BACTERIOLOGICAL PROFILE AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF URINE SAMPLES AMONG ADULT PATIENTS ATTENDING THE OUTPATIENT DEPARTMENT AT A TERTIARY CARE HOSPITAL IN KOLKATA

Debolina Chatterjee[1], Sarthak Kumar Sen[2]

[1]Debolina Chatterjee is pursuing Bachelors degree in the field of Biotechnology from Vellore Institute of Technology, Vellore
[2]Co-author Sarthak Kumar Sen is puruing Bachelors degree in the field of Computer Science and Engineering from Institute of Engineering and Management, Kolkata

Abstract— Urinary tract infections(UTI) are the most common infections of the urinary tract mainly caused by the Gram negative bacteria like *E.coli*, predominantly affecting women atleast once in a lifetime. UTI is a worldwide concern due to the anti microbial resistance conferred by the micro organisms towards the commonly used antibiotics. The objective of the work is to understand the bacteriological profile and the antibiotic susceptibility pattern of the urine samples which are collected from the adult patients with symptoms of UTI attending the out-patient department at a tertiary care hospital in Kolkata, West Bengal(Eastern India). From the study carried out among 310 patients, we have observed that in most of the cases, the microorganisms are resistant to the common orally administered drugs and sensitive towards IV administered antibiotics like Imipenem, Amikacin etc. Inspite of very few oral drugs which are effective towards the uropathogens, Nitrofurantoin shows the highest sensitivity which can be used as an empirical drug for the treatment of UTI in case of outpatients.

Index Terms— *Urinary Tract Infections, E.coli, Antibiotics, Resistance, Out-patients*

————————————— ◆ —————————————

INTRODUCTION

Urinary tract infections are widely considered as a major public health concern considering morbidity as well as financial costs affecting more than 10 million people in India every year. It is very common in outpatients as well as hospitalized patients and it can also lead to death. UTI is mostly caused due to the presence of microbial pathogens also known as uropathogens within the urinary tract such as *E.coli*, *Proteus* sp, *Klebsiella* sp, *Staphylococcus aureus*, *Enterococcus* where *E.coli* is the most commonly obtained microorganism in case of out-patients.

UTI is mostly diagnosed and treated empirically because of socio-economic obstructions, unavailability of appropriate urine culture facilities for large proportion of patients in India. For the initiation of the empirical treatment, it is necessary to have a knowledge about the causative organisms and their antibiotic sensitivity pattern but the most shocking fact is in most of the cases patients do not respond well to the conventional antimicrobial agents.

The present study is carried out with an objective to observe the bacteriological profile in the urine sample and their antibiotic sensitivity pattern obtained from the out patients in a tertiary hospital in Kolkata in 2018. This would be helpful for creating an awareness among patients and the antibiotic use in UTI in this tertiary hospital as well as country level.

MATERIALS AND METHODS

1.1 PLACE OF STUDY: Calcutta National Medical College, Microbiology department

1.2 DURATION: 1st May'18- 15th June' 2018

1.3 SAMPLE COLLECTION : Properly and asceptically collected freshly voided urine of adult patients attending Out Patient Department with symptoms of UTI.

1.4 SAMPLE SIZE: 310 samples were studied in the given time period.

1.5 EXCLUSION CRITERIA: 1. Urine samples of paediatric patients will not be taken for the study.

2. Urine samples of patients admitted in the hospital.

1.6 MATERIALS REQUIRED:

i. Urine sample, ii. Clean sterile urine container, iii. Mac Conkey agar, blood agar, iv. Inoculation loop, v. Peptone water, vi. Glass slide, vii. Crystal violet, viii. Gram's Iodine, ix. Safranin, x. Coverslip, xi. Light microscope, xii. Kovac's reagent, xiii. Plasma , xiv. Distilled water, xv. Bunsen Burner, xvi. Acetone, xvii. TSI Media, xviii. Citrate agar media, xix. Urease agar media, xx. Oxidase reagent (Tetramethyl p-phenylene diamine dihydrochloride).

1.7 METHODOLOGY:

 The patients included in this study were above 18 years of age, who where suspected with UTI due to the presence of the following symptoms including dysuria, frequency, lower abdomen (pelvic) pain, and fever. The patients were advised beforehand not to consume any antibiotic before collection of urine sample Freshly voided mid stream urine sample was collected in a sterile wide mouthed capped urine container after proper asepsis of the perineum at the microbiology department. The specimens are then properly labelled with patient's name, age, date and unique registration number. Any sample collected outside should be transported to the microbiology lab as soon as possible.

1.7.1 Processing of the sample:

 1. Macroscopic analysis:

 Once the urine sample is received in the laboratory, macroscopic urinalysis is carried out to test the urine color, pH, specific gravity, and turbidity.

2. Wet mount preparation and Microscopic Examination:

 The procedure for wet mount preparation is as follows:

 1. Take a clean, dry glass slide

 2. Ensure that the urine sample is in a clean, tightly sealed container, and shake to re-suspend any deposits.

3. The urine is centrifuged at 2000rpm for 5 minutes.

4. Remove most of the supernatant carefully with a pipette (pouring off the supernatant may be easier, but take care not to dislodge the sediment).

5. After the removal of the supernatant carefully, 1 drop of the suspension onto the slide and a cover slip is placed on top.

6. The slide is then observed under the microscope at 10X and 40X objective and the presence of pus cells, crystals and casts are noted down.

1.7.2. Culture of urine samples:

With the help of a bacteriological loop, one loopful urine sample was inoculated on Mac Conkey Agar and blood agar media. The urine is then given some time to get absorbed on the media after which the plates were inverted and incubated aerobically at 37oC for 24h. The plates were next observed for the growth of the microorganisms on them and the colony count was carried by a semi quantitative method and then the number of colonies obtained were multiplied by 1000 to obtain the colony forming units (CFU)/ml. A significant colony count is considered when CFU unit is greater than 105 CFU /ml. Colony characteristics of isolated bacteria was observed and noted down followed by Gram staining was done for the identification of the organisms. Biochemical tests such as catalase test, coagulase test,for gram positive organisms and catalase, citrate, urease, oxidase,indole test, Triple sugar iron test for Gram negative bacteria were also carried out for specimen identification.

1.7.3. Antibiotic Susceptibility Test:

Antibiotic Sensitivity test was carried out by using the Kirby Bauer's disc Diffusion method on Muller Hinton Agar. The antibiotics that are used for the study are as follows Ampicillin, Amoxycillin clavulanic acid, Cefotaxime, Amikacin. Cefoperazone-sulbactum, Ciprofloxacin, Cotrimazole, Doxycycine, Imipenem, Nitrofurantoin, Gentamycin, Cefuroxime, Linezolid, Vancomycin. The results were interpreted on the basis of the size of zone of inhibition according to CLSI guidelines, 2017.[15]

RESULTS

Total number of urine samples that were collected during the time of study with suspected cases of UTI is 310 and among them it has been observed that 10 samples found to be contaminated, 5 samples had shown insignificant colony count and 79 samples had shown a significant colony count greater 10^5 CFU/ml.

1. DISTRIBUTION OF BACTERIAL GROWTH AMONG URINARY SAMPLES
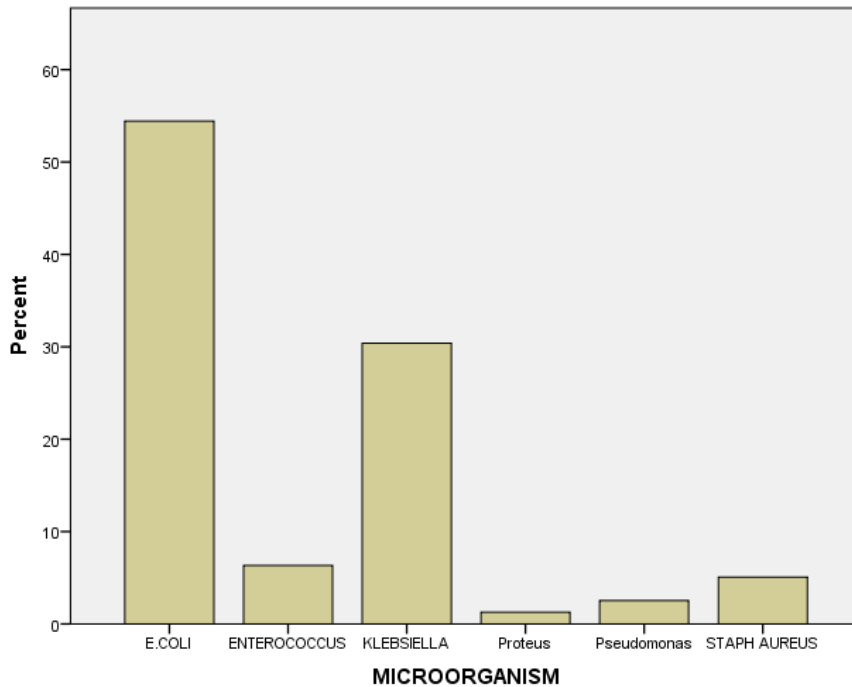
The following bar graph reveals the frequency of occurrence of *E.coli* being the highest (55%) followed by *Klebsiella* sp (30%). Among the Gram positive organisms such as *Enterococcus*(6.5%), *Staphylococcus aureus* (5%), *Pseudomonas* (2.5%) and *Proteus mirabilis* (1%) are least observed in case of the adult out patients.

## 2. UTI FREQUENCY IN MALE AND FEMALES AGE WISE
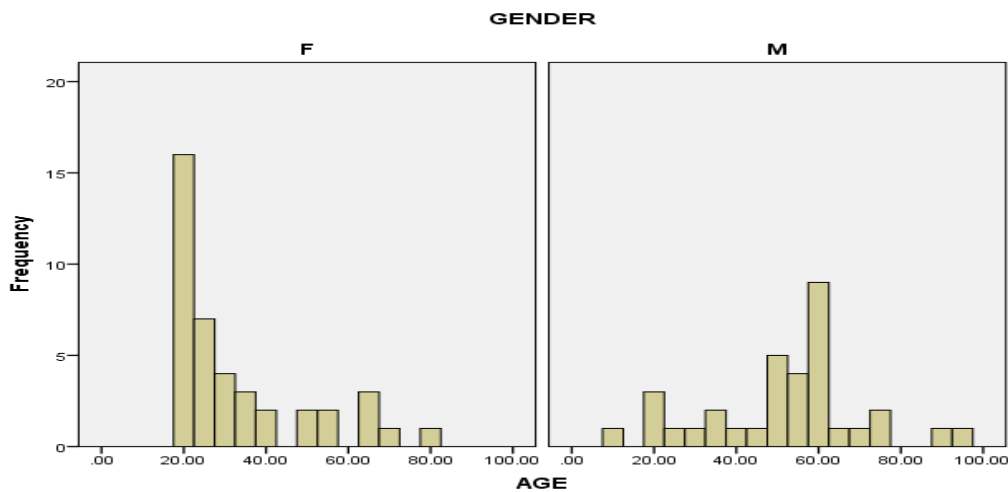
The histogram shows - in case of females, the maximum occurrence of UTI is found at the age group of 20-30 years and in case of males UTI occurs maximum at the age range between 60-70 years.

3. ANTIBIOTIC SENSITIVITY CHART FOR DIFFERENT TYPES OF MICROORGANISMS

| ANTIBIOTICS	E.COLI (N=43)	KLEBSIELLA (N=24)	ENTEROCOCCUS (N=5)	STAPHYLOCOCCUS AUREUS (N=4)
1. AMC	11.63`%	0	20%	75%
2. AK	76.74%	58.33%	0	75%
3. CEF	32.55%	25%	NA	50%
4. CTR	25.58%	8.33%	NA	50%
5. CFS	97.67%	66.67%	NA	NA
6. CIP	30.23%	33.33%	0	100%
7. COT	37.21%	29.17%	NA	NA
8. DOX	44.19%	50%	20%	50%
9. IMP	93.02%	70.83%	NA	NA
10. NT	58.14%	20.83%	20%	75%
11. LZD	NA	NA	100%	100%
12. VA	NA	NA	60%	100%

The table shows the sensitivity pattern for the microorganisms towards the most common antibiotics used for the treatment of UTI. *E.coli* shows the highest sensitivity towards IV administered drugs like Cefoperazone-sulbactum (97.67%), Imipenem (93.02%), Amikacin (76.74%) and among the orally administered drugs Nitrofurantoin (58.14%) is effective. *Klebsiella* sp is highly sensitive towards Imipenem (70.83%), but shows moderate sensitivity to Doxycycine (50%), Cefuroxime(25%) while Nitrofurantoin, the commonly used drug in the out-patient department for treatment of UTI was not found to be effective. *Enterococcus* sp was sensitive to Linezolid(100%) and Vancomycin (100%) and Staph showed higher sensitivity to the orally administered drugs like Ciprofloxacin (100%), Amoxycillin Clavulanic acid(66.7%) , and Nitrofurantoin (66.7%).

3.1 ANTIBIOTIC SENSITIVITY PATTERN FOR GRAM NEGATIVE ORGANISMS

ANTIBIOTIC SENSITIVITY PATTERN FOR GRAM NEGATIVE ORGANISMS
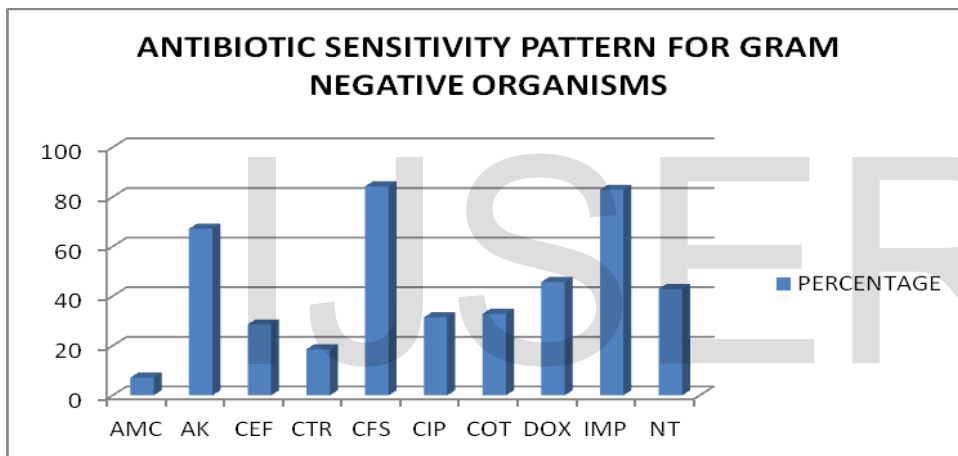
The Gram Negative organisms shows highest sensitivity towards Cefoperazone-Sulbactum (84.29%) and least to   Amoxycillin- clavulanic acid (7.14%)

3.2 ANTIBIOTIC SENSITIVITY PATTERN FOR GRAM POSITIVE ORGANISMS
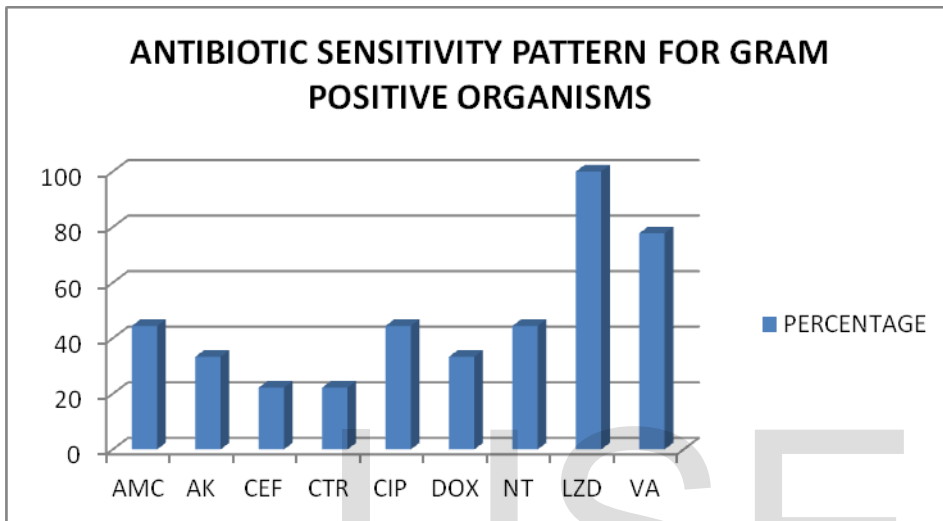
The Gram Positive microorganisms, for example *Enterococcus* Sp, *Saphylococcus aureus* which are isolated from the urine sample shows higher sensitivity to Linezolid (100%) and least to Cefuroxime(22.22%) and Ceftriaxone(22.22%)

DISCUSSION

Urinary Tract Infection is a widespread concern worldwide involving various socio-economic and behavioural factors. An emerging anti microbial resistance problem is affecting both the clinical and the public health scenario because of inappropriate use of antibiotics and this has also effected the changing pattern of sensitivity of pathogens to routinely used anti microbial agents , hence making the situation miserable throughout the globe.

The study demonstrated out of 300 patients who were suspected with UTI, 79 (26.33%) showed significant colony count and among them females between age 20 and 30 years while males between 60-70 years highly affected and the results have relevance with a previously carried out research.[4,6]

UTI affects women more than that of men because of shorter urethral length, delayed micturition, sexual intercourse along with recurrent usage of spermicides and diaphragms. The study has supported the fact with 79 growth cases where less than 30% males are affected with UTI and the result has found relevance with other research studies. [5]

The different uropathogens associated with Urinary Tract Infection in this study revealed *E.coli* (55%) is the most commonly obtained isolate from the urine samples followed by *Klebsiella* sp (30%), *Enterococcus* sp(8%), *Staphylococcus aureus* (5%) and *Proteus mirabilis* (1%) being the least obtained from the urine specimen in the out patients of the tertiary hospital. The result has found relevance with several other studies carried out by John A. *et al* [7,13]

We have observed that the Gram negative organisms are highly sensitive towards the IV administered drugs like Cefoperazone- sulbactum (84.29%), Imipenem(82.86%), Amikacin (67.14%) leading the list whereas the organisms are showing a lower sensitivity pattern towards the most common orally administered drugs viz. Nitrofurantoin (42.86%), Ciprofloxacin (31.43%) and Amoxycillin clavulanic acid (7.14%). Study carried out by *Biswas R.et al* at a tertiary hospital at Dhaka observed similar antibiotic sensitivity pattern towards orally administered drugs like Nitrofurantoin and Amoxycillin clauvanate and higher sensitivity towards Imipenam (98%) followed by Amikacin (86.63%).[1] *Prakash D et al.* published that the sensitivity of the uropathogens towards Imipenam is 84.52% and Meropenam is 92.26% whereas CTZ and CTX are the most resistant drugs. When they studied specifically for E.coli and Klebsiella sp they observed that carbapenems like IMP and MRP showed the maximum sensitivity being 98.45% and 95.45% respectively.[2] *Nalini R et al* had observed at a tertiary care hospital in Tamil Nadu that E.coli shows maximum sensitivity towards Nitrofurantoin (85.19%) followed by Amikacin (66.50%) and maximum resistance is conferred towards Ceftriaxone (82,53%).[3] In a study carried out by *Timothy O.O et al.* the outpatients have tend to manifest the maximum resistance towards Tetracycline, Cotrimazole, Gentamicin, Nitrofurantoin, and Augmentin (amoxicillin clauvanate) but the following drugs such as Ceftriazone, Ofloxacin, Ceftazidime and Ciprofloxacin have shown better activity towards E.coli and Klebsiella sp and they have suggested this antibiotics to be used as empirical drugs.[4] Gram positive microorganisms had shown higher sensitivity to Linezolid (100%) and Vancomycin (77.77%), and in study carried out by *Demille T et al* Levofloxacin, Vancomycin, Ciprofloxacin, and Doxycycine and the highest sensitivity is towards Gentamicin (83.3%), Nitrofurantoin (83.3%), and Amoxicillin clauvanate (100%). [8]

ACKNOWLEDGEMENT

CONCLUSION

The study depicts that there are very few oral drugs which are effective towards the uropathogens among which Nitrofurantoin shows the highest sensitivity which can be used as an empirical drug for the treatment of UTI in case of out-patients. The high degree of resistance pattern that has been observed in the study poses serious implications to the health care and expenses in developing countries like India. This has mostly resulted from frequent hospital admissions and the indiscriminate use of higher generation drugs by the physicians. A proper knowledge of the causative agent and anti biogram along with strict use of antibiotics by the clinicians can surely reduce the burden of antimicrobial resistance.

REFERENCES

1. Eva Hummers-Pradier and Michael M Kochen. "Urinary tract infections in adult general practice patients": *Br J Gen Pract*. 2002. 752-61

2. Daniele Minardi, Gianluca d'Anzeo, Daniele Cantoro, Alessandro Conti, and Giovanni Muzzonigro. " Urinary tract infections in women: etiology and treatment options ": *Int J Gen Med*. 2011. 333-43

3. Farajnia S, Alikhani MY, Ghotaslou R, Naghili B, Nakhlband A. " Causative agents and antimicrobial susceptibilities of urinary tract infections in the northwest of Iran" : *Int J Infect Dis*. 2009. 140-4

4. Yamamoto S1, Tsukamoto T, Terai A, Kurazono H, Takeda Y, Yoshida O. "Genetic evidence supporting the fecal-perineal-urethral hypothesis in cystitis caused by Escherichia coli' *: J Urol*. 1997. 1127-9

5.Patterson TF, Andriole VT. "Detection, significance, and therapy of bacteriuria in pregnancy". *Update in the managed health care era : Infect Dis Clin North Am.* 1997. 593-608.

6. Hill JB, Sheffield JS, McIntire DD, Wendel GD Jr: "Acute pyelonephritis in pregnancy ": *Obstet Gynecol.* 2005. 18-23

7.   Aroutcheva A, Gariti D, Simon M, Shott S, Faro J, Simoes JA, Gurguis A, Faro S: " Defense factors of vaginal lacto-bacilli" : *Am J Obstet Gynecol.* 2001. 375-9.

8. Lona Mody and Manisha Juthani-Mehta. " Urinary tract infections in Older women" : *Jama*. 2014. 844-854.

9. Rama Biswas, Raihan Rabbani, Hasan Shahrear Ahmed, Mohammed Abdus Satter Sarker, Nahida Zafrin, Md. Motla-bur Rahman. " Antibiotic sensitivity pattern of urinary tract infection at a tertiary care hospital" : *Bangladesh Crit Care*. 2014. 21-24.

10. Devanand Prakash and Ramchandra Sahai Saxena. "Distribution and Antimicrobial Susceptibility Pattern of Bacterial Pathogens Causing Urinary Tract Infection in Urban Community of Meerut City, India". *ISRN Microbiology*. 2013

11. Oyekale Oluwalana Timothy, Fadare Joseph Olusesan, Busari Olusegun Adesola, Adeyemo Adeyemi Temitayo, Fayomi Oluwaseun David, Oyekale Oluwatoyin Ige. "Antibiotic resistance pattern of bacterial isolates from cases of uri-nary tract infections among hospitalized and out-patients at a tertiary health facility in South Western Nigeria":*Annals of Tropical Medicine and Health care*. 2014. 130-135.

12. Tazebew Demilie, Getenet Beyene, Selabat Melaku, and Wondewosen Tsegaye. "Urinary Bacterial Profile and Anti-biotic Susceptibility Pattern Among Pregnant Women in North West Ethiopia" : *Ethiopian Journal of Health Science*. 2012. 121-128.

13. Ramesh hotchandani, K.K Aggarwal. "Urinary Tract Infections in Women": *Indian Journal of Clinical Practice*. 2012. 187-192.

14. Mamuye Y. "Antibiotic Resistance Patterns of Common Gram-negative Uropathogens in St. Paul's Hospital Millen-nium Medical College": *Ethiopian Journal of Health Science*. 2016. 93-100.

15. M100 | Performance Standards for Antimicrobial Susceptibility Testing, 28th Edition