

## Original Research Article

# Prevalence of multidrug resistant uropathogens isolated from different age groups in South-India: a cross-sectional study

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## ABSTRACT

**Background:** Urinary tract infections (UTIs) continue to be one of the most common infections encountered by clinicians. The purpose of this study is to identify relevant multidrug resistance (MDR) patterns in South India.

**Methods:** 401 urine culture samples with significant bacteriuria were collected from labs in South India between January 2019 and December 2020. Routine biochemical tests were conducted for primary identification of uropathogens. Antibiotic susceptibility testing was performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Statistical package for the social sciences (SPSS) 16 and Microsoft excel were used to analyse data and determine MDR patterns.

**Results:** The results showed that 54% of cultures with significant bacteriuria exhibited an organism with multidrug resistance. Of these, extended-spectrum beta-lactamases (ESBL) resistance was confirmed in 34.9%. Furthermore, over half (54%) of significant cultures were from outpatients. The incidence of UTIs was highest during the summer and at the lowest in the second phase of the monsoon season.

**Conclusions:** Although the sample collection was limited to a few centres in South India, the results of our study justifies the rationale behind stringent regulation of antibiotic use and careful monitoring of antimicrobial resistance.

**Keywords:** Urinary tract infection, Uropathogens, Antibiotics, Multi-drug resistance

## INTRODUCTION

Urinary tract infections (UTI) are common bacterial infections that can considerably impact quality of life as well as negatively affect economic and public health resources.<sup>1</sup> UTIs can be classified into community-associated UTIs and hospital-associated UTIs. Gram negative bacteria, specifically Enterobacterales are the common cause of UTIs. Antimicrobials have been used for

decades in the treatment of UTIs.<sup>2</sup> Antimicrobial resistance among gram negative bacilli is increasing globally and this poses a considerable challenge for the clinician with limited treatment options.<sup>3,4</sup>

Furthermore, extended-spectrum beta-lactamases (ESBL) producing Enterobacterales limit treatment options. The aim of this study was to determine and evaluate the multidrug resistance patterns of uropathogens.

## METHODS

The present cross-sectional study was conducted between January 2019 and December 2020. 401 isolates from urine samples showing significant bacteriuria ( $>10^5$  CFU/ml) were collected from diagnostic microbiology laboratories in central Kerala. The bacterial isolates were further identified by routine biochemical tests and antibiotic susceptibility was performed at School of Medical Education, Kerala. The *Candida spp.* isolated was only identified morphologically and not processed further.

All bacterial samples which exhibited significant bacteriuria were included in the study; irrespective of age, gender and underlying disease. Cultures that did not show significant bacteriuria were excluded from the study. Antimicrobial susceptibility was performed as prescribed by Clinical & Laboratory Standards Institute (CLSI) guidelines.<sup>5</sup> A separate panel of antibiotics was used for gram negative and gram-positive organisms. ESBL production was detected using cephalosporin/clavulanate combination disks as prescribed by the guidelines of CLSI in M100-S29.<sup>6</sup> Briefly, cefotaxime (30 µg) or ceftazidime (30 µg) disks with and without clavulanate (10 µg) for phenotypic confirmation for the presence of ESBLs. A difference of  $\geq 5$  mm between the zone diameters of either of the cephalosporin disks and their respective cephalosporin/clavulanate disks was taken as phenotypic confirmation of ESBL production. Based on expert consensus, Centers for Disease Control and Prevention (CDC) and European Centre for Disease Prevention and Control (ECDC) isolates were termed multi-drug resistance (MDR) if it exhibited non-susceptibility to at least one agent in three or more antimicrobial categories.<sup>7</sup> The data was analysed using Microsoft excel 2019 and statistical package for the social sciences (SPSS) 16.

This study was approved by the institutional ethical committee (IEC) at the School of Medical Education, Kerala.

## RESULTS

The isolates from samples showing significant bacteriuria ( $>10^5$  CFU/ml) were collected from various private labs in central Kerala between January 2019 and December 2020. 401 samples with significant bacteriuria were obtained during the study period. Of the 401 samples 145 (36.2%) were from males and 256 (63.8%) were from females.

The study population was further divided based on age into 0-7, 8-19, 20-39, 40-60, and  $>60$ . Age wise distribution of positive culture was 23 (6 males and 17 females) for 0-7, 52 (16 males and 36 females) for 8-19, 89 (20 males and 69 females) for 20-39, 68 (28 males and 40 females) for 40-60, 169 (76 males and 93 females) for  $>60$  (Figure 1).

54.9% (n=220) of the 401 positive cultures were from the outpatient unit and 45.1% (n=181) were from inpatient units. 67.27% (n=167.2) of samples were from females and 32.72% (n=72) were from males in the outpatient unit.

In inpatient units, 59.66% (n=108) of the samples were from females and 40.33% (n=73) were from males.

55.61% (n=223) of the 401 isolates were found to be *Escherichia coli*, followed by *Klebsiella pneumoniae* 16.7% (n=67), *Candida spp.* 12.71% (n=51), *Enterococcus faecalis* 3.74% (n=15), *Pseudomonas aeruginosa* 3.74% (n=15), *Enterobacter cloacae* 1.74% (n=7), *Burkholderia cepacia complex* 1.24% (n=5), *Proteus mirabilis* 0.99% (n=4), *Streptococcus agalactiae* 0.74% (n=3), *Klebsiella aerogenes* 0.49% (n=2), *Klebsiella oxytoca* 0.49% (n=2), *Achromobacter spp.* 0.24% (n=1), *Citrobacter spp.* 0.24% (n=1), *Enterococcus faecium* 0.24% (n=1), *Pantoea agglomerans* 0.24% (n=1), *Providencia stuartii* 0.24% (n=1), *Staphylococcus aureus* 0.24% (n=1) and *Staphylococcus hominis* 0.24% (n=1) (Figure 2). Age-wise distribution of uropathogens is described in Table 1.

Prevalence of organisms isolated in the outpatient unit is as follows: *Escherichia coli* 66.81% (n=147) *Klebsiella pneumoniae* 13.63% (n=30), *Candida spp.* 4.54% (n=10), *Enterococcus faecalis* 4.09% (n=9), *Pseudomonas aeruginosa* 1.81% (n=4), *Enterobacter cloacae* 1.81% (n=4), *Burkholderia cepacia complex* 1.81% (n=4), *Streptococcus agalactiae* 1.36% (n=3), *Klebsiella aerogenes* 0.9% (n=2), *Klebsiella oxytoca* 0.9% (n=2), *Achromobacter spp.* 0.45% (n=1), *Citrobacter spp.* 0.45% (n=1), *Staphylococcus aureus* 0.45% (n=1), *Staphylococcus hominis* 0.45% (n=1) (Figure 3).

The prevalence of uropathogens in inpatient units were observed as follows: *Escherichia coli* 41.98% (n=76), *Candida spp.* 22.65% (n=41), *Klebsiella pneumoniae* 20.44% (n=37), *Pseudomonas aeruginosa* 6.07% (n=11), *Enterococcus faecalis* 3.31% (n=6), *Enterobacter cloacae* 1.65% (n=3), *Proteus mirabilis* 1.65% (n=3), *Burkholderia cepacia complex* 0.55% (n=1), *Enterococcus faecium* 0.55% (n=1), *Pantoea agglomerans* 0.55% (n=1), *Providencia stuartii* 0.55% (n=1) (Figure 4).

In the current study, we tried to estimate the seasonal prevalence of UTI. The largest percentage of UTI cases were reported during the summer season and stood at 34.4% (n=138). However, the minimum percentage of 14.7% (n=59) was reported during the second phase of the monsoon season. The first phase of the monsoon accounted for more than a quarter of all cases, 27.2% (n=109). The incidence of UTIs in winter was also relatively significant at 23.7% (n=95) of the total.

The antimicrobial susceptibility of the uropathogens in the current study is given in table 2. In the present study, 54% of the isolates were MDR. Of these 54% (n=102) were from the IP unit while 46% (n=87) were from the OP unit. The prevalence of MDR organisms within the IP and OP unit was 72.9% and 41.4% respectively. 161 non-MDR pathogens were detected, 76.4% (n=123) from the OP and 23.6% (n=38) from IP units. The percentage prevalence within the IP and OP unit was 58.6% and 27.1% respectively (Figure 5). The differences in proportion of

MDR pathogens in IP and OP units were compared using Chi-square test and was found to be significant ( $p<0.001$ ).

The antibiotic sensitivity patterns of *Escherichia coli* and *Klebsiella pneumoniae* were evaluated because they were the significant pathogens in the study using chi-square test ( $p=0.002$ ). In the O P unit, 83.1% were found to be *Escherichia coli* and 16.9% *Klebsiella pneumoniae*. 67.3% of the isolates was *Escherichia coli* and 32.7% were *Klebsiella pneumoniae* in inpatient units (Figure 6).

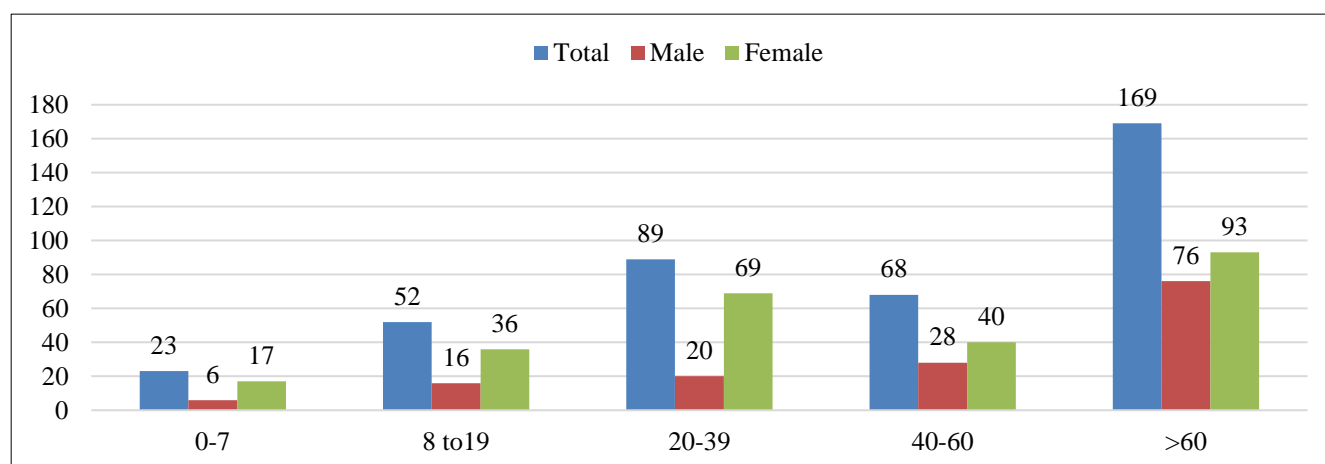
It was observed that the percentage of *Escherichia coli* isolates were higher in OP when compared to IP. Conversely, the percentage of isolates with *Klebsiella pneumoniae* in IP was significantly higher than isolates of the same organism in the OP unit.

A total of 34.9% ( $n=107$ ) isolates were positive for ESBL. ESBL production was detected in 37.31% ( $n=25$ ) *K. pneumoniae*, 35.42% ( $n=79$ ) *E. coli*, 50% ( $n=2$ ) *Proteus mirabilis* and 50% ( $n=1$ ) *K. oxytoca* (Figure 7).

55.1% ( $n=59$ ) ESBL positive isolates were from IP and 44.9% ( $n=48$ ) from the OP unit. The percentage of prevalence within the OP and IP units was 25.8% and 48.8% respectively. A total of 65.1% ( $n=200$ ) of the isolates were negative for ESBL - 69% ( $n=138$ ) from OP unit and 31% ( $n=62$ ) from IP units (Figure 8). The percentage of prevalence of ESBL negative isolates from IP and OP unit was 74.2% and 51.2% respectively. The difference between the prevalence of OP (25.8%) and IP (48.8%) were found significant using Chi-square test ( $p<0.001$ ).

**Table 1: Age wise distribution of uropathogens.**

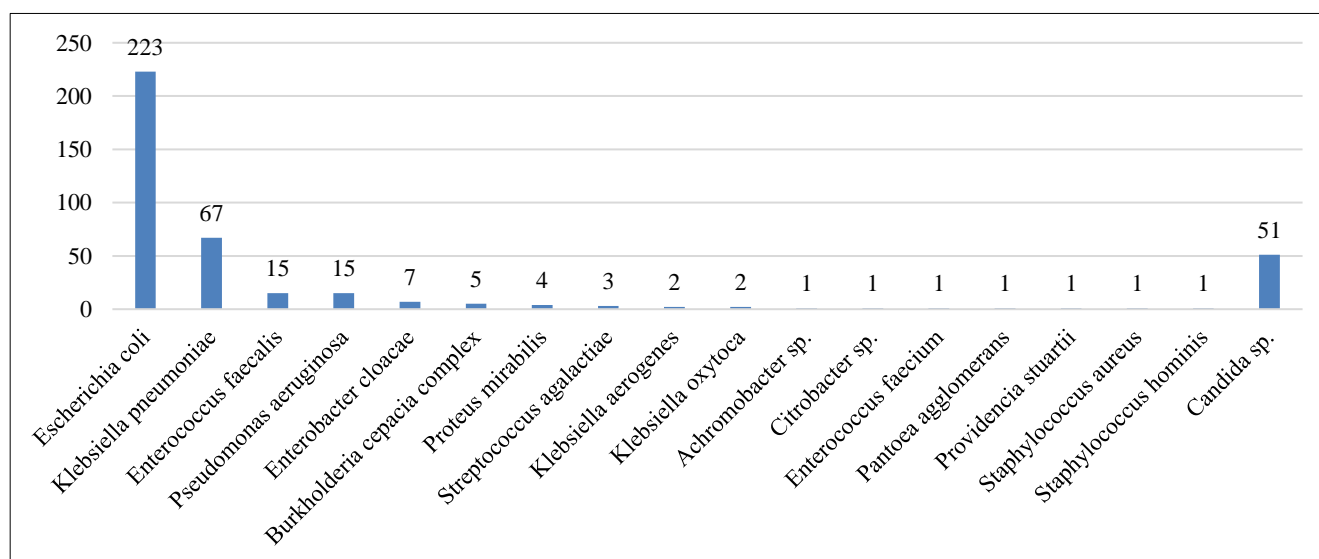
Uropathogen	0-7	8 to 19	20-39	40-60	>60
<i>Escherichia coli</i>	20	42	49	32	80
<i>Klebsiella pneumoniae</i>	2	1	16	16	32
<i>Enterococcus faecalis</i>	0	4	3	1	7
<i>Pseudomonas aeruginosa</i>	0	1	6	1	7
<i>Enterobacter cloacae</i>	0	1	2	2	2
<i>Burkholderia cepacia complex</i>	0	0	0	5	0
<i>Proteus mirabilis</i>	0	0	1	0	3
<i>Streptococcus agalactiae</i>	0	0	2	1	0
<i>Klebsiella aerogenes</i>	0	0	2	0	0
<i>Klebsiella oxytoca</i>	0	0	0	1	1
<i>Achromobacter sp.</i>	0	1	0	0	0
<i>Citrobacter sp.</i>	0	0	0	1	0
<i>Enterococcus faecium</i>	0	0	0	0	1
<i>Pantoea agglomerans</i>	0	0	0	0	1
<i>Providencia stuartii</i>	0	0	0	1	0
<i>Staphylococcus aureus</i>	0	1	0	0	0
<i>Staphylococcus hominis</i>	1	0	0	0	0
<i>Candida sp.</i>	0	1	8	7	35
<b>Total</b>	<b>23</b>	<b>52</b>	<b>89</b>	<b>68</b>	<b>169</b>

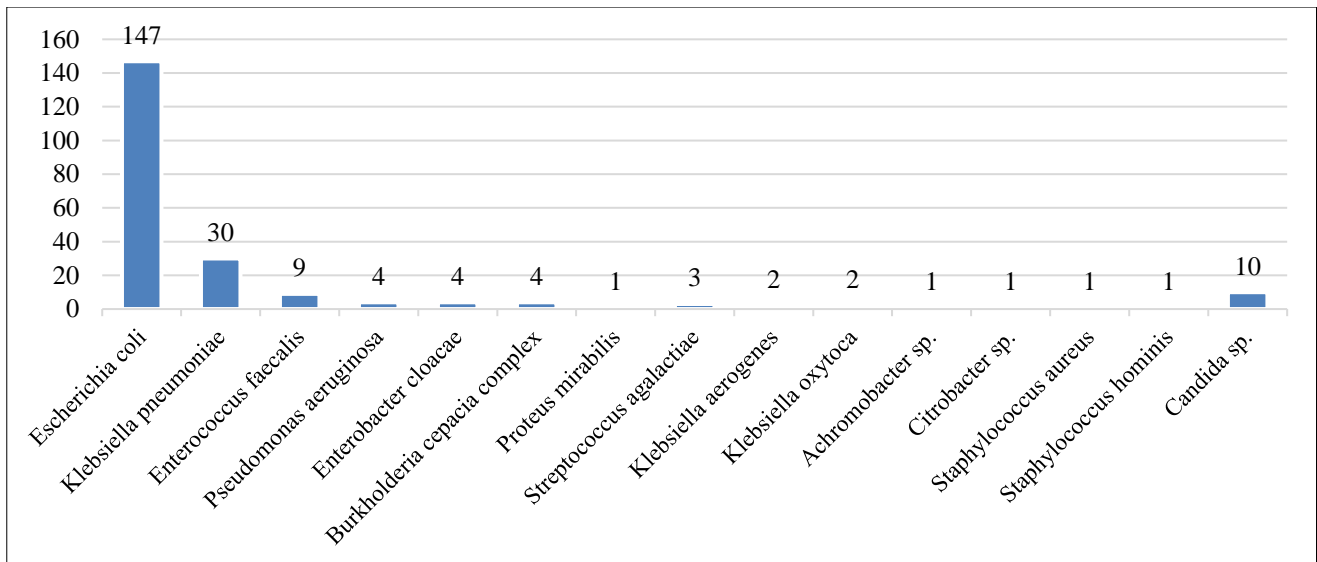


**Figure 1: Age- and gender-wise distribution of study population.**

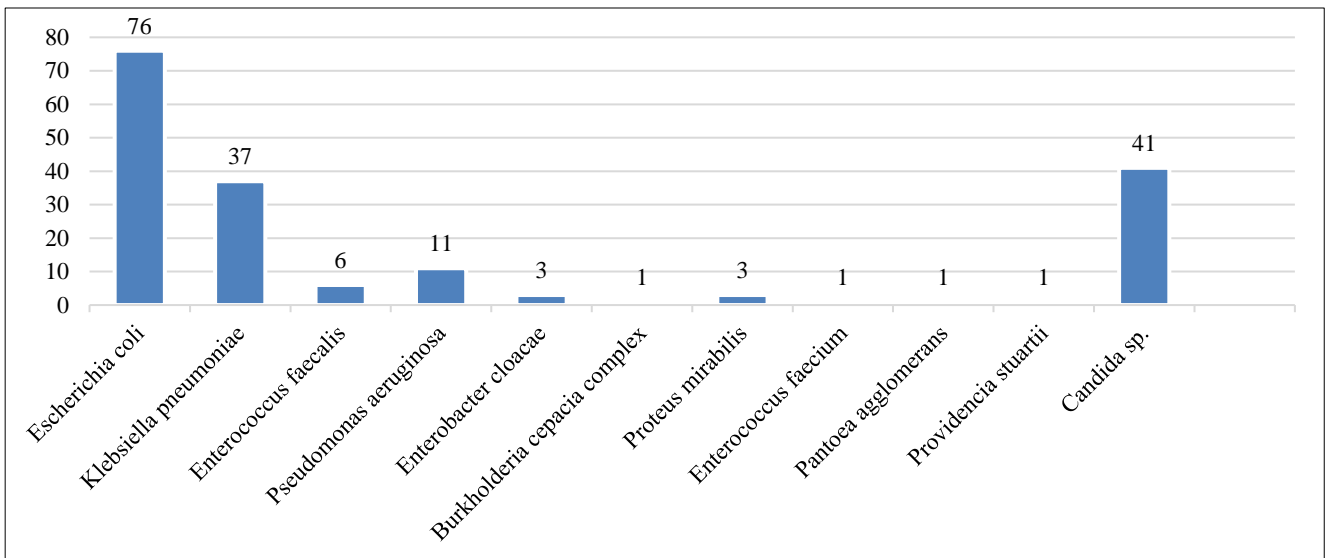
**Table 2: Antimicrobial susceptibility of uropathogens.**

Antibiotic	Sensitive		Resistant		Intermediate		Total
	Frequency	Percent	Frequency	Percent	Frequency	Percent	
Ampicillin	75	29.8	177	70.2	0	0	252
Amoxicillin/clavulanic acid	149	46.6	152	47.5	19	5.9	320
Ampicillin/sulbactam	4	80	1	20	0	0	5
Piperacillin/tazobactam	256	83.7	38	12.4	12	3.9	306
Cefazolin	0	0	3	100	0	0	3
Cephalothin	3	3.9	28	36.4	46	59.7	77
Cefuroxime	150	50	149	49.7	1	0.3	300
Cefoxitin	218	82	41	15.4	7	2.6	266
Cefepime	175	54	142	43.8	7	2.2	324
Cefotaxime	159	54.3	134	45.7	0	0	293
Ceftazidime	181	56.2	137	42.5	4	1.2	322
Ceftriaxone	170	53.6	147	46.4	0	0	317
Ciprofloxacin	188	55.1	143	41.9	10	2.9	341
Levofloxacin	180	59.2	120	39.5	4	1.3	304
Gentamicin	247	72.4	94	27.6	0	0	341
Amikacin	281	92.7	21	6.9	1	0.3	303
Tobramycin	0	0	2	100	0	0	2
Imipenem	291	87.7	28	8.4	13	3.9	332
Meropenem	283	88.7	31	9.7	5	1.6	319
Nitrofurantoin	227	77.7	51	17.5	14	4.8	292
Tigecycline	239	88.8	10	3.7	20	7.4	269
Trimethoprim/sulfamethoxazole	153	48.3	164	51.7	0	0	317
Aztreonam	173	54.7	142	44.9	1	0.3	316
Colistin	239	96.4	9	3.6	0	0	248
Erythromycin	3	100	0	0	0	0	3
Clindamycin	5	83.3	1	16.7	0	0	6
Vancomycin	20	95.2	0	0	1	4.8	21
Teicoplanin	14	100	0	0	0	0	14
Linezolid	21	100	0	0	0	0	21

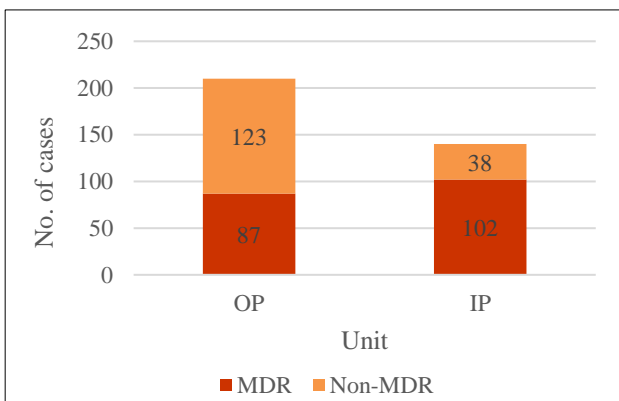
**Figure 2: Total prevalence of uropathogens in UTI.**



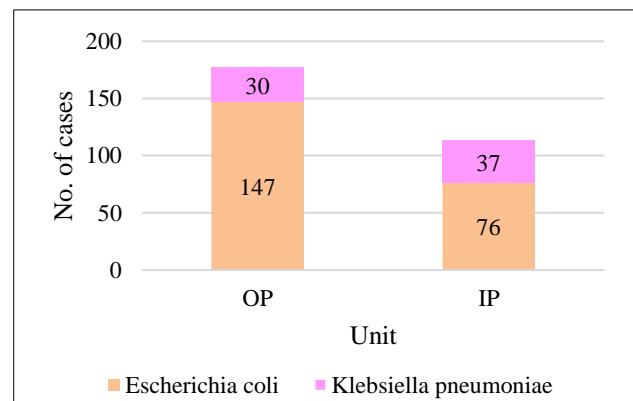
**Figure 3: Prevalence of organisms in OP.**



**Figure 4: Prevalence of organisms in IP.**

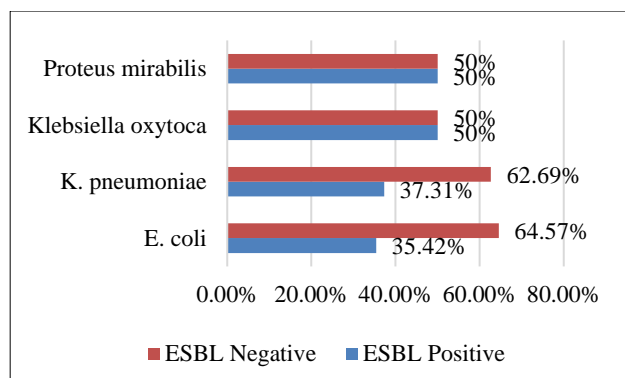


**Figure 5: Distribution and comparison of MDR uropathogens in OP and IP.**

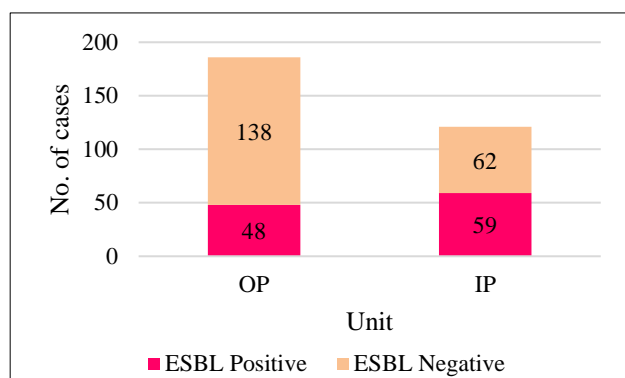


**Figure 6: Comparison of antimicrobial susceptibility of *E. coli* and *K. pneumoniae* from UTI.**





**Figure 7: ESBL production in uropathogens.**



**Figure 8: Distribution and comparison of ESBL positive uropathogens in IP and OP.**

## DISCUSSION

Antimicrobial resistance among uropathogens is increasing globally and considered to be a challenge for clinicians since there are limited treatment options. Management of UTIs in the era of antimicrobial resistance requires a systematic approach to diagnose the type of infection and to select appropriate antimicrobial agent. This current study provides valuable data to compare the status of antimicrobial resistance among uropathogens in community and hospital-setting so as to improve efficient empirical and definitive treatment.

In the present study, the overall prevalence of MDR organisms was found to be 54% which is lesser than 64% reported by Raghavan et al.<sup>8</sup> The higher percentage in the earlier study could be possibly explained by the smaller sample size and single centre involved. In our study, we further grouped the isolates as those from IP units – exhibiting 54% resistance, and the OP unit – exhibiting 46% resistance. This study is in correlation with studies of Bader et al, Flores-Mireles et al and Khoshnood et al studies, in that MDR organisms were isolated mainly from inpatients (percentage prevalence – 58.6%) than outpatients (percentage prevalence – 27.1%).<sup>4,9,10</sup> The difference of isolation of MDR pathogen from IP and OP unit was determined to be significant when compared using the Chi-square test.

The overall ESBL prevalence was 34.9% and it is higher than the 26.9% reported by Kothari and Sagar.<sup>11</sup> This can be explained by the evaluation of both IP and OP isolates in this study which stands in contrast to the other study that considered only OP samples. Our study also compared the ESBL positive isolates by chi-square test in IP and OP settings and found the resistance was significantly higher in IP setting which is in accordance with the study by Ravishankar et al and Gharavi et al.<sup>12,13</sup> Highest rate of ESBL production was seen in *K. pneumoniae* – 37.31% which was closely followed by *E. coli* at 35.42%. Two strains of *P. mirabilis* and one strain of *K. oxytoca* though positive were excluded in the statistical analysis as the number of isolates were too few to be significant.

Highest antimicrobial resistance was exhibited against ampicillin (70.2%) followed by trimethoprim/sulfamethoxazole (51.7%), cefuroxime (49.7%), amoxicillin/clavulanic acid (47.5%), ceftriaxone (46.4%), cefotaxime (45.7%), aztreonam (44.9%), cefepime (43.8%), ceftazidime (42.5%), ciprofloxacin (41.9%), levofloxacin (39.5%), cephalothin (36.4%), gentamicin (27.6%), nitrofurantoin (17.5%), cefoxitin (15.4%), piperacillin/tazobactam (12.4%), meropenem (9.7%), imipenem (8.4%), amikacin (6.9%), tigecycline (3.7%), and colistin (3.6%). Nitrofurantoin, one of the drugs used as an empirical drug for treatment of UTI exhibited lower resistance (17.5%), which is in accordance with study by Uma Shanker et.al, and hence it may be considered as an empirical drug for the treatment of UTI. The other drugs used empirically like trimethoprim/sulfamethoxazole (51.7%) and ciprofloxacin (41.9%) also exhibited high resistance which is also comparable to study by Ravishankar et al, so their role in empirical therapy of UTI may be limited.<sup>12</sup> Further studies with larger sample size including multi-centres will be required to evaluate their role in empirical therapy of UTI. This study also compared the resistance pattern between the two significant uropathogens isolated, i.e. *E. coli* and *K. pneumoniae* using Chi-square test. *E. coli* showed significant resistance in OP patients while *K. pneumoniae* showed higher resistance in IP patients.<sup>12</sup>

In the present study, only samples showing significant bacteriuria was processed because the study was mainly to evaluate antimicrobial resistance in uropathogens. From this data, the overall prevalence of UTI cannot be estimated. During the period of study, 17 bacterial species and one fungal species were obtained from the 401 positive urine cultures. *Candida* was not further processed. The study group was divided into different age groups so as to determine the uropathogens prevalent in these age groups. The highest prevalence of 42.1% was found in the age group greater than 60, followed by 22.2% for the age group between 20 and 39.17 % for 40 to 60. A prevalence of 13 % for the age group between 8 to 9 and 5.7% for ages in between 0 to 7 was also observed. These findings were in concurrence with results obtained by earlier studies.<sup>14-18</sup>

The majority of positive cultures were from the outpatient unit at 54.9% followed by 45.1% in inpatient samples. The

higher prevalence in OP is because UTI is more prevalent in the community than hospital settings. UTI was more prevalent in females in different age groups & IP, OP settings. This was consistent with earlier studies Akhavizadegan et al.<sup>19</sup>

The most commonly isolated organism were *E. coli*-55.61%, followed by *K. pneumoniae*-16.7% and *Candida spp.* – 12.7%. Other bacterial species as shown in the graph were found in less than 3.74% of the total isolates (Figure 2). The results of this study are in accordance with several previous studies.<sup>4,9,13,18,20,21</sup>

The seasonal prevalence of UTI was more during summer, which is similar to other studies by Pardeshi and Simmering et al.<sup>17,22</sup> The mechanism of the supposed seasonal influence in the occurrence of UTI is unclear. Previous studies on UTI suggested that ambient temperature increases incidence. Our study showed a seasonal increase during summer with an average ambient temperature that was higher than other seasons. Previous studies have shown that ambient temperature was associated with increased UTI. A possible explanation of this may be the relative dehydration and insensible fluid loss in environments with higher temperatures. This can cause a decrease in urine output which in turn contributes to increased occurrence of urinary tract infections. A lack of personal hygiene may also favour an increase of UTI during summer. Increased perspiration and sebaceous secretion around the genital area in high temperature provides a beneficial microenvironment for local bacterial colonization thereby defeating the body's natural defences and leading to UTI.

Our study has some limitations as the isolates were collected only from a few centres. These results cannot be easily used to generalise findings from studies with a larger sample size. The correlation of UTI symptoms with underlying disease conditions was also not considered for this study.

## CONCLUSION

Our results suggest that more than half of the urinary tract infections were caused by MDR uropathogens. ESBL production was found predominantly among *K. pneumoniae* followed by *E. coli* species. An intensifying level of resistance to various class of antimicrobial agents was observed among gram negative bacteria in UTI. The importance of this issue in the field of public health and the increased cost associated with it cannot be discounted.

We are of the opinion that the best course of action would be to regulate antibiotic use and carefully monitor antimicrobial resistance. This should prevent inappropriate use of antibiotics that eventually lead to therapeutic failures.

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*Ethical approval: The study was approved by the Institutional Ethics Committee*

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