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ISOLATION AND ANTIBIOTIC SENSITIVITY PATTERN OF MICROORGANISMS FROM URINE SAMPLE CAUSING UTI IN A TERTIARY CARE HOSPITAL, SOUTH KARNATAKA – A RETROSPECTIVE STUDY

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ABSTRACT

Aim and objective: A retrospective study was undertaken to identify the various pathogens isolated from the urine samples and to determine the antibiotic resistance pattern of the major uropathogens causing UTI among patients attending Kasturba Hospital, Manipal. **Materials and Methods:** A total of 96 patients (40-60 years) with UTI, from the period of April to may 2014 , urine samples were collected and examined for microscopic, Gram stain and culture techniques. Isolated microorganisms were identified using microscopically, morphological and biochemical tests. Antibiotic susceptibility tests were done on Mueller-Hinton agar by the Kirby-Bauer disk diffusion method as per Clinical and Laboratory Standard Institute Guidelines. **Results:** The most common organism isolated was *Escherichia coli* 71 *(74%*)followed by *Klebsiella pneumoniae* 16(*17%*). The other organisms isolated were *Pseudomonas* 1 *(2%), Beta-hemolytic streptococci* 1 *(2%), Methicillin resistant Staphylococcus aureus* 2*(2%),Enterococcus* 1 *(1%),Enterobacter* 1*(1%)* and *Proteus* 1 *(1%). Escherichia coli* was sensitive to Amikacin and most resistant to Ampicillin/Amoxicilli 81(84%cases were resistant).*Klebsiella pneumonia* has developed complete resistance to Ampicillin/Amoxicillin96(100%) group of drugs and sensitive to Amikacin.Among the first line drugs, Amikacin and Netilmicin are the most effective whereas the Penicillin group of drugs is the least effective.Imipenem is the most effective reserve drug and high level of resistance is seen against the third degree cephalosporins. **Conclusion:** The study presents a very dismal picture against all the drugs used in empirical therapy for UTI. Both the Penicillin group and the Cephalosporin group of drugs have developed significant resistance against them.The only oral antibiotic effective among the first line drugs is Cotrimoxazole.

KEYWORDS: Urinary tract, Infection, Escherichia coli, Ampicillin, *Klebsiella pneumoniae,* Kirby-Bauer disk diffusion.

INTRODUCTION

Antibiotics provide the main basis for the therapy of microbial (bacterial and fungal) infections. Since the discovery of these antibiotics and their uses as chemotherapeutic agents there was a belief in the medical fraternity that this would lead to the eventual eradication of infectious diseases. However, overuse of antibiotics has become the major factor for the emergence and dissemination of multi-drug resistant strains of several groups of microorganisms.[1] The worldwide emergence of *Escherichia coli, Klebsiella pneumoniae, Haemophilus* and many other ß-lactamase producers has become a major therapeutic problem. Multi-drug resistant strains of E. coli and *K. pneumoniae* are widely distributed in hospitals and are increasingly being isolated from community acquired infections.[2]

The menace of antimicrobial resistance is a growing global and dynamic phenomenon. It's considered to be one of the major threats to public health. Studies from various parts of the world have shown that between 33%-50% of all antimicrobial use does not meet standard implications.[3] The situation is no different here in Southern Karnataka. It becomes even more important now, as there are hardly any new drugs in the

development pipeline and most Indian hospitals have a major problem of multi drug resistance to many bacilli.[4]

Studies carried out in the community have shown that uropathogens such as *Escherichia coli, Klebsiella spp., Proteus spp.*and *Enterococcus spp.* represent the main cause of UTI. Recent studies in North America and Western Europe demonstrated increasing resistance in UTI *E.coli* to ampicillin, trimethoprim and sulfonamides.[5]

Though there have been efforts to improve and optimize appropriateness of the antibiotic usage, latest information on antibiotic sensitivity and resistance patterns of uropathogens implicated in UTI are incomplete and do not consider host factors.[6] Formulating this information becomes very important as it reflects changes over the years. Also, to administer an appropriate empirical therapy it is crucial to know about the most effective antibiotic with respect to the detected organism and also specific host parameters (age and sex of the patient). These must be kept in mind at the moment of empirical prescription of antimicrobials. Formulation of such data analyzed from the reports obtained from patients suffering from UTI will guide us to proper antimicrobial use, thus containing or preventing the menace of resistance which is soon becoming a public health problem worldwide.[7]

For this, we chose to study Urinary Tract Infection (UTI) because it is one of the commonest bacterial infections managed in health practice, accounting for 15% of all community prescriptions for antibiotics.[8] Therefore, it has large socio-economic impacts and may contribute to the emergence of bacterial resistance.[9]

The aim of the study is to determine the antibiotic resistance pattern of the major uropathogens causing UTI among patients attending Kasturba Hospital, Manipal. This helps to analyze the prevalence and the antibiotic susceptibility pattern of the main bacteria responsible for urinary tract infections, so that any change or trend noticed in bacterial resistance is detected and updated recommendations for UTI treatment can be given.

MATERIALS AND METHODS

A retrospective study was carried in the department of microbiology Kasturba Hospital from April 2014 to May 2014. A total of 96 Patients were included in the study. The patients above 18 years of age, attending Kasturba Hospital, Manipal with the clinical symptoms of urinary tract infection and showing a positive urine culture test on urine examination and bacteriology. The patient details were collected from the hospital records and the data regarding the urine culture and sensitivity pattern were obtained from the Microbiology laboratory reports. For patients with more than one sample, only the first positive sample with susceptibility data was included in the study. Samples were excluded if there were duplicate samples with different sensitivities, duplicate samples listed under multiple dates or if samples grew multiple pathogens. For each patient, the hospital IP no., the sample ID, the collection date, age, sex, urine culture results, identification of the bacterial strain responsible for UTI and the corresponding antimicrobial susceptibility test results were registered.

Clean catch, mid-stream urine samples were collected in sterile universal containers. Urine samples were processed within 2 h of collection and, in case of delay, the samples were refrigerated at 2-8°C for up to 6 h.

The samples were plated on Blood Agar (Himedia, Vadhani Ind. Est., LBS Marg, Mumbai, India) and MacConkey Agar media (Himedia, Vadhani Ind. Est., LBS Marg, Mumbai, India) by the semi-quantitative plating method using the calibrated loop technique (0.001 mL). Plates were incubated aerobically overnight at 37°C.

Pure growth of an isolate in a count of $\geq 10^5$ colony forming units (CFU) per milliliter of urine was considered as significant bacteriuria.

Antibiotic susceptibility tests were done on Mueller-Hinton agar by the Kirby-Bauer disk diffusion method as per Clinical and Laboratory Standard Institute Guidelines 2013.

A sample case report (of urine examination and urine bacteriology) is being enclosed with the methodology which includes urine microscopic features (Presence of pus cells and bacteria), Cultural and Antibiotic susceptibility tests reports. On urine bacteriology, the organism isolated and the colony count was noted. Sensitivity or resistance of the bacteria for the antibiotics (as mentioned in the case report) was tested and recorded.

The data collected was treated using the Statistical Package for the Social Sciences SPSS ver. 15 (SPSS, South Asia, Bangalore). All categorical data was summarized using frequency and percentages. Mean and standard deviation was used to summarize the continuous variables. The resistance rate has been expressed as percentage with 95% confidence level.

RESULTS

A total of ninety six patients presenting with clinical symptoms of UTI and having a positive culture report with isolation of one pathogen were considered for this study. Out of which 46 patients were females and 50 were males. The highest number of cases were reported in the age group of [>40 to <=60] years. The age distribution is as presented below (Figure 1).

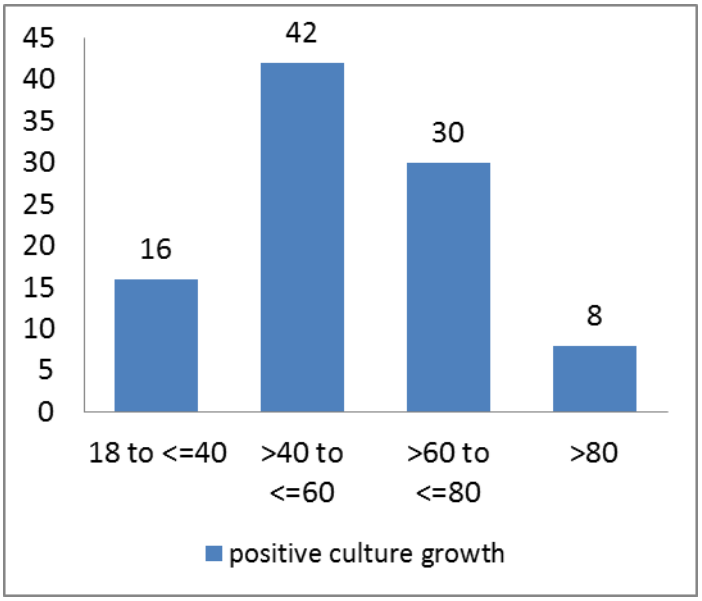

Figure 1: The Age Distribution of Patients.

X axis- Age (in years)Y axis- Number of cases.

Among the 96 samples, 71 cases (74%) showed the growth of *Escherichia coli* followed by *Klebsiella pneumoniae* isolated in 16 samples (17%). The other organisms isolated were *Pseudomonas (2), Beta-hemolytic streptococci (2), Methicillin resistant Staphylococcus aureus (2), Enterococcus (1), Enterobacter (1)* and *Proteus (1)* (Figure 2).

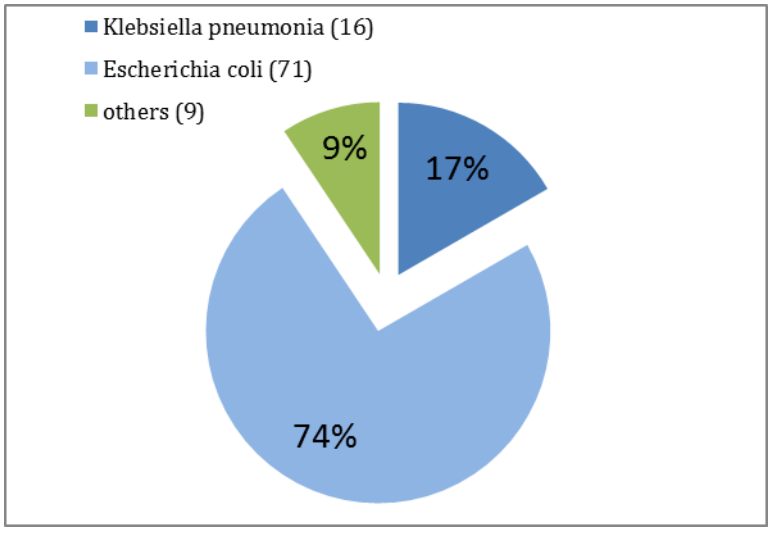

Figure 2: The distribution of different organisms.

The drugs for which the sensitivity patterns of the micro-organisms have been tested are as follows:
First line drugs- Amikacin, Amoxicillin-Clavulanic acid, Ampicillin/Amoxicillin, Cefotaxime/Ceftriaxone, Cefuroxime, Cotrimoxazole, Gentamicin, Netilmicin, Norfloxacin.

Reserve drugs- Aztreonam, Cefoperazone-Sulbactum, Cefpirome/Cefepime, Imipenem, Piperacillin-Tazobactum. The Table 3depicts all the micro-organisms and their sensitivity pattern towards all the antibiotics.

Table 3: Sensitivity pattern showed by the micro-organisms towards the First line antibiotics.

Drugs organism		Amikacin		Amoxicillin-Clavulanic acid.		Ampicillin /Amoxicillin		Cefotaxime /Ceftriaxone	Cefuroxime		Cotrimoxazole			Gentamicin		Netilmicin		Norfloxacin	
	No. of samples	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S
Klebsiella pneumonia	16	7	9	12	4	16	0	12	4	13	3	9	7	10	6	7	9	9	7
Escherichia coli	71	4	67	57	14	60	11	53	18	57	14	40	31	34	37	8	63	54	17
Pseudomonas	1	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0
Beta-hemolytic streptococci	1	0	1	0	1	0	1	1	0	0	1	0	1	0	1	0	1	0	1
Methicillin resistant Staphylococcus aureus	2	2	0	2	0	2	0	2	0	1	1	1	1	1	1	1	1	1	1
Enterococcus	2	0	2	0	2	1	1	0	2	0	2	0	2	1	1	0	2	1	1
Enterobacter	1	0	1	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0
Proteus	2	0	2	0	2	1	1	0	2	0	2	2	0	1	1	0	2	1	1
Total	96	14	82	73	23	82	14	70	26	73	23	54	42	49	47	18	78	68	28

The resistance pattern of the most commonly isolated organism *Escherichia coli* and *Klebsiellapneumoniae*have been analysed. Among the first line drugs, it is seen that *E.coli* had the maximum percentage of resistance against Ampicillin/Amoxicillin 60(84.5% Isolates were resistant) and the maximum sensitivity for Amikacin5 (5% Isolates were resistant) among all the first line drugs. For *Klebsiella pneumoniae*, the drug against which it was the least sensitive was Ampicillin/Amoxicillin (100% were resistant) and the most sensitive was Amikacin and Netilmicin (4.37% cases were resistant). The graphs denoting the sensitivity pattern of the two micro-organisms are depicted below (Figure 4& 5).

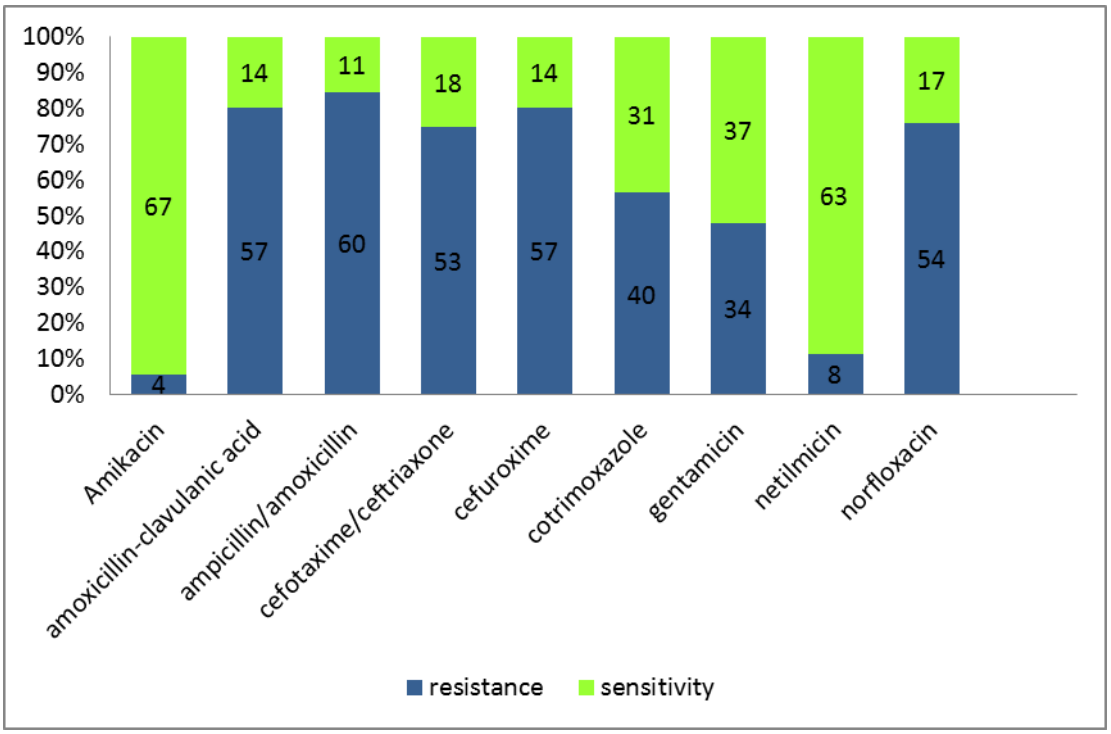

Figure 4: The sensitivity pattern of the Escherichia coli.

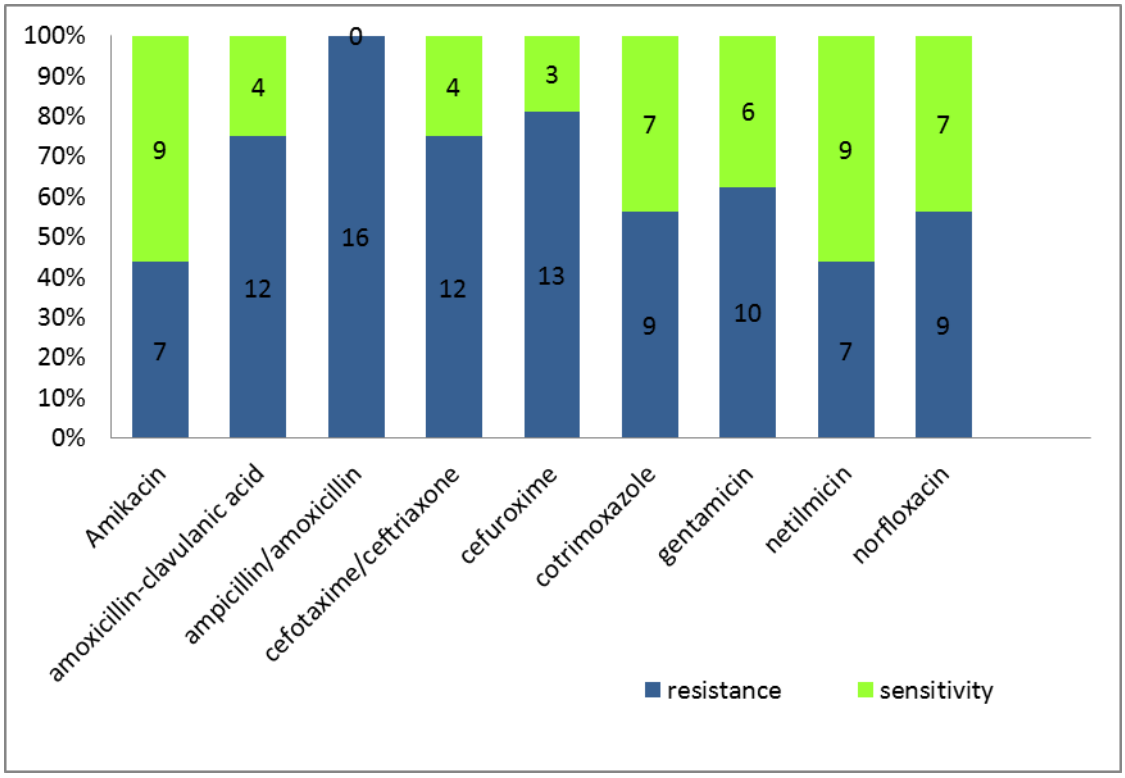

Figure 5: The sensitivity pattern of the Klebsiella pneumonia.

According to the drug sensitivity test by the disk diffusion method, the general efficacy of the drugs against all the micro-organisms (96 samples) is presented below (graph 6& 7). It is seen that Amikacin (85.41% sensitivity) and Netilmicin(81.25% sensitivity) are the drugs that are the most effective among the first line drugs. Among the reserved drugs, Imipenem (92.70% sensitivity) and Cefoperazone-Sulbactum(86.45% sensitivity) are the most effective. The Strains of *E.coli* 60(84.4%) and *Klebsiella pneumoniae* 12(75%) *Pseudomonas 2(2%)Methicillin resistant Staphylococcus aureus2(2%), Enterococcus1 (1%), Enterobacter1(1%)* and *Proteus 1(1%)* isolates shows Multi drug Resistance to first line drugs and were susceptible to reserved drugs.

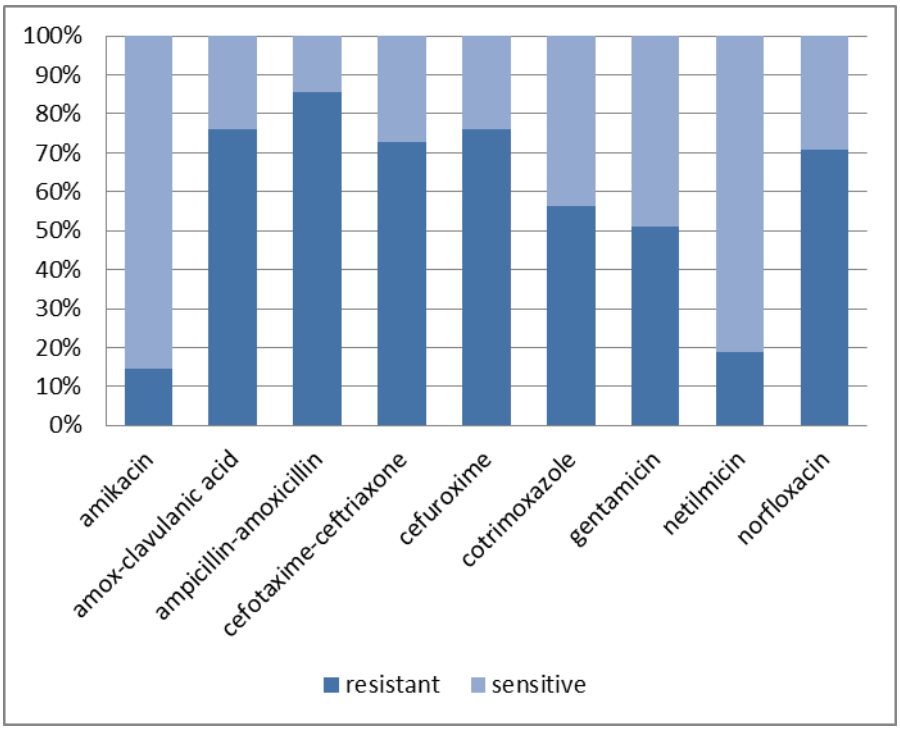

Figure 6: General efficacy of the first line drugs against all the micro-organisms.

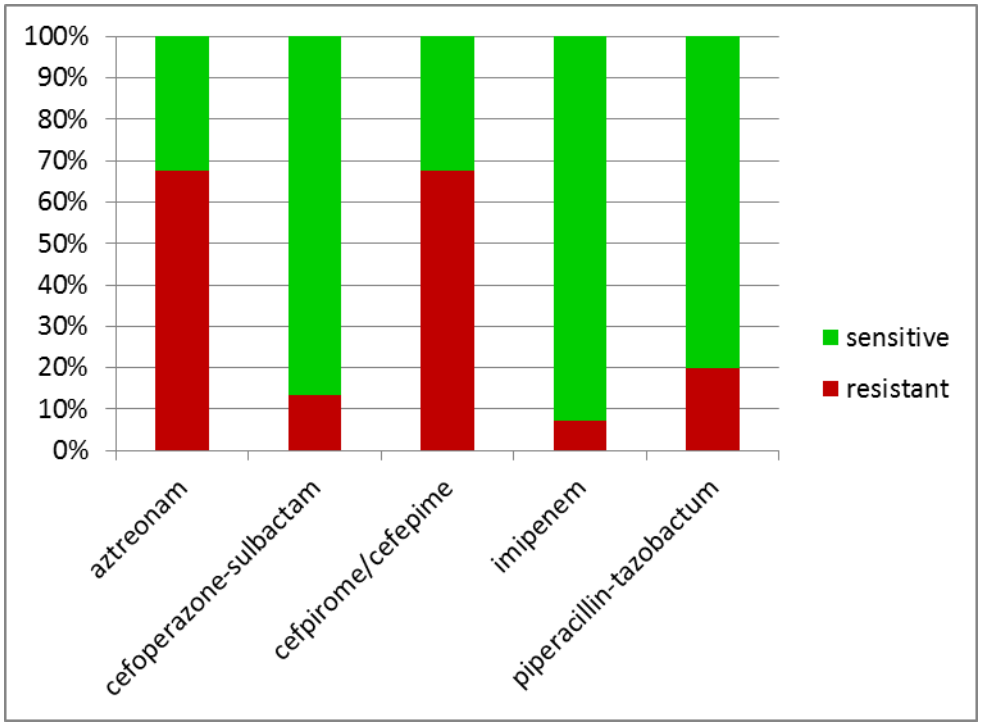

Figure 7: General efficacy of the reserved drugs against all the micro-organisms.

DISCUSSION

In this study, there was no significant difference between the number of male and female patients presenting with UTI (46 females and 50 males). A study in Nigeria in 2007 [3] had 149 female patients and 62 male patients. In a ten year surveillance study conducted in Norway by Linhares et al.[4] 120691 (77.6%) were females and 34898 (22.4%)were males indicating that urinary tract infections are more common in females. The other reports[7,8] in India did not consider the gender as one of the demographic data or showed no significant difference between the number of male and female patients.

The age group reporting with the maximum cases of UTI was between 40 to 60 years.[9]

Among the organisms, all studies showed that *Escherichia coli* was the most frequently isolated organism followed by *Klebsiella pneumonia.* The only exception was the report from Uganda by Kyabaggu D et al.[8] It had *Staphylococcus* species as the most common uropathogen followed by *Klebsiella*species and *E.coli.*

As *E.coli*was the predominant organism isolated (74%), analysis can be focused on its resistance pattern from some of the studies done in different countries over the years.[11]

The resistance of *Escherichia coli* was the highest against the Penicillin group of drugs, (76.05% against Amoxicillin/Clavulanicacid and 84.50% against Ampicillin/Amoxicillin). It was the most susceptible to Amikacin(5.63% resistance) followed by Netilmicin (11.26% resistance). For *Klebsiella pneumonia,* there was complete resistance against Amipicillin/Amoxicillin.[12] Also, *Klebsiella* showed high degree of resistance against the Cephalosporins and other drugs in the penicillin group. It was relatively susceptible to Amikacin (43.75% resistance) and Netilmicin (43.75% resistance).[13]

Now, after comparing the efficacy of the drugs against all the uropathogens isolated , we see that the resistance is maximum against the Penicillin group of drugs (85.41%) and the second generation Cephalosporins (76.04%).[14] The reason for this could be their widespread use by the clinicians as the first line drugs against UTI or other infections. According to the Antibiotic Policy of Kasturba Hospital, 2013[2] any drug with over 70% resistance developed against it should not be used for empirical treatment of any infection (including UTI).[15] The drugs which need parenteral routes of administration such as Amikacin (14.58% resistance) and Netilmicin (18.75% resistance) have retained some sensitivity against them. If the route of administration is to be considered, then according to this study, Cotrimoxazole (56.25% resistance) is the most effective drug among all the oral antibiotics.[16]

Among the reserve drugs, all need parenteral routes ofadministration, have prominent systemic toxicity and can hasten the selection of resistant strains.[17] Therefore, they are used only in life threatening complicated infections. Even among them, we see an appreciably high resistance against Aztreonam (67.7%) and Cefpirome/Cefepime (67.7%).[18]

LIMITATIONS

There were a number of limitations in our study that are intrinsic to a study with a retrospective design. One among them was the inability to know the time period for recovery of the patient and whether there was any change in medication once the antibiotic sensitivity report reached the clinician. However, the Laboratory reports fulfilled most of the data requirements.

Also, it is not assured that all patients reporting with suspected UTI were asked to submit urine samples for microbiological analysis by the healthcare professionals. This might have an impact on the results of our study.

Table 8: Comparison of the urine isolates and drug susceptibility with different studies.

Authors	Total no of isolates	Most common isolate Total num (%)	MDR Total num (%)
Singhal et al (2014,North India)	2653	E.coli 1103(45.7)	960(87)
Monika et al (2017,Maniur,India)	1142	E.coli 696(61)	595(85.9)
Satish Patil et al (2013,North karnataka)	49	E.coli26(53)	24(96)
Prakash et al(2013, Uttar Pradesh, India	155	E.coli60 (42.5)	58(96.9)
Neelima Angaali,et al (2018, Hyderabad,India)	57	E.coli41 (71.92%)	37(90)

CONCLUSION

The study presents a very dismal picture against all the drugs used in empirical therapy for UTI. Both the Penicillin group and the Cephalosporin group of drugs have developed significant resistance against them. The only oral antibiotic effective among the first line drugs is Cotrimoxazole. *Escherichia coli* were the predominant pathogens causing UTI followed by *Klebsiella* species. Antibiotic sensitivity testing is a must for a treatment of UTIs and prevention of drug resistance.

CONFLICTS OF INTEREST

Authors declare no conflict of interest.

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