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PREVALENCE OF PROTEUS SPP AND ITS ANTIBIOTIC RESISTANCE PATTERN IN UTI PATIENTS AT DISTRICT MARDAN, KHYBER PAKHTUNKHWA

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Abstract

Introduction: Urinary tract infections (UTIs) are a significant global health concern, with *Proteus* spp emerging as a key pathogen known for its resistance to antibiotics and association with complications like kidney stones. Despite its clinical importance, data on the prevalence and resistance patterns of *Proteus* spp in District Mardan, Pakistan, remain scarce. This study aimed to determine the prevalence of *Proteus* spp in UTIs and analyze its antibiotic resistance profile to inform better treatment strategies.

Methodology: A cross-sectional study was conducted from June 2024 to May 2025 at Mardan Medical Complex Hospital, involving 684 UTI patients. Urine samples were collected and processed using standard microbiological techniques, including culture on MacConkey and CLED agar, Gram staining, and biochemical tests. Antibiotic susceptibility was assessed via the Kirby-Bauer disk diffusion method against 19 antibiotics, with resistance interpreted per CLSI guidelines. Statistical analysis was performed using SPSS version 23.0.

Results: Out of 684 samples, 48% (n=329) showed bacterial growth, with *Proteus* spp accounting for 17% (n=119) of isolates, making it the second most prevalent pathogen after *Escherichia coli* (22%). The highest resistance was observed against Co-Amoxiclav (93.3%), and Ciprofloxacin (83.2%), while Meropenem (100%) and Fosfomycin (93.3%) were the most effective. Resistance patterns varied significantly by age and gender, with higher resistance noted in middle-aged adults (36–59 years) and males.

Conclusion: The study highlights alarming resistance rates among *Proteus* spp isolates in District Mardan, particularly to first-line antibiotics. These findings underscore the need for antimicrobial stewardship programs and region-specific treatment guidelines to combat the growing threat of multidrug-resistant UTIs. Further research is recommended to explore genetic mechanisms of resistance and evaluate alternative therapies.

Keywords: *Proteus* spp, Urinary tract infections (UTIs), Antibiotic resistance, Multidrug resistance (MDR), Prevalence, Antimicrobial susceptibility, Clinical isolates.

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INTRODUCTION

Proteus is a rod-shaped, Gram-negative bacteria with facultative anaerobic properties. In 1885, Gustav Hauser made the first identification of this motile bacteria [1]. Gustav Hauser originally emphasized its remarkable urease production, unique "swarming" behavior on agar plates, and quick and coordinated multicellular activity [2]. Thirty to forty percent of

women have UTIs repeatedly, and sixty percent of them get one at least once in their lives [3,4]. Using antimicrobial medications that are inefficient or of low quality has several drawbacks, including the development of microbial resistance, treatment failure, illness aggravation, and elevated death rates [5]. The European Commission, WHO, and the US Centers for Disease Control and Prevention (CDCP) have acknowledged the significance of researching resistance's establishment and risk factors as well as the necessity of developing control measures [6]. Urinary tract infections are serious global public health issues. All people, male or female, young or old, are susceptible to these illnesses [7]. In both men and

women, the bacteria often form colonies at the urinary tract entrance. When the bacteria enter the bladder prior to urination and are not removed with urine, infections result. Women are more likely than males to get UTIs, and 81% of all UTIs are documented in women [8]. Because of the vaginal cavity and the near proximity of the rectum to the urethral entrance, women are more likely to acquire bacterial colonies [9]. Depending on the site of infection and the state of the host's body, UTIs are sometimes divided into lower or higher, severe and simple categories [10,11].

Antimicrobial Susceptibility Test the disk diffusion technique on Mueller-Hinton agar, which uses antimicrobials to treat human illnesses in accordance with the Clinical and Laboratory Standards Institute's (CLSI) recommendations [12]. The antimicrobials (Oxoid™, Basingstoke, Hants, UK) were ampicillin (AMP) 10 µg, amoxicillin + clavulanate (AMC) 20/10 µg, cephalothin (CEF) 30 µg, cefoxitin (CFO) 30 µg, ceftazidime (CAZ) 30 µg, ceftriaxone (CRO) 30 µg, cefepime (CPM) 30 µg, nalidixic acid (NAL) 30 µg, norfloxacin (NOR) 10 µg, ciprofloxacin (CIP) 5 µg, sulfamethoxazole-trimethoprim (SUT) 1.25/23.75 µg, aztreonam (ATM) 30 µg, chloramphenicol (CHL) 30 µg, gentamicin (GEN) 10 µg, tobramycin (TOB) 10 µg, amikacin (AMl), fosfomycin (FOS) 200 µg, and ertapenem (ETP) 10 µg. Furthermore, enrofloxacin (ENO), florfenicol (FFC), and ceftiofur (CTF) 30 µg were employed as veterinary antimicrobials [13]. When the isolates showed resistance to three or more distinct types of antimicrobials, they were classified as multidrug-resistant (MDR). In accordance with CLSI guidelines, strains resistant to third-generation cephalosporins were assessed for ESBL production using the combined disk approach. For quality control, *E. coli* ATCC 25922 was employed [14].

According to a recent study on the treatment sensitivity and resistance patterns of patients with complicated UTI's carried out in Peshawar, Pakistan, *Escherichia coli* was the most frequent causative agent, followed by *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* [15]. *Proteus* species were found in about 6% of the mixed flora isolated in a Peshawar investigation on UTIs, but no resistance data were found [16].

Study Rationale and Research Gap

One of the most prevalent clinical bacterial infections that affect patient health and healthcare systems is urinary tract infection (UTI). *Proteus spp* is one of the main organisms that cause UTIs; it not only develops treatment resistance but also causes kidney stones and complicated infections. Treatment outcomes are adversely affected when urease is present and biofilms form, particularly in individuals with recurrent infections or diseases linked to catheter use. The rise in antimicrobial resistance (AMR) worldwide in recent years has made managing UTIs more difficult. In many regions of the world, *Proteus spp*, which has historically

been responsive to a range of first-line medicines, has developed a rising resistance pattern, including multidrug resistance (MDR). The prevalence and resistance profiles of *Proteus spp* are poorly understood globally, especially in District Mardan, Pakistan, and the surrounding regions. Since published research on UTI pathogens primarily looks at broad categories without specifically addressing this bacterium, there is a critical knowledge gap about how the resistance properties of *Proteus spp* differ in local healthcare facilities.

This study must conduct the necessary research to provide the groundwork for future antibiotic treatment strategies since there is a dearth of existing epidemiological data on *Proteus spp* infections with resistance trends. The discrepancies emphasize the necessity of doing region-specific studies to determine the prevalence of *Proteus spp* infections in regional healthcare institutions, such as the Mardan Medical Complex Mardan.

METHODOLOGY

This cross-sectional descriptive study was aimed at establishing the levels of *Proteus spp* in patients with UTIs and analysis of the patterns of antibiotic resistance of the isolated strains. This study is conducted in Mardan, a district located in the Khyber Pakhtunkhwa (KP) province of Pakistan, with a focus on its UTI patient's population. This study was conducted in the microbiology laboratory of the Mardan Medical Complex Hospital in Khyber Pakhtunkhwa. Being a large healthcare facility, this teaching hospital caters to a wide range of patients, including both urban and rural residents. The hospital receives a lot of UTI patients every day, making it a suitable location that may also act as a representative site for our study.

The research period, lasted for one year from June 1, 2024 to May 1, 2025. Data and sample size of (684) were collected from patients of District Mardan, Pakistan. OpenEpi, an open-source program for epidemiological computations, was used to calculate the sample size based on the following criteria: According to recent studies and local health data, 50% of UTI are thought to have *Proteus spp*. To calculate the **sample size** for this study, we can use the standard formula for prevalence studies [17,18].

$$n = \frac{Z^2 \times p \times (1 - p)}{d^2}$$

n = required sample size

Z = Z-score (1.96 for 95% confidence level)

p = estimated prevalence (taken as 0.5 for maximum variability if unknown)

d = desired precision or margin of error (usually 0.05 for 5%)

Using these characteristics, a sample size of 684 was determined, ensuring sufficient statistical power to identify bacterial species and antibiotic resistance estimations. Strict aseptic procedures were followed

for collecting urine samples in order to reduce contamination. Patients were instructed to wash their genital region with clean water to get rid of any commensal organisms before being collected. After discarding the first urine stream, the midstream sample was taken straight into a screw-capped, sterile container with a wide aperture. The samples were sent right away to the lab to be processed. To avoid bacterial overgrowth or specimen degradation, samples were maintained at 4°C and processed within two hours when immediate processing was not feasible.

Urine samples were processed as soon as they arrived at the lab. Specimens were inoculated onto MacConkey agar and Cysteine Lactose Electrolyte Deficient (CLED) agar plates using a sterile calibrated wire loop that held 0.001 mL of urine. To obtain isolated colonies, the wire loop was dipped into the urine sample and the plates were uniformly streaked. For twenty-four hours, the inoculation plates were incubated aerobically at 37°C. Plates were inspected for bacterial growth following incubation. The presence of $\geq 10^5$ colony-forming units (CFU) per milliliter of urine was used to indicate significant bacteriuria [19].

Initially, colonies thought to be *Proteus spp* were recognized by their unique colony form. The colonies were pale on MacConkey agar, suggesting that the bacterium is non-lactose fermenter. One noteworthy observation was the swarming motility, a hallmark of *Proteus species*, on blood agar and other non-selective media. Gram-negative, rod-shaped bacteria were discovered using a Gram stain to verify identification. The urease test, which was part of the biochemical characterization process, revealed that *Proteus spp* had high urease activity, which caused a quick color shift. To distinguish *Proteus spp* from *Proteus vulgaris*, an indole test was performed; *Proteus spp* tested negative. Additional confirmatory tests included the Triple Sugar Iron (TSI) agar test, which revealed high motility and alkaline slant and acid butt with hydrogen sulfide (H₂S) generation [20].

The Kirby-Bauer disk diffusion technique was used to ascertain the antibiotic susceptibility pattern of *Proteus spp* isolates in accordance with the guidelines and standards established by the Clinical and Laboratory Standards Institute (CLSI). A few well-isolated colonies from an overnight culture were chosen, and they were suspended in sterile normal saline to reach turbidity equal to 0.5 McFarland standards in order to create a bacterial suspension. To guarantee equal growth, the standardized inoculum was then uniformly swabbed throughout a Mueller-Hinton agar plate's whole surface. Using sterile forceps, commercially manufactured antibiotic discs were carefully put on the plate's surface at the proper spacing after it had dried for five to ten minutes. For 18 to 24 hours, the plates

were incubated at 37°C. Following incubation, a digital caliper or a ruler were used to quantify the zones of inhibition surrounding the antibiotic discs. The CLSI criteria for Enterobacteriaceae were used to interpret the measurements as Sensitive (S), Intermediate (I) and Resistant (R) [21].

Ethical Approval

This research study, which investigated the prevalence and antibiotic resistance in District Mardan, followed the ethical criteria established by Abdul Wali Khan University Mardan (AWKUM). The Microbiology Department at AWKUM provided ethical approval under the reference number 9449. Before taking part in the study, all subjects provided informed consent. Strict privacy and confidentiality were maintained throughout the data collecting and processing procedure.

Data Collection and Analysis

A systematic data collecting form created especially for this study contained all pertinent clinical and laboratory data. Patient demographics (age, sex), clinical signs and symptoms, culture results, and comprehensive antibiotic susceptibility results were all included in the form. For statistical analysis, data were imported into the SPSS version 23.0. The percentages, proportions, and frequencies were computed using descriptive statistics. *Proteus spp* prevalence among UTI patients was assessed, and rates of drug resistance were examined. To give a clear and thorough picture of the study findings, the results were tallied and, where applicable, shown using graphs and charts.

RESULTS

Prevalence of Bacterial spp in UTI Patients in Mardan

The overall prevalence recorded that in 684 samples 329 (48%) shows bacterial growth on culture medias. The distribution of bacterial species isolated from clinical samples gathered for the current investigation is shown in the Figure 01. In total 684 urine samples, with 22% (n = 151), *Escherichia coli* was the most often isolated pathogen, suggesting that it plays a major role in clinical infections in the study area. *Proteus spp*, which accounted for 17% (n = 119) of isolates, was the second most prevalent organism. Additionally noteworthy was the 04% (n = 25) frequency of *Enterococcus species*, indicating their increasing clinical significance, particularly in nosocomial and urinary infections. *Klebsiella pneumoniae*, a bacterium frequently associated with hospital-acquired infections and antibiotic resistance issues, made up 03% (n = 20). In

this dataset, *Staphylococcus* species which comprise both coagulase-negative and positive strains were found in 2% (n = 14) of patients, indicating a comparatively lower prevalence.

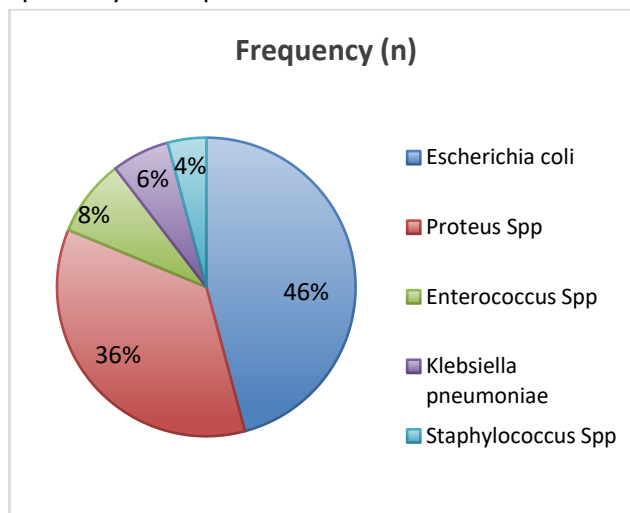


Figure 01: Frequency Distribution of Isolated Bacterial Organisms from Clinical Samples

Gender and Age wise distribution

There were 119 people that took part in the study. There were 70 (58.8%) males and 49 (41.2%) females among them. This suggests that the research cohort was more likely to include male participants than female ones. Figure 02 shows how the study population was distributed by gender.

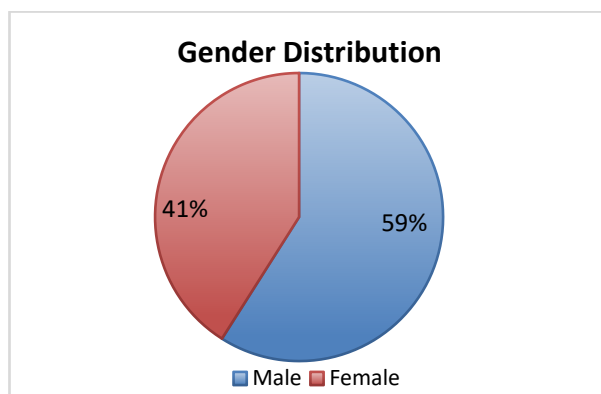


Figure 02: The Gender wise distribution of *Proteus Spp*

The research had 119 participants in all, representing all four age groups. Young Adults (20–35 years old) accounted for the biggest percentage of participants (35.3%), followed by Middle-Aged Adults (36–59 years old), who comprised 31.1% of the sample. Adolescents (10–19 years old) made up 15.1% of the participants, while older adults (60 years and over) made up 18.5%. The valid percent for the age field was 100%, indicating that no data was missing. The increasing number of participants across age categories is reflected in the cumulative percentages as Shown in Figure 03.

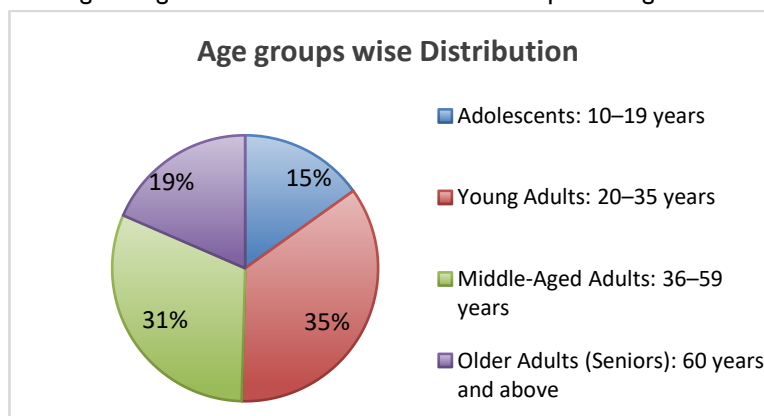


Figure 3: Age groups wise Distribution of *Proteus Spp*

Prevalence of *Proteus spp* UTI Patients and Antibiotics Sensitive and Resistant Pattern

Antimicrobial resistance of isolates to 19 widely used antibiotics was evaluated in the research. Different antibiotics examined showed different levels of sensitivity, resistance, and intermediate responses, according to the data. The most effective antibiotic in this investigation was Meropenem (MRP), as 119 (100.0%) of the isolates showed susceptibility and no instances of resistance or intermediate response. With 111 (93.3%) resistant isolates and only 8 (6.7%) susceptible isolates, with no intermediate responses, Co-Amoxiclav (AMC) had the most concerning resistance rate. As seen in Table 01, our findings highlight the alarmingly high levels of resistance to a number of widely used antibiotics, highlighting the necessity of cautious antibiotic selection and enhanced antimicrobial stewardship initiatives in clinical settings.

Table 01: Antimicrobial Susceptibility Patterns for *Proteus Spp* (n = 119)

Antibiotic	Sensitive n (%)	Resistant n (%)	Intermediate n (%)
Amikacin (AK)	43 (36.1%)	68 (57.1%)	8 (6.7%)
Cefepime (FEP)	61 (51.3%)	57 (47.9%)	1 (0.8%)
Ciprofloxacin (CIP)	20 (16.8%)	99 (83.2%)	–
Co-Amoxiclav (AMC)	8 (6.7%)	111 (93.3%)	–
Colistin (CT)	10 (8.4%)	102 (85.7%)	7 (5.9%)
Doxycycline (DO)	10 (8.4%)	97 (81.5%)	12 (10.1%)
Fosfomycin (FOS)	111 (93.3%)	8 (6.7%)	–
Imipenem (IPM)	99 (83.2%)	8 (6.7%)	12 (10.1%)
Levofloxacin (LEV)	18 (15.1%)	99 (83.2%)	2 (1.7%)
Meropenem (MRP)	119 (100.0%)	–	–
Nitrofurantoin (F)	67 (56.3%)	48 (40.3%)	4 (3.4%)
Norfloxacin (NOR)	41 (34.5%)	70 (58.8%)	8 (6.7%)
Pipemidic Acid	61 (51.3%)	57 (47.9%)	1 (0.8%)
Polymyxin B (PB)	18 (15.1%)	85 (71.4%)	16 (13.4%)
Sulbactam	63 (52.9%)	51 (42.9%)	5 (4.2%)
Piperacillin-Tazobactam	64 (53.8%)	49 (41.2%)	6 (5.0%)
Tigecycline	15 (12.6%)	93 (78.2%)	11 (9.2%)
Co-Trimoxazole	11 (9.2%)	100 (84.0%)	8 (6.7%)
Ceftazidime (CAZ)	58 (48.7%)	54 (45.4%)	7 (5.9%)

Correlation between antibiotic sensitivity in Genders and Different Age Groups

Using the Chi-square test, the study looked at the relationship between gender and antibiotic sensitivity for different antimicrobials. Frequency (n) as Shown in Table 02.

Table 02: Association between Gender and Antibiotic Response

Antibiotic	Total	Male	Female	Sensitive	Resistant	χ^2	p-value
Amikacin	119	70	49	43	68	6.619	0.037
Cefepime	119	70	49	61	57	41.050	0.000
Ciprofloxacin	119	70	49	20	99	16.828	0.000
Co-Amoxiclav	119	70	49	8	111	6.004	0.014
Colistin	119	70	49	10	102	6.063	0.048
Doxycycline	119	70	49	10	97	8.758	0.013
Fosfomycin	119	70	49	111	8	6.004	0.014
Imipenem	119	70	49	99	8	16.828	0.000
Levofloxacin	119	70	49	18	99	5.735	0.057
Meropenem	119	70	49	119	0	—	—

The Chi-square test was used to determine the connection between patient age group and antibiotic response. A total of 119 cases were divided into four age groups: adolescents (10-19 years, n=18), young adults (20-35 years, n=42), middle-aged adults (36-59 years, n=37), and older adults (60+ years, n=22) as shown in Table 03.

Table 03: Association between Age Groups and Antibiotic Response

Antibiotic	Total	Sensitive	Resistant	χ^2	p-value
Amikacin	119	43	68	34.772	0.000
Cefepime	119	61	57	17.073	0.009
Ciprofloxacin	119	20	99	10.391	0.016
Co-Amoxiclav	119	8	111	4.701	0.195

Colistin	119	10	102	1.725	0.943
Doxycycline	119	10	97	8.212	0.223
Fosfomycin	119	111	8	4.701	0.195
Imipenem	119	99	8	14.528	0.024
Levofloxacin	119	18	99	3.165	0.788
Meropenem	119	119	0	—	—

The table 04 shows the mean $\pm$ standard deviation (SD) of antibiotic responses in four age groups: adolescents (10-19 years, n=18), young adults (20-35 years, n=42), middle-aged adults (36-59 years, n=37), and older adults (60+ years, n=22), as well as overall values for the total sample (n=119). Because antibiotic responses were categorized numerically (e.g., 1 for sensitive, 2 for resistant), larger mean values typically indicate better resistance (see table 4).

Table 04: Mean Antibiotic Response by Age Group with Standard Deviation and Frequency

Antibiotic	Mean + SD (10–19) n=18	Mean + SD (20–35) n=42	Mean + SD (36–59) n=37	Mean + SD (60+) n=22	Mean + SD (Total) n=119
Amikacin	1.22+0.55	1.86+0.52	1.89+0.46	1.50+0.67	1.71+0.59
Cefepime	1.11+0.32	1.55+0.50	1.59+0.50	1.55+0.60	1.50+0.52
Ciprofloxacin	1.89+0.32	1.71+0.46	1.97+0.16	1.77+0.43	1.83+0.38
Co-Amoxiclav	1.94+0.24	1.88+0.33	2.00+0.00	1.91+0.29	1.93+0.25
Colistin	2.00+0.34	1.93+0.41	1.97+0.37	2.05+0.38	1.97+0.38
Doxycycline	1.89+0.58	2.07+0.46	2.03+0.29	2.00+0.44	2.02+0.43
Fosfomycin	1.06+0.24	1.12+0.33	1.00+0.00	1.09+0.29	1.07+0.25
Imipenem	1.11+0.32	1.50+0.83	1.03+0.16	1.36+0.73	1.27+0.63
Levofloxacin	1.89+0.47	1.86+0.35	1.86+0.42	1.86+0.35	1.87+0.39
Meropenem	1.00+0.00	1.00+0.00	1.00+0.00	1.00+0.00	1.00+0.00

DISCUSSION

This study offers a thorough examination of antibiotic resistance trends in various age and gender categories. Significant differences in susceptibility to widely used antibiotics are revealed by the results, highlighting the necessity of specialized antimicrobial stewardship tactics. To assess the frequency and trends of antibiotic resistance of bacterial pathogens specifically, *Proteus spp* isolated from clinical samples in District Mardan, the current investigation was carried out. The most common organism among the 384 bacterial isolates examined was *Escherichia coli*, which accounted for 35% of the cases. *Proteus spp* (39%), *Enterococcus species* (15%), *Klebsiella pneumoniae* (6%), and *Staphylococcus species* (5%) were the next most common organisms.

The study's findings showed that there were notable differences in *Proteus spp* antibiotic resistance patterns between different age groups. The overall mean $\pm$ SD for amikacin resistance was 1.89 ± 0.46 for middle-aged groups (36–59 years), and 1.22 ± 0.55 for teenagers (10–19 years). Similarly, cefepime resistance rose with age, reaching 1.59 ± 0.50 in middle-aged adults and 1.11 ± 0.32 in adolescents.

The present study found a substantial age-group difference in antibiotic resistance levels, with middle-aged and older individuals accounting for the higher incidence of resistance to amikacin, cefepime, and ciprofloxacin, among other medications. The findings of a research found that 46.41% of the patients were older than 63 and that their resistance patterns were noticeably greater than those of the younger age groups are in line with these findings [22]. Our study's findings are consistent with a prior study that found that ignorance about antibiotic usage considerably raised the likelihood of inappropriate use, particularly among those aged 21–34 and 35–49 (6.58 and 4.88, respectively). In our data, the highest rates of resistance to popular antibiotics like Amikacin, Ciprofloxacin, and Co-Amoxiclav were found in the same age cohorts, supporting the role that ignorance plays in the rise in antimicrobial resistance. This contrast emphasizes the necessity of focused educational initiatives aimed at the youth and middle-aged population in order to improve antibiotic practices and slow the rate of resistance growth [23]. In contrast to the referred report, our investigation in Mardan revealed a low resistance rate (1.9 and 2.8%),

but the same level of sensitivity to imipenem (83.2%) and Meropenem (100%) across all age categories and both genders. This indicates how well carbapenems work as antibiotics against uropathogens that are resistant to a variety of other medications [24]. Our investigation found that high resistance to many antibiotics, including co-amoxiclav (93.3%), ciprofloxacin (83.2%), and colistin (85.7%), was present in diverse age groups and genders. *P. mirabilis* was extremely resistant to ampicillin (95.5%), nalidixic acid (82.3%), and many cephalosporins (57.8%-79.4%), particularly ampicillin, according to a 2014 research that showed notable levels of resistance [25]. The study's findings indicate a concerning trend of rising antibiotic resistance across the board, but particularly among adults in Mardan who are between the ages of 20 and 59. Antimicrobial stewardship, revised treatment procedures, and region-specific antibiotic prescribing guidelines are all necessary in light of the resistance patterns seen against routinely used antibiotics, such as ciprofloxacin, co-amoxiclav, and colistin. In addition to highlighting the significance of localized data in directing empirical therapy, the consistent resistance profiles when compared with national and international research further support the global nature of antimicrobial resistance. It is advised that ongoing community and hospital-level surveillance of antibiotic susceptibility be conducted, and that future research use genetic testing to identify resistance genes. In addition to improving patient outcomes, putting such approaches into practice will significantly support national efforts to prevent antibiotic resistance.

CONCLUSION

This study demonstrates the notable differences in antibiotic resistance patterns across District Mardan patients according to age and gender. Targeted antimicrobial stewardship efforts are urgently needed, and the results are in line with worldwide trends. It is feasible to stop the spread of antibiotic resistance and preserve the effectiveness of currently available medicines by taking demographic variables into account when prescribing antibiotics and putting comprehensive public health initiatives into place.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

INFORMED CONSENT

Informed consent was obtained from participants.

ETHICAL STATEMENT

The study was approved by a recognized ethics committee.

AUTHOR CONTRIBUTION

Muhammad Junaid (concept, design)
Naeem Ul Haq (concept, data collection)
Hamad Ali (design, corresponding, writing)
Zahid Ullah (analysis, styles)
Saghir Ahmad (design, writing)
Imad Khan (design, review)
Falak Niaz (analysis, statistical analysis)

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