ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24SEP1219

Isolation and Identification of Urinary Tract Infectious Bacteria and their Antibiotic Sensitivity Pattern in Different Age Group

Shilpi Abha Toppo[1]
Research Scholar

Guide: Amia Ekka[2]
Professor, S.o.S Life Science, Pt. Ravishankar Shukla University,
Raipur(C.G), India (492001)

Abstract:- Urinary Tract Infection is the world wide problem. UTIs are common infection that occur when bacteria, infrequently fungi enter the urethra, and infect the urinary tract. This UTIs infection includes the kidney, bladder, uterus and urethra. This is one of the most common complain being form the bambino upto the senior age group. When an infection is limited to the bladder it can only be painful and annoying but serious health problems can affect if UTI spreads to the kidney. Thus, it's salutary to prove the organism which are causing this complaint in the body. In the present study Urine sample of 300 cases were collected from Dr. Bhim Rao Ambedkar Memorial Hospital Raipur, samples were dressed by using(Mac Conkey agar and CLED agar) media by which 9 different species of bacteria were attained. Among 9insulated bacteria only 2 were gram-positive, the remained gram-negative bacteria and 1 fungi (*Candidaalbicance*) was attained. The antibiotic perceptivity pattern was performed to treat these bacteria in which it has been reported that Colistin(CL) and Nitrofurantoin(Nit) are the most effective antibiotic agent against uro-pathogens.

I. INTRODUCTION

Urinary Tract Infection is classified as the most common and being nosocomial bacterial infection in mortal populations around the world. This is a condition caused by pathogenic irruption of the epithelium, which lines the urinary tract from the minor calyx to prostatic urethra (Karzan *et al*., 2017). The proliferation of bacteria in the urothelium can be asymptomatic or characteristic, Cases suffering from characteristic UTI are generally treated with antibiotics (Ana *et al.,* 2015). UTI is common in children and affect both gender joker and womanish but women are more susceptible (Willams *et al.,* 2011) because of their short urethra, and certain behavioral factors which include detention in micturition, sexual exertion and the use of contraceptives which promote colonization of the peri urethral area with coliform bacteria (Hotchandani and Agarwal 2012). UTI are divided into two orders lower urinary tract (the element of the urinary tract below the uretere.g. Bladder and urethra) and upper urinary tract involves the ureter and order. When orders are involved, it's called “acute pyelonephritis”. When bladder inflammation involves it's called “cystitis”. (Balkrishnan and Hill, 2011). Urinary tract infection is a condition in which urinary tract is infected with a pathogen. UTI’s are the most common bacterial infection seen in primary care, second when infection occur to the respiratory tract (Hotchandani and Agarwal, 2012). These are particularly affecting women due to their deconstruction. The predominant pathogen in both complicated and uncomplicated UTI remains pathogenic. UTI is caused by *Escherichia coli*in over 80 of cases and treatment is a course of antibiotics (Williams and Craig2011). UTI is caused by both gram-negative and gram-positive bacteria (Hotchandani and Agarwal, 2012; Ana.,*et al.,* 2015) typically UTI dominated by *E.coli* 75-80 and followed by *Staphylococcus saprophyticus* account about 80 of the community acquired uncomplicated urinary tract infection (Daniele *et al.,* 2011; Karzan *etal.,* 2017; Hotchandani and Agarwal, 2012). But the prevenance of other antibiotic resistance organismssimilar as *Klebsiella, Proteus, Enterobacter, Pseudomonas* increases in complicated UTI (Lakshminarayana*et al.,* 2015)

II. MATERIALS AND METHODS

A. Isolation and Identification:

My examination criteria consisted of those patients who were already on antibiotic treatment. Total 300 samples were collected during this study period. For collection of urine samples patients were advised to collect a clean catch midstream urine specimen in a sterile, wide mouthed container supplied by the laboratory and bring to the laboratory as early as possible. Isolation and identification of bacterial pathogen was done by microscopy and culture methods. Bacterial isolates were identified generally using biochemical methods. The fresh urine samples were streaked out on selective medium CLED agar and MacConkey agar respectively and the plates were incubated for 24 hours at 37°C. Gram staining and biochemical testing of the following organism were done for the identification.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24SEP1219

- **Media Used:** MacConkey Agar Media

✓ Blood Agar Media
✓ Muller Hilton Agar Media
✓ Simmon's Citrate Agar Media
✓ Triple Sugar Iron Agar Media
✓ Christensen's Urease Agar Media
✓ Alkaline Peptone Water
✓ All of the media were autoclaved at 121^0Cfor 20 min.
✓ Carbohydrate Fermentation Broth –
▪ Lactose Broth
▪ Maltose Broth
▪ Glucose Broth
▪ Manitol Broth

- **Chemical Used**: Following chemicals were used

✓ Oxidase Reagent (Tetramethyl paraphenylene dihydrochloride)
✓ Hydrogen peroxide
✓ Ethyl alcohol (95%)
✓ Gram's iodine
✓ Saffrenine
✓ Phenol red

- **Apparatus Used:** Autoclave, Incubator, Hot Air Oven, Laminar Air Flow, WeighingBalance, Microscope.
- **Other Material Required**: Test tubes, Beakers, Conical Flask, Petriplates, Whatman Filter Paper, Inoculating Loop, Culture Tubes, Distilled Water.
- **Sample collection –**Patients were asked to clean their external genitalia with disinfectant and collect their midstream urine in sterilized cap. Samples were kept in ice bag and directly transported to microbiological laboratory.
- **Physical examination** – (ph, color, volume, appearance) parameters of collected urine specimen were analyzed.
- **Urine culturing** -Urine samples were cultured on CLED, MacConkey agar medium.A small amount of urine is placed on the tip of the inoculation loop and it is streaked across the surface of the medium. After the plates were incubated overnight at 37^0C. Significant units CFU/ml of midstream unit.
- **Culture Examination**: After incubation of 24 hours the Petri plates were examined for growth of microorganism and cultural characteristics were noted. The colonies obtained on the media were studied with respect to their number, size, shape and morphology i.e, opaque, transparent, smooth and rough, fermenting and non-lactose fermenting strains, color and consistency were also determined.
- **Microscopically Examination**: The isolated culture was examined microscopically, on the basis of their cell wall composition and presence of capsules. A bacterial culture smear was prepared and stained with crystal violet stain for 30 seconds after this the slide was washed with distilled water, gram iodine was added for 1 minute followed by washing with water and 95% ethanol for 10-20 seconds lastly safranin was added on the slide for 30-60 seconds and rinse by distilled water then the slide was observed under microscope.
- **Disk diffusion antibiotic susceptibility testing-**The standard bacterial isolates is spread onagar plate and then paper disc containing specific concentration of antibiotics are placed and incubated at 37°C overnight,if the isolates are susceptible to the antibiotics, it does not grow around the disc thus forming the zone of inhibition.Strains resist to antibioticsgrow up to the margin of disc.The diameter of zone of inhibition was measured by Kirby Bauer chart.

III. RESULT AND DISCUSSION

A total of 300 patients were randomly assigned after being considered eligible by the doctors. In the end, urine of 300patients were analyzed. The study participants were recalled 1 time per week. There was a significance difference in demographic characteristics such as gender, age, sex and occupation. Sample were cultured and different 9 speciesof bacteria were obtained among 9 isolated bacteria only 2 were gram-positive and cocci shape, the remained gram-negative bacilli shape.

Among 300 urine samples positive result obtained in 249 (83%) samples and rest 51(17%) are found negative. 68.66% females were affected by UTI whereas, 31.33% males were affected by UTI.

It was reported that predominant uro-pathogens are *E.coli* followed by *Enterococcus* and *Klebsiella sp.* which also support our study.The commonest bacteria isolates were *E.coli* which is found to be 38.33% followed by *Enterococcus* 27.33%, *Klebsiella sp.* 8%, *Candida sp.* 7.33%, *Acinetobacter sp.* 5.66%, *Pseudomonas* 2%, *Proteus sp.* 1%, *Citrobactor*, *Straptococcus* and *Styphalococcus* found 0.33%.

This study helps us to find that in the 300 samples 247 samples are gram-negative bacteria and 2 samples are found gram-positive and 51 samples have no growth.Among the positive samples 32 samples are having 2 bacteria in which in 11 samples *E.coli* and *Enterococcus* are found followed by *Klebsiella sp. and Enterococcus* 3 samples, *Enterococcus* and *Candida sp.* 3 samples, *E.coli* and *Klebsiella sp.* 3 samples, *E.coli* and *Pseudomonas sp*. in 3 samples.

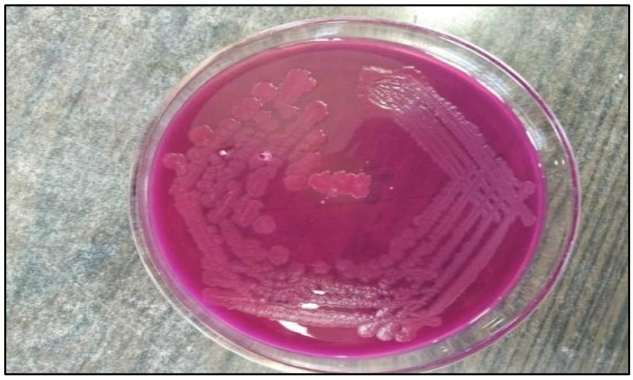
Fig 1: Isolated Colony of E.coli

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24SEP1219

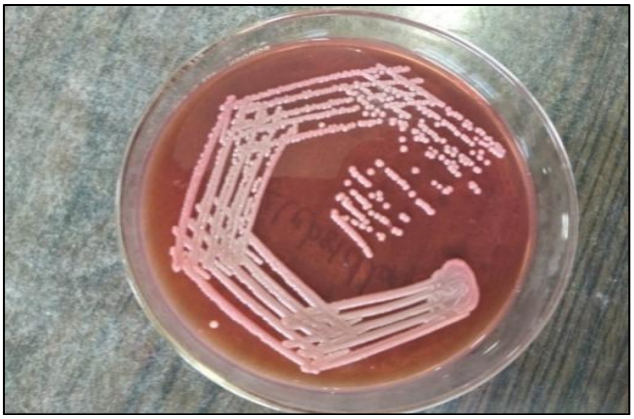

Fig 2: Isolation of Colony of klebsillla

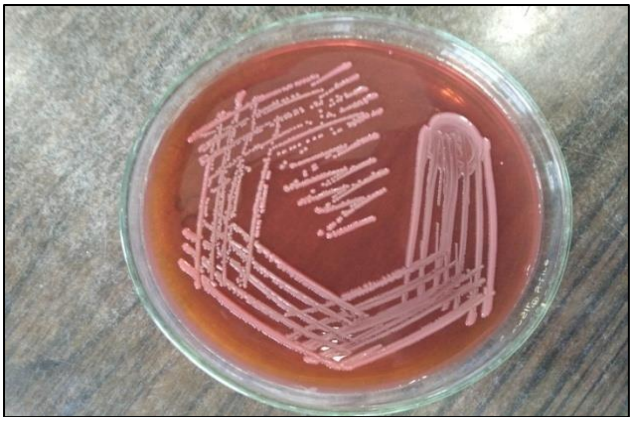

Fig 3: Isolation of Colony of Staphylococcus

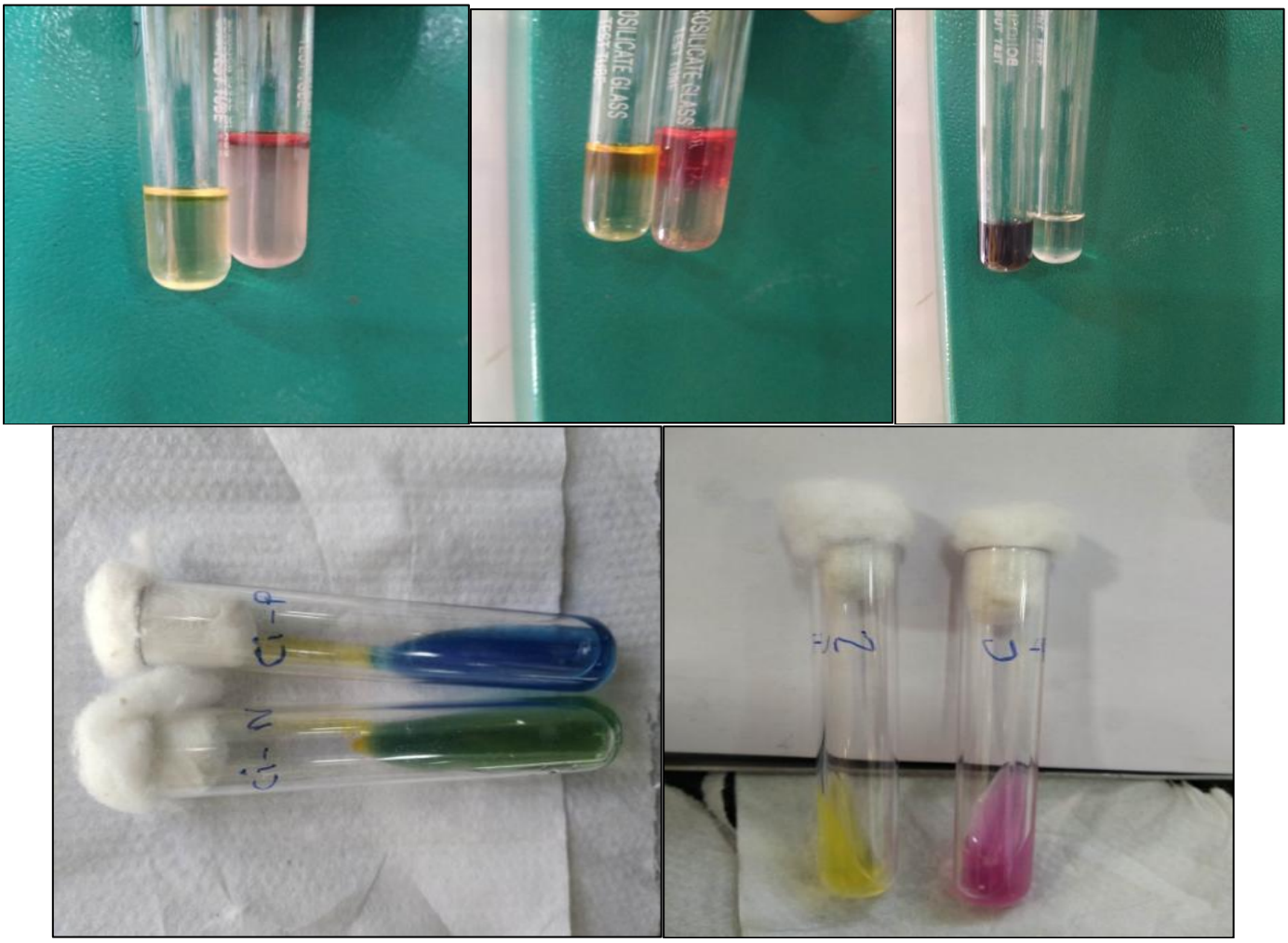

Fig 4: Result Obtained Using Different Biochemical Test

- Indole test result
- MR test result
- Oxidase test result
- Citrate test result
- Urease test result

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24SEP1219

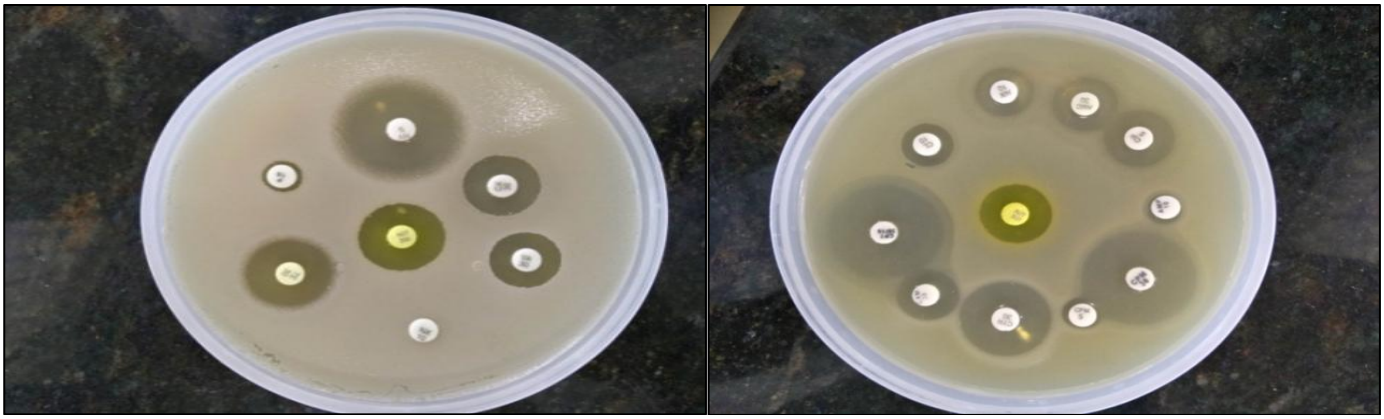

Fig 5: AST Result

Table 1: UTI in Male and Female in Various Age Groups

Age Groups (years)	Male No(%)	Female Nos(%)
10-19	23(7.66%)	24(10.66%)
20-29	10(3.33%)	78(26%)
30-39	13(4.33%)	29(9.66%)
40-49	19(6.33%)	25(8.33%)
50-59	11(3.66%)	15(5%)
60-75	18(6%)	27(9%)
Total UTI case =300	94(31.33%)	206(68.66%)

Table 2: Isolated Bacteria in Urine Sample

Organisms	Total%	Male(%)	Female(%)
E.coli	115(38.33%)	18(6%)	31(10.33%)
Enterococcus	27.33%	24(8%)	58(19.33%)
Klebsiella spp.	8%	8(2.66%)	16(5.33%)
Candida spp.	22(7.33%)	12(4%)	10(3.33%)
Acinetobacter	17(5.66%)	5(1.66%)	12(4%)
Pseudomonas aerugenosa	6(2%)	3(1%)	3(1%)
Staphylococcus aureus	0.33%	1(0.33%)	_
Streptococcus spp.	0.33%	1(0.33%)	_
Proteus spp.	0.33%	_	1(0.33%)
No Growth	49(16.33%)	18(6%`)	31(10.33%)

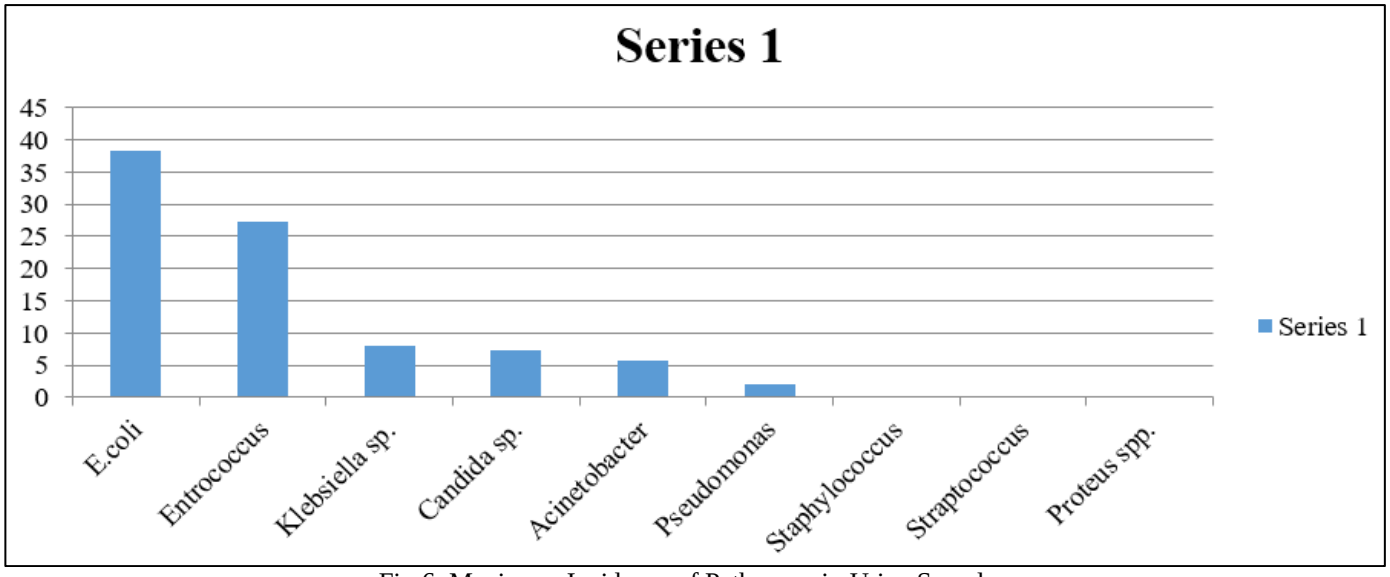

Fig 6: Maximum Incidence of Pathogens in Urine Samples

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24SEP1219

Table 3: Percentage of Sensitive and Resistance Pattern in Antimicrobial Agent.

Antimicrobial agent	Sensitive	Resistance	Percentage resistance (%)	Percentage of sensitivity (%)
Nit	117	12	4	39
Cl	132	20	6.66	44
Va	69	-	-	23
T	12	36	12	4
Do	9	27	9	3
Amc	38	141	47	12.66
Cz	3	117	39	1
Cxm	5	108	36	1.66
Ctr	9	63	21	3
Amp	6	93	31	2
Ctx	15	84	28	5
Cpm	18	93	31	6
Mrp	72	30	10	24
Nx	9	159	53	3
Cot	20	57	19	6.66
HLG	6	33	11	2
Cip	3	27	9	1
GEN	9	20	6.67	3
Cfm	9	63	21	3
Co	3	24	8	1
Cefu	-	12	4	-
Cpt	-	22	7.3	-
P	1	16	5.3	0.33
Lf	4	8	2.67	1.33

IV. SUMMARY

9 isolates of bacteria were identified from 300 patients the sensitivity to different antibiotics was performed and the activity of antibiotics for inhibiting bacterial growth was act different levels, according to their ability. Women are more susceptible to UTI then men in which highest numbers of uro-pathogens are gram-negative. As drug resistance among bacterial pathogen is un evolving process, regular surveillance and monitoring is necessary to provide physician's knowledge on the updated and most effective empirical treatment of UTI's. In order to prevent or decrease resistance to antibiotics the use of antibiotic should be kept under supervision, should be given in appropriate dose for an appropriate period of time.

REFERENCES

[1]. Balakrishnan I and Hill V (2010) Dealing with urinary tract infections. *The Pharm J* 287: 687-690.

[2]. Beyene G. and Tsegaye W. (2011) Bacterial uropathogens in urinary tract infection and antibiotic susceptibility pattern in Jimma University Specialized Hospital, Southwest Ethopia.*Ethopian Journal of Health Science* 21(2): 141-6.

[3]. Chauhan N., (2011) Study of bacterial etiology of infectious disease and there antibiotic sensitivity pattern in paediatric age group.

[4]. Collee J.G., Fraser A.G., Marmion B.P. and Simmin A. (2006) Mackie and McCortney practical medical microbiology, 14th edition, Pearson professional 1996, New York: 84-85, 152.

[5]. Fashsad S., Anvarinejd M., Tavana A.M., Ranjbar R., Japoni A., Zadegan R.M. and Alborzi A. (2011) Molecular epidemiology of Echerichia coli strains isolated from children with community acquired urinary tract infections. *African Journal of Microbiology Research* Vol.5(26)-4476-4483.

[6]. Flores-Mireles, A. L., Walker, J. N., Caparon, M., & Hultgren, S. J. (2015). Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nature reviews. Microbiology*, *13*(5), 269–284.

[7]. Foxmann B. (2014) Urinary tract infection syndroms: occurance common recurrence, bacteriology, risk factor and disease burden. *Infect Dis Clin North Am.* 2014: 28:1-13.

[8]. Gales CA., Jones RN., Gordan KA., Sader SH., Wilke WW., Beach ML., Pfaller MA. And Doern GV., (2000). Sanitary Study Group Latin America, Activity and Spectrum of 22 anti-microbial agent tested against urinary tract infection pathogens in hospitalized patients in Latin America: Report from the 2nd year of the SENTRY Antimicrobial Surveillance programme (1998). *Journal of Antimicrobial Chemotherapy*, 45:295-303.

[9]. Goplakrishnan S., and Muthulakshmi M., (2017) Study on urinary tract infection among females of reproductive age group in a rural area of Kancheepuram district, Tamil Nadu. *Int J Community Med Public Health*. 2017 Oct;4(10):3915-3921.

[10]. Gruneberg RN. 1980. Antibiotic sensitivity of urinary pathogen 1971-1978. *Journal of Clinical Pathlogy*, 33:853-56.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24SEP1219

[11]. Guglietta A (2017) Recurrent urinary tract infections in women: Risk factors, etiology, pathogenesis and prophylaxis. *Future Micro* 12: 239-246.
[12]. Hotchandani R. and Agrawal K.K. (2012). Urinary tract infection in women. *Indian Journal of Clinical Practice*, Vol.23, no.4 187-192.
[13]. Karzan Mohammed K, Faeza Burhan O. and Shahida Nooruldeen Y. (2017). Isolation and identification of urinary tract infectious bacteria and exploring their anti-drug potential against some common antibiotics. *Journal of microbial & biochemical technology* vol. 9(6):285-289.
[14]. Khan IU., Mirza IA., Ikram A. and Ali S. 2014. Antimicrobial susceptibility pattern of bacteria isolated from patients with urinary tract infection. *J Coll Physicians Surg Pak* 24:840-844.
[15]. Khondakar S.S., Satar A.N.I., Saleh A.A., Roy C.K., Ahmed M., Muhammadullah S. and KabirMd..H.,(2016) Study of bacterial pathogens in urinary tract infection and their antibiotic resistance profile in a tertiary care hospital of Bangladesh.*BangladeshJ.MedMicrobiol* 2016:10(01):22-26
[16]. Lakshminarayana S.A., Chavan S.K.D. Prakash R. and Sangeetha S. (2015) Bacterial pathogen in urinary tract infection and antibiotic susceptibility pattern from a teaching hospital, Bangluru, India. *International Journal of Current Microbiology and Applied Sciences* Vol.4 Number 11: 731-736.
[17]. Manikandan C. and Amsath A. (2014). Antibiotic susceptibility pattern of Echerchia coli isolated from urine samples in Pattukkottai, Tamilnadu.*Int.J.Curr.Microbiol.App.Sci*(2014) 3(10) 449-457.
[18]. Mirandi D., D'Anzeo G., Cantoro D., Conti A. and Muzzonigro G. (2011) Urinary tract infection in women: etiology and treatment options. *International Journal of General Medicine* 2011:4 333-343.
[19]. Mireles- A.L.F., Walker J.N., Caparon M. and Hultgren S.J. (2015) Urinary tract infections: epidemiology, mechanism of infection and treatment options. *Nat Rev Microbiol* 2015 May; 13(5): 269-284.
[20]. Raju S.B. and Tiwari S.C. (2001) Urinary tract infection- A Suitable Approach. Lecture notes Department of Nephrology All India Institute Of Medical Science, New Delhi
[21]. Rangari Amit A., Sharma Sachin, Tyagi Nidhi, Singh Paramjeet, Singh Garima and Thakur Rameshwari, (2015). Antibiotic susceptibility pattern of bacterial uropathogens isolated from patients at a Tertiary Care Hospital in western Uttar Pradesh of India. *Int.J.Curr.Microbiol.App.Sci* (2015) 4(10):646-657.
[22]. Rehman H. and Deka M. (2014) Detection and characterization of necrotoxin producing Escherichia coli (NETC) from patients with urinary tract infection (UTI). *Indian Journal of Medical Research* 2014 vol. 139(4): 632-637.
[23]. Sewify M., Nair S., Warsame S., Murad Md., Alhubail A., Behbehani K., Al-Refaei F. and Tiss A. (2015) Prevalence of urinary tract infection and antimicrobial susceptibility among diabetic patients with controlled and uncontrolled Glycemia in Kuwait. *J Diabetic Res.* 2016:6573215
[24]. Vasudevan R (2014) Urinary tract infection: An overview of the infection and the associated risk factors. *J Micro Exp* 1: 8.
[25]. Vijayakumar M. (2011) Revised statement on management of urinary tract infections. *Indian Society of Pediatric Nephrology* Vol. 48 709-717.
[26]. Williams G, Craig JC. (2011) Long-term antibiotics for preventing recurrent urinary tract infection in children. Cochrane Database Syst Rev.