International Journal of Medical and Health Research
ISSN: 2454-9142
Received: 06-11-2019; Accepted: 07-12-2019
www.medicalsciencejournal.com
Volume 6; Issue 1; January 2020; Page No. 25-28

Isolation & identification of pathogenic bacteria cause urinary tract infection and their antibiotic susceptibility pattern in a tertiary care hospital. Jaipur, Rajasthan

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Abstract
Introduction: Urinary Tract Infections is second most common type of infection after upper respiratory tract infections affecting millions of people each year. Common conditions associated with UTI are pyelonephritis, urethritis, cystitis and these conditions are caused by wide range of microorganisms. So this study was done to isolate and identify most common bacterial flora causing UTI and to study their antimicrobial susceptibility pattern.
Material &Methods: This study was done on all Urine samples received in Department of microbiology, National Institute of Medical Sciences and Research, Jaipur over a period of 5 months from July 2018 to November 2018. Samples were received and processed as per the standard guidelines and antimicrobial susceptibility testing was done according to the standard CLSI guidelines.
Result: Out of 120 Urine Samples received in lab, 78 samples were culture positive with majority of samples from female patients than male patients. There was predominance of Gram Negative bacteria in causing UTI than Gram Positive bacteria. Escherichia coli wasthe most common organism causing UTI. Meropenem, Imipenem, Nitrofurantoin, Vancomycin, Linezolid showed great sensitivity pattern while Penicillin, Ampicillin and Cephalosporins showed high resistant pattern.
Conclusion: Current study will be very helpful in initiating proper Empirical treatment therapy of patients suffering from UTI and thus it will be helpful in reducing the morbidity rate and mortality rate.

Keywords: UTI, Escherichia coli, Meropenem, Imipenem, Cephalosporins

Introduction

Urinary Tract Infection (U.T.I.) is one of the most common bacterial infections associated with high morbidity and long term complications. Urinary Tract Infection (UTI) is an infectious disease which is characterized by the presence and growth of microorganism anywhere in the urinary tract which include kidneys, ureters, bladder and urethra [1]. Urinary tract infection (UTI) is a serious health problem affecting millions of people each year. They are the second most common type of infections in the body after upper respiratory tract infections [2].

This problem occurs more often in women than men because a woman's urethra is shorter. The short urethra makes it easier for bacteria from the anus or genital area to reach the bladder [3].

Every year approximately 150 million people are suffering with UTI all over the world. It has been estimated that about six million patients visit outpatient departments and about 300,000 are treated in the world every year for UTI [4, 5]. About 10% of human population gets UTI at some stage during their lives. More than 50% of all women will experience at least one episode of UTI during their life and up to 30% will experience a recurrent infection that may progress to chronic disease [6].

Urinary tract infections (UTI) are serious health problem, due to different resistance mechanisms spreading among uropathogens and leading to multi-resistant strains their treatment is frequently difficult in hospital[7]. The microorganisms can cause UTIs including bacteria, fungi and viruses; bacteria are the major causative organisms and are responsible for more than 95% of UTI cases [8].

Normally UTIs are caused by a variety of Gram-negative and Gram-positive bacteria. The Gram-positive bacteria Includes Staphylococcus sp, Streptococcus sp and Enterococcus sp. Gram-negative includes Escherichia sp, Klebsiellasp, Enterobactersp, Citrobactersp, Proteus sp, Serratiasp, Salmonella sp and Pseudomonas sp. Among this 80-90% of UTI is caused by E. coli [9].

Based on the microbial sensitivity test results, drugs that are usually administered against uropathogens include cotrimoxazole, amoxicillin, ampicillin, aminoglycosides, cephalosporins, nalidixic acid and nitrofurantoin. However, many reports have indicated the presence of multi-drug resistance in organisms causing UTI [10]. Therefore, the aim of this study was to identify the most common bacteria causing UTI and detection of antibiotic susceptibility of isolates.

Material & Methods

The present study was carried out in the department of microbiology of National Institute of Medical Sciences and Research, Shobha Nagar, Jaipur (Rajasthan). This was a cross-sectional observational study. The study was carried out during the period of July 2018 – November 2018, during this period all midstream non repetitive urine samples were collected from the patients suspected of Urinary tract infection. Urine samples were inoculated on Cysteine Lactose Electrolyte Deficient (CLED) agar, MacConkey and Blood agar plates (Hi Media labs Ltd.) and incubated at 35-

37*C for 24 hours using a calibrated loop method delivering 0.001 mL of urine. For gram-negative bacilli, more than 10^5 colonies per mL of urine, whereas for gram positive cocci 10^3 -10^5 colonies per ml was considered significant. The culture isolates were further identified by their morphologies and biochemical characteristics. Antibiotic susceptibility testing was done by Modified Kirby Bauer's disc diffusion method as per CLSI guidelines 2017 using commercially available discs. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as control strains[11].

Results

A Total of 120 midstream urine samples were processed from patient having clinically suspected UTI, attending NIMS University Hospital. Among them 48 (40%) were males and 72 (60%) were female patients. Out of 120 samples 78 (65%) samples were found to be significant bacteriuria and remaining 42(35%) samples were either non-significant or sterile urine. Maximum number of cases were females 72(60%), among which 28 (38.89%) female samples were sterile and 44(61.11%) were positive and male was 48(40%), among which 14(29.17%) male samples were sterile and 34(70.83%) were positive samples.

In the present study, major bacterial isolates was E. coli 50(64.10%), followed by Klebsiella spp. 10 (12.82%), Methicillin-resistant Staphylococcus aureus (MRSA) 11(14.10%), Methicillin-susceptible Staphylococcus aureus (MSSA) 3(3.85%) and Pseudomonas aeruginosa 4(5.13%).

Among the uropathogens, isolated E. coli showed (86%) sensitivity to Imipenem, followed by Nitrofurantoin (80%), Piperacillin/tazobactum (82%), Meropenem (74%), Gentamicin (62%) Amikacin (60%). and lesser sensitivity were seen to Cefixime (40%), Cefepime (38%), Norfloxacin (30%), Aztreonem (22%), Ciprofloxacin (22%) and Ceftriaxone (16%).

Klebsiella species were found (80%) sensitive to Imipenem, followed by Nitrofurantoin (80%), Meropenem (70%), Piperacillin / tazobactum (70%), Gentamicin (60%), Amikacin (60%), and lesser sensitivity were seen to Ciprofloxacin (40%), Norfloxacin (40%), Cefepime (30%), Ceftriaxone (20%), Cefixime (20%) and Azteronam (10%).

Pseudomonas aeruginosa was found to be highly sensitive to Polymyxin-B (100%) to Colistin (100%), Imipenem (75%), Meropenem (75%) Amikacin (75%), Gentamicin (75%), Pipercillin (75%), Piperacillin/ tazobactum (75%), Nitrofurantion (50%), Tobramycin (50%), Ciprofloxacin (50%), Aztronem (50%), Cefepime (50%) and lesser sensitivity was seen to Norfloxacin (25%), Ceftazidime (25%), Ofloxacin (25%).

Among the Gram- positive bacterial isolates MRSA showed high sensitivity to Linezolid (100%), Vancomycin (100%), Nitrofurantoin (72.73%), Chloramphenicol (72.73%), Clindamycin (72.73%), Ampicillin (72.73%), Gentamycin (63.64%) Amikacin (54.55%) and lesser sensitivity were seen to Erythromycin (36.36%), Norfloxacin (27.27%), and Penicillin G (18.18%). Among the Gram- positive bacterial isolates MSSA showed high sensitivity to Linezolid (100%), Vancomycin (100%), Nitrofurantoin (100%), Chloramphenicol (100%), Clindamycin (66.67%), and lesser sensitivity were seen to Erythromycin (33.33%), Ampicillin (33.33%), Gentamycin (33.33%) Amikacin(33.33%), Norfloxacin (0.0%) and Penicillin- G (0.0%).

Table 1: Isolated Uropathogens.

Isolates	Number of isolates in positive samples. (N=78)	Percentage
E. coli	50	64.10%
K lebsiella spp.	10	12.82%
MRSA	11	14.10%
MSSA	3	3.85%
P. aeruginosa	4	5.13%

Table 2: Distribution of Uropathogens according to Sex.

Sex	E. coli	Klebsiella spp.	MRSA	MSSA	P. aeruginosa
Male	24	6	3	1	2
Female	26	4	8	2	2

Table 3: Sensitivity rate of Gram Negative Bacteria. IPM (Imipenem), NIT (Nitrofurantoin), PIT(Piperacillin-Tazobactum), MRP (Meropenem), GEN (Gentamycin), AK (Amikacin), CIP (Ciprofloxacin), CPM (Cefepime), NX (Norfloxacin), AT (Aztreonam).

Drug	Sensitivity of E. coli N=50	Sensitivity % of E. coli	Sensitivity of Klebsiella spp. N=10	Sensitivity% of Klebsiella spp.	Sensitivity of P. aeruginsa N=4	Sensitivity% of P. aeruginosa	Total Sensitivity %
IPM	43	86%	8	80%	3	75%	83.33%
NIT	40	80%	8	80%	2	50%	70%
PIT	41	82%	7	70%	3	73%	75%
MRP	37	74%	7	70%	3	75%	73%
GEN	31	62%	6	60%	3	75%	65.67%
AK	30	60%	6	60%	3	75%	65%
CIP	11	22%	4	40%	2	50%	37.33%
CPM	19	38%	3	30%	2	50%	36%
NX	15	30%	4	40%	1	25%	31.67%
AT	11	22%	1	10%	2	50%	27.33%

Table 4: Sensitivity Pattern of Gram Positive Bacteria.

Drugs	MSSA Sensitivity % N=3	MRSA Sensitivity % N=11	Total Sensitivity of GPC
Linezolid	100%	100%	100%
Vancomycin	100%	100%	100%
Nitrofurantoin	100%	72.73%	86.17%
Chloramphenicol	100%	72.73%	86.17%
Clindamycin	66.67%	72.73%	69.70%
Ampicillin	33.33%	72.73%	53.03%

Gentamycin	33.33%	63.64%	48.49%
Amikacin	33.33%	54.55%	43.94%
Erythromycin	33.33%	36.36%	34.85%
Norfloxacin	0%	27.27%	13.64%
Penicillin G	0%	18.18%	9.9%

Discussion

A total of 120 midstream urine specimens were collected in the study, out of which 72(60%) samples were collected from females and rest 43(40%) samples were from males. Pathogenic bacteria were isolated in 78 samples with a prevalence rate of 65%. The prevalence of female was 44(56.41%) and the prevalence rates in males were 34(43.59%), which was in accordance with the various studies:

Table 5: Sex wise division and its relation with other studies.

SEX	Present Study	Sharminsohely *et al* 2009 [12]	MahajanRuchita *et al* 2014 [13]	Dr. Nerurkaralka *et al* 2014 [14]
Male	34(43.59%)	26(43.30%)	120(40%)	71(42.26%)
Female	44(56.41%)	34(56.70%)	180(60%)	97(57.74%)
Total	78	60	300	168

UTI is most commonly seen in the females of age group 21-40 years as 25(32.05%) of samples were in this age group. This might is due to the fact that, most of the people in this age group are more exposed to agents responsible for causing Urinary tract infection. This is comparable with various studies:

Table 6: Age wise distribution and relation with other studies.

Age group	Present study	MahajanRuchita *et al* study 2014 [13]	Gupta ravi *et al* study 2011[15]
>10	3(13.85%)	10(3.33%)	36(10.40%)
11-20	13(16.67%)	24(12.43%)	50(14.45%)
21-40	25(32.05%)	75(38.86%)	91(26.30%)
41-60	22(28.20%)	40(22.73%)	81(23.41%)
>60	15(19.23%)	44(22.80%)	78(22.54%)

In our study, 64(82.05%) Gram negative bacteria were identified, among which E. coli was the commonest uropathogens which is responsible for causing UTI followed by Klebsiella species 10(15.63%), Pseudomonas aeruginosa 4(6.25%) of isolates. The proportion of bacterial species isolated was similar to those described in previous studies done by Dr. AlkaNerukar *et al*. 2014[14] at Mumbai in India, RuchitaMahajan *et al*., (2014) (102) at Jammu in India.

In this study, Gram positive bacteria are identified 14(17.95%). The frequency of Methicillin resistant Staphylococcus aureus (MRSA) were found 11(14.10%) in total positive uropathogens and 78.57% were found in Gram positive Uropathogens, While The frequency of Methicillin sensitive Staphylococcus aureus (MSSA) were found 3(3.85%) in total positive uropathogens and 21.43% were found in Gram positive Uropathogens. this study was similar to Dr. AlkaNerurkar *et al*., (2012)[14] at Mumbai in India, RuchitaMahajan *et al*., (2014)[13] at Jammu in India.

In present study susceptibility pattern of isolated bacterial strains was also studied, E. coli was showed (86%) sensitivity to Imipenem, followed by Nitrofurantoin (80%), Piperacillin/tazobactum (82%), Meropenem (74%), Gentamicin (62%) Amikacin (60%). and lesser sensitivity were seen to Cefixime (40%), Cefepime (38%), Norfloxacin (30%), Aztreonem (22%), Ciprofloxacin (22%) and Ceftriaxone (16%). Klebsiella species were showed (80%) sensitive to Imipenem, followed by Nitrofurantoin (80%), Meropenem (70%), Piperacillin / tazobactum (70%), Gentamicin (60%), Amikacin (60%), and lesser sensitivity were seen to Ciprofloxacin (40%), Norfloxacin (40%), Cefepime (30%), Ceftriaxone (20%), Cefixime (20%) and Azteronam (10%). Pseudomonas aeruginosa was foundshowed to be highly sensitive to Polymyxin-B (100%) to Colistin (100%), Imipenem (75%), Meropenem (75%) Amikacin (75%), Gentamicin (75%), Pipercillin (75%), Piperacillin/ tazobactum (75%), Nitrofurantion (50%), Tobramycin (50%), Ciprofloxacin (50%), Aztronem (50%), Cefepime (50%) and lesser sensitivity was seen to Norfloxacin (25%), Ceftazidime (25%), Ofloxacin (25%). In present study amongst MRSA, the highest resistance was observed in Penicillin G (81.82%) followed by Norfloxacin (72.73%), Erythromycin (63.64%), Amikacin (45.45%) and the least resistance was seen in Vancomycin, linezolid (0%) followed by Gentamycin (36.36%), Ampicillin, Clindamycin, Chloramphenicol and Nitrofurantoin (27.27%) and in MSSA, the highest resistance was observed in Penicillin G and Norfloxacin (100%) followed by Erythromycin, Amikacin, Gentamycin, Ampicillin (66.67%) and the least resistance was seen in Vancomycin, linezolid, Chloramphenicol and Nitrofurantoin (0%) followed by, Clindamycin (33.33). All these results were comparable with following studies, Dr.AlkaNerukar *et al*., (2012) [14] at Mumbai in India, RuchitaMahajan *et al*., (2014)[13] at Jammu in India, Mahmood K. Salih *et al* (2016) [16] in Iraq.

Conclusion

In this study, higher prevalence rates of urinary bacterial isolates are observed in females the most commonly found organisms were E. coli and Klebsiella. There is an emerging resistance of commonly isolated bacteria to routinely used antibiotics, which can be ascribed to inappropriate antibiotic administration. Important infecting organisms are found to be the commensals of perianal and vaginal regions, emphasizing a need to have proper hygienic practices. As drug resistance among bacterial pathogens is an evolving process, regular surveillance and monitoring is necessary to provide physician's knowledge on the updated and most effective empirical treatment of UTIs. Imepenem, Nitrofurantoin, Polymyxin-B, colistin and meropenem were

effective among all the antibiotics for Gram-negative and Nitrofurantoin, Vancomycin, Clindamycin and linezolid were effective among all the antibiotics for Gram-positive.

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