South Asian Journal of Research in Microbiology

Volume 19, Issue 2, Page 1-13, 2025; Article no.SAJRM.128759
ISSN: 2582-1989

Prevalence of Extended Spectrum Beta-Lactamase Producing Bacteria in Patients with Wound Infections Attending Tertiary Hospitals in Enugu, Nigeria

Agbo, Ifeoma Uzoma [a*], Udeani, Theophilus Kachi [b] and Ngwu, Amauche Martina [c]

[a] Department of Medical Laboratory Science, Enugu State University Teaching Hospital Parklane, Enugu, Enugu State, Nigeria.
[b] Department of Medical Laboratory Science, Faculty of Health Sciences and Technology, University of Nigeria, Enugu Campus, Nigeria.
[c] Department of Medical Laboratory Science, Faculty of Allied Health Sciences, Enugu State University of Science and Technology, Enugu, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author AIU designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author UTK performed the statistical analysis and managed the analyses of the study. Author NAM managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/sajrm/2025/v19i2416

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/128759

Original Research Article

Received: 14/11/2024
Accepted: 18/01/2025
Published: 22/01/2025

**Corresponding author: Email: agboify185@gmail.com;*

Cite as: *Uzoma, Agbo, Ifeoma, Udeani, Theophilus Kachi, and Ngwu, Amauche Martina. 2025. "Prevalence of Extended Spectrum Beta-Lactamase Producing Bacteria in Patients With Wound Infections Attending Tertiary Hospitals in Enugu, Nigeria". South Asian Journal of Research in Microbiology 19 (2):1-13. https://doi.org/10.9734/sajrm/2025/v19i2416.*

ABSTRACT

Background: The problem of antibiotics usage against bacterial infection is the modifications of such antibiotics by the bacteria thereby rendering them ineffective. Extended spectrum β-lactamase (ESBL) producing bacteria invading wound infections may lead to long term hospitalization, financial burden and limited antibiotics for therapy. The goals of this study were to determine the prevalence of ESBL-producing bacteria colonization of wound infections among individuals with non-healing wounds and the antibiotic susceptibility pattern of the ESBL isolates.

Methodology: The study adopted a cross-sectional research design. A total of 266 samples were collected from different wound infections which included diabetic foot ulcers, burn wounds, post-surgical wounds, non-diabetic foot ulcers, pressure ulcers, accident and open cancer wounds that met the inclusion criteria. The patients were consecutively selected. That is, any individual that has a wound and was willing to participate was selected. A structured questionnaire was administered to the patients to obtain information on demographic characteristics, antibiotic usage, and duration of infection, herbal medication, type and site of wound. Identification of bacterial isolates was done using colony/ microscopic morphology, gram stain reaction and standard biochemical tests. Antibiotic susceptibility testing was done using modified Kirby - Bauer disc diffusion method. ESBL detection was done following the recommendations by the Clinical and Laboratory Standard Institute (CLSI) which involves a 2-step approach of initially screening for ESBL producers and phenotypic confirmatory test using a combination disc test method. Molecular screening of the genes encoding for ESBLs was done at the Central Science Laboratory, University of Nigeria, Nsukka.

Results: A total of 196 isolates were recovered from the wound swabs. *Pseudomonas aeruginosa* 56 (28.6%) was the leading organism causing wound infection followed by *Staphylococcus aureus* 24 (12.2%). The bacteria isolates showed that 157 (80.1%) were gram negatives as against 39 (19.9%) that were gram positive bacteria. Among the gram-negative bacteria isolates, 21.7% (34/157) were confirmed as ESBL producers. The ESBL- producers were *Pseudomonas aeruginosa*, *Esherichia coli, Proteus vulgaris, Proteus mirabilis, Klebsiella oxytoca, Klebsiella pneumonia* and *Klebsiella granulomatis* at frequencies of 41.2%, 20.6%,14.7%,11.8%,5.9%,2.9% and 2.9% respectively. This showed that these isolates have the ability to resist penicillins and cephalosporins of the first, second and third generations. The ESBL-producing bacteria isolated exhibited high degree of multidrug resistance especially to tetracyclines, cefuroxime, ceftriaxone, cefotaxime and ceftazidime. The antibiotics amikacin was sensitive to most of the ESBL-producing bacteria isolated. The assessment of the risk factors showed that none of the variables was statistically significant, though those risk factors were still important in evaluating wound infections. Both hospital and community acquired infections showed no statistical significance (*P*= 0.072) which means that both had the same degree of pathogenicity. Among the 10 samples screened for ESBLs genes namely bla SHV, bla TEM, bla CTX-M, bla GES, and bla OXA-50, only bla OXA- 50 was detected in 8 out of the ten samples. Thus, the persistent gene circulating in this region is bla OXA-50, which confers high rate of infection and persistence.

Conclusion: The presence of ESBL-producing bacteria in wounds remains a challenging issue, as the majority of the patients may suffer from long term infected wounds due to treatment failure.

Keywords: Wound infection; ESBL-producing bacteria; phenotypic confirmatory test; PCR; Pseudomonas aeruginosa; Enugu.

1. INTRODUCTION

A wound is said to have occurred when the integrity of the intact skin is compromised. This exposes the skin to colonization by intrinsic and extrinsic organisms (Bowler et al.,2001). When the host natural immune system is overpowered by virulence factors present in one or more microorganisms in a wound, the wound is said to be infected. This leads to invasion and spread of microorganisms in viable tissue, thereby eliciting local and systemic responses. The local responses are a purulent discharge, inflammation, cellulitis and pain around the wound area (Moet, 2007). When a wound is infected, it becomes highly colonized by potentially pathogenic organisms. Healing of the wound tends to delay thereby prolonging hospitalization and invariably increase financial cost. The management of such wound becomes

demanding (Bowler et al., 2001). Antibiotic use in such situations increases, in some cases, if the wound is not properly managed, depending on the location of the wound, it could lead to limb loss. On a global bases, wound infection is responsible for high human morbidity and mortality (Cutting and White,2004). *Staphylococcus, Pseudomonas, Klebsiella, Proteus species, Esherichia coli,* and anaerobes such as *Clostridium* and *Bacteroides* species are among the bacterial agents that are frequently implicated in wound infections (Enweani, 1991; Otokunefo and Datubo-Brown, 1990). Antibiotic resistance by these agents poses a serious challenge in the treatment and healing of infected wounds (Mama,2014). Some of these microorganisms acquire enzymes which modify the antimicrobial substances to their advantage hence presenting a very difficult problem in wound management (Cohen, 2000). Extended Spectrum Beta lactamases (ESBLs) are one of such enzymes produced by some of these organisms which deactivate beta lactam drugs thereby rendering the drugs ineffective and hampering wound treatment. The activities of these ESBLs pose a big challenge to clinicians in management of wounds as their presence also confers resistance to other classes of antibiotics. Extended hospital stays, antibacterial medication, invasive operations, severe co-morbidities, immunosuppression, and intra-abdominal surgery are the main risk factors for infection with ESBL-producing microbes (Asir et al., 2015). It is well accepted that individuals afflicted with infections brought on by organisms that produce Extended Spectrum β-Lactamase are very susceptible to treatment failure when using an Extended Spectrum β-Lactam antibiotic. This is because these germs are becoming more resistant to drugs. In developing countries like Nigeria, regular antimicrobial susceptibility testing cannot identify this kind of medication resistance. The unchecked proliferation of ESBLs is caused by a failure to identify their creators. In addition, some laboratories do not have the facility to detect ESBL-producing organisms in routine laboratory analysis hence this study. The goal of this study was to investigate the colonization of wounds with ESBL-producing bacteria, highlighting the risk factors in treating such infections.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted at two tertiary hospitals; National Orthopaedic Hospital, Enugu (NOHE) and Enugu State University of Science and Technology Teaching Hospital, Parklane (ESUTTHP) between June,2022 and November,2023. These hospitals are well known for handling physical injuries, trauma and infections of the musculoskeletal system, operate special clinics for patients with different wounds.

2.2 Study Population and Design

The study adopted a cross-sectional study design that involved a single collection of samples. The subjects enrolled were individuals with different wound infections which included diabetic foot ulcers, burn wounds, post-operative wounds, non-diabetic foot ulcers, pressure ulcers (bed sores), accident wounds and open cancer wounds. They were selected based on the physical appearance of pus production mixed with a tinge of blood. The individuals consisted of inpatients and those who come from their homes for wound dressing and normal hospital visits. The patients were consecutively selected that is, any individual that has a wound and was willing to participate was selected. The individuals with fresh wounds for example corrective surgery, accident and burn victims were not selected because there was no bacteria colonization of such wounds at the time of collection. A structured questionnaire was administered to the patients to obtain information on sociodemographic characteristics such as age, educational status, occupation and residential areas. The questionnaire also obtained information on antibiotic usage, duration of infection, herbal medication, type and site of wound.

2.3 Sample Collection

Purposive sampling technique was employed in selecting the patients. Those that answered the questionnaire and voluntarily agreed to participate were enrolled in the study. The wound area was wiped first with sterile normal saline. Sterile swab sticks were used to collect pus or wound specimens using the Levine technique which involved rotating the swab stick over a 1cm area of the wound while applying pressure to produce fluid from the wound tissue. Special care was taken during the sample collection to avoid contamination with commensal organisms from the skin. The samples were collected with the help of nurses during wound dressing and were delivered to the laboratory for analysis.

2.4 Bacterial Isolation

The pus cells or tissue exudates collected from the patients were subjected to bacteria culture using standard methods. The pus and wound swabs were inoculated on blood and MacConkey agar plates (Oxoid, England) and incubated at 37°c for 24 hours.

2.5 Identification of the Isolate

Using colony and microscopic morphology, lactose fermentation, the Gram stain response, and the required biochemical tests such as the spot oxidase, citrate utilization, catalase, coagulase, and indole assays, the bacterial isolates were identified (Cheesbrough, 2000).

2.6 Antibiotic Susceptibility Testing of the Isolates

Antimicrobial susceptibility testing was done using a modified Kirby-Bauer disc diffusion method following the guidelines provided by the Clinical and Laboratory Standard Institute (CLSI, 2021). The antibiotic susceptibility testing of the isolates was done using a modified Kirby-Bauer disc diffusion method on Mueller Hinton agar (Oxoid, England) using 0.5 McFarland equivalent. Sterile forceps were used to place the antibiotic discs on the inoculated plates. A commercial antibiotic disc prepared by Biomark laboratory; India was used to ascertain the antimicrobial sensitivity of the identified isolates. The antibiotics were allowed to diffuse properly into the agar before incubation at 37℃ for 18-24 hours. The antibiotic susceptibility pattern of the identified isolates was taken by measuring the zone of inhibition of the antibiotics and the values recorded. The zone diameters were determined using the guidelines provided by the Clinical and Laboratory Standard Institute (CLSI, 2021). This helped to categorize the isolates as susceptible, intermediate and resistant. The resistance, intermediate and sensitivity were interpreted according to the guidelines provided by the Clinical and Laboratory Standard Institute (CLSI, 2021). The antimicrobial discs used included Tetracycline (TET) (10 µg), Co-trimoxazole (COT) (25 µg), Gentamicin (GEN) (10 µg), Cefuroxime (CRX) (30 ug), Chloramphenicol (CHL) (10 µg), Ceftriaxone (CTR) (30 µg), Cefotaxime (CTX) (3 0µg), Ciprofloxacin (CIP) (5 µg), Amikacin (AMK)(30 µg), Vancomycin (VAN) (30 µg), Ceftazidime (CPZ) (30 µg) and Meropenem (MEM) (10 µg). Isolates which were gram-negative and showed resistance to the following third-generation cephalosporins namely cefotaxime (30 µg), ceftazidime (30 µg) and ceftriaxone (30 µg) with a zone of inhibition ≤27 mm for cefotaxime, ≤22 mm for ceftazidime and ≤25 for ceftriaxone were selected as possible ESBL producers and subjected to further studies. All the tests/procedures were performed in compliance with Good Laboratory Practice (GLP) for such procedures, and the procedures were performed using the required Standard Operating Procedures (SOP).

2.7 ESBL Detection

The method recommended by the Clinical and Laboratory Standard Institute (CLSI) which requires a 2-step approach of initially screening for ESBL producers and phenotypic confirmatory tests was adopted in this study for ESBL detection.

2.8 Screening for ESBL Producers

Isolates which were gram-negative and showed resistance to the following third-generation cephalosporins namely cefotaxime (30 µg), ceftazidime (30 µg) and ceftriaxone (30 µg) with zone of inhibition ≤27 mm for cefotaxime, ≤22 mm for ceftazidime and ≤25 for ceftriaxone were selected as possible ESBL producers and subjected to further studies.

2.9 Phenotypic Confirmatory Test

Confirmation of ESBL-producing isolates was done by the phenotypic confirmatory test according to CLSI recommendation. Combination disc test was the method employed. In this experiment, a disc containing ceftazidime 30µg alone was positioned opposite to a disc containing a combination of ceftazidime and clavulanic acid (30/10µg), with a separation distance of 15 mm, on a Muller Hinton agar medium.A positive result was indicated by a difference of ≥ 5 mm between the disc containing ceftazidime plus clavulanic acid and the disc containing ceftazidime alone.

2.10 Molecular Characterization

This was carried out at Central Science Laboratory, University of Nigeria, Nsukka**.** Ten(10) out of the ESBL-positive isolates were screened for the genes blaSHV, blaCTX-M, blaTEM, blaPER, blaGES and the OXA-50 using the polymerase chain reaction technique.The extraction of Gram-negative bacteria DNA was

done using Thermo Scientific GeneJET Genomic DNA Purification Kit following the manufacturer's manual.

2.11 Gel Electrophoresis of Extracted Genomic DNA

To ensure that the DNA was successfully extracted, the genomic DNA of the first 8 samples was run on an agarose gel. Briefly, 7 µl of the extracted genomic DNA was mixed with 3 µl of gel-loading dye in a clean sterile microcentrifuge tube. The mixture was loaded on a 1% agarose gel, which had been pre-stained with 5 µl of ethidium bromide (1µg/mL). A 100 bp DNA ladder (New England Biolabs, USA) was used as the DNA molecular weight marker. The electrophoresis was done at 90 volts until the dye front almost reached the end of the gel. After the electrophoresis run, the gel was viewed on a UV transilluminator, and the gel image was captured.

2.12 Polymerase Chain Reaction (PCR) and Agarose Gel Electrophoresis of PCR Products

The PCR reaction mixture contained 12.5 µl of 1X Master mix with standard buffer, 0.5 µl (10 µM) of each of the forward and reverse primers, 3 µl of the extracted DNA, and 8.5 µl of sterile nuclease-free water to make up to 25 µl of reaction volume.

The polymerase chain reaction (PCR) was carried out using the one Taq Quick load 2X Master Mix with Standard Buffer (New England Biolabs, MA, U.S.A.), which is composed of; 20 mM Tris-HCl, 1.8 mM $MgCl_2$, 22 mM NH_4Cl, 22 mM KCl, 0.2 mM DNTPS, 5% glycerol, 0.06% IGEPAL CA-630, 0.05% Tween 20, Xylene Cyanol FF, Tartrazine and 25 units/ml of Taq DNA polymerase.

This was vortexed at low speed and placed in a thermal cycler machine, with cycling parameters and primers used. Multiplex PCR was used to detect the genes for SHV and CTX-M, and the Bla GES and Bla PER, while conventional linear PCR was done for the BlaTEM type ESBL gene and the OXA-50 gene. The PCR products were analyzed on 1.5% Agarose gel-stained ethidium bromide (1µg/mL) and electrophoresis was carried out at 90 volts for 45 min and visualized under an ultraviolet transilluminator. A 100 bp DNA ladder (New England Biolabs, USA) was used as the DNA molecular weight marker.

2.13 Statistical Analysis

All statistical analyses were performed using SPSS Windows version 22. Categorical variables were described using descriptive statistics (frequencies and percentages). The chi-square test (at 95% confidence interval) was used to test for significant differences in proportion. Statistical significance was set at P-value <0.05.

3. RESULTS

A total of 266 patients with different wound infections were enrolled in the study. The age range of these participants was 15- to 95-year-olds with mean age of 43.6±18.4. Most of the patients were males 175/266 (65.8%) compared to 91/266 (34.2%) of the females with sex ratio of 1.92:1.The study subjects were categorised according to their ages to ascertain those that are vulnerable to wound infections and the modal age range was found to be age group 15-29 accounting for 70 (26.3%) of the study. Out of the 266 study subjects with wound infections, 196 isolates were recovered from their samples.

Table 1 showed frequency distribution of bacterial isolates from wound samples of patients in the tertiary hospital. Of the 196 isolates, 157(80.1%) were Gram negative bacteria while 39(19.9%) were Gram positive bacteria. *Pseudomonas aeruginosa,* 56(28.6%) was the most frequently isolated bacterium, followed by *Staphylococcus aureus,* 24(12.2%) while *Citrobacter freundii* 1(0.5%) and *Providentia spp.* 1(0.5%) were the least isolated bacteria in patients with wound infection.

Table 2 showed distribution of ESBL and non-ESBL-producing Gram-negative bacteria in wound samples. Out of 157 Gram-negative isolates screened, 34(21.7%) were ESBL producers while 123 (78.3%) were non-ESBL producers. The most preponderant ESBL-producing Gram-negative bacteria was *Pseudomonas aeruginosa,* 14(41.2%), followed by *E. coli,* 7(20.6%).

Table 3 showed occurrence of ESBL and non-ESBL- producing bacteria in relation to sources of wound. Out of 66 Gram negative isolates from accident victims, 12(18.2%) were ESBL producers while 54(81.8%) were non-ESBL producers. This happens to be the highest ESBL producers in relation to source of wounds. This was followed by the unknown source where out of 34 Gram negative isolates, 7 (20.6%) were

ESBL producers and 27(79.4%) non-ESBL producers.

Table 4 showed assessment of risk factors associated with ESBL production in wound isolates. Of all the risk factors considered, those currently hospitalized had a p-value of 0.072. Those who had surgery in the past had a p-value of 0.486, self-medication had a p-value 0.447, antibiotics use on doctor's prescription had a p-value of 0.185 and use of herbal therapy had a p-value of 0.149.

Table 1. Frequency distribution of bacterial isolates from wound samples

Isolates	Frequency	Percentage (%)
Acinetobacter baumanni	7	3.6
Citrobacter freundii	1	0.5
E. coli	21	10.7
Enterobacter spp	2	1.0
Enterococcus faecalis	6	3.1
Klebsiella granulomatis	6	3.1
Klebsiella oxytoca	11	5.6
Klebsiella pneumonia	10	5.1
Moraxella catarrhalis	2	1.0
Morganella morganii	2	1.0
Proteus mirabilis	20	10.2
Proteus vulgaris	19	9.7
Providentiaspp	1	0.5
Pseudomonas aeruginosa	56	28.6
Staphylococcus aureus	24	12.2
Staphylococcus epidermidis	3	1.5
Streptococcus pyogenes	5	2.6
Total	**196**	**100**

Table 2. Distribution of ESBL and non-ESBL producing Gram-negative bacteria

Bacteria	N (%)	ESBL (%)	NON-ESBL (%)
Acinetobacter baumanni	7(4.5)	0(0.0)	7(5.7)
Citrobacter freundii	1(0.6)	0(0.0)	1(0.8)
Enterobacter spp	2(1.3)	0(0.0)	2(1.6)
Esherichia coli	21(13.4)	7(20.6)	14(11.4)
Klebsiella granulomatis	6(3.8)	1(2.9)	5(4.1)
Klebsiella pneumonia	10(6.4)	1(2.9)	9(7.3)
Klebsiella oxytoca	11(7.0)	2(5.9)	9(7.3)
Proteus mirabilis	20(12.7)	4**(**11.8**)**	16(13.0)
Proteus vulgaris	19(12.1)	5**(**14.7**)**	14(11.4)
Pseudomonas aeruginosa	56(35.7)	14(41.2**)**	42(34.1)
Moraxella catarrhalis	2(1.3)	0(0.0)	2(1.6)
Morganella morganii	2(1.3)	0(0.0)	2(1.6)
Total	**157**	**34**	**123**

Table 3. Occurrence of ESBL and non-ESBL-producing bacteria in relation to source of wound

Source	N	ESBL%	NON-ESBL%	P-value
Accident	66	12(18.2)	54(81.8)	0.155
Burns	7	1(14.3)	6(85.7)	
Pressure ulcer (bed sores)	11	2(18.2)	9(81.8)	
Diabetic foot ulcer	17	5(29.4)	12(70.6)	
Open cancer wound	8	0(0.0)	8(100.0)	
Non-diabetic foot ulcer	11	6(54.5)	5(45.5)	
Surgery	3	1(33.3)	2(66.7)	
Unknown	34	7(20.6)	27(79.4)	
Total	157	**34(21.7)**	**123(78.3)**	

Table 4. Assessment of risk factors associated with ESBL production in wound isolates

Variable	ESBL Positive%	Non-ESBL producers	P-value
Previous surgery			
Yes	10(25.6)	29(74.4)	0.486
No	24(20.3)	94(79.7)	
Currently Hospitalized			
Yes	20(28.2)	51(71.8)	0.072
No	14(16.3)	72(83.7)	
Self-Medication			
Yes	25(23.4)	82(71.8)	0.447
No	9(18.0)	41(82.0)	
Doctor's Prescription			
Yes	17(18.1)	77(81.9)	0.185
No	17(27.0)	46(73.0)	
Herbal Therapy			
Yes	15(28.3)	38(71.3)	0.149
No	19(18.3)	85(81.7)	

Table 5 showed Distribution of ESBL-producing bacteria among hospitalized and non-hospitalised patients. Extended spectrum β-lactamase (ESBL) producers were recorded more in hospitalised patients 20 than non-hospitalised patients 14. The commonest organism encountered in both hospitalised and non- hospitalised patients were *Pseudomonas aeruginosa* 7(35.5%), 7(50.0%) and *E.coli* 4(20.0%), 3(21.4%) respectively.

Table 6 showed antibiotic susceptibility patterns of the ESBL-producing bacteria. The most active drug against the isolates was Amikacin (78.6-100% susceptibility). Others showed low activity against the isolates. The most susceptible isolate was *Pseudomonas aeruginosa* while *K. granulomatis* was resistant to all the antibiotics used. Of all the antibiotics used to study the susceptibility of the ESBL producers, Amikacin showed maximum activity against the isolates. The least activity was shown by the tetracyclines, cefuroxime, ceftriaxone, cefotaxime and ceftazidime.

Table 7 showed distribution of ESBL genes in wound isolates in the study facilities. Of all the 10 samples screened for ESBL genes, bla TEM, bla CTX-M, bla PER, bla GES, and bla SHV were not detected in any of the isolates. However, the OXA-50 gene was detected in *Proteus vulgaris* (2), *Proteus mirabilis* (2), *Pseudomonas aeruginosa* (2), *E. coli* (1) and *Klebsiella granulomatas* (1).

4. DISCUSSION

The emergence of ESBL-producing bacteria in patients with wound infections poses a serious public health threat especially in the selection of appropriate antimicrobial regimen. Some microorganisms are fast acquiring resistance which results in treatment failures, extended stay in the hospital, amputation and high hospital bills

Table 5. Distribution of ESBL – producing bacteria among hospitalized and non – hospitalized patients

ESBL – producing	Hospitalized (%)	Non- hospitalized (%)	P-value
Esherichia coli	4(20.0)	3(21.4)	0.072
Klebsiella oxytoca	2(10.0)	-	
Klebsiella pneumonia	1(5.0)	-	
Klebsiella granulomatis	-	1(**7.**1)	
Proteus mirabilis	3(15.0)	1(7.1)	
Proteus vulgaris	3(15.0)	2(14.3)	
Pseudomonas aeruginosa	7(35.0)	7(50.0)	
Total	**20**	**14**	

Table 6. Antibiotic Susceptibility Patterns of ESBL – Producing Bacteria (n=33)

	%	TET	COT	GEN	CRX	CHL	CTR	CTX	CIP	AMK	VAN	CPZ	MEM
E-coli(n=7)	S	1(14.3)	1(14.3)	3(42.2)	-(00.0)	5(71.4)	-(00.0)	-(00.0)	-(00.0)	6(85.7)	1(14.3)	-(00.0)	-(00.0)
	I	-	1(14.3)	1(14.3)	1(14.3)	-(00.0)	3(42.9)	2(28.6)	-(00.0)	-(00.0)	-(00.0)	2(28.6)	2(28.6)
	R	6(85.7)	5(71.4)	3(42.2)	6(85.7)	2(28.6)	4(57.1)	5(71.4)	7(100.0)	1(14.3)	6(85.7)	5(71.4)	5(71.4)
K-granulomatis (n=1)	S	-(00.0)	-(00.0)	-(00.0)	-(00.0)	-(00.0)	-(00.0)	-(00.0)	-(00.0)	-(00.0)	-(00.0)	-(00.0)	-(00.0)
	I	-(00.0)	-(00.0)	-(00.0)	-(00.0)	-(00.0)	-(00.0)	-(00.0)	-(00.0)	-(00.0)	-(00.0)	-(00.0)	-(00.0)
	R	1(100.0)	1(100.0)	1(100.0)	1(100.0)	1(100.0)	1(100.)	1(100.0)	1(100.0)	1(100.0)	1(100.0)	1(100.0)	1(100.0)
K-oxytoca (n=2)	S	-(00.0)	-(00.0)	1(00.0)	-(00.0)	-(00.0)	-(00.0)	-(00.0)	-(00.0)	1(50.0)	-(00.0)	-(00.0)	-(00.0)
	I	-(00.0)	-(00.0)	-(50.0)	-(00.0)	-(00.0)	-(00.0)	-(00.0)	-(00.0)	-	-(00.0)	-(00.0)	-(00.0)
	R	2(100.0)	2(100.0)	1(50.0)	2(100.0)	2(100.0)	2(100.0)	2(100.0)	2(100.0)	1(50.0)	2(100.0)	2(100.0)	2(100.0)
Proteus mirabilis (n=4)	S	-(00.0)	2(50.0)	1(25.0)	-(00.0)	-(00.0)	-(00.0)	-(00.0)	-(00.0)	4(100.0)	1(25.0)	-(00.0)	-(00.0)
	I	-(00.0)	-(00.0)	-(00.0)	1(25.0)	-(00.0)	2(50.0)	1(25.0)	-(00.0)	-(00.0)	-(00.0)	-(00.0)	-(00.0)
	R	4(100.0)	2(50.0)	3(75.0)	3(75.0)	4(100.0)	2(50.0)	3(75.0)	4(100.0)	-(00.0)	3(75.0)	4(100.0)	4(100.0)
Proteus vulgaris(n=5)	S	-(00.0)	-(00.0)	-(00.0)	-(00.0)	1(20.0)	-(00.0)	-(00.0)	1(20.0)	1(20.0)	-(00.0)	-(00.0)	-(00.0)
	I	-(00.0)	-(00.0)	-(00.0)	-(00.0)	1(20.0)	3(60.0)	-(00.0)	-(00.0)	-(00.0)	1(20.0)	-(00.0)	-(00.0)
	R	5(100.0)	5(100.0)	5(100.0)	5(100.0)	3(60.0)	2(40.0)	5(100.0)	4(80.0)	4(80.0)	4(80.0)	5(100.0)	5(100.0)
Pseudomonas aeruginosa(n=14)	S	1(7.1)	1(7.1)	4(28.6)	-(00.0)	3(21.4)	1(7.1)	-(00.0)	2(14.3)	11(78.6)	1(7.1)	2(14.3)	2(14.3)
	I	-(00.0)	2(14.3)	-(00.0)	-(00.0)	1(7.1)	1(7.1)	1(7.1)	5(35.7)	-(00.0)	-(00.0)	1(7.1)	-(00.0)
	R	13(92.9)	11(78.6)	10(71.4)	14(100.0)	10(71.4)	12(85.7)	13(92.9)	7(50.0)	3(21.4)	13(92.9)	11(78.6)	12(85.7)

Table 7. Distribution of ESBL genes in Gram negative bacteria isolated from wound patients

Bacterial Isolate	Total N	Bla CTX -M	bla TEM	bla GES	bla PER	bla OXA-50	bla SHV
Proteus vulgaris	4	0	0	0	0	2	0
Proteus mirabilis	2	0	0	0	0	2	0
Pseudomonas aeruginosa	2	0	0	0	0	2	0
E. coli	1	0	0	0	0	1	0
Klebsiella granulomatis	1	0	0	0	0	1	0

Table 8. Cycling parameters and Primers used in the master mix

GENE	Primer	Sequence (5^1 - 3^1)	Amplicon Size (bp)
BlaTEM	F	GAGACAATAACCCTGGTAAAT	459
	R	AGAAGTAAGTTGGCAGCAGTC	
BlaSHV	F	GTCAGCGAAAAACACCTTGCC	398
	R	GTCTTATCGGCGATAAACCAG	
blaCTX-M	F	GAAGGTCATCAAGAAGGTGCG	560
	R	GCATTGCCACGCTTTTCATAG	
Bla GES	F	ATGCGCTTCATTCACGCAC	860
	R	CTATTTGTCCGTGCTCAGG	
Bla PER	F	AATTTGGGCTTAGGGCAGAA	933
	R	ATGAATGTCATTATAAAAGC	
OXA-50	F	GAAAGGCACCTTCGTCCTCTAC	400
	R	CAGAAAGTGGGTCTGTTCCATC	

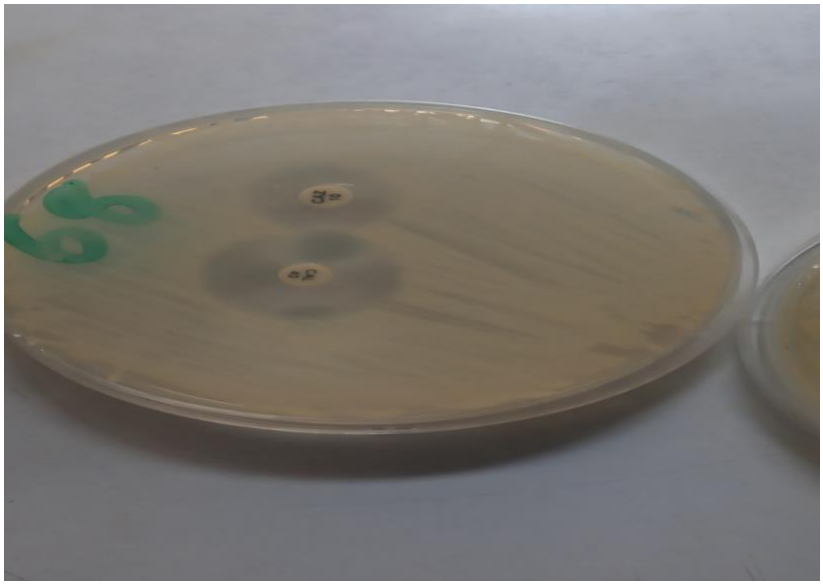

Fig. 1. A positive phenotypic confirmatory test plate

at the end (Chukwunwejim et al*.,* 2018). The purpose of this study was to investigate the prevalence of Extended Spectrum Beta-lactamase producing microorganisms in wound infections. The frequency distribution of the bacterial isolates recovered from the wounds showed that *Pseudomonas aeruginosa* 56(28.6%) was the most prevalent pathogen detected from the swabs followed by *Staphylococcus aureus*24(12.2%). This observation follows the report of Pondei et al*.,* 2013who noted that *Pseudomonas aeruginosa* was the most prevalent pathogen isolated in wound infections. On the contrary, Ohalete et al*.,* 2019, reported *Staphylococcus aureus* as the most predominant pathogen in wound infections. In another study carried out in Nepal by Kabita et al*.,*2020, *E. coli* was found to be the commonest bacteria in wound infection followed by *Staphylococcus aureus.* Earlier work done by Iroha et al., in 2017 at the National Orthopaedic Hospital, Enugu, reported *Klebsiella spp* as having the highest infection rate with a frequency of 59.65%. Although Iroha et al*.,* narrowed their study to *Klebsiella spp* and *E. coli.* This scenario attests to the fact that local and regional variability exists and as such, health institutions have to determine the most common organisms and other related characteristics. As already indicated under the limitation of the study, anaerobic bacteria, which are also incriminated in wound infections, could not be isolated in this work. The prevalence of ESBLs phenotype as obtained in this study is 21.7%. This result is quite similar to the result of the study in Togo by Mlaga et al*.,* 2019 with ESBL Enterobacteriaceae (25.95%) and *E. coli* ESBL production of (17.67%). Another investigation conducted in

Ghana by Oduro-Mensah et al.,2016 revealed an ESBL prevalence of 37.96%. Iroha et al.,2017 reported an ESBL prevalence of 59.6% for *Klebsiella Spp.* The prevalence of ESBLs among the isolates was highest in samples obtained from accident victims while patients with open cancer wound recorded zero prevalence. This may be due to patients waiting for a longer time before accessing medical intervention during which there could be proliferation of bacteria and mixed infection in wounds. A good number of the patients with wounds were accident victims which comprised road accidents, falls and occupational hazards as a result of machines. This is in line with the work done by Iroha et al., 2017 who reported that orthopaedic wounds are more prevalent in people who engage in outdoor job than indoor work. In this study, there was a high prevalence of multi-drug resistance (MDR) in ESBL producers. The highest MDR was found in *Klebsiella spp.* and the lowest level of multi-drug resistance was found in *Pseudomonas aeruginosa*. *K. granulomatis* was 100% resistant to Tetracycline, Co-trimoxazole, Gentamicin, Cefuroxime, chloramphenicol, Cefotaxime, Ciprofloxacin, Amikacin, Vancomycin, Ceftazidime and Meropenem. *K. oxytoca*was also 100% resistant to the above-listed antibiotics apart from Amikacin and Gentamicin in which it showed 50% resistance respectively. In *Proteus spp,* the resistance ranged from 50% to 100%. Apart from Cefuroxime and cefotaxime in which 100% resistance was detected for *Pseudomonas aeruginosa,* the other drugs all had one or more of the *Pseudomonas aeruginosa* sensitive to them. This study finding is in line with what has been observed by Malik and Elhag, 2019. The high level of multi-drug resistance observed in this study corroborates the findings by Nwafia et al., 2019; that the plasmids producing ESBLs can carry resistance to other antibiotics such as aminoglycosides, fluoroquinolones and sulphonamides. The degree of antibiotic consumption was discovered to be greatly related to the degree of antibiotic-resistant infection (Zaman et al., 2017). This could be the possible explanation for the high resistance detected in Cefuroxime and cefotaxime as most of the patients admitted into the orthopaedic hospital were placed on Cefuroxime a 2nd generation cephalosporin and cefotaxime for prophylaxis. Some others were placed on Ceftriaxone and Ceftazidime which are 3rd generation cephalosporin. So many studies have recorded good activity of the carbapenems (meropenem and imipenem) against ESBL. For instance, Mohammad et al., (2021) reported the carbapenems (meropenem) and aminoglycosides (amikacin) as the best treatment options against the ESBL-producing isolates. This study disagrees partially with their finding as it was only amikacin that showed high efficacy to the ESBLs in contrast to meropenem which showed poor effect on the ESBL isolates. The carbapenems are fast losing their efficacy against ESBLs and this is a worrisome development. ESBLs are fast developing great levels of resistance to various classes of antimicrobial agents. The use of carbapenems has increased after the development of resistance to 3rd generation cephalosporins by the ESBLs producing organisms. According to Zaman et al., (2017), the degree of antibiotic utilization determines to a large extent the degree of antibiotic-resistant infections. This could be responsible for the emergence of carbapenem (meropenem) resistant enterobacterial isolates. The molecular screening for the genes responsible for ESBL production in this study revealed that of all the 10 ESBL positive isolates screenedfor blaTEM, blaSHV, blaCTM-X, bla GES, bla PER and bla OXA-50, no ESBL encoding genes were identified in the 10 isolates except bla OXA-50 where 8 samples tested positive. This result is similar to the findings by Agbo et al., (2019), who reported that among the genes screened only OXA-50 genes were able to show positive amplification in eight isolates. However, none of the isolates was positive for the bla PER, bla GES, bla CTX-M, bla SHV and bla TEM. Most studies have reported bla TEM, bla SHV and bla CTX-M as the most prevalent genes in Nigeria. This is in sharp contrast to what we have in this study. This suggests that there may be new ESBL genes responsible for the positive phenotypic test yet to be discovered. Further researches are needed to clarify this. The OXA gene according to Shaikh et al., (2015) mainly occurs in *Pseudomonas aeruginosa* but has been detected in many other gram-negative bacteria. From this study's findings and the report by Agbo et al.,2019, it is like the OXA gene is gradually gaining ground in our locality. There is a dearth of data on the geographical spread of OXA-type ESBLs. The gene was first discovered in Ankara, Turkey (Shaikh et al., 2015).

5. CONCLUSION

Pseudomonas aeruginosa was the predominant pathogen isolated from wound samples 56(28.6%), followed by *Staphylococcus aureus* 24(12.2%). The prevalence of ESBLs as

recorded in this study was 21.7%. *Pseudomonas aeruginosa,*14 (41.2%) *was* the most preponderant ESBL-producing Gram negative bacteria followed by *Escherichia coli,* 7(20.6%). All the isolates in this study were found to be multi-drug resistant. Meropenem which before now was regarded as the gold standard drug for the treatment of patients with resistance to 3rd generation cephalosporins was found less active against both the ESBLs and non- ESBL producers. Amikacin seems to have taken over from meropenem seeing that it was the drug with the highest activity against the isolates. The risk factors associated with ESBL production assessed in this study were not statistically significant. The molecular detection of the OXA gene in 8 out of the 10 samples subjected to molecular studies shows that the OXA gene is gradually gaining ground in our locality. The inability to detect other ESBL encoding genes like TEM, SHV and CTM-X, suggests that there may be new ESBLs genes emerging or a technicality problem in the step-by-step procedure for molecular gene detection. Further researches are needed to investigate the mechanism of resistance conferred by these genes. The high colonization of most of the wounds by *Pseudomonas aeruginosa* indicates the need for improved hand hygiene and changing of gloves in between procedures to help reduce the risk of infection. OXA gene seems to be the commonest gene circulating in our locality. Urgent measures should be put in place to reduce the spread of these resistant genes.

6. LIMITATION OF THE STUDY

Materials used in the microbiological culture are basically for the isolation of aerobic pathogens incriminated in wound infections, and as such may not take into account the anaerobic pathogens.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

SOURCE OF FUNDING

This research did not receive any specific grant from any funding agency in the public, Commercial, or non-profit organizations.

ETHICAL APPROVAL AND CONSENT

The study was conducted at the National Orthopaedic Hospital, Enugu and ESUT Teaching Hospital, Parklane, Enugu. The study protocol was submitted to each of these tertiary hospitals for review and approval. The ethical committee of both institutions after a due review of the protocol approved the study with the following numbers: IRB/HEC NUMBER:3.313/101 and ESUTHP/C-MAC/RA/034/VOL.2/169. Informed consent was duly obtained from the subjects with an indication that the study was voluntary and their non-participation would not affect their visits to the hospital. They were assured of strict confidentiality of their participation and the results obtained. The patients with different categories of wounds were selected for the study, while those that had undergone corrective surgery were excluded due to non-infection of the correction site. In addition, individuals with fresh burn or accident wounds were excluded.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Agbo, M. C., Ezeonu, I. M., Odo, M. N., Ononugbo, C. M., Onodagu, B. O. Eze, C. C., Ezibe, E. N. and Ozioko, C. A. (2019). Phenotypic and molecular characterization of extended spectrum β-lactamases producing *Pseudomonas aeruginosa* in Nigeria. *African Journal of Biotechnology*18 (32):1083-1090.

Asir, J., Nair, S., Devi, S., Prashanth, K., Saranathan, R. and Kanungo, R. (2015). Simultaneous gut colonisation and infection by ESBL-producing *Escherichia coli* in hospitalised patients.*Australas Medical Journal* 8 (6), 200–207.

Bowler, P.G., Duerden, B.I. and Armstrong, D.G. (2001). Wound Microbiology and Associated Approaches to Wound Management. *Clinical Microbiology Review*14: 244-269.

Cheesbrough, M. (2000). Biochemical tests to identify bacteria: In District Laboratory Practice in Tropical Countries. Part 2. Cambridge University Press, pp.62-70.

Cheesbrough, M. (2000). Summary of the clinical and laboratory features of microorganisms: In District Laboratory Practice in Tropical

Countries. Part 2. Cambridge University Press, pp. 157-234.

Chukwunwejim, C. R., Eze, P. M., Ujam, N. T., Abonyi, I. C., Ejikeugwu, C.P., Abonyi, D.O., and Esimone, C. O. (2018). Incidence of community-acquired ESBL-producing bacteria among asymptomatic university students in Anambra state, Nigeria. *European Journal of Biological Research* 8(3): 138 – 147. Doi: 10.5281/zenodo.1314719 10.

Clinical and Laboratory Standards Institute (2021). Performance Standards for Antimicrobial Susceptibility Testing. 31st edition. CLSI Supplement MIOD. Wayne, PA, USA

Cohen, M.L. (2000). Changing patterns of infectious disease. *Nature*.406(6797):762

Cutting, K.F. and White, R. J. (2004). Defined and refined: Criteria for identifying wound infection revisited. *British Journal of community Nursing* 9: S6-S15.

Enweani, U. N. (1991). Surgical Wound Sepsis in Clean Orthopaedic Procedures: Bacteriology and Sensitivity Pattern in a regional Specialist Centre.*Orient Journal of Medicine* 3(1): 16.

Iroha, I. R., Okoye, E., Osigwe, C.A. and Moses, I.B., Ejikeugwu, C. P., (2017). Isolation, Phenotypic Characterization and Prevalence of ESBL-Producing *Escherichia coli* and *Klebsiella species* from Orthopaedic Wounds in National Orthopedic Hospital Enugu (NOHE), South East Nigeria. *Journal of Pharmaceutical Care and Health Systems*4:184

Kabita, A., Shaila, B. and Basudha, S. (2020). Prevalence of multidrug-resistant and Extended Spectrum Beta-Lactamase Producing Bacterial Isolates from infected wounds of patients in Kathmandu Model Hospital, Nepal. *Journal of Science and Technology.*19(1):171-179.

Malik, L. A. and Elhag, K. M. (2019). The First Determination of the Plasmid-Mediated Quinolone Resistance Determinants qnrA and qnrB from the Sudan. *International Journal of Current Microbiology and Applied Sciences* 8(3):1612-1618

Mama, M., Abdissa, A. and Sewunet, T. (2014). Antimicrobial susceptibility pattern of bacterial isolates from wound infection and their sensitivity to alternative topical agents at Jimma University specialized hospital, south-West Ethiopia. *Annals of Clinical Microbiology and Antimicrobials.*13(1): 14

Mlaga, K. D., Salou, M., Dossim, S., Tigossou, S. D., Anago, A. E., Dagnra, A. Y. and Ambahou, S. (2019). Antibiotic Resistance Profile and Molecular Characterisation of *Escherichia coli* Extended Spectrum Beta-Lactamase Producing Isolates from Sylvanus Olympro Teaching Hospital in Lomé, Togo. *Journal of Advances inMicrobiology*14(3):1-7.

Moet, G.J., Jones, R.N., Biedenbach, D.J., Stilwell, M.G. and Fritsche, T.R. (2007) Contemporary causes of skin and soft tissue infections in North America, Latin America, and Europe: report from the SENTRY Antimicrobial Surveillance Program (1998–2004) Diagnostic Microbiology and Infectious Disease 57:7–13.

Mohammad, J. G., Javad, Z., Parisa, R. Zahra, Y., Masoud, Sharif and Niloufar, R. (2021). Comprehensive study of antimicrobial susceptibility pattern and extended spectrum beta -lactamase (ESBL) prevalence in bacteria isolated from urine samples. *Scientific Reports* 11:578

Nwafia, I. N., Ohanu, M. E., Ebede, S. O. and Ozumba, U. C. (2019). Molecular detection and antibiotic resistance pattern of extended-spectrum beta-lactamase producing *Escherichia coli* in a tertiary hospital in Enugu, Nigeria. *Annals of Clinical Microbiology and Antimicrobials.* 18: 41 – 47. Doi: 10.1186/s12941-019-0342-9.

Oduro-Mensah D, Obeng-Nkrumah N, Bonney EY, Oduro-Mensah E, TwumDanso K, Osei YD, Sackey ST. Genetic characterization of TEM-typeESBLassociated antibacterial resistance in Enterobacteriaceae in a tertiary hospital inGhana. 2016;15(1):29. (Retrieved 2 December 2016) https://doi.org/10.1186/s12941-016-0144-2

Ohalete, C.N., Obi, R.K. and Emeakoroba, M.C. (2019). Bacteriology of different wound infection and their antimicrobial susceptibility patterns in Imo state, Nigeria. *World Journal of Pharmacy and Pharmaceutical Sciences*1(3) :1155-1172

Otokunefo, T.V. and Datubo-Brown, D.D. (1990). Bacteriology of Wound Infections in the Surgical Wards of a Teaching Hospital. *West African Journal of Medicine* 9(4): 285-290.

Pondei, K., Fente, B. G. and Oladapo, O. (2013). Current microbial isolates from wound swabs, their culture and sensitivity pattern

at the Niger Delta University Teaching Hospital, Okolobiri, Nigeria. *The Japanese Society of Tropical Medicine* 41(2):49-53

Shaikh, S., Fatima, J., Shakil, S., Mohd, S., Rizvi, D., Kamal, M. A (2015). Antibiotic resistance and extended beta-lactamases: Types, epidemiology and treatment. *Saudi Journal of Biological Sciences* 22: 90-101.

Zaman, S., Hussain, M., Nye, R., Mehta, V., Mamum, T. K. and Hossiah, N. (2017). A Review on Antibiotic Resistance: Alarm Bells are Ringing. *Cureus* 9(6): e1403. DOI 10.7759/*cureus*.1403 *England Journal of Medicine,* York, Plenum Press.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/128759