Urinary Tract Infection and Antibiotics Resistance Pattern among the Escherichia coli and Klebsiella spp. isolated from the patients of urinary tract infections at RIMS, Ranchi, Jharkhand, 834009.

Dr. Manoj Kumar¹, Dr. Bhagan Hembram², Dr. Ashok Kumar Sharma³,
Dr. Amber Prasad⁴.

Abstract: Introduction: Urinary tract infections (UTIs) are amongst the most common infections encountered in clinical practice. Urinary tract infection can be caused by gram negative bacteria such as Escherichia coli, Klebsiella species, Pseudomonas species, Proteus species, Enterobacter species and gram positive bacteria like Staphylococcus aureus, Enterococcus species and coagulase-negative Staphylococcus species. Escherichia coli is the most common organism isolated from the patients of UTI followed by Klebsiella species. Injudicious and irrational use of antibiotics and extended spectrum beta-lactamase enzyme (ESBL) production by Enterobactriacea family may be the main reason for emerging antibiotic resistance. Aims and Objectives: The prospective study was conducted to determine the bacteriology of UTI and antibiotic resistance pattern among Escherichia coli and Klebsiella spp. isolates of urinary samples at Rajendra Institute of Medical Sciences, (RIMS), Ranchi. Materials and Methods: A prospective study from May 2018 to October 2018 was performed after obtaining ethical clearance from Institutional Ethical Committee of Rajendra Institute of Medical Sciences, (RIMS), Ranchi. A total of 300 patients with UTI attended to the bacteriology section and organism identification, culture and sensitivity pattern was carried out as per CLSI standards. Results and discussion: Out of the 300 samples (165 females and 135 males), only 55.8% samples (n=167) showed positive results for urinary tract infections. The isolates were Escherichia coli (57.4%), Klebsiella spp. (19.17%), Staphylococcus aureus (9.8%), Pseudomonas (7.3%), Coagulase-negative Staphylococci (3.9%) and Others (1.9%) respectively. It was also observed that the resistance of gram negative baceteria was much higher than the gram positive bacteria. Conclusion: From this study it is concluded that the sensitivity of Enterobactriacea group of organisms to known antibiotics are decreasing. Even the drugs like Imipenem, Nitrofurantoin and Ceftriaxone are also becoming resistant to Escherichia coli and Klebsiella spp. Escherichia coli isolates showed low resistance to Amikacin and Gentamicin, while Klebsiella isolates showed high resistance to these agents despite being sensitive for many years.

I. Introduction

Urinary tract infections (UTIs) are amongst the most common infections encountered in clinical practice¹. Urinary tract infection can be caused by gram negative bacteria such as Escherichia coli, Klebsiella species, Pseudomonas species, Proteus species, Enterobacter species and gram positive bacteria like Staphylococcus aureus, Enterococcus and coagulase-negative Staphylococcus species². Escherichia coli is the most common organism isolated from the patients of UTI followed by Klebsiella species³. UTI usually affects the lower urinary tract, but sometimes both the lower and upper urinary tract can be involved.

Although UTIs occurs in both men and women, clinical studies suggest that the overall prevalence of UTI is higher in women. Uncomplicated UTIs in healthy women have an incidence of 50/1000/year⁴. An estimated 50% of women experience at least one episode of UTI at some point in their life time and between 20-40% of women have recurrent episodes⁵,⁶. Approximately 20% of all UTIs occur in men⁷. The ability of the bacteria to adhere to host structures is considered essential for the development of infection.

UTI is said to exist when pathogenic organisms are detected in the urine, urethra, bladder, kidney or prostate. In most instances, the growth of more than 10⁵ organisms per ml from a properly collected mid-stream clean catch urine sample indicates infection. However significant bacteriuria is lacking in some cases of true UTI. Particularly, in asymptomatic patients, a small number of bacteria (10² to 10⁵ /ml) may signify infection.

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Injudicious and irrational use of antibiotics in treating UTIs is responsible for the emergence and spread of multi-drug resistance urinary bacterial pathogens, which in recent years, has become a major problem worldwide particularly in visiting countries\(^8\). Apart from these factors extended spectrum beta-lactamase (ESBL) production by Enterobacteriaceae may be the main reason. To ensure appropriate medical treatment, knowledge of the organisms that UTI and their antibiotic susceptibility is mandatory\(^9\). In India, the resistance pattern of community acquired uropathogens has not been extensively studied\(^10\). Since a national surveillance system is lacking in India, there is a great need for long-term studies on the proportions and trends in antibiotic resistance. Several long term studies from India have found high antibiotic resistance proportions, but most of these studies are single-site studies or over shorter periods of time\(^11\)-\(^17\). Recently, a retrospective study over seven years from a private laboratory network in India concluded that there are high proportions of resistant bacterial strains among blood culture isolates from patients across India\(^18\).

II. Aims and Objectives

The study was conducted to determine the bacteriology of UTI and antibiotic resistance pattern among *Escherichia coli* and *Klebsiella* spp. isolates of urinary samples at Rajendra Institute of Medical Sciences, (RIMS), Ranchi, Jharkhand.

III. Materials and Methods

A prospective study from May 2018 to October 2018 was performed after obtaining ethical clearance from Institutional Ethical Committee of Rajendra Institute of Medical Sciences, (RIMS), Ranchi. Urinary samples were collected from patients visiting to clinical microbiology laboratory at Department of Microbiology, RIMS, Ranchi. A total of 300 patients with suspected UTIs attended to microbiology department at RIMS, Ranchi. The organism identification done by culture and biochemical tests and sensitivity pattern was carried out in the bacteriology section of the department of microbiology, RIMS, Ranchi. Only patients who had pyuria (>10 WBC/\(\mu\)l), acute voiding symptoms and significant bacteriuria (>100,000 CFU/ml of urine) were considered for the study as per guidelines of standard operating procedure of urine collection, culture and bacterial identification. All patients had clinical evidence of a urinary tract infections as determined by the treating physicians. Semi-quantitative urine culture was done using a calibrated loop. Samples were inoculated on Blood agar, and MacConkey agar plates. Plates were read after overnight incubation at 37\(^\circ\)C. After incubation, organisms are streaked over a Muller Hinton Agar plate. Antibiotic disc is place on the middle of this Muller Hinton Agar plate and it left for incubation at 37\(^\circ\)C overnight. After overnight incubation, clear zone around the disc is measured to known the sensitivity. All data related to antibiotic sensitivity are kept in a log book in microbiology department. The significant pathogens were identified by standard biochemical procedures. Antimicrobial susceptibility testing was performed using the disc diffusion method as described by Clinical Laboratory Standard Institute (CLSI).

IV. Results

Out of the 300 samples (165 females and 135 males), only 55.8% samples (n=167) showed positive results for urinary tract infections. The isolates were *Escherichia coli* 57.4% (n=96), *Klebsiella* spp. 19.17% (n=32), *Staphylococcus aureus* 9.8% (n=17), *Pseudomonas* 7.3% (n=12), Coagulase-negative Staphylococci 3.9% (n=7) and Others 1.9% (n=3) respectively. It was also observed that the prevalence of gram negative bacteria was much higher than the gram positive bacteria.


**Figure 3.** The pie chart showing the organisms isolated.

Antibiotic susceptibility in *Escherichia coli* and *Klebsiella spp.* isolates of urine samples.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>E. coli</th>
<th>E. coli</th>
<th>E. coli</th>
<th>Klebsiella</th>
<th>Klebsiella</th>
<th>Klebsiella</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistance</td>
<td>Intermediate</td>
<td>Sensitive</td>
<td>Resistance</td>
<td>Intermediate</td>
<td>Sensitive</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.0%</td>
<td>3.7%</td>
<td>96.3%</td>
<td>7.8%</td>
<td>4.3%</td>
<td>87.9%</td>
</tr>
<tr>
<td>Netilimcin</td>
<td>2.3%</td>
<td>1.1%</td>
<td>96.7%</td>
<td>36.7%</td>
<td>0.0%</td>
<td>63.3%</td>
</tr>
<tr>
<td>Cepepime</td>
<td>4.3%</td>
<td>2.5%</td>
<td>93.2%</td>
<td>29.9%</td>
<td>3.7%</td>
<td>66.4%</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>24.5%</td>
<td>2.1%</td>
<td>73.4%</td>
<td>54.3%</td>
<td>13.0%</td>
<td>31.7%</td>
</tr>
<tr>
<td>Amikacin</td>
<td>3.1%</td>
<td>5.6%</td>
<td>91.3%</td>
<td>36.6%</td>
<td>3.3%</td>
<td>60.1%</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>5.8%</td>
<td>3.3%</td>
<td>90.9%</td>
<td>21.9%</td>
<td>6.6%</td>
<td>71.5%</td>
</tr>
<tr>
<td>Co-amoxiclav</td>
<td>59.9%</td>
<td>5.3%</td>
<td>34.8%</td>
<td>69.9%</td>
<td>3.4%</td>
<td>26.7%</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>17.8%</td>
<td>4.9%</td>
<td>77.3%</td>
<td>53.3%</td>
<td>6.5%</td>
<td>40.2%</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>22.1%</td>
<td>7.1%</td>
<td>70.8%</td>
<td>57.3%</td>
<td>6.6%</td>
<td>40.1%</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>23.6%</td>
<td>11.9%</td>
<td>64.5%</td>
<td>46.6%</td>
<td>9.9%</td>
<td>43.5%</td>
</tr>
<tr>
<td>Cotrimoxazone</td>
<td>56.4%</td>
<td>2.9%</td>
<td>40.7%</td>
<td>75.2%</td>
<td>16.5%</td>
<td>8.3%</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>61.9%</td>
<td>2.5%</td>
<td>35.6%</td>
<td>96.6%</td>
<td>3.3%</td>
<td>0.1%</td>
</tr>
</tbody>
</table>

Figure 1. Antibiotic resistance pattern of *Escherichia coli* and *Klebsiella spp.*
Urinary Tract Infection and Antibiotics Resistance Pattern among the Escherichia coli and Klebsiella spp.

V. Discussion

This prospective study was done to determine the bacteriology of UTI and antibiotic susceptibility pattern of Escherichia coli and Klebsiella spp. isolated from urine samples at bacteriology section of Rajendra Institute of Medical Sciences, (RIMS) Ranchi.

In the present setting, a total of 300 samples (165 sample from female and 135 samples from male) of different ages and sexes were collected from the suspected patients referred by treating clinicians of different departments of Rajendra Institute of Medical Sciences, (RIMS), Ranchi. Out of the 300 samples, only 55.8% samples (n=167) showed positive results for urinary tract infections. Among which 54% (n=90) were females and 34% (n=77) were males. The isolates were Escherichia coli 57.4% (n=96), Klebsiella spp. 19.17% (n=32), Staphylococus aureus 9.8% (n=17), Pseudomonas 7.3% (n=12), Coagulase-negative Staphylococci 3.9% (n=7) and Others 1.9% (n=3) respectively. It was also observed that the prevalence of gram negative bacteria was much higher than the gram positive bacteria.

The prevalence of uropathogens found in our study corroborates with a few studies in India, Pakistan and Korea. All of those studies claimed Escherichia coli as the most common uropathogen has also been found to be remarkably consistent with previous studies conducted in Bangladesh.

The antimicrobial agents with highest levels of activity against Escherichia coli isolates were Imipenem and Nitromicin. The highest resistance was shown to Co-amoxyclov (59.90%). Our study, also correlates with a study done by Choudhary V et al. (2016) , that Escherichia coli was the predominant etiologic agent and highest resistance to co-amoxyclov 64.00% as in our study it was 59.90%.

In our study, Klebsiella spp. found to be resistant to co-amoxyclov 69.90%. Such high level of resistance of 50.00% and 76.09% was documented from studies done by Kumar AR et al. in Bhopal, India and Ullah F et al. in North West Pakistan respectively.

From this study, it is clear that the uropathogens are becoming resistant to the most commonly prescribed antibiotics for uncomplicated UTIs treatment. The spectrum of uropathogens isolated from urine samples in this study is very similar to the studies done in different region of India.

It is well proved that the incidence of UTI is more common in females as compared to males which may be anatomical predisposition or other host factors. UTI is more common in females than males, as female urethra structurally found less effective for preventing the bacterial entry. It may be due to the proximity of genital tract and urethra and adherence of uroepithelial mucosa to the mucopolysaccharide lining. The other main factors which make females more prone to UTI are pregnancy and sexual activity. Sexual activity in females increases the risk of urethral contamination as the bacteria could be pushed into the urethra during sexual intercourse as well as bacteria being massaged up the urethra into the bladder during child birth. From our study, highest frequency of infection was observed in females than males which are agreement with this generalization. Among the factors contributing to increased frequency of UTI in women, the major predisposing factors are vaginal colonization with uropathogens, sexual trauma to urethral opening, changes in pH during pregnancy and obstruction. However, uncomplicated UTI may also occur in men because of insertive anal intercourse or lack of circumcision.
or having sexual partner with vaginal colonization with uropathogenic microorganisms or lack of immunity. *Enterobacteriaceae* have several factors responsible for their attachment to the uroepithelium. These gram negative aerobic bacteria colonize the urogenital mucosa with adhesion, pilli, fimbiae and P1-blood group phenotype receptor. The antimicrobial susceptibility pattern of uropathogens varies widely by region.

The various reasons for increasing antibiotic resistance in country like India could be irrational use of antibiotics, over the counter availability of higher antibiotics, poor sanitation, high prevalence of diarrhoea, overcrowding and poor facility to conduct antibiotic sensitivity surveillance in hospitals. Most of hospitals including medical colleges have no proper implementation of antibiotic policy and irrational use of antibiotics in these hospitals is common. After the study by Kumarasamy et al. got published in lancet infectious disease, debate again started in India regarding the problem of antibiotic resistance, especially in Enterobacteriacea family of organisms which was highlighted in the study.

There may be various ways by which Enterobacteriaceae acquire resistance, but production of extended-spectrum beta-lactamase (ESBL) is more important. This emerging trend of resistance in Enterobacteriacea may lead to disastrous consequences with huge economic burden, as in years to come no antibiotics may remain effective.

In our study, *Escherichia coli* is the most common organism isolated and *Klebsiella spp.* is being second from the patients suffering from urinary tract infection. The resistance pattern shows that most of the isolates are resistant against ampicillin, cotrimoxazole, nalidixic acid, and nitrofuratoin. In this study *Klebsiella* isolates shows more resistance than *Escherichia coli*. The major difference was observed for gentamicin and amikacin while klebsiella isolates showed high resistance to gentamicin and amikacin despite being sensitive earlier.

### VI. Conclusion

From this study it is concluded that there is increasing resistance to routinely used antimicrobials. Even the drugs like Imipenem, Ceftriaxone and Nitrofurantoin are also becoming resistant to *Escherichia coli* and *Klebsiella spp.*. Here, *Klebsiella* isolates in this study showed more resistance usual prescribing drugs than *Escherichia coli* isolates. The major difference was observed for Gentimicin and Amikacin and Gentamicin; *Escherichia coli* isolates showed low resistance to Amikacin and Gentamicin, while *Klebsiella* isolates showed high resistance to these agents despite being sensitive for many years.

In our study, culture positive rate for uropathogens was higher, with majority were adult female patients. *Escherichia coli* was the most common etiological agent followed by *Klebsiella spp.*, then *Staphylococcus aureus, Pseudomonas spp.*, *Coagulase-negative Staphylococci* and *Enterococci*. In poor resource settings where the availability of alternative effective antibiotics is limited, serious problems arise in the treatment of multidrug resistant uropathogens. This multidrug resistance problem is not only a challenge for UTIs treatment but also for public health by threatening the lives of individuals. Therefore, it warrants for uniform antibiotic policy in local hospitals as well as in teaching institutions. In institutions one must follow the recommendation laid by the board of antimicrobial policy.

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