ANTIBIOGRAM OF GRAM NEGATIVE UROPATHOGENS IN HOSPITALIZED PATIENTS

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Original Article

Abstract: Background: Urinary tract infections (UTIs) are caused by variety of microorganisms. The frequency of different bacterial isolates and their susceptibility to antibiotics may differ widely, particularly in hospitalized patients. The study of susceptibility pattern becomes obligatory for a proper selection of antibiotics.

Aim: To evaluate antimicrobial susceptibility pattern of the Gram negative organisms isolated from urine cultures in hospitalized patients. **Material and Methods:** The present study was conducted in Yashwantrao Chavan Rural Hospital, Latur over a period of one year from June 2010 to May 2011. A total of 500 urine samples from hospitalized patients which showed significant bacteriuria were studied. Samples were inoculated on Blood agar and MacConckey agar. Further identification of organisms was done by standard Microbiological methods. Antimicrobial Susceptibility pattern was studied by Modified Kirby-Bauer's disc diffusion method with the panel of 15 drugs as per Clinical Laboratories Standard Institute (CLSI) guidelines.

Results: UTIs were found more common in females 296 (59.2%). Commonest organism found was Escherichia coli 252 (50.2%) followed by Klebsiella spp. 123 (24.6%), Pseudomonas spp. 49 (9.8%), Proteus spp. 42 (8.4%), Citrobacter spp. 27(5.4%) and Acinetobacter spp. 8 (1.6%). Majority of the strains were found sensitive to nitrofurantoin 404 (80.8%) followed by amikacin 357 (71.4%), piperacillin-tazobactam 325 (65.2%) and co-trimoxazole 254 (50.8%). Commonly prescribed fluroquinolones were found least effective for treatment of UTI. All the strains were found sensitive to imipenem. Extended spectrum beta lactamase (ESBL) was noted in 169 (67.33%) E.coli and 87 (70.73%) in Klebsiella spp.

Conclusions: To discourage the indiscriminate use of antibiotics and to prevent further development of bacterial drug resistance, proper knowledge of susceptibility pattern of uropathogens in particular area is very important before prescribing empirical antibiotic therapy.

Key-words: Urinary tract infection, Gram negative bacteria, Hospitalized Patients, Antibiogram.

Introduction: Despite the widespread availability of antibiotics, urinary tract infection (UTI) is one of the most important causes of morbidity in general population and also the common cause of nosocomial infection among hospitalised patients.[1]

An indiscriminate use of antimicrobial agents often leads to emergence of resistant microorganisms to one or several of them.[2] Since the pattern of sensitivity is constantly changing, monitoring of the antimicrobial susceptibilities become more important. It provides information on the pathogenic organisms isolated from patients as well assist in choosing the most appropriate antimicrobial therapy till the culture reports become available.[3]

Area specific monitoring studies aimed to gain knowledge about the type of pathogens responsible for UTIs and their resistant patterns may help the clinicians to choose the correct empirical therapy. So, the present study was undertaken to determine the sensitivity profiles of urinary isolates in the hospitalized patients from Yashwantrao Chavan Rural Hospital, Latur which would expectedly indicate the most appropriate antibiotic therapy for the treatment of UTIs.

Material and Methods:

This study was carried out during the period of one year from June 2010 to May 2011 in hospitalized patients of Yashwantrao Chavan

Rural Hospital, Latur. Total of 500 urine samples were studied which showed significant bacteriuria i.e. 10^5 or more cells/ml of urine detected in properly collected 'mid-stream' clean catch urine or from catheter specimen.[4]

Age and sex of the patient were also recorded. Samples were inoculated on Blood agar and MacConkey's agar. The colonies were further processed for identification by standard microbiological methods.[5]

Antimicrobial susceptibility pattern was studied by Modified Kirby-Bauer disc diffusion method with a panel of 15 drugs as per the Clinical Laboratory Standard Institute (CLSI) guidelines and sensitivity pattern was noted. The 15 drugs used were amikacin (30µg), gentamicin (10µg), ciprofloxacin (5µg), ofloxacin (5µg), gatifloxacin (5µg), levofloxacin (5µg), co-trimoxazole (1.25 µg /23.75µg), norfloxacin (10µg), nitrofurantoin (300µg), netilmicin (30µg), piperacillin-tazobactam (100µg+10µg), imipenem (10µg), ceftazidime (30µg), ceftriaxone (30µg), and ceftazidime (30µg) (Hi-Media, India).

The diameter of zone of inhibition of growth was recorded and interpreted by the criteria of CLSI. *E. coli*, *Klebsiella* and *proteus spp*. were screened for extended spectrum beta lactamase (ESBL) by using ceftazidime, ceftriaxone and cefotaxime as screening agents as proposed by CLSI.[6]

Escherichia coli ATCC 25922, *Staphyloccus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 were used as control strains.

Table 1: Sex distribution (Total Positive Samples = 500)

Sex	Number	Percentage
Male	204	40.8
Female	296	59.2
Total	500	100.0

Results:

Out of the total 500 culture positive urine samples from hospitalised patients 296 (59.2%) and 204 (40.8%) were female and male respectively (Table 1). The Gram negative organisms grown on the culture were as follows: *E.coli* 251 (50.2%), *Klebsiella spp.* 123 (24.6%), *Pseudomonas aeruginosa* 49 (9.8%), *Proteus spp*. 42 (8.4%), *Citrobacter freundii* 27 (5.4%) and *Acinetobacter baumanii* 08 (1.6%). (Table 2).

The antibiogram of isolated pathogens is shown in Table 3. Majority of the strains were found to be sensitive to nitrofurantoin (80.8%) followed by amikacin (71.4%) and piperacillin-tazobactam (65.2%). The least sensitivity was for ofloxacin (23.8%) followed by norfloxacin (25.2%), gatifloxacin (25.4%), ciprofloxacin (28%) and levofloxacin (28.8%). Thus, maximum resistance was found to the commonly prescribed fluroquinolone group of antibiotics. *Pseudomonas aeruginosa* strains were found more susceptible to piperacillin-tazobactam (67.3%) followed by amikacin (62.4%). Among most of the hospitalised patients, nearly 50% of strains showed sensitivity to co-trimoxazole. All the strains were sensitive to imipenem. ESBL production was noted in 62.54% of *E.coli* and in 64.22% of *klebsiella spp*.

Discussion:

The prevalence of antimicrobial resistance among microorganisms that cause UTI is increasing worldwide and is a major factor in selecting antibiotics for treatment. There are local variations in the antimicrobial susceptibility among urinary pathogens in different hospitals. Diagnosis of UTI is a good example of the need for close cooperation between the clinician and the microbiologist.

The present study data gives idea about the common trend of increased resistance of uropathogens in this region which may be due to geographica variation or indiscriminate or sublethal use of antibiotics. Among 500 uropathogens isolated from patients with UTI, the commonest isolate was *E. coli* (50.2%) followed by *Klebsiella spp*. (24.6%). These isolation rates of uropathogens are consistent with the study by Tankhiwale *et al*[7], who

reported high incidence of 47.4% for *E. coli* followed by 37.8% for *Klebsiella spp*. It is stated that UTI is more common in females than in males, in our study too there was a female preponderance for this infection and this observation correlates with the previous studies.[8, 9]

Table 2: Gram Negative Bacilli Isolated During the Study

Gram Negative Bacilli	Frequency	
	Number (500)	Percentage
E. coli	251	50.2
Klebsiella Pneumonia	123	24.6
Pseudomonas aeruginosa	49	9.8
Proteus spp.	42	8.4
Citrobacter freundii	27	5.4
Acinetobacter baumanii	08	1.6

E.coli, which was the principal pathogen isolated, showed high susceptibility to nitrofurantoin (82%), amikacin (74.1%) and least susceptible to third generation cephalosporins and fluroquinolones. Similar results are reported from other studies.[10, 11]

Among Gram negative isolates *Pseudomonas aeruginosa* and *Acinetobacter spp*. are known to be associated with hospital infections.[12] *Pseudomonas aeruginosa*, the third commonly isolated organism from hospitalised UTIs was less sensitive to commonly used to antibiotics, but highly sensitive to piperacillin-tazobactam (67.3%) and amikacin (62.4%). Similar results are reported from previous studies by Das *et al*.[13]

Table 3: Overall Sensitivity Pattern of Individual Antibiotic

Antimicrobial Agents	Number of Isolates showing Sensitivity	Percentage of Sensitivity
Amikacin (30µg)	357	71.4
Gentamicin (10µg)	231	46.2
Ciprofloxacin (5µg)	140	28
Ofloxacin (5µg)	119	23.8
Gatifloxacin(5µg)	127	25.4
Leofloxacin (5µg)	144	28.8
Norfloxacin(10µg)	126	25.2
Nitrofurantoin (300µg)	404	80.8
Co-trimoxazole(1.25µg/23.75µg)	254	50.8
Netilmicin (30µg)	282	56.4
Piperacillin- tazobactam (100µg + 10µg)	325	65
Imipenem (10µg)	500	100
Ceftazidime(30µg)	193	38.6
Ceftriaxone(30µg)	188	37.6
Cefotaxime(30µg)	193	38.6

The quinolones viz. norfloxacin, ciprofloxacin, gatifloxacin, ofloxacin and levofloxacin which are most commonly used drugs against UTI were least effective against all the uropathogens encountered during the study period. This observation positively correlates with the previous reports.[14, 15] Since the mechanism of action of these quinolones is almost same, emergence of resistance against one will also decrease the activity of other quinolones. This is contrary to the studies which have reported higher susceptibility to the fluroquinolones.[13]

Table 4: Antibiotic Sensitivity pattern of Gram Negative Bacilli

Isolates	Antimicrobial Agents														
	Ak	**G**	**Cf**	**Of**	**Gf**	**Le**	**Nx**	**Nf**	**CO**	**Nt**	**PT**	**I**	**Ca**	**Ci**	**Ce**
E. coli **(n=251)**	186	121	71	61	62	70	57	206	131	145	166	251	94	87	91
%	74.1	48.2	28.4	24.3	24.7	27.8	22.7	82.0	52.1	57.7	66.1	100	37.4	34.7	36.2
Klebsiella **(n=123)**	88	55	35	28	31	36	30	101	61	72	77	123	39	41	44
%	71.3	44.5	28.5	22.7	25.2	29.2	24.3	82.1	49.5	58.5	62.6	100	31.7	33.3	35.7
Pseudomonas **(n=49)**	29	20	13	11	12	14	16	NA	NA	27	33	49	28	26	27
%	62.4	40.8	26.5	22.4	24.5	28.5	32.6			55.1	67.3	100	57.1	53.0	55.1
Proteus **(n=42)**	29	20	11	10	11	13	12	33	17	20	26	42	19	21	17
%	69.0	47.6	26.1	23.8	26.1	30.9	28.5	78.5	40.4	47.6	61.9	100	45.2	50	40.4
Citrobacter **(n=27)**	20	12	08	07	08	08	09	21	12	14	18	27	11	10	11
%	74.0	44.4	29.6	26.9	29.6	29.6	33.3	77.7	44.4	51.1	66.6	100	40.7	37.0	40.7
Acinetobacter **(n=08)**	05	03	02	02	03	03	02	06	04	05	05	08	02	03	03
%	62.5	37.5	25	25	37.5	37.5	25	75	50	62.5	62.5	100	25	37.5	37.5

Ak- Amikacin, G- Gentamicin, Cf- Ciprofloxacin, Of –Ofloxacin, Gf- Gatifloxacin, Le- Leofloxacin, Nx- Norfloxacin, Nf- Nitrofurantoin CO - Co-trimoxazole, Nt- Netilmicin, PT- Piperacillin – tazobactam, I – Imipenem, Ca – Ceftazidime, Ci- Ceftriaxone ,Ce- Cefotaxime, NA- Not Applicable

Nitrofurantoin was found to be the most effective antibiotic (80.8%), even in strains resistant to majority of the other antibiotics. Similar observation was made by Kader et al.[3] In addition majority of the Gram negative organisms were moderately sensitive to third generation cephalosporins viz. ceftazidime, ceftriaxone and cefotaxime (38.2%). These broad spectrum cephalosporins used indiscriminately in various types of infections caused by different types of microbes which probably lead to emergence of resistance towards these antibiotics within short span of time.

Organisms that produce ESBL have important therapeutic implications as they show resistance to variety of antimicrobial agents including beta lactams, broad spectrum penicillins, third generation cephalosporins and monobactams. As in our study, Allison et al[16] also have shown a high susceptibility rates of ESBL producing organisms to nitrofurantoin, amikacin and imipenem.

To conclude, the uropathogens showed higher sensitivity to the carbapenems. The next best alternative for treating UTI caused by Gran negative organisms is nitrofurantoin even in ESBLs, followed by amikacin, piperacillin-tazobactam, netillin and co-trimoxazole. The emergence and spread of resistance can be reduced through appropriate or careful use of antimicrobial drugs and increasing awareness among the population to the hazards of inappropriate antimicrobial use through public health education campaign.[17] The susceptibility data collected in this study suggest that drug resistance is common problem in uropathogens isolated from hospitalized patients. So, the proper knowledge of sensitivity pattern of uropathogens is very important before prescribing an empirical antimicrobial therapy, this will also discourage the indiscriminate use

of antibiotics and prevent further development of bacterial drug resistance.

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