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Original Article

Microbiology Section

Burden of Multidrug Resistant *Escherichia coli* among Patients with Urinary Tract Infections in a Tertiary Care Centre- A Retrospective Analysis

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ABSTRACT

Introduction: Multidrug Resistant (MDR) *Escherichia coli* isolates causing Urinary Tract Infections (UTIs), capable of producing Extended Spectrum Beta Lactamase (ESBL), AmpC beta lactamase and resistance to carbapenems pose serious challenges to the clinicians causing worse clinical scenarios. The detection of these isolates has prime importance in infection control and improving therapeutic management of patients, as their prevalence is increasing worldwide. Injudicious use of antibiotics has led to selective pressure, resulting in the emergence of antibiotic resistance in gram negative bacteria globally. This rise in antimicrobial resistance has decreased the options for empirical therapy, causing a global health concern in infections associated with ESBL producing and AmpC producing isolates.

Aim: To investigate the burden of ESBL producing, AmpC beta-lactamase producing, carbapenem resistant, and MDR *Escherichia coli* isolated from urine samples in a tertiary care centre.

Materials and Methods: The present retrospective study conducted from January to December 2019. A total of 10,535 urine samples were received during the study period, to find out the burden of drug resistant *Escherichia coli* isolates in urine samples, analyse their antibiogram and patients' socio-demographic information. Records of microscopic observations to antibiogram of each isolate was noted down from the register for urine samples, and further analysed. The data was coded, verified, entered and analysed using Statistical Package for Social Sciences (SPSS) version 18.0.

Results: Among the total urine samples (10,535), 1434 (13.6%) *Escherichia coli* isolates were identified, of which 553 (38.6%) were found to be ESBL producing, 497 (34.6%) AmpC beta-lactamase producing, 172 (12%) Carbapenem resistant, and 765 (53.3%) MDR. Majority of the isolates were from the age group of 51-60 years 288 (20.08%). The least susceptibility of the isolates was detected against penicillin G 1410 (98.3%), followed by ampicillin 1160 (80.9%) and cephazolin 74 (67.9%). Among the MDR isolates, the maximum drug resistance (398, 52%) was seen in penicillin, cephems, quinolones and Folate pathway antagonist group of antimicrobials.

Conclusion: Identification of the drug resistant isolates is of high priority and crucial for therapeutic management of the patients, and infection control. Stringent antimicrobial stewardship policies and judicious use of antimicrobials can decrease the spread of antimicrobial resistant genes in the hospital environment.

Keywords: AmpC β-lactamases, Carbapenem resistance, Co-morbidities, Co-infection, Extended spectrum beta lactamase

INTRODUCTION

The UTI is the most common infection affecting all age groups and is defined as a condition in which bacteria are established and multiply within the urinary tract [1]. Globally, 150 million UTIs are estimated to occur per annum, among which hospital associated UTIs account for 35% [2]. According to many studies it is evident that females are more commonly affected and approximately 60% of women and 12% of men will have at least onetime experienced symptomatic UTI during their lifetime with 24-50% of them will have recurrent events of UTI [3,4].

Urinary tract infections may be classified as community or hospital acquired UTI depending on the place of acquiring the infection. UTI can involve either upper or lower urinary tract or both if extended. Most common organism is P-fimbriated strains of *Escherichia coli* (*E. coli*) which adhere to uroepithelial cells [1,5,6].

Uncomplicated UTIs commonly occur in healthy people, without any physiological abnormalities in the urinary tract. Prevalence of both symptomatic infections and asymptomatic bacteriuria among pregnant women in India varies from 3-24% and asymptomatic bacteriuria ranging between 5-12%, while it ranges from 2-7% among developed countries [5-8]. Complicated UTIs compromise the urinary tract or host defence, which may be due to obstruction by caliculi, stricture, enlargement of prostrate or congenital anomalies and instrumentation and catheterisations. The host risk factors are those having immune suppression as seen in transplants, Human Immuno Deficiency Virus (HIV)/Acquired Immuno Deficiency Syndrome (AIDS) and diabetes mellitus [1,5,9].

Catheter Associated Urinary Tract Infections (CAUTI), account for over one million cases of hospital and nursing homes associated UTI annually, most of the organisms which are biofilm forming in urinary catheters. Cystitis and pyelonephritis can cause severe problems compared to other UTIs [10]. Hospital acquired UTIs have been very commonly isolated from Intensive Care Units (ICUs), wherein the leading aetiological agents include-*Escherichia coli*, followed by *Citrobacter* spp., *Klebsiella* spp., *Candida* spp., *Enterococcus* spp., and *Pseudomonas* spp [9,10].

Antimicrobial resistant organisms are increasing in prevalence especially MDR has become notable over the years, particularly in hospitalised patients. It is evident from various studies that recommended empirical drugs (quinolone family antibiotics) cannot be implemented in all regions since not only bacterial profile, but also antimicrobial resistance varies from one region to another, evidenced through many studies. So, for patient management, good local data is essential. Inappropriate selection of empirical drugs not only increases the cost of treatment but also increases the evolution of drug resistance by selection [11].

Antibiotic resistance of *E. coli* is mostly due to acquired resistant genes obtained through integrons which belong to mobile genetic

elements. Intrinsic resistance due to efflux mechanism is seen against drugs acting on outer membrane like vancomycin and daptomycin. Quinolone resistant gene (qnr) was initially discovered in a strain of *Klebsiella pneumoniae*, recent studies of which indicate a higher prevalence in *E. coli* isolates. The injudicious use of antimicrobials has led to the increasing resistance in *E. coli* isolates. Thus, continuous monitoring of the emerging resistance in *E. coli*, and finding new therapeutic modalities against these organisms is crucial, to reduce the rates of bacteriuria and mortality in patients [12].

This study aims to detect the prevalence of UTIs caused by *E. coli*, analyse the pattern of antimicrobial susceptibility pattern, prevalence of MDR isolates and compare with various studies over a period of 10 years to find the increasing or decreasing pattern in prevalence of MDR isolates, and detect the common co-infections associated.

MATERIALS AND METHODS

A retrospective analysis of prevalence and antimicrobial resistance of *E. coli* isolated from urine samples of patients received in microbiology laboratory, in a tertiary care centre, was performed. All *E. coli* isolated from urine samples for a period of one year, from January to December 2019 were analysed and the collected data was interpreted during the month of January 2020. The data for the retrospective study was taken from the secondary data available as records maintained for isolation of organisms and their antimicrobial susceptibility pattern in urine samples. The Institutional Ethics Committee (IEC) approval was obtained from the Ethical committee with the ethical clearance number 2020/IEC/2020. A total of 10,535 urine samples were received during the study period.

All patients' urine samples received in the microbiology laboratory was processed, following which the respective information regarding age, gender, ward type, clinical conditions, microscopic observation of urine wet mounts were included in the study as well.

Inclusion criteria: All urine samples for culture in the microbiology laboratory were included in the study.

Exclusion criteria: Samples other than urine and isolates other than *E. coli* were excluded from the study.

Microbiological Examination

Clean catch mid-stream urine samples collected under aseptic conditions, were received by the laboratory which were processed with microscopic examination using wet mount method to observe the presence of pus cells, epithelial cells, and the presence of bacteria/yeast, and cultured on UTI CHROMagar (Chromogenic agar) and 5% Sheep Blood Agar (SBA) to isolate the aetiological agents.

The presumptive identification of the isolates was done with colour of colony on UTI CHROMagar and further confirmation was by biochemical tests and antimicrobial susceptibility tests performed as per standard CLSI guidelines [13]. Gram negative organisms were identified by oxidase test, indole test, mannitol motility medium, triple sugar ion test, Simmons' citrate and Christenson's urea agar test as previously mentioned [14].

Significant bacteriuria was considered if the colony forming units in the clean catch mid-stream urine received was $>10^5$ CFU/mL, and 10^3 CFU/mL in case of catheterised patients.

Antimicrobial Susceptibility Tests

Antimicrobial susceptibility test was performed following Kirby-Bauer disc diffusion test and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines except for colistin and polymyxin B (CLSI 2017 because of nonavailability of MIC) [13,15]. The cultured plates were incubated for 16-18 hours at 35°C and the results were interpreted by measuring the inhibition zone around each antibiotic disc.

Screening for ESBL Producing, AmpC Beta-lactamase Producing and Carbapenem Resistant Isolates

The ESBL production was tested in the isolates as per CLSI guidelines 2021 [13]. Screening of carbapenem resistance was performed phenotypically by testing non susceptibility of the isolates to all carbapenems (ertapenem, meropenem, imipenem) as per CLSI guidelines [13]. AmpC beta-lactamase producing isolates were suspected by screening of the isolates using disk-diffusion method in which cefoxitin (30 µg) disk was used. Isolates producing an inhibitory zone diameter of ≤18 mm were suspected as AmpC beta-lactamase producing [16,17].

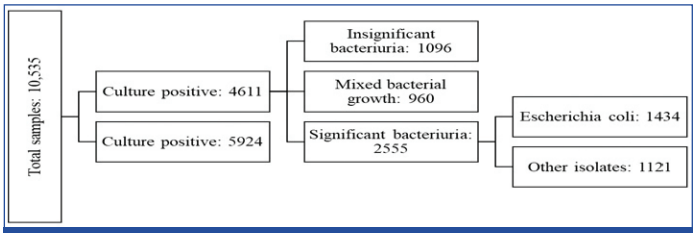
They were further classified as MDR-non susceptible to ≥1 agent in ≥3 antimicrobial categories, and Extensively Drug Resistant (XDR)-non susceptible to ≥1 agent in all but ≤2 categories.

STATISTICAL ANALYSIS

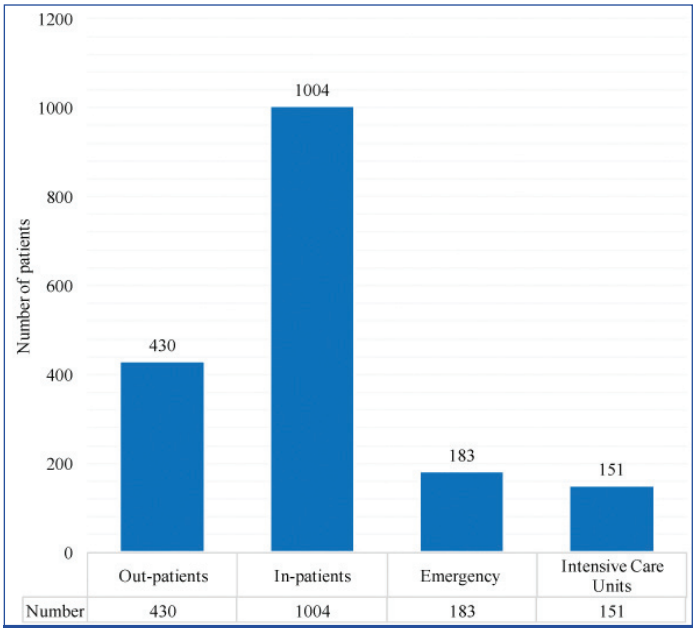
Statistical analysis was done on Microsoft Excel. The data was coded, verified, entered and analysed using Statistical Package for Social Sciences (SPSS) version 18.0.

RESULTS

A total of 10,535 urine samples were received during the study period of one year, among which 4611 (43.7%) samples were found to be culture positive. Among the culture positive samples 2555 (55.4%) samples revealed significant bacteriuria, of which 1434 (56%) isolates were identified as *E. coli* [Table/Fig-1]. Of the study population, outpatients constituted 430 (30%), and inpatients 1004 (70%) [Table/Fig-2]. Most of the isolates were from the age group of 51-60 years (20.08%) and closely followed by 21-30 years (17.9%) predominated by women [Table/Fig-3].



[Table/Fig-1]: Distribution of culture positive and culture negative urine samples.



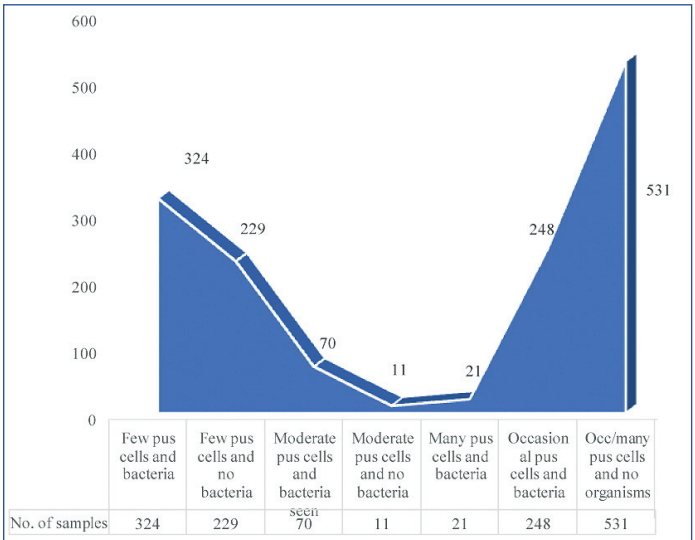
[Table/Fig-2]: Distribution of study population by ward.
Patients from other wards included in Inpatient ward only {urology (92), Nephrology (61), General Medicine (192), General surgery (155), Casualty (80), Obs/Gyn (56), Labour (34)}

Wet mount microscopic observation of urine from study population revealed that, out of 1434, 324 (22.6%) samples had few pus cells

Age group (in years)	Men	Women	Total
<1	5	6	11
1-10	31	34	65
11-20	31	46	77
21-30	61	196	257
31-40	50	78	128
41-50	74	106	180
51-60	138	150	288
61-70	126	102	228
71-80	69	61	130
81-90	34	32	66
>91	3	1	4
Total	622	812	1434

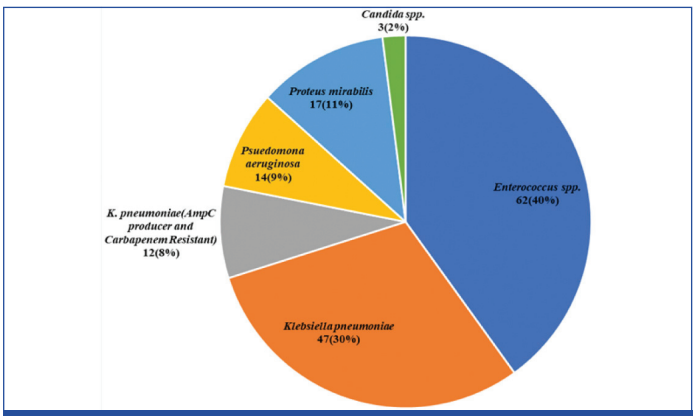
[Table/Fig-3]: Distribution of study population by age and gender.

with bacteria, and majority of samples 531 (37%) showed occasional pus cells without any organisms [Table/Fig-4]. Significant bacteriuria was predominately seen in women 1556 (60.9%) compared to men 999 (39.1%). In present study the number of patients with bacteriuria ≥10^5 CFU/mL was 1170 (81.6%) in which men and women were comparable. Colony counts of 10^4 CFU/mL 102 (7.1%) and 10^3 CFU/mL 152 (10.6%) constituted a lesser percentage of the total patients, mostly predominated by men.

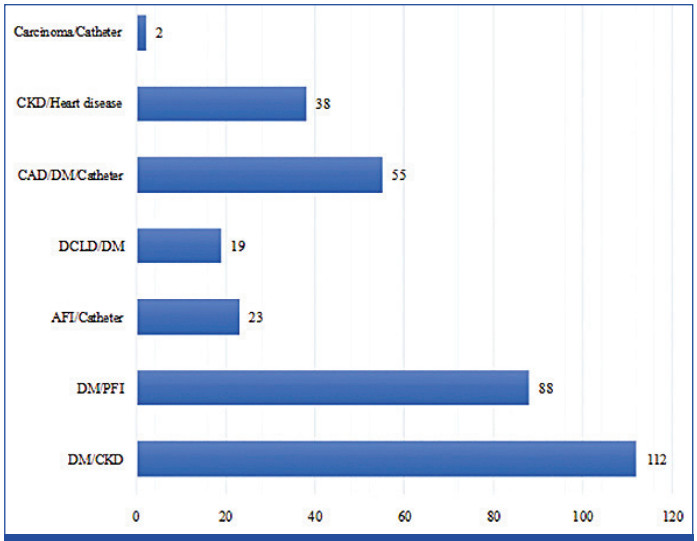


[Table/Fig-4]: Microscopic observation of urine wet mount slides.

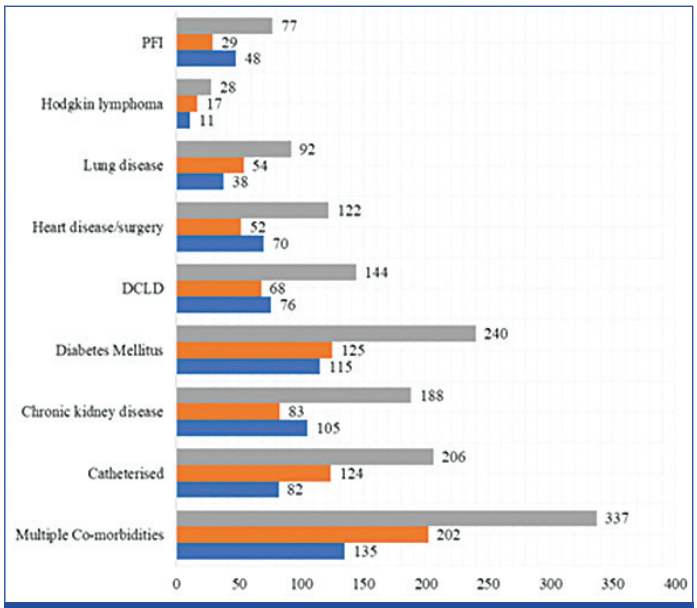
Co-infections were identified among 155 (10.8%) *E. coli* isolates, of which *Enterococcus* spp. predominated, closely followed by *Klebsiella pneumoniae* [Table/Fig-5]. The commonest risk factor in patients with UTI was multiple co-morbidities 337 (23.5%) [Table/Fig-6], followed by diabetes mellitus 240 (16.7%), catheterisation 206 (14.4%), and Chronic Kidney Disease (CKD) 188 (13.1%) [Table/Fig-7].



[Table/Fig-5]: Co-infections associated with *Escherichia coli* in patients.



[Table/Fig-6]: Distribution of multiple co-morbidities among patients.
DM/CKD: Diabetes mellitus associated with chronic kidney disease; DM/PFI: Diabetes mellitus associated with pulmonary fungal infection; AFI/Catheter: Catheterised patients with acute febrile illness; DCLD/DM: Diabetes mellitus associated with decompensated chronic liver disease; CAD/DM/Catheter: Coronary artery disease associated with diabetes mellitus and catheterisation; CKD/Heart disease: Chronic kidney disease associated with various heart diseases; Carcinoma/Catheter: Carcinoma patients with catheterisation



[Table/Fig-7]: Prevalence of co-morbid conditions in the study population.
DCLD: Decompensated chronic liver disease; PFI: Pulmonary fungal infections

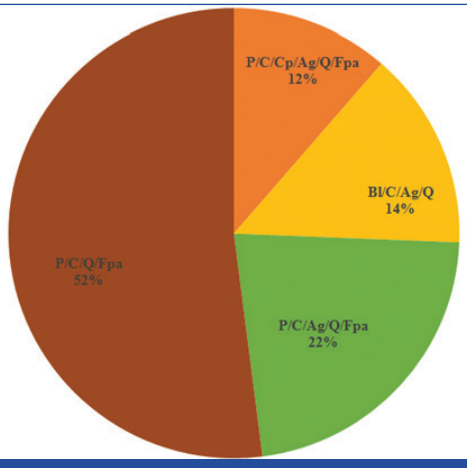
The least susceptibility of the isolates was detected against penicillin G 1410 (98.3%), followed by ampicillin 1160 (80.9%), cefazolin 974 (67.9%), cefotaxime 955 (66.6%), and ceftriaxone 921 (64.2%). Of the total 23 antimicrobial susceptibility discs used, only three drugs were found to be 100% sensitive for all isolates, namely Colistin (CL), Polymyxin B (PB), and Tigecycline (TGC) [Table/Fig-8].

Antimicrobial drug	Abbreviation	Sensitive (%)	Resistant (%)
Ampicillin	AMP	274 (19.1)	1160 (80.9)
Amoxicillin/Clavulanic acid	AMC	480 (33.5)	954 (66.5)
Amikacin	AK	1173 (81.8)	261 (18.2)
Cefazolin	CZ	460 (32.1)	974 (67.9)
Cefepime	CPM	562 (39.2)	872 (60.8)
Cefoxitin	CX	1057 (73.7)	377 (26.3)
Ceftazidime	CAZ	678 (47.3)	756 (52.7)
Ceftriaxone	CTR	513 (35.8)	921 (64.2)
Cefotaxime	CTX	479 (33.4)	955 (66.6)
Chloramphenicol	C	498 (34.7)	936 (65.3)
Ciprofloxacin	CIP	536 (37.4)	898 (62.6)

Gentamycin	GEN	913 (63.7)	521 (36.3)
Tetracycline	TE	559 (39)	875 (61)
Cotrimoxazole	COT	650 (45.3)	784 (54.7)
Penicillin	P	24 (1.7)	1410 (98.3)
Colistin	CL	1434 (100)	0 (0)
Ertapenem	ETP	1253 (87.4)	181 (12.6)
Imipenem	IPM	1301 (90.7)	133 (9.3)
Meropenem	MRP	1275 (88.9)	159 (11.1)
Piperacillin/Tazobactam	PIT	1225 (85.4)	209 (14.6)
Polymyxin B	PB	1434 (100)	0 (0)
Tigecycline	TE	1434 (100)	0 (0)
Nitrofurantoin	NIT	1296 (90.4)	138 (9.6)

[Table/Fig-8]: Distribution of antibiogram of the *E. coli* isolated from urine samples. Maximum number of resistant isolates were found against Penicillin (1410, 98.3%); and zero resistant isolates were found against three drugs namely Colistin, Polymyxin B, and Tigecycline

Among the total isolates, 553 (38.6%) were identified to be ESBL producing, 497 (34.6%) AmpC beta-lactamase producing, 172 (12%) carbapenem resistant, and 765 (53.3%) MDR. Among the MDR isolates, the maximum drug resistance 398 (52%) was seen in penicillins, cephems, quinolones and Folate pathway agonists group of antimicrobials, and the minimum drug resistance (87, 11.4%) was seen in oenicillins, cephems, carbapenems, aminoglycosides, quinolones, and Folate pathway agonists [Table/Fig-9]. No XDR isolates were identified.



[Table/Fig-9]: Distribution of multidrug resistance among antimicrobial classes of drugs (n=765).
P: Penicillins; C: Cephems; Q: Quinolones; Fpa: Folate pathway agonists; Ag: Aminoglycosides; Bl: Beta lactam antibiotics; Cp: Carbapenems

DISCUSSION

Enterobacteriaceae family are known to be the most common aetiological agents in UTIs, of which *E. coli* is known to cause >81% cases, closely followed by *Klebsiella* spp., *Enterobacter* spp., *Staphylococcus saprophyticus*, *Proteus* spp., and *Enterococcus*. The presence of *E. coli* in gut is reported as one of the common factors contributing as a primary source of UTI [11,18,19]. This study focuses on the UTIs caused by *E. coli*, their antimicrobial susceptibility pattern and the contributing factors for the same.

In this study population, women predominated compared to men, with higher incidence of *E. coli*. Although, many studies have similar prevalence in women [2,12,20-22], studies by Karishetti MS, Shaik HB, and Christy VR et al., showed male preponderance [18,23]. Similar to present study, majority of the study population belonged to the age group of 51-60 years in the studies conducted by Karishetti MS, Shaik HB and Nas FS et al., [18,24]; while many studies such as Tada DG et al., Kulkarni SR et al., and Ranjini CY et al., reported higher prevalence of 67%, 81.26% and 56.9% respectively among women in the reproductive age [22,25,26]. The rate of UTIs increases in men with advanced age, prostate enlargement and neurogenic bladder and in females could be to menopause as discussed by Najar MS et al., [1]. Higher incidence of *E. coli* infections was reported among inpatients in the study by Tabasi M et al., (65.4%), similar to present study (70%), while some studies by Gajdacs M et al., and Dehbanipour R et al., have reported higher incidence of 56.75±4.86% and 67.4%, respectively among outpatients [27-29].

Similar to present study, Kwon JH et al., had observed <37% with 10^4 CFU/mL, while most cases were ≥10^5 CFU/mL [30]. They had inferred that patients with colony counts ≥10^5 CFU/mL, were 12.93 times likely to be classified as having UTI. In patients with colony counts <10^5 CFU/mL, the UTI diagnosis was due to catheterisation, under antibiotics, or with co-morbidities. Contrary to this study, where multiple co-morbidities was the most common risk factor in patients, Sasikala G et al., reported diabetes mellitus (46.66%) followed by catheterisation (20%) as the most common risk factors associated with MDR *E. coli* [31].

In this study, the overall resistance of *E. coli* to antimicrobials was high, which was in conformity with that of the study by Kulkarni SR et al., [25]. High resistance rate of *E. coli* to ampicillin and amoxicillin/clavulanic acid in this study was comparable to the study conducted by Sabir S et al., who reported 100% resistant of *E. coli* to beta-lactam drugs [32]. The ampicillin resistance among urinary tract pathogens is probably due to continuous use of it for many years. Earlier, it has been reported that ampicillin had no more effect on urinary tract pathogens [33].

The ESBL producing Enterobacteriaceae are increasing in prevalence and rising global health concern. The prevalence of ESBL producing *E. coli* in present study (38.6%) was comparable to the meta-analysis reports in South-east Asia (37%) and other studies by Koshesh M et al., (37.2%) [34,35]. In one of the recent studies by Abayneh M et al., a very high proportion for *E. coli* isolates (76.5%) were reported to produce ESBL [36]. The incidence of AmpC beta-lactamase (34.6%) producing isolates was significantly high in present study as compared to studies by Koshesh M et al., (2%) and Gajamer VR et al., (1.7%) [35,37]. Carbapenem resistant isolates in present study was of low incidence, similar to the report of Rijal BP et al., (7.6%) [38].

Multidrug resistant reported in this study was 53.3%. Comparison of prevalence of MDR isolates of *E. coli* from UTI from various studies from India and outside India over a period of 10 years (2010-2020; [Table/Fig-10] revealed the trend of increase/decrease in the resistance patterns [26,32,36,39-43]. The studies show an abrupt increase in MDR isolates from the year of 2012, after which a constant range of 82-84% was maintained for five years. The sudden dip in the MDR isolates may be attributed to the increasing awareness of antimicrobial resistance, followed by stringent antimicrobial stewardship policies in hospitals, and judicious use of antimicrobials by the clinicians.

Authors	Study year	Place	MDR *E. coli* isolates
Mehta M et al., [39]	2010-11	Punjab (India)	57%
Niranjan V and Malini A, [40]	2011-12	Puducherry (India)	76.5%
Yerat RC and Rani KL, [41]	2012-13	Bangalore (India)	82.6%
Bijapur GA et al., [42]	2013-14	Kannur (India)	84.4%
Sabir S et al., [32]	2014	Punjab (India)	81%
Ranjini CY et al., [26]	2015	Bangalore (India)	82.6%
Abayneh M et al., [36]	2016-17	Ababa (Ethiopia)	82.4%
Das B et al., [43]	2017-18	Haryana (India)	50%
Present study (Ramakrishna MS et al.,)	2019-20	Potheri (India)	53%

[Table/Fig-10]: Comparison of nultidrug resistant *E. coli* isolated from UTI over a period of 10 years (2010-2020) [26,32,36,39-43].

Limitation(s)

Since, the study was a retrospective one, the minimum inhibitory concentrations of the isolates could not be confirmed by microbroth dilution method. Further investigation of the genes responsible for carbapenem resistance could not be determined. Since, the study was retrospective, the data may be factual, and further prospective studies will help in deriving the actual values of resistant isolates.

CONCLUSION(S)

The present study revealed that the *E. coli* isolates causing UTI were resistant to various drugs, producing ESBL, AmpC-beta-lactamase, showing carbapenem resistance and MDR. The increasing resistance exhibited by these pathogens, pave way for other co-infections, and delay in treatment. Further exploration of virulence factors exhibited by the resistant isolates, will aid in hastening the process of reduction in MDR isolates. Use of antibiotics developing resistance should not be given for empirical therapy, thus routine antibiogram should be conducted to check the development of resistance and prevent further spread in the hospital/community. In the future, phages as a kind of pharmaceutical preparations will be a valuable alternative to commonly used antibiotics to treat UTIs.

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