

A Study of Multidrug resistance bacteria from UTI patients

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Abstract -The present study aimed to ascertain the current situation of antimicrobial resistance of Urinary Tract Infections (UTIs) caused by human pathogens. Twenty midstream urine samples collected from adult patients were analyzed for Multidrug Resistant (MDR) strain isolation and identified. The result was clear that *E.coli* was the predominant pathogen (31.5%) causing UTI, followed by *Staphylococcus aureus* (20.5%), *Klebsiella pneumonia* (15.8%), *Proteus sp.* (7.4%) and *Pseudomonas aeruginosa* (7.5%). The percentages of resistance of all isolates to the antimicrobial agents were: 83.3% to SXT, 80.6% to Nalidixic acid, 67.3% to Amoxycillin, 61% to Cotrimoxazole, 48.8% to Gentamycin, 46% to ciprofloxacin and 43% to Cephalixin. Isolated UTI strains were tested for susceptibility against antibiotics, few of the antibiotics were sensitive but most of antibiotics showed resistant to the MDR strains. Among this, *E.coli*, *K.Pneumoniae* and *P.aeruginosa* were highly resistant to most of the antibiotics, whereas *Staphylococcus Sp.* and *Serratia marcescens* exhibited sensitivity to Cephalixin, Ciprofloxacin and Gentamycin. The present study was evaluated indicating the emergence of multidrug resistant (MDR) among UTI bacterial pathogens.

Key words: Urinary tract infections, multidrug resistant, *Staphylococcus aureus*, ciprofloxacin, nalidixic acid

INTRODUCTION

Urinary Tract Infections (UTIs) are one of the most prevalent extra intestinal bacterial infections. Now-a-days, 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 (Kunin, 1994). Worldwide, about 150 million people are diagnosed with UTI each year (Gupta *et al.*, 2001). 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 (Inabo and Obanibi, 2006). The structure of the females urethra and vagina makes it susceptible to trauma during sexual intercourse as well as bacteria been massaged up the urethra and into the bladder during pregnancy and or child birth (El-Sweih *et al.*, 2008; Kolawole *et al.*, 2009). 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 (Hvidberg *et al.*, 2000).

Now-a-days, drug resistance is a huge growing problem in treating infectious diseases like malaria, Tuberculosis (TB), diarrheal diseases, Urinary Tract Infections (UTIs) etc. As suggested by Goldman and Huskins (1997), the improper and uncontrolled use of many antibiotics resulted in the occurrence of antimicrobial resistance which became a major health problem worldwide. In the past decade, many kinds of resistant strains have been discovered. For example, Methicillin Resistant *Staphylococcus Aureus* (MRSA) (Wagenlehner and Naber, 2004), multidrug resistant *Pseudomonas aeruginosa* (Linuma, 2007) and *Serratia marcescens* (Kim *et al.*, 2006), Vancomycin Resistant *Enterococci* (VRE) (Eliopoulos and Gold, 2001) and Extended Spectrum Beta Lactamase (ESBL) resistant *enterococci* (Bhattacharya, 2006).

Drug resistance of pathogens is serious medical problems because of very fast arise and spread of mutant strains that are insusceptible to medical treatment. Microorganisms use varied mechanisms to acquire drug resistance viz. horizontal gene transfer (plasmids, transposons and bacteriophages), recombination of foreign DNA in bacterial chromosome and mutations in different chromosomal locus (Klemm *et al.*, 2006).

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 (Tenover and McGowan, 1996; Hryniewicz *et al.*, 2001; Kurutepe *et al.*, 2005). The changing patterns in the etiological agents of urinary tract pathogens and their sensitivities to commonly prescribed antibiotics are reported (Jacoby and Archer, 1991; Hryniewicz *et al.*, 2001; Kurutepe *et al.*, 2005; Mordi and Erah, 2006). 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, there is also high prevalence of fake and spurious drugs of questionable quality in circulation. 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

A total of 20 midstream urine samples were collected from the patients (aged 35 -60 years) having UTIs. Each sample was inoculated on both blood agar (with 5% sheep blood) and MacConkey agar plates and incubated at 37°C for 24-48 h and a total 98 number of colonies was counted. Significant growth was identified biochemically and serologically in a systematic way

according to standard methods (Vandepitte *et al.*,1996). Susceptibilities of the common isolated bacteria (*E.coli*, *Enterococcus faecalis*, *Klebsiella pneumonia*, *Serratia marcescens*, *Pseudomonas aeruginosa*, *S. saprophyticus*, *Staphylococcus aureus* and *Proteus sp.*) to certain antimicrobial agents causing UTI were examined.

Antimicrobial sensitivity testing of all isolates was performed on diagnostic sensitivity test plates by the Bauer *et al.* (1966), following the definition of the National Committee of Clinical Laboratory Standards (NCCLS, 1999). Bacterial inoculum was prepared by suspending the freshly-grown bacteria in 25mL sterile nutrient broth. A sterile cotton swab was used to streak the surface of Mueller Hinton agar plates. Filter paper disks containing designated amounts of the antimicrobial drugs obtained from commercial supply firms (Himedia Labs, Mumbai, India) were used. The antimicrobial agents tested were Amoxicillin 10µg, Cephalexin 30 µg, Gentamycin 10 µg, Nalidixic acid 30 µg, Ciprofloxacin 10 µg, Cotrimoxazole 10 µg and Trimethoprim-sulfamethoxazole (SXT) 30 µg.

RESULTS AND DISCUSSION

A summary of the different microorganisms isolated during the study period was shown in Table 1. It is clear that *E. coli* was the predominant uropathogen (31.5%) causing UTI, followed by *Staphylococcus aureus* (20.5%), *Klebsiella pneumonia* (15.8%), *Proteus sp.* (7.4%) and *Pseudomonas aeruginosa* (7.5%). However, *Enterococcus faecalis*, *Staphylococcus saprophyticus* and *Serratia marcescens* were the least dominant uropathogen causing UTI strains. The findings of this study were compared favorably to Akortha and Ibadin (2008) who found that *S.aureus* strains were sensitive and highly resistant to naladixic acid (79.3%). Shittu and Mandara (1999) reported that *S. aureus* as 100% sensitive to gentamycin and cephalosporin. According to this result, major isolates in UTI were *E. coli*, followed by *S. aureus*, *S. saprophyticus*, *Proteus sp.* and *E. faecalis*. These observations were supported by several studies conducted previously. According to Goswami *et al.* (2001). *E. coli* is the most common organism (64.3%), followed by *S.aureus* (21.4%) and *Klebsiellapneumoniae* (14.3%).

The percentages of resistance of all isolates to the antimicrobial agents were: 83.3% to SXT, 80.6% to Nalidixic acid, 67.3% to Amoxicillin, 61% to Cotrimoxazole, 48.8% to Gentamycin, 46% to ciprofloxacin and 43% to cephalixin. The percentages of pathogens resistance varied between 83.3 and 43% to the antimicrobial agents, while in susceptible of the pathogens varied between 56.3 and 16.7% (Table 2). The present study evaluated the prevalence of microorganisms implicated in UTI to ascertain their antimicrobial resistance patterns. In this study, the isolates demonstrating, extremely high percentage of MDR phenotype. A significant increase in resistance of pathogenic strains to SXT, Ampicillin and Cephalothin has been found worldwide (Hooton, 2003) but older agents like Gentamicin that still show high efficacy against UTI pathogens because of its multiple mechanisms of action seem to have enabled it to retain potent activity against

pathogens. The overall rate of resistance to SXT was significant and higher than those reported by Valdivieso *et al.* (1999) and Zhanel *et al.* (2000). For the past decades, SXT or trimethoprim alone has been used widely as an empirical therapy for urinary tract infections caused by *E. coli*. The results of this study indicate that a ciprofloxacin resistant phenotype without concurrent resistance to other antimicrobials was higher than previous reported studies (Sahm *et al.*, 2001a). 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 (Sahm *et al.*, 2001b).

Table 1: Percentage of UTI isolate among the pathogens

Isolates	Percentage
<i>E.coli</i>	31.50
<i>Enterococcus faecalis</i>	5.84
<i>Klebsiella pneumonia</i>	15.86
<i>Proteus sp.</i>	7.47
<i>Pseudomonas aeruginosa</i>	7.64
<i>Staphylococcus saprophyticus</i>	4.15
<i>Staphylococcus aureus</i>	20.50
<i>Serratia marocaena</i>	6.74
Others	0.36

Table 2: Percentage of resistance to the antimicrobial agents among 98 UTIs isolates

Antimicrobial agent	Percentage of Isolates		
	Susceptible	Intermediate	Resistant
<i>Amoxycillin</i>	32.3	0.4	67.3
<i>Cephalexin</i>	56.3	0.7	43.0
<i>Gentamycin</i>	50.9	0.3	48.8
<i>Nalidixic acid</i>	19.4	0.0	80.6
<i>Ciprofloxacin</i>	53.6	0.4	46.0
<i>Cotrimoxazole</i>	38.6	0.4	61.0
SXT	16.7	0.0	83.3

Present study shows that resistance to ciprofloxacin continues to increase. If urgent measures would not be taken to arrest the situation, we may see the return of the era of the search for new drugs to fight bacterial infections.

In this study, we found multidrug resistance strains which are resistant to most of the antimicrobials agent tested. This reflected the fact that ampicillin, tetracycline and streptomycin were the most commonly prescribed antibiotics in the hospital even before the results of urine analyses and also the most easily available in the market without prescription and because they were also very cheap in terms of cost. The widespread use and more often the misuse of antimicrobial drugs has led to a general rise in the emergence of resistant bacteria. Higher resistant strains were reported in USA to ampicillin and cotrimoxazole (Sahm *et al.*, 2001b), whereas few ciprofloxacin resistant strains were found in other countries (Diekema *et al.*, 2004). This study also noticed ciprofloxacin-resistant *E. coli* from UTIs. Ciprofloxacin as an option for therapy to UTIs has been considered, since its multiple mechanisms of

action seem to have enabled it to retain potent activity against *E.Coli*. Ciprofloxacin has high level of activity against UTT isolates of *E.Coli* compared with other commonly used agents, such as Ampicillin and SXT (Gupta *et al.*, 1999).

Table - 3 gives the multi drug resistance profile of various isolates to the routinely used antibiotics. Isolated UTI strains were tested for susceptibility against antibiotics, few of the antibiotics were sensitive but most of antibiotics showed resistant to the MDR strains. Among this *E.coli*, *K.pneumoniae* and *P.aeruginosa* were highly resistant to most of the antibiotics, whereas *Staphylococcus* sp. and *Serratia marcescens* exhibited sensitive to Cephalexin, Cotrimoxazole and Gentamycin. Moreover, most the UTI strains were highly resistant to nalidixic acid and SXT. Drug resistance is one of nature's never ending process by which the organisms develop tolerance to new environmental condition. It may be due to a pre-existing factor in the organisms or result from theacquired factor(s). Rella and Haas (1982) first reported that a nalidixic acid resistant *P.aeruginosa* of UTI showed resistance to β -lactam antibiotics. The finding of this study coincides with Shittu and Mandere (1999) that *S. aureus*strains were highly resistant to nalidixic acid. All the isolates in this study showed resistance to at least 5 different antibiotics, indicating the presence of strong selective pressures from the antibiotics in the community. Brown *et al.* (2003) have reported that horizontal gene transfer is a factor in the occurrence of antibiotic resistance in clinical isolates and suggested that the high prevalence of resistance to a particular antibiotic does not always reflect antibiotic consumption as previously suggested by Nwanze *et al.* (2007).

Table 3: Antimicrobial susceptibility pattern for MDR- UTI isolates

Isolates	Antimicrobial agent						
	AMX	CF	GM	NA	CIP	CO	SXT
<i>E. coli</i>	R	R	S	R	S	R	R
<i>Enterococcus Faecalis</i>	S	R	S	R	S	S	R
<i>Klebsiella pneumonia</i>	R	S	S	R	S	R	R
<i>Proteus sp..</i>	S	S	R	R	R	S	R
<i>Pseudomonas aeruginosa</i>	R	R	S	R	S	R	R
<i>S. saprophyticus</i>	S	S	S	R	R	S	R
<i>Staphylococcus aureus</i>	R	S	R	R	S	S	R
<i>Serratia marcescens</i>	S	S	S	R	S	S	R

AMX = Amoxycillin; CF = Cephalexin; GM = Gentamycin; NA = Nalidixic acid; CIP = Ciprofloxacin; CO = Cotrimoxazole; SXT = Trimethoprim-sulfamethoxazole; R = Resistant; S = Sensitive

According to Mandal *et al.* (2001),*E.coli* as the commonest cause of UTI and antibiotic resistance was high among the strains which emphasize the need for judicious use

of antibiotics. Certain virulence factors like haemolysin production and presence of fimbriae in the *E.coli* may be associated with urovirulence. Moreover, these differences in sensitivity pattern of the isolates could be attributed to time difference between the two studies or environmental factors such as practices of self medication, the drug abuse and indiscriminate misuse of antibiotics among the general population which has favoured the emergence of resistance strains.

CONCLUSION

The susceptibility and resistance profile of all isolates in this study have shown that Trimethoprim-sulfamethoxazole and Nalidixic acid possess the higher efficacy while Ciprofloxacin and Cephalexin possess lower efficacy. Despite this efficacy, there was a general increase in the resistance pattern of isolates to all the antibiotics used in this study. The present study confirms that bacterial resistance would be a greatest problem in the country but, still some bacteria are resistant to antibiotics especially Cephalexin and Ciprofloxacin, frequently used drugs in many parts of the world. These bacterial pathogens may be treated with plant materials to show better results against multidrug resistant (MDR) pathogens.

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