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Antibiotic Susceptibility Pattern of Bacterial Isolates in Wound Infections

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Abstract

Background: Wound infections caused by bacteria like *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are a major health risk, especially for immunocompromised individuals. Antibiotic overuse has increased resistance, complicating treatment. Ongoing monitoring and species-specific antibiotic use are vital for effective treatment.

Objective(s): To determine the prevalence of micro-organisms responsible for wound infections, identifying the most common pathogens involved, assess the antibiotic susceptibility patterns of these microorganisms to guide effective treatment and prevent the spread of resistant strains.

Methodology: In this study, bacterial isolates from wound infections were collected in a clinical setting, and species were identified using common microbiological protocols. Using the disk diffusion method, antibiotic susceptibility and resistance was evaluated by measuring the zone of inhibition. Bacterial species and resistance patterns were compared using statistical techniques such as Chi-Square testing. The therapeutic effect of antibiotics for various isolates was evaluated using descriptive statistics.

Results: The study found *S.aureus (59%),* and *E.faecalis (42%)* were common in wound infections, after *P.aeruginosa(30%), S.pyogens(29%), E.coli(20%) and K.pneumoniae(19%)*. Meropenem and Imipenem was the most effective antibiotic, while Penicillin and Amikacin showed high resistance. Vancomycin and Ceftriaxone had moderate efficacy with variable susceptibility. The results highlight the importance of species-specific antibiotic selection for treating wound infections.

Conclusion(s): The study on the antibiotic susceptibility pattern of bacterial isolates in wound infections reveals important findings regarding the prevalence of pathogens and their resistance to commonly used antibiotics. S. aureus, E. faecalis, and P. aeruginosa are the main causes of wound infections, and their resistance to widely used antibiotics is growing. Penicillin and Amikacin shown great resistance, although Meropenem and Imipenem were the most effective. To improve treatment outcomes and fight resistance, species-specific antibiotic tactics must be tailored.

Key words: Wound infections, Bacterial species, Antibiotic susceptibility, Meropenem, Penicillin, Ceftriaxone, Antibiotic resistance, species-specific therapy, resistance trends, infection control.

Introduction

Any opening or injury to the skin caused by trauma, accident, surgery, or burn that allows microorganisms to enter the body is called a wound infection. Infection of the wound is the outcome of successful arrival and spread of one or more species of microbes, occasionally producing pus creation.[1]A wound is the disruption in the continuity of soft parts of the body structure.[2] A wound offers a warm, humid, and nutrient-rich environment that is ideal for the colonization, growth, and infection of microorganisms.[3] Successful invasion and proliferation of microorganisms within sterile body tissues, anywhere in the body, defines wound infection. This process can lead to pus formation.[4]

The presence of infection in wounds becomes a significant barrier to healing because it creates adverse impacts that reduce both patient quality of life and wound healing rate. Patients with infected wounds experience greater discomfort because these wounds become hypersensitive along with odor production which leads to an increase in patient disturbance.[5] Human skin hosts various different bacterial species at any given time. The human skin remains vulnerable to pathogen colonization because it exists in contact with outside agents along with sustaining skin injuries and surface abrasions.[6]

There exist two fundamental categories of wounds known as acute and chronic. The healing process of acute wounds occurs according to standard wound repair stages while resulting from external causes such as cuts, burns, abrasions and surgical procedures. Postoperative wound infections represent nearly one-third of hospital-acquired infections that lead to 70–80% fatal results.[7,8] Six types of wound infection include surgical site infection alongside Bite wound infection and Burn wound infection with Acute soft tissue infection and diabetic foot ulcer infection as well as leg and decubitus (pressure) ulcer infection.[9]

One of the primary bacterial causes of wound infections include Pseudomonas aeruginosa and Staphylococcus aureus as well as Klebsiella pneumoniae and Enterococcus faecalis together with Acinetobacter baumannii. The initial period of infection showcases S. aureus as the main colonizing bacteria that belongs to the gram-positive species.[10] Gram-negative bacteria, like P. aeruginosa and A. baumannii, begin to colonize the wound around the start of the second week.[11]

S. aureus maintained the highest isolation rate among chronic leg ulcers bacterial species during the research period and was found in 93.5% of patients. The isolation rate of S. aureus exceeded other research findings by being found in 93.5% of investigated ulcers yet matched rates obtained by Madsen et al.[12] Having a prevalence rate of 60% in acute wounds and 100% in chronic wounds, the biofilm is one of the most intricate components involved in wound healing. Due to their difficulty in healing, these wounds present a significant problem for both public and military medical facilities.[13]The vital approach to sustain healing processes consists of eliminating microbial infections from the damaged tissue.[14]

The widespread use of antibiotics continues to become less effective against bacterial pathogens because multiple antibiotic resistances are now present in many bacteria populations. Bacterial drug resistance developed because antibiotics received an indiscriminate and widespread use led by overatedacment, self-medication practices and dental prescriptions of illogical drugs combined with antibiotic use extended beyond medical standards.[15]

Hospitals around the world encounter hospital-acquired infections as a major health threat that poses serious risks to patients.[16] The WHO has provided a description of this issue that wounds represent a main source of infectious diseases because they lead to substantial economic loss with high rates of disease occurrence and death. The development of infection control strategies has failed to eliminate wound infections due to drug-resistant microorganisms.[17]

Treatment of wound infections faces significant challenges from the presence of two major antimicrobial substances: Methicillin Resistant Staphylococcus aureus (MRSA) as well as Extended Spectrum Beta Lactamase (ESBL) production.[18] Therefore, choosing the right medications based on antibiotic sensitivity testing is crucial. So, appropriate drugs selected by antibiotic sensitivity testing have great importance.[19] Having acquired resistance to standard antibiotics proved challenging for healthcare providers while treating bacterial wound infections. A crucial part of this investigation examined how Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes, Enterococcus faecalis and Klebsiella pneumoniae bacterial strains responded to commonly administered antibiotics from wound infection sites. The research purpose is to detect both bacterial species and susceptibility patterns against commonly used therapeutic antibiotics in wound infection.

Material and Methods

Study Design: It was a descriptive cross-sectional study.

Settings: This retrospective study was conducted to investigate the antibiotic susceptibility pattern of bacterial isolates from wound infections at Shaikh Zayed Hospital.

Duration of Study: The study was carried out over a period of 4 to 6 months(Oct-March) after the synopsis had been approved.

Sample Size: 199

Formula: n = (Z^2 * p * (1-p)) / E^2.

Sampling Technique: Random conventional sampling was used to select participants who met the inclusion criteria and were available during the study period.

Sample Selection

Inclusion Criteria

- Presence of a wound infection (e.g., surgical site infections, bite wound infections, burn wound infections, acute soft tissue infections, diabetic foot ulcer infections, leg and decubitus (pressure) ulcer infections).
- Wound duration of at least 30 days.
- Clinical signs of infection (e.g., redness, swelling, warmth, purulence).

Exclusion Criteria

- Wounds with no visible signs of infection.
- Patients with severe immunosuppression (e.g., HIV/AIDS, cancer).
- Patients on antibiotics or antimicrobial therapy within the past 2 weeks.

Equipment: In this study, bacterial cultures were processed using the BACTEC FX system (BD) for initial growth and the VITEK 2 Compact (bioMérieux) for identification and antibiotic susceptibility testing. Cultures were incubated at 37°C in Heratherm incubators, utilizing Blood Agar and MacConkey Agar as standard culture media. An autoclave sterilized equipment and media, while a microscope facilitated the examination of colony morphology for preliminary identification of the isolate.

Scanning Technique

- A sterile swab was used to collect surface samples from the wound area.
- Specimens were inoculated on Blood Agar and MacConkey Agar.
- Cultures were incubated at 37°C for 24–48 hours.
- Colony morphology was observed, and biochemical tests (e.g., oxidase, urease) were performed along with specific identification kits for confirmation.
- Antibiotic Testing was performed.
- The Kirby-Bauer disk diffusion method was used, following CLSI guidelines, to assess antibiotic susceptibility profiles.
- Results were categorized as Susceptible, Intermediate, or Resistant.

Data Collection Procedure
This study was done in Lahore. According to the medical records verified by the assigned research assistant, wound infection patients were selected for the study. The following data was collected from hospital laboratories.

1. Sample Collection

- **Patient Samples**: Wound swabs were collected from patients

2. **Sample Processing and Bacterial Identification**

- **Culture**: Wound samples were inoculated on **blood agar** and **MacConkey agar**, and incubated at **37°C** for 24–48 hours.

Colony Morphology:

- *Staphylococcus aureus*: Golden/yellow colonies
- *Streptococcus pyogenes*: Small, translucent, or greyish colonies
- *Escherichia coli*: Colorless or light pink colonies
- *Pseudomonas aeruginosa*: Green/blue colonies due to pyocyanin pigment
- *Klebsiella pneumoniae*: Light pink/colorless colonies
- *Enterococcus faecalis*: White or cream-colored colonies

- **Identification**: The bacterial isolates were identified using:
  - **Gram Staining**: Gram-positive cocci (S. aureus, S. pyogenes, E. faecalis) and Gram-negative rods (E. coli, P. aeruginosa, K. pneumoniae).
  - **Biochemical Tests**: Standard tests were performed, such as:
  - **Catalase Test**: (S. aureus is catalase positive, while S. pyogenes is catalase negative)
  - **Oxidase Test**: (P. aeruginosa is oxidase positive)
  - **Citrate Utilization**: (K. pneumoniae is citrate positive)
  - **Indole Test**: (E. coli is indole positive)

3. Antimicrobial Susceptibility Testing (AST)

- **Kirby-Bauer Disk Diffusion Method**:

o Inoculate Mueller-Hinton agar plates with a standardized inoculum of bacterial isolates obtained from wound infection.

o Place antibiotic disks containing various antibiotics (e.g., amikacin, gentamicin, imipenem, meropenem, piperacillin/Tazobactam, Cefepime, ciprofloxacin, etc.) onto the agar plates.

o Incubate the plates and measure the zones of inhibition around each disk.

o Interpret the results according to Clinical and Laboratory Standards Institute (CLSI) guidelines to determine susceptibility or resistance to each antibiotic.

Data Analysis Procedure
For the data analysis, statistical software was used, such as SPSS (Statistical Package for the Social Sciences), version 28.0.

1. Descriptive Statistics

- **Frequency Distribution:** To summarize categorical variables like gender, bacteria and antibiotic resistance profiles.

2. Test of Significance

- **Chi-square Test:** Test to compare the distribution of bacterial species across different antibiotics and to assess associations between antibiotic resistance.

RESULTS
A total of 199 wound infection patients were evaluated and Six (6) bacteria were isolated from them, such as Gram-negitive bacteria (E. coli, P. aeruginosa, and Klebsiella pneumonia) and Gram-positive bacteria (E. faecalis, S. aureus, S. pyogenes) The Gram staining results showed that all were positive.

1.Gender Distribution of Bacterial Isolates in Wound Infection

Table 1: Gender distribution of Wound Infection

Gender	Frequency	Percentage
Female	103	52.0
Male	96	48.0
Total	199	100.0

A total 199 samples were investigated in this study. The positive cultures were predominantly found in female patients (n= 103), while male patients (n=96). This suggests a higher occurrence of Wound infections in females in hospitalized settings.

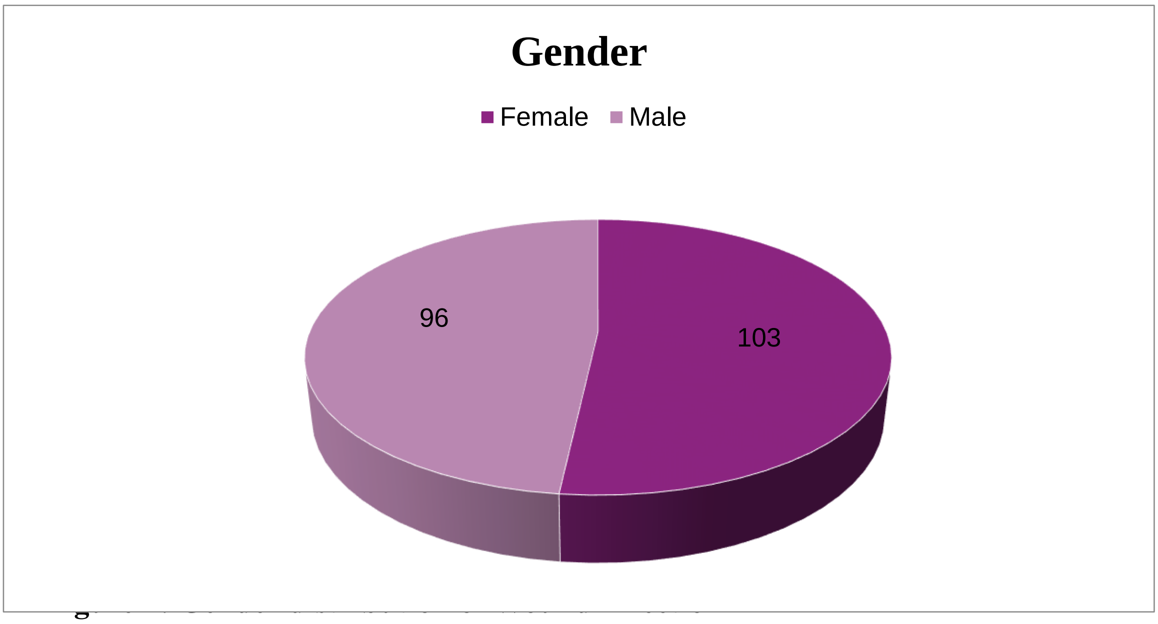
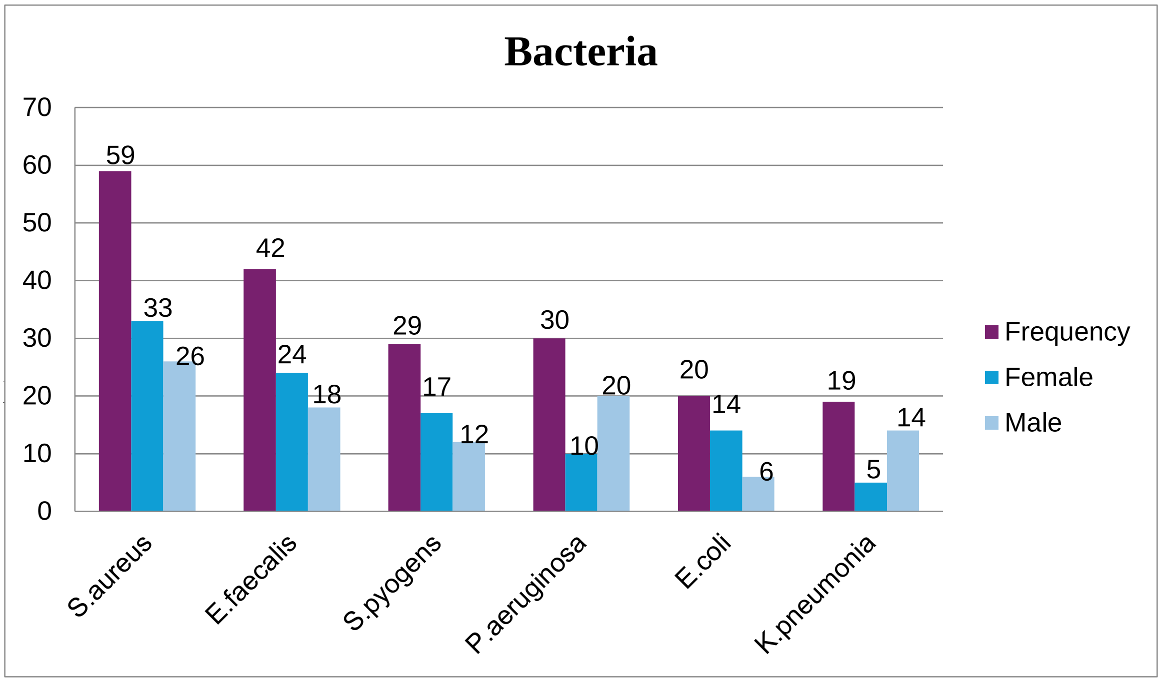


Table no 2: Distribution of different bacteria across Gender

Bacteria	Frequency	Female	Male
S. aureus	59	33	26
E. Faecalis	42	24	18
S. pyogens	29	17	12
P. aeruginosa	30	10	20
E. coli	20	14	6
K. pneumoniae	19	5	14

This table presents the distribution of various bacterial types across male and female patients. S. aureus showed the highest frequency, with a female majority (55.9%). E. coli also had a higher prevalence in females (70%). E. faecalis and S. pyogenes were more evenly distributed but still slightly higher in females. In contrast, K. pneumoniae and P. aeruginosa were more common in males, indicating gender-based variation in bacterial infections.



2. Antibiotic Susceptibility Testing (AST) Result

S. aureus, E. faecalis, S. pyogens, E. coli, K. pneumoniae, P. aeruginose isolates were tested against various antibiotics to determine their susceptibility patterns.

Table3: Antibiotic resistance rates of E. coli, Entero, Klebsiella, Pseudo isolated from various drugs

	Antibiotics									
Bacteria	**CEF**	**Vanco**	**Cipro**	**Mero**	**AK**	**AUG**	**CN**	**Metro**	**pencillin**	**IMP**
E. coli	7	14	7	3	4	14	2	8	16	2
E. faecalis	19	9	24	5	26	4	4	32	26	33
Klebsiella	9	14	3	3	1	16	16	10	1	5
Pseudo	11	22	2	6	6	23	24	6	6	6
S. aureus	19	18	8	31	35	11	21	13	35	17
S. pyogene	4	4	3	5	24	5	5	3	24	2

Antibiotic Resistance Rates:

Highest Resistance Rates:

- Penicillin (40%), Amikacin (35%), and Metronidazole (32%) showed the highest resistance, especially against S. aureus and E. faecalis, making them less effective treatment options.
- Gentamicin (24%) and Augmentin (23%) also exhibited high resistance in P. aeruginosa, indicating reduced efficacy.

Moderate Resistance Rates:

- Vancomycin (14–22%), Ceftriaxone (up to 19%), and Ciprofloxacin (up to 24%) showed moderate resistance, suggesting their effectiveness may vary and should be guided by susceptibility testing.
- K. pneumoniae and E. coli showed moderate resistance to Metronidazole and Amikacin.

Lowest Resistance Rates (Most Effective Antibiotics):

- Imipenem (2–6%) and Meropenem (3–6%) had the lowest resistance rates across all isolates, indicating Carbapenems as the most effective treatment choices.
- Ciprofloxacin (2–7%) and Gentamicin (2–6%) also showed low resistance in E. coli, S. pyogenes, and K. pneumoniae, supporting their potential use, especially in less severe infections.

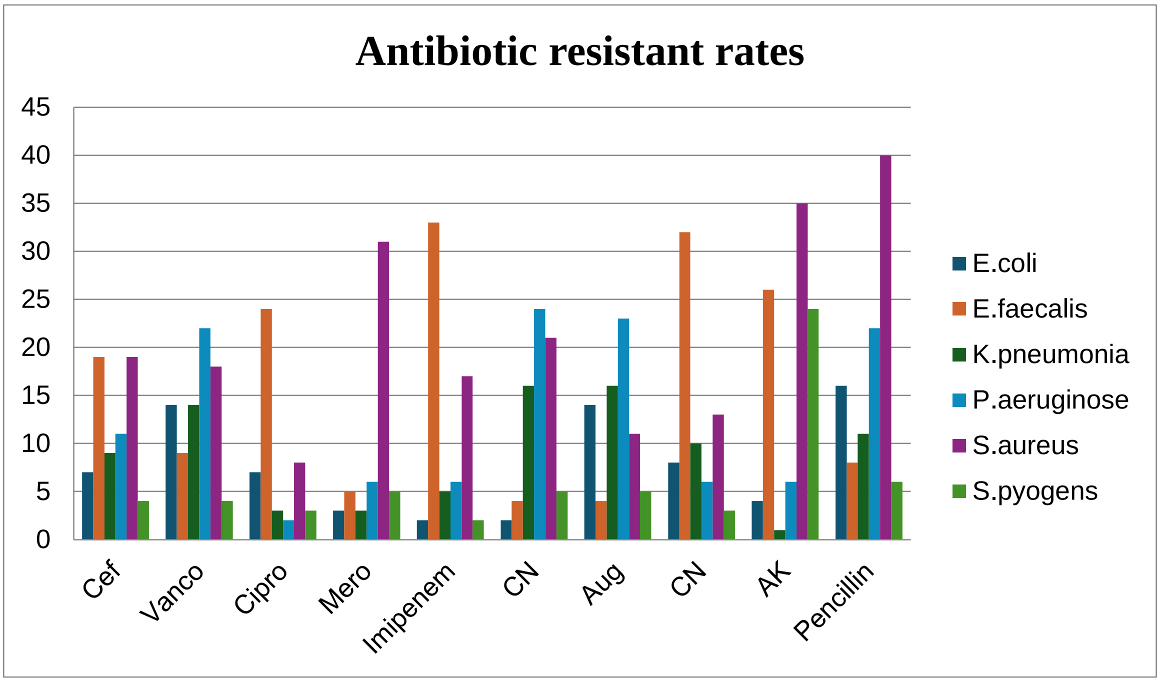


Figure3: Chart show Antibiotic resistance rates of E. coli, E. faecalis, K. pneumoniae, P. aeruginose, S. aureus, S. pyogens species

1.Penicillin Resistance in Various Bacteria

Table 4: Anti-microbial resistance of Penicillin in different bacteria

	Penicillin	
Bacteria	**R**	**I**
E.coli	16	4
E.Faecalis	8	34
k.Pneumoniae	11	8
P.aeruginosa	22	8
S.aureus	40	19
S.Pyogens	6	23
Total	**103**	**96**

This table presents the counts for each bacterial type's response to the antibiotic Penicillin categorized as "Resistant," "Sensitive."

❖ It helps to visualize the distribution of antibiotic responses among different types of bacteria.

Chi-Square Test

Test	Value	Df	P-value
Pearson Chi-Square	47.555[a]	5	.000

❖ The chi-square value is 47.555, with a p-value of 0.000

Interpretation

The Chi-Square test shows a highly significant association between the variables (p = 0.000), indicating a strong relationship. The low p-value confirms that the observed differences are unlikely due to chance.

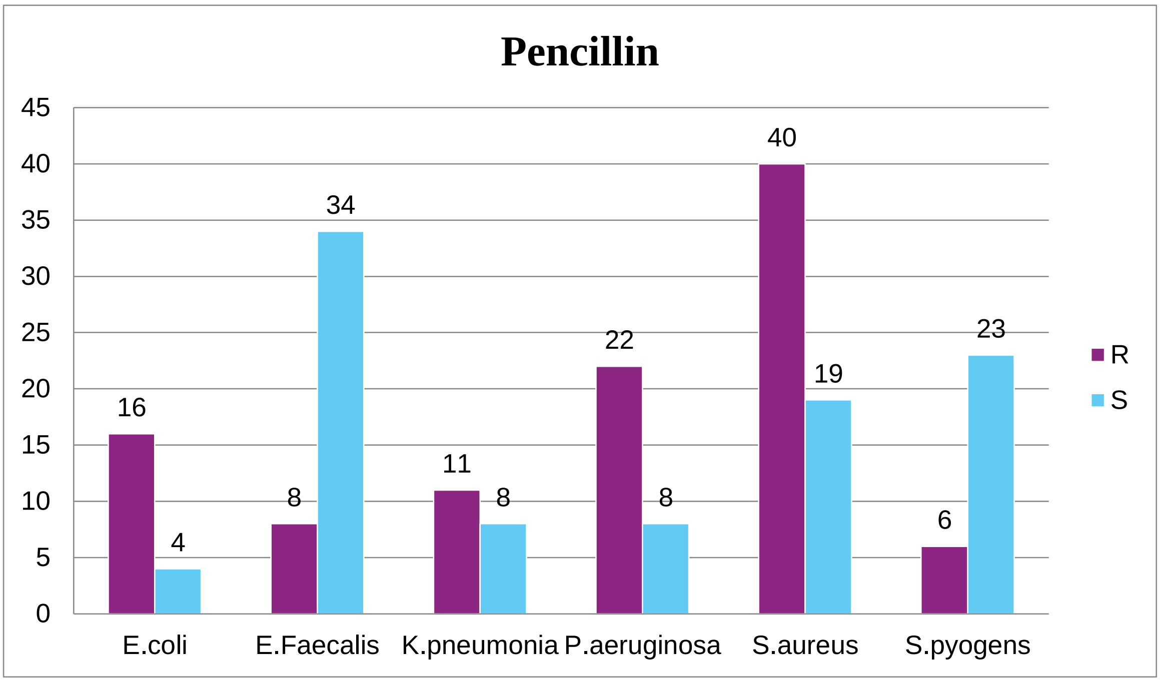


Figure 4: Chart showing Resistivity and Sensitivity patterns of Pencillin in different bacteria

The majority of bacteria, particularly S. aureus (40R), P. aeruginosa (22R), and E. coli (16R), showed high resistance to Penicillin, indicating it is less effective against these pathogens. In contrast, E. faecalis (34S) and S. pyogenes (23S) exhibited greater sensitivity, suggesting better treatment outcomes with Penicillin for these species.

AK Resistance in Various Bacteria

Table 5: Anti-microbial resistance of AK in different Specimens

	Amikacin		
Bacteria	**R**	**S**	**I**
E.coli	4	15	1
E.Faecalis	26	16	0
k.Pneumoniae	1	4	14
P.aeruginosa	6	24	0
S.aureus	35	22	2
S.Pyogens	24	5	0
Total	**96**	**86**	**17**

❖ This table presents the counts for each bacterial type's response to the antibiotic AK categorized as "Resistant," "Sensitive and Intermediate"

❖ It helps to visualize the distribution of antibiotic responses among different types of bacteria.

Chi-Square Tests

Test	Value	Df	p-value
Pearson Chi-Square	152.455[a]	10	.000

❖ The chi-square value is 152.455 with a p-value of 0.000

Interpretation

The Chi-Square test shows a highly significant association between the variables (p = 0.000), indicating a strong relationship. The results suggest that the observed differences are unlikely due to chance, confirming statistical significance.

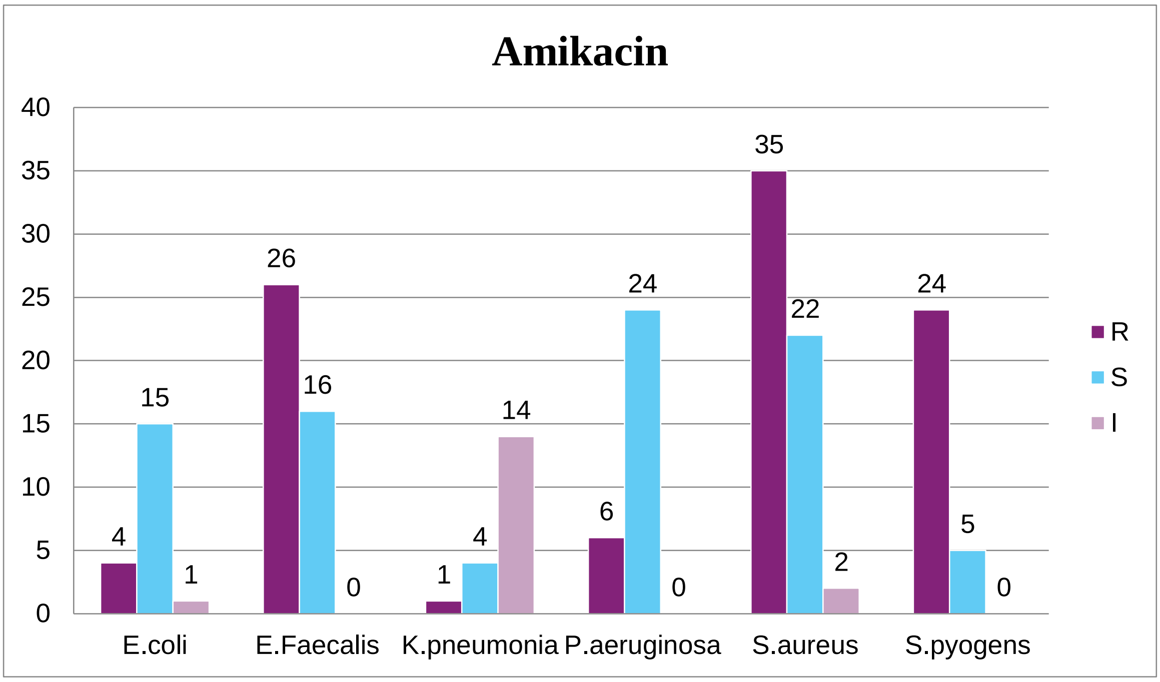


Figure 5: Chart showing Resistivity and Sensitivity patterns of AK in different bacteria

E. faecalis and S. aureus exhibit high resistance to Amikacin, with 26 and 35 resistant cases, respectively.

K. pneumoniae and P. aeruginosa show more sensitivity, particularly P. aeruginosa with 24 sensitive isolates.

CN Resistance in Various Bacteria

Table 6: Anti-microbial resistance of CN in different bacteria

	Gentamicin		
Bacteria	**R**	**S**	**I**
E.coli	2	18	0
E.Faecalis	4	9	29
k.Pneumoniae	16	3	0
P.aeruginosa	24	6	0
S.aureus	21	16	22
S.Pyogens	5	5	19

Total	72	57	70

❖ This table presents the counts for each bacterial type's response to the antibiotic CN categorized as "Resistant", Sensitive and Intermediate."

❖ It helps to visualize the distribution of antibiotic responses among different types of bacteria.

Chi-Square Tests

Test	Value	Df	p-value
Pearson Chi-Square	118.880[a]	10	.000

❖ The chi-square value is 118.880 with a p-value of 0.000

Interpretation

The Pearson Chi-Square test indicates a highly significant association between the variables (p = 0.000.This suggests a strong relationship between bacterial types and their response patterns. The result is statistically reliable and unlikely due to chance.

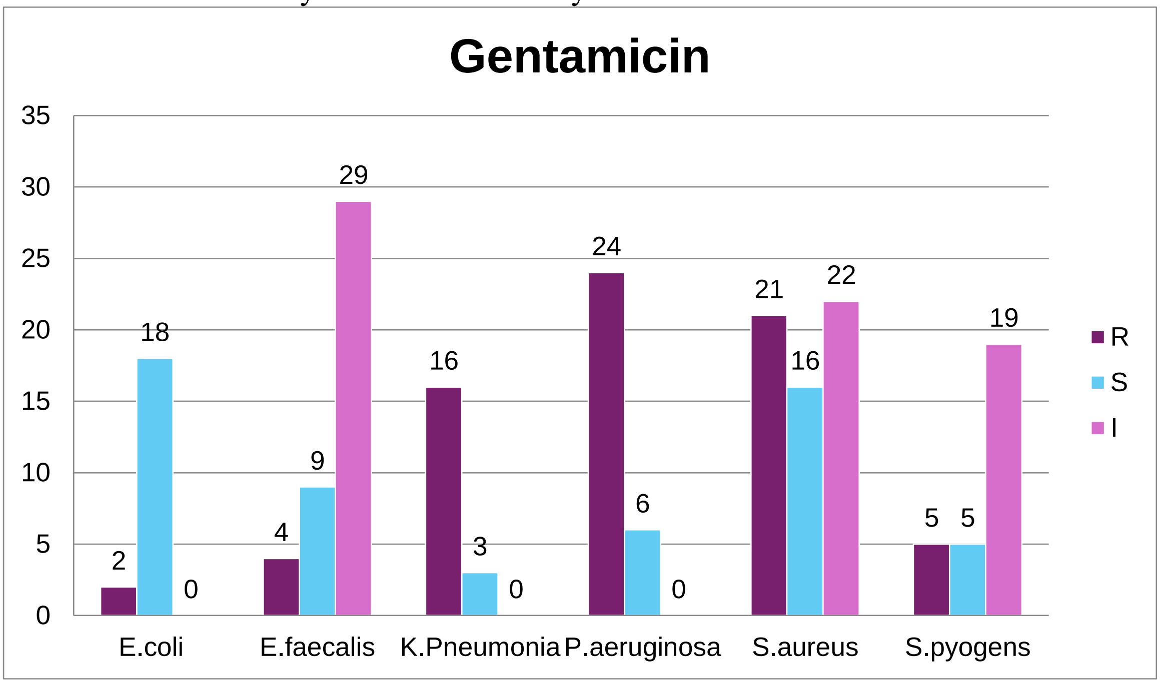


Figure 6: Chart showing Resistivity and Sensitivity patterns of CN in different bacteria

Overall, with 72 resistant, 57 sensitive, and 70 intermediate isolates, Gentamicin shows mixed performance across different bacterial species.

Augmentin Resistance in Various Bacteria

Table 7: Anti-microbial resistance of AUG in different bacteria

	Augmentin		
Bacteria	**R**	**S**	**I**
E.coli	14	6	0
E.Faecalis	4	10	28
k.Pneumoniae	16	3	0
P.aeruginosa	23	7	0

S.aureus	11	8	40
S.Pyogens	5	10	14
Total	**73**	**44**	**82**

❖ This table presents the counts for each bacterial type's response to the antibiotic Augmentin categorized as "Resistant , Sensitive and Intermediate."

❖ It helps to visualize the distribution of antibiotic responses among different types of bacteria.

Chi-Square Tests

Test	Value	Df	p-value
Pearson Chi-Square	97.948[a]	10	.000

❖ The chi-square value is 97.948 with a p-value of 0.000

Interpretation

The Chi-Square test shows a highly significant association between the variables (p = 0.000). This means the differences observed are statistically meaningful and not due to chance. The test confirms a strong relationship between the categories analyzed.

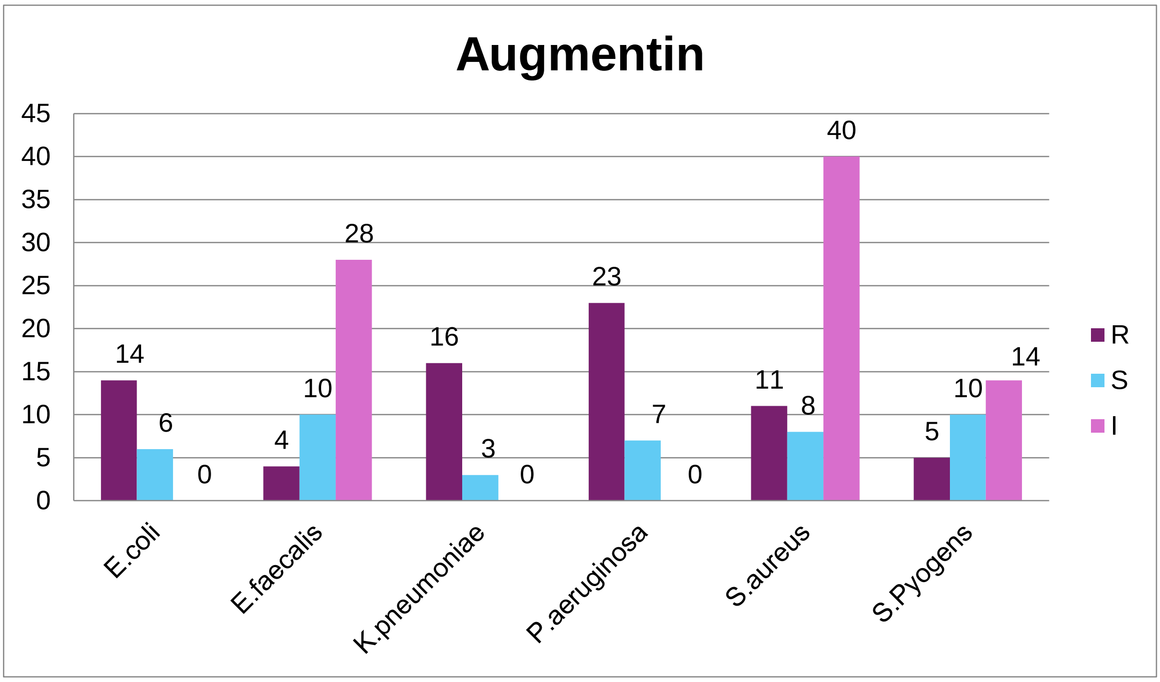


Figure 7: Chart showing Resistivity and Sensitivity patterns of Aug in different bacteria

This chart shows the resistance (R), sensitivity (S), and intermediate (I) patterns of various bacterial types to Augmentin (Aug).

Overall, out of 199 total isolates, 73 were resistant, 44 intermediate, and 82 sensitive, reflecting mixed effectiveness of Augmentin depending on the bacterial species.

Discussion

Wound infections, caused by a variety of bacterial pathogens, are a significant concern in clinical practice, particularly in settings involving chronic wounds, burns, or postoperative infections. The identification of the causative bacteria and their antibiotic susceptibility profiles is essential for effective treatment and management. This comparison discusses the findings of the present study with previous studies to assess the consistency, deviations, and trends in bacterial pathogens and antibiotic resistance patterns, with a particular focus on Gram-positive and Gram-negative organisms, multi-drug resistance, and the role of geographical region.

The primary bacterial cause of wound infections according to this study is Staphylococcus aureus at 50% while Escherichia coli remains at 22.5% and Pseudomonas aeruginosa stands at 17.5%. The identification of S. aureus as the most common infection pathogen in wounds matches previous research findings by Kariuki et al. (2022) as well as Puca et al. (2021). The study conducted by Puca et al. (2021) showed S. aureus was detected in 79.4% of Gram-positive cases and the primary Gram-negative bacteria included P. aeruginosa at 40.2% and E. coli at 20.7% respectively.[20]

According to Lipsky et al. (2016), S. aureus emerged as the dominant pathogen (37%) at level of P. aeruginosa (17%) and E. coli (6%) which validates the ongoing significance of S. aureus in wound infections throughout worldwide regions.[21] Studies such as this one with S. aureus and P. aeruginosa as main pathogens together with actual differences in antibiotic resistance based on geographical locations are important research elements. Studies by Kariuki et al. (2022) show increased ampicillin and oxacillin resistance in East Africa yet Puca et al. (2021) and Lipsky et al. (2016) found different resistance patterns in Western countries since these patterns are potentially shaped by local antibiotic use and infection prevention methods as well as microbial communities in specific geographic areas.[22-24] The present research shows that S. aureus isolates demonstrate low resistance toward both vancomycin and methicillin even though other studies reported higher levels such as Abdalla et al. (2015) who observed 90.5% vancomycin resistance. The healthcare facilities in this region utilize particular antibiotic therapies and enforce antibiotic stewardship protocols leading to low resistance rates.[25]

Conclusion

In this study the antibiotic susceptibility pattern of bacterial isolates in wound infections reveals important findings regarding the prevalence of pathogens and their resistance to commonly used antibiotics. A total of 199 wound infection patients were evaluated, with bacterial isolates showing a notable gender distribution. Females accounted for 52% (103 patients), while males made up 48% (96 patients), indicating a slightly higher prevalence of wound infections in females. Among the six bacterial species isolated, S. aureus was the most frequent (29.6%), followed by E. faecalis (21.1%) and S. pyogenes (14.6%). Antibiotic susceptibility testing revealed high resistance to Penicillin (40%), Amikacin (35%), and Metronidazole (32%) across various bacteria, with S. aureus and E. faecalis showing the highest resistance. However, Carbapenems (Imipenem and Meropenem) showed the lowest resistance rates (2–6%), making them the most effective treatment options.The study underlined how important it is to choose antibiotics based on species for treating wound infections.

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