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PATTERNS OF BACTERIAL ISOLATES AND ANTIMICROBIAL SUSCEPTIBILITY IN CLINICAL MICROBIOLOGY CULTURES

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Abstract
Globally, bacterial infections remain a major contributor to morbidity, and the increasing emergence of antimicrobial resistance has complicated effective clinical management. Continuous monitoring of bacterial distribution and antimicrobial susceptibility patterns is essential to support appropriate antibiotic selection and antimicrobial stewardship. The distribution of bacterial isolates from culture-positive clinical specimens and an assessment of their antibiotic susceptibility patterns were the objectives of this investigation. Forty culture-positive bacterial isolates were subjected to descriptive cross-sectional research in the lab. In compliance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI), the bacteria were identified using Gramme staining, colony morphology, and biochemical testing, and their antibiotic susceptibility was assessed using the Kirby-Bauer disc diffusion technique. Microsoft Excel was used to analyse the data and summarized using frequencies and percentages. The most frequent source of specimens was urine, which was followed by sputum and pus or wound swabs. Most of the bacteria were Gram-negative, and E. coli, S. aureus, and K. pneumoniae were the most frequently isolated species. E. coli was mainly isolated from urine specimens, whereas S. aureus predominated in wound specimens. Reduced susceptibility to ampicillin was observed among several Gram-negative isolates, while amikacin and meropenem showed high effectiveness. Vancomycin and linezolid continued to be completely effective against gram-positive isolates. The results underline the significance of regular culture and antibiotic susceptibility testing for evidence-based treatment and antimicrobial stewardship, as well as the prevalence of Gram-negative bacteria.

Keywords: Bacterial isolates, Antimicrobial susceptibility, Gram-negative bacteria, Kirby–Bauer method, Microbial surveillance.

Introduction

Bacterial infections are still a serious global public health issue that greatly increases morbidity, death, and medical expenses. They cause infection in all age groups and in almost every anatomical location such as the urinary tract, respiratory tract, bloodstream, skin, soft tissue, and surgical wounds. Proper identification of the causative bacterial pathogens is an essential part of good clinical management because a timely diagnosis will allow proper choice of antimicrobial therapy and minimize the risk of complications. Clinical microbiology labs are crucial to this process because they isolate and identify bacterial pathogens from clinical specimens and evaluate their susceptibility to antibiotics. The data obtained from microbiological culture and antimicrobial susceptibility testing (AST) are not only used for the care of individual patients, but also for

infectious disease surveillance and antimicrobial resistance (AMR) surveillance (Moremi et al., 2016; Wasihun et al., 2015).

AMR is becoming one of the biggest challenges faced by modern healthcare systems. Overuse and misuse of antibiotics in the community and healthcare settings have caused bacterial strains to become resistant and the effectiveness of commonly used antibiotics to decline. Infections with resistant microorganisms are thus linked to lengthier hospital stays, higher cost of care, treatment failure, and death. Thus, regular surveillance of antibiotic susceptibility is critical to the rational use of empirical antibiotic therapy and to ensure the continued utility of existing antimicrobial drugs. Routine laboratory surveillance has become an important part of antimicrobial stewardship programs, as it gives real-time data on local trends in antimicrobial resistance patterns to help make evidence-based decisions about prescribing (Moremi et al., 2016; Amsalu et al., 2017).

Clinical microbiology cultures still form the basis of laboratory diagnosis of bacterial infections. After isolation, a conventional microbiological identification method and then AST give clinicians information specific to the organisms, which is important in terms of knowing how they are going to be treated. These laboratory investigations help provide effective antimicrobial treatment, decrease unnecessary administration of broad-spectrum antimicrobials, and enhance treatment outcomes. In addition, routine microbiological data collected in laboratories can be usefully analysed for epidemiological information of the occurrence of the different species and the development of resistance to the antibiotics used. These surveillance data can be used to inform institutional antibiotic policy and enhance infection prevention and control (Bhat et al., 2016; Dessie et al., 2016).

Bacterial pathogens recovered from clinical specimens are distributed in a wide variety and vary depending on the type of specimen, geographical region, healthcare environment, patient characteristics and local antimicrobial prescribing habits. E. coli, Klebsiella pneumonieae and Pseudomonas aeruginosa are common isolates from urinary, respiratory and wound samples, while Gram-positive organisms like S. aureus and Enterococcus faecalis remain significant agents of device-associated infections, soft tissue and bloodstream infections. The ability to resist antimicrobial agents is variable, and there is a need for periodic analysis of the bacterial profile and antimicrobial resistance patterns to ensure that empirical treatment is always suitable and effective (Trojan et al., 2016; Khan et al., 2014; Gitau et al., 2018).

Various studies have shown marked variations in the dispersal of isolates of bacteria and antimicrobial resistance in numerous clinical settings. Moremi et al. (2016) found that the species of bacteria and their resistance patterns were significantly different between clinical samples, highlighting the need for ongoing microbiological surveillance for antimicrobial stewardship. Likewise, Gram-negative organisms were significantly more abundant in wound infections, and had differential sensitivity to the antibiotics commonly used, showing the importance of antibiotic susceptibility testing in wound infections (Mohammed et al., 2017). Similar studies conducted in Ethiopia by Wasihun et al. (2015), Dessie et al. (2016) and Amsalu et al. (2017) also reported the emergence of altered bacterial epidemiology and rising AMR among clinical isolates. There has also been significant inter-isolate variability reported in susceptibility of Staphylococcus aureus and methicillin-resistant S. aureus (MRSA) isolates in investigations targeting specific pathogens, highlighting the need for frequent monitoring of resistance in the laboratory to allow for the timely detection of any emergence of resistance, which will help guide appropriate antibiotic use (Dilnessa & Bitew, 2016; Gitau et al., 2018). Similarly, Khan et al. (2014) showed that the urinary bacterial isolates had different susceptibility patterns to the commonly used antibiotics, thus highlighting the importance of regular surveillance of local urinary antimicrobial susceptibility patterns.

Despite the fact that several studies have been conducted to characterise bacterial pathogens and antibiotic resistance, the dispersal of isolates of bacteria and AMR continues to change due to continuous changes in antimicrobial use, infection control measures and adaptation of the bacteria.

Therefore, past results might not represent present microbiological changes. Continuing laboratory-based testing of recovered bacterial isolates from various clinical samples is thus crucial to provide new evidence to support effective antimicrobial use, initiatives for infection control and antimicrobial stewardship.

Study Objectives:

1. To govern the distribution of isolates of bacteria recovered from culture-positive clinical specimens.
2. To assess the retrieved bacterial isolates' patterns of antimicrobial susceptibility.
3. To examine the distribution of bacterial isolates according to Gram-staining characteristics and clinical specimen type.

2. Methodology

2.1 Research Design

The distribution and pattern of bacterial isolates' sensitivity to antibiotics were ascertained by a laboratory-based descriptive cross-sectional investigation. The study's bacterial isolates were microbiologically identified and subjected to laboratory tests for antibiotic susceptibility.

2.2 Study Samples

In all, there were 40 bacterial isolates that were collected from culture-positive samples. In this regard, only bacterial isolates that have both a full bacterial identification and full antibacterial sensitivity were used in the study. The duplicate isolates of the same bacteria from the same samples and contaminated cultures were not included in order to maintain the integrity of the data.

2.3 Data Collection

The data collected from the microbiological testing were recorded through a standardized data recording form within the laboratory. The data collected were the clinical sample type, the bacteria isolated, Gram reaction, and the outcomes of the antibiotic sensitivity test.

2.4 Bacterial Identification

Clinical samples were grown on suitable microbiological media under normal laboratory conditions. Identification of bacteria was carried out based on the growth appearance, gram stain properties, and biochemical tests according to microbiology standards.

2.5 AST

The Clinical and Laboratory Standards Institute (CLSI) recommendations were followed in conducting the AST test using Mueller-Hinton Agar and the Kirby-Bauer disc diffusion technique. The inhibition zones were analysed and classified as Susceptible, Intermediate, or Resistant using the most recent CLSI interpretative criteria.

2.6 Study Variables

The independent variables were type of clinical specimen, Gram stain result, and bacteria involved. Dependent variables were sensitivity to antibiotics and were categorised as resistant, intermediate, or susceptible to each antibiotic.

2.7 Statistical Analysis

Data analysis was done using Microsoft Excel programme. The distribution of bacterial isolates, sample type, Gramme stain response, and antibiotic resistance pattern were all described using descriptive statistics in terms of frequency counts and percentages. Antibiotic susceptibility was

determined in terms of percentage of susceptible, intermediate, and resistant isolates. Chi-square test was conducted wherever necessary to test for statistical significance at $p < 0.05$.

3. Results

3.1 Clinical Specimen Types' Distribution

The present investigation involved forty positive clinical specimens in total. The isolated specimens came from various clinical sources that are normally seen during microbiological tests. Evaluation of the distribution of clinical specimen sources gives an idea about the sources of the bacteria isolated for analysis of their susceptibility to antimicrobial drugs. The distribution of clinical specimen sources is shown in Figure 1.

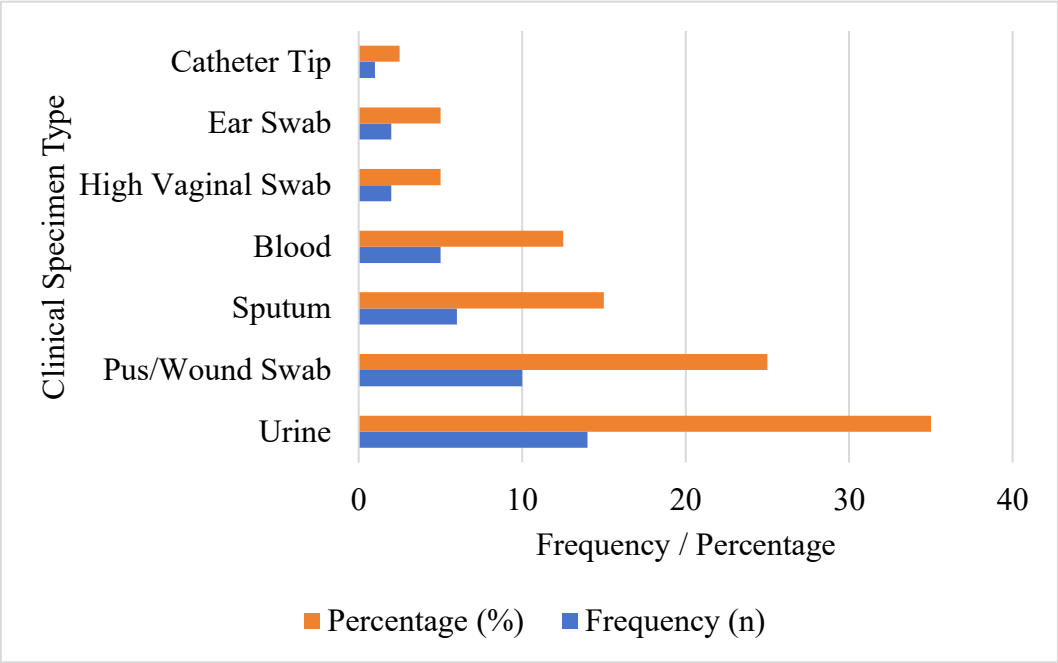


Figure 1. Distribution of Clinical Specimen Types Among Culture-Positive Samples

Urine samples had the highest proportion of cultures compared to pus, wound swabs, and sputum, as shown in Figure 1. The blood samples contributed moderately while high vaginal swabs, ear swabs, and catheter tips were the least number of isolates. Overall, the findings showed that the main sources of bacterial isolates were urine and wound samples.

3.2 Distribution of Bacterial Isolates

Eight bacteria species were cultured out of all the culture positive samples. Frequency of individual bacteria isolate gives very important information about the main bacteria that have been isolated in various clinical samples. Distribution of bacteria isolate in laboratory is shown in Table 1 below.

Table 1. Bacterial Isolate Frequencies

Isolates of Bacteria	Frequency (n)	Percentage (%)
Escherichia coli	11	27.5
Staphylococcus aureus	8	20.0
K. pneumoniae	7	17.5
P. aeruginosa	5	12.5

E. faecalis	3	7.5
Proteus mirabilis	3	7.5
A. baumannii	2	5.0
Citrobacter freundii	1	2.5

Table 1 makes clear that the most prevalent isolates include *E. coli*, *S. aureus*, and *K. pneumoniae*, while other bacteria were found with relatively low frequency. All of them together made up the microbial spectrum detected in this study. These results prove that the study included both Gram-negative and Gram-positive microorganisms.

3.3 Distribution of Gram-Negative and Gram-Positive Isolates of Bacteria

In order to determine the total makeup of bacteria in the experiment, bacteria from the culture-positive samples were identified using the Gramme reaction. The frequency of Gram-negative and Gram-positive bacteria in the isolated bacteria may be determined by using the Gramme reaction for identification of the bacteria. The distribution of bacteria based on Gram reaction is shown in Figure 2 below.

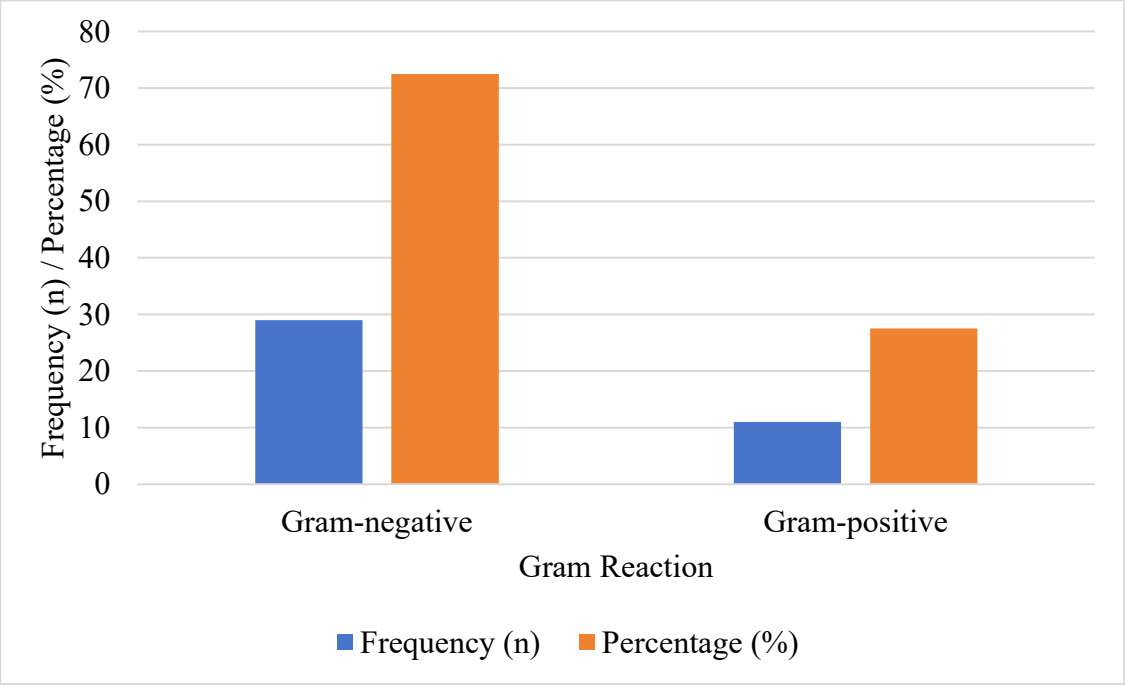


Figure 2. Distribution of Gram-Negative and Gram-Positive

Figure 2 revealed that Gram-negative bacteria made up the larger portion of the isolated colonies while Gram-positive bacteria made up only a smaller fraction of the entire bacterial population. The prevalence of Gram-negative bacteria implies that enteric and opportunistic pathogens comprised the predominant types of bacteria isolated from the samples.

3.4 AMR Patterns of Major Bacterial Isolates

A variety of commonly used antibiotics were employed to investigate the antimicrobial susceptibilities of the most common bacterial strains. Reporting the susceptibilities of bacteria by individual strain helps provide more insight into their individual reactions to the antimicrobial drugs. The susceptibility patterns are shown in Table 4.

Table 2. Species-wise Antimicrobial Susceptibility Profile of Major Bacterial Isolates

Antibiotic	E. coli (n=11)	K. pneumoniae (n=7)	P. aeruginosa (n=5)	S. aureus (n=8)	E. faecalis (n=3)
Ampicillin	27.3	14.3	-	25.0	66.7
Ceftriaxone	63.6	57.1	40.0	-	-
Ciprofloxacin	72.7	57.1	60.0	62.5	66.7
Gentamicin	81.8	71.4	80.0	75.0	66.7
Amikacin	90.9	85.7	100.0	-	-
Meropenem	100.0	85.7	100.0	-	-
Vancomycin	-	-	-	100.0	100.0
Linezolid	-	-	-	100.0	100.0

Note: "-" indicates that the antimicrobial agent is not routinely tested or reported for the respective bacterial species.

Differences in susceptibility patterns were noted for the various bacterial species tested (Table 2). Gram-positive bacteria were shown to be more responsive to vancomycin and linezolid, but Gram-negative bacteria were more susceptible to amikacin and meropenem than to ampicillin and ceftriaxone. This study underscores the need for performing antibiotic susceptibility testing, especially in view of species-specific responses to antibiotics.

3.5 Bacterial Isolate Distribution by Type of Clinical Specimen

In addition, the dispersal of bacteria was also analyzed based on the clinical specimen from which the bacteria were isolated. Analysis of the distribution of bacteria across different types of specimens is important for understanding the epidemiology of infection and the typical organisms found at various anatomical locations. Figure 3 shows the distribution of isolates of bacteria by specimen types.

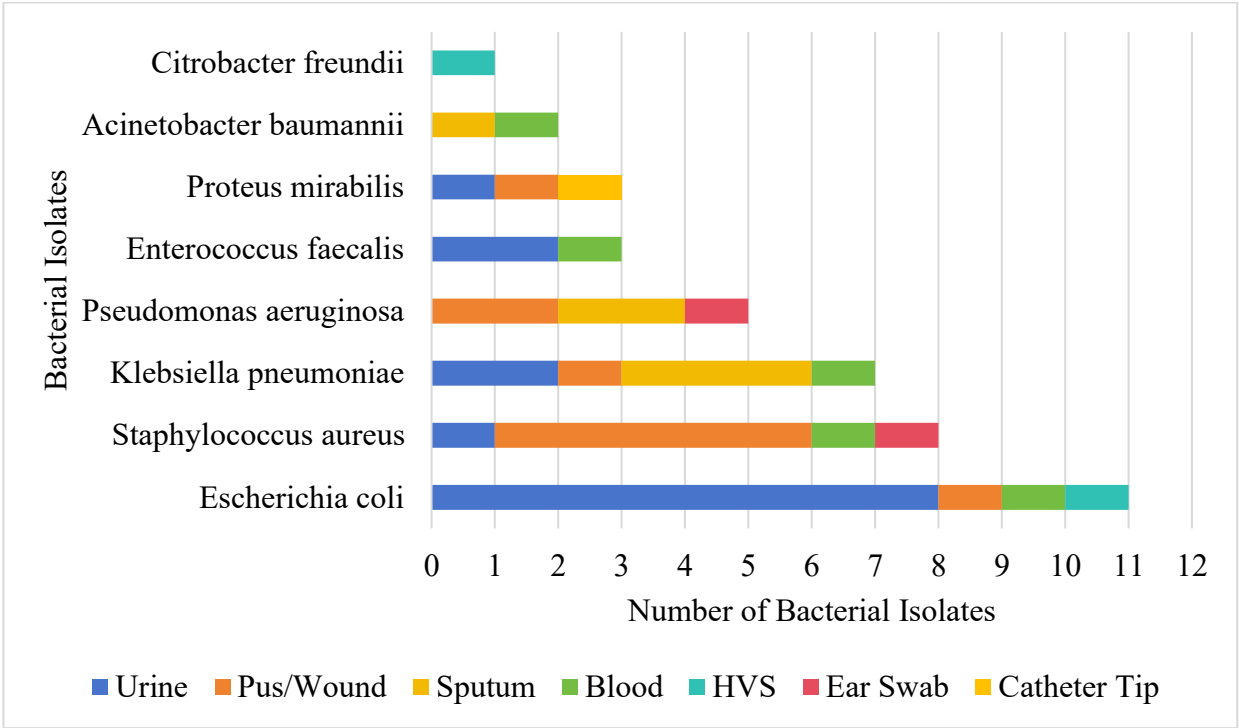


Figure 3. Bacterial Isolate Distribution by Clinical Specimen Type

Figure 3 illustrated how different clinical specimens had different bacterial isolate dispersion patterns. The isolates of *E. coli* were mostly recovered from urine specimens, while *S. aureus* were predominantly isolated from pus and wound specimens. *K. pneumoniae* and *P. aeruginosa* were mostly associated with sputum specimens, while *E. faecalis* was mostly recovered from urine and blood specimens. *P. mirabilis* was recovered from urine, pus/wound, and catheter tip specimens, while *A. baumannii* was isolated from sputum and blood specimens. *C. freundii* was isolated only from HVS specimens. Generally, the results show that the distribution of bacterial isolates differed by specimen type.

3.6 Association Between Clinical Specimen Type and Bacterial Isolates

To determine if the distribution of isolates of bacteria varied significantly depending on the kind of clinical material, the chi-square test for independence was used. The two factors were shown to have a substantial association, which means that some bacterial species were isolated more often from certain clinical specimens (Table 3).

Table 3. Association Between Clinical Specimen Type and Bacterial Isolates

Statistical Test	**Value**
Chi-square (χ^2)	48.62
Degrees of freedom (df)	42
p-value	0.021

The notable correlation noted above ($\chi^2 = 48.62$, $p = 0.021$) implies that the presence of the bacterial strains depended on the source of specimen (Table 3). The bacterium *Escherichia coli* were mainly detected in urine specimens, while the bacterium *Staphylococcus aureus* was more commonly found in pus and wound swabs. Likewise, the respiratory specimens were mainly characterized by the higher occurrence of the bacteria *K. pneumoniae* and *P. aeruginosa*.

4. Discussion

The current study sought to evaluate the distribution and patterns of AMR of forty isolates of bacteria from diverse clinical samples. Urine was the most frequent source of specimens, followed by sputum and pus or wound swabs. Gram-negative organisms predominated and demonstrated their significant role in the bacterial flora. The most prevalent organism identified was *E. coli*, followed by *S. aureus*, *K. pneumoniae,* and *P. aeruginosa*. Organisms differed in their distribution: *E. coli* was predominantly recovered from urine, *S. aureus* was mainly isolated from pus or wound specimens, and *K. pneumoniae* and *P. aeruginosa* were frequently accompanying with sputum specimens.

AST showed comparatively limited activity of ampicillin against several major isolates. Amikacin and meropenem, instead, were active against the Gram-negative organisms. Vancomycin and linezolid were quite operative in contradiction of the tested Gram-positive isolates. The results show that the distribution and susceptibility patterns of these bacteria are highly dependent on the species of the bacteria and the source of the specimens.

The current study's prevalence of Gram-negative bacteria is consistent with a number of other investigations conducted in diverse clinical contexts. It was reported that there is a significant proportion of Gram-negative organisms in bloodstream isolates which showed significant resistance to commonly used antibiotics highlighting the need for regular AST to guide the selection of appropriate antibiotic therapy (Negussie et al., 2015). Similarly, Prakash Simkhada et al. (2016) reported that the most frequent bacterial infections found in blood culture isolates from tertiary care hospitals were gram-negative bacteria, which highlighted the significance of ongoing microbiological surveillance for empirical therapy..

Escherichia coli was the most common bacteria isolated from urine samples in the present study which is in accordance with the study conducted by Bitew et al. (2017) that identified *E. coli* as the main uropathogenic bacteria found in people suffering from UTI. Similarly, Derese et al. (2016) found *E. coli* to be the most common cause of UTIs among pregnant women and to be sensitive to different antimicrobial drugs, which further made it advisable to use culture-based treatment. This is in keeping with the known epidemiology of bacterial UTI, where urinary isolates are predominant.

The high prevalence of *Staphylococcus aureus* confirmed by Bessa et al. (2015) in pus and wound samples is similar to that observed here, where both gram-positive and gram-negative bacteria with different antimicrobial susceptibility profiles were frequently found from infected wounds. The same was observed by Rai et al. (2017) who found the major bacterial pathogens in wound swabs as *S. aureus*, *E. coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* by conventional culture and susceptibility testing. The similarities highlight the microbiologic diversity of wound infections and the need for laboratory confirmation to treat them with the right antimicrobials.

Additionally, the antimicrobial susceptibility profile seen in this study is in line with other research. Resistance to ampicillin has consistently been reported in several studies and is considered a common form of resistance of Gram-negative organisms. Amikacin and the carbapenems, on the other hand, had relatively high activity against the Gram-negative isolates, findings which were consistent with those of Singh et al. (2017), who found that almost all Gram-negative isolates were more susceptible to aminoglycosides and other antimicrobial agents reserved for their treatment, compared to those routinely used in the treatment of bacterial infection in burn patients. Likewise, Fayyaz et al. (2015) highlighted the different antimicrobial susceptibility patterns of clinical isolates and urged routine susceptibility testing for selection of the most appropriate antimicrobial agents and for prevention of inappropriate empirical therapy.

The recent investigation indicated that vancomycin and linezolid were 100% effective against the Gram-positive isolates, which has been confirmed by other studies demonstrating continued efficacy of these agents against susceptible Gram-positive pathogens. Similarly, Shiferaw et al. (2015) reported that the antimicrobial susceptibility patterns were found to be different for bacteria isolated from external ocular infections and emphasized the point of choosing treatment based on the laboratory results of the bacteria instead of empirical assumptions.

The current results also highlight the need for ongoing surveillance of AMR in various types of specimens. Gashe et al. (2018) showed that clinical isolates are becoming increasingly resistant to third generation cephalosporins, highlighting the escalating problem of AMR in hospital and community associated pathogens. Similarly, Malik et al. (2018) reported on the different susceptibility patterns of respiratory bacterial isolates isolated from patients in intensive care unit, demonstrating that resistance profiles vary depending on the clinical source and species of the bacteria. Moreover, standardized identification methods and AST are foundational pillars in clinical microbiology laboratories to enable proper diagnosis, correct antibiotic selection and surveillance of emerging resistance patterns, as noted by Gajdács et al., 2017. In general, the present results confirm those reported in the literature that the epidemiological profile of these bacteria and their susceptibility to antimicrobial agents still remains dynamic from one geographic area to another and from one type of specimen to another, requiring regular local surveillance to ensure appropriate and effective use of antimicrobial agents.

The study's findings have consequences for the choice of antibiotics. This high *E. coli* prevalence in urine samples has suggested that *E. coli* should be considered a significant organism to investigate a possible urinary infection. Likewise, the isolation of *S. aureus*, *P. aeruginosa* or other gram-negative organisms from wound culture should be treated with appropriate coverage until the culture report comes back. Low susceptibility to ampicillin indicates that there may be some organisms commonly recovered that are not sensitive to the drug, and so empirical use may not be effective. It was discovered that meropenem, amikacin, vancomycin, and linezolid were effective medications but

they require caution as they can lose their efficacy. Culture-based identification and susceptibility testing can assist with the identification of targeted treatment, minimize unnecessary exposure to broad-spectrum antimicrobials and play a role in antimicrobial stewardship.

The inclusion of isolates from several specimen types was one of the study's advantages, enabling the bacterial distribution to be analyzed in relation to various clinical specimens. The identification of the bacteria and their species-level susceptibility to antibiotics were also included into the investigation. The Kirby-Bauer disc diffusion method and standardised culture protocols contributed to the uniformity of laboratory interpretation.

Only 40 isolates were available for the study, and results may not be generalizable. Detailed species-level comparisons were not possible for some bacterial species as very few individuals were present. Multidrug resistance, production of extended-spectrum beta-lactamase, methicillin resistance, minimum inhibitory concentration, and the molecular mechanisms of resistance were not evaluated in the investigation. Furthermore, not all antimicrobials were tested against all isolates as the choice of antibiotic depended on the bacterial group.

Larger numbers of isolates from various laboratory sources and longer-term monitoring should be included to detect shifts in bacterial distribution and resistance in future studies. Major resistance mechanisms should also be tested phenotypically and/or molecularly. Preparing local antibiograms and the improvement of laboratory input into antimicrobial stewardship are recommended to guide effective antimicrobial therapy.

5. Conclusion

The identification of the bacteria and their species-level susceptibility to antibiotics were also included into the investigation. The most common organisms isolated were Gram-negative bacteria, in particular *E. coli*, *S. aureus* and *K. pneumoniae*. The results also showed that the most common bacterial species in urine and pus/wound samples differed significantly, where *E. coli* predominated in urine samples and *S. aureus* in wound/pus samples. There was decreased activity for ampicillin against certain large gram-negative isolates, and high activity for amikacin and meropenem against the gram-negative organisms, as determined by AST. Gram-positive isolates exhibited strong resistance to linezolid and vancomycin, suggesting that these drugs are still useful in the treatment of such infections, where clinically indicated. This observed interpatient susceptibility variation underscores the need to proceed with culture-based bacterial identification and with regular AST to inform antibiotic use decisions. Empirical therapy, or using antibiotics based on clinical criteria before laboratory confirmation, could lead to improper use of antibiotics could accelerate the development of AMR. Therefore, it is recommended that there is regular monitoring of local bacterial epidemiology and trends of resistance that can help to guide the selection of antimicrobials and enhance stewardship efforts. To sum up, the study offers insightful information on the distribution of the primary bacterial pathogens and their patterns of resistance, highlighting the need for ongoing microbial monitoring and thoughtful use of antibiotics for better patient care and for implementing appropriate infection prevention measures.

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