

Bacteriological Profile and Antimicrobial Resistance of Urine Culture Isolates

Sadhvi Paliwal¹, Manoj Kumar Mishra², Kalpana Sharma³

^{1,2}Research Scholar, Motherhood University, Roorkee, India

³Professor, Faculty of Science, Motherhood University, Roorkee, India

Abstract - Urinary tract infection (UTI) is an important cause of mortality and morbidity and among the most common healthcare associated infections. In this study we described the frequency of occurrence and antimicrobial susceptibility patterns of nosocomial and community acquired UTI isolates from a Novus Path Labs, India and Nepal and its Associates Hospitals. All patients with a positive urine culture were enrolled. Antimicrobial susceptibility testing was performed with disk diffusion and E-test MIC. During the study period, 456 isolates were obtained from urine cultures, from a total of 8818 collected sets, among which 291 were felt to represent true bacteremia and 98 were nosocomial. *Acinetobacter* spp. were the most frequently isolated agents in the hospital and community acquired UTIs (32%), followed by *Escherichia coli* (13.7%) and *Klebsiella* spp. (12%). The most effective antibiotics for gram-negative and gram-positive bacteria were ciprofloxacin (13% resistance rate) and vancomycin and oxacillin (with 13% resistance rate), respectively. Analysis of antibiotic resistance pattern showed that 20.43% of *Acinetobacter* spp. and 15.4% of *Pseudomonas aeruginosa* were multi drug resistant (MDR), while 48.7% of *Klebsiella* spp were ESBL-producing isolates and 15% of *Staphylococcus aureus* were oxacillin-resistant. Not observe any vancomycin-resistant strains among isolates of *S. aureus*.

Index Terms - Urinary tract infection, bacterial infection, antibiotics, antimicrobial susceptibility pattern.

INTRODUCTION

Urinary tract infection (UTI) is an important cause of mortality and morbidity and among the most common healthcare associated infections. Wide spectrums of organisms have been described and this spectrum is subject to geographical alteration (Diekma DJ, 2003). In a prospective multicenter study of UTI, Weinstein et al. noted substantial changes in the microbiology, epidemiology and clinical and prognostic significance

of positive urine cultures over a 20-year period. They found that *Staphylococcus aureus* and *Escherichia coli* continued to be the most common etiologic agents of UTI and noted important increases in UTI due to coagulase-negative staphylococcus, fungi, and *Pseudomonas aeruginosa* (community acquired) (Winn W, 2006).

One of the more alarming recent trends in infectious diseases is the increasing frequency of antimicrobial resistance among microbial pathogens causing nosocomial and community-acquired infections. Numerous classes of antimicrobial agents have become less effective as a result of the emergence of antimicrobial resistance, often as result of selective pressure of antimicrobial usage (Garg A, 2007).

These resistance trends and the clinical significance and changing spectrum of microbial pathogens argue strongly for antimicrobial resistance surveillance. Such a program will play a critical role in guiding physicians toward appropriate agents for use in the treatment of both community- and hospital-acquired UTI, as well as identifying changing patterns of etiologic agents and drug susceptibility. Most previous studies of UTI have been performed in temperate developed countries. These studies have mainly focused on nosocomial infections, especially those acquired in an intensive care unit (Tien HC, 2007).

In the present study we described the frequency of occurrence and antimicrobial susceptibility patterns of nosocomial and community-acquired UTI isolates from a Novus Path Labs, India and Nepal and its Associates Hospitals (Munoz-Price L, 2008).

MATERIALS AND METHOD

All clinical and laboratory data were prospectively collected. The initial data including age, sex, underlying disease, source of infection, nosocomial

and previous antibiotic use were gathered by a prepared questionnaire. Laboratory data including culture and sensitivity results from urine were also recorded. Antimicrobial susceptibility testing was achieved with disk diffusion and E-test MIC. The antimicrobial agents tested were as follows: amikacin; ampicillin; cephalothin, ceftriaxone, ciprofloxacin, ceftazidime, trimethoprim-sulfamethoxazole, gentamicin, imipenem, and cefixime. Urine cultures were taken on a routine basis when sepsis was suspected on clinical ground such as fever, tachycardia, tachypnea, or leukocytosis/leucopenia (Cheng B, 2015).

All urine samples were processed in microbiology laboratory according to the standard procedures. Indeed, 5 ml of urine was obtained from each adult patient and inoculated immediately into 50 ml of 'Bone broth. The broths were subculture on 5% sheep urine agar and MacConkey agar after overnight incubation. Subcultures were performed on days 1, 2, 3, 5, 7 and 10. Positive growth was identified by gram staining, colony characteristics, and standard biochemical tests (Sucu N, 2005).

Disk diffusion testing was performed by standard NCCLS methodology (17), using Muller-Hinton plates supplemented with 5% added sheep urine inoculated with a 0.5 Mcfarland suspension. After overnight incubation in both air and 5% CO₂ at 35°C, zone diameters were measured with calipers (Douglas MW, 2004).

Standard methodology was used to determine E-test MICs (18). Muller-Hinton plates supplemented with added 5% sheep urine were inoculated with a 0.5 Mcfarland suspension scraped from plates, and E-test strips (AB Biodisk, Solna, Sweden) were placed on each plate. After overnight incubation at 35°C, the MIC was read as the intersect where the ellipse of growth inhibition intersects the strip. E-test MICs were determined both in air and in CO₂. The breakpoints used to define susceptible, resistant and intermediate categories for each antimicrobial agent were those recommended by the National Committee for Clinical Laboratory Standards (NCCLS). *E. coli* ATCC 25922, *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, and *P. aeruginosa* ATCC 27853 were used as control (Karlowsky JA, 2003).

A urine culture was considered to be contaminated if one or more of the following organisms were identified in only one of a series of urine culture specimens: coagulase-negative *Staphylococcus*

species, *Propionibacterium acnes*, *Micrococcus* species, viridians-group streptococci, *Corynebacterium* species, or *Bacillus* species (Bekeris LG, 2005).

RESULTS

During the study period, there were 456 (5.17%) episodes in which an isolate was obtained from urine cultures, from a total of 8818 collected sets. Of these, 291(3.3%) were felt to represent true bacteremia, and 98(33.7%) of these were nosocomial and 166 patients (57%) had no underlying disease. Characteristic of 291 patients with true bacteria are presented in table 1. Antibiotic treatment during 4 weeks preceding hospital admission. Long-term oral corticosteroid use was defined as administration of corticosteroids (≥ 20 mg/day) for ≥ 1 month during the previous 3 months. Considered comorbidities included the presence of diabetes mellitus, malignant neoplasm, renal failure and IV abusing. The bacterial isolates were considered nosocomial isolates if they were cultured more than 48 hours after admission or within 30 days of hospital discharge (Weinstein MP, 2001). Otherwise, the isolates were considered community acquired. The sources of infections were classified as one of the following: respiratory tract, genitourinary tract, intra-abdominal, unknown (when no obvious source of bacteremia was identified), or others. *KleUTiella* spp. isolates with increased MIC results (≥ 2 µg/ml) for ceftazidime and/or ceftriaxone were considered as potential ESBL (extended-spectrum β -lactamas)-producing isolates.

The study population included 157 males and 134 females with the mean age (\pm SD) of 46.0 \pm 29.6 years (a range, 1-98 years). Sources of bacteremia included respiratory tract (18.9%), genitourinary tract (10.7%), and intra-abdominal (8.2%). *Acinetobacter* spp. (32%; 21 *A. baumannii* and 72 *A. lowfii*), *E. coli* (13.7%), *KleUTiella* (12%; 13 *K. pneumoniae* and 22 others), *Pseudomonas aeruginosa* (12%), *Alkaliginosa* (7.2%), *Enterobacter* (6.9%), *Staphylococcus aureus* (6.9%), *Moraxella* (5.5%), *Serratia* (2.7%), *Enterococcus* (1.7%), *Proteus* (0.7%), group-D *Streptococcus* (0.7%), *Viridanse Streptococcus* (0.3%), *Strptococcus pneumoniae* (0.3%), and *Hafnia* (0.3%).

Among gram-negative isolates, 13% were resistant to ciprofloxacin, nevertheless, ciprofloxacin was superior to all antimicrobial agents ($p < 0.001$). Similarly, among gram-positive isolates, 13.8% were

resistant to oxacillin and 13.4% to vancomycin, even though, oxacillin and vancomycin were superior to penicillin G, ceftriaxone, and cefixime ($p < 0.001$, 0.03, and 0.001, respectively), with a trend towards imipenem, gentamicin, and erythromycin superiority that was not statistically significant ($p < 1.00$, 0.50, and 0.25, respectively). All *S. aureus* isolates were sensitive to vancomycin. Rifampin and oxacillin were superior to penicillin G, cefixime, and ceftriaxone ($p < 0.001$, 0.001 and 0.03, respectively), with a trend towards ciprofloxacin, erythromycin, and meropenem that was not statistically significant ($p < 0.12$, 0.25 and 1.00, respectively) for *S. aureus* isolates.

Analysis of antibiotic resistance pattern showed that 20.4% of *Acinetobacter* spp. and 15.4% of *P. aeruginosa* were MDR, while 48.7% of *KleUTiella* spp were ESBL-producing isolates. The rate of ESBL-producing *KleUTiella* spp. was higher among young patients (≤ 18 y) at rate of 80%. Moreover, patients aged 60-79 years were more likely to be infected with an oxacillin-resistant strain of *S. aureus* (50%) than patients in the other age groups (0-33.3%) (table 4).

Patients in ICUs were at a higher risk for acquiring a UTI caused by MDR-*P. aeruginosa* (31.8%), ESBL-producing *KleUTiella* spp. (77.8%), and oxacillin-resistant *S. aureus* (60%) compared to patients hospitalized in a non-ICU setting, where these pathogens were isolated at rates of 26.8%, 50%, and 0%, respectively (table 2).

Patients who derived their infection from the hospital environment were at a higher risk for sepsis by a pathogen with a resistant phenotype compared to patients with community-acquired infections. MDR-*acinetobacter*, MDR-*P. aeruginosa*, ESBL-producing *KleUTiella* spp., and oxacillin-resistant *S. aureus* were more common among nosocomial isolates (22.2%, 34.5%, 62.5%, and 37.5%, respectively) compared to strains acquired from community infections (11.8%, 14.1%, 52.9%, and 0%, respectively) (table 2). Similar scenario was found for a history of antibiotic and corticosteroid use. Oxacillin-resistant *S. aureus* was more common among patients with diabetes mellitus (66.7%) compared to non-diabetics (6.3%).

DISCUSSION

In this study, 63% of positive urine cultures were felt to represent true bacteremia, which is near to Douglas

et al. (52%) (21) but more than Usman et al. (38%) (6) and Sucu et al. (46%) (22).

The most commonly isolated group in most of the prior studies was gram-positive organisms (1,6,8), although the range of organisms causing bacteremia differ widely. *S. aureus* was the most common in some (1,3,4,5,7,9,21) and *E. coli* in others (6,8). In our setting, *Acinetobacter* spp. was more frequently isolated. Although the trend of these infections has been focused on hospitalized patients, there is another patient population that may be affected by this important pathogen; namely, patients in community setting that have some form of morbidity, especially in the tropical and sub-tropical climates (24,28).

Similarly to others, the most common source of UTI was respiratory tract (18.9%), followed by genitourinary tract (10.7%). Moreover, DM was the most frequently reported underlying disease (8,21).

The percentage of carbapenem-resistant *Acinetobacter* spp. isolates (approximately 70%) was substantially more than other reports, in which this figure ranged 3-30% (7,20,30-33), however, in Ranjbar study it all isolates were resistant to carbapenems. Carbapenem resistance among *acinetobacter* isolates appears to be increased, partly because of wide-spread unnecessary use of carbapenem.

In our study, the resistance rate of *acinetobacter* and *P. aeruginosa* (non-fermenting bacteria) to ciprofloxacin was relatively low (8.6% and 11%, respectively). This is in agreement with studies performed in Far East and United Kingdom (32,34, 35). Nevertheless, a much higher rate of ciprofloxacin resistance was reported from North America and north European countries where resistance ranged from 33 to 92% (7,20,30,31,36) for *acinetobacter*. Furthermore, in America (3,7,20) and India (23) *P. aeruginosa* resistance to ciprofloxacin ranged from 20 to 50%. Resistance of non-fermenting bacteria to fluoroquinolones is a major problem in many parts of the worlds. It appeared that the selection pressure caused by the indiscriminate use of fluoroquinolones was responsible for the persistence and spread of resistant *acinetobacter*. This selection pressure is much stronger when the antimicrobial agents are given intravenously than when they are given orally. Although oral forms of fluoroquinolones are used frequently in Iran, its only available intravenous agent (ciprofloxacin) is rarely used.

Not surprisingly, amikacin was more active than gentamicin in non-fermenting bacteria. Accordingly, in a study from USA, amikacin resistance rate (11.3-15.7%) was significantly lower than gentamicin (44.4-51.9%). Moniri et al reported similar findings, in which *P. aeruginosa* resistance to amikacin (17%) was lower than gentamicin (31%) (38). Superiority of amikacin was also reported by others (3,7,39).

The rate of MDR-acinetobacter is increasing in many parts of the world and poses a serious therapeutic dilemma. In some institutes, the treatment of MDR-acinetobacter is being limited to polymixin B (24,30). In this study, the rate of MDR-acinetobacter isolates was low (20.43%) compared to other study from Iran (100%) (29), but the same as Halstead et al. study (29.3%) (40). MDR-*P.aeruginosa* were slightly isolated (15.4%) compared to other study from Iran (73.9%) (41). This discrepancy could be in part explained by different definitions for MDR.

There were no significant differences in resistance pattern of *E.coli* and *KleUTiella* spp. Carbapenems and amikacin were the most active agents against these organisms, a finding that was demonstrated by prior investigators (3,7,41).

In this study, the resistance rate of *E.coli* to ciprofloxacin (47.5%) was similar to another study in Iran (40.2%)(42), however, the resistance rate of *E.coli* and *KleUTiella* to ampicillin, cefixime, and trimethoprim-sulfamethoxazole were high. This is in agreement with other studies (3,7,23). These drugs have been commonly overused in outpatients for many years, hence, high resistance rate is expected.

Prior investigators have proposed high resistance of *S.aureus* to penicillin (7,23), for example, in USA, the incidence of resistance of *S.aureus* from urine cultures to penicillin was 90% (3). Our results revealed more or less the same resistance rate (95%).

Antimicrobial resistance to erythromycin, gentamicin, ciprofloxacin, meropenem were above 30%, but none of the strains showed resistance to vancomycin, therefore, vancomycin could be safely used in multidrug resistant strains. Similar results have been reported by other researchers (7,23,43).

Although high rates of antimicrobial resistance were observed in this study, there were several encouraging observations regarding specific antimicrobial agents. Firstly, we did not observe any vancomycin-resistant or –intermediate strains among isolates of *S. aureus*. Secondly, rifampin had a good activity against most

of gram-positive organisms so could help us in the treatment of severe and life-threatening gram-positive infections. Thirdly, ciprofloxacin had a good activity against most of gram-negative organisms, and finally, carbapenems (imipenem and meropenem) were strongly active against strains of Enterobacteriaceae (*E. coli*, *KleUTiella*) that were resistant to third generation of cephalosporines.

In conclusion, our data demonstrate an unusual range of organisms causing urinestream infections, which differs significantly from previously published data. Of particular interest is the high rate of *Acinetobacter* spp. These results highlight the important role of local microbiology laboratories to address appropriate empiric antibiotic therapy. Prompt, effective therapy requires up to date knowledge of locally prevalent organisms, and ongoing surveillance for emerging antibiotic resistance. The rise in antibiotic resistance in urine isolates emphasis the importance of hospital infection control, rational prescribing policies, and need for new antimicrobial drugs and vaccines. Our results seem helpful in providing useful guidelines for choosing an effective antibiotic in cases of septicemia and salvage therapy against hospital resistant strains. Lastly, we emphasize that empiric therapy should be guided by local susceptibility data when available, however, in the absence of such information, surveillance data can help with therapeutic choices.

Our results should be interpreted cautiously since this study included a single referral hospital with few numbers of bacteremia, as well as a short study period. Table 1. Characteristics of 291 patients with true bacteremia admitted in Rasul Akram hospital

Sex	Number (%)
Male	157(54.0)
Female	134(46.0)
Age (years)	
0-18	72(24.0)
19-39	51(17.0)
40-59	42(14.4)
60-79	30(10.3)
80-90	36(12.4)
With comorbidity	
Diabetes mellitus	45(15.5)
Corticosteroid use	45(15.5)
Malignant neoplasm	25(8.6)
IV addiction	20(6.9)
Renal failure	19(6.5)
Source of infection	
Unknown	111(38.3)
Respiratory tract	55(18.9)

Genitourinary tract	39(10.7)	Emergency	45(15.5)
Intra-abdominal	24(8.2)	Pediatric	30(1.03)
Others	70(24.0)	Surgery	25(8.6)
Ward			
Internal	116(39.9)		
ICU	75(25.8)		

Table 2. Resistance among gram-negative isolates by E. test.

agent	MIC ₅₀ /MIC ₉₀ [†]	%R [‡]	MIC ₅₀ /MIC ₉₀	%R	MIC ₅₀ /MIC ₉₀	%R	MIC ₅₀ /MIC ₉₀	%R	%R
β-lactams	256/256	81.7	256/256	87.5	256/256	94.3	256/256	76.2	77.9
Ampicillin									
Cephalothin	256/256	92.5	256/256	60.0	256/256	68.8	256/256	76.9	79.8
Ceftazidime	6/256	30.1	6/256	20.0	0.5/256	37.1	4/256	26.9	28.6
Cefixime	256/256	92.5	8/256	53.8	1/256	54.3	256/256	69.2	72.1
Ceftriaxone	256/256	91.4	96/256	50.0	4/256	51.4	256/256	65.4	69.5
Imipenem	32/32	73.1	0.19/0.75	2.5	0.25/0.75	5.7	4/32	42.3	35.5
Meropenem	32/32	62.4	0.047/0.25	5.0	0.047/0.125	5.7	1.5/32	30.8	29.8
Aminoglycosides	64/256	50.5	2/16	2.5	4/48	14.3	33/256	46.2	29.8
Amikacin									
Gentamicin	32/256	67.7	64/256	70.0	64/256	57.1	24/256	57.7	61.1
Others									
Trimethoprim-sulfamethoxazole	0.5/32	18.3	32/32	67.5	2/32	42.9	1.5/32	34.6	34.4
Ciprofloxacin	2/32	8.6	0.38/32	47.5	0.094/16	17.1	0.25/2	11.5	13.7

Table 3. Patient risk factor assessment for resistant phenotypes among bloodstream infection pathogens

1-18 19-39			40-59	60-79	>80	Yes	No	NI	Community-Yes acquired		No	Yes	No	Yes	No
MDR-P. aeruginosa [‡]	15.4	0	0	25.0	0	11.1	17.6	22.2	11.8	0	16.0	28.6	10.5	50.0	9.1
MDR-	16.7	14.3	23.5	20.0	28.6	31.8	26.8	34.5	14.1	22.2	20.0	30.8	13.0	38.5	17.5
Acinetobacter															
spp [‡]															
ESBL-	80.0	66.7	40.0	25.0	100	77.8	50.0	62.5	52.9	26.0	64.3	64.3	52.5	85.7	50.0
phenotype															
Klebsiella spp [#]															
Oxacillin-resistant S. aureus	0	0	33.3	50.0	0	60.0	0	37.5	0	66.7	6.3	50.0	6.7	66.7	6.3

REFERENCES

- [1] Diekma DJ, Beekman SE, Chapin KC, Morel KA, Munson E, Doern GV. Epidemiology and outcome of nosocomial and community onset urinestream infection. J Clin Microbiol 2003;41:3655-60.
- [2] Weinstein MP, Towns ML, Quartey SM, Mirrett S, Reimer LG, Parmigiani G, et al. The clinical significance of positive urine cultures in the 1990s: a prospective comprehensive evaluation of microbiology, epidemiology, and outcome of bacteremia and fungemia in adults. Clin Infect Dis 1997;24:584-602. Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, et al. 2001
- [3] Winn W, Allen S, Janda W, Koneman E, Procop G, Schreckenber P, Woods G, editors. Koneman's color atlas and textbook of diagnostic microbiology. 6th ed. Philadelphia: Lippincott Williams & Wilkins. 2006;p: 100-2.
- [4] Bekeris LG, Tworek JA, Walsh MK, Valenstein PN. Trends in blood culture contamination. Arch Pathol Lab Med 2005;129:1222-25.

- [5] Karlowsky JA, Draghi DC, Jones ME, Thornsberry C, Friedland IR, Sahm DF. Surveillance for antimicrobial susceptibility among clinical isolates of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* from hospitalized patients in the United States, 1998 to 2001. *Antimicrob Agents Chemother* 2003; 47(25):1681-88.
- [6] Douglas MW, Lum G, Roy J, Fisher DA, Anstey NM, Currie BJ. Epidemiology of community-acquired and nosocomial bloodstream infections in tropical Australia: a 12-month prospective study. *Trop Med Int Health* 2004; 9(7):795-804.
- [7] Sucu N, Caylan R, Aydin K, Yilmaz G, Aktoz B, Koksall I. Prospective evaluation of blood cultures in medical faculty hospital of Blacksea Technical University. *Mikrobiyoloji Bulteni* 2005;39:455-64.
- [8] Garg A, Anupurba S, Garg J, Goyal RK, Sen MR. Bacteriological profile and antimicrobial resistance of blood culture isolates from a university hospital. *JIACM* 2007; 8(2):139-43.
- [9] Munoz-Price L, Weinstein RA. *Acinetobacter* infection. *N Eng J Med* 2008;358:1271-81.
- [10] Tien HC, Batted A, Bryce EA, Fuller J, Mulvey M, Bernard K, et al. multi-drug resistant *acinetobacter* infections in critically injured Canadian forces soldiers. *BMC Infect Dis* 2007;7:95.
- [11] Cheng B, Xie G, Yao S, Wu X, Guo Q, Gu M, et al. Epidemiology of severe sepsis in critically ill surgical, 2015, PP:123-196