TRENDS IN ANTIMICROBIAL SUSCEPTIBILITY OF BACTERIAL ISOLATES FROM HOSPITALIZED PATIENTS WITH URINARY TRACT INFECTION

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ABSTRACT: Urinary Tract Infection (UTI) is one of the most common infectious diseases, ranking next to upper respiratory tract infection in their incidence and a foremost cause of morbidity and mortality in humans. The study aimed to determine the antibiotic resistance patterns of the most common bacteria, isolated from nosocomial urinary tract infections (i.e., cystitis, urethritis, and pyelonephritis). A total of 428 urinary isolates from hospitalized patients were identified and antimicrobial susceptibility pattern to various clinically important antibiotics was determined using Kirby Bauer's disk diffusion method and interpreted according to CLSI guidelines. The gram-negative and positive bacteria accounted for 75.5 % and 5.6% respectively, and the remaining 18.9 % were yeasts. The frequency of *Escherichia coli* was highest at 240 (56.1 %) followed by *Candida* 81 (18.9 %), *Klebsiella species* 51 (11.9 %), *Pseudomonas* 16 (3.7 %) *Enterococci* 16 (3.5 %), *Proteus* 9 (2.1 %), and *Coagulase-negative staphylococci* 5 (1.2 %). The *in vitro* susceptibility rate of gram-negative isolates was 90.1 % to imipenem, 80.5 % to Amikacin, and 73.7 % to Piperacillin/ tazobactam. Among gram-positive isolates, the rate of susceptibility was higher for Vancomycin (95.8 %), Teicoplanin (91.7 %), Nitrofurantoin (83.3%) and Imipenem (66.7 %). This high prevalence of antimicrobial resistance among urinary tract pathogens, particularly against amoxicillin/clavulanic acid, Cephalosporins, fluoroquinolones, and co-trimoxazole advocates thecareful use of antibiotic therapy. It is recommended that effective empirical antibiotic therapy should be based on local prevalence data for the disease-causing pathogen and their antibiotic susceptibilities rather than following universalguidelines.

KEY WORDS: Antimicrobial, Bacteria, Hospital, Clinical isolates

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INTRODUCTION

Hospital-acquired infections (HAIs) or nosocomial infections are the ones that are acquired during a hospital stay. These are usually defined as infections identified at least 48-72 hours after admission to health care institutions (1). HAIs are also an important public health problem worldwide (2). Among HAIs, the most frequent types are urinary tract infections, surgical wound infections, pneumonia, and bloodstream infections (BSI) (3, 4). UTI is broadly defined as the infection of the urinary system while symptomatic UTI requires the presence of significant bacteriuria with a quantitative count of 10^5 colony-forming units/mL of bacteria from urine specimens (5). The most frequent bacterial pathogen associated with UTI is *Escherichia coli*, followed by other *Enterobacteriaceae,* while gram-positive organisms like Staphylococcus aureus, Coagulase-Negative Staphylococci (CoNS), and enterococci are less frequent (6). The clinical significance of UTI is due to its high mortality rate, and chronic pyelonephritis that leads to chronic renal failure (7, 8). The severity of UTI depends on the susceptibility of the host and bacterial virulence. Children and old agepeople are more prone to develop UTIs. UTI is alsomore common in women than in males. Patients with genitourinary abnormalities are also at high risk for developing UTI (9, 10).

The extensive use of antibiotics is also playing an important role in shifting nosocomial

pathogens from easily treatable to more resistant ones. This change is a major problem for hospital infection control and prevention measures (11). The change in etiology and antibiotic resistance of urinary pathogens is now a major problem worldwide (12, 13). Several reasons associated with the emergence of antibiotic resistance include inappropriate antibiotic prescription, poor infection control strategies, and the use of antibiotics in animal and poultry feed. The constantly variable antibiotic sensitivity pattern among bacterial isolates, the determination of sensitivity pattern, and continued monitoring of antibiotic resistance are recommended.

The present study aimed to determine the antibiotic susceptibility pattern of the most common bacterial isolates from hospital-associated urinary tract infections over six months.

MATERIALS AND METHODS

Collection of Isolates: The urinary isolates were collected from hospitalized patients with clinical symptoms of UTI at the Pakistan Institute of Medical Sciences (PIMS), Islamabad, Pakistan from November 2013 to May 2014. The isolates were considered significant according if the bacterial count was equal to or more than 10^5 CFU/ ml of a single potential pathogen. Furthermore, only one isolate from one patient was considered. Cysteine Lactose Electrolyte Deficient (CLED) agar was used for the primary isolation and quantitation of microorganisms.

Identification: Gram staining and other routine biochemical tests were used to identify the bacteria. Furthermore, in addition to the conventional biochemical tests, Analytical Profile Index (API) 20E/NE strips were inoculated and recorded according to the instructions of the manufacturer.

Antimicrobial Susceptibility Testing: Different antibiotic discs i.e. Susceptibility testing was performed on Mueller Hinton agar (Oxoid, UK) by Kirby-Baur Method. The following antibiotic discs (Oxoid, UK) were used in the study: Amoxicillin, Amoxicillin/ clavulanic Acid, Piperacillin+ tazobactam, Ceftazidime, Ceftriaxone, Gentamicin, Amikacin, Imipenem, Nalidixic Acid, Ciprofloxacin, Levofloxacin, Nitrofurantoin, Co-trimoxazole were used for antimicrobial susceptibility testing for all isolates whereas Vancomycin, Teicoplanin, and Erythromycin were used for gram-positive isolates only. The susceptibility or resistance criteria were interpreted according to the Clinical Laboratory Standards Institute (CLSI) criteria.

Data Analysis: The data was analyzed by putting all the results in Microsoft Excel 2007. The percentage susceptibility and resistance werecalculated.

RESULTS

A total of 347 random bacterial isolates that were obtained from UTI patients were analyzed. Among these 139 (40.1%) were obtained from males and 208 (59.9%) were from females. The majority of UTI isolates were Gram-negative rods (93.1%) while Gram-positive cocci were 6.9%

E. coli (69.2%) and *Klebsiella* (14.7%) were the most common causative agent followed by *Pseudomonas* (4.6%), *Enterococcus* (4.3%), *Proteus* (2.6%), CoNS (1.4%), *Enterobacter* (1.4%), *S. aureus* (1.2&) and *Citrobacter* (0.9%). The gender-wise frequency of urinary isolates is shown in **Table 1**.

The antimicrobial pattern showed that imipenem (92.9%) and amikacin (82.5%) were the most effective against E. coli isolates. Vancomycin and Teicoplanin were found effective against most of the gram-positive isolates. The overall susceptibility pattern shows 88.5% and 77.4% of urinary isolates were sensitive to imipenem and

amikacin respectively whereas Nitrofurantoin and Piperacillin/ tazobactam were also

effective against these isolates. The overall susceptibility pattern of urinary tract pathogens is shown in **Table 2**.

DISCUSSION

In the present study, the most common uropathogenic found was *E. coli* (56%) followed by *Candida spp* (19%) and *Klebsiella* (12%).

A study in the Asia-Pacific region in 2009-2010 published similar findings and reported *E. coli* as the most common uropathogenic i.e. 56.5% while *Klebsiella*as 13.8% (14). Najmul et. al. 2013 also reported that gram-negative bacteria are more commonly uropathogenic as compared to gram-positive and the prevalence of *E. coli* is highest (43.3%) (15). The prevalence of UTI was morein females (57.7%) than in males (42.3%) which is similar to other reports from Pakistan. A study from Lahore, Pakistan reported the prevalence of UTI in females as 73% and 36% in males however the gender difference in the prevalence of UTI was less marked in our study. Further, the prevalence of *E. coli* was 66.97% followed by *Enterococci* (8.26%), *Candida,* and *Pseudomonas spp* (7.34%) (16).

Our study has shown comparatively less prevalence of gram-positive isolates (6%) than gram-negative (75.5%). Prevalence of gram-positive bacteria found in *Enterococcus* (4%),*CoNS* (1%) and *S. aureus* (1%). A study from Iran has reported the prevalence of *CoNS*(2.3%) and Aminoglycosides as the most effective drugs against gram-negative isolates (17). Another study from Iraq reported the prevalence of gram-negative isolates (84%) and gram- positive isolates (16%) (18). In our study,the second most common uropathogenic reported is *Candida* species (19%) followed by *Klebsiella* (12%). *Candida* is mostly isolated as a causative pathogen in diabetic patients and its prevalence is reported to be 8.3% in diabetic patients (19). A current study in China also has reported a high prevalence of *Candida spp* of about 15% and also reported that the frequency of *Candida* is higher in non-urologic departments (20). One study has reported the occurrence of *Candida* species in urinary isolates to be 12.96% (21).

Hameed et. al (2012). has reported the sensitivity of Piperacillin/tazobactam (94.4%), Amikacin (90.5%), and Imipenem (62.65%) which shows variations from our results (22). Another study has reported high sensitivity rates of Imipenem (100%), Piperacillin/tazobactam (97%) and Amikacin (90%) (23).

Another study from Pakistan reported a higher level of resistance to beta-lactam antibiotics among *E. coli* and *Klebsiella* and the least resistance to Piperacillin/Tazobactam (10.3% and 17.6%) and Nitrofurantoin (27.6% and 28.5%) respectively has been reported that is similar to our results (24, 25). A study from Iran has reported the sensitivity rates among *E. coli* to Imipenem and Meropenem(100%), Amikacin (94.4%), and resistance rates to Ampicillin (100%), Amoxicillin/Clavulanic acid (63.8%), 3rd generation Cephalosporins (61.1%). This study also shows similarity with our results and found Imipenem and Amikacin as the most effective drugs (26). A recent study in Pakistan has reported a high prevalence of *Klebsiella pneumonia* (40%) and *E. coli* (32.6%) in children and resistance of Piperacillin + Tazobactam and Meropenem is reported as least of about 5.4% and 14.1% respectively (27). A study from Iraq has reported Amikacin as the most effective drugand Ampicillin resistance is highest among all isolates (18).

A current study in t h e Netherlands has reported *E. coli* is the most common UTI pathogen and Carbapenems and Amoxicillin + Clavulanic acid asthe most effective drugs (28). A Swedish study also has reported *E. coli* as most common UTI pathogen having the least resistance rate for Nitrofurantoin while for *Klebsiella,*

Ciprofloxacin and Cefadroxil are reported as the most effective drugs (29). A study from the United States also reported similar results, *E. coli* and *Klebsiella* as the most common uropathogenic and Amikacin and Imipenem as the most effective drugs (30). A similar prevalence of UTI pathogens and their susceptibilities to our studies are reported by another study in India (31). A recent study from Nepal has reported high resistance ratesof *E. coli* for Co-trimoxazole, Fluoroquinolones, and 3rd generation Cephalosporins whereas least resistant rates for Amikacin (6.2%) and Nitrofurantoin (7.9%) (32).

A ten-year surveillance study (2000-2009) from Portugal has shown that the resistance rate of *S. aureus* is 2.5% for Vancomycin and 3.1% for Teicoplanin and the resistant pattern of *Enterococcus spp* is 1.1% for Vancomycin and 4% for Teicoplanin (33). In other words, these drugs havehigh sensitivity that by our results. Hameed et. al. (2012) reported the prevalence of gram-positive isolates to be 8.6% having *S. aureus* (6.45%) and *Enterococcus spp* (2.15%) and higher sensitivity of gram-positive isolates for Vancomycin, Amikacin and Piperacillin/ tazobactam while maximum resistance to Erythromycin, Cephalosporins, Ampicillin, and Gentamicin (22). According to this study, the susceptibility of Amikacin is reported as 86.6% and Imipenem 80.5% against gram-negative bacteria which is comparable to our findings indicating the Imipenem (90.1%) and Amikacin (80.5%) as the most effective drugs against gram-negative bacteria(14). According to the findings of our study, the differences in antimicrobial susceptibilities based on gender are not very prominent. A recent study also reported a lack of significant differences in susceptibilities on gender basis among the patients (34).

The extent of antibiotic resistance among urinary tract pathogens in the present study is quite frighteningas routinely used antimicrobial agents were found ineffective against most of the isolates. Antibiotic resistance is an immense problem in healthcare settings threatening the lives of hospitalized individuals. Therefore, it is imperative t o issue to focus on the policymakers and demandfor the formulation of a strict antibiotics prescription policy in Pakistan. Moreover, it is concluded that Imipenem, Amikacin, and Nitrofurantoin showed better *in vitro* efficacy against urinary tract isolates compared with other antimicrobials, therefore, signifying their therapeutic role in the empirical therapy of urinary tract infections.

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Table 1: Gender-based distribution frequency of isolated pathogens

Bacterial Isolates	Male	Female	Total
	n (%)	n (%)	n (%)
E. coli	88 (36.7%)	152 (63.3%)	240 (69.2%)
Klebsiella sp.	23 (45.1%)	28 (54.9%)	51 (14.7%)
Pseudomonas sp.	10 (62.5%)	6 (37.5%)	16 (4.6%)
Enterococcus sp.	9 (60.0%)	6 (40.0%)	15 (4.3%)
Proteus sp.	4 (44.4%)	5 (55.6%)	9 (2.6%)
CoNS*	0 (0.0%)	5 (100.0%)	5 (1.4%)
Enterobacter sp.	1 (25.0%)	3 (75.0%)	4 (1.2%)
S. aureus	2 (50.0%)	2 (50.0%)	4 (1.2%)
Citrobacter sp.	2 (66.7%)	1 (33.3%)	3 (0.9%)
Total	139 (40.1%)	208 (59.9%)	347 (100.0%)

*CoNS: Coagulase-negative Staphylococci

Table 2: Frequency distribution with respect to types of injuries.

imicrobialAgents	E. coli	Klebsiella	Pseudomonas	Enterococcus	Proteus	CONS	Enterobacter	Staph. aureus	Citrobacter	Total
Amoxicillin	S (%)	S (%)	S (%)	S (%)	S (%)	S (%)	S (%)	S (%)	S(%)	S (%)
Amoxicillin/ clavulanic acid	5 (2.1%)	0 (0.0%)	0 (0.0%)	7 (46.7%)	0 (0.0%)	1 (20%)	0 (0%)	1 (25%)	0(0.0%)	14 (4%)
Piperacillin/ tazobactam	95 (39.6%)	16 (31.4%)	1 (6.3%)	7 (46.7%)	5 (55.6%)	5 (100%)	1 (25%)	2 (50%)	2 (66.7%)	134 (38.6%)
Ceftazidime	179 (74.6%)	38 (74.5%)	9 (56.3%)	8 (53.3%)	9 (100%)	5 (100%)	1 (25%)	2 (50%)	2 (66.7%)	253 (72.9%)
Ceftriaxone	72 (30.0%)	15 (29.4%)	7 (43.8%)	3 (20%)	4 (44.4%)	5 (100%)	1 (25%)	2 (50%)	2 (66.7%)	111 (32%)
Gentamicin	70 (29.2%)	14 (27.5%)	3 (18.8%)	2 (13.3%)	4 (44.4%)	5 (100%)	1 (25%)	2 (50%)	2 (66.7%)	103 (29.7%)
Amikacin	89 (37.1%)	20 (39.2%)	4 (25.0%)	0 (0%)	4 (44.4%)	1 (20%)	2 (50%)	3 (75%)	1 (33.3%)	124 (35.7%)
Imipenem	198 (82.5%)	43 (84.3%)	8 (50.0%)	0 (0%)	6 (66.7%)	1 (20%)	3 (75%)	4 (100%)	2 (66.7%)	265 (76.4%)
Nalidixic Acid	223 (92.9%)	45 (88.2%)	9 (56.3%)	7 (46.7%)	8 (88.9%)	5 (100%)	3 (75%)	4 (100%)	3 (100.0%)	307 (88.5%)

Ciprofloxacin	6 (2.5%)	2 (3.9%)	0 (0.0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0(0.0%)	8 (2.3%)
Levofloxacin	59 (24.6%)	13 (25.5%)	5 (31.3%)	1 (6.7%)	3 (33.3%)	0 (0%)	1 (25%)	1 (25%)	2 (66.7%)	85 (24.5%)
Nitrofurantoin	67 (27.9%)	14 (26.9%)	6 (37.5%)	1 (6.7%)	3 (33.3%)	0 (0%)	1 (25%)	1 (25%)	2 (66.7%)	95 (27.4%)
Co-trimoxazole	179 (74.6%)	30 (58.8%)	3 (18.8%)	13 (86.7%)	8 (88.9%)	4 (80%)	2 (50%)	3 (75%)	2 (66.7%)	244 (70.3%)
Vancomycin	48 (20.0%)	8 (15.1%)	1 (6.3%)	1 (6.7%)	3 (33.3%)	0 (0%)	1 (25%)	0 (0%)	0(0.0%)	62 (17.9%)
Teicoplanin	NA	NA	NA	14 (93.3%)	NA	5 (100%)	NA	4 (100%)	NA	23 (6.6%)
Erythromycin	NA	NA	NA	13 (86.7%)	NA	5 (100%)	NA	4 (100%)	NA	22 (6.3%)
	NA	NA	NA	2 (13.3%)	NA	2 (40%)	NA	2 (50%)	NA	6 (1.7%)