30</sup> and present study deals with AMR of the most predominant uropathogen.

#### Qualitative and semi-quantitative Scoring by Urine Microscopy

Ten millilitres (ml) of urine were centrifuged at 1500 rpm for 5min, urine sediment was placed on a glass slide and coverslip was gently applied. The unstained slide was observed in light microscope under high power field (40X) and analysed for red and white blood cells, renal tubular epithelial cells (RTEC), granular cast and hyaline casts. RTEC are defined for “size variation (11 to 15 nm in diameter), shape variation (round to columnar), and a well-evident nucleus with nucleoli”. Granular casts are defined as “fine or coarse granules contained within a cast matrix, whereas hyaline casts are defined as cast matrix without cells and are colourless. Granular casts and RTEC per high-power field were recorded on the data collection sheet as present or absent and, when present, were also quantified as the number counted: one to five, six to 10, and >10 and one to five, six to 20, >20, respectively”.<sup>28</sup> (Table 1)

Uropathogenesis of *E. coli* was confirmed by demonstration of various phenotypic studies like mannose sensitive hemagglutination for Type 1 Fimbriae, mannose resistant hemagglutination for P Fimbriae, crystal violet method for biofilm formation, hemolysin production and serum resistance. *E. coli* from culture broth were studied for presence of mutant genes involved in its pathogenesis like factors associated with bacterial adhesion namely fimH, papA, iutA, and csgA, and bacterial toxicity namely hlyA, cnf1 and iss. The data is being analyzed for association of virulence factors with blood group secretor status and antibiotic resistance and is part of the larger study.

#### Blood Sample Collection and testing for Blood sugar

Two ml of whole blood was collected in clot activator tubes from all the study participants in fasting and 2 hours post-prandial state. Blood sugar was assessed using chemiluminescence

assay by Glucose oxidase-peroxidase method and serum creatinine by modified Jaffe reaction in Beckman Coulter autoanalyzer. WHO guidelines were followed for identifying patients with diabetes mellitus. AKI was defined by “kidney disease improving global outcomes (KDIGO) 2012 guidelines”.

#### Secretor status assessment by adsorption-inhibition method

Saliva from the patient was collected from study participants and used for assessment of secretor status by adsorption-inhibition method.<sup>31</sup> Secretor status assessment was repeated twice and concordant results were taken for assessment. Antimicrobial sensitivity testing

All patients had single *E. coli* isolates and they were processed for antimicrobial sensitivity testing in Muller Hinton agar with the following antimicrobials which are selected by agents from different antimicrobial classes like Aminoglycosides (amikacin 30µg), Penicillin (ampicillin 10µg), Fluoroquinolones (levofloxacin 5µg, norfloxacin 10µg), Nitrofurans (nitrofurantoin 300µg), Phosphonic acids (fosfomycin 200µg), Folate pathway inhibitors (co-trimoxazole 1.25/23.75µg), Third generation cephalosporins (cefotaxime 30µg, ceftazidime 30µg, ceftriaxone 30µg), Penicillins with β-lactamase inhibitors (piperacillin-tazobactam 100/10µg), and Carbapenems (meropenem 10µg). *Escherichia coli* ATCC 25922 strain was used as control. Clinical and Laboratory Standards Institute (CLSI) 2019 guidelines<sup>32</sup> was executed in interpretation of results. AMR was compared in upper UTI patients with blood group secretor in comparison with non-secretors and similarly, diabetics in comparison with non-diabetics.

**Table 1.** Perazella scoring system based on number of granular cast and RTEC seen under high-power field

Score	Description
1	RTE cells 0 and granular casts 0
2	RTE cells 0 and granular casts 1 to 5 or RTE cells 1 to 5 and granular casts 0
3	RTE cells 1 to 5 and granular casts 1 to 5 or RTE cells 0 and granular casts 6 to 10 or RTE cells 6 to 20 and granular casts 0

Note: Score of ≥2 represents strong predictor of Acute Kidney Injury.

**Table 2.** Primer and Probe sequence of genes for Real Time PCR

No.	Gene	Primer
1	blaTEM-70	Thermofisher Scientific assay ID Ba04646128_s1
2	blaSHV	Forward CGGTCAGCGAAAAACACC Reverse CGCAAAAAGGCAGTCAATC probe CCGCCATTACCATGAGCGATAACAG
3	blaCTX-M	Forward GACGTTAAACACCGCCATTC Reverse GGTGGTATTGCCTTTCATCC probe CACTTCACCTCGGGCAATGGCG
4	NDM1	Forward GTCTGGCAGCACACTTCCTA Reverse CGCCATCCCTGACGATCAAAC probe TCTCGACATGCCGGTTTCGG

### Screening and confirmation of ESBL producers

*E. coli* isolates showing resistance to ceftazidime (30µg), cefotaxime (30µg), and aztreonam (30µg) while being susceptible to carbapenems (meropenem) were considered Potential ESBL producers. Those isolates were recruited for ESBL confirmation test by combination disk method based on CLSI 2019 guidelines.<sup>32</sup> As defined by CLSI 2019 guidelines, “the disk used for study of ESBL production was cefotaxime and ceftazidime alone and cefotaxime and ceftazidime in combination with clavulanic acid. A  $\geq$  5 mm increase in growth inhibition zone for any antimicrobial associated with clavulanic acid, in comparison with the inhibition zone of antibiotic tested alone, confirmed ESBL production” by *E. coli* in our study. ESBL was differentiated from Amp C-type  $\beta$ -lactamases, when the isolates were resistant to cephalosporins but sensitive to clavulanic acid in combination with cephalosporins and are resistant to carbapenem.<sup>7-9</sup>

### Genotypic characterisation of Anti-microbial resistance

Bacteria grown overnight in MacConkey and blood agar were inoculated and further grown in LB broth. 1.6 ml of culture broth was used for DNA isolation with Qiagen bacterial DNA isolation kit QIAamp UCP Pathogen Mini kit (catalogue No. 50214) as per the manufacturer’s protocol. Isolated DNA was quantified using Qubit 2.0 fluorometer. Commercial internal control provided by Helini Biomolecules India, were used along with the samples for normalization of the DNA extraction and amplification.

Genes regulating AMR in *E. coli* isolates were detected using Real Time PCR (RT PCR). The genes bla CTX-M, bla<sub>TEM</sub>, bla<sub>SHV</sub> genes and NDM1 gene associated with metallo  $\beta$ - lactamases were studied. TaqMan probes were sourced from Helini, Biomolecules, India for bla CTX-M, bla<sub>SHV</sub> and NDM1 genes while probe for bla TEM was sourced from Thermofisher Scientific (assay ID Ba04646128\_s1) and RT PCR was performed with Applied Biosystem Quant Studio 3 instrument. All the primers and probes (Table 2) were selected from the National Centre for Biotechnology Information website (NCBI; <http://www.ncbi.nlm.nih.gov>) and synthesized by Helini Biomolecules, India. The primers were checked for specificity in a BLAST search available through the NCBI website (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Experiments are repeated thrice to check accuracy and reliability and mean Ct value of less than 30 was used to assess the presence of gene. All PCR assays were run with in-built commercial controls in each run which were run in parallel along with the samples right from isolation step. We used RT PCR for detection as we had used Taqman probes for better sensitivity and specificity of detection. Quantification was not a part of the study design. The base pair sequence mentioned in Table 2.

The sensitivity of the Taqman probes for each antimicrobial resistance gene was determined by serial dilution of the DNA elute from the culture isolates. DNA isolation was done using the QIAprep mini prep system. Overnight *E. coli* culture was inoculated into Luria Bertani broth and turbidity matched with 0.5 Mac Farland standards and

**Table 3.** Demographic distribution of patients in the study with Diabetic and Secretor status

Age group (Years)	Total number (%)	Male number (%)	Female number (%)	Non-secretor number (%)	Secretor number (%)	DM number (%)	Non-DM number (%)
14-25	22(7.3)	7(31.8)	15(68.2)	8(36.4)	14(63.6)	2(9.1)	20(90.9)
26-65	194(64.7)	82(42.3)	112(57.7)	122(62.9)	72(37.1)	73(37.6)	121(62.4)
>66	84(28)	39(46.4)	45(53.6)	53(63.1)	31(36.9)	36(42.9)	48(57.1)

1.5ml of broth is used for DNA isolation. The DNA isolated was quantified using Qubit 2 fluorimeter from ThermoFisher. The average quantity of DNA isolated in our study samples was 12 micrograms with DNA concentration ranging from 10 - 14 micrograms.

Ten microlitres of DNA was used for further amplification with the Taqman probe. To determine the sensitivity of the probe, we used serial dilution of the DNA elute from the culture broth as well as the reference standard in range from 1:100, 1:1000 and 1:10,000 and 1:100,000. The standards were replicated thrice to ensure higher replicate number and to ensure the sensitivity at a lower copy number input. The R2 value for the standard curve ranged from 0.98 to 0.99.

For each RT PCR run, the threshold line was set above the background fluorescence, intersecting at the reaction curve at the beginning of its exponential phase. The RT PCR was set for 40 cycles of amplification. Ct value less than 30 was determined as the cut off for detection of antimicrobial resistance for our target genes.

Specificity was determined by comparing the results of the Taqman probe amplification of the antimicrobial resistance with ATCC *E.*

*coli* 25922 and in-built commercial controls. In our assays, we did not have any false positive amplification of the genes

#### Statistical Analysis

Statistical interpretation of data was done by analysis with STATA software. Pearson Chi2 test was used to analyze categorical variable and p-value of less than 0.05 is interpreted as statistically significant.

#### RESULTS

A total of 2758 patients were studied, of whom 1711 were male and 1047 were female. Age group of patients ranged from 14-97 years. Significant bacteriuria was observed in 529 (19.2%) samples of which 427 (80.7%) were Gram negative bacilli. The most commonly isolated organism was *E. coli* (56.7%), followed by *Klebsiella* (18.1%), Enterococci (10.8%), *Pseudomonas* (5.8%) and *Staphylococcus aureus* (4.3%), other Gram-negative bacilli (2.3%) and yeasts (1.8%).

Our study focused on the patients with upper UTI, caused by *E. coli*, who had at least two weeks follow up and 300 patients fulfilled the criteria. The distribution of age, gender and diabetes mellitus in UTI patients with *E. coli* infection were studied. UTI is common in females

**Table 4.** Association of Diabetic and Secretor status of the patient with AKI at presentation and at 14 days follow up

Diabetes mellitus	AKI at presentation		Pearson Chi <sup>2</sup> P value	AKI at 14 days follow up		Pearson Chi <sup>2</sup> P value
	Absent	Present		Absent	Present	
Absent	140(73%)	49(26%)	0.001	162(85.7%)	27(14.3%)	0.001
Present	61(55%)	50(45%)		78(70.3%)	33(29.7%)	
Total	201(67%)	99(33%)		240(80%)	60(20%)	
Secretor status	Absent	Present	0.000	Absent	Present	0.005
Nonsecretor	104(56.8%)	79(43.17)		137(74.9)	46(25.1)	
Secretor	97(82.9%)	20(17.09)		103(88.03)	14(11.97)	
Total	201(67%)	99(33%)		240(80%)	60(20%)	



**Table 5.** Correlation of secretor status with sex, diabetic status and AKI in patients with UTI

Demographic and Clinical Parameters	Non-secretor N (%)	Secretor N (%)	Pearson Chi <sup>2</sup> P value
Sex			
Male	83(64.8%)	45(35.2%)	0.239
female	100(58.1%)	117(41.9%)	
Diabetes mellitus			
Non-DM	97(51.3%)	92(48.7%)	0.000
DM	86(77.5%)	25(22.5%)	
AKI			
Absent	104(51.7%)	97(48.3%)	0.000
present	79(79.8%)	20(20.2%)	

**Table 6.** Antibiotic resistance pattern in UTI by *E. coli* n=300 and in ESBL producer

Antimicrobials	<i>E. coli</i> n(%) n= 300 Resistance	ESBL producer n(%) n=160 Resistance
Ceftazidime	199 (66.3)	160 (100)
Cefotaxime	206 (68.7)	160 (100)
Ceftriaxone	206 (68.7)	160 (100)
Ampicillin	209 (69.7)	160 (100)
Piperacillin-Tazobactam	95 (31.6)	52 (32.5)
Meropenem	42 (14)	-
Norfloxacin	266 (88.7)	146 (91.2)
Levofloxacin	262 (87.3)	132 (82.5)
Amikacin	51 (17)	27 (16.9)
Cotrimoxazole	204 (68)	127 (79.4)
Nitrofurantoin	40 (13.3)	22 (13.7)
Fosfomycin	0 (0)	0 (0)

172 (57.3%) across all age groups, while diabetics constituted 37% of the study population (Table 3). In our study population 77.5% of diabetics were non-secretors and blood group non-secretors were significantly associated with type II diabetes mellitus ( $p < 0.000$ ). Diabetes mellitus (DM) was not a statistically significant predisposing factor for upper UTI in our patient population. DM was significantly associated with AKI, at presentation as well as at the end of two weeks ( $p = 0.001$ ), (Table 4) while non-secretors had significant association with AKI at presentation ( $p < 0.000$ ) as well as at the end of two weeks follow-up ( $p = 0.005$ ). (Table 4,5)

Urine microscopy semi-quantitative score was found to be significant in identification of AKI

at the time of diagnosis and predicted persistence of AKI at 14 days follow up. ( $p < 0.000$ )

Patients in the study had no history of antibiotic intake in the past 48 hours, before onset of symptoms. All the studied isolates were resistant to at least two of the tested antibiotics. Maximum resistance was found for fluoroquinolones (87%), third generation cephalosporins (68%) and cotrimoxazole (68%). Maximum sensitivity was found for fosfomycin (100%) followed by nitrofurantoin (86%), meropenem (86%) and amikacin (83%) (Table 6).

*E. coli* culture resistant to meropenem (42/300) were excluded in the ESBL confirmation test as there is a chance that AmpC beta lactamases may mislead the test and gives false positive. About 160/258 (53.3%) of *E. coli* isolates were identified as ESBL producers by combination disk (phenotypic) method. ESBL-producing *E. coli* isolates were more resistant to antimicrobials as in Table 6. MDR was noted in 127/160 (79.4%) of ESBL producers.

On correlation of AMR in *E. coli* with secretor status in saliva, non-secretors were found to have significant association with resistance to ampicillin ( $p < 0.000$ ), third generation cephalosporins ( $p < 0.000$ ). About 124/187 (66.3%) of MDR *E. coli* were from non-secretor patients. Diabetes mellitus in patients did not have any statistical correlation with AMR. Table 7

Molecular detection of ESBL-producing *E. coli* isolates revealed high prevalence of bla CTX-M gene in 88.7% of *E. coli* followed by bla SHV (34.4%) and bla TEM (11.25%). The simultaneous presence of all the three ESBL were seen in 5%

**Table 7.** Association of Resistance to antibiotics in non-secretor and diabetic patients

Antibiotic resistance	Non-secretors n(%) Total N=183 Resistance	Pearson Chi <sup>2</sup> value (P value)	Diabetic patients n(%) Total N=111 Resistance	Pearson Chi <sup>2</sup> value (P value)
Ampicillin	147(80.3%)	25.2379 (<0.001)	80(72.1%)	(0.487) 0.4824
Amikacin	29(15.8%)	0.4421 (0.506)	13(11.7%)	3.4921 (0.062)
Cefotaxime	145(79.2%)	24.3582 (<0.001)	78(70.3%)	0.2106 (0.646)
Ceftriaxone	145(79.2%)	24.3582 (<0.001)	78(70.3%)	0.2106 (0.646)
Cotrimoxazole	130(71%)	1.9906 (0.158)	77(69.4%)	0.1518 (0.697)
Fosfomycin	0	-	0	-
Nitrofurantoin	23(12.6%) (0.626)	0.2377	10(9%)	2.8512 (0.091)
Norfloxacin	159(86.9%)	1.4818 (0.223)	98(88.3%)	0.0251 (0.874)
Meropenem	40(21.9%)	1.0123 (0.314)	25(22.5%)	0.7007 (0.403)
ESBL production	115(62.8%)	23.2722 (<0.001)	62(55.9%)	0.6971 (0.706)

**Table 8.** Frequency of extended-spectrum beta-lactamase genes (n=160) and *NDM1* gene (n=300), isolated in *E. coli* isolates

Name of gene	Frequency (%)
<i>bla</i> <sub>CTX-M</sub>	142 (88.7)
<i>bla</i> <sub>TEM</sub>	18 (11.25)
<i>bla</i> <sub>SHV</sub>	55 (34.4)
<i>bla</i> <sub>TEM</sub> + <i>bla</i> <sub>CTX-M</sub>	18(11.25)
<i>bla</i> <sub>CTX-M</sub> + <i>bla</i> <sub>SHV</sub>	20(12.5)
<i>bla</i> <sub>CTX-MV</sub> , <i>bla</i> <sub>TEM</sub> and <i>bla</i> <sub>SHV</sub>	8 (5)
<i>NDM1</i>	18 (6)
<i>NDM1</i> + <i>bla</i> <sub>CTX-M</sub>	15(83.3)

of the isolates. When we studied *NDM1* gene, it was seen in 6% of *E. coli* isolates from patients with upper UTI, which reflected carbapenem resistance. Most of the isolates encoding *NDM1* (15/18) coexist with *bla* CTX-M (Table 8). All the *NDM1* positive isolates except one was found to be multi-drug resistant 94% (17/18).

Genotypic detection of ESBL producing *E. coli* isolates also revealed significant association of *bla* CTX-M ( $p < 0.000$ ), *bla* TEM ( $p = 0.01$ ) and *NDM1* ( $p = 0.012$ ) genes with non-secretors while *bla* SHV was not found to be significant associated

with non-secretors in our study subset. (Table 8)

AMR in bacterial culture and its genotypic characterization were not significantly associated with AKI in our study.

## DISCUSSION

UTI is a significant cause for AKI thereby leading to morbidity and AMR is a constant threat to severe and persistent AKI. AMR is also constantly evolving due to differences in prescription practice, patient's co-morbidities and disease manifestations in different clinical settings. Secretor status is known to influence bacterial adhesion and we found that non-secretors had strong association with AKI and diabetes mellitus in UTI patients. Semi-quantitative urine microscopy scoring was useful in identification of AKI and is a potentially inexpensive tool, meriting further study.

The study attempts to address the deficiency on the association of secretors with UTI and AKI. We studied the AMR in urine culture and presence of genes associated with the resistance properties and correlated the same with AKI and secretor status. This study highlights the significant association of non-secretors with AMR.



A study from Egypt shows low resistance to quinolones and this may be due variations in prescription and resistance pattern.<sup>33</sup> In our study, fluoroquinolones show maximum resistance followed by cephalosporins and cotrimoxazole which was similar to other studies.<sup>4,34</sup>

Though there is significant discrepancy in relative distribution of ESBL producing Enterobacteriaceae among different regions in the world, there has been an overall exponential increase recently adding a tremendous risk of patients with UTI who do not respond to treatment.

Several studies from India have reported 60-80% ESBL production of *E. coli* in UTI.<sup>35-38</sup> Whereas in a multicentric study conducted across various regions of India by Gautham V et al.<sup>39</sup> found ESBL production in about 35% of study population. Studies across the globe showed distribution of ESBL prevalence from 8% to 62% due to varying prescription practices.<sup>40-42</sup> Our study reports a higher level of ESBL production of about 62%.

Increase in ESBL producing *E. coli* as a cause of UTI is attributed to several factors such as antibiotic policy and multiple other risk factors including common infections in different geographic areas and hospitalization for various indications.<sup>3</sup> Also, there are various risk factors acquired from community conferring the risk factor for bla CTX-M production in this subset.<sup>43,44</sup>

In early 1960s the plasmid-mediated  $\beta$ -lactamase was first described in Gram-negative bacteria and named TEM 1, followed by SHV-2 enzyme which was capable of hydrolysing these antibiotics in Germany.<sup>45,46</sup> An enzyme which hydrolyses Cefotaxime was identified and named CTX-M47 and since 2000, this is predominant in different settings like Republic of Korea<sup>48</sup> and Morocco<sup>49</sup> having surmounted other ESBL enzymes viz., TEM and SHV having around 40 CTX-M subtypes.<sup>7</sup> Studies conducted in different parts of India also reported a high prevalence of CTX-M-type ESBL<sup>3,50,51</sup>, which was reflected in the findings of present study and referred this current explosive spread of CTX-M as "CTX-M pandemic".<sup>52</sup>

In our study bla CTX-M gene was detected in 88.7% followed by bla SHV and bla TEM and the findings are comparable with Canton R et al.<sup>53</sup>, but not in agreement with the results of studies by Hassuna NA et al.<sup>33</sup>, Gautham V et al.<sup>39</sup>, Bajpai

T et al.<sup>54</sup>, Jena J et al.<sup>55</sup> as bla<sub>TEM</sub> gene was the predominant one. However, the combination of ESBL genes differed in studies between study centres. High prevalence of coexistence of two genes bla<sub>CTX-M</sub> and bla<sub>TEM</sub> was reported by Hassuna NA et al.<sup>33</sup> and Kammili N et al.<sup>36</sup> which correlates with our results whereas Al-Jamei SA et al.<sup>42</sup> reported higher prevalence in combination of bla CTX-M and bla<sub>SHV</sub>. This highlights the emerging complexity of antibacterial resistance in different regions of the world and warrants further studies.

In present study, maximum sensitivity was found for fosfomycin followed by nitrofurantoin, meropenem and amikacin. Fosfomycin and nitrofurantoin were the oral antimicrobials with broad spectrum of activity. In 2011 Infectious Diseases Society of America (IDSA) established guidelines for use of nitrofurantoin and fosfomycin (single-dose) as first-line treatment for uncomplicated UTI<sup>56</sup>. Several studies in India have reported around 95% sensitivity for Fosfomycin and also reported its utility in MDR *E. coli*.<sup>5,37,38,57</sup> Studies across different countries shows nearly 85-100% susceptibility to fosfomycin and minimal propensity for collateral damage.<sup>14,40,41,58</sup> In our series, we found 100% sensitivity for fosfomycin due to this drug not being in common therapeutic use.

Previously, nitrofurantoin and amikacin were thought to be contraindicated in renal impairment. But recently risk-benefit analysis recommends cautious, short-term use in mild renal impairment patients, if there are no alternative antimicrobials in MDR settings.<sup>59-61</sup> Amladi AU et al., shows only 56% sensitivity to nitrofurantoin<sup>14</sup> whereas in our study, it shows 87% sensitivity highlighting that it can be a reserve drug when other drugs are resistant for the organism.

Patients with diabetes mellitus did not show any statistical association with AMR in our study and the results were similar to findings by Meiland R et al.<sup>62</sup> Ghenghesh KS et al.<sup>63</sup> However, Malmartel A reported increased AMR in UTI patients with diabetes mellitus.<sup>64</sup>

We found significant association of AMR with non-secretors, particularly with beta lactam group of antimicrobials and with ESBL producers and related genes. Non-secretors are thus found to be vulnerable for antimicrobial resistance. In our study, non-secretors had higher predisposition

of upper UTI infection with *E. coli*. These findings correlated with the findings from Stapleton<sup>21</sup> and Dahash SL<sup>23</sup> where they had found correlation with persistence and recurrence of UTI due to *E. coli*. Our study highlights that non-secretor were at higher risk of antibiotic resistance in *E. coli*. Similar observations have been found for non-secretors in association with antibiotic susceptibility.<sup>25,65-66</sup> We did not find much cross-reference linking AMR and AKI, with blood group secretor status. This is an important observation requiring further study in a larger subset. AMR leading to AKI significantly increases the cost of treatment at every level from investigation workup required, need and duration of hospitalization and need for multiple different groups of medications with follow up visits due to frequent recurrences and visceral damage due to resistant organisms.<sup>67</sup>

Diabetes mellitus was associated with AKI in our study and other groups have found similar results.<sup>21</sup> Semi-quantitative urine microscopy has been studied by other authors from different geographic settings to assess AKI and our study results are similar in detection and prediction of persistence of AKI in the first two weeks.<sup>68</sup>

In our study, we did not study the single nucleotide polymorphisms (SNPs) associated with blood group secretor status, as studied by many of the recent studies due to the possibility of variations in the SNPs in different populations. However, this is a limitation in our study as we have studied only the phenotypic expression of blood group antigens in saliva and this needs to be explored further to understand the pathogenesis of increased risk for AMR among non-secretors. Lack of long-term clinical follow-up in our study is another major limitation, as we could follow up the patients only for an average of two weeks from the time of diagnosis.

To conclude, we studied patients with upper UTI due to *E. coli* in a tertiary care institution for AMR along with risk factors. Women were at a higher risk of upper UTI across all age groups. Fluoroquinolones, third generation cephalosporins, and cotrimoxazole had maximum resistance while older antibiotics such as fosfomycin, nitrofurantoin, meropenem and amikacin had maximum sensitivity to *E. coli*. With increasing resistance against the current treatment options, older drugs may emerge as effective

options. We also detected higher sensitivity to yesteryear antibiotics such as fosfomycin. We did not find any association of AMR with diabetes mellitus but non-secretor assessed by phenotypic method was found to be significantly associated with resistance to beta lactam antibiotics as well as with ESBL producers and AKI.

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#### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

#### AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

#### FUNDING

None.

#### DATA AVAILABILITY

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

#### ETHICS STATEMENT

The study protocol was approved by the Institute Ethical Committee (No.GHEIC/2016 dated 22/12/2016) of Indira Gandhi Government General Hospital and Postgraduate Institute - 605001. Informed written consent was received from all the participants.

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