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26 **Discussion Points:** Did you know the antibiotics we rely on to treat urinary tract infections are losing their
27 effectiveness? Picture a world where our go-to antibiotics can no longer tackle UTIs – it's a scary thought!
28 A recent study has revealed an alarming trend of increasing antibiotic resistance among the bacteria
29 responsible for these pesky infections. According to the study Gram-negative organisms, such as the
30 notorious E. coli, are showing resistance to commonly used UTI antibiotics like trimethoprim-
31 sulfamethoxazole and ciprofloxacin. On the other hand, gram-positive organisms are becoming less
32 susceptible to antibiotics like fluoroquinolones, leaving us with limited options for effective treatment. All
33 this can be a serious threat in future. It's time to take action and prevent a world where UTIs become
34 untreatable monsters! Let's combat antibiotic resistance and ensure a future where our trusty antibiotics
35 can still kick UTIs.

36 #AntibioticResistance #UTI #PublicHealth #WakeUpCall
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1 **ABSTRACT.**

2 **Background:** Bacterial resistance against antibiotics has become a global challenge, and measures are
3 needed to stop this. The aim of this study is to highlight this problem and to determine the antibiotic
4 susceptibility pattern of organisms in this area.

5 **Method:** This descriptive cross-sectional study was conducted in Sheikh Zayed Medical Hospital, Rahim Yar
6 Khan. The urine samples obtained from 4 different wards were sent for culture and sensitivity analysis. 9
7 antibiotics (Nitrofurantoin, Fosfomycin, Ciprofloxacin, Ceftriaxone, Trimethoprim-Sulfamethoxazole,
8 Norfloxacin, Linezolid, Amoxicillin, and Imipenem) were tested against 5 isolated strains of uropathogens using
9 Kirby Bauer disk diffusion test. The sensitivity reports were obtained, and data points were entered into a
10 spreadsheet and analysed using SPSS.

11 **Results:** Out of 101 samples of uropathogens that showed positive growths (42.08%), 53 (52.4%) were from
12 male patients and 48 (47.5%) positive growths were from females. *Escherichia Coli* had the highest positive
13 growths (58%) followed by *Pseudomonas* (19%) *Klebsiella* (13%), *Staphylococcus Aureus* (7%) and
14 *Coagulase-negative staphylococci* (3%). Imipenem was the most sensitive drug whereas the highest resistance
15 by organisms was developed against TMP-SMX. No significant association ($p>0.05$) was found between any of
16 the anti-microbial drugs and *Escherichia coli*, Gram-Positive Uropathogens, and Gram Negative Uropathogens.

17 **Conclusion:** The high increasing rate of broad-spectrum antibiotics resistance suggests that diagnostic and
18 culture tests should be encouraged in hospitals. Based on these test results, appropriate antibiotics should be
19 prescribed. The limitations include the inability to distinguish between nosocomial and community-acquired
20 urinary tract infections and also did not consider other demographic factors like age.

21
22 **Key Words:** Urinary tract infection (UTI); Antibiotic resistance; Drug Resistance, Bacterial; *Escherichia coli*;
23 Imipenem
24

1 INTRODUCTION.

2

3 Antibiotics play a vital role in the treatment of infectious diseases by bacterial stasis or lysis. However, the
4 increased and improper use of antibiotics has led to the development of antibiotic resistance, where bacteria
5 acquire the ability to survive antibiotic treatment.¹ Bacteria employ various mechanisms to develop resistance,
6 including enzyme production, reduced drug sensitivity, and possession of numerous mobilizable genes within
7 bacterial populations. Additionally, patient self-medication, over-prescribing, and incomplete dosing significantly
8 contribute to bacterial resistance.²

9 In the context of antibiotic resistance, Pakistan, as a developing nation, faces the challenge of multiple drug-
10 resistant and extensively drug-resistant bacteria.³ An example of this issue is the synergistic action of
11 Azithromycin and fluoroquinolones in treating co-infections alongside COVID-19, which has led to the
12 associated overuse of antibiotics and the potential for antimicrobial resistance.⁴ Given the prevalent misuse and
13 overuse of antibiotics in Pakistan, understanding susceptibility patterns and developing effective strategies are
14 imperative, especially since there has been no research of this kind conducted in the lower Punjab region,
15 necessitating immediate action to establish efficient measures.

16 Urinary tract infections (UTIs) are prevalent infectious diseases, particularly in developing countries. The
17 emergence of drug resistance among bacterial uropathogens has further complicated the problem, giving rise
18 to antibiotic-resistant species.⁵ The 2017-2018 GLASS (Global Antimicrobial Resistance and Use Surveillance
19 System) report indicated over 70% resistance to ceftriaxone and ciprofloxacin in *Escherichia coli* in Pakistan.
20 To tackle this issue effectively, it is crucial to determine the susceptibility patterns of uropathogens to specific
21 antibiotics to minimize exacerbation of resistance. Moreover, the implementation of antibiotic stewardship
22 programs is essential to enhance healthcare quality and ensure appropriate antibiotic use.⁶ These collective
23 measures will enable healthcare professionals to manage urinary tract infections more effectively, employing a
24 targeted approach that reduces recurrence rates and achieves higher cure rates within shorter durations.

25 The primary objective of our study is to identify UTI causing pathogens and their antimicrobial susceptibility
26 patterns based on gender, gram-staining, and hospital ward. This research aims to improve treatment efficacy
27 and reduce recurrence rates. Additionally, our study seeks to promote culture testing and the practice of
28 appropriate prescription of antibiotics based on culture and susceptibility patterns. By addressing these
29 objectives, we aim to contribute valuable insights that will aid in the development of more effective treatment
30 strategies for UTIs, while also improving appropriate antibiotic prescribing practices.

31

1 **METHODS**

2

3 Study Design and Setting

4 The study was a descriptive cross-sectional study conducted at Sheikh Zayed Medical Hospital in Rahim Yar
5 Khan, Punjab, Pakistan from May to July 2022. The purpose of the study was to investigate the prevalence of
6 urinary tract infections (UTIs) in patients admitted to the medicine, gynecology, surgery, and nephrology wards.
7 These departments were chosen due to the high burden of UTI patients in them, as observed by the researchers
8 during their hospital rotations. The study was evaluated using the STROBE checklist for cross sectional studies.
9 Ethical clearance was obtained from the Institutional Research Board Sheikh Zayed Medical College/Hospital,
10 with the reference No: 479/IRB/SZMC/SZH, and written permission was obtained from the head of each
11 department. The researchers had no potential conflicts of interest, and no external funding for the study.

12 Participants

13 Patients presenting with uncomplicated UTI symptoms (abdominal pain, burning micturition, cloudy or foul-
14 smelling urine) were included in the study, while those at high risk of complications or in critical condition were
15 excluded. Immunocompromised, septic, and patients with other comorbidities like diabetes and those who had
16 taken antibiotics in the last 24 hours were also excluded. This was done as such patients may require different
17 management approaches that may confound the results. This exclusion criterion resulted in a higher proportion
18 of female patients in the study, as the gynecology ward primarily admitted female patients. Simple random
19 sampling was employed.

20 Data Collection

21 Patients included in the study provided informed consent, and their data was collected using a self-developed
22 questionnaire, which included variables such as name, age, gender, and ward name. The questionnaire was
23 pre-tested through trial interviews to improve question-asking methods and variables. Based on previous studies
24 and hospital records, a study sample size of more than 200 was expected between the period of study from
25 May till July 2022.

26 Confirmation of Diagnosis

27 Early morning mid-stream urine samples were collected from the patients and stored in sterile urine collection
28 containers. The urine culture samples were sent to the microbiological culture sensitivity laboratory for analysis.
29 Positive results were determined when significant bacterial growth $> 10^5$ CFU/ml was observed. Colony study
30 and biochemical tests were performed to identify the microorganisms. Some of the disks didn't show positive
31 bacterial growth, which could be due to other microorganisms like fungi, however only UTI causing bacteria
32 were studied in this study.

33 Antibiotic Sensitivity Testing

34 MacConkey agar was used to subculture the colonies and obtain pure growth of the microorganisms. The Kirby
35 Bauer disk diffusion test was conducted to assess the sensitivity of the isolates to ten different antibiotics. The
36 ten antibiotics used in the procedure were furnished separately as discs to the laboratory by the researchers,
37 exclusively for use on these urine samples. The measurement of the zone of inhibition of bacterial growth was
38 performed, and the results were compared with the guidelines of the Clinical and Laboratory Standards Institute
39 (CLSI). All intermediate results were considered as sensitive too.

40 Antibiotics and Sensitivity Reports

1 The organisms were subjected to various groups of antibiotic discs from Oxoid, including Nitrofurantoin (300µg),
2 Fosfomycin (50µg), Ciprofloxacin (5µg), Ceftriaxone (30µg), Trimethoprim/Sulfamethoxazole (1:19 and 25µg),
3 Norfloxacin (5µg), Linezolid (30µg), Amoxicillin (30µg), and Imipenem (10µg). The sensitivity reports of all
4 patients were individually studied. Then the data points were entered into a spreadsheet.

5 Statistical Analysis

6 The data was analyzed using IBM's Statistical Package for Social Sciences (SPSS version 26). Descriptive
7 statistics, including numbers, frequencies, and percentages, were used to describe the data. There was no
8 missing data. Age was the only quantitative variable. Tables and charts were utilized to display the data, and
9 the association between categorical variables was assessed using the Chi-square test. A standard p-value of
10 <0.05 was considered statistically significant. The chi-square test was also conducted to assess the significance
11 of antimicrobial drugs against Gram-positive cocci (GPC) and Gram-negative bacteria separately. No sensitivity
12 analysis was applicable.

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Accepted, in-progress

1 RESULTS.

2
3 The study analyzed 240 urine samples from suspected urinary tract infection cases admitted to the departments
4 of medicine, gynecology, surgery, and nephrology. There was no attrition and no missing data or loss of patients
5 to follow-up between admission and diagnosis and arrival of antimicrobial culture and sensitivity report. Among
6 the 101 positive growths for uropathogens, 53 were male patients, and 48 were female patients. The most
7 common organism was *Escherichia Coli* (*E. coli*) with a growth rate of 58%, followed by *Pseudomonas* (19%),
8 *Klebsiella* (13%), *Staphylococcus Aureus* (*S. Aureus*) (7%), and *Coagulase-negative staphylococci* (*CoNS*)
9 (3%). **Table 1** presents the growth rate of uropathogens and the drugs to which they are sensitive. **Figure 1**
10 illustrates the distribution of microorganisms by gender of patients. The medicine ward had the highest growth
11 rate (45%), followed by gynecology (41.86%), surgery (41.17%), and nephrology (38.75%). The uropathogens
12 were further tested for antimicrobial susceptibility patterns. The results of urine culture analysis from the samples
13 collected in these wards are summarized in **Figure 2**, presenting the growth rates and distribution of these
14 organisms.

15 In this study, a total of 9 antibiotics, including Nitrofurantoin (NF), Ciprofloxacin (CIP), Fosfomycin (FOS),
16 Ceftriaxone (CRO), Trimethoprim-Sulfamethoxazole (SXT), Norfloxacin (NOR), Linezolid (LNZ), Amoxicillin
17 (AMC), and Imipenem (IMP), were checked for their sensitivity and resistance against the 5 organisms: *E. coli*,
18 *Pseudomonas*, *Klebsiella*, *S. Aureus*, and *CoNS*.

19 Among gram-negative organisms, Imipenem showed the highest sensitivity (76%), while the highest resistance
20 was observed against TMP-SMX (74%). The findings of resistance and sensitivity to these antibiotics by all
21 uropathogens in general are summarized in **Figure 3**. Gram-positive cocci demonstrated the highest sensitivity
22 to Imipenem (80%) and the highest resistance to TMP-SMX (80%). Their sensitivity and resistance to other
23 antibiotics is depicted in **Figure 4**. Notably, there was a difference in sensitivity between gram-positive cocci
24 and gram-negative uropathogens, particularly in relation to linezolid, as well as quantitative differences among
25 Amoxicillin, Fosfomycin, and Ceftriaxone.

26 Regarding specific organisms, *E. coli* exhibited the highest sensitivity to IMP (78%) and the highest resistance
27 against SXT (73%). *Pseudomonas* displayed the highest resistance against SXT (79%), while it showed high
28 sensitivity to both LNZ (63%) and IMP (68%). *S. Aureus* isolates exhibited the highest resistance against CIP,
29 SXT, and NOR (71%), while its sensitivity was highest against IMP (71%), followed by FOS and CRO (50%).
30 *Klebsiella* showed the highest sensitivity to IMP (76.92%), followed by LNZ (53.8%), CRO, AMC (46.15%), and
31 NF (38.46%). It displayed the highest resistance against SXT, CIP, and FOS (69.24%). *CoNS* showed maximum
32 sensitivity to IMP (100%) and maximum resistance to SXT (100%) among the few samples that showed its
33 growth. The antimicrobial sensitivity pattern of *E. coli* based on the tissue culture sensitivity report is depicted
34 in **Figure 5**, while **Figure 6** presents the antimicrobial sensitivity pattern for *Pseudomonas*, and **Figure 7** reports
35 the susceptibility pattern of *S. Aureus*.

36 With the chi-square test, no significant association was found between any of the antimicrobial drugs and *E.*
37 *coli* growth or sensitivity to Imipenem. No statistically significant findings were also observed in the efficacy of
38 antimicrobial drugs against Gram-positive cocci (GPC) and Gram-negative bacteria separately.

39

1 DISCUSSION.

2
3 In the present study, we observed an increasing trend of resistance to commonly used antibiotics among
4 uropathogens. The culture of bacteria revealed a positivity ratio of approximately 42%, with multiple isolated
5 strains exhibiting resistance to the tested drugs. The susceptibility pattern differed between gram-positive and
6 gram-negative organisms. Gram-negative organisms showed increasing resistance to traditional urinary tract
7 infection antibiotics such as trimethoprim-sulfamethoxazole, norfloxacin, and ciprofloxacin, while most of them
8 remained susceptible to ceftriaxone, linezolid, and imipenem. Gram-positive organisms demonstrated moderate
9 susceptibility to ceftriaxone, fosfomycin, and amoxicillin, with imipenem being the most effective.
10 Fluoroquinolones and other antibiotics had reduced effectiveness against most gram-positive strains.
11 Coagulase-negative staphylococci were only found in women, and the medicine ward exhibited a higher
12 incidence of urinary tract infections compared to other wards. *E. coli* affected more females than males,
13 indicating a higher risk for females.⁷ Amoxicillin and fosfomycin showed relatively better efficacy against *E. coli*
14 compared to other gram-negative bacteria, while linezolid was less effective. These findings are consistent with
15 previous research conducted in Europe.⁸ *Pseudomonas* displayed high resistance to all antibiotics except
16 imipenem and linezolid. The resistance observed can be attributed to various factors, including self-medication,
17 overprescription, insufficient doses, and inappropriate antibiotic usage.

18 Limitations of this study should be acknowledged. Firstly, the study was unable to differentiate between
19 nosocomial (hospital-acquired) urinary tract infections and those caused through other colonization routes. As
20 the urine cultures were obtained from hospital patients, the identified microbes could represent multi-drug
21 resistant strains present in the hospital environment, acquired by the patients due to stress, immunocompromise
22 states, or other hospital-related factors.⁹ This could give rise to selection bias as the selected population was
23 from hospital settings and did not include patients treated in community settings. Additionally, the male-to-
24 female patient ratio is not truly representative due to some data being primarily collected from the gynaecology
25 ward, which predominantly caters to women patients. The study also did not consider factors such as age,
26 patient awareness, and habits related to self-medication, immune status, socio-economic status, hospital stay
27 and other co morbidities. These variables can act as confounding factors and may create a bias. Human errors
28 during antimicrobial disc sensitivity testing are also possible. Although the chi-square test showed no
29 significance between the usage of any drug and the bacteria's sensitivity or resistance, further analysis with a
30 larger sample size is warranted to confirm these findings. Additional studies should also be undertaken of
31 patients with UTI with comorbidities, to study the disease trends in them.

32 Although the study was well-designed and conducted with ethical considerations, there is a chance of error in
33 the procedures used, such as the disc sensitivity test and the delay between disc application and incubation
34 while measuring the reading zone, creating a risk of measurement bias, This could be reduced by proper placing
35 of the disk and timely measurements of the reading zone.

36 The growth positivity rate observed in the study is within a reasonable range, indicating the proficiency of the
37 antimicrobial laboratory technique with minimal chances of missing potential organism growth in the culture. *E.*
38 *coli* and other gram-negative bacteria are commonly associated with UTIs due to their virulence factors that
39 facilitate colonization and ascending infection in the urinary tract. *E. coli*, in particular, possesses specific Type
40 I fimbriae and P pili containing hemolysin and other toxins, contributing to its pathogenicity in causing urinary
41 tract infections. On the other hand, fewer gram-positive organisms were isolated in urine cultures of UTIs. It is

1 important to note that UTIs can occur in patients within hospitals, even if they were not part of the initial
2 presenting complaint.¹⁰ For instance, post-operative patients in surgery or gynaecology wards, particularly those
3 who underwent caesarean section, were screened for urine culture, and some were found to have infections.
4 The reduced resistance to Imipenem across all bacteria indicates appropriate prescription practices for this drug
5 and suggests a lower prevalence of carbapenemase-producing bacteria, especially among *E. coli* and
6 *Klebsiella*, in the region. However, this also suggests a higher occurrence of extended-spectrum beta-lactamase
7 (ESBL)-producing bacteria, rendering commonly used antibiotics for other infectious diseases less effective.
8 Previous in-vitro studies have also shown increased effectiveness of Imipenem,¹¹ but it is worth noting that
9 research from India indicates resistance to multiple carbapenems.¹² It is important to reconsider the use of
10 Trimethoprim-sulfamethoxazole (TMP-SMX) as an antibiotic choice due to the high rate of resistance against it,
11 despite its supposed activity against *Methicillin Resistant Staphylococcus Aureus* (MRSA) and gram-negative
12 bacteria causing UTIs. Fluoroquinolones, especially the newer generations, were previously considered first-
13 line therapy for complicated UTIs and pyelonephritis. However, a considerable amount of literature now refutes
14 their use in UTIs for various reasons.¹³ This overprescription of fluoroquinolones may have led to the
15 development of resistance not only against these drugs but also against nitrofurantoin, which was commonly
16 used in cystitis. Commonly used drugs like ceftriaxone and amoxicillin show average rates of sensitivity and
17 resistance, indicating that these drugs could become ineffective if they continue to be self-medicated and
18 prescribed without appropriate history and microbial culture. Fosfomycin, on the other hand, still retains some
19 effectiveness against gram-positive strains, possibly due to its lesser usage and lower prevalence.¹⁴ The
20 increasing resistance to linezolid may be attributed to the high prevalence of *methicillin-resistant*
21 *Staphylococcus aureus* (MRSA) and the drug being the first-line treatment for multi-drug resistant organisms,
22 leading to its overuse.

23 The findings from this study have significant implications and can be generalized, particularly among hospital
24 populations worldwide. By looking at the susceptibility pattern of UTI causing pathogens various hospitals in the
25 region should monitor their prescribed antibiotics and reassess their usage. A need for more culture and
26 sensitivity based practice would also be felt to reinforce the better prescription of antibiotics. Such practices will
27 be particularly useful in low income areas or in populations struggling with hygiene, both of which will have
28 significant overlap with the area where the study was conducted as well. *E. coli*, *Klebsiella*, and *Pseudomonas*,
29 which were studied for their antimicrobial susceptibility patterns in urine cultures, are causative agents of both
30 community-acquired and hospital-acquired infections.¹⁵ The increasing resistance to most antibiotics observed
31 in these pathogens not only affects patients with UTIs but also those with other infectious diseases such as
32 pneumonia, diarrhea, and sepsis, who may be prescribed commonly used antibiotics like ceftriaxone,
33 amoxicillin, and linezolid without experiencing improvement due to resistance against them. To further explore
34 these correlations and trends, future studies can investigate the antimicrobial susceptibility patterns of other
35 frequently encountered infectious diseases. It would be valuable to isolate and identify specific strains of
36 microorganisms with these resistance patterns and conduct genomic studies to explore the underlying
37 mechanisms of resistance. Antimicrobial stewardship programs in hospitals should be promoted and
38 implemented, along with systematic reviews of antibiotic prescriptions across all hospital wards.¹⁶ It is crucial to
39 increase awareness among healthcare professionals regarding the importance of microbial culture and
40 sensitivity evaluation and to educate the general population about the risks of self-medication and microbial
41 resistance.¹⁷ Additionally, there is a need for the development of improved diagnostic features and testing

1 methods that can differentiate not only between bacterial and viral infections but also among different bacterial
2 infections. Global collaboration is essential to establish effective management plans based on accurate
3 diagnosis.

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Accepted, in-press

1 SUMMARY - ACCELERATING TRANSLATION

2

3 **Title:** Assessment of Antimicrobial Resistance and Susceptibility Pattern of UTI-causing microorganisms in
4 Southern Punjab, Pakistan.

5 **Main Problem to Solve:** As use of antibiotics becomes commonplace without complete adherence or protocol,
6 bacteria have started to emerge that are resistant to traditionally prescribed antibiotics. This study determines
7 how some urinary tract infection causing organisms have become resistant to some common antibiotics.

8 **Aim of Study:** The aim of the study is to enhance treatment approaches, improve prescription practices,
9 encourage microorganism culture and antibiotic sensitivity testing in hospital settings as well as encouraging
10 antibiotic stewardships. Our goal is to provide valuable statistics regarding resistance patterns of UTI-causing
11 microorganisms in the region as well as raise awareness and encourage health professionals to exercise
12 caution in their prescription decisions and self-prescription among patients.

13 **Methodology:** Early morning mid-stream urine samples were collected from different wards of Sheikh Zayed
14 Hospital, Rahim yar Khan. Informed consent and data like age, and ward name was collected through a self-
15 developed questionnaire. Among those with positive growths were sent to microbiological culture sensitivity
16 laboratory for analysis against 5 common antibiotics: Nitrofurantoin, Ciprofloxacin, Norfloxacin, TMP-SMX,
17 Imipenem, Amoxicillin, Ceftriaxone, Fosfomycin, Linezolid. Kirby Bauer disk diffusion test was conducted to
18 assess the sensitivity of the isolates. Data was analyzed using SPSS and the association between categorical
19 variables was assessed using the Chi-square test. Descriptive statistics, including numbers, frequencies, and
20 percentages, were used to describe the results obtained from culture and sensitivity tests.

21 **Results:** Out of the 101 urine samples tested, 42.08% showed positive growth of bacteria causing urinary tract
22 infections. The most common bacteria found was *Escherichia coli* (*E. coli*), which accounted for 58% of the
23 positive growths followed by *Pseudomonas* (19%) and *Klebsiella* (13%), *Staphylococcus aureus* (*S. aureus*)
24 (7%), and *Coagulase-negative staphylococci* (*CoNS*) (3%).

25 Imipenem was found to be the most effective drug, with the highest sensitivity against bacteria causing urinary
26 tract infections. It showed a sensitivity rate of 80% against Gram-Positive Uropathogens, 78% against *E. coli*,
27 and 76% against gram negative uropathogens. On the other hand, the bacteria showed the highest resistance
28 to TMP-SMX, with resistance rates of 80% in Gram-Positive Uropathogens, 74% in gram-negative
29 uropathogens, and 73% in *E. coli*.

30 No significant association of sensitivity or resistance was found between any of the antimicrobial drugs and *E.*
31 *Coli* or Gram-Positive Uropathogens or Gram Negative Uropathogens

32 **Conclusion:** The study concluded that urinary tract organisms displayed escalating resistance to commonly
33 used antibiotics and antimicrobial stewardship programs are needed in hospitals along with development of
34 improved diagnostic features and testing methods to differentiate among bacterial infections and eventually
35 prescription of antibiotics accordingly.

1 **REFERENCES.**

- 2
- 3 1. Sulis G, Adam P, Nafade V, Gore G, Daniels B, Daftary A, et al. Antibiotic prescription practices in
4 primary care in low- and middle-income countries: A systematic review and meta-analysis. *PLoS Med.*
5 2020;17(6):e1003139.
 - 6 2. Abdelrazik E, El-Hadidi M. Tracking Antibiotic Resistance from the Environment to Human Health.
7 *Methods Mol Biol.* 2023;2649:289–301.
 - 8 3. Bilal H, Khan MN, Rehman T, Hameed MF, Yang X. Antibiotic resistance in Pakistan: a systematic
9 review of past decade. *BMC Infect Dis.* 2021;21(1):244.
 - 10 4. UI Mustafa Z, Salman M, Aldeyab M, Kow CS, Hasan SS. Antimicrobial consumption among
11 hospitalized patients with COVID-19 in Pakistan. *SN Compr Clin Med.* 2021;3(8):1691–5.
 - 12 5. Muzammil M, Adnan M, Sikandar SM, Waheed MU, Javed N, Ur Rehman MF. Study of culture and
13 sensitivity patterns of urinary tract infections in patients presenting with urinary symptoms in a tertiary
14 care hospital. *Cureus.* 2020;12(2):e7013.
 - 15 6. Alemkere G, Tenna A, Engidawork E. Antibiotic use practice and predictors of hospital outcome among
16 patients with systemic bacterial infection: Identifying targets for antibiotic and health care resource
17 stewardship. *PLoS One.* 2019;14(2):e0212661.
 - 18 7. Tamadonfar KO, Omattage NS, Spaulding CN, Hultgren SJ. Reaching the end of the line: Urinary tract
19 infections. *Microbiol Spectr.* 2019;7(3).
 - 20 8. Kot B. Antibiotic Resistance Among Uropathogenic Escherichia coli. *Pol J Microbiol.* 2019;68(4):403–
21 15.
 - 22 9. Sante L, Lecuona M, Jaime-Aguirre A, Arias A. Risk factors to secondary nosocomial bacteremia to
23 UTI in a tertiary hospital. *Rev Esp Quimioter.* 2019;32(4):311–6.
 - 24 10. Kranz J, Schmidt S, Wagenlehner Florian and Schneidewind L. Catheter-associated urinary tract
25 infections in adult patients. *Dtsch Arztebl Int.* 2020;117(6):83–8.
 - 26 11. Karlowsky JA, Lob SH, Kazmierczak KM, Young K, Motyl MR, Sahm DF. In vitro activity of
27 imipenem/relebactam against Enterobacteriaceae and Pseudomonas aeruginosa isolated from
28 intraabdominal and urinary tract infection samples: SMART Surveillance United States 2015-2017. *J*
29 *Glob Antimicrob Resist.* 2020;21:223–8.
 - 30 12. Ravishankar U, P S, Thayanidhi P. Antimicrobial resistance among uropathogens: Surveillance report
31 from south India. *Cureus.* 2021;13(1):e12913.
 - 32 13. Chao YS, Farrah K. Fluoroquinolones for the Treatment of Urinary Tract Infection: A Review of Clinical
33 Effectiveness, Cost-Effectiveness, and Guidelines. Ottawa (ON): Canadian Agency for Drugs and
34 Technologies in Health; 2019.
 - 35 14. Ten Doeschate T, Kuiper S, van Nieuwkoop Cees and Hassing RJ, Ketels T, van Mens Suzan P and
36 van den Bijllaardt W, van der Bij AK, et al. Fosfomycin vs ciprofloxacin as oral step-down treatment for
37 Escherichia coli febrile urinary tract infections in women: A randomized, placebo-controlled, double-
38 blind, multicenter trial. *Clin Infect Dis.* 2022;75(2):221–9.
 - 39 15. Miyoshi-Akiyama T, Tada T, Ohmagari N, Viet Hung N, Tharavichitkul P, Pokhrel BM, et al. Emergence
40 and Spread of Epidemic Multidrug-Resistant Pseudomonas aeruginosa. *Genome Biol Evol.*
41 2017;9(12):3238–45.

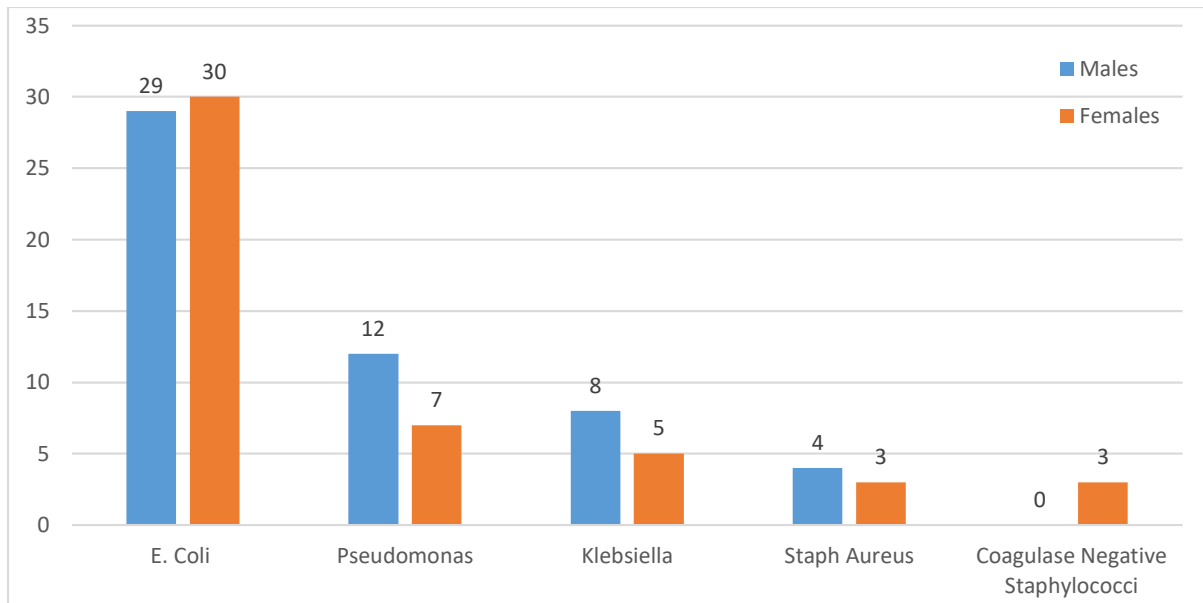
- 1 16. Majumder MAA, Rahman S, Cohall Damian and Bharatha A, Singh K, Haque M, Gittens-St Hilaire M.
2 Antimicrobial stewardship: Fighting antimicrobial resistance and protecting global public health. Infect
3 Drug Resist. 2020;13:4713–38.
4 17. Redfern J, Bowater L, Coulthwaite L, Verran J. Raising awareness of antimicrobial resistance among
5 the general public in the UK: the role of public engagement activities. JAC Antimicrob Resist.
6 2020;2(1):dlaa012.

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1 **FIGURES AND TABLES.**

2

3 **Figure 1.** Distribution of the Grown Microorganisms by Gender of Patients.

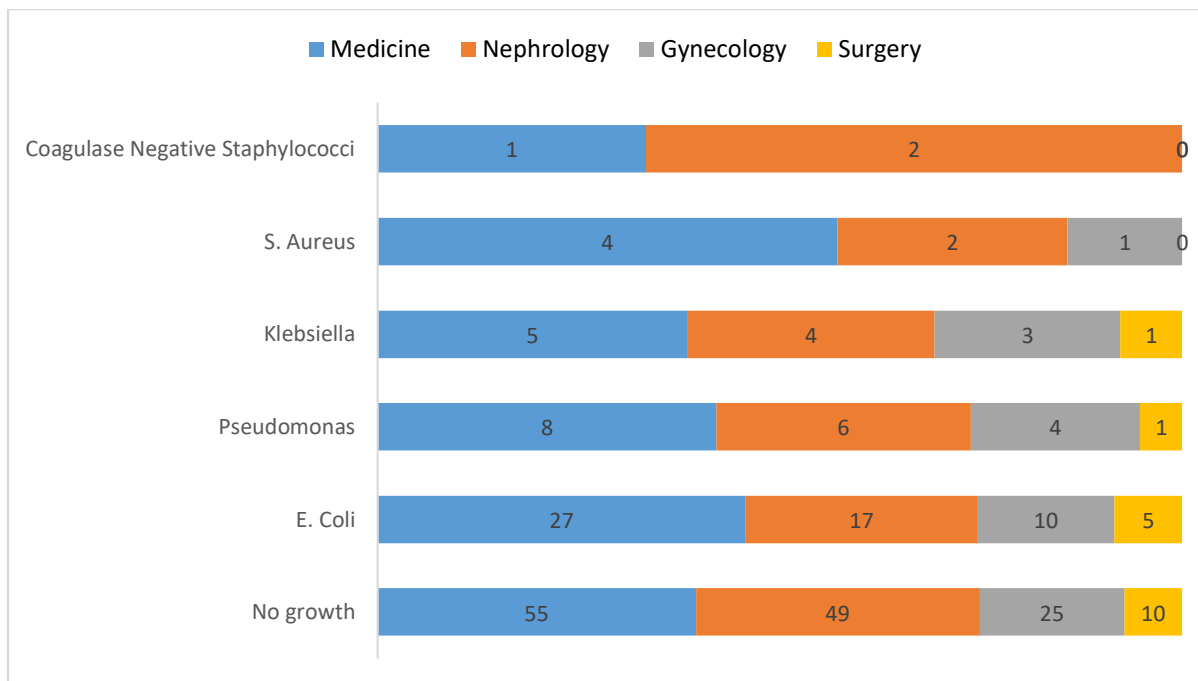


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5 There were 59 patients with the growth of Escherichia Coli (E. coli) out of which 29 were males and 30 were
 6 females. 19 patients with the growth of Pseudomonas out of which 12 were males and 7 were females. 13
 7 patients with the growth of Klebsiella out of which 8 were males and 5 were females. 7 with the growth of
 8 Staphylococcus Aureus out of which 4 were males and 3 were females. 3 with the growth of Coagulase Negative
 9 Staphylococci out of which 0 were males and 3 were females.

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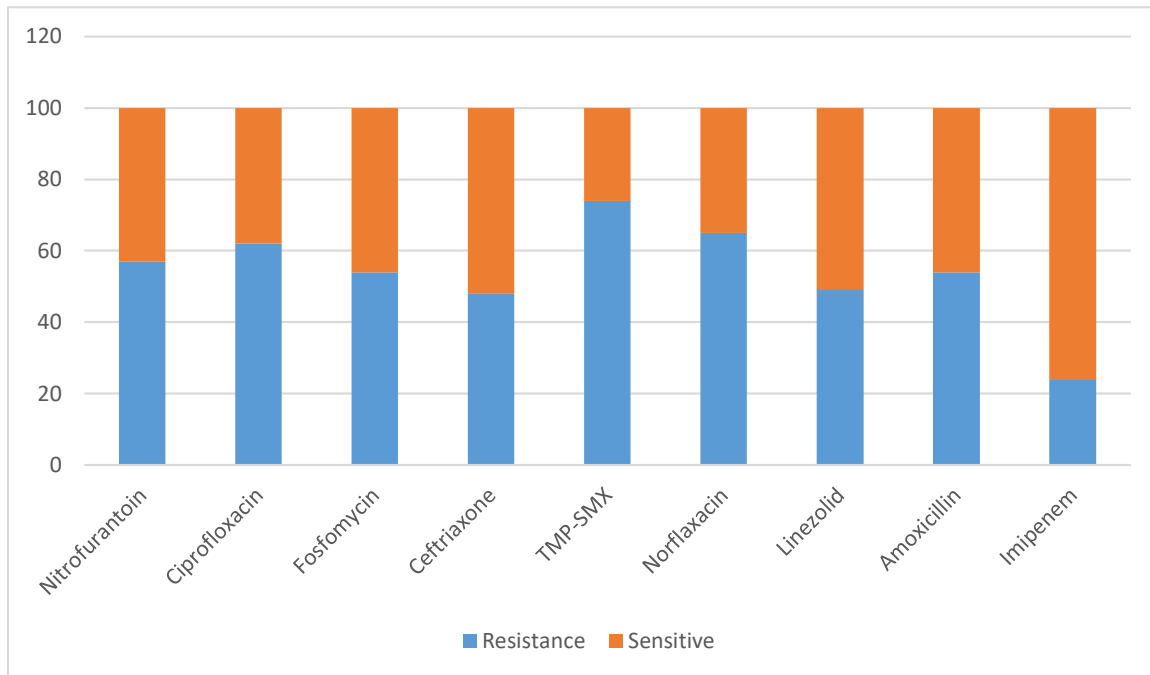
1 **Figure 2.** Growth Results Classified by each Ward from where Samples were Taken.



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3 This table shows the name and the number of uropathogens isolated and grown from urine cultures in each
4 ward. Medicine ward had a total of 45 positive growths, out of which 27 were E. Coli, 8 were Pseudomonas, 5
5 were Klebsiella, 4 were Staphylococcus Aureus and 1 was Coagulase Negative Staphylococci. The zero at the
6 end of Coagulase Negative Staphylococci show that there was none of it in Gynecology or Surgery ward, and
7 no Staphylococcus Aureus causing UTI in Surgery ward.

8

1 **Figure 3.** Effectiveness of Various Antimicrobial Drugs Against Common Gram-Negative Pathogens.



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4 Nitrofurantoin showed 57% resistance and 43% sensitivity. Ciprofloxacin showed 62% resistance and 38%

5 sensitivity. Fosfomycin showed 54% resistance and 46% sensitivity. Ceftriaxone showed 48% resistance and

6 52% sensitivity. Trimethoprim-sulfamethoxazole (TMP-SMX) showed 74% resistance and 26% sensitivity.

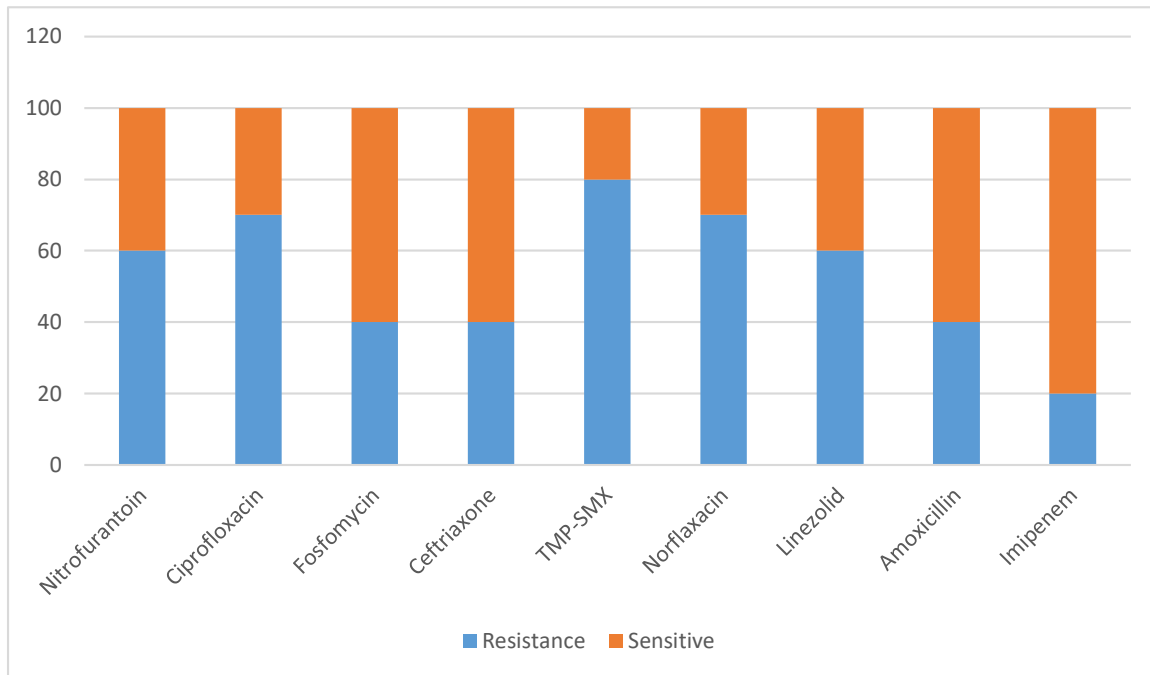
7 Norfloxacin showed 65% resistance and 35% sensitivity. Linezolid showed 49% resistance and 51% sensitivity.

8 Amoxicillin showed 54% resistance and 46% sensitivity and Imipenem showed 24% resistance and 76%

9 sensitivity.

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1 **Figure 4.** Effectiveness of Various Antimicrobial Drugs Against Common Gram Positive Uropathogens.



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4 Nitrofurantoin showed 60% resistance and 40% sensitivity. Ciprofloxacin showed 70% resistance and 30%

5 sensitivity. Fosfomycin showed 40% resistance and 60% sensitivity. Ceftriaxone showed 40% resistance and

6 60% sensitivity. Trimethoprim-sulfamethoxazole (TMP-SMX) showed 80% resistance and 20% sensitivity.

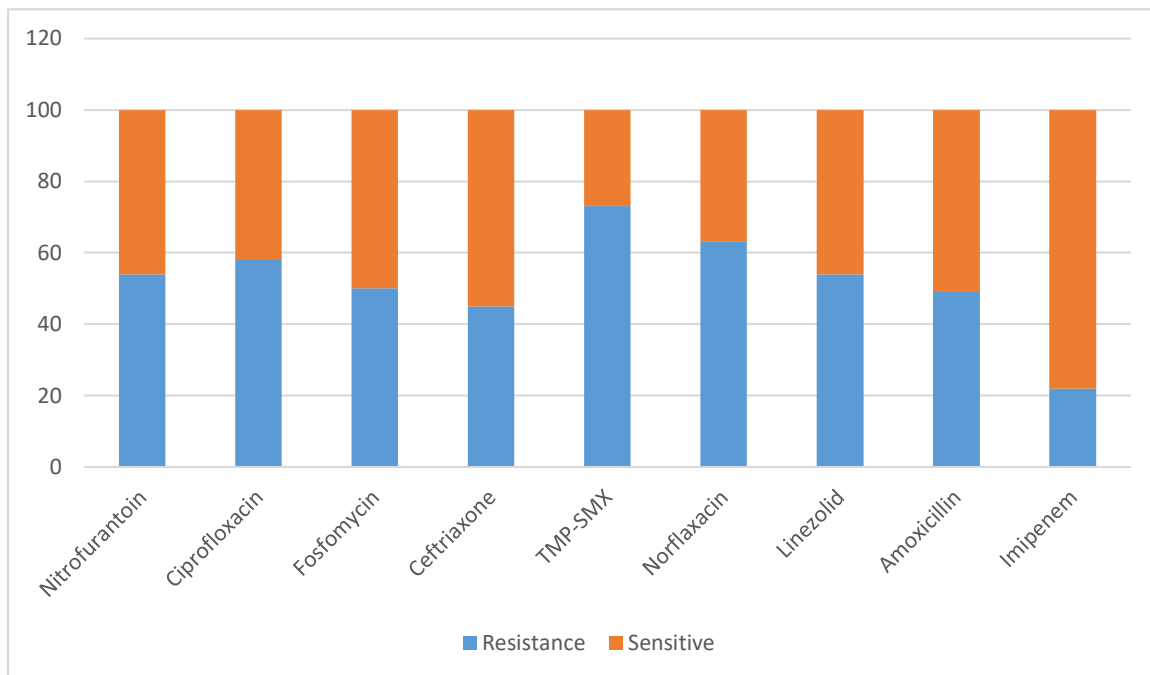
7 Norfloxacin showed 70% resistance and 30% sensitivity. Linezolid showed 60% resistance and 40% sensitivity.

8 Amoxicillin showed 40% resistance and 60% sensitivity and Imipenem showed 20% resistance and 80%

9 sensitivity.

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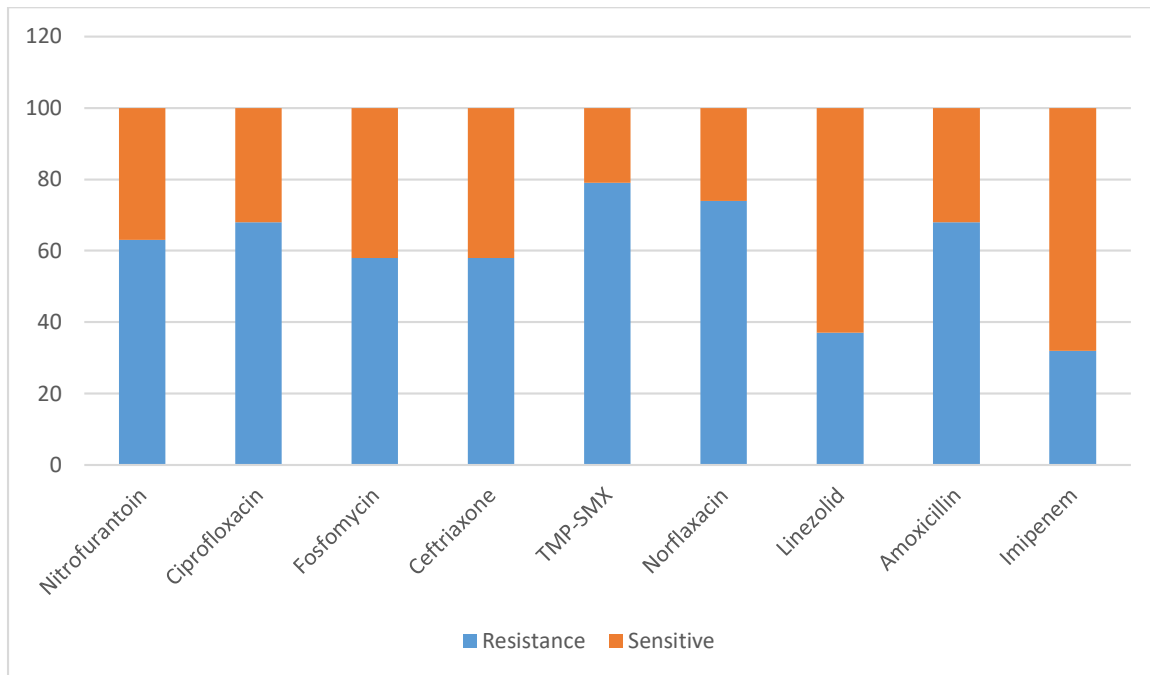
1 **Figure 5.** Antimicrobial Drug Sensitivity and Resistance Pattern for E. Coli.



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Nitrofurantoin showed 54% resistance and 46% sensitivity. Ciprofloxacin showed 58% resistance and 42% sensitivity. Fosfomycin showed 50% resistance and 50% sensitivity. Ceftriaxone showed 45% resistance and 55% sensitivity. Trimethoprim-sulfamethoxazole showed 73% resistance and 27% sensitivity. Norfloxacin showed 63% resistance and 37% sensitivity. Linezolid showed 54% resistance and 46% sensitivity. Amoxicillin showed 49% resistance and 51% sensitivity. Imipenem showed 22% resistance and 78% sensitivity.

1 **Figure 6.** Antimicrobial Drug Sensitivity and Resistance Pattern for Pseudomonas.



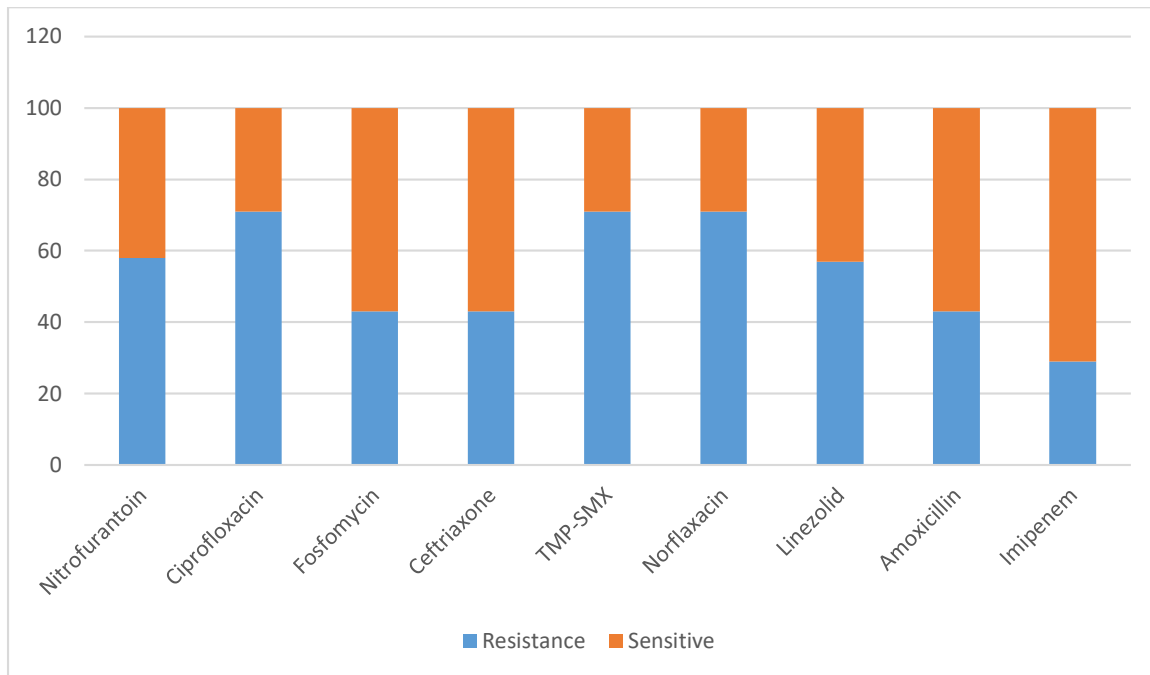
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4 Nitrofurantoin showed 63% resistance and 37% sensitivity. Ciprofloxacin showed 68% resistance and 32%
 5 sensitivity. Fosfomycin showed 58% resistance and 42% sensitivity. Ceftriaxone showed 58% resistance and
 6 42% sensitivity. Trimethoprim-sulfamethoxazole (TMP-SMX) showed 79% resistance and 21% sensitivity.
 7 Norfloxacin showed 74% resistance and 26% sensitivity. Linezolid showed 37% resistance and 63% sensitivity.
 8 Amoxicillin showed 68% resistance and 32% sensitivity and Imipenem showed 32% resistance and 68%
 9 sensitivity.

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1 **Figure 7.** Antimicrobial Drug Sensitivity and Resistance Pattern for *S. Aureus*.



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4 Nitrofurantoin showed 58% resistance and 42% sensitivity. Ciprofloxacin showed 71% resistance and 29%

5 sensitivity. Fosfomycin showed 43% resistance and 57% sensitivity. Ceftriaxone showed 43% resistance and

6 57% sensitivity. Trimethoprim-sulfamethoxazole (TMP-SMX) showed 71% resistance and 29% sensitivity.

7 Norfloxacin showed 71% resistance and 29% sensitivity. Linezolid showed 57% resistance and 43% sensitivity.

8 Amoxicillin (AMC) showed 43% resistance and 57% sensitivity and Imipenem showed 29% resistance and 71%

9 sensitivity.

10

1 **Table 1.** Microorganisms and their Sensitivity in Percentage to Various Antibiotics.

Microorganism	Total Growth	Antibiotic used								
		NF ^a	CIP ^b	FOS ^c	CRO ^d	SXT ^e	NOR ^f	LNZ ^g	AMC ^h	IMP ⁱ
Escherichia Coli	59	45.76	42.37	50.84	55.93	27.11	37.28	45.76	50.84	77.96
Pseudomonas	19	36.84	31.57	42.10	42.10	21.05	26.31	63.15	31.57	68.42
Klebsiella	13	38.46	30.76	30.76	46.15	30.76	38.46	53.8	46.15	76.92
S. Aureus	7	42.85	28.57	57.14	57.14	28.57	28.57	42.85	57.14	71.42
CoNS ^j	3	33.33	33.33	66.66	66.66	0	33.33	33.33	66.66	100
	101									

- 2
- 3 ^a Nitrofurantoin, ^b Ciprofloxacin, ^c Fosfomycin, ^d Ceftriaxone, ^e Trimethoprim-Sulfamethoxazole,
- 4 ^f Norfloxacin, ^g Linezolid, ^h Amoxicillin, ⁱ Imipenem, ^j Coagulase-negative staphylococci (CoNS).
- 5 The numbers indicate in percentage the sensitivity of that uropathogen to the antibiotic used. For instance, in
- 6 E. Coli 45.76% percentage of the positive growth cultures were sensitive to Nitrofurantoin and so on.

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