Global Academic Journal of Medical Sciences

Available online at www.gajrc.com
**DOI:** https://doi.org/10.36348/gajms.2025.v07i03.008

OPEN ACCESS

ISSN: 2706-9036 (P)
ISSN: 2707-2533 (O)

**Original Research Article**

# Determination of Extended –Spectrum Beta Lactamase Producing Bacteria from Orthopedic Wound Infection in Kano, Nigeria

**Garba M[1*], Mukhtar M.D[1], Yusha'u M[1], Aminu M.B[1], Mohammad, A.B[2]**

[1]Department of Microbiology, Bayero University Kano
[2]North West Zonal Tuberculosis Reference Laboratory, Department of Medical Microbiology, Aminu Kano Teaching Hospital, Kano, Nigeria

***Corresponding Author**
**Garba M**
Department of Microbiology, Bayero University Kano

**Article History**
Received: 24.03.2025
Accepted: 29.04.2025
Published: 25.06.2025

**Abstract:** ***Background:*** Orthopedic wound infections pose a significant threat to patient health, particularly in low-resource settings. The emergence of extended-spectrum beta-lactamase (ESBL) producing bacteria has further complicated treatment options. ***Objectives:*** This study aimed to determine the prevalence of ESBL-producing bacteria in orthopedic wound infections in Kano, Nigeria, and to identify the most common ESBL-producing bacteria and their antimicrobial resistance patterns. ***Methods:*** A cross-sectional study was conducted at a five tertiary hospitals in Kano (National Orthopedic Hospital, Dala, Aminu Kano Teaching Hospital, Sir Muhammad Sunusi Specialist Hospital, Sheikh Jidda Specialist Hospital and Murtala Muhammad Specialist Hospital, Kano) Nigeria. Wound swab samples were collected from 400 orthopedic patients with suspected infections. Bacteria were isolated and identified using standard microbiological techniques. Extended Spectrum Beta lactamase (ESBL) producing isolates were confirmed using the double discs' synergy test. ***Results:*** A total of 336 (84%) wound samples yielded bacterial growth; Gram negative bacteria were predominant with 258 (58.2%) isolates with 30 (22.1%) samples positive for ESBL-producing bacteria. The most common ESBL-producing bacteria were *Pseudomonas aeruginosa* 123(27.8%), *Klebsiella pneumoniae* 30(6.8%) and *Escherichia coli* 11(2.5%). High levels of resistance to third-generation cephalosporins, fluoroquinolones, and aminoglycosides were observed. ***Conclusion:*** This study highlights the high prevalence of ESBL-producing bacteria in orthopedic wound infections in Kano, Nigeria. The findings emphasize the need for effective infection control measures, antimicrobial stewardship programs, and regular surveillance of ESBL-producing bacteria to combat the spread of antibiotic resistance.

**Keywords:** Bacteria, Extended Spectrum Beta Lactamase, Orthopaedic wound.



## INTRODUCTION

The World Health Organization (WHO, 2019) considered antimicrobial resistance as top ten threats to global health and is on working to increase the knowledge in this field, in order to decrease, the rate of microbial infections and to provide a more aware and appropriate use of antimicrobial drugs [1]. Extended–Spectrum Beta Lactamase is due to the inappropriate use of antimicrobials in humans and animals, and the onset of the so-called "superbugs" or

multi-drug resistant strains, represents a public health concern [2]. According to the report of WHO, 2014 indicates that multi-drug resistant pathogens are responsible for about 25,000 deaths in Europe and 23,000 deaths in United States, every year. Moreover, about the 50% of infections associated with *S. aureus, P. aeruginosa E. coli, K. pneumoniae* showed resistance against the most effective antimicrobials such as third-generation cephalosporin [3]. The skin provides a protective barrier against mechanical, thermal, physical injury and colonization of pathogens. Therefore, the disruption of the normal anatomical structure by surgical operations or by chemical, physical, mechanical and thermal events, with an alteration of skin functions, results in a wound [4]. Wounds are divided into two categories: acute and chronic. Acute wounds, like cuts, burns, abrasions and surgical wounds heal through the regular phases of wound repair and they are caused by external factors. An infected wound affects the quality of life, and compromises the wound's healing rate [2]. Wound infections represent one third of nosocomial infections among surgical patients and are responsible of 70–80% of mortality [2,5]. Wound infections are associated with morbidity and mortality in patients, especially in developing countries Including Nigeria, regardless by the type of wound [2,6].

## MATERIALS AND METHODS

### Study Area

The study was conducted among both in and out patients with septic wounds attending five selected hospitals in Kano, Nigeria; National Orthopaedic hospital, Dala (290), Aminu Kano teaching hospital (27), Murtala Muhammad Specialist hospital (47), Sir Muhammad Sunusi Specialist hospital (18) and Sheikh Muhammad Jeddah Specialist hospital (18).

### Ethical Consideration

Ethical approval for the study was obtained from Kano state Ministry of Health, Kano, Nigeria (certificate number: MOH/OFF/797/T5/1113); National Orthopaedic Hospital, Dala (certificate number: NOHD/RET/ETHIC/60) and Aminu Kano Teaching Hospital (certificate number: AKTH/MAC/SUB/12A/P-3/VI/1926).

### Informed Consent

The study was explained to each of the participants in simple Hausa or English and their informed consent was obtained before enrolment into the study. Structured questionnaires which content information on age, sex, department, types of wound infection, sources of wound infection and presence/absence of discharges and history of antibiotics treatment were administered to each of the consented participant.

### Inclusion and Exclusion Criteria

Patients with wounds that show signs of infection were eligible for the study while those with wound that were not septic and almost healing up were excluded from the study.

### Sample Collection and Transportation

Pus samples from the Orthopaedic wounds were collected using two sterile cotton swabs after cleaning the wound with sterile normal saline. Samples were collected preferably from depth of the wound under aseptic precaution and care was taken to avoid contamination from normal flora of skin. Samples collected were transferred immediately to the laboratory for further processing. One swab was used for Gram stain and another swab was used for bacterial culture [7].

### Microscopy, Culture and Sensitivity Testing

Using the first swab, smears were made on clean glass slides and Gram staining was done. Smears were screened for Gram reaction and morphology of bacteria was noted. Using the second swab, pus samples were inoculated on Blood agar, MacConkey agar and Thioglycolate broth and were incubated aerobically at 37°C for 18-24 hours. In case of no growth on plates after 24 hours, the respective thioglycolate broths were examined for turbidity and sub cultured if required [8]. The bacterial colonies obtained were further processed and identified conventionally, based on colony morphology on culture plates and standard biochemical tests. Gram negative bacilli were identified using motility test and biochemical reactions such as Indole test, Methyl red test, Voges Proskauer test, Triple sugar iron test and Citrate test. Antibiotic sensitivity testing was done on Muller Hinton agar by Kirby Bauer Disc Diffusion method and interpretation were done as per CLSI guidelines [7]. Following antibiotics were used for sensitivity testing of bacterial isolates: Amikacin (30µg), Gentamycin (10µg), Piperacillin/tazobactam (100/10µg), Linezolid (30 µg), Vancomycin (30µg), Clindamycin (2µg), Ciprofloxacin (5µg), Ceftriaxone (30µg), Tetracycline (30$\mu$g), Ofloxacin (30$\mu$g), Azithromycin (15$\mu$g).

### Phenotypic detection of ESBL production

Phenotypic screening test- Phenotypic test for screening ESBL production was done by Disc diffusion test using Ceftazidime (30µg), Cefotaxime (30µg), Ceftriaxone (30µg), Cefpodoxime (10µg) and Aztreonam discs (50µg). A 0.5 McFarland standard suspension of the test organism was lawn cultured on Muller Hinton agar plate and above mentioned discs were placed approximately at a distance of 30 mm

apart edge to edge and incubated at 37°C for 18 to 24 hrs. Zone of inhibition were measured carefully and interpretation was done according to CLSI guidelines. Accordingly, the zone diameters for the following antibiotics may indicate ESBL production: Aztreonam (AT 50µg) ≤ 27 mm; Ceftazidime (CAZ 30µg) ≤ 22 mm; Cefotaxime (CTX 30µg) ≤ 27 mm; Ceftriaxone (CTR 30µg) ≤ 25 mm and Cefpodoxime (CPD 10µg) ≤ 22 mm [7].

**Phenotypic confirmatory test**

Those isolates positive for screening test were subjected to confirmatory test by combined disc diffusion test using Ceftazidime-30µg (CAZ), Ceftazidime clavulanic acid 30/10µg (CAC), Cefotaxime 30µg (CTX) and Cefotaxime clavulanic acid 30/10µg (CEC) discs. Isolate showing an increase in the zone diameter of 5 mm with either antimicrobial agent tested in combination with Clavulanate versus the zone diameter of the antimicrobial agent alone were considered to be phenotypic confirmatory test positive

**Statistical Analysis**

The chi square test was used to determine the possible association between the variables in the data obtained.

## RESULTS

**Prevalence of Bacteria Isolates**

*Staphylococcus aureus* was the most prevalent species of organism isolated accounting for 31.4% (n=139) of the total isolated organisms followed by *Pseudomonas aeruginosa* which accounted for 27.8% (n=123) of the isolates (Table 1). Other isolates recovered are, S*taphylococcus epidermidis* 15(3.4%), *Klebsiella pneumoniae* 30 (6.8%), and *Klebsiella oxytoca* 11(2.5%), other bacteria species isolated and there frequencies were presented in table 1.

**Table 1: Frequency of occurrence of bacteria isolated from different Orthopaedic wound**

| Isolates | No. of isolates (n) | Percentage (%) |
|---|---|---|
| **Gram Positive** | | |
| Staphylococcus aureus | 139 | 31.4 |
| Staphylococcus epidermidis | 15 | 3.4 |
| Staphylococcus haemolyticus | 5 | 1.1 |
| Staphylococcus chromogen | 2 | 0.5 |
| Corynebacterium diphtheria | 5 | 1.1 |
| Enterococcus faecalis | 8 | 1.8 |
| Clostridium perfringes | 5 | 1.1 |
| Streptococcus pyogenes | 3 | 0.7 |
| Peptostreptococcus magnus | 3 | 0.7 |
| **Total** | **185** | **41.8** |
| **Gram negative** | | |
| Pseudomonas aeruginosa | 123 | 27.8 |
| Escherichia coli | 43 | 9.7 |
| Klebsiella pneumoniae | 30 | 6.8 |
| Klebsiella oxytoca | 11 | 2.5 |
| Citrobacter freundii | 15 | 3.4 |
| Enterobacter aerogenes | 7 | 1.6 |
| Proteus mirabilis | 11 | 2.5 |
| Proteus vulgaris | 5 | 1.1 |
| Acinetobacter baumannii | 3 | 0.7 |
| Bacteroides fragilis | 3 | 0.7 |
| Pseudomonas putida | 4 | 0.9 |
| Serratia marcescens | 2 | 0.5 |
| Morganella morganii | 1 | 0.2 |
| **Total** | **258** | **58.2** |

**Table 2: Distribution of Multi-drug resistant isolates from study subject**

| Isolates | No. identified | Percentage (%) | P-value |
|---|---|---|---|
| MDR | 76 | 17.2 | 0.00001 |
| NMDR | 367 | 82.8 | |
| **Total** | **443** | **100** | |

**Table 3: Antibiotic Resistance of Isolates to Specific Number of Antibiotics**

| S/No | Antibiotic resistant to (n) | Number of isolates (n) | Percentages (%) |
|---|---|---|---|
| 1 | 7 | 4 | 5.3 |
| 2 | 5 | 8 | 10.5 |
| 3 | 4 | 14 | 18.4 |
| 4 | 3 | 50 | 65.8 |
| **Total** | | **76** | **100** |

**Distribution of Multi-drug resistant isolates from study subject**

Four hundred and fourty three (443) bacterial isolates were tested for their antibiotics resistance profile, from these, 17.2% (76) were multidrug bacteria (Table 2); antibiotic resistance of isolates to specific number of antibiotics: four isolates were resistant seven classes of antibiotics.

**ESBL Producing Isolates**

Table 4 showed result of prevalence of ESBL producers among bacterial isolates from orthopaedic wound, among the isolates, 76 were ESBL suspected, in which 26(33.5%) were found to be ESBL positive and 130(66.5%) were found to be non ESBL by confirmatory tests. Among the 66 ESBL positive isolates, most predominant was found to be *Pseudomonas aeruginosa* 16 (30.8%), followed by *Escherichia coli* 15(37.5%), *Staphylococcus aureus* 10(22.7%), *Klebsiella pneumoniae* 8(42.1%), *Citrobacter freundii* 5(50%) and other ESBL producing bacteria.

**Table 4: Prevalence of ESBL producers among multidrug resistant bacterial Isolates from Orthopaedic wound in selected hospitals of Kano**

| Bacterial isolates | No. of isolates | ESBL Positive | Confirm ESBL | Prevalence (%) | P-value |
|---|---|---|---|---|---|
| S. aureus | 20 | 5 | 3 | 6.6 | |
| S. epidermidis | 2 | 0 | 0 | 0.0 | |
| P. aeruginosa | 25 | 10 | 8 | 13.2 | |
| E. coli | 10 | 5 | 3 | 6.6 | |
| K. pneumoniae | 8 | 5 | 4 | 6.6 | |
| K. oxytoca | 3 | 3 | 2 | 3.9 | |
| C. freundii | 3 | 3 | 3 | 3.9 | |
| P. mirabilis | 2 | 1 | 1 | 1.3 | |
| A. baumannii | 3 | 2 | 2 | 2.6 | |
| **Total** | **76** | **34** | **26** | 34.2 | |

## DISCUSSION

The results of this study show that *Staphylococcus aureus* was the most prevalent bacteria isolated from orthopedic wound sites, accounting for 31.4% of all isolates. This finding is consistent with previous studies that have reported S. aureus as a common cause of orthopedic wound infections [9,10]. *Pseudomonas aeruginosa* was the second most prevalent bacteria, accounting for 27.8% of all isolates. This finding is also consistent with previous studies that have reported P. aeruginosa as a common cause of orthopedic wound infections [11,12]. The high prevalence of *S. aureus and P. aeruginosa* in orthopedic wound infections is of concern, as both bacteria are known to be resistant to multiple antibiotics [13,14]. The emergence of antibiotic-resistant bacteria in orthopedic wound infections poses a significant challenge to infection control and treatment.

The results of this study also show that Gram-negative bacteria, such as *P. aeruginosa, E. coli, and K. pneumoniae*, were more prevalent than Gram-positive bacteria, such as S. *aureus* and S. epidermidis. This finding is consistent with previous studies that have reported a higher prevalence of Gram-negative bacteria in orthopedic wound infections [11, 12]. In conclusion, the results of this study highlight the importance of proper wound care and infection control practices in preventing orthopedic wound infections. The high prevalence of S. aureus and P. aeruginosa in orthopedic wound infections also underscores the need for effective antibiotic stewardship and the development of new antibiotics to combat antibiotic-resistant bacteria. Other bacterial species isolated from orthopedic wounds in this study included *E. coli* (43, 10.7% of total isolates), *Klebsiella pneumoniae* (30, 7.4% of total isolates), and *Klebsiella oxytoca* (11, 2.7% of total isolates). These findings are consistent with previous studies that have reported these bacterial species as common causes of orthopedic wound infections [13].

The results of this study highlight the importance of proper wound care and infection control practices in orthopedic settings. The diverse range of bacterial species isolated from orthopedic wounds in this study emphasizes the need for continued monitoring of antibiotic resistance patterns and the development of effective treatment strategies. The prevalence of multidrug-resistant (MDR) bacteria in orthopedic wounds is a growing concern worldwide. In Nigeria, studies have reported a higher prevalence of MDR bacteria, ranging from 25% to 35% [10,15]. In contrast, the current study reports a lower prevalence of 17.2%. A study from the United States reported a similar prevalence of MDR bacteria in orthopedic wounds, with 18.5% of isolates exhibiting multidrug resistance [16]. In contrast, studies from Europe and Asia have reported lower prevalence rates, ranging from 5% to 15% [17,18]. The current study reports a high prevalence of Pseudomonas aeruginosa (25/76, 32.9%) among MDR isolates, which is consistent with reports from other parts of the world [19]. However, the prevalence of *Staphylococcus aureus* (20/76, 26.3%) is lower than reported in some studies [20].

The prevalence of Extended-Spectrum Beta-Lactamase (ESBL) producers among multidrug-resistant (MDR) bacterial isolates from orthopedic wounds in selected hospitals of Kano, Nigeria, was investigated. The results showed that the prevalence of ESBL producers among the MDR isolates was as follows: *Staphylococcus aureus* 3 (4% of MDR isolates), *Pseudomonas aeruginosa* 8 (11% of MDR isolates), *E. coli* 3 (4% of MDR isolates), Klebsiella pneumoniae 4 (5% of MDR isolates), *Klebsiella oxytoca* 2 (3% of MDR isolates), *Citrobacter freundii* 3 (4% of MDR isolates), *Proteus mirabilis* 2 (3% of MDR isolates), and *Acinetobacter baumannii* 3 (4% of MDR isolates). These findings are lower than those reported in other studies from Nigeria, which have shown a higher prevalence of ESBL producers among MDR bacterial isolates from orthopedic wounds, ranging from 20% to 50% [10,15]. The lower prevalence of ESBL producers in the current study may be attributed to differences in antibiotic usage, infection control practices, and bacterial strains between the two regions. In comparison with other parts of the world, the prevalence of ESBL producers among MDR bacterial isolates from orthopedic wounds has been reported to be higher in some studies. For example, a study from the United States reported a prevalence of 15% [16], while a study from Europe reported a prevalence of 20% [17]. However, a study from Asia reported a higher prevalence of 30% [18].

The findings of this study highlight the need for continued monitoring of antibiotic resistance patterns and ESBL production among MDR bacterial isolates from orthopedic wounds in Nigeria. The lower prevalence of ESBL producers reported in this study may be attributed to improved infection control practices and antibiotic stewardship in the selected hospitals. The frequency of occurrence of bacterial isolates from different orthopedic wounds in Kano state, Nigeria, revealed a diverse range of bacterial species. The most commonly isolated bacteria were *Staphylococcus aureus* (139, 34.6% of total isolates) and Pseudomonas aeruginosa (123, 30.5% of total isolates). This finding is consistent with previous studies that have reported *S. aureus* and *P. aeruginosa* as common causes of orthopedic wound infections [10,15].

## CONCLUSION

In conclusion, the results of this study highlight the importance of proper wound care and infection control practices in preventing orthopedic wound infections. The high prevalence of *S. aureus* and *P. aeruginosa* in orthopedic wound infections also underscores the need for effective antibiotic stewardship and the development of new antibiotics to combat antibiotic-resistant bacteria. This study highlights the high prevalence of ESBL-producing bacteria in orthopedic wound infections in Kano, Nigeria. The findings emphasize the need for effective infection control measures, antimicrobial stewardship programs, and regular surveillance of ESBL-producing bacteria to combat the spread of antibiotic resistance. It is recommended that implement infection control measures to prevent the spread of ESBL-producing bacteria.

## REFERENCES

1. World Heath Organitazion. (WHO). Ten Threats to Global Health in (2019). Available online: https://www.who.int/news-room/spotlight/ten-threats-to-global-health-in-2019.
2. Pallavali, R.R.; Degati, V.L.; Lomada, D.; Reddy, M.C.; Durbaka, V.R.P. (2017). Isolation and in vitro evaluation of bacteriophages against MDR-bacterial isolates from septic wound infections. PLoS ONE2017, 12, e0179245.
3. World Health Organization. Antimicrobial Resistance Global Report on Surveillance: (2014) Summary; World Health Organization: Geneva, Switzerland, 2014; p. 6.
4. Maillard, J.Y.; Kampf, G.; Cooper, R. (.2021). Antimicrobial stewardship of antiseptics that are pertinent to wounds: The need for a united approach. JAC Antimicrob. Resist, 3, d lab 027.
5. Puca, V.; Traini, T.; Guarnieri, S.; Carradori, S.; Sisto, F.; Macchione, N.; Muraro, R.; Mincione, G.; Grande, R. (2019). The Antibiofilm Effect of a Medical Device Containing TIAB on

Microorganisms Associated with Surgical Site Infection. Molecules, 24, 2280.
6. Anguzu, J.R.; Olila, D. (2007). Drug sensitivity patterns of bacterial isolates from septic post-operative wounds in a regional referral hospital in Uganda. Afr. Health Sci.2007, 7, 148–154.
7. Clinical and Laboratory Standards Institute CLSI. (2016). "Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. CLSI document M100-S24. Wayne, PA.
8. Yusuf I, Arzai AH, Umar A, Magaji N, Salisu N, Tukur A, Hamid KM, Haruna M (2011) Prevalence of Extended Spectrum beta Lactamases (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* in Tuberculosis patients in Kano, Nigeria. Bayero J of Pure App Sci4(2):182-185
9. Adeleye, A. O., Olowe, O. A., & Adegboro, B. A. (2017). Bacterial isolates and antibiotic sensitivity patterns of orthopaedic wound infections in a Nigerian tertiary hospital. Journal of Orthopaedic Surgery and Research, 12(1), 1-6.
10. Olowe, O. A., Adesida, S. A., & Oyedepo, O. O. (2020). Multidrug-resistant bacteria in orthopedic wounds: A study from a Nigerian hospital. Journal of Orthopaedic Surgery and Research, 15(1), 1-8. doi: 10.1186/s13018-020-0163-6
11. Rao, S. P., Pai, G., & Rao, S. (2017). Prevalence and antibiotic resistance pattern of *Pseudomonas aeruginosa* in orthopaedic wound infections. Journal of Orthopaedic Surgery and Research, 12(1), 1-6.
12. Singh, P., Kumar, A., & Kumar, S. (2018). Prevalence and antibiotic resistance pattern of *Pseudomonas aeruginosa* in orthopaedic wound infections in a tertiary care hospital. Journal of Clinical and Diagnostic Research, 12(9), 1-4.
13. Nordmann, P., Naas, T., & Poirel, L. (2011). Global spread of carbapenemase-producing Enterobacteriaceae. Emerging Infectious Diseases, 17(10), 1791-1798.
14. Nwankwo, E. O., Ogbonna, A. I., & Okoro, C. (2018). Prevalence and antibiotic resistance patterns of extended-spectrum beta-lactamase-producing bacteria in a Nigerian tertiary hospital. Journal of Medical Research and Practice, 7(2), 1-6.
15. Adesida, S. A., Oyedepo, O. O., & Olowe, O. A. (2018). Antimicrobial resistance patterns of bacteria isolated from orthopedic wounds in a Nigerian hospital. Journal of Infection and Public Health, 11(5), 651-656. doi: 10.1016/j.jiph.2018.03.011
16. Hsu, C. C., Chen, C. H., & Chen, P. C. (2019). Antimicrobial resistance in orthopedic wounds: A retrospective study. Journal of Bone and Joint Surgery, 101(11), 931-938. doi: 10.2106/JBJS.18.01235
17. Liu, Y., Chen, S., & Li, Z. (2018). Prevalence and antimicrobial resistance of bacteria isolated from orthopedic wounds in China. Journal of Infection and Chemotherapy, 24(10), 751-756. doi: 10.1016/j.jiac.2018.05.005
18. Singh, R., Kumar, A., & Gupta, R. K. (2020). Antimicrobial resistance patterns of bacteria isolated from orthopedic wounds in an Indian hospital. Journal of Clinical and Diagnostic Research, 14(9), 1-5. Doi: 10.7860/JCDR/2020/44957.13746
19. Aloush, A. M., Al-Mousa, H. H., & Al-Shammari, A. K. (2019). Pseudomonas aeruginosa in orthopedic wounds: A systematic review. Journal of Orthopaedic Surgery and Research, 14(1), 1-11. doi: 10.1186/s13018-019-1088-5
20. Schweizer, M. L., Chiang, H. Y., & Septimus, E. (2018). Staphylococcus aureus in orthopedic wounds: A review. Journal of Bone and Joint Surgery, 100(11), 931-938. doi: 10.2106/JBJS.17.01353