



## AGE AND GENDER-WISE OCCURRENCE OF PSEUDOMONAS AERUGINOSA IN MINGORA, SWAT, AND THE EFFECTIVENESS OF COMMONLY USED ANTIBIOTICS FOR ITS TREATMENT

Muhammad Sohail Khalil<sup>1a</sup>, Musharaf Khan<sup>1b</sup>, Hasham Ali Khan<sup>1c</sup>, Fawad Khan<sup>1d</sup>, Inam Ullah<sup>1e</sup>, Babar Khan<sup>1f</sup>, Muhammad Izazullah<sup>2</sup>

<sup>1a,1b,1c,1d,1e,1f</sup>Centre for Biotechnology and Microbiology (CB&M), University of swat, Pakistan  
<sup>2</sup> Swat Medical College, Saidu Sharif Swat, Pakistan

ARTICLE INFO	ABSTRACT
<p><b>Keywords:</b> Age, Gender, Antibiotics, Multidrug Resistant And Microorganism</p> <p><b>Corresponding Author:</b> Muhammad Sohail Khalil, Centre for Biotechnology and Microbiology (CB&amp;M), University of swat, Pakistan <b>Email:</b> <a href="mailto:sohailkhalilswat@gmail.com">sohailkhalilswat@gmail.com</a></p>	<p><i>Pseudomonas aeruginosa</i> is a gram-negative, aerobic, non-spore-forming rod that causes various infections in both immunocompetent and immunocompromised hosts. It is commonly found in the environment and is an opportunistic pathogen in hospital settings. Its unique ability to form biofilms increases antibiotic resistance and resists host defenses. <i>Pseudomonas</i> infections can occur in various parts of the body. It is the second most common cause of nosocomial pneumonia, urinary tract infection, surgical-site infection, and fifth most common isolate overall. <i>Pseudomonas</i> infections are increasingly resistant to certain antibiotics and a 2-drug regimen is recommended for severe sepsis, septicemia and inpatient neutropenia. This study aims to investigate the age- and gender-wise occurrence of <i>P. aeruginosa</i> in Mingora, Swat, KP, and evaluate the effectiveness of commonly used antibiotics in its treatment. The obtained results revealed the highest occurrence rate of 40 % in age group 18-30 years, followed by 34% in the age group of 32-42 years and 26% in elders of 41-52 years' age. Regarding the gender distribution, the occurrence rates of 58% and 42% were observed in males and females respectively. The antimicrobial susceptibility results showed Ciprofloxacin as the most effective antibiotic against <i>P. aeruginosa</i> isolates. The rates of susceptible isolates were ciprofloxacin (CIP) 72%, amikacin (AK) 70%, ceftazidime (CAZ) 63%, imipenem (IPM) 52%, piperacillin/tazobactam (TZP) 50%, cefepime (FEP) 46%, aztreonam (ATM) 45%, tobramycin (TOB) 42% and levofloxacin (LEV) 33%. 58% of isolates were resistant to more than two groups of antibiotics and were regarded as multidrug resistant (MDR).</p>

## 1. Introduction

*Pseudomonas aeruginosa* is a gram-negative, opportunistic bacterium that poses a significant challenge to public health due to its high adaptability, natural resistance to many antibiotics, and ability to thrive in various environments (Mangi, Phulpoto, Qazi, & Kanhar, 2016; Uddin, Jabeen, Saadat Mehmood, & Rizwan, 2018). Known for causing a wide array of infections, it is particularly dangerous for immunocompromised individuals, including patients with cancer, cystic fibrosis, diabetes, AIDS, and those undergoing organ transplants or chemotherapy (A. Ahmad et al., 2021). The bacterium's unique capacity to form biofilms and resist both immune responses and antibiotic treatment has made it a major cause of concern in hospital and community settings alike (Ali Syed et al., 2024; Rehman et al., 2023). Infection by *P. aeruginosa* is often nosocomial in nature, being one of the leading pathogens in hospital-acquired pneumonia, urinary tract infections (UTIs), sepsis, and wound infections. Its transmission is facilitated through contaminated surfaces and water sources such as hospital sinks, medical equipment, and humidifiers (J. Ahmad & Ahmad; Khan et al., 2023). The organism is also commonly found in community water sources like public swimming pools, Jacuzzis, and spas, further contributing to its spread. Individuals using ventilators, urinary catheters, or undergoing frequent medical procedures are particularly vulnerable due to compromised physical and immune defenses (Javed et al., 2023). Globally, the rise of multidrug-resistant (MDR) strains of *P. aeruginosa* has added complexity to its management. The bacterium's mechanisms of resistance include the production of beta-lactamases and extended-spectrum beta-lactamases (ESBLs), reduced permeability of the outer membrane, efflux pumps, and genetic mutations (Aziz et al., 2022; Shah et al., 2023; URREHMAN, NAILA, & JUNAID AHMAD). These factors significantly limit the effectiveness of commonly used antibiotics like ciprofloxacin, levofloxacin, gentamicin, and certain cephalosporins (Kumari, Narwal, & Agrawal, 2020). The World Health Organization and Centers for Disease Control and Prevention have classified MDR *P. aeruginosa* as a serious public health threat, especially in intensive care settings. In Pakistan, and particularly in the region of Mingora, Swat (Khyber Pakhtunkhwa), there is a scarcity of localized, evidence-based research on the epidemiology of *P. aeruginosa* infections (Jamal et al., 2024; Mahaseth, Chaurasia, Jha, & Sanjana, 2020). The patterns of infection in relation to age and gender, as well as the antibiotic susceptibility profiles specific to the local population, remain poorly understood. This lack of data presents a challenge for clinicians and public health professionals attempting to implement effective treatment strategies and antibiotic stewardship programs (Singh). Understanding the age and gender-wise occurrence of *P. aeruginosa* infections in Mingora is crucial for identifying high-risk populations and tailoring targeted interventions. Moreover, evaluating the efficacy of commonly used antibiotics in the region is essential for guiding empirical therapy and reducing the spread of resistant strains. This study aims to fill the gap in regional data by investigating the distribution of *P. aeruginosa* infections across different demographic groups and analyzing the effectiveness of commonly prescribed antibiotics, including fluoroquinolones and beta-lactam-based therapies.

## 2. Materials and Methods

### 2.1 Materials

This study used MacConkey agar for bacterial isolation and Muller Hinton agar (MHA) for antibiotic sensitivity testing. Laboratory tools included sterile Petri dishes, swabs, loops, test tubes, and filter paper. Biochemical reagents such as crystal violet, iodine, ethanol, safranin, oxidase reagent, hydrogen peroxide, bromothymol blue, Ehrlich's reagent, and Kovac's reagent

were used. Eleven antibiotic discs, including ciprofloxacin, levofloxacin, gentamicin, and imipenem, were applied. Standard lab equipment like an autoclave, incubator, refrigerator, Bunsen burner, and light microscope were also used.

## **2.2 Data Collection**

The data for this study was collected from Saidu Teaching Hospital, Swat, after receiving ethical clearance and written permission from the hospital's ethical committee and the Department of Pathology. Patient records were reviewed, and data from 240 patients with culture-confirmed *Pseudomonas aeruginosa* infections were selected. These patients were admitted to the hospital with clinical signs and laboratory confirmation of infection. Only those individuals who met the defined inclusion criteria were considered for analysis.

## **2.3 Media Preparation**

Two different culture media were prepared and used throughout the study. MacConkey agar was utilized for the isolation of *P. aeruginosa* from patient samples, as it allows for the selective growth of gram-negative bacteria. Muller Hinton agar (MHA) was prepared for antibiotic sensitivity testing following the standard protocol. Both media were autoclaved at 121°C for 15 minutes to ensure sterility before use.

## **2.4 Identification of *P. aeruginosa***

Samples from various clinical sources were cultured. Identification was done using biochemical tests. Gram staining showed gram-negative rods. Positive catalase and oxidase tests confirmed the species. The urease and indole tests were negative, while citrate and TSI tests confirmed further characteristics typical of *P. aeruginosa*.

## **2.5 Antimicrobial Susceptibility Assay**

Antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion method on Muller Hinton agar plates. A pure colony of *P. aeruginosa* was inoculated into a saline suspension adjusted to 0.5 McFarland standard. The bacterial suspension was then evenly swabbed across the MHA plate surface. Once dried, antibiotic discs were carefully placed on the agar surface and the plates were incubated at 37°C for 24 hours in a microaerophilic environment. After incubation, the diameters of the zones of inhibition around each antibiotic disc were measured and interpreted according to guidelines from the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The results provided insight into the resistance and susceptibility patterns of the isolated *P. aeruginosa* strains

## **3. Results**

### **3.1. Age-wise Distribution of *P. aeruginosa* Patients**

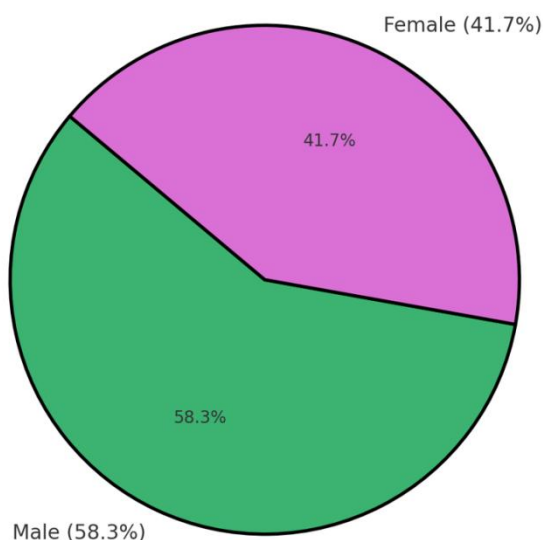
A total of 240 *Pseudomonas aeruginosa* positive patient records were collected from Saidu Teaching Hospital, Swat, and analyzed for age-wise distribution. Patients were categorized into three age groups: 18–30 years, 31–40 years, and 41–50 years. The highest number of cases, 97 patients (40.4%), was recorded in the 18–30 years age group. This was followed by 81 patients (33.8%) in the 31–40 years group, and 62 patients (25.8%) in the 41–50 years group. These findings indicate that younger adults, particularly those aged 18–30, are more frequently affected by *P. aeruginosa* infections. The higher infection rate in this group may be linked to increased exposure to public settings, hospital visits, or weakened immunity due to lifestyle or occupational factors. It also suggests that preventive strategies should focus more on younger adults who may not be traditionally viewed as high-risk (Table 1). Further investigation is needed to understand the behavioral or environmental causes of this trend

**Table 1:** Show the Ag-wise distribution of *P. aeruginosa* patients

Age distribution	Number of patients (n=240)	Percent
18 to 30 years	97	40.4
31 to 40 years	81	33.8
41 to 50 years	62	25.8

### 3.2. Gender-wise Distribution of *P. aeruginosa* Patients

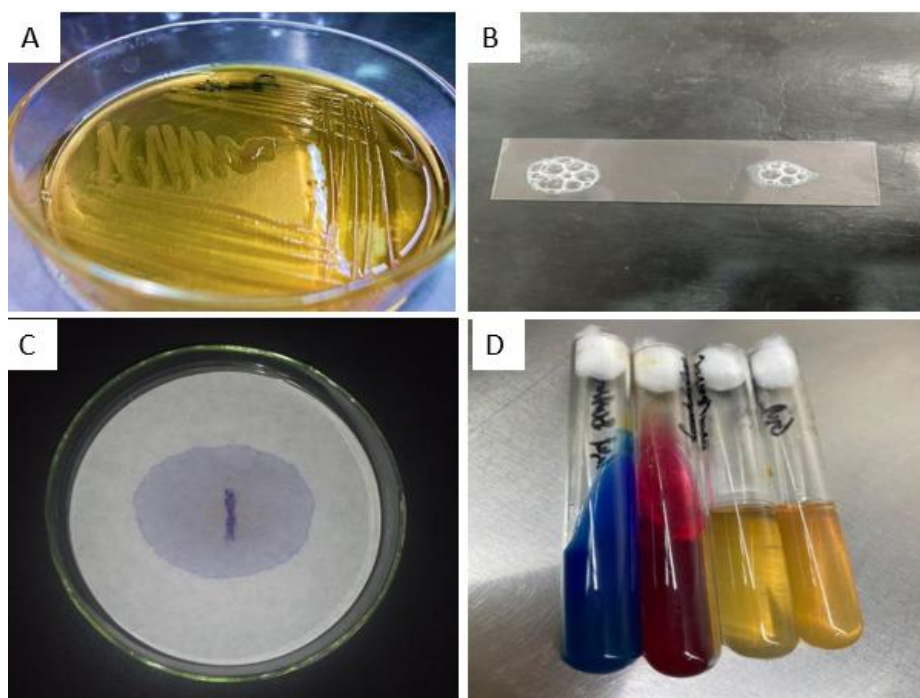
Among the 240 *Pseudomonas aeruginosa* positive cases analyzed at Saidu Teaching Hospital, Swat, gender-wise distribution revealed a higher incidence in males compared to females. Of the total patients, 140 (58.3%) were male, while 100 (41.7%) were female. This noticeable difference suggests that males may be at greater risk of acquiring *P. aeruginosa* infections. The higher prevalence in males could be influenced by a variety of factors, including greater occupational exposure, more frequent interaction with contaminated environments, or increased likelihood of undergoing invasive medical procedures. Additionally, lifestyle habits and healthcare-seeking behavior may contribute to this disparity. Understanding gender-specific trends is essential for developing targeted infection control measures and raising awareness in high-risk groups. Further investigation is recommended to determine the precise factors driving the higher infection rate among males. (Fig.1).



**Figure 1:** Pie chart showing gender-wise distribution of *Pseudomonas aeruginosa* patients

### 3.2. Biochemical Test

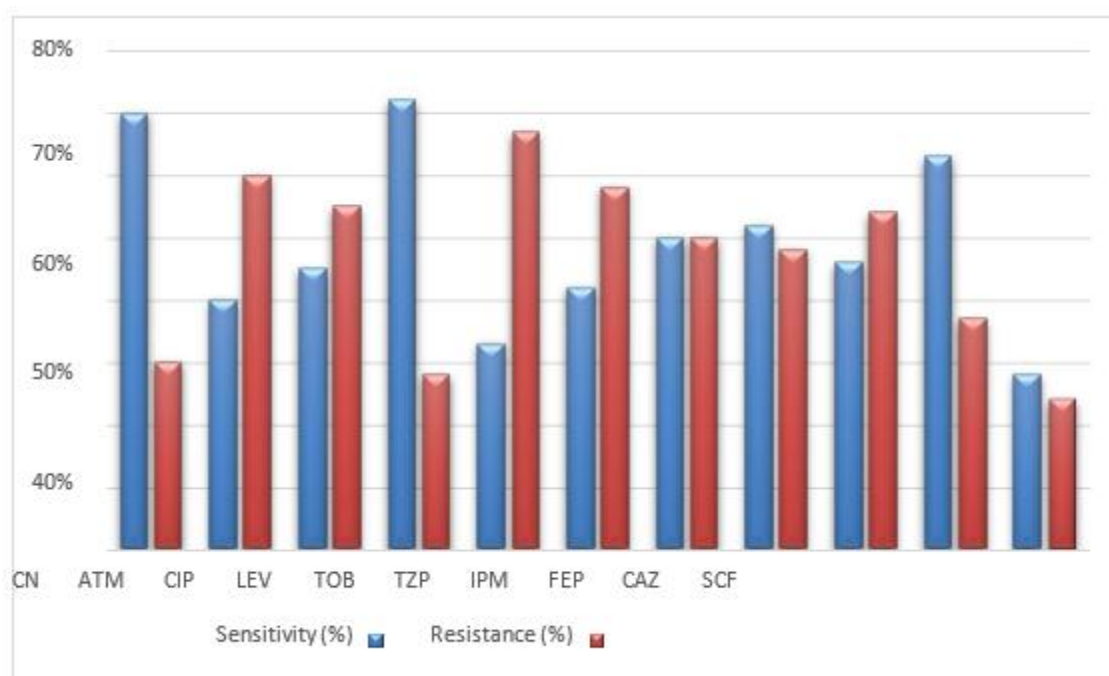
The results of the identification tests confirmed the presence of *Pseudomonas aeruginosa* in all 240 clinical isolates. Gram staining showed gram-negative rod-shaped bacteria. The catalase and oxidase tests were positive, supporting the identification of *P. aeruginosa*. The urease and indole tests were negative, differentiating it from other similar organisms. The citrate test showed a positive result, indicated by a color change from green to blue. In the Triple Sugar Iron (TSI) test, an alkaline slant with no sugar fermentation was observed, confirming non-fermentative characteristics. These biochemical test results collectively verified the identity of *P. aeruginosa* in the collected samples. (Fig. 2).



**Figure 2.** Show the biochemical test result (A) MacConkey agar (B) catalase test (C) Oxidase test (D) Urease, TSI, citrate and indole

### 3.3. Antimicrobial susceptibility of *P. aeruginosa* Isolates

The antimicrobial resistance (AMR) patterns of *Pseudomonas aeruginosa* isolates tested against eleven commonly used antibiotics revealed important insights. Ciprofloxacin showed the highest in vitro activity, with 72% of isolates being susceptible, followed closely by amikacin (70%) and ceftazidime (63%). Other antibiotics with moderate effectiveness included imipenem (52%), piperacillin/tazobactam (50%), cefepime (46%), and aztreonam (45%). Tobramycin showed 42% susceptibility, while sulzone and levofloxacin had notably lower rates at 28% and 33% respectively. More than half of the isolates (58%) exhibited resistance to multiple classes of antibiotics, highlighting the significant presence of multidrug-resistant strains. The highest resistance rates were observed for levofloxacin (67%) and gentamicin (60%). Despite this, many levofloxacin-resistant isolates remained susceptible to ciprofloxacin and amikacin, suggesting these antibiotics may still be effective alternatives in cases of resistance (Fig. 3). These findings underscore the need for ongoing antimicrobial surveillance and informed antibiotic prescribing practices to manage *P. aeruginosa* infections effectively.



**Figure 3:** Antibiotic sensitivity and resistance profile of *P. aeruginosa* Isolates

#### 4. Discussion

The findings of this study reveal significant insights into the antimicrobial resistance (AMR) patterns of *Pseudomonas aeruginosa* in clinical isolates from Saidu Teaching Hospital, Swat. Among the eleven antibiotics tested, ciprofloxacin emerged as the most effective agent, with a susceptibility rate of 72%, followed by amikacin (70%) and ceftazidime (63%). These results suggest that fluoroquinolones and aminoglycosides still hold considerable therapeutic value in treating *P. aeruginosa* infections in this region, especially when guided by susceptibility profiles. The observed multidrug resistance rate of 58% aligns with global concerns about the increasing prevalence of MDR *P. aeruginosa*. High resistance levels were recorded against levofloxacin (67%) and gentamicin (60%), which is consistent with similar studies conducted in other parts of Pakistan and internationally. For instance, a study by (Sujatha, Afaq, & Sameer) reported ciprofloxacin resistance of 54% and levofloxacin resistance of 66% among *P. aeruginosa* isolates, which closely matches our findings. Similarly, (Khatri, Bharty, Prakash, & Chourasiya, 2021) found 54% of isolates resistant to ciprofloxacin, but also noted that approximately 46% remained sensitive, highlighting the regional variability in susceptibility trends. In contrast, a study from India by (Damrolien) noted even higher resistance rates to fluoroquinolones and beta-lactam antibiotics, emphasizing the need for local AMR surveillance. The moderate susceptibility to imipenem (52%) and piperacillin/tazobactam (50%) in our study is slightly lower than reported in earlier studies, suggesting a possible trend of declining carbapenem and extended-spectrum beta-lactam activity. These findings raise concerns about the narrowing spectrum of effective treatment options and reflect the need to preserve the efficacy of last-resort antibiotics through stewardship programs (India & Ramanath; Ojha, Sah, Karn, & Parajuli, 2024). Interestingly, despite high resistance to levofloxacin, many isolates remained susceptible to ciprofloxacin and amikacin. This observation may point to differences in resistance mechanisms and supports the use of combination therapy, particularly in severe or hospital-acquired infections (Siddiquie & Mishra, 2014). Similar patterns were observed by (Ahmed, Kamal, Kamran, & Zaidi, 2016), who emphasized the value of dual-antibiotic strategies in combating

resistant *P. aeruginosa* strains. The resistance pattern observed in this study reinforces the importance of routine culture and sensitivity testing before initiating empirical therapy (Ahmed et al., 2016; Thukral & Saxena, 2019). Empirical use of antibiotics without laboratory confirmation can contribute to the rise of MDR organisms. Therefore, continuous regional monitoring of AMR trends and incorporation of susceptibility data into treatment guidelines are essential for optimizing patient outcomes and minimizing the development of resistance.

## 5. Conclusion

This study highlights the concerning prevalence of multidrug-resistant *Pseudomonas aeruginosa* among clinical isolates in Saidu Teaching Hospital, Swat. While antibiotics such as ciprofloxacin, amikacin, and ceftazidime showed relatively high effectiveness, a significant proportion of isolates exhibited resistance to multiple antibiotic classes, particularly levofloxacin and gentamicin. These findings emphasize the urgent need for regular antimicrobial susceptibility testing, rational antibiotic prescribing, and robust infection control practices. Furthermore, the high rate of resistance underscores the importance of local surveillance data to guide appropriate empirical therapy. Continued monitoring and awareness are essential to combat the growing threat of antimicrobial resistance and ensure effective treatment of *P. aeruginosa* infections in both hospital and community settings.

## Reference

- Ahmad, A., Mustaan, S., Wahab, F., Ayyaz, M., Sadiq, A., & Hussain, I. (2021). Pattern of Antibiotic Resistance in Salmonella Typhi Isolates with Special Consideration to Extended Drug Resistant Typhoid. *Journal of Saidu Medical College*, 11(2), 103-107.
- Ahmad, J., & Ahmad, S. Simultaneous Application Of Non-Antibiotics With Antibiotics For Enhanced Activity Against Multidrug Resistant Pseudomonas Aeruginosa.
- Ahmed, N., Kamal, M., Kamran, A., & Zaidi, A. H. (2016). FREQUENCY OF EXTENDED SPECTRUM BETA LACTAMASES IN ENTEROBACTERIACEAE IN URINARY ISOLATES RELATED TO AGE AND GENDER. *Medical Channel*, 22(2).
- Ali Syed, I., Alvi, I. A., Fiaz, M., Ahmad, J., Butt, S., Ullah, A., . . . Hayat, S. (2024). Synthesis of silver nanoparticles from Ganoderma species and their activity against multi drug resistant pathogens. *Chemistry & Biodiversity*, 21(4), e202301304.
- Aziz, A., Zahoor, M., Aziz, A., Asghar, M., Ahmad, J., & Islam, G. (2022). Identification of Resistance Pattern in Different Strains of Bacteria causing Septicemia in Human at Lady Reading Hospital of Khyber Pakhtunkhwa. *Pakistan Journal of Medical & Health Sciences*, 16(08), 687-687.
- Damrolien, S. Epidemiology and antibiotic resistance pattern of gram-negative bacteria induced urinary tract infections (UTIs) in Manipur, North-East India.
- India, P., & Ramanath, K. Secondary Bacterial Infection in COVID-19 Patients and Associated Risk factors.
- Jamal, A., Ullah, A., Jamal, T., Jamal, A., Muhammad, F., Ullah, S., . . . Basit, A. (2024). Antimicrobial Resistance Pattern of Pseudomonas aeruginosa Isolated from Urine Specimen in Peshawar, Pakistan: Resistance Pattern of Pseudomonas aeruginosa. *Pakistan BioMedical Journal*, 03-08.
- Javed, S., Ahmad, J., Zareen, Z., Iqbal, Z., Hubab, M., Rehman, M. U., . . . Ahmed, S. (2023). Study on awareness, knowledge, and practices towards antibiotic use among the educated and uneducated people of Khyber Pakhtunkhwa Province, Pakistan. *ABCS Health Sciences*, 48, e023218-e023218.
- Khan, S., Fiaz, M., Alvi, I. A., Ikram, M., Yasmin, H., Ahmad, J., . . . Ahmad, A. (2023). Molecular profiling, characterization and antimicrobial efficacy of silver nanoparticles synthesized from



- Calvatia gigantea and Mycena leaiana against multidrug-resistant pathogens. *Molecules*, 28(17), 6291.
- Khatri, S., Bharty, S., Prakash, B., & Chourasiya, K. (2021). Prevalence of Pseudomonas Aeruginosa in Clinical Isolates. *People*, 14(1), 32.
- Kumari, S., Narwal, A. V. S., & Agrawal, S. K. (2020). TO EVALUATE THE PREVALENCE OF PSEUDOMONAS AERUGINOSA AND ITS ANTIMICROBIAL SENSITIVITY PROFILE AMONG POST OPERATIVE WOUND INFECTIONS. *Int J Acad Med Pharm*, 2(3), 334-338.
- Mahaseth, S. N., Chaurasia, L., Jha, B., & Sanjana, R. K. (2020). Prevalence and antimicrobial susceptibility pattern of Pseudomonas aeruginosa isolated from various clinical samples in a tertiary Care Hospital. *Janaki Medical College Journal of Medical Science*, 8(2), 11-17.
- Mangi, S., Phulpoto, A. H., Qazi, M. A., & Kanhar, N. A. (2016). Antibiotic resistance pattern and gender wise prevalence of Pseudomonas aeruginosa strain isolated from the tertiary health care units. *International Journal of Bioscience*, s, 9(5), 173-182.
- Ojha, T. R., Sah, A. K., Karn, G. L., & Parajuli, P. (2024). Antibiotic Susceptibility Pattern of Pseudomonas aeruginosa and Detection of Virulence Genes in a Tertiary Care Hospital, Kathmandu, Nepal. *Nepal Medical College Journal*, 26(3), 188-197.
- Rehman, A., BASHIR, K., FAROOQ, S., KHAN, A., HASSAN, N., SHERAZ, M., . . . ULLAH, A. (2023). Molecular analysis of aminoglycosides and  $\beta$ -lactams resistant genes among urinary tract infections. *Bulletin of Biological and Allied Sciences Research*, 2023(1), 56-56.
- Shah, R., Sarosh, I., Shaukat, R., Alarjani, K. M., Rasheed, R. A., Hussein, D. S., . . . Khan, M. K. (2023). Antimicrobial activity of AgNO<sub>3</sub> nanoparticles synthesized using Valeriana wallichii against ESKAPE pathogens. *Pakistan Journal of Pharmaceutical Sciences*, 36.
- Siddiquie, M. D., & Mishra, R. P. (2014). Age and gender wise distribution pattern of typhoid causing bacteria Salmonella serovars in Mahakaushal region. *World Journal of Pharmaceutical Research*, 3(4), 1183-1203.
- Singh, K. Retrospective study on prevalence of Bacterial isolates from patients of Blood Stream Infection admitted in a tertiary care center.
- Sujatha, R., Afaq, N., & Sameer, D. The Rising Threat of Drug Resistant in Pseudomonas (P. Aeruginosa) in Patients Attending a Tertiary Care Hospital, Kanpur.
- Thukral, S., & Saxena, S. (2019). PREVALENCE OF LOWER RESPIRATORY TRACT INFECTION AMONG PATIENTS IN A TERTIARY CARE HOSPITAL IN NORTH INDIA. *Plant Archives*, 19(2), 2191-2194.
- Uddin, M. N., Jabeen, B., Saadat Mehmood, M., & Rizwan, M. (2018). 15. Characterization of isolated bacteria from burn patients and its susceptibility against different antibiotics in district Swat. *Pure and Applied Biology (PAB)*, 7(1), 121-132.
- URREHMAN, A. B., NAILA, I. U., & JUNAID AHMAD, Z. A. A Review of Global Epidemiology and Antibiotic Resistance of Staphylococcus Aureus.