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Original Research Article

Isolation, Identification and Antibiotics Susceptibility Patterns of Bacteria Isolated from Wound of Patients Attending Ahmad Sani Yariman Bakura Specialist Hospital, Gusau

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Abstract: Wound infections are significant challenge in clinical practice, predominantly caused by bacterial colonization, which delay healing and increase patient morbidity. This study aimed to isolate, identify and determine the antibiotics susceptibility patterns of bacteria isolated from wound of patients attending Ahmad Sani Yariman Bakura Specialist Hospital, Gusau. Fifty wound samples were collected and processed microbiologically including culture, gram staining, biochemical test and serological test. The antibiotics susceptibility testing was carried out using the Kirby–Bauer's disc diffusion method. The bacterial species isolated were *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Klebsiella pneumonia.* The antibiotics susceptibility testing consistently yielded high susceptibility to amikacin across all the bacterial isolates, indicating the effectiveness of amikacin towards each isolate. However, high resistance was observed against meropenem across all the isolated species, posing a significant challenge in the effective management of wound infections. These findings highlight the prevalence of bacterial pathogens in wound infections and emphasize the growing challenge of antibiotics resistance, particularly to meropenem.

Keywords: Antibiotics Susceptibility, Isolation, Identification, Wound.

INTRODUCTION

Wound infections remain a significant cause of morbidity and prolonged hospital stays globally, particularly in developing countries where resources and infection control measures may be limited. A wound is essentially a disruption in the integrity of the skin and underlying tissues, often resulting from trauma, surgery, burns, or chronic conditions such as diabetes. Once the protective barrier of the skin is compromised, it becomes susceptible to microbial invasion, which can lead to infection if not properly managed (Hurlow *et al.,* 2022).

The diverse microbial flora of the skin, coupled with environmental contaminants, ensures that most wounds are exposed to microorganisms. Common bacterial culprits implicated in wound infections include Gram-positive cocci such as *Staphylococcus aureus* (including Methicillin-Resistant *S. aureus* - MRSA) and *Streptococcus pyogenes*, as well as Gram-negative bacilli like *Pseudomonas aeruginosa, Escherichia coli,* and *Klebsiella pneumoniae* (Bucataru *et al.,* 2023). The spectrum of bacteria involved in wound infections is dynamic and can vary based on geographical location, hospital setting, patient demographics, and the type of wound (Worku *et al.,* 2024).

Wound infections can be classified into acute and chronic types. Acute wound infections usually occur shortly after the wound is inflicted and may heal within a short time if appropriately managed. In contrast,

chronic wound infections persist over a longer period and are often associated with biofilm formation, which complicates treatment due to increased resistance to antibiotics (Durand *et al.*, 2022). Common chronic wounds include diabetic foot ulcers, pressure ulcers, and venous leg ulcers, all of which are challenging to manage due to the multifactorial nature of their etiology and healing process.

Antibiotic susceptibility in wound infections is a critical aspect of effective treatment and infection control. When a wound becomes infected, the choice of antibiotics depends on the sensitivity of the causative microorganisms. Susceptibility testing helps determine which antibiotics are most effective against specific bacterial strains isolated from the wound (Nobel *et al.,* 2022). This ensures targeted therapy, minimizes treatment failure, and prevents the misuse of broad-spectrum antibiotics that can lead to resistance.

Monitoring antibiotic susceptibility patterns also aids in developing local antibiotic guidelines, ultimately improving patient outcomes and reducing healthcare costs. This study aimed to isolate, identify and determine the antibiotics susceptibility patterns of bacteria isolated from wound of patients attending Ahmad Sani Yariman Bakura Specialist Hospital, Gusau.

MATERIALS AND METHODS

Study Area

Ahmad Sani Yariman Bakura Specialist Hospital is located in Gusau, the capital city of Zamfara State, Northwestern Nigeria. The hospital is a major tertiary healthcare facility that provides comprehensive medical and surgical services to a large and diverse population from Gusau and surrounding local government areas. As a referral center, the hospital handles a significant number of cases involving traumatic injuries, surgical wounds, diabetic ulcers, and burn infections, making it an ideal location for the collection of wound swab samples. The hospital operates various departments including Surgery, Internal Medicine, Paediatrics, Obstetrics and Gynaecology, and Accident and Emergency, which collectively cater to both in-patients and out-patients presenting with various types of wounds (Hunte *et al.,* 2023).

Ethical Approval

Ethical Clearance was obtained from Ahmad Sani Yariman Bakura Specialist Hospital, Gusau ethics committee.

Inclusion Criteria: Those with infected wound that were willing to participate in the study.

Exclusion Criteria: Those who are not willing to participate in the study.

Sample Collection and Study Population

A total of fifty (50) wound swabs were aseptically collected using sterile swab sticks moistened with saline, from patients attending Ahmad Sani Yariman Bakura Specialist Hospital (ASYBH), Gusau, Zamfara State. Samples were transported to the Microbiology Laboratory Federal University Gusau (FUG) within one hour for analysis (Muhibi *et al.,* 2022).

Isolation of Bacteria

The swab sticks containing the wound swab were streaked on nutrient agar plates and incubated at 37^0C for 24 hours. After incubation period, colonies obtained were further sub cultured to obtain a pure culture. The obtained pure culture was preserved on agar slant and refrigerated at 4^0C for further analysis (Mushtaq *et al.,* 2023).

Biochemical Identification of the Isolates

Pure cultures of the isolated organisms were used for the biochemical testing. Biochemical tests that were carried out for the identification of the organisms include Citrate test, Coagulase test, Catalase test, Urease test, Methyl-red test, Voges Proskauer test, Indole test and Triple sugar iron test.

Citrate Test

The test organism was inoculated into the test-tube containing the simmons citrate agar and incubated at 37°C for 24 hours. A change in colour from green to blue and the presence of growth indicates a positive result (Basak *et al.,* 2021).

Coagulase Test

This test was performed by preparing a thick suspension of bacterial cells mixed into a drop of human plasma on a microscope slide. Visible clumping of bacterial cells indicates a positive result, while the absence of coarse clumping indicates negative result (Mahmood *et al.,* 2022).

Catalase Test

A drop of hydrogen peroxide was added at the centre of a clean grease free slide. The discrete colonies of the isolates were collected with a sterile wire loop and emulsified in the drop of hydrogen peroxide (H_2O_2). Presence of bubbles indicates a positive result (Jannesari *et al.,* 2023).

Urease Test

This test is used to detect the presence of urease enzyme activity in microorganisms. Christensen's urea agar was prepared and 2 drops of urea solution was added and left to solidify. It was then inoculated with the test organism and incubated at 37℃ for 24 hours. A color change from yellow to pink indicates a positive result (Pih *et al.,* 2022).

Methyl Red Test

Methyl-red Voges-Proskauer (MR-VP) broth was prepared, 10 ml of the broth was dispensed into test tubes and sterilized. The test isolates were inoculated into the test tubes and was incubated at 37°C for 24 hours. After incubation, a few drops of methyl red indicator were added to the culture and a resultant red coloration indicates a positive result (Ikram *et al*., 2022)**.**

Voges Proskauer Test

Two milli-liter of sterile Methyl red-Voges Proskauer broth was inoculated with test organism and incubated at 37°C for 24 hours. 10% α-naphthalol was added and then mixed, about 3mls of KOH was added and shaken. The set up was then left for an hour at room temperature. A change from pink to red color indicates a positive result (AL-Joda *et al.,* 2021).

Indole Test

The test isolate was inoculated using a sterile wire loop into 5ml of sterilized peptone water which was contained in different test tube. The test tube containing the organisms was incubated at 37°c for 48 hours. After the Incubation period, 3-4 drops (0.5ml) of indole reagent known as Kovac's reagent was added and shaken gently. A positive result yielded a red-colored ring at the top layer of the medium (Salwan *et al.,* 2023).

Triple Sugar Iron (TSI) Test

A discrete bacterial colony was picked using a sterile wire loop and inoculated into TSI agar slant by stabbing the bottom (butt) and streaking on the surface. It was then incubated at 37°C for 24 hours. After incubation, the following reactions were observed which indicates a positive result: presence of yellow color in the slant or bottom indicates acid production from sugar fermentation, presence of cracks or lifting of the agar indicates gas production while a black precipitate (blackening of the agar) in the butt indicates hydrogen sulfide production (Bainbridge and Abigail, 2023).

Serological Test

Escherichia Coli

Serological confirmation of *E. coli* was carried out using the slide agglutination test with commercially available polyvalent and monovalent antisera. A loopful of the colony from a pure culture was emulsified in a drop of sterile saline on a clean slide. A drop of specific O antisera was then added and gently mixed. Visible clumping within 1 minute indicates a positive reaction and confirms the organism (Debnath *et al.,* 2024).

Staphylococcus Aureus

Staphylococcus aureus was confirmed serologically using the latex agglutination test. The latex particles coated with human IgG and fibrinogen were mixed with the bacterial suspension on a card, *S. aureus* then produced protein A and clumping factor, which binds to the IgG and fibrinogen on the latex beads and resulted in a visible agglutination within seconds (Kabak and Ekaterina, 2022).

Pseudomonas Aeruginosa

Serological identification of *P. aeruginosa* was based on its O-antigen serogroups, a suspension of the isolate was mixed with specific antisera on a slide and visible clumping indicates a positive reaction, which confirmed that the strain belongs to a particular serogroup (Nasrin *et al.,* 2022).

Klebsiella Pneumoniae

The serological confirmation of *K. pneumoniae* was performed by detecting capsular (K) antigens, whereby the colony suspension was mixed with specific antisera on a slide for latex agglutination. Positive agglutination indicates the presence of capsular antigen as well as *K. pneumoniae* serotype (Hamzah *et al.,* 2020).

Preparation of 0.5 McFarland Standard

A 1% solution of sulphuric acid was prepared by adding 1 ml of concentrated H_2SO_4 to 99 ml of water and mixed. A 1% w/v of $Bacl_2$ was also prepared by dissolving 0.5 g of dehydrated $Bacl_2$ in 50 ml of distilled water. 0.6 ml of $Bacl_2$ solution was added to 99.4 ml solution of sulphuric acid and mixed. A small volume of turbid solution was transferred to screw capped bottle and used for preparing the inoculum. A loopful of each of the test organism was transferred into bottles containing normal saline and compared with 0.5 Macfarland standard until it's equivalent.

Antibiotics Susceptibility Test

The test was carried out using the Kirby–Bauer's / disc diffusion method. 0.5 Macfarland standard of each isolate were spread on the prepared Muller Hinton agar and allowed to dry. The antibiotics disc containing amikacin (30 µg), tigecycline (15 µg), chloramphenicol (30 µg), erythromycin (15 µg) and meropenem (10 µg) was added to the plates using a sterile forceps and were incubated at 37^0C for 24 hours (Kumar and Keshav, 2024). After incubation period, the zone of inhibition was measured using transparent ruler by taking the distance between the edge of the antibiotic disc and the edge of the zone boundary of bacterial growth around the antibiotic in millimeters (Joseph *et al.,* 2023). It was then compared with the Clinical Laboratory and Standard Institute.

RESULTS

Microscopic and Biochemical Characteristics of the Isolates

Table 1 Presents the morphological and the biochemical characteristics of the isolates. *Escherichia coli* appeared Gram negative and reacted positively to indole, Methyl-red, catalase, Lactose, glucose, gas, motility and negatively to citrate, Voges-Proskauer, urease, coagulase, sucrose and hydrogen sulfide. *Klebsiella pneumoniae* appeared Gram negative and reacted positively to citrate, urease, Voges-Proskauer,

catalase, lactose, sucrose, glucose, gas, and negatively to indole, Methyl red, coagulase, hydrogen sulfide and motility. *Pseudomonas aeruginosa* appeared Gram negative and reacted positively to citrate, catalase, glucose, motility and negatively to indole, urease, Methyl red, Voges-Proskauer, coagulase, lactose, sucrose, gas, and hydrogen sulfide. *Staphylococcus aureus* appeared Gram positive and reacted positively to coagulase, Voges-Proskauer, catalase, glucose, sucrose and negatively to indole, citrate, urease, Methyl red, lactose, gas, hydrogen sulfide and motility.

Table 1: Microscopic and biochemical characteristics of the isolates

Gram Mrph.	Ind.	Cit.	Urea	MR	VP	Coa.	Cat.	TSI						Prob org.
								Lac.	Glu.	Suc.	Gas	H_2S	Mot.	
-ve Rod	+	-	-	+	-	-	+	+	+	-	+	-	+	*E. coli*
+ve Cocci	-	-	-	-	+	+	+	-	+	+	-	-	-	*S. aureus*
-ve Rod	-	+	-	-	-	-	+	-	+	-	-	-	+	*P. aeruginosa*
-ve Rod	-	+	+	-	+	-	+	+	+	+	+	-	-	*Klebsiella* pneumoniae

Key: TSI = Triple Sugar Iron, Lac. = Lactose, Glu. = Glucse, Suc. = Sucrose, Gas = Gas, H2S = Hydrogen sulfide, Mot. = Motility, Ind. = indole, Cit. = Citrate, MR = Methyl red, VR = Voges-procrs Coa. = Coagulase, Cat. = Catalase, Mrph. = Morphology, Prob org.= Probable organisms, Positive = +ve, Negative = -ve.

Serological Identity of Bacterial Isolate

Table 2 Presents the serological characteristics of the bacteria isolate. *Escherichia coli* reacted positively to the polyvalent and monovalent antisera agglutination kit. *Staphylococcus aureus* reacted positively to the latex agglutination test showing a visible clumping. *Pseudomonas aeruginosa* reacted positively to the O antigen serogroups antisera. *Klebsiella pneumoniae* also reacted positively to the capsular (K) antigens detector, mixed with specific antisera on a slide for latex agglutination.

Table 2: Serological Identity of Bacterial Isolate

Bacteria isolate	Serology Laboratory Report	Reactions
Escherichia coli	PVA and MVA	Positive
Staphylococcus aureus	LATO	Positive
Klebsiella pneumoniae	OAS	Positive
Pseudomonas aeruginosa	KA	Positive

Key: PVA and MVA = Polyvalent Antisera and Monovalent Antisera, LATO = Latex Agglutination Test, OAS = O Antigen Serogroups, KA = Capsular Antigens.

Frequency of Occurrence of the Bacteria Isolates

Table 3 Presents the frequency of occurrence of the bacteria isolates in which *Escherichia coli* has the highest frequency of occurrence (30%), followed by *Staphylococcus aureus* (25%), *Klebsiella pneumoniae* (25%) and *Pseudomonas aeruginosa* has the lowest frequency of occurrence (20%).

Table 3: Frequency of Occurrence of the Bacteria Isolates

Bacteria isolate	Frequency of Occurrence	Percentage (%) of Occurrence
Escherichia coli	6	30
Staphylococcus aureus	5	25
Klebsiella pneumoniae	5	25
Pseudomonas aeruginosa	4	20
Total	20	100

Antibiotics Susceptibility Pattern of the Bacteria Isolated

Table 4 Presents the antibiotic susceptibility pattern of the isolates. *Escherichia coli* was susceptible to amikacin, chloramphenicol, intermediate to tigecycline, erythromycin and resistant to meropenem. *Staphylococcus aureus* was susceptible to amikacin, tigecycline, intermediate to chloramphenicol, erythromycin and resistant to meropenem. *Pseudomonas aeruginosa* was susceptible to amikacin, tigecycline, chloramphenicol, erythromycin and resistant to meropenem, while *Klebsiella pneumoniae* was susceptible to amikacin, tigecycline, erythromycin, intermediate to chloramphenicol and resistant to meropenem.

Table 4: Antibiotics Susceptibility Pattern of the Bacteria Isolated.

		Zone of inhibition (mm) (CLSI 29th edition)				
S/N	ID	AK (30µg)	C (30µg)	TGC (15µg)	MEM (10µg)	E (15µg)
1.	*E. coli*	18(S)	20(S)	15(I)	0(R)	17(I)
2.	*S. aureus*	20(S)	15(I)	20(S)	10(R)	17(I)
3.	*Kleb.*	25(S)	15(I)	20(S)	10(R)	20(S)
4.	*Pseudo.*	30(S)	20(S)	20(S)	0(R)	25(S)

Key: AK = Amikacin, C = Chloramphenicol, TGC = Tigecycline, MEM = Meropenem, E = Erythromycin, mm = Millimeter, µg = Micro gram, R = Resistant, S = Susceptible, I = Intermediate, CLSI = Clinical Laboratory and Standard Institute.

Sensitivity based on CLSI = Clinical Laboratory and Standard Institute (29th edition).

DISCUSSION

The finding of this study revealed the presence of *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* from wound swab samples collected among patients attending Ahmad Sani Yariman Bakura Specialist Hospital. This is in consistent with the finding of Onyango *et al*., (2023) who also isolated *E. coli* from wound infections due to poor hygiene and contamination. Similarly, the presence of *Staphylococcus aureus* agrees with the study of Tiagabu *et al*., (2021), who reported *S. aureus* as a common cause of skin and soft tissue infections due to its colonization ability. The isolation of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* is consistent with the study of Olatunji *et al*., (2024), who indicated that these opportunistic pathogens are often associated with hospital-acquired infections, particularly in immunocompromised patients. The presence of these organisms in wound infections may be attributed to fecal contamination, poor hygiene practices, inadequate wound care and impaired immune system that can enhance their ability to invade tissues, influence the development and persistent of wound infections, thereby making them more resistant to antibiotics and host immune responses.

The frequent isolation of *Escherichia coli* (30%) is notable and can be oftentimes linked to its fecal origin, poor hygiene practices as well as improper wound care. However, this is in contrast with the recent study by Karungamye and Petro, (2024) who reported lower prevalence of *E. coli* (27.9%) from wound. The observed universal resistance to meropenem across all isolates is alarming but not unprecedented. Study by Mohammed *et al*., (2025) reported similar resistance, possibly due to misuse or overuse of carbapenems in clinical settings. The high susceptibility to amikacin is in consistent with finding of Abdullahi and Aliyu. (2018) who reported that amikacin remains effective against multidrug-resistant Gram-negative bacteria due to its limited use and stability against aminoglycoside-modifying enzymes. Additionally, the intermediate resistance to tigecycline and chloramphenicol in some isolates aligns with observations from Orji *et al*., (2021), who noted declining efficacy of older antibiotics due to gradual resistance development.

In essence, these findings align with other regional studies, highlighting the growing challenge of multidrug resistance and offering scientific rationale to explain the outcome. The resistance patterns observed may be attributed to poor antibiotic stewardship and the presence of resistance mechanisms such as carbapenemases and efflux pumps. Continuous surveillance and strict infection control practices are crucial to managing wound infections effectively.

CONCLUSION

This study successfully isolated and identified *Escherichia coli, Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* as significant bacterial pathogens associated with wound infections at Ahmad Sanni Yariman Bakura Specialist Hospital, Gusau. The dominance of *Escherichia coli* (30%) in the wound infections reflects its common fecal origin as well as patient susceptibility. However, the lower prevalence of *Pseudomonas aeruginosa* (20%) suggest different hospital flora, patient demographics and infection control effectiveness. A critical finding was the high

resistance observed against meropenem across all isolated species, highlighting a significant challenge in the effective management of these infections. While amikacin and chloramphenicol largely retained their effectiveness, the intermediate susceptibility to tigecycline and erythromycin in some isolates signals an evolving resistance landscape. It is recommended that Enhanced infection control practices, including strict hand hygiene and proper wound care techniques should be enforced in hospital settings to reduce the incidence and spread of wound infections.

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