■ *Original Article*

Study of antimicrobial susceptibility pattern of Gram positive organisms causing UTI in a tertiary care hospital in eastern region of Nepal

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Abstract

Background: Urinary tract infections (UTIs) are the most important cause of mortality and morbidity affecting all age groups with an estimated 150 million cases occurring globally per year. Resistance to antibiotics is highly prevalent in bacterial isolates causing UTI. **Objectives**: To observe the isolation of gram positive bacteria causing UTIs and determine their resistance pattern to antibiotics. **Methods:** A retrospective study was conducted in BPKIHS from August 2009- August 2010. A total of 11022 urine samples from patients who visited BPKIHS were analyzed. All specimens were inoculated on routine culture media. Bacterial isolates were identified by conventional bacteriological methods. Susceptibility testing was performed by standard methods as recommended by clinical laboratory standard institute. **Results:** A total of 459 gram positive uropathogens were isolated. Altogether 5 different gram positive bacteria were isolated among which Staphylococcus aureus (47%) was the most predominant organism followed by Enterococcus species (34%), Enterococcus faecalis (18%), and Coagulase-negative Staphylococci (1%). UTI caused by gram positive uropathogens was seen in 68.8% females as compared to 31.2% males. Multidrug resistance (MDR) isolates accounted for 308 out of 459 isolates (67.10%). Multidrug resistance was commonest with Enterococcus spp (71.5%) followed by Streptococcus spp (66.6%). Drugs, which retained usefulness for Gram-positive isolates were vancomycin, nitrofurantoin, ciprofloxacin and norfloxacin. **Conclusion**: The study revealed that bacterial resistance in gram positive uropathogens in tertiary hospital in eastern region continues to be a great problem. So, regular monitoring of emergence of resistance is highly recommended and specific antibiotics should be given only after the laboratory results are available.

Keywords: BPKIHS, Urinary tract infection, Multidrug-resistance.

Introduction

Urine located within the urinary tract, excluding the distal region of the urethra is considered sterile in healthy individuals, as indicated by the absence of cultivable bacterial cells. UTI describes a condition in which there are micro-organisms established and multiplying within the urinary tract. On the basis of the work done by Kass, 10^5 colony forming units of a single species per milliliters in a clean catch midstream sample of urine is considered as significant bacteriuria.[1] While this threshold still holds in asymptomatic patients, in many cases a lower threshold is considered significant for symptomatic patients.

Urinary tract infections are one of the most common types of bacterial infections in humans occurring both in the community and the health care settings and ranks high amongst the most common reasons that

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compel an individual to seek medical attention[2]. UTIs encompass a spectrum of clinical entities ranging in severity from asymptomatic infection to acute cystitis, prostatitis, pyelonephritis and urethritis. It represents one of the most common diseases encountered in medical practice today, affecting people of all ages, from the neonate to the geriatric age group[3]. Most infections are caused by retrograde ascent of bacteria from the faecal flora via the urethra to the bladder and kidney especially in the females who have a shorter and wider urethra and are more readily transversed by microorganisms. There are urinary pathogen virulence factors that promote adherence to mucosal surfaces and subsequent infections.[4] Host factors such as the epithelial cell receptivity are also important in the infection process. Although fungi and viruses are occasional etiological agents, UTIs are predominantly caused by bacteria. The most common bacteria implicated as causative agents of UTI generally originate in the intestine and include but not limited to Escherichia coli, Pseudomonas spp, Streptococcus spp, Proteus spp, Klebsiella spp, Staphylococcus spp, Enterococcus spp, Neisseria gonorrhoeae, Chlamydia trachomatis, Candida spp, Mycoplasma spp. Extremes of age, female gender, pregnancy, instrumentation, urinary tract infection, neurologic dysfunction, renal disease, and expression of A, B and H blood group oligosaccharides on the surface of epithelial cells are predisposing factors for the development of UTIs.[5]

One woman in five develops a UTI during her lifetime; UTI in men are less common. Nearly 20% of women who have a UTI will have another and 30% of those will have yet another episode of UTI. Worldwide, about 150 million people are diagnosed with UTI each year, costing the global economy in excess of six billion US dollars. UTIs occur at the rate of 2 - 3% of hospital admission and account for 35 - 40% of all nosocomial infections.[6]

UTI is a common disease among Nepalese population as well as one of the commonest nosocomial infections. According to the annual report published by Department of Health Services (2059/60), morbidity of UTI in Nepal was 1, 25,058. Today, antimicrobial drugs remain the front line therapy for conquering bacterial infection.[7] For the successful treatment, culture and sensitivity test is essential which is lacking in many parts of Nepal.

Studies aimed at gaining knowledge about the type of pathogens responsible for UTIs and their susceptibility patterns may help the clinicians to choose the right empirical treatment. In the last three decades, there have been a lot of reports in the scientific literature on the inappropriate use of antimicrobial agents and the spread of bacterial resistance among microorganisms causing urinary tract infections. Knowledge of etiological agents of UTIs and their sensitivities to available drugs is of immense value to the rational selection and use of antimicrobial agents and to the development of appropriate prescribing policies.[8] It is hoped that the results will provide useful information which would be used in the formulation of policies for the rational and effective use of antimicrobial agents.

Methods

This is a retrospective study of one year (August 009 – August 010) in the midstream urine specimens from outpatient and inpatient of our hospital. A total of 11022 samples were processed. There were 7197 females and 3825 males in the study. The urine specimens were processed within one hour of collection. Bacterial culture was done by semi-quantitative method using calibrated loop delivering 0.001 ml of urine on McConkey agar, Blood agar, and cysteine lactose electrolyte deficient agar (CLED) following incubation at 37°C overnight and the observation was made the next day. Unlike Gram negative bacilli that needs more than 100 colonies corresponding to 10^5 cfu/ml to be significant, Gram positive cocci were considered as significant irrespective of the colony count.[9] The Gram positive bacterial isolates were identified using standard bacteriological tests.[10] Antimicrobial susceptibility pattern was studied on Mueller Hinton Agar (MHA) or MHA + 5% sheep blood by Bauer Kirby disc diffusion method recommended by CLSI guidelines.[11] For Gram positive isolates antibiotic discs put up were amikacin (30 ì g), ampicillin (30 ì g), nalidixic acid (30 ì g), norfloxacin (300ìg), penicillin (10 IU), cefotaxime (30 ì g), ciprofloxacin (30 ì g), co-trimoxazole (25 ì g), gentamicin (120 ì g), nitrofurantoin (100 ì g) and vancomycin (30 ì g).

Results

Of the total 11022 urine specimens analyzed for the study; 8448 (76.66%) revealed culture negative whereas 2574 (23.34%) showed significant bacteriuria among which 2115 (19.18%) revealed GNB bacteriuria and 459 (4.16%) revealed GPC bacteriuria (Table 1). Among the patients with significant bacteriuria by Gram positive bacteria, 316 (68.8%) were female and remaining 143 (31.2%) were males.

Table 1: Patterns of Culture results

Gram positive organisms	Gram negative organisms	No Growth	Total
459(4.16%)	2115(19.18%)	8448(76.64%)	11022

As shown in Table 2, altogether 5 different gram positive bacteria were isolated among which Staphylococcus aureus (47%) was the most predominant organism followed by Enterococcus species (34%), Enterococcus faecalis (18%) and Coagulase-negative Staphylococci (1%).

Table 2: Distribution of gram positive bacterial isolates

Organism	Percentage
Staphylococcus aureus	47%
Enterococcus Spp	34%
Enterococcus faecalis	18%
CONS(Coagulase negative Staphylococci)	1%

The antibiotic susceptibility profile showed that most of urinary bacterial isolates were resistant to almost all the common antibiotics tested. Among the common antibiotics tested against all Gram positive bacteria, the most effective antibiotic was found to be nitrofurantoin (83.45%) followed by ciprofloxacin (74.95%) and gentamycin (68.13%). Most of the Gram positive bacteria were resistant to Penicillin (94.33%) and nalidixic acid (91.06%). Among the 5 different Gram positive bacteria, 100 percent of the isolates were susceptible to vancomycin. The percentage resistance of Gram positive bacterial isolates to each antibiotic tested was as shown in Table 3.

Table 3: Antimicrobial drugs susceptibility profile of Gram positive bacteria

Antimicrobial agents	Various Gram positive isolates				
	CONS (n =6)	*E. feacalis* (n = 83)	*Enterococcus. Spp.*(n = 156)	*S.aureus* (n = 214)	Total (459)
Amikacin (30 ìg)	0	80.72%	74.35%	7.94%	41.17%
Ampicillin (10 ìg)	83.30%	89.15%	79.48%	55.14%	67.53%
Cefotaxime (30 ìg)	100%	38.55%	77.77%	45.79%	56.42%
Ciprofloxacin (30 ìg)	0	40.96%	28.20%	15.88%	25.05%
Co-trimoxazole (25 ìg)	16.66%	93.97%	87.17%	71.96%	80.39%
Gentamicin (120 ìg)	33.33%	66.26%	71.79%	14.95%	31.87%
Nalidixicacid (30 ìg)	100%	98.80%	94.87%	86.44%	91.06%
Nitrofurantoin(100 ìg)	0	32.53%	23.07%	7.47%	16.55%
Norfloxacin (300 ìg)	50.00%	78.31%	82.05%	43.45%	59.69%
Penicillin (10 IU)	50.00%	96.40%	98.29%	93.92%	94.33%
Vancomycin (30 ìg)	0	0	0	0	0

Multidrug resistance (MDR) isolates accounted for 308 out of 459 (67.10%) isolates which was commonest with Enterococcus spp (71.5%) followed by Streptococcus spp (66.6%) (Table 4).

Table 4: Resistance pattern and distribution of MDR gram positive bacterial isolates

Gram positive bacterial isolates	Total no. of isolates	Sensitive to all	Resistant to 1 drug	Resistant to 2 drugs	Resistant to ≥3 MDR Strains	drugs %
CONS	6	0	2	0	4	66.66%
E. feacalis	83	0	6	18	59	71.08%
Enterococcus spp.	156	5	9	39	103	66.02%
S. aureus	214	9	13	51	141	65.88%
Total	459	14	30	108	307	67.00%

Discussion

This study shows the pattern of UTI prevalent according to sex, organisms affecting and the antimicrobial drugs susceptibility pattern of Gram positive bacterial isolates isolated from patients with UTI in Microbiology Department of B.P Koirala Institute of Health Sciences, Dharan, Nepal. Out of 11022 urine samples, 2574 (23.34%) showed the growth of significant bacteriuria. Several other studies from the country also observed such a low rate of growth positivity for UT.[12,13] The possible cause of low rate of growth positivity might be due to urine samples obtained from patients under treatment, infection due to slow growing organisms or due to those organisms that were not able to grow on the routine media we used. Our finding was also lower than those reported by other investigators in the country. Study carried out in TUTH has shown bacterial growth in 44.5% and 42.8% of outpatients and inpatients respectively.[14] However, very low growth rate (4.6%) has been reported from elsewhere.[15]

Mostly, UTI is originated from colonic bacteria, which comprise mainly Gram-negative bacteria. In our study, cases of significant bacteriuria caused by Gram positive bacteria were found to be 17.83% (459/2574). Among the patients with significant bacteriuria by Gram positive bacteria, 316 (68.8%) were female and remaining 143 (31.2%) were males. Similar type of findings was reported in a study done by Shrestha et al, 2007.[16] This higher growth positivity seen in females was found to be statistically significant (pÂ0.05) and may be attributed to their anatomical structure (short urethra and proximity to anal orrifica). This was in agreement with the findings of other investigators from Nepa[17, 18] and elsewhere.[19]

Among gram-positive bacteria, S. aureus was the most common. In our study, the incident was found to be 47% of the total Gram positive bacterial isolates. Isolation of S. aureus from the urine should around suspicion of bacteremic infection of the kidney acquired by haematogeous spread so a pure culture of S. aureus is considered to be significant regardless of the number of colony forming unit. In this study, significant bacteriuria caused by Enterococcus species was found to be 34%. Quite contrast results have been reported in India (8%) [20] and Iran (10.78%).[21] Enterococcus faecalis accounted for 18% of total gram positive bacterial isolates. Similar isolation rate for E. faecalis was reported in Nigeria. UTI due to Enterococcus faecalis is usually associated with the use of instruments or catheterization.[22] Our study revealed the isolation of Coagulase-negative Staphylococci to be 1%. Isolation of 2.8% for S. saprophyticus was reported in a study from Nigeria, 1.07% for S. epidermidis from Iran[21] and 2.3% from India.[23]

In our study, vancomycin was found to be the most effective drug against gram positive bacteria. However, nitrofurantoin against gram positive bacteria was found to be 83.45% sensitive which is similar various studies done previously.[24, 25, 16] Nitrofurantoin should be considered as drug of choice for acute, uncomplicated UTI particularly in view that it continues to show low in resistance.[26] On the other hand, penicillin and nalidixic acid used in routine test were found to be least effective drugs against gram positive bacteria (94.33% and 91.06% resistant respectively. Resistance to penicillins may be determined by the organisms due to the production of penicillin destroying enzymes such as beta-lactamase.[27] It was found to be 74.95% of gram positive bacteria were sensitive to ciprofloxacin and 68.13% to gentamycin. Gram positive bacteria accounted for 308 out of 459 (67.10%) were found

to be multi drug resistant. In a similar type of study, 45% MDR bacterial strains were detected.[16]

Higher resistance rates to all antibiotics tested except vancomycin and nitrofurantoin may be explained due to high and uncontrolled consumption of these antibiotics during the past decade. These antibiotics were prescribed not only for UTI, but also for infections in other body sites. Various previous reports have indicated that the high resistance of uropathogenic bacteria to antimicrobial agents in developing countries is often due to self-medication, the sub-optimal quality of antimicrobial drugs and poor community and patient hygiene.[28] This widespread and inappropriate use of antibiotics is recognized as a significant contributing factor to the spread of bacterial resistance and the development of resistance to antimicrobial agents.[29]

Conclusion

The study revealed that the problem of the bacterial resistance to antimicrobial drugs is more troublesome to developing countries like Nepal. So, regular monitoring of emergence of resistance is highly recommended and specific antibiotics should be given only after the laboratory results are available. There should be definite hospital antimicrobial drug policy in order to prevent emergence of multi drug resistance organisms and the study should be continued for detection of MDR strains, ESBL producing strains and further studied up to genetic level. In addition, a regular feedback and antibiogram should be given to the clinicians for effective management of UTI.

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