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Bacteriological Profile and Antibiotic Sensitivity Pattern of Uropathogens from a Tertiary Care Hospital in Kashmir

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ABSTRACT

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Urinary tract infection is one of the most common bacterial infections worldwide and is defined as the bacterial infiltration of otherwise sterile urinary tract. This includes both the upper and the lower urinary tract including urethra (urethritis), bladder (cystitis), ureters (ureteritis) and kidney (pyelonephritis). Common uropathogens are *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumonia*, *Pseudomonas* spp. and *Proteus mirabilis*. These infections have risk of high recurrence rates and increasing antimicrobial resistance among uropathogens has enhanced economic burden. In this study, we intend to analyze frequency of occurrence and antibiotic sensitivity pattern of bacteria isolated from the urine samples of symptomatic patients. Clean catch midstream urine samples were processed by semi quantitative culture techniques and the growths obtained were further identified by standard microbiological techniques. Kirby-bauer disc diffusion test was used to study the antibiotic sensitivity profile and data analyzed for a period of two years from January 2017 to December 2018. A total of 4512 samples were studied. *E coli* was the most frequent isolate. Most of the isolates were sensitive to nitrofurantoin

Introduction

Urinary tract infections reportedly affect about 150 million people across the globe each year and are considered one of the most common human infections caused by bacteria.^[1] This accounts for about one fifth of emergency visits in out-patient department in one study.^[2] These infections are a remarkable cause of morbidity especially in females of reproductive age groups and elderly males. A history of a minimum of one

episode of urinary tract infection is experienced by at least 40-50% of all females in the age group between 15-49 years.^[3] The vulnerability of this population group is attributed to the anatomical, physiological and metabolic factors in them.^[4] A worldwide emergence of antimicrobial resistance among uropathogens has subject them to subsequent changes in their pathogenic characteristics.^[4]

The urinary tract infections are categorized as complicated and uncomplicated based on the

clinical conditions of the patient. Factors that lead to incompetent host defences or a compromise in structure or function of the urinary tract may cause complicated urinary tract infections. These may include obstruction caused by calculi, foreign bodies like indwelling catheters responsible for 70-80% of complicated urinary tract infections in USA^[5] and causing increased hospital stay that accounts for nearly one million cases every year^[6], neurological compromise causing urinary retention, immune-suppression, pregnancy and renal failure.^[7,8]

Uncomplicated urinary tract infections on the other hand are not generally associated with structural and functional abnormalities of urinary tract and the persons affected are otherwise considered normal.^[9,10] The differentiation of these infections into lower urinary tract infections and upper urinary tract infections is based on anatomic considerations^[9,11] and is generally associated with one or more risk factors such as recent history of sexual activity, diabetes, a prior episode of UTI or genetic vulnerability.^[11,12]

Urinary tract infection is caused by a wide range of bacteria including both Gram negative and gram positive bacteria and some fungi. More frequent causes are uropathogenic *Escherichia coli* (UPEC) followed by *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, Group B streptococcus, *Proteus mirabilis*, *Pseudomonas aureginosa*, *Staphylococcus aureus* and candida species.^[10,12,13,14] The order of prevalence of agents other than UPEC in causing complicated urinary tract infections as evidenced by some studies is *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Candida* spp., *Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aureginosa* and Group B streptococcus.^[8,15,16,17] Notably all these organisms are important pathogens in

the hospital settings as well. The enhanced selection pressures in these environments lead to an increase in the emergence of drug resistant strains and treatment failures. The normal microbiota of the vagina and the gut may be altered due to injudicious use of antibiotics and may further accelerate the emergence of multidrug resistant microorganisms.^[18]

In the present study we analyzed all cases of urinary tract infection over a period of two years (2017-2018) for the causative pathogen and their antimicrobial sensitivity profile in a tertiary care hospital in Kashmir and observed the change in the pattern over time. The urine samples collected from in-patients and patients attending the out-patient department were also compared.

The main aim of this study includes to study the bacteriological profile of urine samples from cases of symptomatic urinary tract infections. And also to study and compare the antibiotic sensitivity profile of various uropathogens isolated.

Materials and Methods

Study site and type

The present study is a retrospective observational study conducted in the Department of Microbiology in Sher-I-Kashmir Institute of Medical Sciences (SKIMS), Medical college hospital, Bemina.

Study period

The study was conducted over a period of two years from January 2017 to December 2018.

Samples

All urine samples from symptomatic patients were included in the study.

Collection and processing of samples

Midstream clean catch urine was collected from all symptomatic patients suspected of urinary tract infection under strict sterile precautions over a two years period.

Urine culture was done using Cysteine Lactose Electrolyte Deficient (CLED agar) (Hi-Media, India) by semiquantitative method.^[19]

A colony count amounting to greater than 10^5 cfu/ml was considered significant.^[20] A repeat urine culture was performed for all symptomatic cases with lower colony count.

A final identification of isolates was done by standard microbiological techniques.^[21]

Antimicrobial susceptibility testing of all identified isolates was done by Kirby-Bauer disc diffusion method according to clinical laboratory standards institute (CLSI) guidelines 2017.^[22]

Yeast cells isolated from urine samples were included and identified by Gram stain and Germ tube formation.^[23]

Results and Discussion

A total of 4512 (2172 for year 2017 and 2340 for year 2018) urine samples from symptomatic urinary tract infection patients of all age groups were studied in the department of Microbiology in a tertiary care hospital during two years.

More samples were received from the out-patient department than the in-patients during both the years. Among these, culture showed growth in only 952 cases accounting for 21.09% and the rest 3560 samples with no observable growth even on repeat culture were reported sterile following routine diagnostic methodology.

A comparison of isolates from out-patients and in-patients was done for the frequency of isolation of uropathogen. *Escherichia coli* followed by *Enterococcus faecalis* was isolated maximum number of times during these two years with a combined culture positivity rate of 81.71% during 2017 and 82.12% during 2018. However *E coli* was most frequently isolated from urine samples of out-patients (69.28% during 2017 and 69.08% during 2018) whereas *Enterococcus faecalis* was isolated more from urine of hospitalized patients (67.36% during 2017 and 75.24% during 2018). The other uropathogens isolated during these two years mostly from urine samples of hospitalized patients were *Klebsiella* spp., *Pseudomonas* spp., *Acinetobacter* spp. and *Candida* spp. revealing the importance of these organisms in the hospital settings. A considerable percentage of *Proteus* spp., *Staphylococcus* spp. and *Citrobacter* spp. were however isolated from the community patients (Table 1).

A male to female ratio of 1: 1.49 was observed in reference to the total samples received during two years. Of the 4512 samples, 1810 (40.11%) were collected from male patients and 2702 (59.88%) were collected from female patients. Culture positivity rates were more in females (Table 2).

Gender variations were also observed in our study with respect to the age distributions of urinary tract infections. In females maximum number of cases was observed in the age group 20-40 yrs whereas most males having urinary tract infection belonged to 50 yrs and above.

The sensitivity patterns of Enterobacteriaceae, nonfermenters and Gram positive cocci are given below in Tables 3, 4 and 5 respectively.

Statistical analysis

Chi-square test was applied for analysis of categorical data. P-value <0.05 was taken as significant.

Urinary tract infection is commonly encountered entity globally, affecting both genders belonging to all age groups^[24] (Fig. 1). It is rapidly gaining concerns in both community and hospital settings. This is related to the increasing antibiotic resistance among uropathogens that brings unpredictable outcomes by empirical treatments.^[25] The

antibiotic susceptibility patterns keep on changing both in relation to time and the geographical locations of the world, yet the spectrum of various uropathogens remains more or less the same with *E. coli* being the most frequent isolate.^[26] This remains consistent with most of the other studies including the present study with an isolation rate of 61.34% for *Escherichia coli* during a period of two years. This was followed by an isolation rate of 20.58% for *Enterococcus faecalis*. However the rates of isolation among these two uropathogens were different among out-patients and in-patients.

Table.1 Uropathogens isolated from urine samples during 2017 and 2018 in Department of Microbiology, SKIMS, MCH

Organism Isolated	Year 2017; 2172 urine samples received				Year 2018; 2340 urine samples received			
	No. of isolates	% isolated	OPD (1267)	IPD (905)	No. of isolates	% isolated	OPD (1345)	IPD (995)
<i>Escherichia coli</i>	267	60.27%	185 (69.28%)	82(30.71%)	317	62.27%	219(69.08%)	98(30.91%)
<i>Enterococcus faecalis</i>	95	21.44%	31 (32.63%)	64(67.36%)	101	19.84%	25 (24.75%)	76(75.24%)
<i>Proteus spp.</i>	16	3.61%	12 (75%)	4 (25%)	12	2.35%	7(58.33%)	5(41.66%)
<i>Pseudomonas spp.</i>	14	3.16%	2 (14.28%)	12(85.71%)	10	1.96%	2(20%)	8(80%)
<i>Klebsiella spp.</i>	12	2.70%	0 (0.00%)	12 (100%)	17	3.33%	0(0.00%)	17(100%)
<i>Acinetobacter spp.</i>	9	2.03%	0 (0.00%)	9 (100%)	15	2.94%	1(6.66%)	14(93.33%)
<i>Citrobacter spp.</i>	5	1.12%	2 (40%)	3 (60%)	7	1.37%	4(57.14%)	3(42.85%)
<i>Staphylococcus aureus</i>	4	0.90%	2 (50%)	2 (50%)	5	0.98%	2(40%)	3(60%)
<i>Candida spp.</i>	18	4.06%	3 (16.66%)	15(83.33%)	21	4.12%	4(19.04%)	17(80.95%)
Others	3	0.67%	3 (100%)	0 (0.00%)	4	0.78%	2(50%)	2(50%)
Total samples with Culture positive	443 (20.39%)		240	203	509 (21.75%)		266	243

Table.2 Gender distribution for frequency of isolation of uropathogens from urine samples

Gender	Year 2017			Year 2018		
	Total number of samples	Uropathogen isolated	No growth on culture	Total number of samples	Uropathogen isolated	No growth on culture
Male	917	305 (33.26%)	612 (66.73%)	893	277 (31.01%)	616 (68.98%)
Female	1255	612 (48.76%)	643 (51.23%)	1447	682 (47.13%)	765 (52.86%)
Total	2172	443 (20.39%)	1729 (79.60%)	2340	509 (21.75%)	1831 (78.24%)

Table.3 Antibiotic sensitivities of Gram negative Enterobacteriaceae

Organism		No. of isolates	AMP %S	CAZ %S	CIP %S	TOB %S	PIT %S	AK %S	AMC %S	CFS %S	CTR %S	IPM %S	MRP %S	NX %S	PB %S	NIT %S
<i>Escherichia coli</i>	IPD	180 (30.82%)	17	14	16	60	51	70	6	22	26	91	30	20	100	88
	OPD	404 (69.17%)	25	18	22	67	74	73	8	26	30	94	38	31	100	92
<i>Klebsiella spp.</i>	IPD	29 (100%)	18	20	38	56	54	64	20	20	15	82	40	31	100	10
	OPD	0 (0.0%)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Proteus spp.</i>	IPD	9 (32.14%)	11	22	45	45	67	78	56	34	22	78	56	34	100	0
	OPD	19 (67.85%)	21	26	47	53	68	84	39	32	26	84	58	37	100	0
<i>Citrobacter spp.</i>	IPD	6 (50%)	0	0	34	16	50	67	0	16	16	84	67	33	100	84
	OPD	6 (50%)	16	16	50	34	50	84	16	34	34	84	67	50	100	84

AMP: Ampicillin, CAZ: Ceftazidime, CIP: Ciprofloxacin, TOB: Tobramycin, PIT: Piperacillin tazobactam, AK: Amikacin, AMC: Amoxycylav, CFS: Cephoperazone sulbactam, CTR: Ceftriaxone, IPM: Imipenem, MRP: Meropenem, NX: Norfloxacin, PB: Polymyxin B, NIT: Nitrofurantoin, spp.: species, IPD: inpatient department, OPD: outpatient department, %S: percentage sensitivity

Table.4 Antibiotic sensitivity pattern of non fermenters

Organism		No. of isolates	PIT %S	CAZ %S	GEN %S	TOB %S	IPM %S	MRP %S	A/S %S	CFS %S	AK %S	CIP %S	NX %S	TGC %S	PB %S	NIT %S
<i>Pseudomonas spp.</i>	IPD	20 (83.33%)	75	35	55	45	60	50	35	35	55	45	60	95	100	0
	OPD	4 (16.66%)	75	25	50	50	75	50	25	25	50	25	75	100	100	25
<i>Acinetobacter spp.</i>	IPD	23 (95.83%)	74	0	9	4	83	17	0	0	0	0	0	96	100	0
	OPD	1 (4.16%)	100	0	0	0	100	0	0	0	0	0	0	100	100	0

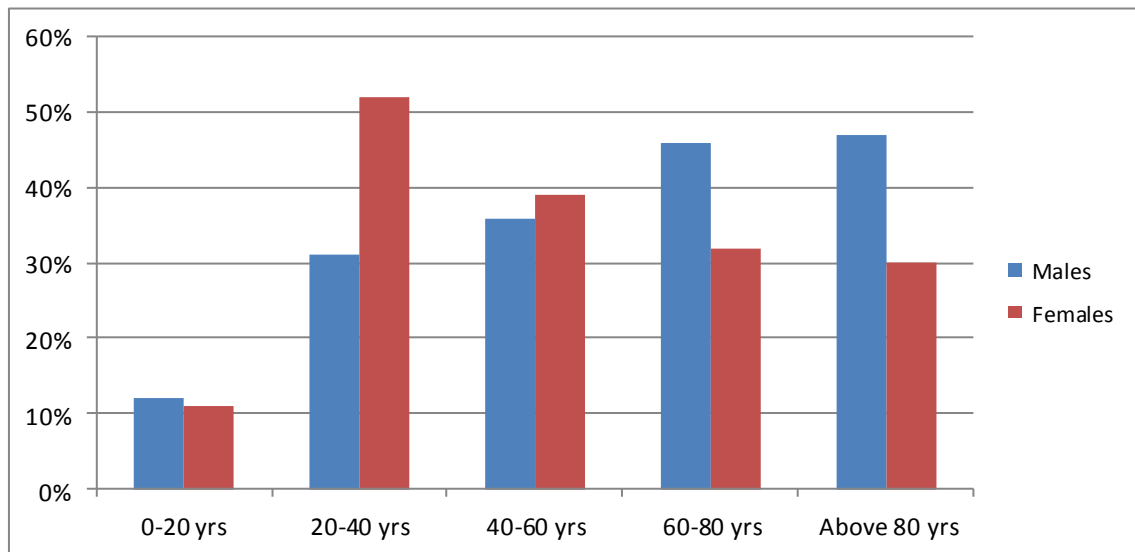
PIT: Piperacillin tazobactam, CAZ: Ceftazidime, GEN: Gentamicin, TOB: Tobramycin, IPM: Imipenem, MRP: Meropenem, A/S: Ampicillin Sulbactam, CFS: Cephoperazone sulbactam, AK: Amikacin, CIP: Ciprofloxacin, NX: Norfloxacin, TGC: Tigecyclin, PB: Polymyxin B, NIT: Nitrofurantoin, spp.: species, IPD: inpatient department, OPD: outpatient department, %S: percentage sensitivity

Table.5 Antibiotic sensitivity pattern of Gram positive cocci

Organism		No. of isolates	P %S	AMP %S	CX %S	CD %S	E %S	LZ %S	VA %S	OF %S	T %S	CIP %S	AK %S	NX %S	COT %S	NIT %S
<i>Enterococcus faecalis</i>	IPD	140 (71.42%)	0	11		8	8	100	97	14	20	18	56	78	67	75
	OPD	56 (28.57%)	0	7		15	10	100	96	25	25	36	64	82	74	95
<i>Staphylococcus aureus</i>	IPD	5 (55.55%)	0	20	60	40	40	100	100	40	20	60	60	60	60	80
	OPD	4 (44.44%)	0	25	50	50	50	100	100	50	25	50	50	50	50	75

P: Penicillin, AMP: Ampicillin, CX: Cefoxitin, CD: Clindamycin, E: Erythromycin, LZ: Linzolid, VA: Vancomycin, OF: Ofloxacin, T: Tetracyclin, CIP: Ciprofloxacin, AK: Amikacin, NX: Norfloxacin, COT: Cotrimoxazole, NIT: Nitrofurantoin, spp.: species, IPD: inpatient department, OPD: outpatient department, %S: percentage sensitivity

Fig.1 Prevalence of UTI in various age groups of symptomatic patients in SKIMS MCH; variations among males and females



In our study, it was observed that *E coli*, *proteus* spp. and *Staphylococcus* spp. were isolated more frequently from urine samples of out-patients and *Enterococcus faecalis*, *Pseudomonas* spp., *Klebsiella* spp., *Acinetobacter* spp. and *Candida* spp. from urine of in-patients. The frequency of isolation of less common isolates varies in different studies.

In the present study, isolation of *Candida* spp. was observed to be 4.06% and 4.12% during 2017 and 2018 respectively with 82.14% isolates cultured from the urine samples of in-patients. A similar isolation rate was reported in a study on hospitalized patients in Goa.^[27] Factors in the hospital settings like immune-compromise, Cancer, use of steroids and broad range of antibiotics for chronic illness predispose for fungal infections.

We observed an overall isolation rate of 47.94% among females of reproductive age group compared to an isolation rate of 32.13% among males. This observation correlates well with the findings in a study done by Deshpande *et al.*^[28]

In our study *Escherichia coli* isolates showed marked susceptibility to nitrofurantoin (88% for in-patients and 92% for out-patientst) whereas more isolates showed resistance to ciprofloxacin (16% for in-patients and 22% for out-patients) and norfloxacin (20% for in-patients and 31% for out-patients). Considerable isolates were susceptible to aminoglycosides (71.5% to amikacin and 64.72% to tobramycin). These observations showed marked discordance with another study from south India by Arjunan *et al.*, for nitrofurantoin and fluoroquinolones, but concordant results for aminoglycosides^[24]

A high percentage of resistance in *E. coli* isolates was found to certain β lactam antibiotics like ampicillin, amoxyclav, ceftazidime, ceftriaxone and cefoperazone-sulbactam, though a considerable number of isolates were sensitive to piperacillin-tazobactam (51% for in-patients and 74% for out-patients). Sensitivity to imipenem was 91% for in-patients and 94% for out-patients. This should however act as a reserve drug for the treatment of complicated urinary tract infections. A lower sensitivity rates were

observed to meropenem. The sensitivity rates was slightly lesser in the *E coli* isolates from hospital samples than in out-patient samples for most of the antibiotics but nowhere the difference was found statistically significant ($p>0.05$). Some studies however report much higher amikacin resistance among hospital samples compared to those from community^[27,29]

Klebsiella spp., *Acinetobacter* spp. and *Pseudomonas* spp. are important causes of bacterial infections in hospital settings. The strains of these bacteria encounter intense selection pressure and spread in hospital environments through improper hand hygiene and contaminated equipments.^[30] All isolates of *Klebsiella* spp. were cultured from urine samples of hospitalized patients and showed considerable resistance to most first line antibiotics including nitrofurantoin. Higher sensitivity rates were however reported for tobramycin (56%), piperacillin-tazobactam (54%), Amikacin (64%), Imipenem (82%) and polymyxin B (100%). A similar antibiotic pattern was observed for *Pseudomonas* spp. and *Acinetobacter* spp. where all the isolates from hospital samples were reported resistant to nitrofurantoin. A very high sensitivity rates to polymyxin B, tigecycline, piperacillin-tazobactam and imipenem was observed among most of the isolates of these bacteria.

All isolates of *Enterococcus faecalis* and *Staphylococcus aureus* were found sensitive to Linezolid and resistant to penicillin. Vancomycin sensitivity rate was 100% for *Staphylococcus aureus* and 96-97% for *Enterococcus faecalis*. A high sensitivity rate was also reported in our study for these bacteria to nitrofurantoin, Cotrimoxazole and Amikacin that was in concordance to some other studies.^[24,27,31,32,33]

A high percentage of isolates in our study were reported resistant to fluoroquinolones

and cephalosporins which may be because of their indiscriminate use for treating all bacterial infections in this part of our country. On the other hand a large number of isolates were reported sensitive to nitrofurantoin, an antimicrobial agent with local activity in urinary tract. This drug is observed to be a better option for empirically treating urinary tract infections.^[9,34,35]

In conclusion, the variations in the spectrum of uropathogens and the increasing antimicrobial resistance among organisms that encounter intense selection pressure demands a consistent evaluation of these bacteria. A constant monitoring of sensitivity pattern of uropathogens to commonly used antibiotics is essential. Empirical treatments be strictly according to the sensitivity patterns of uropathogens isolated in that area. There is a need to develop hospital guidelines on catheter use and infection control policies. This will certainly lower the economic burdens caused by these infections.

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