

## Estimation of Antimicrobial Resistance from Clinical Samples in a Tertiary Care Hospital Settings

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### Abstract:

Antimicrobial resistance (AMR) has emerged as a global public health threat, posing significant challenges to the effective treatment of infectious diseases. An accurate estimation of AMR prevalence is crucial for guiding appropriate antibiotic therapy and implementing effective infection control measures. The methodology involves the isolation and identification of bacteria from clinical samples, followed by susceptibility testing using standard microbiological techniques. The study also includes the identification of commonly encountered bacterial pathogens and their resistance profiles against a panel of antibiotics, encompassing a range of drug classes. Data is analysed to identify trends in resistance, including variations among different pathogens, clinical sample types, and patient demographics. Preliminary findings indicate a concerning increase in AMR, particularly in Gram-negative bacteria such as *Escherichia coli* and *Klebsiella pneumoniae*. Extended-spectrum beta-lactamase (ESBL) and carbapenemase-producing strains are notably prevalent. These findings underscore the urgency of implementing effective infection control measures, antibiotic stewardship programs, and novel therapeutic strategies to combat AMR in the hospital setting. Understanding the dynamics of AMR in a tertiary care hospital is crucial for optimizing treatment protocols and preserving the efficacy of antibiotics. This research contributes to the body of knowledge on AMR and provides valuable insights for healthcare professionals, policymakers, and researchers working towards addressing this critical issue. It is imperative that we continue to monitor and respond to AMR to ensure the sustainability of healthcare delivery and patient safety in the face of evolving resistance patterns. This research paper aims to assess the extent of antimicrobial resistance in clinical samples obtained from a hospital setting and provide insights into the patterns and trends of resistance among common pathogens. The study highlights the need for robust surveillance programs and targeted interventions to combat AMR in hospitals.

**Keywords:** Antimicrobial Resistance; Antimicrobial Stewardship; Antibiotic Susceptibility Test; Hospital-Acquired Infections; Infection Control; Surveillance.

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### Introduction

The increasing prevalence of AMR necessitates continuous monitoring and surveillance of antimicrobial susceptibility patterns in clinical settings. Hospital-acquired infections are of particular concern due to their potential association with multidrug-resistant organisms. This study focuses on estimating the prevalence and patterns of AMR among clinical samples collected from patients in a hospital setting. AMR microbes evolve mechanism that protects them from the effect of antimicrobials. All the classes of microbes can evolve resistance where the drugs are no longer effective. Fungus develops antifungal resistance,

virus develops antiviral resistance, and bacteria develop antibiotic resistance, while protozoa develop antiprotozoal resistance respectively. All these come under antimicrobial resistance. Microbes' resistance to multiple antimicrobials are called multiple drug resistance (MDR).[1] The ability to cure common diseases is becoming danger due to the evolution and spread of multi drug-resistant (MDR) microorganisms that have developed new resistance mechanisms. The rapid global spread of MDR bacteria (superbugs) that cause infections that is not treatable with existing antimicrobial medicines such as antibiotics. In the

clinical pipeline, there are no new antibiotics. In 2019, just six of the 32 antibiotics that treat the priority infections identified by WHO and listed as being in clinical development were deemed new. Access to high-quality antimicrobials continues to be a significant problem. Antibiotic shortages have an impact at all stages of development, but particularly on health care systems. Antibiotics are becoming less and less effective as drug resistance spreads globally, making diseases more difficult to treat and eventually increasing mortality.[2] The prevalence of infectious diseases is among the highest in the world in India, and these conditions are made worse by the country's poor sanitation and nutritional status. There are specific policies or guidelines for the appropriate use of antibiotics currently under various national health programs, such as Integrated Management of Neonatal and Childhood Illness (IMNCI), but these are not available for other diseases of public health importance, such as enteric fever and others.[3][4] National regulations were developed and put into place addressing the restricted use and sale of oseltamivir in the nation during the most recent H1N1 pandemic.[5] Another significant problem is the lack of national data on antibiotic resistance in many infections, with the exception of those for which there is a particular public health program. The country's National Tuberculosis Elimination Program (NTEP) networked its laboratories, which produced some important information on drug resistance in tuberculosis. More recently, the National AIDS Control organization built a network of laboratories for antimicrobial testing of HIV.[6] AMR is a global problem that transcends national boundaries. Infectious diseases can easily spread across countries through travel and trade, making AMR a concern for the entire global community.[7] Addressing AMR requires international collaboration, coordination, and surveillance to prevent the spread of resistant strains. Studying AMR helps inform policy development and interventions.[8] By understanding the factors contributing to AMR, researchers can provide evidence-based recommendations for antibiotic stewardship, infection prevention and control measures, surveillance systems, and the development of new antimicrobial agents. Effective policies and interventions can slow down the emergence and spread of resistant strains.[9]

#### **Aims & Objectives:**

1. To estimate in antimicrobial resistance pattern in hospital setting.
2. Understanding antimicrobial resistance pattern using WHONET software.
3. Preparation antibiogram and its implication.

4. To promote antimicrobial stewardship programs to ensure the appropriate and responsible use of antimicrobial.
5. To encourage the development and implementation of guidelines for prescribing and dispensing antimicrobials to minimize unnecessary or inappropriate use.

#### **Material and methods:**

##### **Study design:**

A retrospective study design to analyse clinical samples obtained from patients admitted to the Shri Vinoba Bhawe Civil Hospital, Silvassa, U.T. of Dadra and Nagar Haveli over a specified period. Data on the identified pathogens and their susceptibility profiles to various antimicrobial agents will be collected from laboratory records. Susceptibility testing will be performed following standardized procedures, such as disk diffusion or broth microdilution, in accordance with established guidelines.

##### **Sample collection and laboratory methods:**

Sample collection in AMR surveillance typically involves the collection of biological specimens from individuals suspected of having infections caused by bacteria or other microorganisms.[10] The goal is to obtain samples that can be tested for the presence of antimicrobial-resistant strains. The specimens include blood, urine, respiratory secretions- sputum or swabs, wound swabs, and stool samples.

Samples are collected different location such as IPD, OPD, ICU using sterile techniques.

##### **Types of samples:**

- Blood- collection of blood through venipuncture using sterile needles and tubes.
- Urine- Mid stream urine sample in a sterile container.
- Sputum- Collected by asking the patient to cough deeply and expectorate into a sterile container.
- Wound swabs- Sterile swabs are used to collect material from the infected area.
- Stool samples- Patients provides stool sample in a sterile container.

##### **Culture Methods:**

Appropriate culture methods applied as per samples following standard culture SOP.

##### **Disc diffusion method:**

Kirby Bauer approach [11] Mueller-Hinton is used in the disc diffusion test. As per Clinical and Laboratory standard Institute (CLSI) and European committee of Antibiotic Susceptibility Test (EUCAST) provide standards. The method of susceptibility testing known as disc diffusion is

said to be the simplest and least expensive. It may also be easily modified to test newly developed antibiotics or formulations.[12]

If the antibiotic or extract is effective against bacteria at a certain concentration, no colonies will grow where the concentration in the agar is greater than or equal to the effective concentration. This is the zone of inhibition. In general, larger zones of inhibition correlate with lower minimum inhibitory concentrations (MICs) of antibiotic or extract for that bacterial strain.

#### Agar plates and inoculum preparation:

MH agar is usually prepared for disc diffusion method. MH agar is poured into the petri plates in sterile condition and allowed to solidify. For inoculum preparation, bacterial culture is inoculated in the saline water using nichrome wire loop according to McFarland Standard which is equal to 150 million cells/ml (approximately). [13]

Under aseptic condition, organism is collected with a sterile swab and streaked across the plates in one direction, rotated 60° and streaked again, rotated another 60° and streaked. Using Flame sterilized forceps, specific antibiotic discs are placed onto the plates and pressed gently to ensure its attachment. These plates are incubated overnight at 35°C.[14]

#### Data analysis Using WHONET:

WHONET is a software tool developed by the World Health Organization (WHO) for the surveillance and analysis of antimicrobial resistance (AMR) in bacteria. It is widely used in

clinical and research settings to manage and analyse data related to AMR. Data entry, maintenance, cleaning, and exploration are the steps for data analysis in WHONET for AMR.

Explore the data with WHONET's built-in capabilities. Summary data, trend visualisation, and simple reports may be needed. Analyse the dataset's antibiotic resistance trends.

After analysis of data, create visualizations such as charts, graphs, and maps to present the findings followed by preparation of comprehensive reports to summarize the findings. Continuous Monitoring and surveillance is essential to regularly update the database.

Use the analysis results to inform decisions about antimicrobial prescribing, infection control measures, and public health interventions.

By following these steps, you can effectively analyse AMR data using WHONET and contribute to the understanding and management of antimicrobial resistance.

#### Results:

Total no. of isolates was 1,453. Out of which 628 (43.2%) and 825 (56.8%) were from female and male patients respectively. Maximum no. of patients was from age group of 25-34 and 15-24 i.e., 14.9% and 13.4% respectively. Out of 1453 isolates 622 (42.80%) were from intensive care unit, 660 (45.40%) were from In-patient department and 171 (11.80%) were from Out-patient department as mentioned Table 1.

**Table 1: Number of isolates related to different parameters**

| Variables             | No. of isolates | Percentage (%) |
|-----------------------|-----------------|----------------|
| <b>Age (in years)</b> |                 |                |
| <1                    | 396             | 27.3           |
| 1-4                   | 31              | 2.1            |
| 5-14                  | 58              | 4.0            |
| 15-24                 | 194             | 13.4           |
| 25-34                 | 217             | 14.9           |
| 35-44                 | 166             | 11.4           |
| 45-54                 | 111             | 7.6            |
| 55-64                 | 123             | 8.5            |
| 65-74                 | 111             | 7.6            |
| 75-84                 | 41              | 2.8            |
| 85+                   | 05              | 0.3            |
| <b>Location</b>       |                 |                |
| ICU                   | 622             | 42.80          |
| IPD                   | 660             | 45.40          |
| OPD                   | 171             | 11.80          |
| <b>Sample</b>         |                 |                |
| Blood                 | 437             | 30.1           |
| Cerebrospinal fluid   | 01              | 0.1            |
| Fluid                 | 121             | 8.3            |
| Gastric fluid         | 05              | 0.3            |
| Pus                   | 290             | 20.0           |

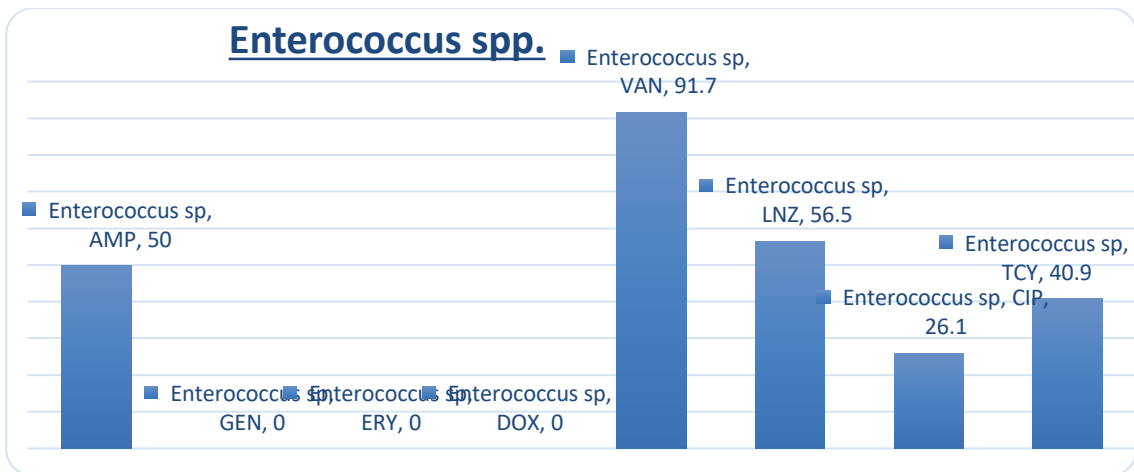
|        |     |      |
|--------|-----|------|
| Sputum | 86  | 5.9  |
| Stool  | 41  | 2.8  |
| Swab   | 126 | 8.7  |
| Tissue | 14  | 1.0  |
| Urine  | 327 | 22.5 |

**Organism frequencies**

The graph given below represents the average no. of isolates of gram-positive organisms and the result of AST.

1. **Enterococcus spp.** There were 24 total isolates (1.4%) from 24 patients (14 females and 10 males).

ICU, IPD, and OPD isolates obtained 8, 13, and 3 isolates, respectively. Enterococcus isolates were showing high susceptibility towards vancomycin (91.7%), followed by linezolid (56.5%), ampicillin (50%) and 100% resistant to gentamycin, erythromycin, doxycycline followed by ciprofloxacin (73.9%), tetracycline (59.1%).



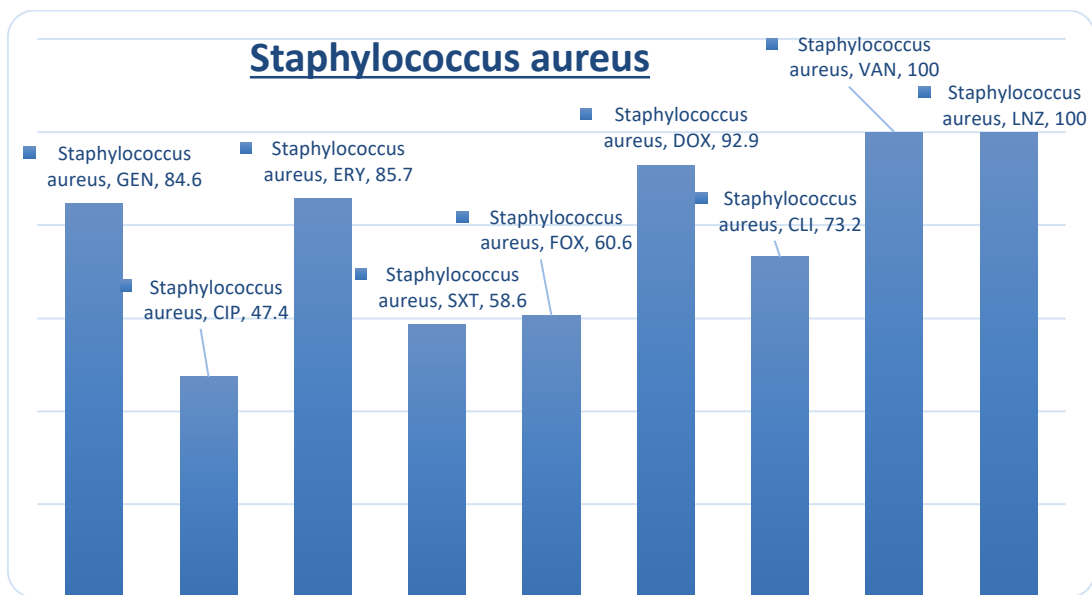
**Graph 1: AST representation of Enterococcus sp.**

**2. Staphylococcus aureus**

A total of 99 (6.8%) isolates were obtained from 90 patients (38 female and 53 male).

Isolates from the IPD, OPD, and ICU were 19,60,15. Staphylococcus aureus showed 100%

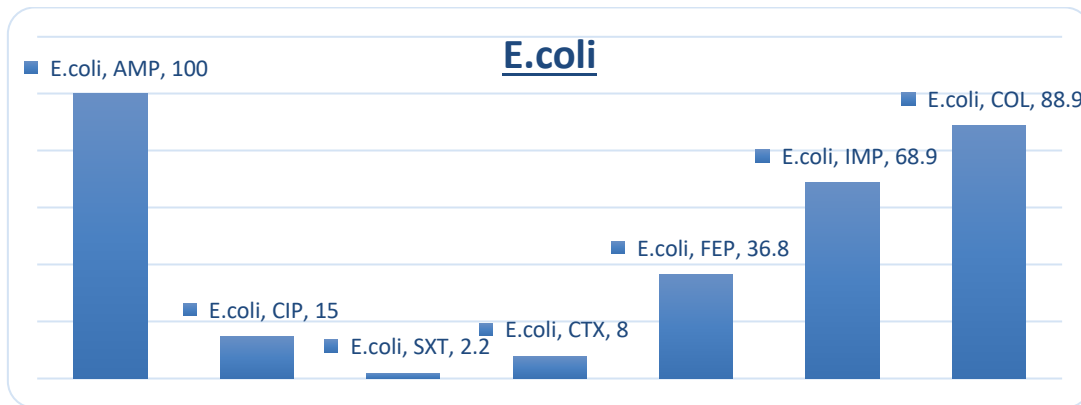
high susceptibility towards vancomycin, linezolid followed by doxycycline (92.9%), erythromycin (85.7%), gentamycin (84.6%), clindamycin (73.2%), Fosfomycin (60.6%), trimethoprim sulfamethoxazole (58.6%) and resistant to ciprofloxacin (52.6%).



**Graph 2: AST representation of Staphylococcus aureus.**

**3. E. coli:** There were 89 total isolates (6.1%), from 83 patients (47 females and 36 males). Isolates from the ICU, OPD, and IPD were 11,11,67. E. coli showed 100% sensitivity towards ampicillin

followed colistin (88.9%), imipenem (68.9%) and resistant to trimethoprim sulfamethoxazole (97.2%), cefotaxime (92%), ciprofloxacin (85%), ceftazidime (63.2%).

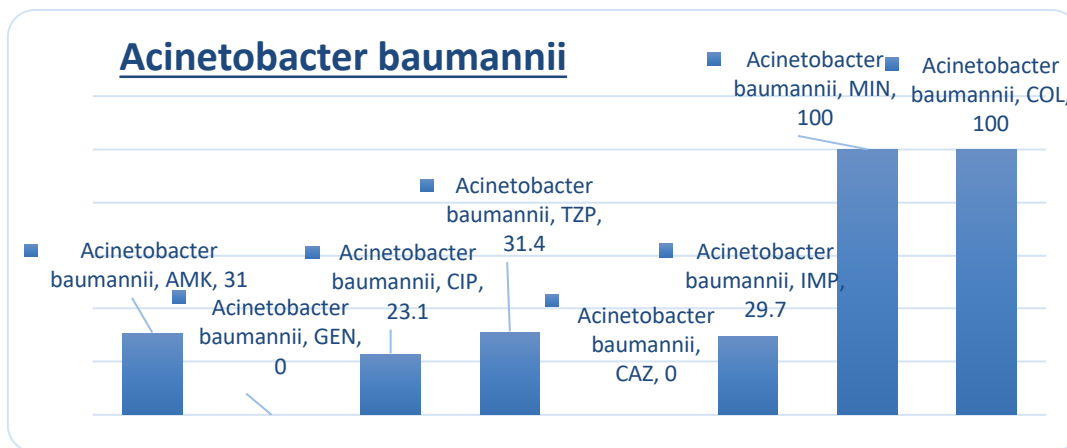


Graph 3: AST representation of E. coli

**4. Acinetobacter sp.**

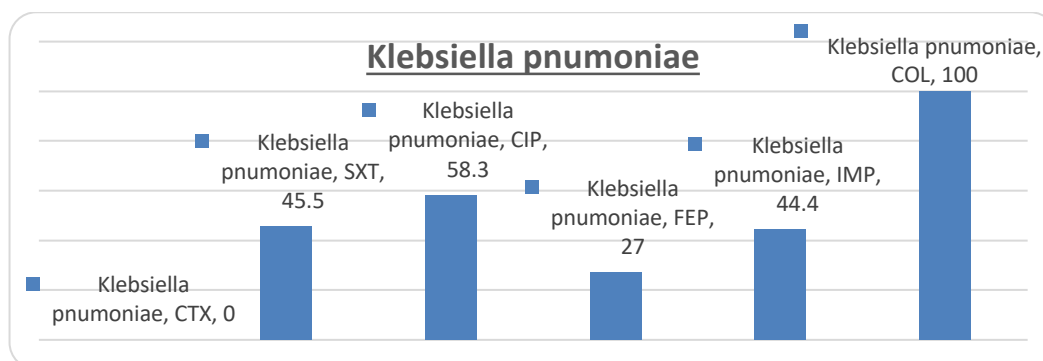
There were 83 patients (47 females and 36 males), resulting in 89 isolates overall (6.1%). There were 11,11,67 isolates from the ICU, OPD, and IPD. Acinetobacter baumannii Showed 100%

susceptibility towards Minocycline and colistin and high resistant towards gentamycin, ceftazidime followed by ciprofloxacin (76.9%), imipenem (70.3%), amikacin (69%), piperacillin tazobactam (68.6%)



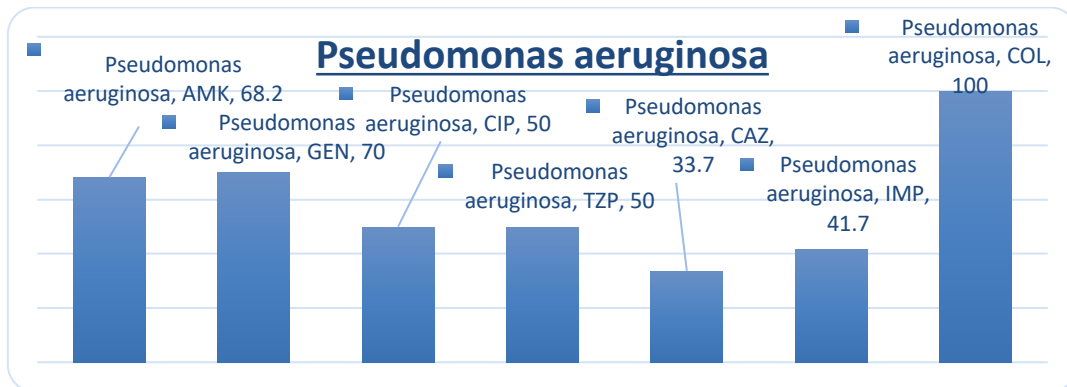
Graph 4: AST representation of Acinetobacter baumannii

**5. Klebsiella pneumoniae:** There were 81 (5.6%) total isolates from 76 patients (33 females and 43 males), with 25,47,6 of these isolates emerging from the ICU, IPD, and OPD, respectively. Klebsiella pneumoniae have shown 100% sensitivity to Colistin and ciprofloxacin (58.3%) and high resistance towards ceftazidime (73%), imipenem (55.6%), trimethoprim sulfamethoxazole (54.5%)



Graph 5: AST representation of Klebsiella pneumoniae

**6. Pseudomonas aeruginosa:** Total number of isolates was 58 (4%), with 13 female and 39 male patients. Isolates from the ICU, IPD, and OPD, respectively, were 11, 26, and 15. Pseudomonas aeruginosa showed susceptibility towards colistin (100%), gentamycin (70%), amikacin (68.2%), ciprofloxacin (50%), piperacillin tazobactam (50%) and resistant to ceftazidime (66.3%) and imipenem (58.3%).



**Graph 6: AST Representation of Pseudomonas aeruginosa**

## Discussion

Antimicrobial resistance (AMR) is a serious global health threat that occurs when bacteria, viruses, fungi, and parasites evolve to resist the drugs used to treat infections. This phenomenon has led to the emergence of "superbugs" that are increasingly difficult, if not impossible, to treat. AMR is driven by the overuse and misuse of antibiotics and other antimicrobial drugs in both humans and animals. In addition, poor infection prevention and control practices, inadequate sanitation and hygiene, and lack of access to clean water also contribute to the spread of AMR. The consequences of AMR are dire, including increased morbidity and mortality, longer hospital stays, higher healthcare costs, and reduced productivity. Without urgent action, AMR could lead to a future where common infections are no longer treatable, and routine medical procedures become life-threatening.

This study examined prevalence of antimicrobial resistant among main pathogenic organisms isolated from different locations of the hospital. The result of this study revealed that among 1,453 cultures from different samples, 657 and 82 cultures have shown no growth and no pathogen respectively. 575 cultures were positive from which bacteria were isolated. In this study, Staphylococcus aureus 99(6.8%) and E.coli 89(6.1%) were most frequent pathogens. This lines up with the study conducted by Mahmoudi et al., 2017[14]. Ciprofloxacin was the only drug common in all the isolates which showed 85% resistance to E. coli, 76.91% resistance to Acinetobacter baumannii, 73.9% resistance to Enterococcus spp., 52.6% resistance to Staphylococcus aureus, 58.3% resistance to Klebsiella pneumoniae and 50RS% to Pseudomonas aeruginosa. Our study revealed that Pseudomonas aeruginosa have shown resistance towards maximum number of antibiotics and

susceptible to only colistin (100%) and gentamycin (70%) whereas Staphylococcus aureus was resistant to only ciprofloxacin (52.6%) and 100% susceptible to vancomycin and linezolid which is similar to the study conducted by (Pelegrin et al., 2021) and (Diekema et al., 2001). [15] [16]

Enterococcus spp. Showed 100% resistant towards gentamycin, erythromycin and doxycycline and shown higher susceptibility towards vancomycin (91.7%). In other hand Acinetobacter baumannii have also shown 100% resistance to gentamycin and ceftazidime and 100% susceptible to colistin and minocycline which line up with the study conducted by (Kateete et al., 2019) and (Okasha, Asser and Kholeif, 2023). [17] [18]

E.coli showed 100% susceptibility towards ampicillin followed by colistin (88.9%), imipenem (68.9%) and showed high resistant towards trimethoprim/sulfamethoxazole (97.8%), cefotaxime (92%) followed by ciprofloxacin (85%) and cefepime (63.2%). Klebsiella have also shown high resistant towards cefotaxime (100%), cefepime (73%) followed by imipenem (55.6%) and high susceptibility towards colistin (100%) followed ciprofloxacin (58.3%). This result correlates with the study conducted by (Karki et al., 2021). [19] The assessment of antimicrobial resistance (AMR) from clinical samples within a tertiary care hospital setting reveals a challenging landscape that demands immediate attention and intervention. Our study has illuminated the increasing prevalence of resistant microorganisms, particularly among Gram-negative bacteria, such as Escherichia coli and Klebsiella pneumoniae. The emergence of extended-spectrum beta-lactamase (ESBL) and carbapenemase-producing strains poses a significant threat to patient care, as it limits the effectiveness of conventional antibiotics.

These findings emphasize the critical need for a comprehensive, multifaceted approach to combat AMR in the hospital environment. In this battle against resistance, infection control measures must be stringent, and antibiotic stewardship programs should be actively implemented to ensure the responsible use of antimicrobial agents. Moreover, the development of novel therapeutic strategies, including the exploration of new antibiotics and alternative treatment modalities, is imperative to address the evolving resistance patterns.

Continued surveillance and research are essential to monitor and understand the dynamics of AMR within healthcare institutions. Such insights provide the foundation for informed decision-making by healthcare professionals and policymakers. They are also essential for the development of strategies that can mitigate the impact of AMR, preserve the efficacy of antibiotics, and ultimately enhance patient safety.

### Conclusion

In conclusion, the study underscores the urgency of addressing AMR as a priority in healthcare. Failure to do so not only jeopardizes patient well-being but also poses a substantial economic burden. Tertiary care hospitals must remain vigilant in the face of this global health crisis, as effective management of AMR requires a concerted effort involving healthcare providers, researchers, and policymakers.

By adopting a proactive and collaborative approach, we can hope to curb the rising tide of antimicrobial resistance and secure a safer future for patient care.

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