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### ANTIBIOGRAM OF BACTERIAL ISOLATES FROM PATIENTS OF UTI IN B AND C TEACHING HOSPITAL

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#### **ABSTRACT**

The main objective of this study was to investigate the bacterial infections of urinary tract in patients with urolithiasis. The patients were referred to and/or visiting the Urology Department in B & C Teaching Hospital in Birtamode city. The present study was carried out from March 2017 until November 2017. One hundred and sixty patients with urolithiasis were included in the present study whose ages ranged from 12 to 70 years. Male to female ratio was almost 2:1. The control group consisted of two groups; group one consisted of 40 persons (20 males and 20 females) who were selected randomly from Biratmod city during the period of this study and they had no previous personal nor family history of urolithiasis, while group two consist of 40 persons (20 males and 20 females) infected with urinary tract infection. Urine culture was done for all the patients and the control group. 45% (45/160) of these had UTI's. Six patients revealed mixed bacterial growth. 84% (38/45) of patients with UTI were infected with gram negative organisms whereas only 16% (7/45) of patients were infected with gram positive organisms. The majority of the gram negative bacteria isolated belonged to the family Enterobacteriaceae particularly E. coli as it was isolated from 14 (31%) cases. The urease producing bacteria were isolated from 31 cases (50%). UTI's in urolithiasis was more frequent in females than males and the percentages were 28/45 and 17/45 respectively. The most effective antimicrobial agent was amikacin and the least effective one was ampicillin, whereas ciprofloxacin, cefoxitin, levofloxacin, nitrofurantoin, nalidixic acid, chloramphenicol, amoxycillin and gentamicin were effective at different levels.

**KEYWORDS:** Antibiotic Resistance, UTI, Urolithiasis.

#### INTRODUCTION

Antibiotic resistance is a specific type of drug resistance when a microorganism has the ability of withstanding the effects of antibiotics. Urinary Tract Infections (UTIs) are one of the most prevalent extra-intestinal bacterial infections. Nowadays, it represents one of the most common diseases encountered in medical practice affecting people of all ages from the neonate to the geriatric age group.<sup>[1]</sup> Worldwide, about 150 million people are diagnosed with UTI each year. [2] 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 transferred by microorganisms.[3] The structure of the females urethra and vagina makes it susceptible to trauma during sexual intercourse as well as bacteria being massaged up the urethra and into the bladder during pregnancy and/or child birth. [4,5] Majority of UTIs are not life threatening and do not cause any irreversible damage. However, when the kidneys are involved, there is a risk of irreparable tissue damage with an increased risk of bacteremia. [6] The emergence of antibiotic resistance in the management of UTIs is a serious public health issue, particularly in the developing world where apart from high level of poverty, ignorance and poor hygienic practices. 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.

#### MATERIALS AND METHODS

#### **Source of Specimens**

This study was conducted in the Urology Department in B & C Teaching Hospital as a part of research programs. The number of the patients was 160 and they were recruited from March 2017 until November 2017. All have urolithiasis including 104 males and 54 females. Patients aged between 12 to 70 years. The control group composed of 80 subjects who were looked healthy and had comparable criteria to the patients and composed of 40 males and 40 females.

#### **Laboratory Methods**

Culture of Urine Specimens Media were prepared and sterilized according to the manufacturer's instruction. The prepared media used for isolation, determination of the viable count, identification and susceptibility testing were carried out after being solidified.<sup>[7]</sup>

## **Preparation of the Culture Medium for Antibiotic Sensitivity Testing**

AST of all isolates was performed on Mueller-Hinton medium by the Kirby Bauer method (1996) following the definition of the National Committee of Clinical Laboratory Standard (NCCLS, 1999). The medium was allowed to cool at 45°C and poured into Petri dishes to about 4 mm thickness of medium. The solidified plates were incubated at 37°C for 15 - 30 minutes to let the excess moisture to evaporate (Fisher scientific, USA). [7]

#### **Inoculation and Incubation**

The plates were inoculated by dipping a sterile swab into the inoculum, the excess inoculum was removed by pressing and rotating the swab firmly against the side wall of the tube above the level of fluid, then the swab was rubbed all over the surface of the medium, rotating the plate 3 times at an angle of 60 degree after each application and finally the swab passed around the edge of agar surface. The plate was left to dry at room temperature with the lid closed for few minutes. After 15 minutes of inoculation, the antibiotic discs were applied and the plates were inverted for incubation to avoid accumulation of moisture on the agar surface. [8] Maximum 5 antibiotic discs were selected and placed onto each plate using flamed forceps for application of the discs on the plate and each disc pressed down gently to ensure even contact with the medium. After overnight incubation at 37°C the diameter of each zone including the diameter of zone inhibition was measured and recorded in mm and compared with the standard inhibition zone. For motile organisms, e.g. Proteus spp. the swarming haze was ignored and zones were measured at the point where growth was obviously inhibited.[8]

#### **Statistical Analysis**

Statistical analyses in the present study were done by using Microsoft Office Excel 2007, SPSS version 12 (Statistical Package for Social Sciences).

#### **RESULTS**

Urine culture had been done for all the 160 patients who were included in this study. Only 45 patients had positive urine culture. 28 of them were females and 17 were males. The most common organism was E. coli which was isolated from 14 patients with percentage of 31.1%. E. coli was the most prevalent followed by Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae, Staphylococcus Saprophyticus and Serratia marcescenes and the frequencies of isolation were 22.2%, 15.5%, 11.1%, 7.4%, 13.3%, 4.4% and 2.2% respectively). The

antibiotic sensitivity test was carried out using disc diffusion technique for all the bacterial isolates to the most commonly antibiotic agents that used in the treatment of UTI. It was found that different bacterial pathogens were highly sensitive to ciprofloxacin and most of them were also highly sensitive to nitrofurantoin, nalidixic acid and cefotaxin. The present study also shows that the most resistant bacteria were Pseudomonas aeruginosa and almost 80% of its isolates were still sensitive to amikacin and ciprofloxacin. Almost all isolates (100%) of Escherichia coli were highly sensitive to levofluxacin, cefoxitin and chloromphenicol. E. coli isolates were resistant to nalidixic acid and almost 3% only of them were sensitive to nalidixic acid. Most of the pathogens isolated were moderately sensitive to gentamicin. Staph. aureus isolates were highly resistant to ampicillin and amoxycillin.

Table 1: Total Sensitivity testing of overall antibiotics used in this test.

Name of Antibiotic	Sensitivity percentage
Ampicillin	3.5
Amoxicillin	9.2
Amikacin	95.7
Gentamicin	53.5
Ciprofloxacin	80.7
Nalidixic acid	36.4
Levofloxacin	57.8
Cefoxitin	57.8
Chloramphenicol	65
Nitrofurantoin	55

Table 2: Percentage of isolated bacteria from Urine culture of patients.

Bacterial Isolate	Percentage
Escherichia coli	31.1
Proteus mirabilis	22.2
Pseudomonas aeruginosa	15.5
Klebsiella pneumonia	13.3
Staphyloccocus aureus	11.1
Staphylococcus saprophyticus	4.4
Serratia marcescens	2.2

#### DISCUSSION

Urine culture for all 160 patients was made, which was positive for 28% patients. In contrast, the present results were almost different from those reported by Al-Jebouri<sup>[11]</sup> and Al-Jebouri and hasen.<sup>[12]</sup> However, E. coli, P. mirabilis and P.aeruginosa were the most prevalent organisms and K.pneumoniae, Staphylococcus species and S.marcescens were the least causing UTI among patients studied (Table 2). E. coli as a commonest cause of UTI may be due to certain virulence factors like hemolysin production and presence of fimbriae. Moreover, Serratia marcescens was rarely isolated 2.2% from the UTI cases investigated here and almost the same pattern of isolation was reported by Manikandan et

al. in India. [13] The present study revealed other organisms to be associated with Nepalin patients who suffered from UTI and these organisms were reported previously by Al-Jebourians Hasen. [12] The most organisms caused UTI in this study were belonging to gram negative bacteria which were isolated from 84.4% patients. These results were almost similar to those of Al-Jebouri, [11] Al-Rawi [14] and Navaneeth et al. [15] It was shown that 4.3% of the patients with infection stones were having UTI while only 24% of patients with noninfection stone were having UTI as was proven by urine culture. Statistically, there was no significant difference in distribution of positive urine culture between infection and noninfection stones. These results were almost comparable with those found by Al-Jebouri.[11] The prevalence of UTI was higher among females than male patients (in a ratio of 6:1) and this was almost similar to that of Al-Nass et al. [16] Women are more prone to have UTI than men because in females, the urethra is much shorter and closer to the anus than in males, and they lack the bacteriostatic properties of prostatic secretions.<sup>[17]</sup> The overall percentages of resistance of all isolates to the antimicrobial agents were 66.5 to ampicillin, 90.8 to amoxycillin, 4.3 to amikacin, 46.5 to gentamicin, 19.3 to ciprofloxacin, 63.6 to nalidixic acid, 42.8 levofloxacin, 42.2 to cefoxitin, 35 to chloromphenicol and 55 to nitrofurantoin. (Table 1) The present study showed a high elevation in antibiotic resistance of pathogens isolated from the Nepalese patients compared to previous years.<sup>[18]</sup> This might be due to misuse of antibiotics, usage of antibiotics from unknown origin, i.e. from uncontrol source of production, utilizing of inactivated antimicrobials, selective pressure of antibiotics and lacking of quality control on some sources of antibiotics entering Nepal especially by the private sector. However, the present study showed that almost all types of pathogens causing UTI were resistant to ampicillin and most of them were highly resistant to amoxycillin. Moreover, the study conducted by Manikandan et al.<sup>[13]</sup> revealed that almost 60% of the pathogens causing UTI were resistant to amoxycillin. In contrast, Akortha and Ibadin found that most of their isolates were sensitive to nalidixic acid. [19] However, a significant increase in resistance of pathogenic strains to SXT, ampicillin and cephalothin has been found worldwide<sup>[20]</sup> but certain agents like gentamicin and nitrofurantoin still show a moderate efficacy against UTI pathogens because of its multiple mechanisms of action seem to have enabled it to retain potent activity against pathogens.<sup>[21]</sup> Furthermore, E. coli, Proteus mirabilis, Ps. aeruginosa, S. aureus, K. pneumoniae and S. marcesens were still highly sensitive to amikacin, ciprofloxacin and chloromphenicol. However, chloromphenicol is not preferred to be commonly used for medication as it might because aplastic anemia. Furthermore, the most common UTI pathogens and highly resistant to antibiotics emphasize the need for judicious use of antibiotics.

#### CONCLUSION

The susceptibility and resistance profile of all isolates in the current study have shown that ampicillin and amoxycillin possess the lower efficacy while amikacin and ciprofloxacin possess the higher efficacy. There was a general increase in the resistance pattern of isolates to all the antibiotics used in this study. The current findings confirm that bacterial resistance would be the greatest and frightening problem in our country. A new generation of antibiotics is still locally working in a higher rate. The usage of antibiotics in our locality of the world needs to be under health assessment and control with more laboratory investigations to explore the intrinsic and extrinsic parameters led to conclude high rate of resistance occurred in the local pathogens that might be disseminated to other geographical areas.

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