

Research Article

# Prevalence and antibiotic sensitivity pattern of bacterial isolates from catheterized UTI patients: a local study

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**Abstract:** Prevalence and multidrug resistance among bacteria in catheter-associated urinary tract infections (CAUTIs) has been surging recently. Hence, the bacterial prevalence rate and antibacterial susceptibility in CAUTIs among ICU patients was evaluated in this study. A total of 120 patients admitted to the ICU of Nishtar Hospital, Multan, were selected for this purpose. Both gram-positive and gram-negative bacterial isolates were characterized based on the biochemical tests including catalase test, oxidase test, indole test, TSI test, citrate test, coagulase test and growth on 6.5% NaCl agar. The prevalence of bacterial species was estimated as *Escherichia coli* (32%), *Staphylococcus aureus* (26%), *Pseudomonas* spp. (18%), *Proteus* spp. (14%) and *Enterococcus* spp. (2%). A considerable degree of resistance against commonly prescribed antibiotics was observed. Gram negative bacteria showed resistance to ciprofloxacin, piperacillin-tazobactam and amikacin but susceptibility to imipenem, tigecycline and polymyxin. Gram positive bacteria showed resistance to antibiotics such as piperacillin-tazobactam, ampicillin, gentamicin, oxacillin and ceftazidime. These observations point towards the ineffectiveness of these antibiotics for treating bacterial infections among CAUTI patients and demonstrate the latest trends in antimicrobial drug resistance profile among local population.

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## 1. Introduction

Urinary tract infections (UTIs) are the most common type of hospital acquired infections caused by bacteria which may involve any part of the urinary system such as urethra, ureter, bladder and kidney. These are equally common in primary and secondary care [1]. Frequency of UTI has been found to increase gradually in recent times amongst ICU patients having complications such as diabetes mellitus, renal disease, hypertension and liver disease. Approximately 21-50% patients receive urinary catheters during their stay in hospital, and among those, almost 75% experience catheter-associated urinary tract infection (CAUTI). It places them at high risk as microorganisms enter catheters, promote colonization and cause mucosal irritation [2,3]. CAUTI are mainly caused by gram-negative bacteria belonging to Enterobacteriaceae such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Acinetobacter baumannii*, and *Serratia* spp. as well as some gram-positive bacteria such as *Staphylococcus aureus* and *Enterococcus* spp. [4]. The health consequences of short-term catheterization pertain to fever, acute pyelonephritis, bacteremia, and death whereas long-term catheterization can potentially lead to catheter obstruction, urinary tract stones, local peri-urinary infections, chronic renal inflammation, renal failure and bladder cancer [5,6].

A number of patients might suffer from the financial burden due to relapse of UTI, use of antimicrobial drugs, long stay in hospital, adverse drug reactions, and unsatisfactory or insufficient therapeutic options, which is also one of the causes of antimicrobial resistance becoming a global threat. It has been reported that antimicrobial resistance is increasing due to greater availability as well as excessive and improper use of antibiotics in many developing countries [7-10]. The main objective of this study was to characterize and identify microorganisms associated with ICU catheterized patients so as to determine the prevalence and latest trends in antibacterial susceptibility profile.

## 2. Materials and Methods

### 2.1 Data collection and ethical considerations

A hospital-based study was carried out at the Department of Microbiology, Nishtar Hospital, Multan, Pakistan between January to June, 2018 with the approval of Institutional Research Ethics Committee. Data collection was done after securing informed, written and voluntary consent from each of the participants and their information was kept confidential. Furthermore, for appropriate treatment and management, the positive results were reported to the attending physicians. Out of 270 admitted patients, 120 catheterized patients from Intensive Care Unit (ICU) were selected for this study. Urine samples were collected in tightly closed containers to avoid any air contamination and were transported to the Microbiology laboratory of Nishtar Hospital for further processing. The impact of comorbidity was determined by data collected which indicates the presence of one or more co-morbidities that often accompany a primary condition.

### 2.2 Bacterial isolation and phenotypic characterization

Bacterial isolation and characterization were performed with the help of recommended culturing conditions and biochemical tests. Cysteine lactose electrolyte deficient (CLED) medium plates were used to inoculate each urine sample with the help of a calibrated loop (0.001 mL). The plates were aerobically incubated at 37°C for 24 hours while the phenotypic characterization of isolates was done using colony characteristics as well as a series of biochemical tests, including coagulase, triple sugar-iron (TSI), indole, catalase, oxidase, citrate utilization test as well as growth on 6.5% NaCl. A culture growth of  $\geq 10^5$  CFU/mL accompanied by signs and symptoms was categorized as UTI. For every patient, a single positive culture was considered for the analysis only.

### 2.3 Antibacterial susceptibility testing

Kirby-Bauer disc diffusion method was used for the evaluation of antimicrobial susceptibility testing based on the Clinical and Laboratory Standards Institute (CLSI) recommendations [11]. Fresh (24-hr old), pure colonies (3-5) were suspended in 5 mL of saline (0.85% NaCl) in order to prepare the bacterial inoculum equivalent to 0.5 McFarland standard. Mueller-Hinton agar plates were prepared and used for lawn preparation by using a sterile cotton swab. Plates were incubated at 37°C for 24 hours and examined. Antimicrobials tested included Vancomycin (30µg), Ampicillin (10µg), Oxacillin (1µg), Gentamicin (10µg), Imipenem (10µg), Fosfomycin (50µg), Ceftazidime (30µg), Nitrofurantoin (300µg), Co-trimoxazole (25µg), Tazobactam/Piperacillin (100/10µg), Colistin (10µg), Tigecycline (15µg), Ciprofloxacin (5µg), Linezolid (30µg), Amikacin (30µg), Polymyxin B (300 units) and Ceftriaxone (30µg). The disks were placed onto the agar surface and incubated at 37°C for 24 hours so as to measure the zones of inhibition later on. Isolates were recorded as sensitive, intermediate sensitive or resistant following the CLSI guidelines.

## 3. Results

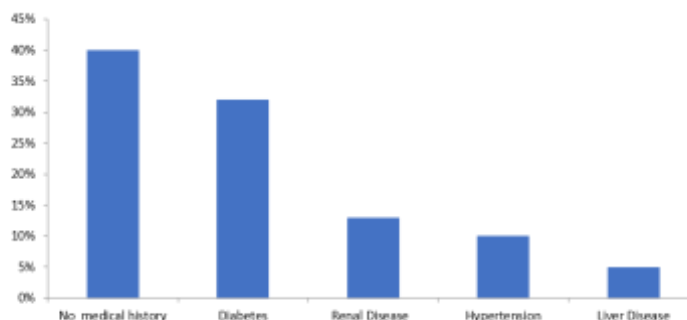
During the study period, a total of 120 patients out of 270 hospital admissions received urinary catheters during their stay in Nishtar Hospital, Multan, who volunteered to participate in this study. Out of 120 samples, 65 (54.2%) were positive for microbial pathogens while the remaining 55 (45.8%) were sterile. The frequency of microbes was

higher among male patients (N=72, 60%) compared to females (N=48, 40%) as most participants were male due to their greater likelihood of visiting the hospital (Table 1). In this

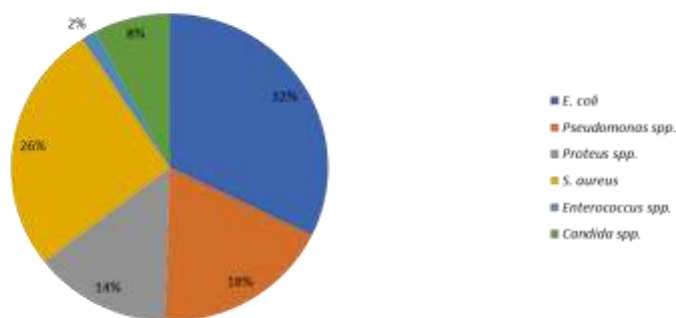
**Table 1.** Characteristics of Catheterized UTI patients admitted to ICU at Nishtar Hospital, Multan, and selected as study participants.

Characteristic	Category	N (%)
Gender	Male	72 (60%)
	Female	48 (40%)
Average Age (years)	Male	39 ± 10
	Female	37 ± 8
Current Marital Status	Unmarried	37 (30.8%)
	Married	71 (59.2%)
	Divorced/Widowed	12 (10%)

study, 40% of patients had no previous medical history whereas the remaining 60% had common comorbidities such as diabetes (32%) which seemed to be the second most common factor associated with CAUTI and liver disease (5%) that remained least common as shown in Figure I. Out of the 65 positive cultures, 60 (92.3%) were bacterial isolates and 5 (7.7%) were fungal isolates. Among the bacterial isolates, 42 (70%) were gram negative and 18 (30%) were gram positive. The majority of bacterial species were gram negative including *E. coli* (32%), *Pseudomonas* spp. (18%) and *Proteus* spp. (14%) as shown in Figure II. Gram positive isolates from patients included *S. aureus* (26%) and *Enterococcus* spp. (2%). Only bacterial samples were selected for further analysis.



**Figure 1.** Co-morbidities prevailing among catheterized UTI patients at Nishtar Hospital, Multan, 2018.



**Figure 2.** Frequency of bacterial and fungal isolates among catheterized UTI patients in ICU at Nishtar Hospital, Multan, 2018.

Out of all the bacterial isolates, 88.2% of *S. aureus* isolates from catheterized UTI patients were resistant to ampicillin followed by 35.2% resistance to piperacillin-tazobactam. On the other hand, all *S. aureus* isolates were susceptible to ceftazidime. All of the *Enterococcus* spp. from CAUTI patients were resistant to gentamicin, oxacillin, ceftazidime and piperacillin-tazobactam as shown in Table 2. In contrast, all of the *E. coli* isolates were susceptible to imipenem followed by 90.5% susceptibility to both tigecycline and polymyxin as shown in Table 3. 83.3% of *P. aeruginosa* isolates were resistant to both piperacillin-tazobactam and amikacin followed by 75% resistance to ciprofloxacin. Similarly, all of the *P. vulgaris* and *P. mirabilis* isolates were resistant to ciprofloxacin and amikacin. On the other hand, all *P. mirabilis* isolates were sensitive to ceftriaxone.

**Table 2.** Antimicrobial susceptibility pattern of the gram-positive bacterial isolates amongst catheterized UTI patients at Nishtar Hospital, Multan, Pakistan, 2018.

Bacteria	Number of Isolates	Antibiotic sensitivity Pattern	Antibiotic								
			VA	NIT	CN	IMP	FOS	OX	CAZ	AMP	SXT
<i>S. aureus</i>	17	R, N (%)	2 (11.8)	3 (17.6)	0 (0)	2 (11.8)	3 (17.6)	7 (41.1)	13 (76.4)	15 (88.2)	6 (35.2)
		I, N (%)	2 (11.8)	4 (23.5)	1 (5.9)	0 (0)	0 (0)	1 (5.9)	4 (23.5)	1 (5.9)	1 (5.9)
		S, N (%)	13 (76.5)	10 (58.8)	16 (94.1)	15 (88.2)	14 (82.3)	9 (52.9)	0 (0)	1 (5.9)	10 (58.8)
<i>Enterococcus spp.</i>	1	R, N (%)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)	1 (100)
		I, N (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
		S, N (%)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)

S: sensitive; I: intermediate sensitive; R: resistant; VA: vancomycin; NIT: nitrofurantoin; CN: gentamicin; IMP: imipenem; FOS: fosfomycin; OX: oxacillin; CAZ: ceftazidime; AMP: ampicillin; SXT: co-trimoxazole

**Table 3.** Antimicrobial susceptibility pattern of the gram-negative bacterial isolates amongst catheterized UTI patients at Nishtar Hospital, Multan, Pakistan, 2018.

Bacteria	Number of Isolates	Antibiotic sensitivity Pattern	Antibiotic								
			TPZ	CT	TGC	IMP	CIP	CRO	AMI	LNZ	PB
<i>E. coli</i>	21	R, N (%)	2 (9.5)	9 (42.9)	0 (0)	0 (0)	4 (19)	0 (0)	0 (0)	4 (19)	2 (9.5)
		I, N (%)	10 (47.6)	1 (4.8)	2 (9.5)	0 (0)	7 (33.3)	8 (38)	5 (23.8)	5 (23.8)	0 (0)
		S, N (%)	9 (42.8)	11 (52.3)	19 (90.5)	21 (100)	10 (47.6)	13 (61.9)	16 (76.1)	12 (57.1)	19 (90.4)
<i>P. aeruginosa</i>	12	R, N (%)	10 (83.3)	4 (33.3)	6 (50)	5 (41.7)	9 (75)	0 (0)	10 (83.3)	4 (33.3)	2 (16.7)
		I, N (%)	0 (0)	0 (0)	2 (16.7)	2 (16.7)	1 (8.3)	5 (41.7)	1 (8.3)	3 (25)	0 (0)
		S, N (%)	2 (16.7)	8 (66.7)	4 (33.3)	5 (41.7)	2 (16.7)	7 (58.3)	1 (8.3)	5 (41.7)	10 (83.3)
<i>P. vulgaris</i>	5	R, N (%)	3 (60)	3 (60)	0 (0)	3 (60)	5 (100)	0 (0)	5 (100)	3 (60)	1 (20)
		I, N (%)	1 (20)	0 (0)	3 (60)	2 (40)	0 (0)	1 (20)	0 (0)	1 (20)	0 (0)
		S, N (%)	1 (20)	2 (40)	2 (40)	0 (0)	0 (0)	4 (80)	0 (0)	1 (20)	4 (80)
<i>P. mirabilis</i>	4	R, N (%)	1 (25)	2 (50)	2 (50)	1 (25)	4 (100)	0 (0)	4 (100)	2 (50)	3 (75)
		I, N (%)	2 (50)	0 (0)	2 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
		S, N (%)	1 (25)	2 (50)	0 (0)	3 (75)	0 (0)	4 (100)	0 (0)	2 (50)	1 (25)

S: sensitive; I: intermediate sensitive; R: resistant; TPZ: piperacillin-tazobactam; CT: colistin; TGC: tigecycline; IMP: imipenem; CIP: ciprofloxacin; CRO: ceftriaxone; AMI: amikacin; LNZ: linezolid; PB: polymyxin

#### 4. Discussion

CAUTI is considered as the second most common hospital acquired infection and is more frequently found in women as nearly half of all women experience at least one episode during their lifetime [12-14]. The higher prevalence of CAUTI in females compared to males may be due to their anatomical and physiological differences [12]. An increased anus to urethra distance and antimicrobial activity performed by prostate secretions are among the factors responsible for prevalence difference between two sexes [15]. Common

comorbidities include diabetes mellitus, renal disease, hypertension and liver disease. In this study, diabetes (32%) seemed to be the second most common factor associated with CAUTI while prolonged catheter usage remained the first. This is in line with the finding of a study which showed 44% prevalence of diabetes in CAUTI patients [16]. Diabetic nephropathy is one of the main factors that increase susceptibility of the patients to UTI when compared with non-diabetic patients. Also, the immunity is reduced in diabetic patients enhancing the risk for acquiring UTI [17].

The prevalence rate of bacteria in this study was significantly higher than previously reported studies for Iranian and Ethiopian populations which was 3% and 22.7%, respectively [18,19]. Gram-negative microorganisms were more prevalent than gram-positive bacteria and this observation was in agreement with previously reported study in an Ethiopian population [20,21]. The highest prevalence rate in this study was for *E. coli* followed by *S. aureus*, *Pseudomonas* spp., *Proteus* spp., and *Enterococcus* spp. which is in accordance with the previous findings of Niveditha et al in 2012 and Ronald in 2002 [16,22]. In a survey of nosocomial infections, CDC indicated that *E. coli* is primarily responsible for hospital acquired UTI while *Enterococcus* spp. accounted for 13.9% of total infections [23]. Bacterial species isolated from positive urinalysis in another population were *E. coli* (73%), *Klebsiella* spp. (6%), *Proteus* spp. (4%), *Pseudomonas* spp. (2%), *Enterococcus* spp. (7%) and *Staphylococcus* spp. (3%) [24]. *Staphylococcus* spp. were the second most prevalent among isolated pathogens in this study that is in accordance with a previously reported study from Ethiopia showing 20% prevalence for *Staphylococcus* spp. [25].

The first-line treatment for UTI is recommendation of suitable and appropriate antibiotics. Another effective measure is the identification of bacteria and their antibiotic susceptibility pattern analysis [10]. In recent decades, antimicrobial resistance has risen in CAUTI isolates from patients treated in ICU. Data collected between 2006 and 2007 described in a report from CDC indicated that 24.8% of all *E. coli* isolated from the CAUTI patients show resistance against fluoroquinolones. In addition, among Extended Spectrum  $\beta$ -Lactamase (ESBL) producing Enterobacteriaceae, 5.5% of *E. coli* and 21.2% of *K. pneumoniae* isolated from CAUTI patients were resistant to ceftazidime [26]. The risk of MDR colonization increases due to indwelling urinary catheters among ICU patients. Therefore, limiting the use of indwelling devices and reducing the use of broad-spectrum antibiotics are important strategies in MDR prevention [27]. High rate of resistance that has been reported in the present study against the most commonly prescribed antibiotics might be due to easy availability, low cost and improper use of these antimicrobials. All of the *E. coli* isolates were susceptible to imipenem followed by 90.5% susceptibility to both tigecycline and polymyxin which is in accordance with the studies from Ethiopia which showed susceptibility of *E. coli* to ceftriaxone [28]. *P. aeruginosa* isolates in this study were 83.3% resistant to both piperacillin-tazobactam and amikacin followed by 75% resistance to ciprofloxacin which is in contrast to previous study where *P. aeruginosa* was susceptible to imipenem and piperacillin-tazobactam [29]. *Pseudomonas* and *Proteus* spp. are mostly associated with the biofilm growth on catheters. Some *Proteus* spp. increase urinary pH by hydrolyzing urea present in biofilm which results in mineral precipitation causing mineral encrustations in renal calculi or along the surface of catheter [30]. Furthermore, *P. vulgaris* and *P. mirabilis* strains both were completely resistant to ciprofloxacin and amikacin.

In this study, *Enterococcus* as common commensal in the intestine was resistant to nitrofurantoin, oxacillin and ceftazidime showing ineffectiveness for treating UTI. This is in line with previous study from Southwestern Nigeria where all of the isolates were resistant to ceftazidime, ampicillin, oxacillin and gentamicin. In addition, they also observed resistance against co-trimoxazole and vancomycin [31]. All other tested antibiotics were effective while a Nigerian study has reported complete susceptibility of *Enterococcus* spp. to vancomycin and ampicillin but high level of resistance to gentamicin [32]. *S. aureus* isolates from catheterized UTI patients also showed resistance to ampicillin and piperacillin-

tazobactam. Majority of the isolates in the present study were found as being resistant to two or more antimicrobials.

The study was limited by sample size and future studies in a relatively large population may reveal a clearer picture. However, the alarming increase in drug resistance among pathogens across the globe has already become a major threat to public health. To combat this situation, focus should be directed towards adoption of preventive strategies for UTIs, development of anti-virulence therapies for microbial pathogens as well as adoption of clinical guidelines for appropriate use of antibiotics.

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