Original Article

A RETROSPECTIVE STUDY ON PREVALENCE OF MULTI DRUG RESISTANT STRAINS OF UROPATHOGENS IN KATHMANDU MODEL HOSPITAL

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Abstract

Background

The emergence of multidrug resistance strains of uropathogens may greatly increase the difficulty of urinary tract infection. Our study was conducted to analyze the antimicrobial susceptibility pattern of uropathogens and to determine the occurrence of multidrug resistant strains among the isolates.

Method

All mid stream urine samples were cultured by using semi-quantitative method on 5% Blood Agar and Mac Conkey Agar plates. All isolated uropathogens were identified by using Gram stain and biochemical methods. Sensitivity tests were performed on Mueller Hinton agar plate by Kirby Bauer's Disc Diffusion Technique.

Result

During the study period (December 2006-December 2007), a total of 3648 samples were analyzed. Among 3648 samples, 864(23.6%) showed significant growth. Greater prevalence of urinary tract infection was found in July and August and in 20-29 years age group.*E.coli* (89.6%) was the most common among the gram negative isolate and Coagulase negative staphylococci was the most common (37.5%) in gram positive isolates. Among the total isolates multidrug resistance was the most commonly associated with *Enterococcus faecalis* (75.0%) followed by *E.coli* (68.7%), *Pseudomonas aeruginosa* (66.6%) and *Coagulase negative staphylococci* (61.5%).Nitrofurantoin(81.1%) was the most effective first line antibiotic for gram negative isolates and Ciprofloxacin(93.7%) and cefotaxime(93.7%) were for the gram positive isolates.Amikacin(80.0%) was the most effective antibiotic tested as extended panel for multidrug resistance strains.

Conclusion

Multidrug resistance strains of uropathogens are emerging very rapidly which may create serious problem in proper management of urinary tract infection in near future.

Keywords

Uropathogen, Urinary tract infection, Multidrug resistance

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Introduction

Urinary tract infection (UTI) is defined as bacteriuria i.e. multiplication of the organisms in urinary tract and the presence of more than a hundred thousand (10^5) organisms per milliliter in the mid stream sample of urine (MSU)[1].Although the widespread availability of antibiotics, UTI remains the most common bacterial infection in the human[2]. UTI can occur at anytime in the life of an individual[3].According to the annual report published by Department of Health Services, Kathmandu(1996/1997), the morbidity rate of UTI is 0.42% of total population(21,216,638) and urine samples appear as the second commonest sample(18%) to be submitted to the laboratory after blood(48%) for examination. The incidence of UTI is high in female population about 2% at the age of 15-24 years and increasing 1-2% every decade to a prevalence rate of 10% in 55-64 years[4].Antibiotics today's are the frontline therapeutic means for the medical intervention in an infection, which plays a central role in the control and management of infectious diseases. The current knowledge of antibiotic sensitivity profile of urinary pathogens is mandatory for the proper management of urinary tract infection[5].Since antibiotic resistance pattern may vary in a wide variety of pathogenic organisms even over a short period and depend on site of isolation and on different environments, periodic update in the knowledge about antibacterial activity is needed[6,7].

The present study was conducted to determine the antimicrobial susceptibility pattern of uropathogens and to analyze the Multidrug resistant strains of uropathogens from patients suffering with urinary tract infection in Kathmandu Model hospital, Kathmandu.

Materials and Methods

3648 urine samples were examined from clinically suspected urinary tract infection in patients attending Kathmandu Model Hospital during this research period of thirteen months (December 2006- December 2007) using culture and sensitivity tests. All the patients were instructed carefully for collection of mid stream urine specimens. They were given a sterile, dry and clean collection bottles for urine collection .All the urine specimens were processed within 30 minutes of collection.

Culture of all urine specimens was done on 5% Blood Agar and Mac Conkey Agar plate utilizing semi-quantitative culture method. Shaking with hand to ensure a uniform suspension of bacteria vigorously mixed the urine specimens. Then an inoculating loop of standard dimension was used to take up approximately fixed and known volume (0.001ml) of mixed urine and placed on the center of the plate. The drop was spread in a line and then over the entire surface of the agar plate. After inoculation, the culture plates were incubated in an inverted position

at 37°C for 24 hours[8]. After 24 hours, the number of colonies were counted on each plate, which was multiplied by 1000 to calculate the number of organisms per ml in the specimen. Samples showing 10^5 or more organisms per ml of urine were taken as significant. Colony counts less than this was considered as non-significant[9].

Gram staining and various biochemical tests were used to identify pure culture of bacterial growth. Different biochemical media used were Triple Sugar Iron Agar,Sulphite Indole motility Agar ,Urease agar ,Simmons's Citrate Agar ,MRVP and Oxidation Fermentation medium.Catalase, Coagulase and Oxidase Tests were also performed.The primary panel antibiotics used for Gram negative bacteria were Amoxycillin (10mcg),Cefotaxime (30 mcg), Ciprofloxacin (5mcg),Cefixime (5 mcg) , Cotrimoxazole (1.25/23.75mcg), Norfloxacin (10mcg), Nitrofurantoin (300 mcg) and Ofloxacin (5 mcg) and those used for Gram positive bacteria were Amoxycillin (10mcg), Cefotaxime (30 mcg), Ciprofloxacin (5mcg), Cephalexin (30 mcg) , Cotrimoxazole (1.25/23.75mcg) and Cloxacillin (5 mcg). Amikacin(30 mcg), Ceftriaxone 30 mcg), eftazidime(30 cg), hloramphenicol 30 mcg) and Gentamycin 10 mcg) were used as extended panel for Multi Drug Resistance (MDR) Gram negative isolates. All the antibiotics were tested by Kirby Bauer's Disc Diffusion Technique.The colonies picked up and suspended in Nutrient broth and adjusted turbidity to 0.5 Mc Farland Standard. Within 15 minutes a sterilized cotton swab was dipped into the adjusted suspension. Carpet culture was done by streaking the swab over the entire sterile. The antibiotic impregnated discs were placed on the surface of the agar plate and then incubated at 37°C for 18 hours. The different inhibition zones were measured and interpreted the results on the basis of zone size compared with standard interpretive table given by manufacturer.

Result

Table 1: Source wise and gender wise Distribution of Urine Samples Requested for Culture

S.No.	Result	OPD				In Patient				Total No.	%
		Male		Female		Male		Female			
		No	%	No	%	No	%	No	%		
1	Significant Growth	230		547		40		47		864	23.6
2	No significant Growth	198		493		30		58		779	21.3
3	No Growth	674		1059		130		142		2005	54.9

A total of 3648 urine samples, 3201 (87.7%) from Out Patient Department and 447(12.2%) from admitted patients. 864 (23.6%) showed the significant growth and 2005 (54.9%) samples were sterile and 779 (21.3%) samples showed insignificant growth. Urinary Tract Infection was more common in female (68.7%) compared to males (31.2%)

Table 2: Month wise Distribution of Urine Samples and uropathogens

Year	Month	Total samples processed	Total isolates		
		No.	%	No	%
2006	December	160	4.3	44	5.0
2007	January	201	5.5	42	4.8
2007	February	210	5.7	29	3.3
2007	March	265	7.2	57	6.5
2007	April	302	8.2	70	8.1
2007	May	322	8.8	72	8.3
2007	June	303	8.3	80	9.2
2007	**July**	**385**	**10.5**	**120**	**13.8**
2007	**August**	**384**	**10.5**	**115**	**13.3**
2007	September	338	9.2	65	7.5
2007	October	251	6.8	60	6.9
2007	November	261	7.1	50	5.7
2007	December	266	7.2	53	6.1
Grand Total		**3648**	**100.0**	**864**	**100.0**

The no. of samples was significantly higher from July to September and a corresponding higher isolation rates was also observed in this season.

Table 3: Profile of Gram negative and Gram positive uropathogens

S.No.	Uropathogens	No. of isolates	%
Gram negative			
1	*E.coli*	**746**	**89.6**
2	*Citrobacter freundii*	36	4.3
3	*Enterobacter aerogens*	26	3.1
4	*Proteus mirabilis*	9	1.0
5	*Morganella morganii*	8	0.9
6	*Proteus mirabilis*	3	0.3
7	*Pseudomonas aeruginosa*	3	0.3
8	*Salmonella .typhi*	1	0.1
Gram positive			
1	*Coagulase negative staphylococci*	**12**	**37.5**
2	*Staphylococcus saprophyticus*	9	28.1
3	*Staphylococcus aureus*	7	21.8
4	*Enterococcus faecalis*	4	12.5

Among the total bacterial isolates (864),gram negative encountered the highest position 832(86.3%)followed by gram positive 32 (13.7%).Out of 832 gram negative uropathogens, the highest numbers of isolates were found to be *E.Coli* 746 (89.6%) followed by *Citrobacter freundii* (4.3%) and *Enterobacter aerogens* (3.1%).Among the total 32 gram positive isolates, Coagulase Negative Staphylococci (37.5%) was the most prevalent followed by *Staphylococcus saprophyticus* (28.1%), *Staphylococcus aureus* (21.8%) and *Enterococcus faecalis* (12.5%).

Table 4: Age wise distribution of the uropathogens

Bacteria	0-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80-89	90-99
E.coli	22	59	**243**	98	82	85	77	50	26	4
Citrobacter freundii	1	6	11	7	5	2	2	3	-	-
Enterobacter aerogens	1	1	18	2	-	-	-	3	1	-
Proteus mirabilis	-	-	4	3	1	1	-	-	-	-
Morganella morganii	-	1	3	-	1	1	3	-	-	-
Proteus vulgaris	-	-	1	1	-	1	-	-	-	-
Pseudomonas aeruginosa	-	-	-	1	-	-	-	1	1	-
Salmonella .typhi	-	-	1	-	-	-	-	-	-	-
Coagulase negative staphylococci	1	1	3	4	-	-	1	2	-	-
Staphylococcus saprophyticus	-	-	3	2	1	2	1	-	-	-
Staphylococcus aureus	-	-	1	-	1	4	1	-	-	-
Enterococcus faecalis	-	-	-	-	1	1	2	-	-	-

Maximum infection was found in the age group of 20-29 years followed by age group of 30-39 years. Most of the uropathogens were isolated in these age groups.

Table 4: Antibiotic sensitivity profile of gram negative isolates

S.No.	Antibiotic	Isolate No.	Sensitive		Intermediate		Resistant	
			No.	%	No.	%	No.	%
1	Amoxycillin	832	306	36.7	6	0.7	520	**62.5**
2	Cefotaxime	832	**615**	**73.9**	4	0.4	216	25.9
3	Ciprofloxacin	832	491	59.0	2	0.2	339	**40.7**
4	Cotrimoxazole	832	472	56.7	2	0.2	358	**43.0**
5	Cefixime	832	543	65.2	1	0.1	288	34.6
6	Nitrofurantoin	832	**675**	**81.1**	-	-	157	18.8
7	Norfloxacin	832	502	60.3	4	0.4	326	39.1

8	Ofloxacin	832	509	61.1	1	0.1	322	38.7

Among the 832 gram negative isolates, Nitrofurantoin was the most effective antibiotic in 675 (81.1%) isolates followed by Cefotaxime (75.9%), Cefixime (75.9%), (Norfloxacin (63.2%), Ofloxacin (61.1%), Ciprofloxacin (59.0%) and Cotrimoxazole (56.7%).Amoxycillin (36.7%) was the least effective antibiotic.

Table 6: Antibiotic sensitivity profile of gram positive isolates

S.No.	Antibiotic	Isolate No.	Sensitive		Intermediate		Resistant	
			No.	%	No.	%	No.	%
1	Amoxycillin	32	21	65.6	-	-	11	34.4
2	Cefotaxime	32	**30**	**93.7**	-	-	2	6.3
3	Ciprofloxacin	32	**30**	**93.7**	-	-	2	6.3
4	Cotrimoxazole	32	25	78.1	5	15.6	2	6.3
5	Cephalexin	32	29	90.6	-	-	3	9.4
6	Cloxacillin	32	22	68.7	5	15.6	5	15.7
7	Erythromycin	32	20	62.5	-	-	12	37.5
8	Norfloxacin	32	25	78.1	-	-	7	21.9

Among the 32 gram positive isolates, Cefotaxime and Ciprofloxacin were the most susceptible antibiotics in 30(93.7%) isolates, followed by Cephalexin (90.6%), Cotrimoxazole (78.1%), Norfloxacin (78.1%), Amoxycillin (65.6%) and Erythromycin (62.5%).

Table 7: Antibiotic Resistance Pattern of Uropathogens

Organisms	Total Isolate No.	Resistance to						
		0 drug	1 drug	2 drugs	MDR strains			
					3 drugs	>3 drugs	Total	%
E.coli	746	75	83	75	136	377	513	**68.7**
Citrobacter freundii	36	12	10	10	2	2	4	11.1
Enterobacter aerogens	26	6	10	2	5	3	8	30.7
Morganella morganii	8	2	2	2	1	1	2	25.0
Pseudomonas aeruginosa	3	-	-	1	-	2	2	**66.6**
Salmonella .typhi	1	-	-	2	-	-	-	-
Coagulase negative staphylococci	12	3	1	-	3	5	8	61.5
Staphylococcus aureus	7	2	2	1	2	-	2	28.5
Enterococcus faecalis	4	-	-	1	2	1	3	**75.0**

Among the total isolates multidrug resistance (MDR= resistance≥3drugs) was the most commonly associated with *Enterococcus faecalis* (75.0%) followed by *E.coli* (68.7%), *Pseudomonas aeruginosa* (66.6%), *Coagulase negative staphylococci* (61.5%), *Enterobacter aerogens* (30.7%), *Staphylococcus aureus* (28.5%) and *Morganella morganii* (25.0%).

Table 8: Extended Panel of Antibiotics for MDR Gram Negative Uropathogens

S.No.	Antibiotic	Isolate No.	Sensitive		Intermediate		Resistant	
			No.	%	No.	%	No.	%
1	Amikacin	350	**280**	**80.0**	13	3.7	57	16.2
2	Ceftriaxone	350	225	64.2	-	-	125	35.7
3	Gentamycin	350	206	58.8	-	-	144	41.1
4	Chloramphenicol	350	156	44.5	12	3.4	194	55.4
5	Ceftazidime	350	123	35.1	14	4.0	227	64.8

Among 350 Multi Drug Resistance gram negative uropathogens, Amikacin (78.9%) was the most effective drug followed by Ceftriaxone (62.4%) and Gentamycin (56.7%).

Discussions

Urinary tract infection is a common cause of morbidity and can lead to a significant mortality. Careful diagnosis and treatment result in successful resolution of infection in most instances. Bacteriological examination of the urine is major aid in the diagnosis of infection.

During the study period (December 2006-December2007), 3648 urine samples were analyzed. Out of 3648 samples 864 (23.6%) showed significant growth. The rate of growth positivity was found to be 68.7% in female whereas it was found to be 31.2% in males. Such high rate of growth possibility in women is reported by other investigators[10, 11, 12.] The higher no. of requests and higher growth positivity in females is attributed to their anatomical structure (short urethra and proximity to anal orifice).In this study, the highest percentage of the infected patients (30%) belonged to age group 20-29 year. This age group of the patient is sexually active and is of childbearing age in case of female. This finding corresponds to other results [10, 12].

Reports from world wide suggest a significant peak of UTI for a few months in post summer season each year. They attribute this to hot and humid conditions during these months. We have observed that the samples were maximum in July and August with corresponding high recovery of isolates during this period[13,14].

E.coli (86.3%) was the commonest isolate resembles to various other reports[10, 12.]*E.coli* is the most important pathogen for uncomplicated as the complicated UTI and has shown a slow and steady increase in resistance to several antibiotics over the past decade[15,16]. Nitrofurantoin was found to be the most effective for gram negative isolates (81.1%).Similar data is presented in other reports[10,12,17].

In our study, *Enterococcus faecalis* (75.0%) was found to be the most common Multi Drug Resistance (MDR) uropathogens followed by *E.Coli (*68.7%) and *Pseudomonas aeruginosa* (66.6%).MDR was defined as resistance to 2 or >2 classes of the antimicrobials (≥3drugs) of first line tested in this study.*Enterococcus faecalis* and *Pseudomonas aeruginosa* were commonly

associated with the patients admitted in the hospital, in whom there is increase intake of antibiotics and increased urological manipulation. According to Hasan et al, multidrug resistance was the commonest with *Enterococcus faecalis* (78.8%) *followed* by *Pseudomonas aeruginosa* (65.1%) [14].

Infectious Diseases Society of America (IDSA) recently recommended that each hospital should establish mechanisms to determine the local resistance rates among uropathogens and that the standard antimicrobial regimens for empirical treatment of UTIs should be reassessed periodically in light of changing susceptibility pattern. Clinicians should be aware of current antimicrobial susceptibility patterns for *E.coli* and other uropathogens in their local communities as antimicrobial susceptibility changes over time[18].The overall high resistance to Amoxycillin and Cotrimoxazole found in this study resembles to other studies[18,19,20].Similar to our study the resistance rate of Ciprofloxacin in other studies[18,19].In *E.coli* uropathogen, a correlation between Ampicillin, Cotrimoxazole and Ciprofloxacin resistances has previously reported[21].A decline in the activity of Ciprofloxacin would be especially problematic in view of the ability of gram negative bacilli to acquire resistance to all other classes of antimicrobials[22]. Among the total isolates multidrug resistance (MDR= resistance≥3drugs) was the most commonly associated with *Enterococcus faecalis* (75.0%) followed by *E.coli* (68.7%), and *Pseudomonas aeruginosa* (66.6%). This study is closely related to the study done by Hasan et.al[14].The most useful first line antibiotic for the treatment of urinary tract infection is Nitrofurantoin which is also reported to other studies[10, 12].In extended panel of antibiotics for the treatment of MDR uropathogens, Amikacin was the most effective antimicrobial agent and this was also shown in this study[14].

Conclusion

In conclusion, the alarming finding of this study was the high prevalence of MDR uropathogens which are resistant to almost all antimicrobial agents. Higher resistance rates to all antibiotics tested in our study may be due to high and uncontrolled consumption of these antibiotics. All the antibiotics are easily available in the market and can be purchased without requiring the doctor's prescription in our country. Further study is needed to find out the clear reason of such high prevalence of MDR uropathogens.This is an urgency to recognize all the factors involved in the prevalence of MDR, control and regular monitoring of the usage of antimicrobial agents and select the best strategies for the prevention and control the development of resistance.

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