

## Original Research Article

# PREVALENCE OF EXTENDED SPECTRUM BETA-LACTAMASE PRODUCING BACTERIA IN PATIENTS WITH WOUND INFECTIONS ATTENDING TERTIARY HOSPITALS IN ENUGU, NIGERIA.

## ABSTRACT

### Background and aims

The problem of antibiotics usage against bacterial infection is the modifications of such antibiotics by the bacteria thereby rendering them ineffective. Extended spectrum  $\beta$ -lactamase producing bacteria invading wound infections may lead to long term hospitalization, financial burden and limited antibiotics for therapy. The goal of this study was to determine the prevalence of ESBL-producing bacteria colonization of wound infections among individuals with non-healing wounds. **Materials and Methods:** This was a cross-sectional study. Individuals with chronic wound infections were recruited from tertiary hospitals in Enugu. They were interviewed and administered with a structured questionnaire to obtain information on sociodemographic characteristics and hospitalization periods within a period of one year and those that voluntarily agreed to participate were enrolled in the study. The patients had pus samples collected with sterile swab sticks. The pus samples were analysed bacteriologically and the bacteria growth identified using standard methods. The gram-negative bacterial isolates were confirmed as ESBL-producing bacteria by their resistance to cefotaxime, ceftazidime and ceftriaxone and phenotypic typing.

### Results

The age group of  $\leq 29$  years formed the majority of the subjects with 26.3% (175/266). *Pseudomonas aeruginosa* 56(28.6%) was the leading organism causing wound infection followed by *Staphylococcus aureus* 24(12.2%). The prevalence of ESBLs among the gram-negative isolates was 21.7% (34/157). The males had higher frequency of ESBL producers than the females. The highest prevalence of ESBL was observed in *Pseudomonas aeruginosa* 41.2% (14/34). The least prevalence was seen in *Klebsiella pneumoniae* (2.9%) and *Klebsiella granulomatis* (2.9%). Sociodemographic characteristics of the subjects in relation to ESBL production showed no statistical significance. The risk factors assessed showed no statistical significance. The ESBL-producing bacterial isolates from the community and hospital environments indicated a frequency 58.8% (20/34) and 41.2% (14/34) respectively,  $P=0.072$ .

**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 typing, *Pseudomonas aeruginosa*, Enugu.

## INTRODUCTION

### Background to the Study

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  $\beta$ -Lactamase are very susceptible to treatment failure when using an Extended Spectrum  $\beta$ -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.

Moreso, 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.

## **MATERIALS AND METHODS**

**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.

### **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.

### **Ethical Issues**

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.

**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.

**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.

### **Identification of the Isolates**

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).

### **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°C for 18 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 (10µg), Co-trimoxazole (25µg), Gentamicin (10µg), Cefuroxime (30µg), Chloramphenicol (10µg), Ceftriaxone (30µg), Cefotaxime (30µg), Ciprofloxacin (5µg), Amikacin (30µg), Vancomycin (30µg), Ceftazidime (30µg) and Meropenem (10µg). Isolates which were gram-negative and showed resistance to the following third-generation cephalosporins namely cefotaxime (30µg), ceftazidime (30µg) and ceftriaxone (30µg) with a zone of inhibition  $\leq 27$ mm for cefotaxime,  $\leq 22$ mm for ceftazidime and  $\leq 25$  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).

### **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.

### **Screening for ESBL Producers**

Isolates which were gram-negative and showed resistance to the following third-generation cephalosporins namely cefotaxime (30µg), ceftazidime (30µg) and ceftriaxone (30µg) with zone of inhibition  $\leq 27$ mm for cefotaxime,  $\leq 22$ mm for ceftazidime and  $\leq 25$  for ceftriaxone were



selected as possible ESBL producers and subjected to further studies.

### **Phenotypic Confirmatory Test**

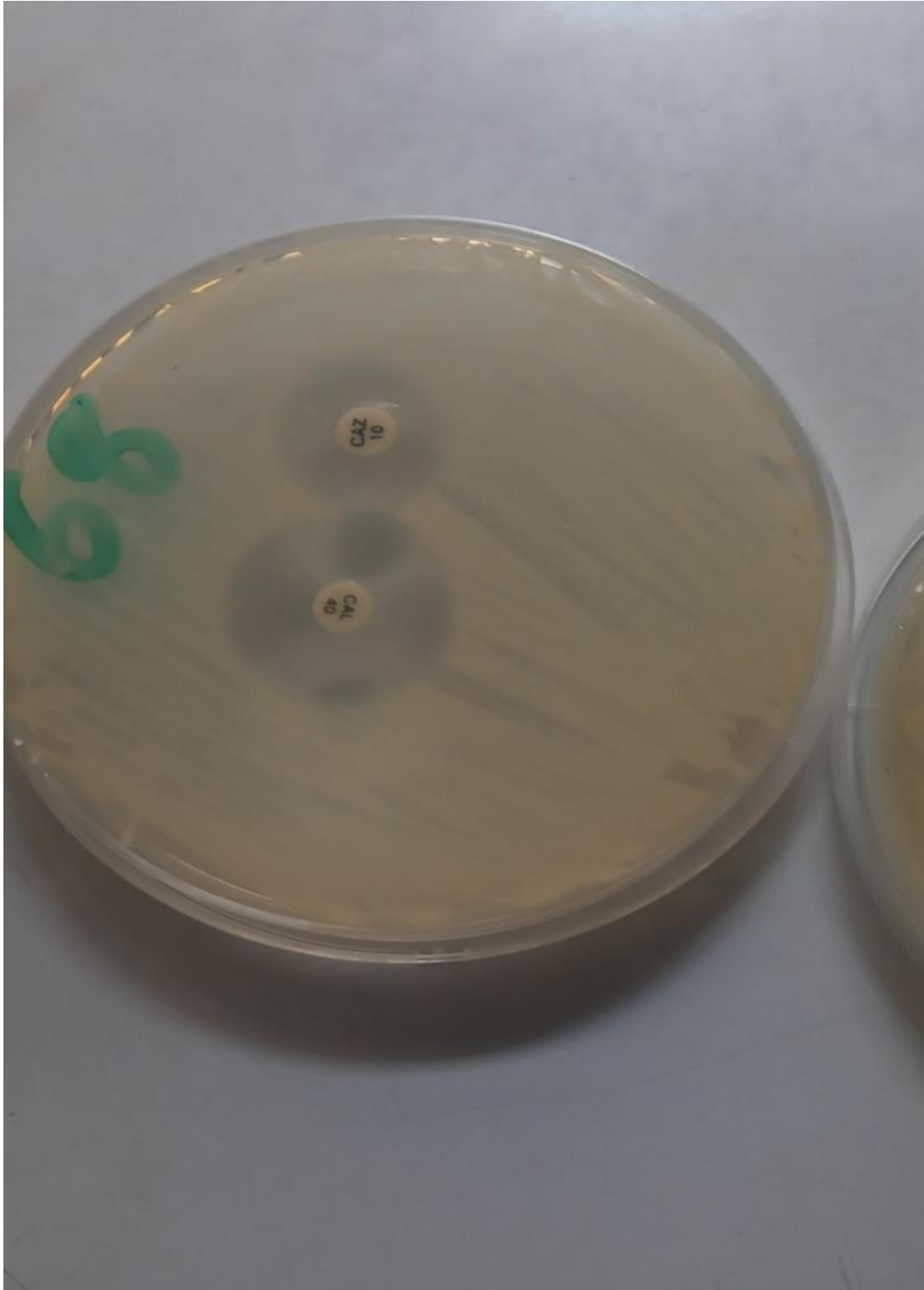
Confirmation of ESBL-producing isolates was done by the phenotypic confirmatory test according to CLSI recommendation. Combination disc test was the method employed. In this experiment, a disc containing ceftazidime 30 $\mu$ g alone was positioned opposite to a disc containing a combination of ceftazidime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

### **Statistical Analysis**

All statistical analysis 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  $<0.05$ .

### **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.



**Figure 1: A positive Phenotypic confirmatory test plate**

## RESULT

Table 1: shows the sociodemographic characteristics of the study subjects. There were 175(65.8%) males and 91(34.2%) females that participated in this study. The age ranges from 15 years to 95 years with mean age of  $43.61 \pm 18.4$ . Those in the age category of  $\leq 29$  had the highest number of orthopaedic wounds 70(26.3%). A total of 167 participants representing 62.8% of the study population are married while 99(37.2%) are single. Out of the 266 patients, 100 (37.6%) live in the urban area, 72(27.1%) live in the semi- urban and 94(35.3%) live in the rural area. Among the study participants, 18(6.8%) had no formal education while 87(32.7%) studied up to the tertiary level of education. Regarding the occupation of the study population, 44 (16.5%) were not gainfully employed while 107(40.2%) were traders. A total of 120(45.1%) were hospitalized while 146(54.9%) were not hospitalized. Those who had surgery in the past were 60(22.6%) while 206(77.4%) have never had surgery. Those who practice self-medication were 167(62.8%) while those who do not practice self-medication were 99 (37.2%). Subjects who take antibiotics based on doctor's prescription were 175 (65.8%) while 91(34.2%) take antibiotics without doctor's prescription. Among the participants, 94(35.3%) admitted that they use herbal medication whereas 172 (64.7%) do not use herbal medication. Table 2: shows the isolates of ESBLs and non-ESBLs. Thirty-four (21.7%) of the isolates were found positive following preliminary screening. The distribution is as shown in table 2. Out of the 21 isolates of *E. coli*, 7 (20.6%) were found to be positive for ESBL, *K. oxytoca*, *K. pneumonia*, *K. granulomatis* all had 2(5.9%), 1(2.9%) and 1 (2.9%) positive ESBLs respectively. *Proteus mirabilis* had 4 (11.8%) while *Proteus vulgaris* had 5 (14.7%) positive ESBLs. *Pseudomonas aeruginosa* had the highest number of ESBL positive isolates 14 (41.2%). Table 3: occurrence of ESBL-producing bacteria in relation to sources of wound. Accident victims have the highest frequency of ESBL-producers while open cancer wound patients had none. Table 4: shows the sociodemographic characteristics of the subjects in relation to ESBL- producing bacteria. Of all the variables like sex, age, educational level, occupation, marital status and residential area examined at  $p\text{-value} \leq 0.05$ , none was statistically significant. Table 5: Shows the assessment of the risk factors with ESBL producers. Of all the risk factors considered, those currently hospitalized had a  $p\text{-value}$  0.072. Those who had surgery in the past  $p\text{-value}$  0.486, self-medication had a  $p\text{-value}$  0.447, antibiotics use on doctor's prescription had a  $p\text{-value}$  0.185 and use of herbal therapy had a  $p\text{-value}$  0.149. Table 6: represents the distribution of ESBL producing bacteria among hospitalized and non-hospitalized patients. ESBL producers were

more in organisms isolated from hospitalized patients 20(58.8%) than non-hospitalised14(41.2%).

**Table 1: Sociodemographic characteristics of the study subjects**

<b>Variable</b>	<b>Frequency</b>	<b>Percentage (%)</b>
<b>Sex</b>		
Male	175	65.8
Female	91	34.2
<b>Age group (years)</b>		
≤ 29	70	26.3
30 – 39	50	21.8
40 – 49	38	14.3
50 – 59	38	14.3
60 – 69	33	12.4
≥ 70	29	10.9
<b>Marital Status</b>		
Single	99	37.2
Married	167	62.8
<b>Residential Area</b>		
Semi – urban	72	27.1
Urban	100	37.6
Rural	94	35.3
<b>Educational level</b>		
No formal education	18	6.8
Primary	69	25.9
Secondary	92	34.6
Tertiary	87	32.7
<b>Occupation</b>		
Farming	17	6.4
Civil servant	30	11.3
Artisan	39	14.7
Driver	24	9.0
Unemployed	44	16.5
House wife	5	1.9

Business/trader	107	40.2
<b>Are you currently hospitalized?</b>		
Yes	120	45.1
No	146	54.9
<b>Have you had surgery in the past?</b>		
Yes	60	22.6
No	206	77.4
<b>Self -Medication</b>		
Yes	167	62.8
No	99	37.2
<b>Doctor's Prescription</b>		
Yes	175	65.8
No	91	34.2
<b>Herbal Medication</b>		
Yes	94	35.3
No	172	64.7

UNDER PEER REVIEW

**Table 2: Isolates of ESBL and non-ESBL producing bacteria**

<b>Bacteria</b>	<b>ESBL (%)</b>	<b>NON-ESBL (%)</b>
<i>Acinetobacter baumannii</i>	0(0.0)	7(5.7)
<i>Citrobacter freundii</i>	0(0.0)	1(0.8)
<i>Enterobacter spp</i>	0(0.0)	2(1.6)
<i>Esherichia coli</i>	7(20.6)	14(11.4)
<i>Klebsiella granulomatis</i>	1(2.9)	5(4.1)
<i>Klebsiella pneumonia</i>	1(2.9)	9(7.3)
<i>Klebsiella oxytoca</i>	2(5.9)	9(7.3)
<i>Proteus mirabilis</i>	4(11.8)	16(13.0)
<i>Proteus vulgaris</i>	5(14.7)	14(11.4)
<i>Pseudomonas aeruginosa</i>	14(41.2)	42(34.4)
<i>Moraxella catarrhalis</i>	0(0.0)	2(1.6)
<i>Morganella morganii</i>	0(0.0)	2(1.6)
<b>Total</b>	<b>34</b>	<b>123</b>

**Table 3: Occurrence of ESBL-producing bacteria in relation to sources of wound**

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

**Table 4: sociodemographic characteristics of the subjects in relation to ESBL – producing bacteria**

<b>Variable</b>	<b>No. of ESBL Isolates%</b>	<b>No. of Non-ESBL Isolates%</b>	<b>P-value</b>
<b>Sex</b>			
Male	23(21.5)	84(78.5)	0.943
Female	11(22.0)	39(78.0)	
<b>Age group (years)</b>			
≤ 29	6(14.3)	36(85.7)	0.108
30 – 39	6(21.4)	22(78.6)	
40 – 49	8(42.1)	11(57.9)	
50 – 59	6(24.0)	19((76.0)	
60 – 69	5(21.7)	18(78.3)	
≥ 70	3(15.0)	17(85.0)	
<b>Marital Status</b>			
Single	11(19.6)	45(80.4)	0.648
Married	23(22.8)	78(77.2)	
<b>Residential Area</b>			
Semi – urban	11(23.9)	35(76.1)	0.889
Urban	12(20.0)	48(80.0)	
Rural	11(21.6)	40(78.4)	
<b>Educational level</b>			
No formal education	2(22.2)	7(77.8)	0.929
Primary	7(18.4)	31(81.6)	
Secondary	14(24.1)	44(75.9)	
Tertiary	11(21.2)	41(73.8)	
<b>Occupation</b>			
Farming	3(27.3)	8(72.7)	0.724
Civil servant	5(27.8)	13(72.2)	
Artisan	4(18.2)	18(81.8)	
Driver	4(30.8)	9(69.2)	
Unemployed	4(13.3)	26(86.7)	
House wife	0(0.0)	4(100.0)	
Business/trader	14(23.7)	45(76.3)	



**Table 5: Assessment of risk factors associated with ESBL production in wound isolates**

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

**Table 6: Distribution of ESBL – producing bacteria among hospitalized and non –**

**hospitalized patients**

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

## DISCUSSION

The purpose of this study was to investigate the prevalence Extended spectrum beta-lactamase producing microorganisms in wound infections. The investigation recruited a cohort of 266 participants, and the incidence of wound infection was found to be higher in males (65.8%) than in females (34.2%). The findings of Ohalete *et al.*, 2019, which revealed a statistically significant disparity in the prevalence of wound infection between males (59.3%) and females (60.7%), are consistent with the results of this study. The reason for this is not far-fetched since the majority of the males in Nigeria traditionally engage in occupations such as transportation, farming, industrial work, mechanic work and trading which may expose them to traumatic conditions. As can be seen from this study, the majority of the participants live in urban and semi-urban areas where a host of activities like trading, industrial work, and construction work take place. 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 jobs than indoor work. This study also shows that males and females aged  $\leq 29$  years and 30 to 39 years old had the highest prevalence of orthopaedic wounds. This may be because these age groups are very active and energetic as such can engage in any activity with ease.

The frequency distribution of the bacteria isolates recovered from the wounds showed that *Pseudomonas aeruginosa* (28.6%) was the most prevalent pathogen detected from the swabs followed by *Staphylococcus aureus* (12.2%). This observation follows the report of Pondei *et al.*, 2013 and Mehta *et al.*, 2007 who noted that *Pseudomonas aeruginosa* was the most prevalent pathogen isolated in wound infections. On the contrary, Ohalete *et al.*, 2019; Wariso and Nwachukwu, 2003; Egbe *et al.*, 2011 reported *Staphylococcus aureus* as the predominant pathogen in wound infections. 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. Earlier work done by Iroha *et al.*, in 2017 at the NOHE, reported *Klebsiella spp* as having the highest infection rate with a frequency of 59.65%. 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 and such a situation could lead to heavy growth of bacteria and mixed infection in wounds.

The prevalence of ESBLs – phenotype as obtained in this study is 21.7%. This is low when compared to the rates obtained in studies done by Iroha *et al.*, 2017 that recorded an ESBL prevalence of 59.6% for *Klebsiella Spps.*

Although the prevalence of ESBLs in this study is low when compared to the values obtained by the aforementioned, it is still higher than the values namely 20.0% and 16.0% as obtained from the southwest and southeastern Nigeria by Aibinu *et al.*, 2003 and Akujiobi and Ewuru, 2010 respectively. According to Dejenie *et al.*, 2019, the combination disk test (CDT) used for the confirmation of ESBLs in this study is better than the Double Disc Synergic Test (DDST) used in the detection of ESBLs. The reason for the low prevalence obtained in this study could not have been a result of the method of ESBL detection used in this study. The CLSI suggested the use of cefpodoxime, ceftazidime, aztreonam, cefotaxime or ceftriaxone disks for the performance of antibiotic susceptibility testing for ESBLs. The argument here is that since the affinity of ESBLs for different substrates is variable, the use of more than one of these agents for screening improves the sensitivity of detection of likely ESBL producers. Livermore and Patterson, 2006 opined that it is suitable to use cefotaxime which has been noted for its consistency when it comes to CTX-M susceptibility and ceftazidime which has been proved over time to be a good substrate for TEM and SHV variants. The antibiotic disk used in this study to screen for ESBL production met the above-mentioned requirements. For the phenotypic confirmatory tests for ESBL production, CLSI advocated the use of cefotaxime (30µg) or ceftazidime (30µg) disk with or without clavulanate (10µg) for phenotypic confirmation of the presence of ESBLs in *Klebsiella species*, *E. coli*, *Proteus mirabilis* and *Salmonella species*. CLSI also recommended that the disk tests be performed with confluent growth on Mueller Hilton Agar and a difference of  $\geq 5$ mm between the zone diameters of either the cephalosporin disks and their respective cephalosporin/clavulanate disks is taken to be phenotypic confirmation of ESBL production. This study still satisfied the above conditions. The low prevalence of ESBLs in this study cannot be attributed to a shortfall in standard operating procedures. Other factors other than methodology

could have contributed to the low prevalence of ESBLs in this study. For instance, the coexistence of AmpC type  $\beta$ -lactamases and ESBLs in the same organism not only results in decreased cephalosporin zone diameter but may also give false negative test results for the detection of ESBLs. The probable explanation is that AmpC-type  $\beta$ -lactamase resists inhibition by clavulanate and therefore blocks the synergetic effect of clavulanate and cephalosporins against ESBLs. This AmpC-type  $\beta$ -lactamase effect may have contributed to the low prevalence of ESBLs in this study. This and other factors may have been responsible for the low prevalence of ESBLs in this study. The association of ESBLs with the age of the participants, sex, educational level, occupation and marital status showed that there was no statistical significance between ESBLs and these variables. Although some studies have observed a statistical significance in some of these variables. The risk factors for the acquisition of ESBLs assessed was also not statistically significant although hospitalization had a p-value of 0.072.

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