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ANTIMICROBIAL RESISTANCE PROFILES AND MULTIDRUG-RESISTANCE PATTERNS OF BACTERIAL ISOLATES FROM CLINICAL SAMPLES

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Abstract
Antimicrobial resistance (AMR) and multidrug resistance among clinical bacterial isolates represent an increasing challenge for effective infection management. Understanding local resistance patterns is essential for appropriate antimicrobial selection and stewardship. This study aimed to evaluate antimicrobial-resistance profiles and multidrug-resistance patterns among bacterial isolates obtained from different clinical samples. A laboratory-based descriptive cross-sectional analysis was conducted using 40 bacterial isolates recovered from urine, blood, sputum, wound, and pus samples. Bacterial species, Gram reaction, patient characteristics, hospital unit, antimicrobial-susceptibility profiles, resistant-drug count, resistant-class count, and MDR status were assessed. Frequencies, percentages, chi-square tests, independent-samples t-tests, correlation analysis, and binary logistic regression were used for statistical analysis. Gram-negative organisms predominated, with Escherichia coli identified most frequently. High resistance was observed against ampicillin, tetracycline, cefotaxime, and ceftriaxone, whereas meropenem retained the greatest activity. Multidrug resistance was present in 82.5% of isolates and was particularly prominent among Acinetobacter baumannii, Klebsiella pneumoniae, and Pseudomonas aeruginosa. Patient age showed positive correlations with resistant-drug and resistant-class counts and emerged as a significant predictor of MDR status in logistic regression. The findings demonstrate a substantial burden of antimicrobial and multidrug resistance among clinical bacterial isolates. Routine susceptibility testing, local antibiograms, infection-control measures, and strengthened antimicrobial-stewardship programmes are essential for improving treatment decisions and limiting further resistance.

Keywords: Antimicrobial resistance; Multidrug resistance; Clinical isolates; Antibiotic susceptibility; Bacterial infections

Introduction

Bacterial infections still cause a significant amount of disease, both in the community and in hospital settings, and are still a burden on healthcare systems. The urinary tract, bloodstream, respiratory tract, wounds, and other body sites are all susceptible to infection by a variety of bacterial pathogens, and prompt diagnosis and appropriate antimicrobial treatment are critical in the management of patients. Antibiotics have contributed significantly to the management of bacterial diseases, but the rise and spread of antimicrobial resistance (AMR) is now threatening their efficacy. Resistance is the development or acquisition of resistance mechanisms in bacteria that enable them to resist the action of antimicrobial agents that inhibit or kill them. Consequently, resistant infections can take longer for treatment, may need other or more expensive medications, may necessitate longer hospital stays and more intensive clinical observation. Resistance has been seen to be high with several widely-used antimicrobial drugs in long-term surveillance of clinical isolates and thus, the need for continual monitoring of AMR in healthcare environments has been highlighted (Regassa et al., 2023).

Improper or overuse of antibiotics, using antibiotics based on estimated organisms rather than actual susceptibility testing, poor infection control measures, and the spread of antibiotic resistance within the healthcare environment can speed up the development of AMR. The emergence of resistance to broad-spectrum antibiotics is a particular cause for concern as these are commonly used to treat severe bacterial infections. The

clinical isolates tested indicated a high level of resistance to third-generation cephalosporins, indicating the decreased effectiveness of these widely used antimicrobial agents (Gashe et al., 2018). As a result, a rise in AMR may result in treatment failure, longer hospital stays, and increased health care costs, complications, and patient outcomes.

Bacterial organisms are often recovered in clinical microbiology laboratories from urine, blood, sputum, wound, pus, and other clinical specimens. The distribution of the bacterial species and the level of antimicrobial susceptibility can be patient-specific, site-specific, hospital unit-specific, antimicrobial use patterns, and local epidemiology dependent. Several gram-negative and gram-positive bacteria are a major source of infection in routine clinical samples, and there is a wide range of resistance to the widely used antibiotics (Hailemariam et al., 2021). In the same vein, a long-term study of clinical samples obtained from a referral hospital showed that there is a considerable variation in antimicrobial-resistance patterns of bacterial isolates (Abebe et al., 2019).

Gram-negative bacteria are of special interest as they have several intrinsic and acquired resistance mechanisms, including decreased membrane permeability, efflux pumps, enzymatic drug inactivation, and resistance determinants. Gram-positive organisms continue to be clinically relevant, especially in healthcare-associated, bloodstream and wound infections. Bacterial distribution in various specimen types has revealed that the distribution of the organisms is not equal and that the antimicrobial susceptibility patterns are also different among various clinical samples, indicating the importance of locally generated antimicrobial-susceptibility data (Shebl & Mosaad, 2019). Another significant clinical issue is surgical-site infections, as bacteria may contaminate and lead to treatment challenges and postoperative complications. Data from multiple centres have shown that there is a significant level of AMR in the organisms causing surgical site infections, and there is an increasing need for laboratory-directed antimicrobial therapy and infection-prevention measures for surgical site infections (Worku et al., 2023).

One of the most serious problems of AMR is multidrug resistance (MDR), which usually means resistance to drugs from more than one drug group. MDR organisms could mean loss of treatment options and potentially the use of reserve or last-line antibiotics. Infections in the hospitalised and critically ill are especially dangerous as they are likely to receive repeated or long courses of antimicrobial drugs, invasive procedures, and have a higher risk of exposure to HAPs. One study that followed the outcomes of patients in the intensive care units revealed an unexpected burden of MDR infections, as well as the clinical difficulties of treating these infections in high-risk hospital settings (Fahim, 2021).

Multidrug resistance has also been reported in the case of hospital-associated bacterial pathogens; however, patterns of resistance and prevalence may differ depending on the bacterial species and clinical setting (Jara et al., 2021). Other significant sources of resistant organisms are wound infections. Multiple antibiotic-resistant bacterial isolates have been found in infected wounds, highlighting the need for antibiotic susceptibility testing in the treatment of infected wounds and minimising the unnecessary use of antibiotics (Nobel et al., 2022). Similarly, in Bangladesh, a study of infected wound patients found significant levels of MDR bacterial isolates, highlighting the public-health significance of MDR surveillance in clinical settings (Alam et al., 2021).

Although several studies have been carried out on the issue of AMR, the assessment of the distribution of the bacteria, individual antimicrobial susceptibility and multidrug resistance pattern in various clinical specimens is important. Generalised treatment decisions are less reliable because resistance may vary with species of bacteria, Gram reaction, source of sample, and unit within the hospital. Furthermore, the ability to detect antimicrobial agents that are highly resistant and remain more active can aid in making course-of-treatment decisions and the use of antimicrobial stewardship. An integrated assessment of these characteristics can help to better understand local burden and clinical characteristics of AMR. Hence, the present study is designed to find out the resistance pattern and multiple drug resistance of the bacterial isolates isolated from various clinical samples.

Objectives

1. To determine the distribution of bacterial isolates across different clinical sample types.
2. To assess the susceptibility and resistance profiles of bacterial isolates against selected antibiotics.
3. To determine the prevalence and distribution of MDR isolates according to bacterial species, Gram reaction, clinical sample, and hospital unit.

2. Materials and Methods

2.1 Study Design and Samples

40 clinical bacterial isolates from urine, blood, sputum, wound, and pus samples were used in the descriptive cross-sectional study carried out in the laboratory. Samples were collected from the ICU, medicine, surgery, and outpatient units. Bacterial isolates with only partial antimicrobial-susceptibility data were not recorded.

2.2 Bacterial Identification and Antimicrobial-Susceptibility Testing

Standard microbiological methods, such as colony morphology, Gram stain, biochemical or automated identification methods, were used to identify the bacterial isolates. There were 8 species of bacteria that were analyzed. Antimicrobial susceptibility testing was performed by the Kirby–Bauer disk-diffusion method or its equivalent, and interpreted based on the CLSI or EUCAST interpretation guidelines. The results were grouped into susceptible, intermediate, and resistant.

2.3 Antimicrobial Agents Evaluated

Susceptibility was tested against nine antibiotics: ampicillin, amoxicillin–clavulanate, ciprofloxacin, gentamicin, cefotaxime, ceftriaxone, meropenem, tetracycline, and trimethoprim–sulfamethoxazole.

2.4 Definition of Multidrug Resistance

Multidrug resistance was defined as resistance to antimicrobial agents in 3 or more categories. Each isolate was classified as MDR or non-MDR, and the number of resistant antimicrobial classes and resistant drugs were determined.

2.5 Study Variables

Patient age, sex, sample type, hospital unit, bacterial species, whether the bacteria were Gram-positive or Gram-negative, susceptibility results for each antibiotic, number of antibiotics the bacteria were resistant to, number of classes of resistant antibiotics, and MDR status were all included as variables.

2.6 Statistical Analysis

Data were analyzed using Python. Frequency and percentage were used to summarize categorical variables, and mean, standard deviation, median, and range were used for continuous variables. Antibiotic-specific resistance and susceptibility rates, as well as the overall prevalence of MDR, were determined. The associations of MDR status with categorical variables (sex, Gram reaction, sample type, hospital unit, and bacterial species) were evaluated using the chi-square test. An independent-samples t-test was used to compare the mean ages of the MDR and non-MDR groups. Pearson or Spearman correlation was used to look at the relationship between age, resistant-drug count, and resistant-class count. Selected demographic and clinical variables were explored for associations with MDR status using binary logistic regression. Odds ratios, 95% confidence intervals, and p-values were used to report regression findings. Significant differences were considered at a $p < 0.05$ statistical level.

2.7 Ethical Considerations

Confidentiality of the patients was ensured using coded sample identification numbers, and no personally identifying information was provided. The presence of institutional ethical approval is to be reported if the dataset is real patient-derived clinical samples.

3. Results

3.1 General Characteristics of the Clinical Samples

The bacterial isolates were obtained from patients of different ages with an equal ratio of males to females. The Gram-positive organisms were outnumbered by the Gram-negative organisms, and most of the isolates were multidrug-resistant. Both the resistant-drug and resistant-class counts also suggested a significant overall burden of AMR. Table 1 shows the general characteristics of the study samples and isolates.

Table 1. General characteristics of the clinical samples and bacterial isolates

Characteristic	Result
Age, years, mean ± SD	50.80 ± 15.06
Female	20 (50.0%)
Male	20 (50.0%)
Gram-negative isolates	27 (67.5%)
Gram-positive isolates	13 (32.5%)
MDR isolates	33 (82.5%)
Non-MDR isolates	7 (17.5%)
Resistant-drug count, mean ± SD	5.75 ± 2.63
Resistant-class count, mean ± SD	4.45 ± 1.91

3.2 Distribution of Clinical Samples and Bacterial Isolates

Urine, blood, sputum, wound and pus specimens were collected as clinical specimens. The commonest specimen was urine and the least common specimen was pus. The distribution of the various clinical specimens is shown in the table below and in Figure 1. Samples were also collected from a number of hospital units, primarily from the surgical and intensive care units.

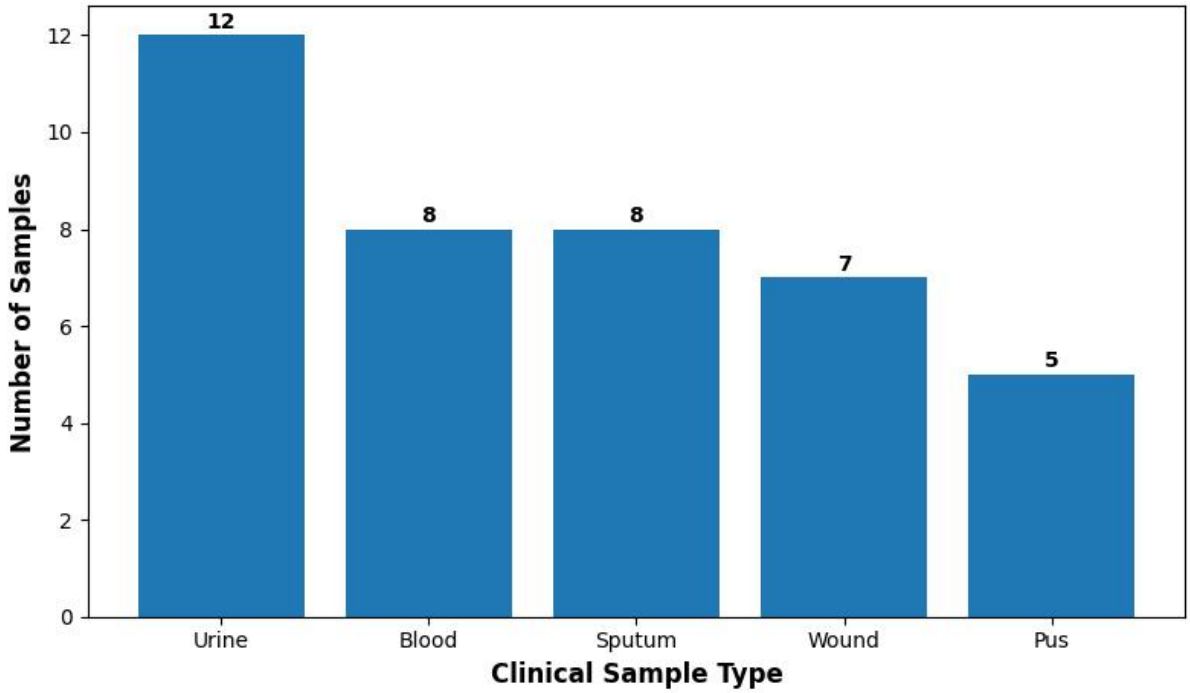


Figure 1. Distribution of clinical sample types

There was significant variation between the clinical specimens in the bacterial profile. Escherichia coli was the most frequently isolated organism, followed by *Klebsiella pneumoniae* and Staphylococcus aureus. Other clinically important Gram-negative and Gram-positive organisms were also included. The complete distribution of the bacterial isolates is shown in Table 2.

Table 2. Distribution of bacterial isolates

Bacterial isolate	Frequency	Percentage (%)
Escherichia coli	9	22.5
Klebsiella pneumoniae	7	17.5
Staphylococcus aureus	7	17.5
Pseudomonas aeruginosa	4	10.0
Enterococcus faecalis	4	10.0
Proteus mirabilis	4	10.0
Acinetobacter baumannii	3	7.5
Streptococcus pneumoniae	2	5.0

3.3 Overall Antimicrobial-Resistance Profile

The antimicrobial susceptibility results showed that there was a large difference between the various antimicrobial agents. Higher resistance was seen for ampicillin and high resistance for tetracycline and the tested 3rd generation cephalosporins. By comparison, meropenem was the most active of the tested drugs. Intermediate susceptibility was rarely seen and occurred only with ceftriaxone. Overall resistance pattern as shown in Figure 2.

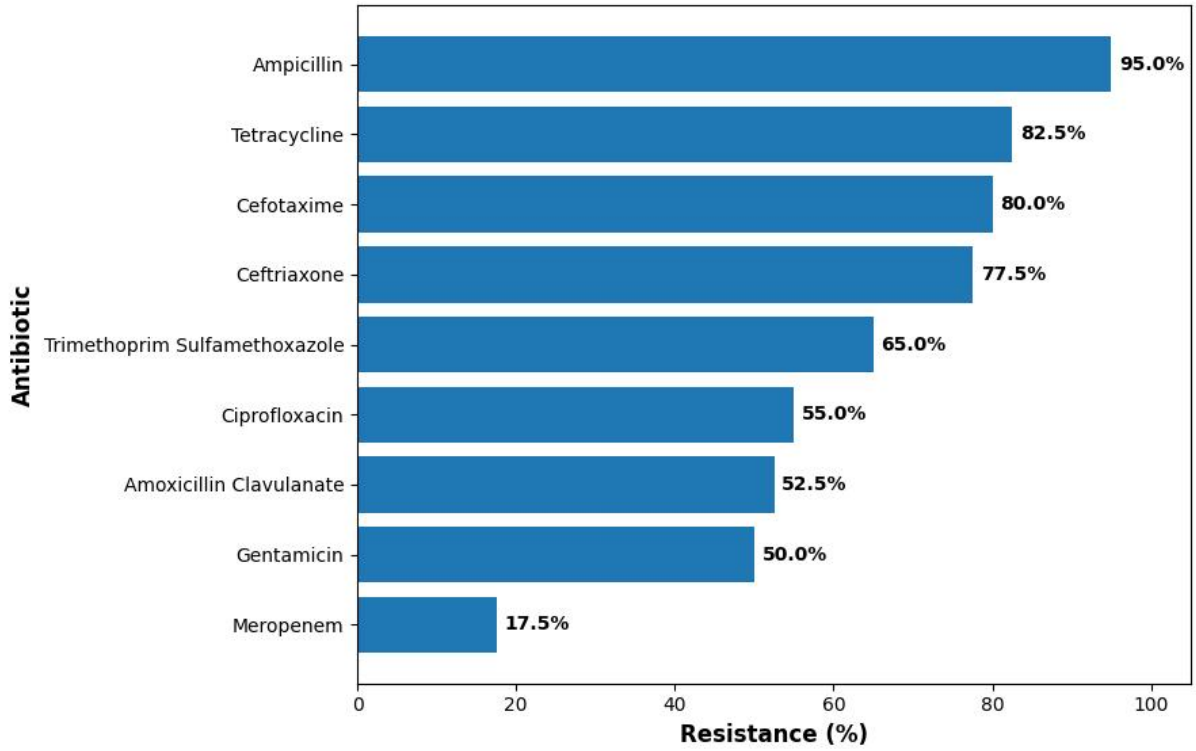


Figure 2. Overall resistance percentages of the tested antimicrobial agents

3.4 Prevalence and Distribution of Multidrug Resistance

The distribution of multidrug resistance varied significantly among bacterial species, but the majority of isolates were multidrug resistant. Several important Gram-negative organisms were found to have complete MDR, while no MDR isolates were found in *Streptococcus pneumoniae* in this data set. Other species showed intermediate to high occurrence of MDR. Species specific results for the MDR are included in Table 4.

Table 4. Distribution of multidrug resistance according to bacterial species

Bacterial species	MDR	Non-MDR	Total	MDR (%)
Acinetobacter baumannii	3	0	3	100.0
Enterococcus faecalis	3	1	4	75.0
Escherichia coli	7	2	9	77.8
Klebsiella pneumoniae	7	0	7	100.0
Proteus mirabilis	3	1	4	75.0
Pseudomonas aeruginosa	4	0	4	100.0
Staphylococcus aureus	6	1	7	85.7
Streptococcus pneumoniae	0	2	2	0.0

3.5 Association of Clinical and Microbiological Factors With MDR

The association analysis showed that there was no significant association between sex, Gram reaction, sample type, hospital unit, and bacterial species with MDR at the significant level of 0.05. However, the categorical variables that were examined had the strongest statistical trend for bacterial species. Complete results of the chi-square analysis are shown in Table 5. There was a tendency for patients with MDR isolates to be older than patients with non-MDR isolates, but this difference was not statistically significant at the predetermined level of significance.

Table 5. Statistical associations between selected characteristics and MDR status

Variable	Statistical test	Test statistic	df	p-value
Sex	Chi-square	0.000	1	1.000
Gram reaction	Chi-square	1.184	1	0.276
Sample type	Chi-square	2.548	4	0.636
Hospital unit	Chi-square	5.500	3	0.139
Bacterial species	Chi-square	12.899	7	0.075
Age: MDR vs non-MDR	Independent-samples t-test	2.307	—	0.0507

Figure 5 depicts the MDR occurrence in each hospital unit separately for visual comparison of the relative burden in each clinical setting.

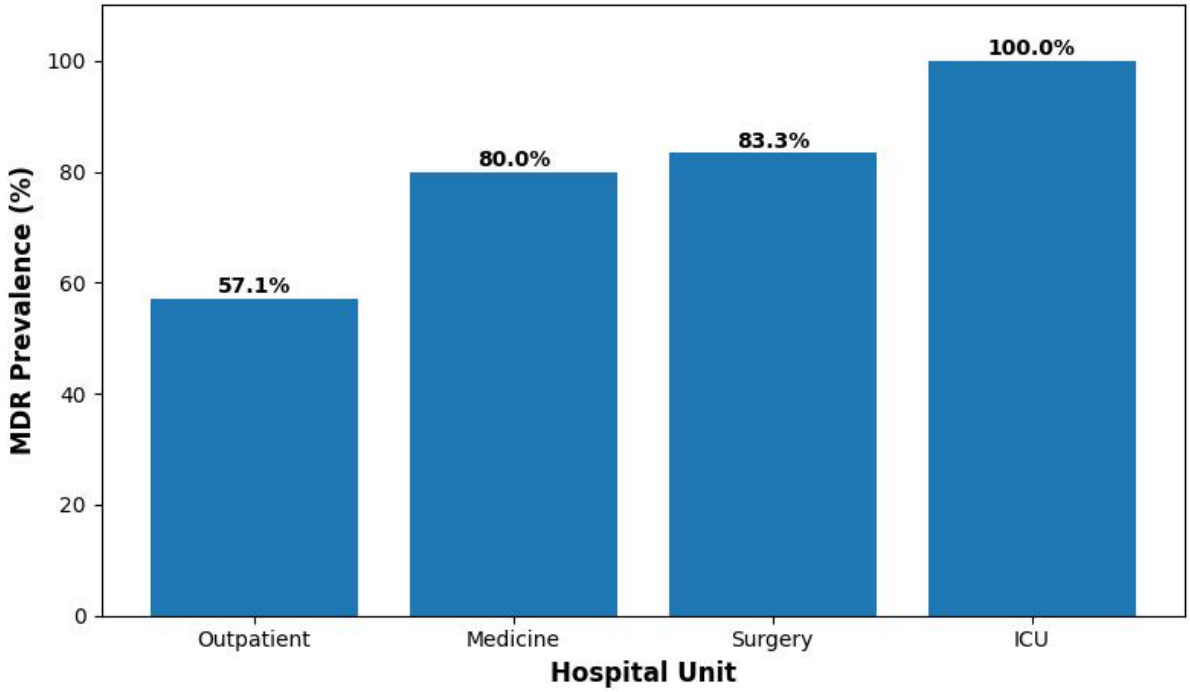


Figure 5. Multidrug-resistance prevalence according to hospital unit

3.6 Correlation Analysis

The continuous resistance-related variables showed strong and positive correlations between each other. The patient age was significantly and positively correlated with resistant-drug count and resistant-class count. The best correlation was found between resistant-drug count and resistant-class count, where resistant isolates to more individual drugs were also resistant to more classes of drugs. The details of the correlation coefficients and significance values are available in Table 6.

Table 6. Pearson correlation analysis of patient age and antimicrobial-resistance measures

Variable 1	Variable 2	Pearson r	p-value
Patient age	Resistant-drug count	0.712	<0.001
Patient age	Resistant-class count	0.683	<0.001
Resistant-drug count	Resistant-class count	0.984	<0.001

3.7 Logistic Regression Analysis

To explore if age, sex, or Gram reaction was independently related to MDR, binary logistic regression was performed. The overall model was statistically significant and it explained 65.6% of the variance in the data. The variables included in the model, age of the patient was the only statistically significant predictor of MDR, while sex and Gram reaction were not independent predictors of MDR. The full estimates, adjusted for this change, are shown in Table 7.

Table 7. Binary logistic regression analysis of factors associated with MDR status

Predictor	Odds ratio	95% CI	p-value
Patient age	1.111	1.013–1.220	**0.026**
Male sex	0.460	0.062–3.384	0.446
Gram-positive reaction	0.253	0.033–1.975	0.190

The overall regression model was statistically significant (likelihood-ratio p = 0.021) and the selected predictors explained the variation in MDR status, as determined by pseudo-R^2.

4. Discussion

The present study showed considerable AMR among bacterial isolates from clinical samples. Gram-negative organisms were more common than Gram-positive, with Escherichia coli being the most common Gram-negative species, followed by *Klebsiella pneumoniae* and Staphylococcus aureus. The resistance of ampicillin, tetracycline, cefotaxime and ceftriaxone was high, while meropenem had comparatively good activity. A

multidrug resistance (MDR) pattern was also frequently observed with A. baumannii, K. pneumoniae and P. aeruginosa, emphasising the importance of ongoing surveillance of resistance and judicious choices of the most effective antimicrobial agents.

Intrinsic and acquired mechanisms of AMR of Gram-negative organisms are clinically significant, as they are more likely to be resistant to antimicrobial agents. The same is true of the Gram-negative clinical isolates in hospital settings, where resistance has been shown to be common for each of the major classes of antimicrobials (Said et al., 2021). The frequent isolation of E. coli might also indicate its significance in urinary and other infections. Studies in hospitalized patients have documented decreased susceptibility in bacterial uropathogens, especially in Gram-negative organisms (Golli et al., 2019). Similarly, MDROs have been reported in urinary tract infections (UTIs) and empirical treatment should be guided by susceptibility rather than giving blanket treatment with multiple antimicrobials (Prastiyanto et al., 2024).

The high resistance against beta-lactams and third-generation cephalosporins observed in the present study may limit commonly available treatment options. Production of extended-spectrum beta-lactamase and production of carbapenemases have been linked to Gram-negative bacilli resistance in postoperative infections (Tilahun, 2022). AMR has also been detected in environmental hotspots, indicating the potential for the spread of resistance between clinical, community, and environmental settings (Geta & Kibret, 2022). However, the relatively high activity of meropenem in the current study indicates that the activity will persist as long as it is used prudently to prevent further resistance.

Another significant finding that needs to be highlighted is the high burden of MDR. *Acinetobacter spp*. are of note because of their capacity to gain resistance and be present in healthcare settings. The presence of high levels of multidrug resistance (MDR) among clinical Acinetobacter isolates has been shown in previous trend analyses (Ayenew et al., 2021). In addition, plasmid-mediated resistance can also contribute to the transfer and maintenance of multidrug-resistance determinants between bacteria from various clinical samples (Okoye et al., 2022).

Another significant source of resistant organisms is wound infections. Various bacteria species have been found in infected wounds and showed high resistance to various antimicrobials (Prastiyanto et al., 2024). Similar results have been found for Gram-negative wound isolates, with resistance potentially complicating treatment and highlighting the importance of using laboratory information to inform antimicrobial selection (Bandy et al., 2022). The observations of the present invention are consistent with the observations of the present invention that multi-resistant organisms are present in multiple clinical specimen types.

The small number of *Streptococcus pneumoniae* isolates in this data set was non-MDR. However, this finding must be interpreted with caution, due to the small number of isolates. Clinical development of multidrug-resistance patterns have been previously documented with S. pneumoniae (Sharew et al., 2021). Resistance also varies among patient populations, with bacterial isolates from cancer patients exhibiting significant antimicrobial susceptibility differences, possibly due to differences in healthcare exposure and clinical susceptibility (Worku et al., 2022).

The blood samples collected in the current study also had a high MDR burden. In earlier studies, bloodstream infections were also shown to have high levels of AMR, reflecting the challenges of treating antimicrobial resistant bloodstream infections (Tsegaye et al., 2021). In systemic infections it is therefore crucial to identify and treat the infection promptly and according to susceptibility. The statistical analysis also revealed that age of the patient was positively correlated with counts of resistant-drug and resistant-class and was a significant predictor of MDR in logistic regression. This association could be because of increased cumulative health care exposure, previous antimicrobial use, or comorbidity in older patients, but was not directly measured. In particular, similar studies on MDR E. coli have highlighted patient- and healthcare-related factors as a contributing factor to AMR (Mousa et al., 2025).

The overall results do support the use of regular institutional antibiograms, culture-driven antibiotic use, enhanced infection control protocols and antimicrobial stewardship programmes. Results are not generalizable due to small sample sizes and few isolates within bacterial species, and molecular resistance mechanisms were not evaluated. Further, larger multicentre studies with MIC testing and molecular characterisation of resistance genes are recommended to gain a more detailed understanding of the epidemiology of MDR.

5. Conclusion

The present study showed that there is high burden of AMR and multidrug resistance among bacterial isolates from various clinical samples. Gram-negative organisms were more prevalent than Gram-positive organisms and Escherichia coli was the most common organism found. Significant resistance was noted towards many commonly used antimicrobial agents with ampicillin, tetracycline, cefotaxime, ceftriaxone, showing relatively high resistance, while meropenem showed relatively high antimicrobial activity. The multidrug resistant pattern was observed in all bacterial isolates and was more prevalent in the bacteria *Acinetobacter baumannii*,

Klebsiella pneumoniae and *Pseudomonas aeruginosa*. The results also showed that age of patients was positively correlated with the development of AMR and was a significant factor in determining MDR status, whereas sex and Gram reaction did not independently influence MDR status. The results highlight the value of the regular antimicrobial susceptibility testing and monitoring of local resistance trends. Inappropriate antibiotic use can be reduced and further emergence of resistant organisms prevented if institutional antibiograms are prepared regularly, antimicrobial therapy is guided by microbiological culture, infection-control measures are adequate, and antimicrobial-stewardship programmes are strengthened. There is a need for larger multicentre studies using more antimicrobial panels, MIMs and molecular characterization of resistance mechanism to better understand the problem of MDDRI.

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