Microbial Etiology and Resistance Patterns of Urinary Tract Infection at a Tertiary Care Centre – A Hospital based Study

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Abstract

Urinary tract infections (UTIs) are among the most common infections diagnosed in clinical practice. Treatment is often initiated before microbiological confirmation and anti-microbial susceptibility testing. With the rapidly rising antibiotic resistance treatment failures are not uncommon. Beta-lactamase production by gram-negative bacteria causing UTI is the commonest mode of drug resistance. The aim of current study was to detect and determine the hospital based prevalence of UTI, causative uropathogens and their antimicrobial susceptibility patterns. A total of 9,518 clean catch, mid stream urine samples were processed over 2 years. Semi-quantitative urine cultures and AST were performed. Diverse underlying resistance mechanisms were determined by detecting ESBLs, Carbapenemases, AmpC β-Lactamases, and Metallo-β-Lactamases through various standardized phenotypic methods.

Out of the 9,518 samples tested 1171 (12.3%) were culture positive. Majority (66.7%) were from female patients. Highest prevalence (60%) was seen in patients > 40 years of age. E. coli (48%) was the predominant causative organism, followed by Enterococcus spp. (23%). Among GNB high resistance rates were observed against Beta-lactams, Beta-lactam/β-lactamase inhibitor combinations, and fluoroquinolones. 34.5% of GNB were confirmed as ESBL, 40% as carbapenemase, 36.5% as AmpC β-Lactamase, and 41.5% as MBL producers. We found very high levels of resistance against a broad range of antibiotics including the most widely used β-lactam group. With the resistance slopes getting steeper and steeper empirical treatment of UTIs might be fraught with the danger of many failures. Culturing and performing AST for all patients with UTI might be a prudent step for their rationale treatment and a step forward in halting the emergence of further resistance.

Keywords: UTI, Anti-microbial Resistance, ESBLs, Carbapenemases
INTRODUCTION

Urinary tract infections (UTIs) are among the most prevalent bacterial infections in both community and hospital settings worldwide. UTIs occur following a series of complex interactions between the host biologic/behavioral and bacterial virulence factors. Infections are primarily established via the ascending route; however, hematogenous spread is not uncommon. Though majority of the infections occur in the lower urinary tract, a considerable number involve the upper tract. While lower (ascending) UTIs are relatively common, uncomplicated and respond to empirical therapy, the upper (descending) UTIs are less common, complicated and much difficult to treat. UTIs occurs across age groups, with frequency in infants around 1% to 2%. It’s much more common in males during the initial 3 months of life often in association with congenital urologic abnormalities and thereafter occurs more often in girls throughout childhood. Once adulthood is reached, UTIs occur more frequently in women. High prevalence of UTI among females is due to an interplay of multiple factors including shorter urethra, nearness of the urethral meatus to warm, moist vulvar / perianal areas, frequent sexual intercourse, pregnancy, and estrogen deficiency in postmenopausal period. In general, about 50-70% of women will have a UTI sometime during their lifetime, and 20-30% of women who have a UTI will have a recurrent UTI. The prevalence of uncomplicated UTI in women over 65 years of age is approximately 20%, compared with approximately 11% in the overall population globally. In India the overall prevalence of UTI was found to be around 33%, while in Kashmir its around 27-29%. UTI is most commonly caused by Escherichia coli followed by other gram-negative organisms like Klebsiella, Proteus, Enterobacter, Pseudomonas aeruginosa, Acinetobacter spp. and some gram-positive organisms like Enterococcus spp, Staphylococcus aureus, and Coagulase negative staphylococci. While Staphylococcus saprophyticus has a tendency to cause infections in sexually active young females.

UTI is becoming increasingly difficult to treat because of emergence of anti-microbial resistance among routinely isolated bacteria, especially among the Enterobacteriales. Almost all resistance seen to β-lactam antibiotics in Enterobacteriaceae is mediated by acquired or chromosomal β-lactamases or through the transfer of R plasmids. Some members possess chromosomally determined inducible AmpC β-lactamases. Other β-lactamases capable of hydrolyzing penicillin's, first three generations of cephalosporins and monobactams, are designated extended spectrum Beta-lactamases (ESBLs). Carbapenem hydrolyzing isolates producing Carbapenemases are referred to as carbapenem-Resistant Enterobacteriaceae. Metallo-β-lactamases (MBLs) is a group of β-lactamases that hydrolyze carbapenems but have poor ability to neutralize monobactams. These are not inhibited by inhibitors like clavulanic acid or Tazobactam, but inhibited by Ethylenediamine tetraacetic acid (EDTA), and dipicolinic acid.

Easy transmission of plasmids coding ESBLs between organisms has led to a major problem globally, particularly in patients that are hospitalized and often such strains lead to frequent outbreaks. Carbapenemases are the mainstay of treatment against complicated infections caused by ESBL producers. However over the years, resistance to carbapenems due to carbapenemase and MBL has been frequently seen among bacterial isolates from all round the globe. Metallo-β-lactamase (MBL) require zinc ions for their activation and carbapenem resistance is chiefly mediated by this enzyme.

More recently highly resistant uropathogenic organisms such as Acinetobacter species and ESBL, carbapenemase, and AmpC β-lactamase producing Enterobacteriaceae have been observed frequently as causing complicated hospital associated UTIs.

Anti-microbial treatment choices for infections due to β-lactamase positive bacteria are difficult, sparse, often resulting in treatment failure. The multidrug resistant bacterial isolates in the ICUs and hospital environment pose major therapeutic problems and also have far reaching consequences for infection control management.

Aim & objective
• The current study was undertaken to detect and determine UTI prevalence, its etiological
profile, drug susceptibility, and resistance pattern among patients attending a tertiary-care hospital in Kashmir.

MATERIALS AND METHODS

This hospital based, cross-sectional study was carried out in the Postgraduate department of Microbiology, GMC Srinagar, J&K, India, from July 2018 to June 2020. During this time 9,518 clean catch, mid-stream urine samples were collected and immediately transported in sterile, dry, screw capped, leak proof containers to the Bacteriology lab. A sterile 4 mm Nichrome wire loop which delivers 0.001 mL of urine was used for the isolation of pathogenic bacteria. A loopful of urine was inoculated on MacConkeys agar and HiCrome UTI agar. All the plates were incubated at 37°C for 24 hours. The cultures were reported as sterile, positive, or contaminated. Pure growth was further identified with the help of various traditional biochemical tests and also by VITEK 2 compact GN/GP ID card (BioMerieux, France). AST was done by disc diffusion on Muller Hinton agar (MHA) by Kirby-Bauer’s method and individual zone sizes were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines, 2020.

Anti-microbial susceptibility
AST was done interchangeably on MHA by disc diffusion (n= 760) method or VITEK 2 (n = 411) with susceptibility cards (AST 235, N405, N406, and P628 BioMerieux, India) as per as CLSI M100-S-30. The antibiotic discs were chosen according to the latest CLSI guidelines. These included, Ampicillin, Gentamicin, Amikacin, Amoxycillin/clavulanic acid, Piperacillin/Tazobactam, Cefixime, Cefoxitin, Cefotaxime, Ceftriaxone, Ciprofloxacin, Levofloxacin, Tobramycin, Trimethoprim/sulfamethoxazole, Ceftazidime, Cefepime, Aztreonam, Cefoperazone/sulbactam, Imipenem, Meropenem, Fosfomycin, Nitrofurantoin, Tetracycline, Tigecycline, Colistin, Penicillin, Oxacillin, Clindamycin, Erythromycin, Vancomycin, and Linezolid. E. coli ATCC 25922, P. aeruginosa ATCC 27853 and S. aureus ATCC 25923 were used as controls. Bacterial isolates displaying resistance to at least three classes of antibiotics were designated as multidrug resistant isolates (MDR).

Screening for ESBL production
All the isolated gram-negative organisms were initially screened for ESBL production by the ceftazidime (30µg) and ceftriaxone/cefotaxime (30µg) discs. In accordance with CLSI guidelines, isolates that showed decreased susceptibility to at least one of the drugs with zone of inhibition for ceftazidime ≤ 22 mm, cefotaxime ≤ 27 mm, and ceftriaxone ≤ 25 mm were designated as possible ESBL producing strains (screen positive).

Double disc synergy test for ESBL confirmation
All screen positive ESBL strains were confirmed by double-disc assay for ESBL production using discs of ceftazidime (30µg) and ceftazidime-clavulanic acid (30/10µg) and cefotaxime (30µg) and cefotaxime-clavulanic acid discs (30/10µg). The zones of inhibition for ceftazidime and cefotaxime discs were then compared against the ceftazidime-clavulanic acid and cefotaxime-clavulanic acid discs, respectively. An increase in the zone diameter of ≥ 5 mm for either agent tested in combination with clavulanic acid vs its zone when tested alone was confirmed as positive for ESBL production.

Screening for carbapenemase production
Bacterial isolates that tested intermediate or resistant to one or more carbapenems and/or resistant to one or more 3rd generation cephalosporins were considered as possible carbapenemase producers (screen positive) in accordance with CLSI guidelines.

Confirmatory test for carbapenemase production (MHT)
All screen positive bacterial isolates were tested by Modified Hodge test (MHT) for confirmation or otherwise of carbapenemase production. A suspension of 0.5 McFarland standard of E. coli ATCC 25922 of 1:10 dilution in saline was lawn cultured on MHA plate and a meropenem disk (10µg) was placed at the centre. Then the test organism was streaked out from the edge of the meropenem disk in a straight line to the edge of the MHA plate. On one MHA
plate, four strains were tested and incubated for 16-20 hours at 37°C. On interpretation, a positive MHT showed enhanced growth as clover leaf like indentation of the E. coli ATCC 25922 growing along the test organism growth streak within the meropenem disk inhibition zone indicating carbapenemase production and a negative MHT showed no enhanced growth of the E. coli ATCC 25922 along the test organism growth streak within the meropenem disk inhibition diffusion zone.

Detection of Metallo-β-lactamases (MBL)

This was done by Imipenem-EDTA (10/750µg) combined disc test. Bacterial isolates were inoculated onto MHA plates as per the laid CLSI guidelines. Then a 10µg Imipenem disk and a Imipenem - EDTA (10/750µg) combined disk were placed on the inoculated MHA plate and further incubated aerobically for 16-18 hrs at 37°C. Inhibition zones were measured and a difference of >7 mm between the Imipenem and Imipenem – EDTA was considered positive for MBL production.15

Screening for AmpC β-lactamases

AmpC β-Lactamase production screening was done using a 30µg disc of Cefoxitin and isolates with inhibition zones of ≤ 14 mm were considered resistant according to CLSI guidelines.

Statistical analysis

The study data was drafted on Microsoft Excel software and further analyzed with the help of SPSS 27 (statistical product and service solutions) software.

RESULTS

Of the total 9,518 non-duplicate urine samples processed, 1171 (12.3%) samples were culture positive (>10^3 CFU/ml of single pathogen or each of 2 pathogens), while 305 (3.2%) samples were contaminated (≥3 organisms with no one predominating)16 (Table 1). Of the 1171 uropathogens recovered, 781 (66.7%) were from female patients, while 390 (33.3%) were from male patients (range = 1 month to >60 years). Most of the culture positive samples (n=702, 60%) were from the patients who were > 40 years of age. The most susceptible age group among UTI patients irrespective of gender was 40-59 years (39.5%) followed by 20-39 years (30.5%), ≥ 60 years (20.5%) and then between 0-19 years (9.5%) [Figure 1].

E. coli 561 (48%) was the most predominantly isolated organism, followed by Enterococcus spp. 270 (23%), K. pneumoniae 153 (13%), Pseudomonas aeruginosa 82 (7%), Staphylococcus aureus 47 (4%), Proteus spp. 33 (3%), and Acinetobacter baumannii 25 (2%). Overall, gram negative bacteria (73%) were isolated much frequently than gram positive bacteria (27%) [Figure 2].

Antimicrobial susceptibility pattern of the isolated uropathogens is given in details in Table 2. Intrinsically resistant antimicrobials for a particular organism were neither tested nor reported. Among the Enterobacteriaceae (n=747) and other isolated gram negative non-fermenting bacilli (n=107), high resistance rates were seen towards Beta-lactams, Beta-lactam/β-lactamase inhibitor combinations, and Fluoroquinolones, while the lowest rate of resistance was seen towards amikacin, carbapenems, nitrofurantoin, fosfomycin and tigecycline. Among gram positive organisms (n=317) high resistance rates were seen with β-lactams, Macrolides, and Fluoroquinolones. While testing Staphylococcus aureus isolates, mecA mediated Oxacillin/methicillin resistance (MRSA) using Cefoxitin disk was observed in 48.5% isolates.

<table>
<thead>
<tr>
<th>Table 1. Culture profile and bacterial organisms isolated</th>
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</thead>
<tbody>
<tr>
<td>Total specimen (n=9518)</td>
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<tr>
<td>Culture - 8042 (84.5%)</td>
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<tr>
<td>Culture + 1171 (12.3%)</td>
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<tr>
<td>Contaminated 305 (3.2%)</td>
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<tr>
<td>Organisms isolated N (%)</td>
</tr>
<tr>
<td>Gram negative organisms</td>
</tr>
<tr>
<td>E.coli 561 (48%)</td>
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<tr>
<td>Klebsiella spp. 153 (13%)</td>
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<tr>
<td>Pseudomonas spp. 82 (7%)</td>
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<tr>
<td>Proteus mirabilis 33 (3%)</td>
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<tr>
<td>Acinetobacter spp. 25 (2%)</td>
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<tr>
<td>Gram positive organisms</td>
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<tr>
<td>Enterococcus spp. 270 (23%)</td>
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<tr>
<td>Staphylococcus aureus 47 (4%)</td>
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</table>
All MRSA isolates were considered resistant to other Beta-lactams, Beta-lactam/β-lactamase inhibitor combinations, cephems (except 5th generation, e.g.: ceftaroline, ceftabiprole), and carbapenems. All gram positive isolates showed excellent susceptibility towards gentamicin, nitrofurantoin, tigecycline, vancomycin, and linezolid. While 12% of isolated Enterococcus spp. were resistant to vancomycin (VRE), fosfomycin susceptibility was 100%.

Out of the 854 isolated GNB, 309 (36.2%) were suspected ESBL producing strains (all ESBL screen positive isolates), among which 107 (34.5%) were confirmed as ESBL producers. ESBLs were highest among Klebsiella pneumoniae (38.9%) followed by Proteus spp. (36%), E. coli (31.3%), Pseudomonas aeruginosa (26.8%), and Acinetobacter baumannii (25%).

Out of the 854 GNB isolated, 342 (40%) were found to be carbapenemase producers by MHT. Klebsiella pneumoniae accounted for (31.9%), followed by Pseudomonas aeruginosa (26.8%), Proteus spp. (26%), Acinetobacter baumannii (15%) and E. coli (12.3%).

Out of the total 342 carbapenem resistant GNB, a total of 142 (41.5%) were found to be MBL producing isolates, with the highest number in K. pneumoniae (54.3%) followed by P. aeruginosa (41.2%), A. baumannii (35.6%), P. mirabilis (26.4%), and E. coli (21.3%). Cefoxitin resistance used for screening of Amp C beta-lactamases among GNB was observed in 312 (36.5%) isolates, with the highest found among Pseudomonas aeruginosa (64.5%) followed by Klebsiella pneumoniae (57.9%), Acinetobacter baumannii (44%), E. coli (42.2%), and Proteus spp. (26%) (Table 3).
DISCUSSION

A considerable proportion of OPD patients have an underlying urinary tract infection. UTIs are the most common nosocomial infections especially so in catheterized patients. The collective burden is large enough to warrant not only surveillance but actively looking for resistance. Doing so ensures an appropriate antibiotic to the patient, lesser treatment failures and a step taken in the right direction to contain the menace of rising drug resistance.

The overall prevalence of UTI in our study was 12.3%. The prevalence rate is similar to a research from northern India by Akram et al. 10.8%. and another study from southern India by Eshwarappa et al. 9.1%. The prevalence is lower when compared to studies by Sneka et al. 33.1% and Puneeta et al. 24.1%. Higher prevalence rates of UTI in some studies can be attributed to higher symptomatic patients tested, more female participants, and hospital based studies. A significant number (66.7%) of patients with UTI in our study were females, which is consistent with other studies like 61.8% as observed by Puneeta et al. Higher prevalence rates of UTI among females is attributed to shorter urethra, nearness of the urethral meatus to warm,

### Table 2. Antibiotic susceptibility pattern of isolated uropathogens (percentage)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>AST pattern (%)</th>
<th>E. coli</th>
<th>Klebsiella</th>
<th>Proteus</th>
<th>Pseudomonas</th>
<th>Acinetobacter</th>
<th>Enterococcus</th>
<th>S. aureus</th>
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<td>78.8</td>
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<td>93.9</td>
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<td>81.4</td>
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<td>85.5</td>
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*Not tested, AMC=Amoxicillin/clavulanic acid, PIT=Piperacillin/Tazobactam, COT=Co-trimoxazole, CFS=Cefoperazone/sulbactam, Cefoxitin was used for AmpC detection in gram negative isolates
moist vulvar and perianal areas, frequent sexual intercourse, pregnancy, and estrogen deficiency in postmenopause period.\textsuperscript{19}

In our study, gram-negative bacteria constituted 73%, while gram-positive constituted 27% of the total bacterial isolates. The most common isolated bacteria was \textit{E. coli} (48%) followed by \textit{Enterococci spp.} (23%), \textit{K. pneumoniae} (13%), \textit{Pseudomonas aeruginosa} (7%), \textit{Staphylococcus aureus} (4%), \textit{Proteus mirabilis} (3%), and \textit{Acinetobacter spp.} (2%). These findings are consistent with observations made by Kumari et al.\textsuperscript{21} and Karlowsky et al.\textsuperscript{22} They found \textit{E. coli} as the most common bacteria isolated among people diagnosed with UTI. However, in our study, \textit{Enterococci spp.} (23%) was the second most common bacteria isolated, while as in studies,\textsuperscript{17,19,20} it was \textit{Klebsiella pneumoniae}. This can be explained by the nature of studies, since ours was a hospital based study the prevalence of some organisms varies with those that were conducted at the community level.

Antimicrobial drug resistance is an area of major concern in the treatment of UTIs, both in community and hospital settings. With every passing year the prevalence of MDR, XDR and PDR bacteria is steadily increasing and this requires close monitoring by microbiology labs to reduce this eminent threat which is now a global phenomenon.\textsuperscript{23} In our study, most of the isolated strains of family \textit{Enterobacteriaceae} (e.g. \textit{E. coli}, \textit{Klebsiella spp.}, and \textit{Proteus spp.}) and the non-fermenting GNB (e.g. \textit{Pseudomonas aeruginosa} and \textit{Acinetobacter baumannii}) were found resistant to more than one class of antibiotics. \textit{E. coli} was highly resistant to ampicillin (91.4%), followed by ciprofloxacin (78.1%), cefixime (70%), and amoxyclav (65.5%). Whereas, tigecycline (2%), nitrofurantoin (4.8%), fosfomycin (6.5%), amikacin (7.7%), and meropenem (9%) were the least resistant drugs. \textit{Klebsiella spp.} showed high rates of resistance towards cefixime (78.4%), amoxyclav (71.7%), and ciprofloxacin (63.7%). Whereas, Tigecycline (16.5%), Amikacin (16.7%), and gentamicin (22.8%) had the least resistance rates. For \textit{Proteus spp.} high rate of resistance was seen with levofloxacin (86.9), Co-trimoxazole (69.8%), and third generation cephalosporins. Whereas, cefoperazone/sulbactam (3.9%), amikacin (5%), and PIT (9%) were the least resistant. Resistance rates of co-trimoxazole (COT), a commonly prescribed empirical antibiotic for UTI were 34.9% for \textit{E. coli} and 27.9% for \textit{Klebsiella spp.}. For \textit{Pseudomonas aeruginosa} levofloxacin (71.2%) and ciprofloxacin (60.7%) were highly resistant, while meropenem (21.6%) and imipenem (26.8) were least resistant. Anti-pseudomonal antibiotics such as, PIT (32.8%), cefepime (33.7%), ceftazidime (28.7%), and tobramycin (45%) showed variable rates of resistance. \textit{Acinetobacter baumannii}

\begin{table}[h]
\centering
\caption{Prevalence of β-Lactamases in isolated gram negative organisms}
\begin{tabular}{cccc}
\hline
ESBL & Carbapenemase & AmpC β-lactamase & MBL \\
\hline
34.5\% & 40\% & 36.5\% & 16.6\% \\
\hline
\end{tabular}
\end{table}
strains were found highly resistant to ceftriaxone (60%), and levofloxacin (55%), while meropenem (10.6%), amikacin (12.5%), and imipenem (15%) were found to be least resistant. Alarming rates of resistance were also reported in studies by Sneka et al., Puneta et al., Kothari et al., Shailaja et al., and Hasan et al.

In our study, ESBL production was 34.5% with the highest prevalence of ESBLs observed in *Klebsiella pneumoniae* (38.9%) followed by *Pseudomonas aeruginosa* (31.3%), *Acinetobacter baumannii* (25%). Similar ESBL production rates were seen by Nepal et al. in their study. However, in the year 2015 Raut et al. observed prevalence of ESBL producing isolates of *E. coli* and *Klebsiella pneumoniae* to be 18.2 and 4.1%, respectively and as high as 90.9% for *Klebsiella pneumoniae* and 80% for *E. coli* in another study by Pathak et al. The global ESBL prevalence among clinical bacterial isolates ranges anywhere from < 1 to 74%. Infections that are caused by ESBL, AmpC β-lactamase expressing *Enterobacteriaceae* are often treated by carbapenems. Despite being stable to most Beta-lactamases, their utilization as a last choice antibiotic has been severely jeopardized by the emergence of new class of microbial enzymes well capable of hydrolyzing carbapenems, now referred to as carbapenemases. Carbenapenem resistance is attributed primarily to expression of carbapenemases and also to over expression of β-lactamases, efflux pumps, and impermeability due to porin loss. However, carbapenemase production is the major mechanism of resistance in *Enterobacteriaceae*, associated with multidrug, extensive, and also pan-drug resistance. Presently carbapenemases such as KPC, MBL (NDM-1), IMP, VIM, and OXA-48 pose a substantial threat in *Enterobacteriaceae*. These enzymes get quickly disseminated between patients and do not recognize political boundaries. In India, negligible data on KPC producing isolates exist, however it’s the main hub for NDM type MBL carbapenemases.

Among GNB, 342 (40%) were found to be Carbapenemase producers. Highest prevalence was found in *Klebsiella pneumoniae* (31.9%) followed by *Pseudomonas aeruginosa* (26.8%), *Proteus spp.* (26%), *Acinetobacter baumannii* (15%) and *E. coli* (12.3%). Similar observations were made by Patidar et al., who detected 43% carbapenemase producers among 107 CRE by MHT. Sathya et al. found 62% of carbapenem resistant isolates tested positive for carbapenemases production, which is greater than our observations. Highly fluctuating rates of carbapenem resistance in *Enterobacteriaceae* (5.75% to 51%) has been observed by Gupta et al., and Wattal et al. Amp C β-lactamase production was found in 312 (36.5%) isolates, with the highest among *Pseudomonas aeruginosa* (64.5%) followed by *Klebsiella pneumoniae* (57.9%). Carbapenems have been the drugs of choice for the treatment of various infections due to ESBL producing organisms, but carbapenemase producers are being increasingly reported globally.

In this study 41.5% of carbapenem resistant isolates were also found to be MBL producers, with highest prevalence among *K. pneumoniae* (54.3%) followed by *P. aeruginosa* (41.2%). MBL production in our study was lower when compared to observations made by Bora et al. (*E. coli* = 18.98%, *K. pneumoniae* = 21.08%). However, in another study the MBL production among *Enterobacteriaceae* was found to be 18%.

**CONCLUSION**

We found high levels of resistance against a battery of indispensable antibiotics which is a matter of great concern not only for the treating clinicians but also for infection control practitioners. With such a resistance scenario treatment failures would not be an uncommon occurrence in these settings, resulting not only in increased morbidity and cost but also contributing to the slope of rising resistance trends. While it’s a common practice to treat uncomplicated UTIs empirically such an endeavor might prove unrewarding in the near future with the backdrop of alarming resistance that we have. It might be prudent to culture all suspected UTIs and determine anti-microbial susceptibility for effective treatment of patients and saving the utility of current antibiotics for our future generations. This becomes especially important, with no newer antibiotics on the seeable horizon.
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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

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was approved by the Institutional Review Board, Government Medical College, Srinagar, with reference number IRBGMC/ C5-26.

REFERENCES

19. Sneka P, Mangayarkarasi V. Bacterial pathogens causing


