



EVALUATION OF ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF BACTERIAL PATHOGENS IN CLINICAL SPECIMENS.

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ABSTRACT

BACKGROUND: The prevalence of bacterial resistance to antibiotics has escalated in recent years. Resistance against Gram-negative bacteria (GNB) and Gram-positive bacteria (GPB) has been documented. **OBJECTIVE:** This study investigated the frequency, antibiotic susceptibility, and resistance mechanisms of clinical sample isolates from Department of Pathology. **MATERIAL AND METHOD:** This study investigated the frequency, antibiotic susceptibility, and resistance mechanisms of clinical sample isolates from Department of Pathology. Aseptically collected 793 bacterial isolates from wound swabs, urine, blood, central venous pressure line tips, and respiratory specimens to grow on CLED or Blood agar for 24 hours at 37°C. The isolate will be identified using microbiological methods after culture. **RESULT:** From wound samples, which included pus, swabs, and tissue, 237 bacterial isolates were identified: *Enterococcus* species (n=59, 24%), *Enterobacterales* (n=148, 62%), and *Pseudomonas aeruginosa* (n=30, 12%). The urine sample produced a uniform distribution and quantity of isolates. In blood and CVP line tip cultures, 168 bacterial isolates were detected, including *Acinetobacter* species (n=37, 22%), *Enterobacterales* (n=56, 33%), and *Salmonella Typhi* (n=75, 44%). Included were *Escherichia coli* (n=8, 5.2%), *Acinetobacter* species (n=46, 30%), *Klebsiella pneumoniae* (n=20, 13.2%), and *Pseudomonas aeruginosa* (n=77, 50%). Whereas Ciprofloxacin resistance varied from 65–68% across locations, pus swab and urine isolates were resistant to Penicillin (75%) and Ampicillin (97%). Respiratory samples were moderately resistant to Ciprofloxacin (65.2%) and Gentamicin (52.8%) but low to Polymyxin B (6.2%) and Tigecycline (13.7%). These results show extensive first-line antibiotic resistance and continued vulnerability to last-resort medicines. **CONCLUSION:** This study shows that diverse clinical specimens often resist common antibiotics. The effectiveness of last-resort medicines like Polymyxin B and Tigecycline shows their importance in treating multidrug-resistant diseases.

KEYWORDS: Drug Resistance, Bacterial, Microbial Sensitivity Tests, Gram-Negative Bacterial Infections

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INTRODUCTION

Microbial infectious illnesses profoundly impact human health. Fortunately, antibiotic medications have alleviated human bacterial illnesses since the discovery of penicillin by Alexander Fleming in 1928¹. Antimicrobial resistance (AMR) has emerged as a significant global health problem, particularly in sub-Saharan Africa, where the prevalence of infectious illnesses is substantial. Prevalent diseases such as pneumonia, diarrhoea, sexually transmitted infections, surgical infections, and TB are progressively growing resistant to treatment. Recent evidence indicates that around 700,000 deaths yearly are related to antimicrobial resistance (AMR) infections, with projections suggesting a rise to 10 million deaths per year by 2050 if current trends continue².

Antibiotic resistance (AR) arises from natural, acquired, or clinical mechanisms and refers to the capacity of pathogenic bacteria to withstand the effects of antibiotic drugs³. Natural or intrinsic resistance refers to the inherent ability of bacteria to withstand antibiotics without previous exposure or horizontal gene transfer, whereas acquired resistance arises from intrinsic gene mutations following exposure to specific mutagens, antibiotics, or through horizontal genetic exchange⁴. The clinical emergence of antimicrobial resistance (AMR) is predominantly caused by non-adherence to recommended antimicrobial medication, which fosters spontaneous mutations in chromosomal or regulatory genes, resulting in the development of novel mutant pathogens under selection pressure from antimicrobials⁵. Need the use of broad-spectrum antibiotics as the definitive

option. The advent and proliferation of multi-drug resistant bacteria, which are difficult to treat with existing medicines, is particularly concerning. This encompasses the advent and proliferation of extended-spectrum beta-lactamases (ESBL), AmpC beta-lactamases, and carbapenemase-producing Gram-negative bacteria (i.e., carbapenem-resistant Enterobacteriaceae - CRE) and *Staphylococcus aureus* (MRSA), which are fast escalating globally⁶.

A study conducted by the Centers for the Control of Diseases (CDC) revealed that there are an estimated 50,985 cases of *P. aeruginosa* infections associated to healthcare in the United States annually. Out of these cases, over 6,100 (13%) are caused by strains of the bacteria that are resistant to multiple drugs⁷. Denmark, the UK, and Australia have colonistin resistance. Multiple treatments were used to treat *P. aeruginosa* infections. As a result, giant studies has been committed to the improvement of novel antibiotic combos for the remedy of multidrug resistant *P. aeruginosa* disease and infection⁸. Patients admitted to a room previously occupied by an individual infected with *Clostridium difficile*, *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Acinetobacter baumannii*, or vancomycin-resistant *Enterococci* (VRE) face up to a 70% likelihood of acquiring these pathogens during their hospital stay⁹.

Due to the significant prevalence of diseases, inappropriate antibiotic usage, the over-the-counter accessibility of medications, and constraints in antimicrobial susceptibility testing, drug resistance poses a substantial concern in

low-income nations¹⁰. The majority of the isolates exhibited resistance to ampicillin, amoxicillin combined with clavulanic acid, amoxicillin, tetracycline, and cotrimoxazole antimicrobials. The prevalence of multidrug-resistant isolates is exceedingly high. Consequently, it is advisable to implement stringent antibiotic utilisation rules within the hospital and to assist doctors in the judicious selection of antibiotic therapy, while routinely updating the list and credible sources of medications^{11,12}. This study aims to analyze the antibiogram profiles of bacterial isolates from various clinical specimens such as pus, urine, blood, and respiratory tract samples—to ascertain the prevalence of multidrug-resistant organisms and facilitate the formulation of evidence-based antibiotic stewardship strategies in clinical practice.

MATERIAL AND METHOD

The cross sectional study of antibiotic resistance patterns of bacterial pathogens isolated from clinical specimens including urine, blood and CVP line tips, and respiratory samples (sputum, tracheal aspirate, endobronchial (EB) washings, non-bronchoscopic lavage (NBL), and bronchoalveolar lavage (BAL), gathered from patients at Department of Pathology. Patients of all ages and genders from whom clinical specimens were taken; urine, blood, pus, swabs, tissue, and respiratory samples (sputum, tracheal aspirate, EB washes, NBL, BAL) after informed consent and Specimens with no bacterial growth or those contaminated during processing and Isolates exhibiting inadequate antibiotic susceptibility data was excluded from the study after approval from Institutional Ethics Review Committee. A total of 793 bacterial isolates were obtained using WHO sample size calculator by putting the variable the confidence level taken is 95% (26) from various specimen types, including wound/pus swabs, urine, blood, central venous pressure line tips, and respiratory specimens. From pus/pus swab tissue and Urine sample, 237 bacterial

isolates. From blood and CVP line tip cultures, 168 bacterial isolates were generated. Comprising sputum, tracheal aspirate, endobronchial (EB) washing, nasopharyngeal lavage (NBL), and bronchoalveolar lavage (BAL), and respiratory specimens produced 151 bacterial isolates overall. Samples were cultivated on Blood Agar, MacConkey Agar and CLED media then incubated for 24 to 48 hours at 37°C. Standard biochemical assays and selective media verified individual bacterial pathogen.

In compliance with CLSI 2023 guidelines, the disc diffusion method employs a 0.5 McFarland turbidity standard and inoculation on Mueller-Hinton agar (BD, USA) or blood-enriched Mueller-Hinton agar¹³. Among the antibiotics examined were **Ampicillin** (AMP), Amoxicillin-Clavulanate (AMC), Cefixime (CFM), Ceftriaxone (CRO), Ceftazidime (CAZ), Piperacillin-Tazobactam (TZP), Imipenem (IMP), Meropenem (MEM), Ciprofloxacin (CIP), Trimethoprim-Sulfamethoxazole (SXT), Gentamicin (GEN), Amikacin (AK), Tigecycline (TGC), Polymyxin B (PB), Doxycycline (DOX), Linezolid (LNZ), Vancomycin (VAN), Nitrofurantoin (NIT), and Fosfomycin (FOS).

All gathered data were originally inputted and sanitised with Epi Info™ version 7, a statistical software suite created by the Centers for Disease Control and Prevention (CDC) for epidemiological assessment. The dataset was meticulously examined for completeness, consistency, and precision. Duplicates, missing values, and anomalies were found and rectified throughout the cleaning process to assure data integrity. The cleansed data then were imported into RStudio version 3.6.0 for advanced statistical analysis and data visualization. To aggregate the resistance patterns of bacterial isolates to different antibiotics, descriptive statistics comprising frequencies, percentages, means, standard deviations, minimum and maximum values, were computed. Analyzed across several

specimen types (e.g., urine, blood, respiratory samples) and bacterial species were antibiotic susceptibility profiles. Histogram and boxplots visualized resistance data helped to clearly show patterns and variations in antibiotic resistance. To further grasp the spread and strength of antibiotic resistance, the study additionally stratified by organism type and specimen source.

RESULT

A total of 237 bacterial isolates were isolated from pus, swabs, and tissue: *Enterococcus* species (n=59, 24%), *Enterobacterales* (n=148, 62%), and *Pseudomonas aeruginosa* (n=30, 12%). The urine sample yielded a consistent

distribution and number of isolates. A total of 168 bacterial isolates were identified in blood and CVP line tip cultures, comprising *Acinetobacter* species (n=37, 22%), *Enterobacterales* (n=56, 33%), and *Salmonella Typhi* (n=75, 44%). A total of 151 isolates were acquired from respiratory specimens, including sputum, tracheal aspirate, endobronchial wash, nasopharyngeal lavage, and bronchoalveolar lavage. Included were *Escherichia coli* (n=8, 5.2%), *Acinetobacter* species (n=46, 30%), *Klebsiella pneumoniae* (n=20, 13.2%), and *Pseudomonas aeruginosa* (n=77, 50%) shown in the table 1.

Table 5: Frequency and percentage distribution of bacterial isolates across pus/tissue swabs, blood and CVP line tips, and respiratory specimens

Sampling isolation	Bacterial isolates	Frequency and Percentage
Pus, swabs, and tissue	<i>Enterococcus</i> species	24% (n=59)
	<i>Enterobacterales</i>	62% (n=148)
	<i>Pseudomonas aeruginosa</i>	12%(n=30)
Blood and CVP line tip cultures	<i>Acinetobacter</i> species	22% (n=37)
	<i>Enterobacterales</i>	33% (n=56)
	<i>Salmonella Typhi</i>	44% (n=75)
Respiratory specimens	<i>Escherichia coli</i>	5.2% (n=8)
	<i>Acinetobacter</i> species	30% (n=46)
	<i>Klebsiella pneumoniae</i>	13.2% (n=20)
	<i>Pseudomonas aeruginosa</i>	50% (n=77)

The data were distributed in four age groups in which minimum age was 12 while maximum age was 80 and the mean of age were 56.8. The age group distribution is highest among the 61 to 80 years with 452 patients, followed by the 41 to 60 years with 222 patients, and the 21 to

40 years with 71 patients. The least cases were observed in the 1 to 20 years age group, consisting of 48 patients (Table 1).

Table 1. Age-wise Distribution of the Samples

Age Group	No. of Patients
1 – 20	48 (6%)
21- 40	71 (8%)
41-60	222 (28%)
61-80	452 (57%)

Gram-positive isolates of *Staphylococcus aureus* showed high resistance to penicillin (98%), ampicillin (95%), and erythromycin (85%), while MRSA strains were intrinsically resistant to β -lactams and exhibited significant resistance to ciprofloxacin (76%) and erythromycin (54%). Both organisms were very sensitive to linezolid ($\leq 5\%$) and vancomycin ($\leq 2\%$). Different species of Gram-negative

microbes had different antibiotic resistance patterns. *Pseudomonas aeruginosa* was antibiotic-resistant, especially to Ciprofloxacin, Piperacillin–Tazobactam, and Meropenem. *Klebsiella pneumoniae* and *Escherichia coli* showed resistance to β -lactam antibiotics - cefixime (CFM), ceftriaxone (CRO), and amoxiclav (AMC) - but less so to polymyxin B (PB) and tigecycline (TGC). *Acinetobacter* species repeatedly showed strong resistance to most antibiotics, notably amoxiclav, cefixime, and cotrimoxazole (SXT), indicating its multidrug resistance. Several isolates responded better to aminoglycosides like amikacin (AK) and carbapenems like imipenem (IMP) shown in the figure (1, 2).

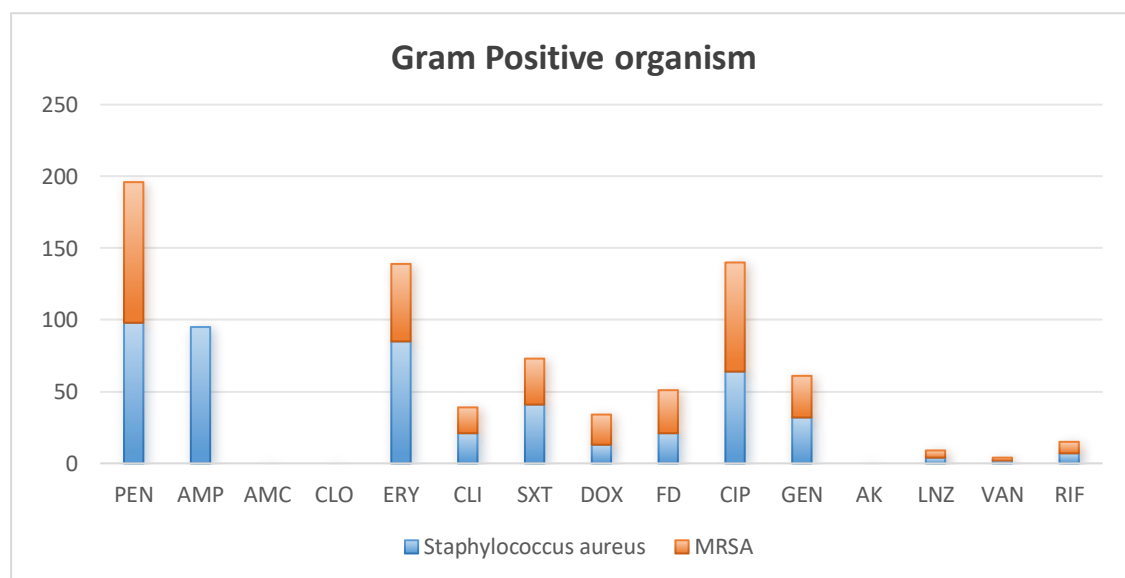


Figure 1. Gram Positive organism isolated from pus swab tissue and Inclusive pattern of antibiotic resistance

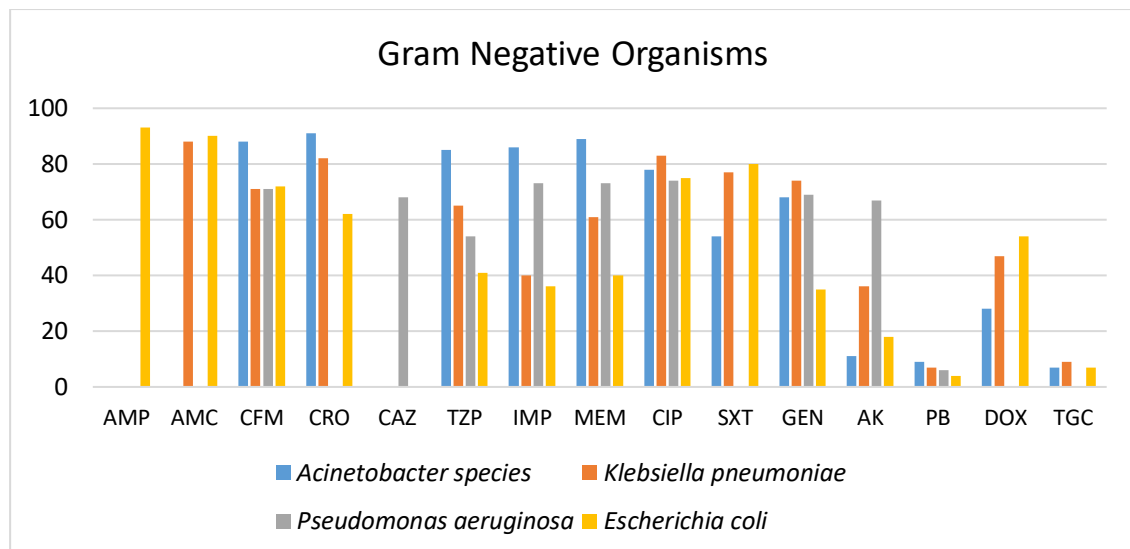


Figure 2. Antibiotic resistance distribution among Gram-negative organisms isolated from pus swab tissue.

The analysis of antibiotic resistance patterns in urine isolates demonstrated differing degrees of resistance among seven frequently used drugs. Penicillin (PEN) had the highest mean resistance at 75%, accompanied by a standard deviation (SD) of 28.3%, signifying diversity across the examined isolates. Ciprofloxacin (CIP) demonstrated significant resistance, averaging 65.5% with reduced variability (SD = 6.36%). Ampicillin (AMP) and

Fosfomycin (FOS) exhibited modest resistance rates, averaging 50% and 51%, respectively. Linezolid (LNZ) exhibited a mean resistance of 44.7%, accompanied by considerable variability (SD = 42.2%), indicating inconsistent resistance among isolates. Vancomycin (VAN) and Nitrofurantoin (NIT) exhibited mean resistance rates of 17.7% and 20%, respectively shown in the figure (3) and on the basis bacteria figure (4).

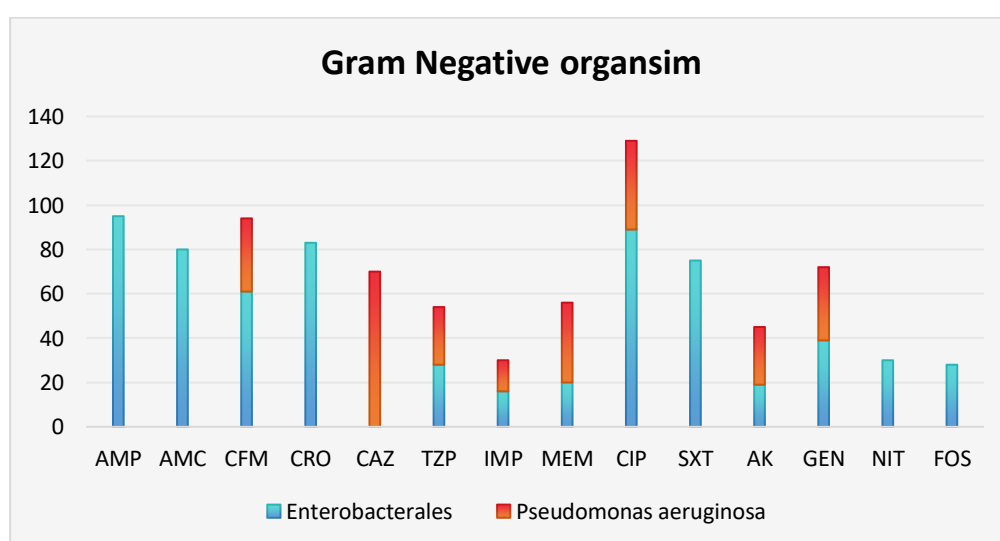


Figure 3. Gram negative organisms and there pattern of antibiotic resistance in urinary isolates

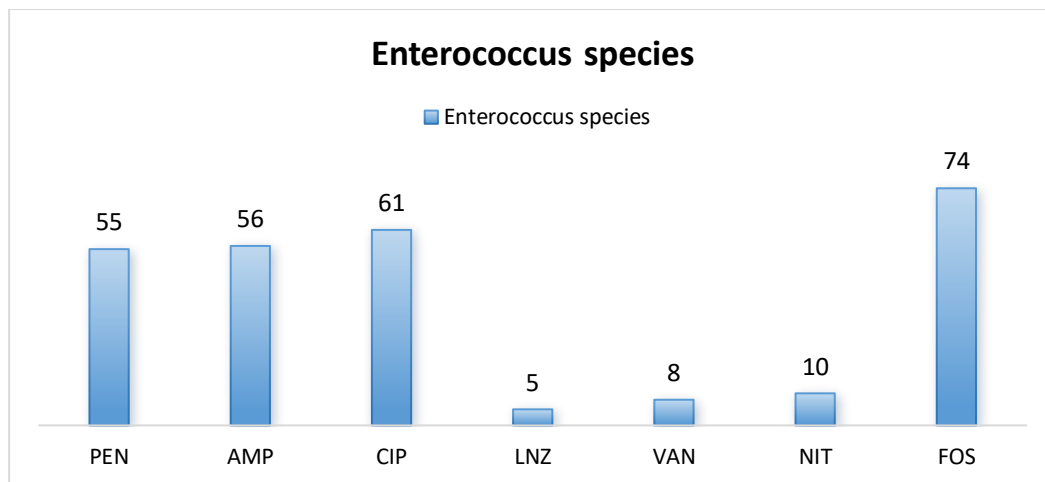


Figure 4. Gram Positive Organism of Enterococcus species and there antibiotic resistance pattern isolated from urine.

The antibiotic resistance patterns identified in blood and central venous catheter line tip cultures demonstrate a notable degree of resistance to many antibiotics. Penicillin (AMP) exhibited the greatest resistance, with an average resistance rate of 97%. Tigecycline (TGC) and Polymyxin B (PB) demonstrated the lowest resistance rates, recorded at 8.5% and 5%, respectively.

Additional antibiotics, including Cefepime (CFM), Ceftriaxone (CRO), and Imipenem (IMP), exhibited moderate resistance, with resistance rates between 37% and 72%. Ciprofloxacin (CIP) and Sulfamethoxazole-trimethoprim (SXT) exhibited resistance rates of 68% and 59.7%, respectively shown in the figure (5).

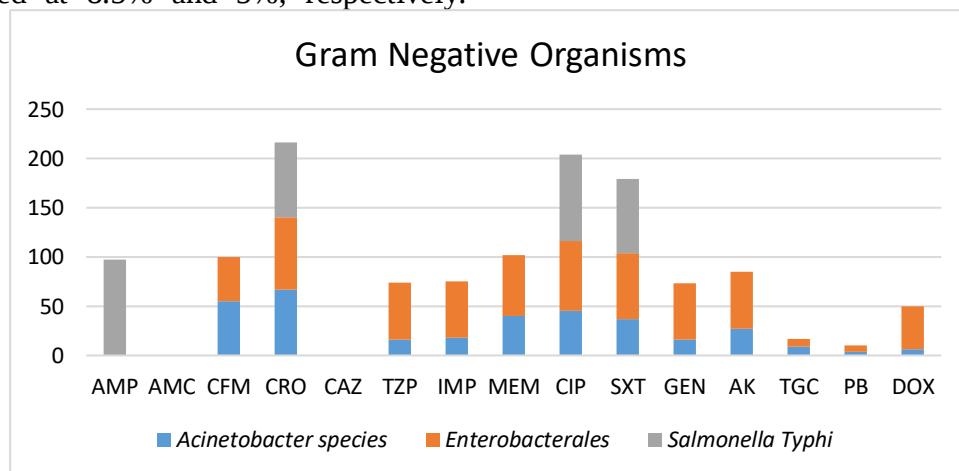


Figure 5 Antibiotic resistance rates across various antibiotics in blood CVP line culture samples.

The study of respiratory data revealed substantial variability in antimicrobial resistance patterns across various medications. The highest average resistance was recorded for Ceftriaxone (CRO) at 84.7%, followed by Ceftazidime (CAZ) at 74%, and Trimethoprim-Sulfamethoxazole (SXT) at 67.3%, signifying restricted effectiveness of these

drugs against respiratory infections. Moderate resistance was seen in Ciprofloxacin (CIP) at 65.2%, Cefixime (CFM) at 66.5%, Gentamicin (GEN) at 52.8%, Imipenem (IMP) at 47.8%, and Meropenem (MEM) at 48%. Doxycycline (DOX) exhibited a resistance rate of 49.3%, Amikacin (AK) 41.8%, and Piperacillin-Tazobactam (TZP) 45.2%. Tigecycline

(TGC) and Polymyxin B (PB) had the lowest resistance rates at 13.7% and 6.2%, respectively, indicating their potential efficacy as treatment alternatives for

multidrug-resistant respiratory infections shown in the figure (6).

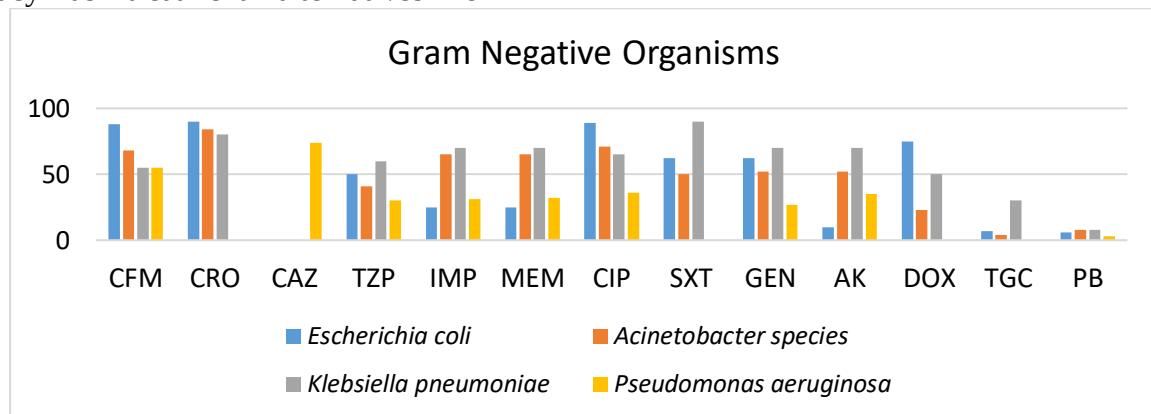


Figure 6 displaying, among respiratory bacteria extracted from sputum, tracheal aspirate, bronchal lavage, and other specimens, the average proportion of antibiotic resistance. Higher resistance percentages of antibiotics point to lower effectiveness.

DISCUSSION

A serious global health concern, antimicrobial resistance (AMR) impairs the effective therapy of bacterial infections and increases morbidity, mortality, and healthcare expenditures¹⁴. AntibioGrams are therefore very important tools in clinical microbiology to monitor local sensitivity trends and guide empirical antibiotic therapy. Processed in the Department of Pathology, CMH Peshawar, this antibiogram provides a whole picture of antimicrobial sensitivity patterns of bacterial isolates acquired from several clinical specimens including pus, urine, blood, and lung samples.

Other study reported that attributable to a higher number of specimens in this investigation originating from urine, since females have a greater susceptibility to urinary tract infections (UTIs). In this investigation, Gram-negative bacteria constituted the primary isolates. This aligns with other results from various regions of Ethiopia, India, and China, where Gram-negative bacteria were more commonly identified than Gram-positive isolates^{15, 16}. Antimicrobial susceptibility profiles across clinical specimens showed diverse resistance patterns. Wound isolates (pus,

swab, and tissue) were resistant to Penicillin (75%) and Ciprofloxacin (65.5%), but sensitive to Vancomycin (82.3%) and Nitrofurantoin (80%). Ampicillin resistance was 97% in urine isolates, whereas Cefuroxime (50%), Ciprofloxacin (68%), and Trimethoprim-Sulfamethoxazole (59.7%) were moderate. Polymyxin B (95%), Tigecycline (91.5%), and Imipenem (62.5%) had the highest sensitivity. Blood and CVP line tips isolates displayed similar resistance trends to urine isolates, resisting Ampicillin (97%) and Ciprofloxacin (68%) while being more sensitive to Polymyxin B (95%) and Tigecycline (91.5%). Sputum, tracheal aspirate, endobronchial washing, non-bronchoscopic lavage, and bronchoalveolar lavage were resistant to Cefuroxime (66.5%), Ciprofloxacin (65.2%), and Gentamicin (52.8%), but Polymyxin B (6.2%) and Tigecycline (13.7%) were not. These findings highlight Gram-negative isolates' high antibiotic resistance, notably against commonly used antibiotics, although Polymyxin B and Tigecycline remain effective.

The Gram-negative bacterial isolates in this investigation were resistant to ampicillin (100%), amoxicillin+clavulanic acid

(92.2%), amoxicillin (90.6%), tetracycline (84%), cotrimoxazole (78.8%), and erythromycin (77.1%). The extensive usage of these antibiotics in the research area may explain this. These antibiotics are widely used because they are inexpensive and accessible. Ghanaian Gram-negative isolates were 94.4% ampicillin-resistant, while India had substantial ampicillin and cotrimoxazole resistance. However, it was greater than the Debre Markos, Ethiopia research¹⁷. Bacterial isolates exhibited resistance to penicillin (71%), ampicillin (71%), amoxicillin (62.9%), cotrimoxazole (58.1%), and tetracycline (64.6%)¹⁸.

Resistance was also noted against third-generation cephalosporins (Ceftriaxone and Ceftazidime), although Clindamycin and Meropenem are critical antibiotics designated for use in tertiary institutions as outlined in the WHO antibiotic stewardship programs¹⁹. In our investigation, both *E. coli* and *Klebsiella* spp. isolates shown significant resistance to several tested antibiotics, including Ciprofloxacin, a frequently used treatment for urinary tract infections. *P. mirabilis*, recognised for causing urinary tract infections, was less commonly isolated from urine but was prevalent in ear and wound swabs. To optimize patient management, clinicians ought to prescribe antibiotics for urinary tract infections based on whether the infection is uncomplicated (lower-tract UTI) or complicated (associated with conditions such as structural or functional abnormalities of the genitourinary tract or the presence of underlying diseases^{20, 21}).

The genus *Staphylococcus* have forty different species, amongst which *S. aureus* is most dangerous. Methicillin-Resistant *Staphylococcus aureus* (MRSA) is the pathogen that is present in different types of infections including burn infections²². Patients with severe burns are particularly vulnerable to MRSA infection due to immunological abnormalities and the loss of the skin's functional barrier. *S. aureus* also makes up around 50% of a healthy person's natural microbiome. The bacteria

are associated with a variety of pyogenic, community-acquired, healthcare-associated, superficial, and deep skin illnesses²³⁻²⁷.

The predominant bacteria included *S. aureus*, *E. coli*, *P. aeruginosa*, *P. mirabilis*, *V. cholerae*, *Klebsiella* sp., *Streptococcus* sp., *Salmonella* sp., *Citrobacter* sp., *N. gonorrhoeae*, *S. aureus*, *E. coli*, *P. aeruginosa*, *Klebsiella* spp., *Salmonella* spp., and *Citrobacter* spp. The majority of these isolates are included in the WHO's list of priority bacterial pathogens for research, discovery, and the development of novel antibiotics^{23,24}. The most commonly analyzed clinical specimens were ear pus, urine, wound pus, faeces, and blood. The abundance of ear pus specimens indicates the prevalence of suppurative otitis conditions, particularly among children and adolescents under 15 years of age, especially in sub-Saharan Africa²⁵.

LIMITATION

The limited number of isolates for some taxa and specimen types restricted the generalizability of the findings. Molecular testing to identify individual resistance genes was not conducted, which may have yielded more profound insights. The data were obtained from a single center, hence the findings may not reflect broader regional resistance patterns. Furthermore, patient clinical outcomes were not assessed, and certain antibiotic findings were either missing or inaccessible, therefore impacting the thoroughness of the resistance profile.

CONCLUSION

Antimicrobial resistance is a contemporary issue needing quick and educated clinical response, not only a danger from the future. Guiding doctors towards more focused and successful antibiotic treatments, our antibiogram offers a strong picture of resistance trends among clinical isolates gathered at the Department of Pathology, CMH Peshawar. Staying ahead of developing resistance trends and guaranteeing the effectiveness of the currently used antibiotics depend on

ongoing surveillance and regular antibiogram updates. Including such data-driven insights into regular clinical decisions not only improves patient care but also is very important for the worldwide battle against antibiotic resistance.

RECOMMENDATION

Establish rigorous antibiotic stewardship initiatives to reduce the development of multidrug resistance. Improve infection control protocols in hospital environments to mitigate the transmission of resistant strains. Advocate for the advancement and use of novel antibiotics effective against multidrug-resistant bacteria. Perform systematic surveillance and molecular analysis of resistance mechanisms to guide treatment strategies.

ETHICS APPROVAL: The ERC gave ethical review approval.

CONSENT TO PARTICIPATE: written and verbal consent was taken from subjects and next of kin.

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AUTHORS' CONTRIBUTIONS:

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated in the work to take public responsibility of this manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST: No competing interest declared

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