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***Research Article** (Open Access)*

PREVALENCE OF MICROORGANISMS CAUSING URINARY TRACT INFECTION AND ITS ANTIMICROBIAL SUSCEPTIBILITY PATTERN

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ABSTRACT
Urinary tract infection is one of the most common bacterial infections seen in clinical practice particularly in developing countries. The changing pattern of antimicrobial susceptibility of bacterial pathogens causing acute Urinary tract infection (UTI) is a growing problem. The retrospective cross-sectional study was carried out for duration of six months, from April 2022 to September 2022. A total of 200 positive Urine samples data were scrutinized for the study. The rate of culture positivity in females was 54% and in males was 45%. *E-coli* was the most frequently isolated urinary pathogen (54.5%), followed by *Klebsiella* sp. (13%), *Enterococcus* sp. (9%) and *Enterobacter* sp. (5%). The most susceptible age group in females was 20-60 years and for males was 0-5 years' age group. *E-coli* was highly sensitive to Amikacin, Imipenem and Meropenem and exhibited high resistance to Ampicillin (83.4%) and Ciprofloxacin (40.36%) Cefexime (43.11%). *Klebsiella* sp. was highly sensitive to Gentamycin, Amikacin, Meropenem and Imipenem and showed highly resistant to Ampicillin and Cefotaxime. Higher prevalence of UTI was seen in females. *E.coli* was the most commonly isolated organisms in UTI. Urinary pathogens showed resistance to commonly used antibiotics like Ampicillin, ciprofloxacin. Antibiotic susceptibility testing revealed that 46% (92/200) of the pathogenic bacteria isolated from urine samples were Multi drug resistant organisms. It was found that 54.1% (59/109) of *E. coli* and 42.3% (11/26) of *Klebsiella sp.* were multi drug resistant followed by *Enterococcus sp., Morganella sp., Enterobacter sp., Acinetobacter sp., Staphylococcus sp., Streptococcus sp. and Corynebacterium sp.*

Keywords: Urinary tract Infections, Antibiotic resistance, Multi drug resistance

INTRODUCTION
Urinary tract infections (UTI) are considered to be one of the common infections affecting the community. It is the most routine clinical scenario observed in Physician's desk as well in Gynecology department. UTI is regarded as significant if the microorganisms out number 10^5 organisms per milliliter of urine in two consecutive mid-stream urine samples. Clinical symptoms may be varied depending upon the site of infection, etiological agents, severity as well the immune status of patients. The prevalence was high from old days and females are having higher prevalence rate than males due to their anatomical and physiological peculiarities.

Antibiotic resistance is a global issue in the current scenario. Most of the common pathogens exhibit antibiotic resistance against the commonly used drugs because of inappropriate use of antibiotics, availability of antibiotics through pharmacy without any restriction and the prescription of antibiotics by Physician without proper antibiotic susceptibility testing. Over the past few Years, there is a dramatic increase in the rate of multi drug resistant organisms among uro-pathogens. The community must be aware about the depth of the problem so as to have the precautionary measures in place. This current study is aimed to determine the prevalence of the UTI-causing pathogens, and their antimicrobial susceptibility pattern among individuals with suspected UTIs.

Kanika *et al.,* (2022). conducted a study on "Bacterial profile and antibiotic susceptibility pattern of uropathogens causing urinary tract infection in the eastern part of North India''. The study was carried out between November 2018 and December 2019. A total of 427 samples were collected and processed. Out of

427 samples 333 were found positive, where 287 were Gram-negative bacteria (GNB), and 46 were Gram-positive bacteria (GPB). Females had a higher prevalence of UTI (60.7%) than males (39.3%). The most susceptible age group in females was 18–50 years as compared to males, whereas at the age of 51–80 years and >80 years males were more susceptible than females. The most prevalent pathogen identified were *Escherichia coli* (55.0%), followed by *Proteus sp.* (6.9%), *Klebsiella pneumoniae* (6.6%), *Pseudomonas aeruginosa* (6.3%), of which 96.0% were Multi drug resistant bacteria (MDR) bacteria.

Shamataj *et al.,* (2012) carried out a study on "Bacteriology of Urinary Tract Infection and Antibiotic Susceptibility Pattern in a Tertiary Care Hospital in South India". The study was done from January 2011 to December 2011. A total of 573 mid-stream urine samples from the suspected UTI patients were tested microbiologically and antimicrobial susceptibility test were performed. The rate of culture positivity in females was 87.82% and in males was 27.92%. *E. coli* was the most frequently isolated urinary pathogen (37.95%), followed by *Klebsiella spp* (21.41%) and *Acinetobacter spp* (10.94%). *E.coli* was highly sensitive to Nitrofurantoin (81.92%) and Amikacin (69.88%) and it was highly resistant to Ampicillin (1.0%). *Klebsiella sp.* was highly sensitive to Imipenem and it was highly resistant to Ampicillin.

Madhu *et al.,* (2020) did a study on "Prevalence and antibiotic susceptibility pattern of pathogens in children with urinary tract infection in a tertiary care hospital". A retrospective cross-sectional study was conducted in 208 children of 1-18 years age group with suspected UTI infection who were admitted in KIMS hospital, Bangalore from January to December 2018. Overall 208 children between 1-18 years with suspected UTI were screened. Out of which 48 were culture positive, with a prevalence of 23%. Culture positive UTI was predominantly found in males in 1-5 years age group as against female predominance in 6-18 years age group. *E.coli* (45.83%) was the commonest organism isolated in our study, followed by *Enterococcus spp* (31.25%), *Klebsiella spp* (16.67%), *Proteus spp* (4.17%) and *Acinetobacter spp* (4.17%). Antibiotics with highest sensitivity to *E.coli* were Amikacin (91%) and Gentamycin (77%). *Klebsiella sp.were* most sensitive to Gentamicin (87.5%) and Piperacillin (75%). *Enterococcus spp* has highest sensitivity to Vancomycin (67%) and Linezolid (60%).

Poonam *et al.,* (2020) performed a study on "Prevalence and in vitro antibiotic susceptibility pattern of bacterial strains isolated from tribal women suffering from urinary tract infections in District Anuppur , Madhya Pradesh, India''. Urine samples were collected from 550 tribal women and samples were cultured. Out of 550 urine samples, 360 (65.45%) were culture-positive and 190 (34.55%) were culture-negative. *Klebsiella pneumonia, Proteus mirabilis, Escherichia coli* & *Pseudomonas aeruginosa* have been identified as major uropathogens. *Klebsiella pneumoniae* is the most prevalent bacteria followed by *P. mirabilis, E. coli* and *P. aeruginosa*. The antibiotic susceptibility profiling showed that most of the isolates of *K. pneumoniae, P. mirabilis* and *E. coli* were resistant against penicillin (86.67-100%) and Rifampicin (72.73-97.67%). Gentamicin, Kanamycin and Streptomycin were the most active antibiotics against the isolates of *P. aeruginosa, P. mirabilis, K. pneumonia* and *E. coli* respectively.

A retrospective analysis of culture results of urine samples was conducted by Pritam Pardeshi at Microbiology department of tertiary health care hospital in Mumbai. A total of 1741 data collected and out of it 584 showed positive with various pathogens. The overall prevalence of UTI was 33.54% of which 66.78% were females and 33.22% were from males. High prevalence was observed in females as compared to males (2:1). Though the overall prevalence was high in old aged (>45 years) patients, in females high prevalence was seen among middle-aged (31 to 45 years) patients and in male high prevalence was seen among old age (>45 years) patients. From total 584 uropathogens, *E.coli* (53.77%) was the commonest isolate causing UTI followed by *Klebsiella pneumoniae* (27.40%). The most effective antimicrobial agents in their study were Meropenem, Gentamicin, Nitrofurantoin and Cotrimoxazole whereas higher resistance was observed among Fluoroquinolones, Amoxicillin and third generation Cephalosporin; these are the drugs which are commonly given empirically for UTI (Pritam, 2018).

In the study conducted by Mohammad *et al.,* (2015) during June to December 2020 titled, "Prevalence and Antimicrobial Susceptibility pattern of pathogens Isolated from different age groups with Urinary Tract Infection in Bangladesh", identified pathogens were mostly E. coli (82.41%) followed by *Enterococcus*

faecalis, Klebsiella sp, Pseudomonas aeruginosa and *Proteus sp*. Most of the bacteria showed high (≥ 98.25%) sensitivity to Meropenem, moderate susceptibility to Amoxicillin, Azithromycin, Ciprofloxacin, Gentamicin, Levofloxacin, Ceftriaxone, Cefepime and Nitrofurantoin and least (<20%) sensitivity to Cefixime, Cephradine, Cefuroxime, Clindamycin, and Trimethoprime.

Pandey *et al.,* (2020) from Tribhuvan University Teaching Hospital (TUTH), Nepal conducted a study on "Antimicrobial Susceptibility pattern of pathogenic bacteria causing urinary tract infection in tertiary care hospital in Kathmandu". A prospective observational study was conducted from April 2017 to September 2017. 209 sample was taken for the study out of it 191(91.40%) were gram-negative bacteria, and 18(8.60%) were Gram positive. *E. coli* (52.5%) was found to be the predominant organism of UTI and showed high sensitivity to Nitrofurantoin (93.3%), Meropenem (92.9%), and Colistin (82.3%).The most effective antimicrobial agent against Gram-positive *Enterococcus spp* species were linezolid (100%), Vancomycin (100%) and Nitrofurantoin (100%). A very high rate of resistance was seen against Amikacin (75%), Nalidixic acid (68.7%), and Cotrimoxazole (63.9%) in Gram-negative bacilli isolates of *E. coli*. *Escherichia coli*, the most common uropathogens isolated more commonly from the female (66%) patients compared to the male (34%) patients and isolation of *E. coli* among female patients is statistically significant.

Muhammad *et al.,* (2020) carried out a study on "Prevalence and antibiotic susceptibility pattern of uropathogens in outpatients at a tertiary care hospital". A cross-sectional study was carried out over 7 months (January to July 2019). In total, 804 urine samples were collected from individuals with suspected UTIs and inoculated on recommended media. Among the tested specimens, 290 (36.1%) had significant bacterial growth and 147 (50.7%) of the strains were isolated from female patients. The frequently identified isolates were *Escherichia coli (*68.9%), followed by *Klebsiella pneumoniae* (8.9%) and *Staphylococcus aureus* (6.7%). The highest percentages of resistance have been observed against tested antibiotics. The majority of the isolates were extended spectrum β-lactamase producers (85.2%) and multidrug-resistant (98.3%). They observed that Gram-negative bacteria were the main cause of UTIs where the predominant microorganism was *E. coli.*

Akshatha *et al.,* (2021) conducted a study on "Antimicrobial Susceptibility Pattern of Urinary Pathogens in a Tertiary Care Hospital". A total of 200 urine samples were obtained from patients with signs and symptoms suggestive of UTIs attending Bapuji and Chigateri General Hospital for a period of 3 months that is from May 2016 to July 2016. There were 170 urinary isolates from 200 samples. Children less than 10 years were more affected 54 (27 %). Female patients were more, 119 (59.5 %) compared to males 81 (40.5 %). *Escherichia coli* was the most predominant isolate, 71 (41.8 %) followed by *Enterococcus species* 22 (1.9 %). Organisms were resistant to commonly used antibiotics i.e., Cotrimoxazole, Ciprofloxacin, Ciprofloxacin and Amoxicillin. Both Gram negative and Gram-positive isolates were sensitive to Amikacin and Nitrofurantoin.

Gholamreza *et al.,* (2017) conducted a study on "Antibiotic susceptibility patterns of uropathogens among children with urinary tract infection in Shiraz". This cross sectional study was performed among 202 children with UTI, aged 2 months to 18 years old, between August and November 2014 in pediatric medical centers of Shiraz. The results showed that the frequency of UTI was significantly higher in girls (70.3%) than in boys. The most commonly discovered pathogens were *Escherichia coli* (51.5%), followed by *Klebsiella spp.* (16.8%), and *Enterococcus spp.* (9.9%). Overall susceptibility test showed the highest resistance to Ampicillin (81.2%) and Cotrimoxazole (79.2%), and the highest sensitivity to Imipenem (90.1%) and Gentamicin (65.3%). Gram negative and positive bacteria showed the highest antibiotic resistance to Amoxicillin (83.8%) and Clindamycin (100%), respectively. In addition, production of extended spectrum beta lactamase (ESBL) was 69.2% and 30.8% in *E. coli* and *Klebsiella sp* respectively.

Aim and Objectives

Aim

The study was aimed to determine the prevalence of microorganisms causing UTI and its antibiotic susceptibility pattern.

Objectives
1. Prevalence of microorganisms causing UTI.
2. Evaluate antibiotic susceptibility pattern on isolated organisms.
3. To find MDR organisms in UTI samples.

MATERIALS AND METHODS

Study design
A retrospective cross sectional study design was implemented and carried out for duration of six months, from April 2022 to September 2022. It was carried out at Ahalia Hospital, Palakkad, Kerala. The hospital provides tertiary care to the patients in and around Palakkad .This was conducted based on in-patient and out-patient medical records of patients admitted at Ahalia Hospital. The samples were processed in the Microbiology section at NABL accredited Ahalia Central Laboratory attached to Ahalia Hospital. In the current study, study age, sex, the organism isolated and the antimicrobial susceptibility profiles were collected from the records. A total of 200 patients data were collected and entered into excel for analysis and statistical analysis. Ethical clearance was obtained from Ahalia Institutional Ethical Committee.
Inclusion and Exclusion Criteria
Patients of all age groups were included in this study. The records of patients' data which contains the patient's age, sex, urine culture result including antimicrobial susceptibility test (AST) results for significant bacteriuria (10^5CFU/ml) of monomorphic organisms which have been processed and recorded from April 2022 to October 2022 were included. However, records lacking at least one of the variables age, sex, urine culture results, and AST results of cultures with significant bacteriuria were excluded.
Processing Samples
The processing of samples including isolation, identification and antibiotic susceptibility patterns were practiced using Vitek 2 Compact system using Advanced Colorimetry™

RESULTS AND DISCUSSION

A total of 200 samples were included in the current study. Out of the positive isolates, 172 (86%) samples yielded the growth of Gram negative bacteria, 24 (12%) samples yielded the growth of Gram positive bacteria and 4 (2%) samples yielded the growth of *Candida sp*. *E.coli* was the most frequently isolated urinary pathogen (54%), followed by *Klebsiella sp* (13%), *Enterococcus sp* (9%) followed by *Morganella sp, Acinetobacter sp, Proteus sp, Pseudomonas sp, Morganella sp, Citrobacter sp, Candida sp, Corynebacterium spp , Enterobacter sp* and *Streptococcus sp*. The isolation rates of organisms and overall distribution of the pathogens causing UTI is shown in the Figure1.
The majority of the causative agents of urinary tract infection in the study were Gram-negative bacteria 176 (88%) which was comparable with previous studies conducted by Tigist Mechal *et al.,* (2021) 71.1%, Sanjib Adhikari *et al.,* (2019) 72.9%, Alka Nerurkar *et al.,* (2012) 76.78%, Sneka and Mangayarkarasi (2019), 70.6%, Veronica Folliero *et al.,* (2020) 78.5% and Desie Kasew *et al.,* (2022) 82%. Although *E. coli* was the commonest organism isolated in the present study (54.5%), the prevalence was varied in different studies such as that of A. Muhammad *et al.,* (2020) (68.3%). However, some studies, such as, Islam Shaifali**,** *et al.,* (2012) (33.1%), Desie Kasew *et al.,* (2022) (37.7%), Akram M et al (21.4%) Pina Caput 53.5%, Gholamreza Pouladfar *et al.,* (51.5%), have shown less prevalence of *E. coli* as compared to the present study.
In the current study, *Klebsiella sp* and *Enterococcus sp* were more common in females as compared to *Pseudomonas sp* and *Moragnella sp* which were more common in males.
The reason why *E-coli* and *Enterococcus sp* cause UTI more commonly in females is might be due to the migration of bacteria from the anal canal to the lower urinary tract due to lack of cleanliness, faulty toilet cleaning and contaminations from used diapers (Madhu *et al.,* 2020)
Data was classified based on the gender and there were 92 males and 102 females and study showed that 46% (92/200) of males and 54% (102/200) of females were affected from UTI (Table 2 and Figure 2). The prevalence of UTI in females was higher than the males.

The findings of the study were similar to the study conducted by Kanika Bhargava et al. during 2022 in the eastern part of north India. Females had a higher prevalence of UTI (60.7%) than males (39.3%). The study of Devanand Praksh *et al.*, (2013) study showed a high prevalence of UTI in females (73.57%) than in males (35.14%) which correlates with other findings which revealed that the frequency of UTI is greater in females as compared to males.

In the present study, the data was classified based on the age group. The most susceptible age group of UTI was between 20 and 60 (37.5%) followed by 0-5(29%),>60(20%) and 6-20 (14%). Among females UTI was commonly seen in the age group of 20-60 years and in males it was common in between 0 -5 years. The age wise distribution of UTI is shown in Table 2 and Figure 3.

The proximity of the urethral meatus to the anus, the shorter urethra, sexual intercourse, incontinence, and improper toilet habits may contribute to a higher rate of UTI in females than in males. Along with that the hormonal changes also plays an important determinant of UTI. Estrogen decline during menopause will lead to the reduction in Lactobacillus Sp. and elevation of *E,coli* and this shift of vaginal flora in turns increases the incidence of UTI. The highest susceptible age group of male was 0-5 years; this result was in line with the study done by Yamirot *et al.*, (2018), which showed urinary tract infections tend to occur in smaller children, that is in the age group 1-5 years. The reason behind this result might be due to the fact that the smaller children are still not trained properly to urinate and have not been able to maintain proper genital area hygiene, along with the increased practice of keeping diapers for a prolonged duration which might be the reason behind increased prevalence. Lower prevalence in 6-18 years' age group may be due to improvement in their immunity, improved personal hygiene and quitting the practice of using diapers.

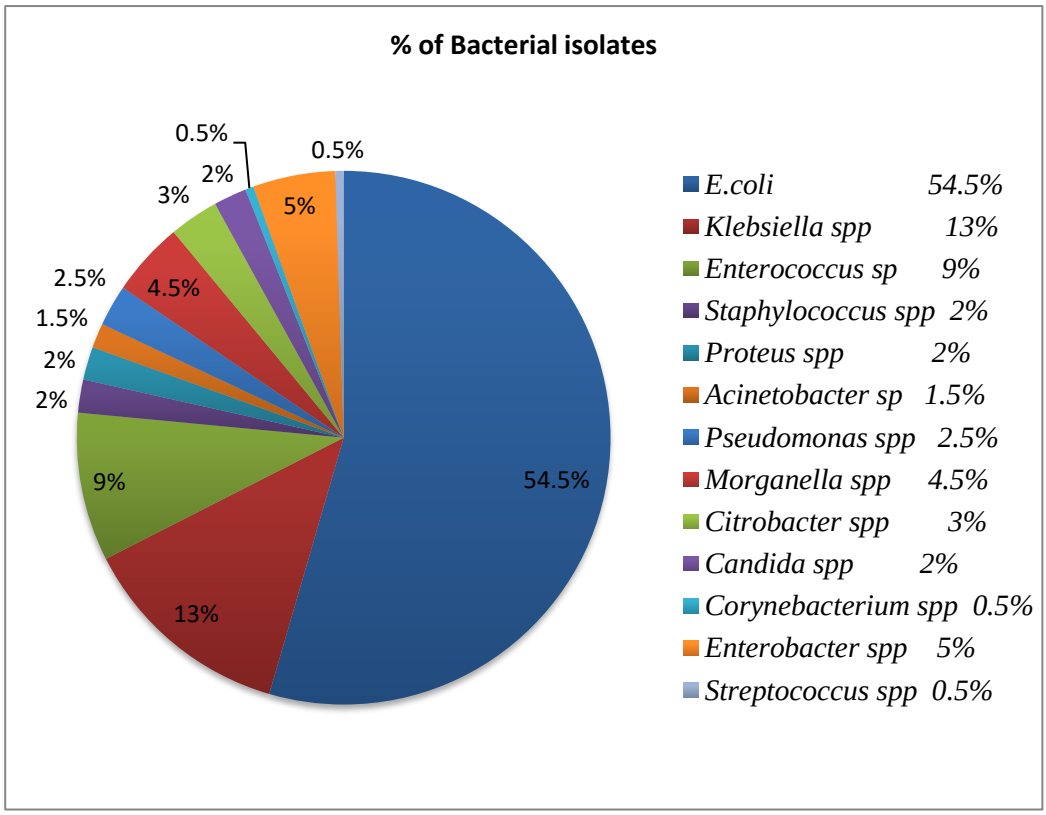

Figure 1: Percentage of Bacterial Isolates in UTI (N=200)

Antibiotic susceptibility results showed the resistant and susceptible antibiotics for the tested uropathogens Antibiotics with highest sensitivity to *E. coli* were Amikacin (76.14%), Imipenem (71.55%) and Meropenem (71.55%), Gentamicin (66.9%), highest resistance is seen for Ampicillin (83%), Cefexime (43%),

Ciprofloxacin (40%) and Ceftriaxone (40%). A Study conducted by *Madhu et al., (2020)*, showed that Amikacin as the most sensitive antibiotic and Ampicillin the most resistant antibiotic to *E. coli.*
Klebsiella sp was highly sensitive to Gentamicin, Amikacin, Meropenem and Imipenem and it was highly resistant to Ampicillin and Cefotaxime. The antibiotic resistance of mostly isolated five organisms is shown in the tables, 4, 5, 6, 7 and 8.

Table 1: Prevalence of UTI Based On Gender

Sex	Number of samples	Percentage of samples (%)
Male	92	46%
Female	108	54%
Total	200	100%

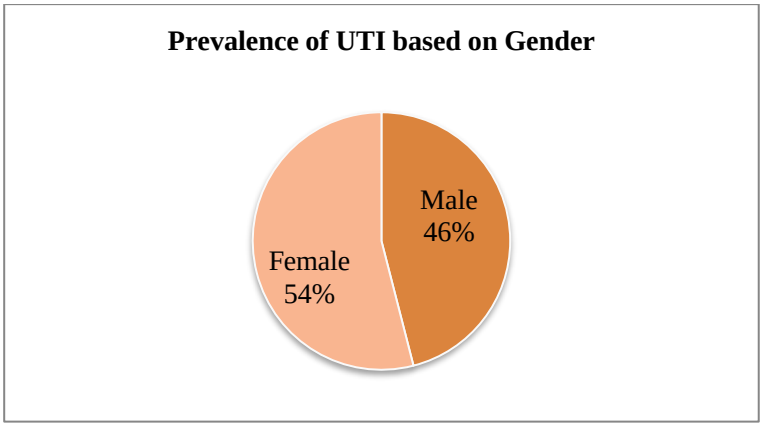

Figure 2: Prevalence of UTI Based On Gender

Table 2: Age wise distribution in relation to positive urine culture

Age	Total number of patients	Male	Percentage	Female	Percentage
0-5	58	35	63.3 %	23	39.6%
6-20	28	10	35.7%	18	64.2%
20-60	75	22	29.3%	53	70.6%
>60	39	18	46.1%	21	53.8%

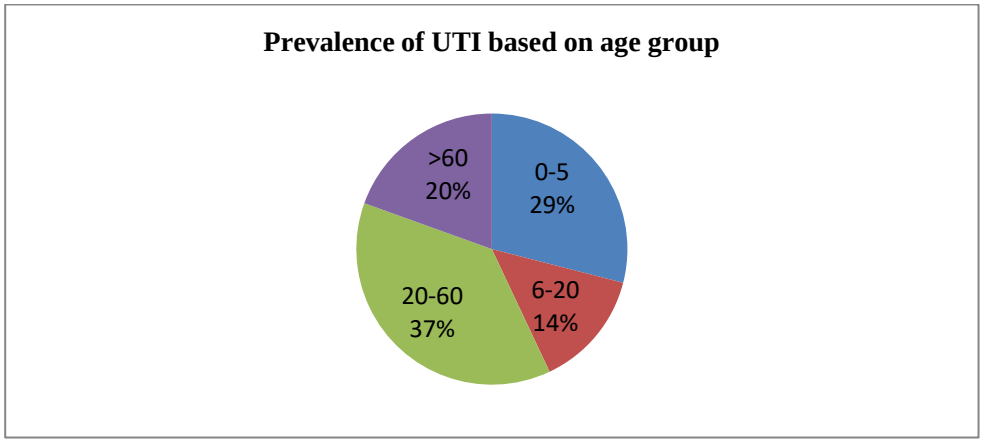

Figure 3: Age Distribution in Relation to Positive Urine Culture

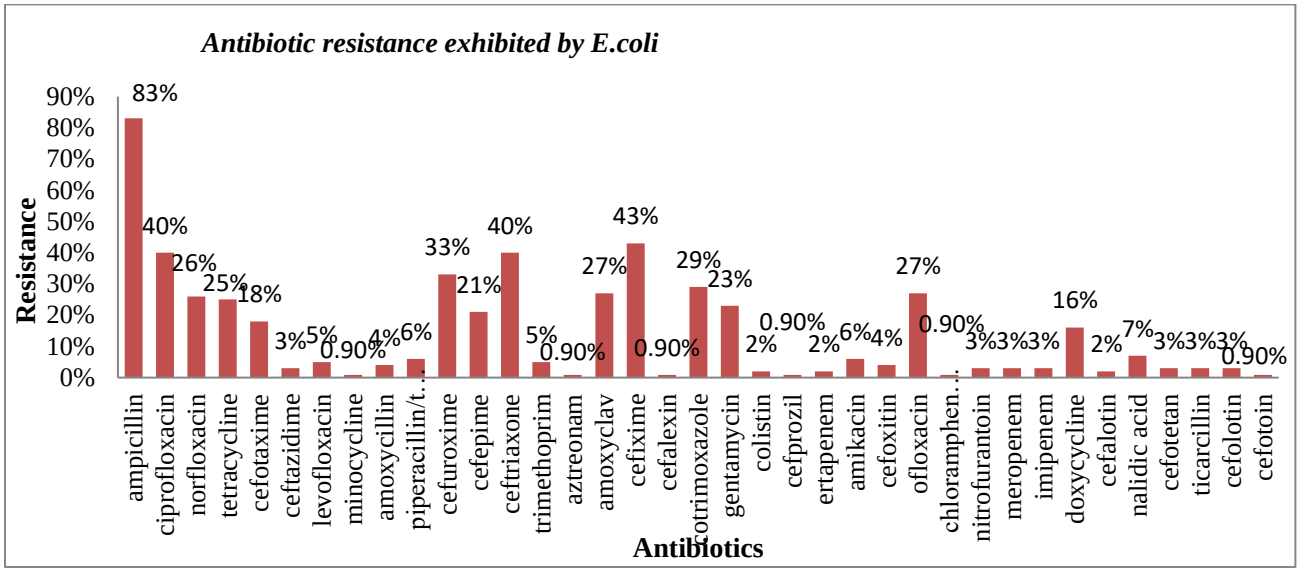

Figure 4: Antibiotic resistance chart of *E.coli*

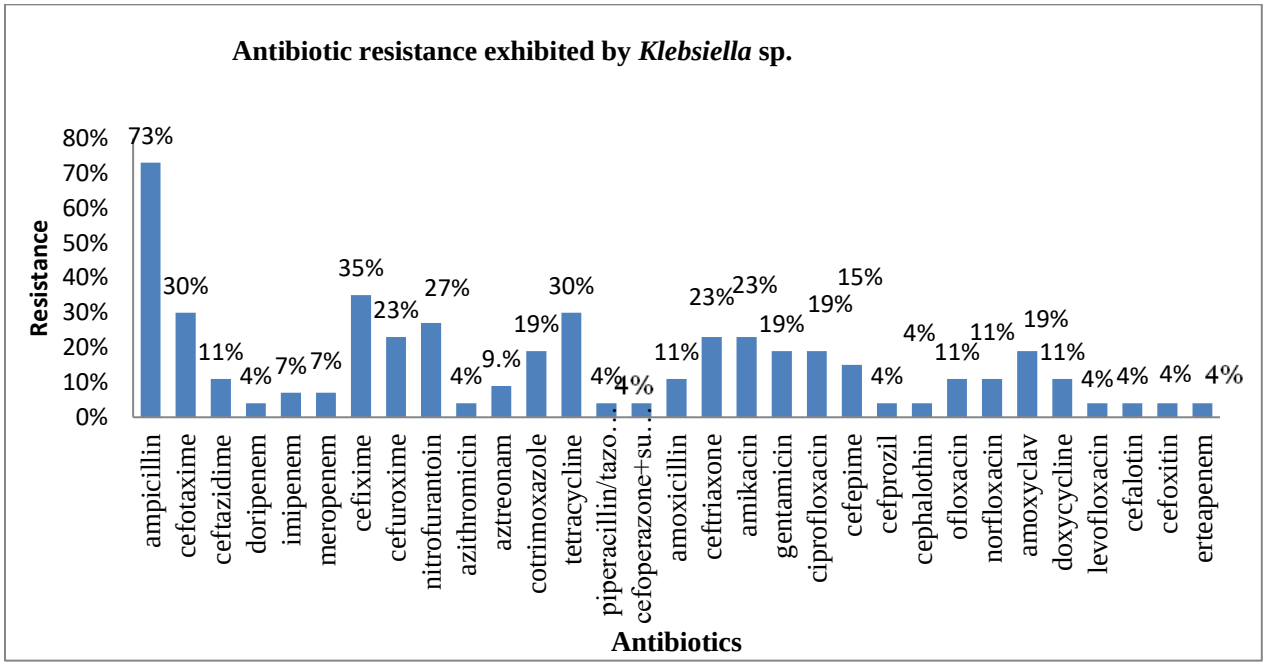

Figure 5: Antibiotic resistance chart of *Klebsiella sp.*

Antibiotic susceptibility testing revealed that 46% (92/200) of the pathogenic bacteria isolated from urine samples were Multi drug resistant organisms. It was found that 54.1% (59/109) of *E. coli* and 42.3% (11/26) of *Klebsiella sp.* were multi drug resistant followed by *Enterococcus sp., Morganella sp., Enterobacter sp., Acinetobacter sp., Staphylococcus sp., Streptococcus sp. and Corynebacterium sp.*

Only 2 % of the isolates were Staphylococci and out of them a quarter were MDR. One organism each were identified under Streptococci and *Corynebacterium* species, but both turned to be MDR. Multidrug resistance was evident in all the bacterial isolates types and no species turned out to be exceptional.

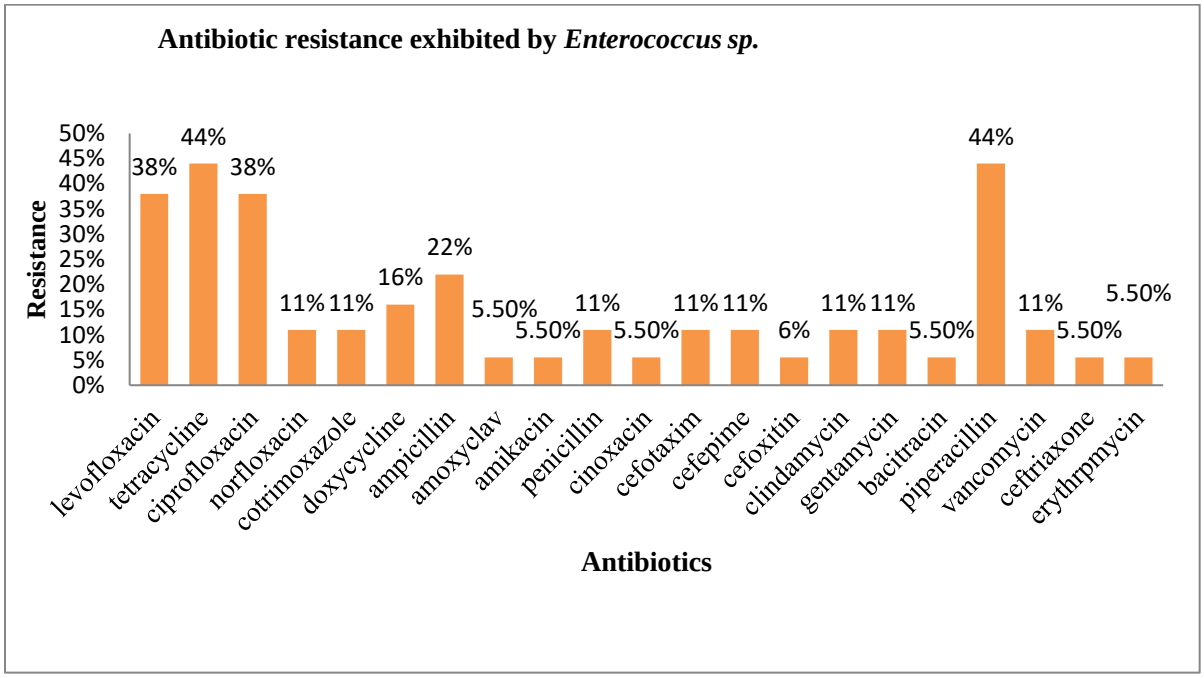

Figure 6: Antibiotic resistance chart of *Enterococcus* sp.

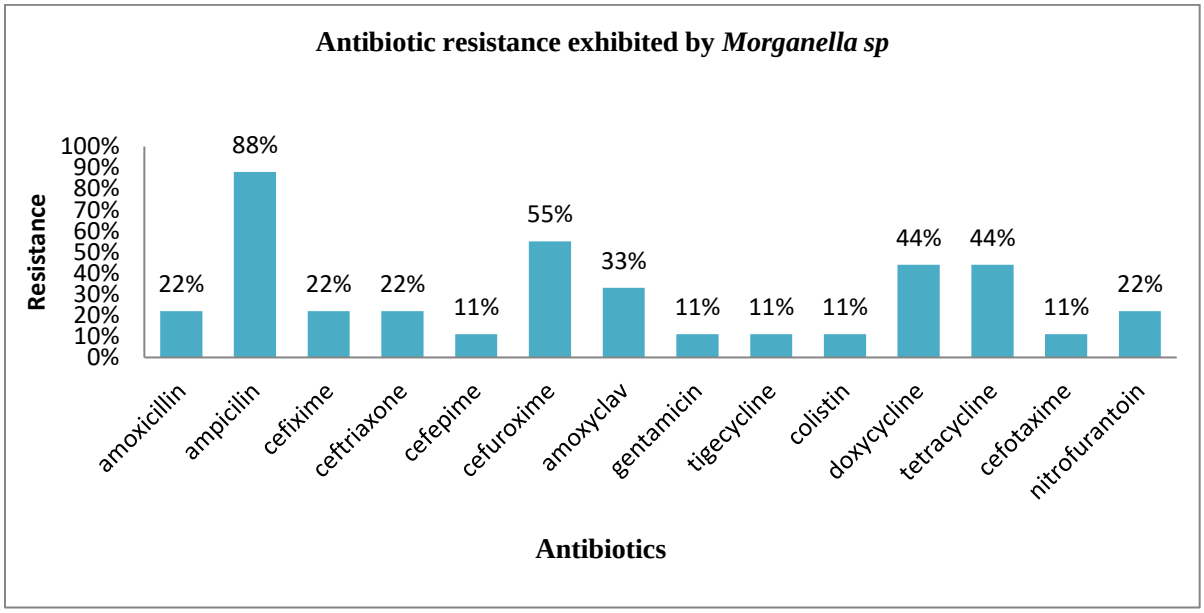

Figure 7: Antibiotic resistance chart of *Morganella* sp.

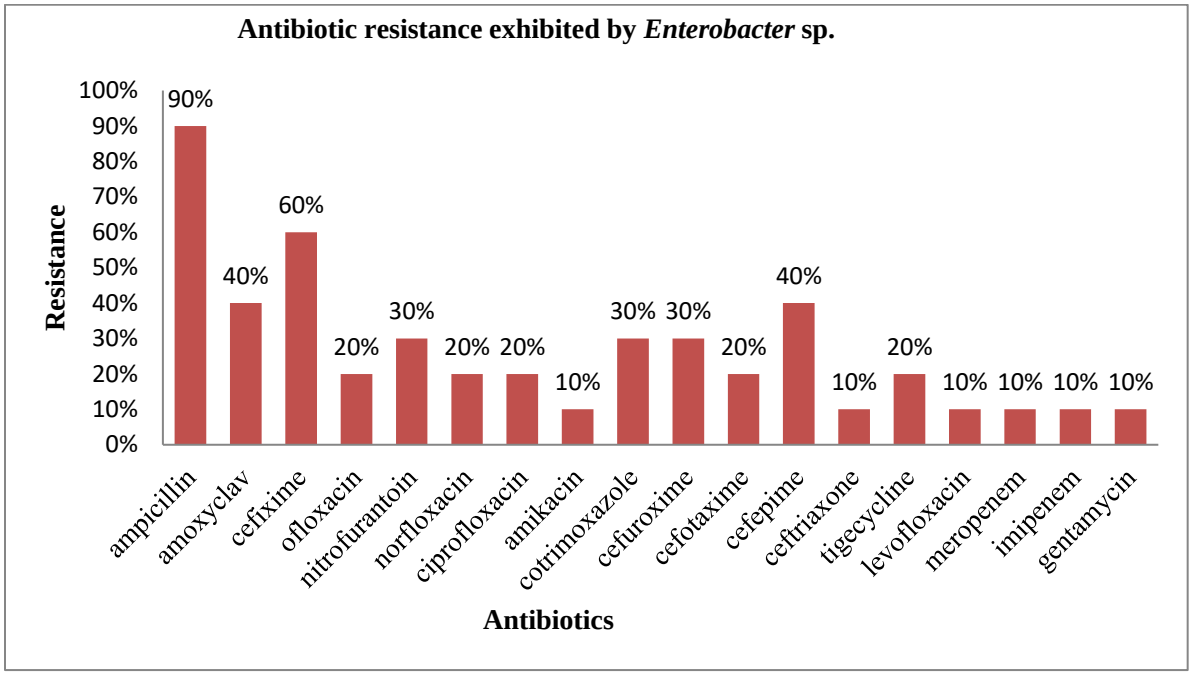

Figure 8: Antibiotic resistance chart of *Enterobacter* sp.

Table 3: MDR organisms from positive isolates

Bacterial pathogen	Total no. of organisms	MDR organisms
Escherichia coli	**109 (54.5%)**	**59 (54.1%)**
Klebsiella sp	**26 (13%)**	**11 (42.3%)**
Staphylococcus sp	**4 (2%)**	**1(25%)**
Enterococcus sp	**18 (9%)**	**9(50%)**
Acinetobacter sp	**3(1.5%)**	**2(66.6)**
Morganella sp	**9 (4.5%)**	**4(44.4%)**
Citrobacter sp	**6(3%)**	**1(16.6%)**
Corynebacterium sp	**1 (0.5%)**	**1(100%)**
Enterobacter sp	**10(5%)**	**3(30%)**
Streptococcus sp	**1(0.5%)**	**1(100%)**

The high MDR in this study might be due to widespread misuse of antibiotics, inappropriate prescription of drugs, and lack of knowledge about drug resistance in the study area. The study showed that urinary pathogens are still sensitive to commonly used antibiotics, particularly in community infections, but species distribution and their susceptibility to antibiotics are changing. It requires regular monitoring to determine the current status of resistance against antimicrobial agents.
The high MDR in this study might be due to widespread misuse of antibiotics, inappropriate prescription of drugs, and lack of knowledge about drug resistance in the study area.

CONCLUSION

The present situation is alarming, because of emergence of resistance against commonly used antibiotics in UTI. Different factors are attributable for emergence of resistance like high consumption of antibiotics, irrational use, incomplete course of therapy, and self-medication by patients. If this continues an effective antibiotic would be failed to treat even simple or minor infections. Hence, to determine the current status of

resistance against antimicrobial agents, routine antimicrobial susceptibility testing must be timely performed. Each country or region has epidemiological data on the bacteria that cause UTIs and different antibiotic sensitivity patterns which is important for guidance in UTI therapy. The pattern of bacterial sensitivity to antibiotics is rapidly changing over time, especially in developing countries so knowledge of hospital sensitivity pattern is at most important for proper selection of antibiotics in UTI therapy.

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