Vol. 5 No. 1 (2026)

Pakistan Journal of Medical & Cardiological Review

https://pakjmcr.com/index.php/1/about

Online ISSN 3007-2387

Print ISSN 3007-2379

Orakzai et al., 2026

Antibiogram Profile and Biofilm Development of Clinical Gram-Negative Bacterial Isolates from Urinary Tract Infection

Muhammad Ismail Orakzai
Medical Lab Technologist at Rehman Medical Hospital Peshawar, Pakistan
Muzamil Arshad
Lecturer Sarhad University Peshawar, Pakistan
Summayya Kanwal
Department of Microbiology, Women University Mardan, Pakistan
Kaleem Ullah*
Senior Lecturer Medical Lab Technology Sarhad University Peshawar, Pakistan. Corresponding Author Email: kaleemullah@suit.edu.pk
Musadiq Khan
Assistant Professor , Cardiology, RCAHS, RMI, Peshawar.

Author Details

Received on 25 Feb 2026

Accepted on 22 March 2026

Published on 23 March 2026

Corresponding E-mails & Authors*:
Kaleem Ullah
kaleemullah@suit.edu.pk

Abstract

A urinary tract infection affects mostly the urinary system including urethra, bladder, kidneys, and ureters primarily caused by Gram positive and negative bacteria and poses a major health issue nowadays. The current study aim was to determine the antibiotic susceptibility/resistance profiles of gram-negative bacterial isolates as well as the biofilm formation ability of Gram -negative bacteria obtained from UTIs patients. This was basically a cross-sectional study, and the duration of the study was from July 2024- December 2024. A total of 80 urine samples of urinary tract infection patients were collected from Rehman Medical Institute Peshawar. Isolates were identified using Gram staining procedures, selective and differential media and through various biochemical tests. Antibiotic susceptibility assay was done using Kirby Bauer disk diffusion method. A total of 80 isolates were preceded in the current study, out of the total isolates 40 were E. coli, 32 were K. pneumonia, 7 were P. aeruginosa while only one isolate was identified as Proteus mirabilis. In E. coli isolates the highest resistivity was observed against ceftazidime and ceftriaxone (67.5%) each, while highest sensitivity was observed against Fosfomycin in 90% of isolates, while in the case of K. pneumonia the highest resistivity was observed against ceftazidime and cefotaxime (71.87%) each, while highest sensitivity was observed against meropenem

and tigecycline in 87.5% of isolates. In P. aeruginosa the highest resistivity was observed against ceftazidime (57.14%), while highest sensitivity was observed against colistin in 100% isolates. The highest resistance pattern against commonly used antibiotics may pose a great challenge to medical practitioners. Other than the antibiotic resistance the biofilm-forming ability of these Resistant strains also creates a challenge for clinicians.

Keywords: Antibiotic Resistance, Biofilm formation, K. pneumonia, P. aeruginosa

INTRODUCTION

AMR represents one of the greatest public health threats of the twenty first century with a real threat to human health, health care systems and economic development. Antimicrobial resistance, the ability of bacteria to withstand the effects of an antibiotic, has quickly been developed because of irresponsible and inappropriate use of antibiotics in human medicine, agriculture, and animal husbandry. Other factors, such as inaccurate or delayed diagnosis, infection prevention and control practices, antimicrobial stewardship, and limited public awareness, have also played a role in the rapid spread of antimicrobial resistance. This has made infections that were once easily treated more difficult to control and led to longer hospitalizations, higher health care costs, treatment failures, and deaths (1,2). South Asia has been identified as one of the areas most impacted by antimicrobial resistance, owing to the high prevalence of infectious diseases, ease of accessibility of antibiotics, and poor surveillance systems. The rise of multi-drug resistant (MDR) and extensively drug resistant (XDR) bacterial pathogens to a growing extent is a serious problem in Pakistan. Multidrug-resistant bacteria associated with standard infections such as those of the genital tract, urinary tract infections (UTIs), and sepsis have been seen in healthcare settings across the country. The occurrence of these resistant organisms has made it more difficult to devise empirical treatment plans and underscored the critical need for ongoing surveillance of bacterial pathogens and patterns of antimicrobial resistance (3,4). UTI is one of the most prevalent bacterial infections in the world and has been seen in all age groups but due to anatomical and physiological reasons, women are more prone to UTI. A UTI is a bacterial infection of any portion of the urinary tract, which consists of the kidneys, ureters, bladder and urethra. Estimated to affect around 150 million people each year globally, UTIs are a significant cause of outpatient attendance and hospital admission. UTIs are more common in women because of the proximity of the urethral meatus (opening) to the anus, pregnancy, sexual activity, menopause and other predisposing factors. Recurrent infections also occur fairly frequently; in addition to repeated courses of antibiotics, there is a risk of the emergence of resistant bacterial strains, which makes it a significant clinical burden (5–9). Gram-negative bacteria,

especially members of the Enterobacteriaceae family are responsible for most UTIs, with Escherichia coli responsible for the bulk of the community and hospital acquired UTIs. Additional significant uropathogen are Klebsiella pneumoniae, Proteus mirabilis, Morganella morganii, Pseudomonas aeruginosa, Enterobacter spp. and Staphylococcus saprophyticus. Distribution of these pathogens can be influenced by patient demographics, geographic location, medical setting, and other medical conditions, including diabetes mellitus, urinary catheterization, spinal cord injury and immunocompromised patients. Multidrug-resistant (MDR) Gram-negative uropathogen are becoming a primary treatment challenge due to their resistance to antibiotics that are often used to treat these infections (4,10). The development of urinary tract infection (UTI) includes the colonization of the periurethral area and the subsequent spread of the infection up the urethra and into the bladder, and in more severe infections, also the kidneys. There are a number of bacterial virulence properties associated with successful colonization, such as fimbriae, adhesins, motility, toxins, and biofilm formation. Biofilm formation is regarded as one of the most significant processes involved in the persistence and resistance of bacteria to antimicrobial agents. Bacterial cells within a biofilm are surrounded by a naturally occurring extracellular polymeric substance they produce to shield them from host immune responses, and they are extremely difficult for antibiotics to penetrate. As a result, biofilm-associated bacteria are more resistant to antimicrobial therapy, have increased ability to cause recurrent infections, and can persist and cause prolonged colonization. Biofilm is of special concern in CAUTI, because the bacteria are able to adhere to the surface of the catheter, leading to chronic infection and treatment failures (17–20). Laboratory diagnosis is crucial to the successful treatment of urinary tract infections. Clinical signs like dysuria, urgency, increased frequency, suprapubic pain, hematuria and foul urine are highly suggestive of UTI, but laboratory confirmation is required especially in complicated and recurrent UTI. Urine microscopy, bacterial culture, biochemical identification and antimicrobial susceptibility testing are conventional methods of diagnosis.

Of these, urine culture is still considered the gold standard to identify the organism(s) causing the UTI and to determine which antibiotics to use. Standard culture-based testing takes several days, however, delaying the start of specific therapy. The use of molecular diagnostic methods is more sensitive and rapid for the detection of bacterial pathogens and antimicrobial resistance genes but has been limited in many developing countries due to the cost and infrastructure availability (11–13). A rise in the frequency of antimicrobial resistant uropathogen, and the need to monitor the local antibiogram patterns on a regular basis. Bacterial isolates possess susceptibility and

resistance patterns that are useful in determining which type of treatment will be most effective for the initial patient care and can be used to inform clinical decision making prior to culture findings. In addition, monitoring of antimicrobial resistance patterns helps to implement antibiotic stewardship programs to inform treatments and infection control practices, and to guide evidence-based treatment recommendations. The resistance pattern is different in various geographical areas and health care centers and periodically evaluation of the bacterial isolates and their antibiogram profile is necessary to optimize patient management and minimize the transfer of resistant bacterial strains. As the incidence of UTI continues to rise, multidrug resistant (MDR) Gram-negative bacteria have emerged and biofilm formation is an important factor in UTI, continual surveillance of bacterial pathogens, antimicrobial susceptibility profiles, and biofilm producing capacity has become more important. This information is vital to better antibiotic use in empirical antibiotic therapy, infection control measures, and antimicrobial stewardship programs to mitigate the effects of antimicrobial resistance and enhance clinical outcomes.

METHODS AND MATERIAL

The descriptive cross-sectional study was conducted in Microbiology Laboratory Rehman Medical Institute Peshawar. The study was conducted after approval from the institutional research board from July until December 2024. The total number of urine samples in the study was 80. Urine samples were obtained from the patients who were clinically diagnosed as Urinary tract infections (UTIs). Urine cultures were included if they were suspected to be a UTI and excluded if they contained less than 10^5 CFU/mL and mixed bacteria growth on the preserved urine samples. Collected samples of urine were cultured in Cystine Lactose Electrolyte Deficient (CLED) agar, MacConkey agar and Pseudomonas cetrimide agar and allowed to incubate overnight at 37C. The bacteria strains were first determined by regular microbiological practices used in the hospital laboratory. They were identified by Gram staining and biochemical tests (citrate utilization, indole production, urease test and oxidase test) for Gram-negative isolates. Antimicrobial susceptibility testing (AST) was conducted using the manufacturer's instructions and in accordance with the Clinical and Laboratory Standards Institute (CLSI) (2024). The chemical composition of CLED agar consisted of Lab Lemco powder (3.0 g/L), tryptone (4.0 g/L), peptone (4.0 g/L), lactose (10.0 g/L), L-cystine (0.128 g/L), bromothymol blue (0.02 g/L), and agar (15.0 g/L). MacConkey agar contained peptone (20.0 g/L), lactose (10.0 g/L), bile salts (1.5 g/L), sodium chloride (5.0 g/L), neutral red (0.03 g/L), crystal violet (0.001 g/L), and agar (15.0 g/L). The composition of Pseudomonas cetrimide agar contained magnesium chloride (1,4 g/L), gelatin peptone

(20,0 g/L), cetrimide (0,03 g/L) and agar (15,0 g/L). The bacterial culture was smeared on a clean glass slide, air-dried and heat-fixed for Gram staining. It was stained for one minute with crystal violet and then washed off with tap water. Iodine solution of Gram was then applied for 1 minute and then washed with water. The smear was decolored with 95% ethyl alcohol for a few seconds and then counterstained with neutral red for 30 seconds. The slide was washed and air dried before it was looked at under the oil immersion microscope. Klebsiella pneumoniae citrate utilization test was carried out by streaking a pure isolated colony on Simmons citrate agar slants. Inoculated slants were then left to incubate aerobically at 37°C for 24 hrs. The change of medium color from green to blue was interpreted as a positive change. The antibiotics administered to patients for Gram-negative bacterial isolates were ceftazidime, ceftriaxone, amoxicillin/clavulanic acid, piperacillin-tazobactam, cefepime, meropenem, amikacin, co-trimoxazole, nitrofurantoin, imipenem, ciprofloxacin, tigecycline, tetracycline, gentamicin, colistin and aztreonam. The inhibition zones were measured and interpreted following the guidelines set out by the CLSI (2024). Kirby Bauer disc diffusion method was used for antimicrobial susceptibility testing. Mueller–Hinton agar plates were made and prepared as per manufacturer's guidelines. A sterile tube was used to dispense 2 mL of sterile saline (0.9% NaCl) in which four to five pure isolated colonies were suspended to create a bacterial inoculum. The turbidity of the suspension was adjusted to match the turbidity of the 0.5 McFarland. A sterile cotton swab was dipped in the suspension, and excess fluid was removed by pressing the swab against the inside wall of the tube and then the inoculum was spread evenly over the surface of Mueller–Hinton (MH) agar plates, creating a lawn culture. The sterile forceps were used to deposit antibiotic discs on the surface of the agar plates separated by about 24 mm. Plates were allowed to incubate for 24 hours at 37°C. The diameters of the inhibition zone formed were recorded with a ruler after incubation and interpreted based on the CLSI guidelines (2024). The crystal violet microtiter plate assay was used to determine biofilm formation. In brief, 200 µL of a bacterial culture grown in the broth was seeded into each well of an ELISA microtiter plate and the plates were then incubated for 24 hours. The culture broth was aspirated, and the wells were rinsed 3 times with water to remove non adherent cells. Twenty minutes later, 200 µl of crystal violet was poured into each well and left to stand for 30 minutes. The excess stain was thrown away, and the wells were washed three times with water. Then, to each well 200µL of 95% ethanol was added and allowed to stand for 5 minutes to solubilize the bound crystal violet (C.V) for further analysis.

RESULTS

Demographic Distribution of Patients

In this study a total of 80 isolates were obtained. Standard microbiological procedures were applied to identify the organisms. The UTIs infections were dominant in the case of females as compared to male. Out of 80 individuals 51 were female while the rest were male (29). The UTI infections were found in various age groups, the most infected group age was 61-70, followed by 51-60 and 21-30, the rest of the details are shown in Table 3.1. All the isolates were obtained from urine samples. Descriptive statistics were applied to find the mean and standard deviation. The mean age was 51.88 years. And the standards deviation of the ages is ± 19.40.

Table No 1 Shows the UTI infections in various age groups

S. No	Age group	Number/frequency	Percentage
1	0-10	1	1.25%
2	20-Nov	3	3.75%
3	21-30	13	16.75%
4	31-40	8	10%
5	41-50	8	10%
6	51-60	13	16.75%
7	61-70	19	23.75%
8	71-80	8	10%
9	81-90	6	7.50%

Distribution of Gram-Negative Isolates

Initially the isolates were identified using Gram staining, selective and differential media and using various biochemical tests. The most dominant bacteria in the UTI infection were *E. coli* followed *by K. Pneumonaie,* the rest of the details are shown in Table 2.

Table No: 2 Shows the distribution of Gram- negative bacteria in UTI Infections

S. No	Bacterial isolate	Number/frequency	Percentage
1	*E. coli*	40	50%
2	*K. Pneumonaie*	32	40%
3	*Pseudomonas aeruginosa*	7	8.75%
4	*Proteus Mirabilis*	1	1.25%

Initial identification through Gram staining

All the isolates were confirmed as Gram negative bacteria after performing the Gram staining, the image shows the pink colony of gram- negative bacteria

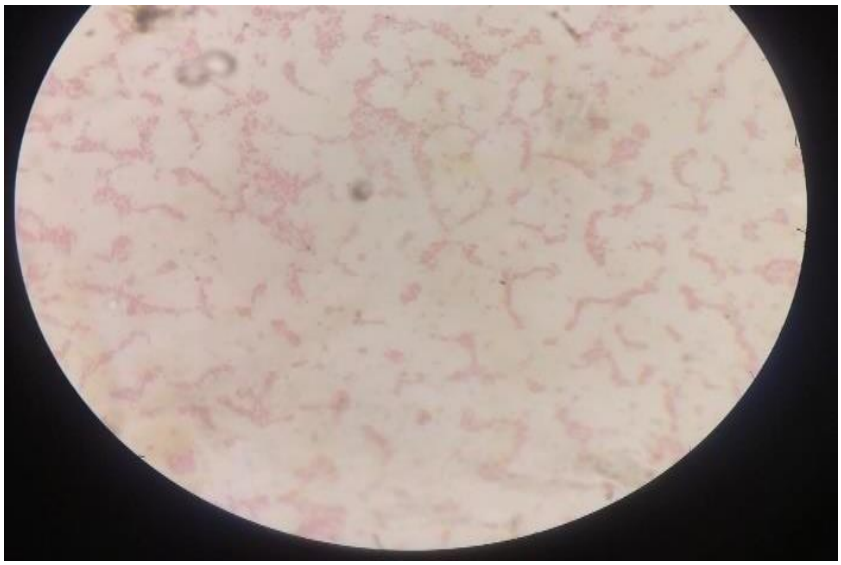

Figure no. 1 shows the gram staining of Isolates

Identification through selective and differential media:

We proceeded with 8o urine samples for our current study, in which 40 were confirmed as *E. coli* based on selective media known as eosin methylene blue (EMB), as shown in figure no. 2.

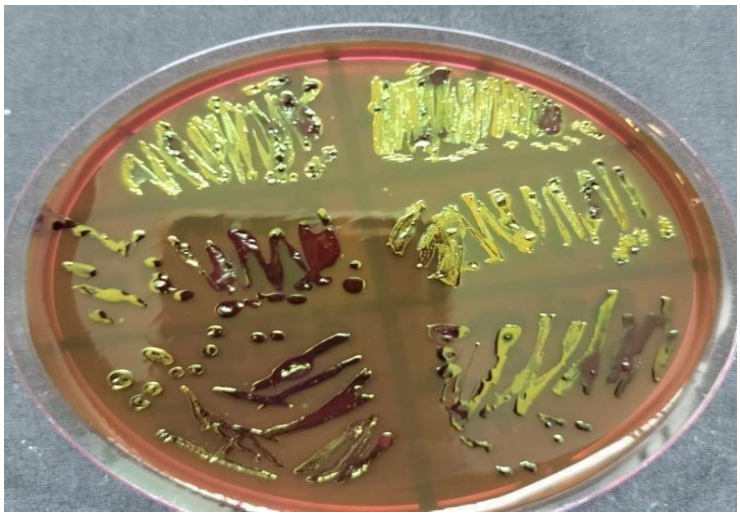

Fig no. .2 Shows the Growth of E. coli on eosin methylene blue Agar The metallic green colony confirms the growth of E. coli.

Antibiotic Susceptibility profiles of gram-negative isolates

Out of total of 80 isolates, 40 were characterized as *E. coli*. The Antibiotic susceptibility profiles of *E. coli* isolates were determined using disk diffusion method. The highest resistivity was observed against ceftazidime and ceftriaxone (67.5%) each, while highest

sensitivity was observed against Fosfomycin in 90% of isolates, while rest of the details of susceptibility profile of E. coli isolates are shown in Table 4.4.

Table 4.3 Shows the antibiotic susceptibility profiles of *E. coli* isolates

	Antibiotic	Resistant		Sensitive	
S. No		Frequency/	percentage	Frequency/	percentage
1	Ceftazidime	27	67.50%	13	32.50%
2	Ceftriaxone	27	67.50%	13	32.50%
3	Amoxillin/ Clavulanic acid	30	75%	10	25%
4	Pipracilin-Tazobactum	16	40%	24	60%
5	Meropenem	5	12.50%	35	87.50%
6	Cefepime	15	37.50%	25	62.50%
7	Amikacin	11	27.50%	29	72.50%
8	Co-trimoxazole	21	52.50%	19	47.50%
9	Nitrofurantoin	9	22.50%	31	77.50%
10	Fosfomycin	4	10%	36	90%
11	Ciprofloxacin	18	45%	32	55%
12	Ceftazidime	6	15%	34	85%
13	Imipenem	4	10%	34	90%
14	Gentamicin	7	17.50%	33	82.50%

Antibiotic Susceptibility profiles of *K. pneumoniae* isolates

Out of total of 80 isolates, 32 were characterized as *K. pneumoniae*. The Antibiotic susceptibility profiles of *K. pneumoniae* isolates were determined using disk diffusion method. The highest resistivity was observed against ceftazidime and cefotaxime (71.87%) each, while highest sensitivity was observed against meropenem and tigecycline in 87.5% of isolates, while rest of the details of susceptibility profile of *K. pneumoniae* isolates are shown in Table no. 4

Table No.4 Shows the antibiotic susceptibility profiles of *K. pneumoniae* isolates

S. No	Antibiotic	Resistant		Sensitive	
		Frequency/	percentage	Frequency/	percentage
1	Ceftazidime	23	71.87%	9	28.12%
2	Cefotaxime	23	71.87%	9	28.12%
3	Ceftriaxone	22	68.75%	10	31.25%
4	Amoxicillin-Clavulanic acid	22	68.75%	10	31.25%

5	Pipracilin-tazobactum	20	62.50%	12	37.50%
6	Meropenem	4	12.50%	28	87.50%
7	Tigecycline	4	12.50%	28	87.50%
8	cefepime	3	9.37%	29	90.62%
9	tetracycline	20	62.50%	12	37.50%
10	Amikacin	8	25%	24	75%
11	Cotrimoxazole	17	53.12%	15	46.87%
12	Nitrofurantoin	4	12.50%	28	87.50%
13	Fosfomycin	1	3.12%	31	96.87%
14	Ciprofloxacin	19	59.37%	13	40.62%
15	Ceftazidime-avibactam	6	18.75%	26	81.25%
16	Imipenem	4	12.50%	28	87.50%
17	Gentamicin	4	12.50%	28	87.50%

Antibiotic Susceptibility profiles of *P. aeruginosa* isolates:

Out of total of 80 isolates 7 were characterized as *P. aeruginosa*. The Antibiotic susceptibility profiles of *P. aeruginosa* isolates were determined using disk diffusion method. The highest resistivity was observed against ceftazidime (57.14%), while highest sensitivity was observed against colistin in 100% of isolates, while rest of the details of susceptibility profile of *P. aeruginosa* isolates are shown in Table no. 5.

Table No. 5 Shows the antibiotic susceptibility profiles of *P. aeruginosa* isolates

S. No	Antibiotic	Resistant		Sensitive	
		Frequency/ percentage n (%)		Frequency/ percentage n (%)	
1	Ceftazidime	4	57.14%	3	42.85%
2	Piperacillin - tazobactam	2	28.57%	5	71.42%
3	Aztreonam	2	28.57%	5	71.42%
4	Meropenem	3	42.85%	4	57.14%
5	Colistin	-	0%	7	100%
6	Cefepime	3	42.85%	4	57.14%
7	Amikacin	3	42.85%	4	57.14%
8	Ciprofloxacin	2	28.57%	5	71.42%
9	Ceftazidime-avibactam	2	28.57%	5	71.42%

10	Imipenem	3	42.85%	4	57.14%
11	Gentamicin	3	42.85%	4	57.14%

Biofilm Assay:

Out of total 80 isolates biofilm assay was done for some selected isolates, in case of E. coli isolates 70% of isolates were strong biofilm former, 15% were weak while 15% of the isolates were in the form of moderate biofilm formation. In case of *K. pneumonia,* 75% were strong, 12.5 were weak and moderate biofilm former. In case of Pseudomonas aeruginosa, 71.42% were strong while 28.57% were moderate. In case of *Proteus mirabilis* only one isolate showed the moderate biofilm formation as shown in Figure 4.5

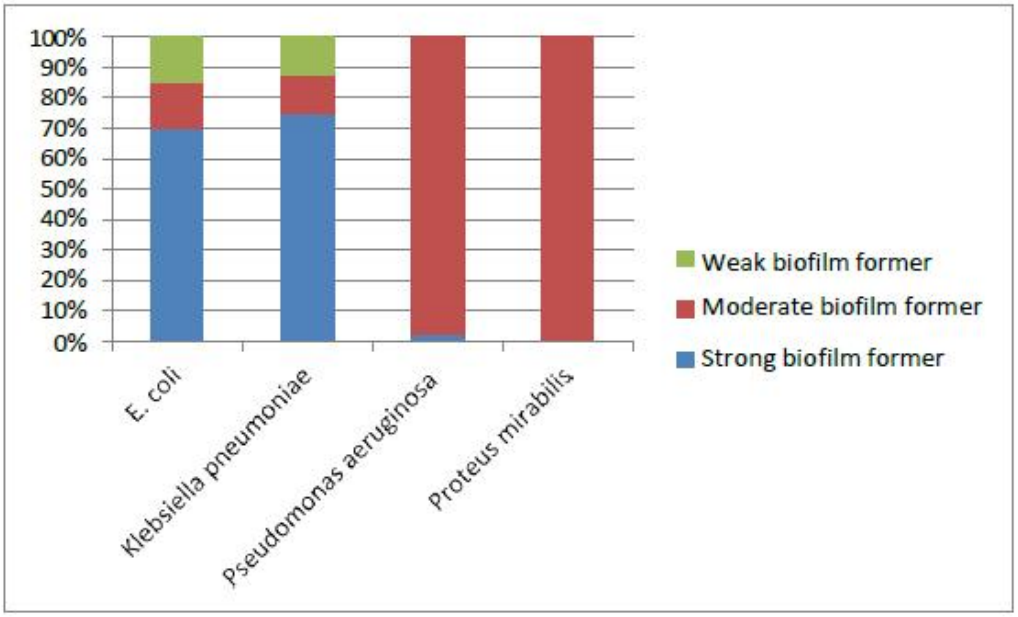

Figure 4.5 shows biofilm formation of study isolates

DISCUSSION

This study describes the prevalence and antibiotic susceptibility profiles of Gram-negative pathogens isolated from Urinary tract infection patients. In the current investigation he UTIs infections were dominant in the case of females as compared to male. Out of 80 individuals 51 were female while the rest were male. Another study also reported that UTIs infections were more dominant in females than male [23]. A recent report from Pakistan by Khatoon *et al.*, [24] also screened the UTIs in which the infection was dominant in case of female as compared to male. The most dominant bacteria like *E. coli* are supported by literature review as we know that E. coli is the commensal of the

human body as well their opportunistic nature showed their presence in UTIs infection. The same study also reported the various age groups having these infections in the age group of 26-35 [24], the findings of this study are not similar to the current study as the UTI infections were found in various age groups, the most infected group age was 61- 70, followed by 51-60 and 21-30. Another study reported that the mean age group in UTIs patients were 52.18, while in the current study the mean ages is 51.88 years. As in the current study *E. coli* was the most dominant organism isolated from UTI, another reports [23, 24] from Pakistan also reported the similar findings as the *E. coli* was found the most common pathogen causing UTIs. Various studies reported the bacterial incidence in various age groups its prevalence in various age groups depends upon the patient condition catheter associated infection and number of sample size. The pattern of bacteria causing UTI in this study is the *E. coli* the most prevalent one, followed by *K. Pneumonaie. aeruginosa* and *Proteus mirabilis* [23], another report Pakistan noticed the different bacteria pattern as *E. coli* the most dominant one followed by enterococcus, streptococcus agalactiae and *K. pneumonia*. Another study from Iran by [25] reported the similar pattern of *E. coli* isolates as in the current study. Another investigation by Rafalskiy et al [26] in Russia reported the similar distribution of Gram- negative isolates as noticed in the current study. Various studies reported the different patterns of Gram-negative pathogens circulating in various regions with different patterns of bacteria. In *E. coli* isolates the highest resistivity was observed against ceftazidime and ceftriaxone (67.5%) each, while highest sensitivity was observed against Fosfomycin in 90% of isolates, another study from Iran by [25] reported that 61.8%, resistance against ceftazidime and 48.5% in the case of ceftriaxone which are the highest resistance against *E. coli* isolates. In the case of *Pneumonaie* the highest resistivity was observed against ceftazidime and cefotaxime (71.87%) each, while highest sensitivity was observed against meropenem and tigecycline in 87.5% of isolates. Resistance towards Amoxicillin-Clavulanic acid in this study was 68.75%, another study reported the same resistance pattern in 68% of isolates from Pakistan by (24). In the current study, 12.5% isolates were showed resistance to tigecycline in case of K. pneumonia isolates, while another report from Pakistan by (24) noticed the tigecyclines resistance in 0% of isolates. Similar resistance pattern was also observed in the case of meropenem that is 0% of isolates were imipenem resistant but in the current study 12.5% isolates were imipenem resistant. In the current study the *E. coli* isolates showed more susceptibility towards Fosfomycin, and imipenem, similar resistance pattern was observed in another study from the *E. coli* was highly susceptible to imipenem, Fosfomycin, amikacin and nitrofurantoin with 0.7%, 0.9%, 4.5%, and 1.2% of isolates respectively. In the current study, the most active

antimicrobial was Fosfomycin, imipenem and colistin with lowest percentages of resistance; similar resistance pattern was observed by another study that *Klebsiella pneumoniae* showed resistance to colistin, imipenem, and piperacillin/tazobactam with percentages of 0.5%, 6.8% and 4.2% respectively (26). The various resistant patterns in different studies might be due to number of sample size, study duration and the geographical location. In the current study, in the case of E. coli isolates 70% of isolates were strong biofilm former, 15% were weak while 15% of the isolates were in the form of moderate biofilm formation. In case of *K. Pneumonaie* 75% were strong, 12.5 were weak and moderate biofilm former. In case of Pseudomonas aeruginosa, 71.42% were strong while 28.57% were moderate. In case of *Proteus mirabilis,* only one isolate showed the moderate biofilm formation. In another study 30% of isolates were strong, 40% were moderate, while 15% were weak in case of E. coli, 25%, were strong, and 35% each were moderate and weak biofilm former in case of *K. Pneumonaie* (27).

CONCLUSION

From the current it was concluded that the highest resistance pattern against commonly used antibiotics may pose a great challenge to medical practitioners. Other than the antibiotic resistance the biofilm-forming ability of these Resistant strains also creates a challenge for clinicians.

REFERENCES

1. Din M, Babar KM, Lehri Ahmed S, Aleem A, Shah D, Ghilzai D, et al. Prevalence of extensive drug resistance in bacterial isolates harboring blaNDM-1 in Quetta, Pakistan. Pak J Med Sci. 2019;35(4):1155–60.
2. Hussain T, Moqadasi M, Malik S, Salman Zahid A, Nazary K, Khosa SM, et al. Uropathogens antimicrobial sensitivity and resistance pattern from outpatients in Balochistan, Pakistan. Cureus. 2021;13(8).
3. Bilal H, Khan MN, Rehman T, Hameed MF, Yang X. Antibiotic resistance in Pakistan: A systematic review of past decade. BMC Infect Dis. 2021;21(1):1–19.
4. Öztürk R, Murt A. Epidemiology of urological infections: A global burden. World J Urol [Internet]. 2020;38(11):2669–79.
5. Iqbal Z, Sheikh AS, Basheer A, Hafsa H Tul, Ahmed M, Sabri AN, et al. Antibiotic drug resistance pattern of uropathogens in pediatric patients in Pakistani population. Antibiotics. 2023;12(2).
6. Tan CW, Chlebicki MP. Urinary tract infections in adults. Singapore Med J. 2016;57(9):485–90.
7. John AS, Mboto CI, Agbo B. A review on the prevalence and predisposing factors responsible for urinary tract infection among adults. 2016;6(4):7–11.

8. Yang X, Chen H, Zheng Y, Qu S, Wang H, Yi F. Disease burden and long-term trends of urinary tract infections: A worldwide report. Front Public Heal. 2022;10.
9. Hosseinpour M, Pezeshgi A, Mahdiabadi MZ, Sabzghabaei F, Hajishah H, Mahdavynia S. Prevalence and risk factors of urinary tract infection in kidney recipients: A meta-analysis study. BMC Nephrol [Internet]. 2023;24(1):1–13.
10. Kaur R, Kaur R. Symptoms, risk factors, diagnosis and treatment of urinary tract infections. Postgrad Med J. 2021;97(1154):803–12.
11. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol. 2019;37(8):852–7.
12. Cason C, D'Accolti M, Soffritti I, Mazzacane S, Comar M, Caselli E. Next-generation sequencing and PCR technologies in monitoring the hospital microbiome and its drug resistance. Front Microbiol. 2022;13(July):1–10.
13. Anjum MF, Zankari E, Hasman H. Molecular methods for detection of antimicrobial resistance. Microbiol Spectr. 2017;5(6):1–17.
14. Alves MJ, Barreira JCM, Carvalho I, Trinta L, Perreira L, Ferreira ICFR, et al. Propensity for biofilm formation by clinical isolates from urinary tract infections: Developing a multifactorial predictive model to improve antibiotherapy. J Med Microbiol. 2014;63(3):471–7.
15. Hawaldar P, Patil K. Study of biofilm formation and antibiotic resistance in urinary isolates at a tertiary care hospital in South India. AcademiaEdu [Internet]. 2017;7(April):107–12.
16. Ledesma JR, Ma J, Zhang M, Basting AVL, Chu HT, Vongpradith A, et al. Global, regional, and national age-specific progress towards the 2020 milestones of the WHO End TB Strategy: A systematic analysis for the Global Burden of Disease Study 2021. Lancet Infect Dis. 2024;1–28.
17. Maione A, Galdiero E, Cirillo L, Gambino E, Gallo MA, Sasso FP, et al. Prevalence, resistance patterns and biofilm production ability of bacterial uropathogens from cases of community-acquired urinary tract infections in South Italy. Pathogens. 2023;12(4).
18. Marma CC, Ahmed S, Akhter F, Keya SA, Ullah P, Barua S. Detection of biofilm producing uropathogenic bacteria and their antibiotic sensitivity pattern. Sch J Appl Med Sci. 2022;10(8):1244–51.
19. Shah TA, Preethishree P, Ashwini, Pai V. Bacterial profile of urinary tract infections: Evaluation of biofilm formation and antibiotic resistance pattern of uropathogenic Escherichia coli. J Pure Appl Microbiol. 2020;14(4):2577–84.

20. Sharma P, Dogra S, Mahajan B, Sharma SS. Biofilm formation by uropathogens and its impact on epidemiology. Soc Heal Rev. 2022;4(2):40–7.
21. Shrestha B, Shrestha B, Poudel A, Lekhak B, Upreti MK. In-vitro biofilm detection among uropathogens and their antibiogram profile. Tribhuvan Univ J Microbiol. 2018;5(1):57–62.Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. American journal of clinical pathology. 1966 Apr 1;45(4_ts):493-6.
22.Aamir AH, Raja UY, Asghar A, Mahar SA, Ghaffar T, Ahmed I, Qureshi FM, Zafar J, Hasan MI, Riaz A, Raza SA. Asymptomatic urinary tract infections and associated risk factors in Pakistani Muslim type 2 diabetic patients. BMC infectious diseases. 2021 Dec;21:1-6.
23.Khatoon I, Khanam S, Azam A, Qadeer S, Naz S, Hassan NU. Incidence pattern, antibiotic susceptibility pattern and associated risk factors of bacterial Uropathogens among general population of Pakistan. Infection and Drug Resistance. 2023 Dec 31:4995-5005.
24.Shirvani M, Keramati A, Esmaeli M. Evaluating the pattern of antibiotic resistance of urinary tract infection (UTI)-causing bacteria in the urine culture samples of patients in the infectious ward of Imam Khomeini Hospital, Kermanshah, in Iran from 2016–2018. African Journal of Urology. 2023 Jun 26;29(1):32.
25.Rafalskiy V, Pushkar D, Yakovlev S, Epstein O, Putilovskiy M, Tarasov S, Glazunov A, Korenev S, Moiseeva E, Gorelysheva N. Distribution and antibiotic resistance profile of key Gram-negative bacteria that cause community-onset urinary tract infections in the Russian Federation: RESOURCE multicentre surveillance 2017 study. Journal of global antimicrobial resistance. 2020 Jun 1;21:188-94.
26.Maione A, Galdiero E, Cirillo L, Gambino E, Gallo MA, Sasso FP, Petrillo A, Guida M, Galdiero M. Prevalence, resistance patterns and biofilm production ability of bacterial uropathogens from cases of community-acquired urinary tract infections in South Italy. Pathogens. 2023 Mar 29;12(4):537.