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Original Article

First Report of Molecular Characterization of Extended Spectrum Beta-Lactamase-Producing *Acinetobacter baumannii* among Debilitated Patients in Tertiary Care Hospital, Ebonyi State, Nigeria

Ikechukwu Herbert Egwu[a], Modesta Mmaduabuchi Egwu-Ikechukwu[b*], Boniface Oke[a], Charity Chinyere Nnabugwu[b]

[a]*Department of Applied Microbiology, Faculty of Science, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria,*
[b]*Department of Microbiology, Faculty of Biological Sciences, Alex Ekwueme-Federal University Ndufu- Alike Ikwo, Abakaliki, Ebonyi State, Nigeria*

Abstract

Extended Spectrum Beta Lactamase (ESBL)-Producing *Acinetobacter baumannii* has threatened patients' optimal healthcare in various tertiary care hospitals globally. The paucity of information regarding this life-threatening organism in Nigeria necessitated this research. Hence, this study aimed to molecularly characterize ESBL-producing *A. baumannii* among debilitated patients in a tertiary care hospital in Ebonyi State, Nigeria. Debilitated patients admitted in the intensive care unit (ICU), medical, surgical, and orthopedic wards were sampled, and 385 clinical samples were obtained from them over a six-month study period. Standard microbiological methods were used to identify *A. baumannii* isolates, while 16S rRNA PCR was used to confirm the isolates molecularly. The Kirby-Bauer disc diffusion techniques were used to ascertain the antibiotic sensitivity profiles of *A. baumannii* isolates. The double disc synergy test (DDST) method was employed to determine ESBLs production among the isolates, while to determine *A. baumannii* isolates harboring bla_{TEM}, bla_{SHV}, and bla_{OXA} genes, PCR techniques were used. A total of 23 (6%) *A. baumannii* isolates were recovered from 385 clinical samples. The isolated *A. baumannii* exhibited multidrug resistance (MDR) traits, while 43.5% of the isolates were ESBL-producing *A. baumannii*. Also, 9 (90%) and 3 (30%) of the isolated *A. baumannii* harbored bla_{TEM} and bla_{OXA} genes, respectively, while no isolate harbored the bla_{SHV} gene. The isolated *A. baumannii* were observed to harbor ESBL genes. Importantly, this is the first report of ESBL-producing *A. baumannii* in Ebonyi State, Nigeria.

Keywords: *Acinetobacter baumannii*, bla_{TEM}, *bla*OXA, bla_{SHV} genes, Multidrug resistance.

Corresponding Author: Dr. Egwu-Ikechukwu, Modesta, Department of Microbiology, Alex Ekwueme Federal University Ndufu-Alike Ikwo, P.M.B 1010, Ebonyi State, Nigeria. E-mails: agwu.modesta@yahoo.com

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1. Introduction

Acinetobacter baumannii has become one of the most worrisome pathogens confronting healthcare institutions globally, and this is a result of its ability to easily acquire resistance genes, among other factors [1, 2]. *A. baumannii*

is a notorious pathogenic organism that troubles both healthcare institutions and community as a result of its capability to acquire resistant determinants. Once established in a healthcare institution, it becomes a significant threat as it becomes challenging to get rid of, thus spreading quickly in the hospital environment, contaminating hospital equipment, and causing various infections, especially in debilitated patients whose immune system has seriously been jeopardized. Resistance of pathogenic microorganisms such as *A. baumannii* to commonly used antibiotics has become a global problem with serious and overwhelming consequences on the healthcare of patients [3, 4]. *A. baumannii* has become a significant emerging opportunistic nosocomial pathogen and has also been reported to cause various degrees of community-acquired infections worldwide [5].

In this present antibiotic era, *A. baumannii* has become one of the major threats to antibiotics as it jeopardizes virtually all antibiotic classes, including cephalosporins, monobactams, fluoroquinolones, carbapenems, penicillins, aminoglycosides. A major class of antibiotics known as β-lactams is the most commonly used against many bacterial infections caused mainly by Gram-negative bacteria such as *A. baumannii*. However, resistance to this vital group of life-saving antibiotics has been reported globally [6-8].

An essential mechanism through which most pathogenic organisms resist β-lactam antibiotics is the inactivation of antimicrobials by enzymes, by producing extended-spectrum beta-lactamases [9]. Generally, extended-spectrum beta-lactamases (ESBLs) are plasmid-mediated enzymes that hydrolyze extended-spectrum beta-lactams, except for carbapenems and cephamycins [9,10]. ESBLs are the commonest type of β-lactamase enzymes that have become a crucial mechanism of resistance to β-lactam antibiotics, mainly amongst the Enterobacteriaceae family and *Pseudomonas aeruginosa,* but also been reported in *A. baumannii* [11, 12].

Considering the ambler molecular classification of β-lactamase genes, the majority of ESBLs are commonly the members of the bla_{SHV}, bla_{TEM}, or bla_{CTX-M} (class A) families while other minor ESBL types are members of $bla_{BES-11,}$ bla_{PER-1}, bla_{OXA}, and bla_{VEB-1} [13,14]. Many subtypes of each ESBL type exist, and several ESBL variants numbering above 300 have also been described and reported worldwide [13]. However, one of the essential and significant ESBL gene families is the bla_{CTX-M}, further divided into five phylogenetic groups concerning amino acid sequence identity [15, 16]. Furthermore, over 128 variants of bla_{CTX-M} have already been identified and were grouped into five major classes: CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25 [17]. Thus far, the prevalence of ESBL-producing organisms such as *A. baumannii* is rapidly increasing geometrically, and patients with this important pathogenic organism are seriously faced with an overwhelming health treatment challenge including but not limited to the high cost of medical treatment, and prolonged hospital stay. They may eventually lead to death as a result of treatment failures [18,19]. Infections due to ESBL-producing- *A. baumannii* can frustrate clinicians, patients, and caregivers, rendering treatments ineffective.

The importance of screening for ESBL-producing pathogenic microorganisms in medical practice and for the overall treatment of patients cannot be overemphasized. It is necessitated by their ability to produce antibiotic-resistant strains capable of resisting the most commonly used antibiotics in hospitals, including cephalosporins, which usually serve as first-line antibiotics in treating various bacterial infections.

The paucity of information regarding this life-threatening microorganism in Nigeria, especially the South-Eastern part of Nigeria, necessitated this research; hence, this study aimed to molecularly characterize ESBL-producing *A. baumannii* among debilitated patients in Alex Ekwueme-Federal University Teaching Hospital, Abakaliki, Ebonyi State, Nigeria.

2. Materials and Methods

2.1. Study Area

The study area of this research is Alex Ekwueme-Federal University Teaching Hospital, Abakaliki (AE-FUTHA), previously known as Federal Teaching Hospital, Abakaliki (FETHA), Ebonyi State, Nigeria. AE-FUTHA is a tertiary healthcare hospital located in the South Eastern Nigeria. It is a referral hospital for several private missions and general hospitals in the State and other neighboring States around Ebonyi State, Nigeria.

2.2 Inclusion Criteria

A random sampling of all critically ill/debilitated patients who consent to participate in this research, aged 20 years and above, also have spent at least ten days in the hospital's medical, surgical, and orthopedic wards was carried out. At the same time, those sampled in the intensive care unit were patients who had spent at least 72 hours in the ICU.

2.3 Exclusion Criteria

Exclusion criteria include patients who are not critically ill/debilitated patients. Also, patients under 20 years and/or have not spent at least ten days in medical, surgical, and orthopedic wards and at least 72 hours on admission to the ICU. Lastly, patients admitted to AE-FUTHA who did not consent to participate in this study were also not sampled.

2.4 Ethical Issues

Ethical clearance was sort and obtained from the Research and Ethical Committee of Federal Teaching Hospital, Abakaliki (FETHA), now known as Alex Ekwueme-University Teaching Hospital Abakaliki (AE-FUTHA), with REC approval number FETHA/REC/VOL1/ 2017/581. Both written informed consent and oral consent were obtained from each patient or caregiver of such patient prior to sample collection.

2.5 Study Design, Sample Collection, and Bacteriological Analysis

A total of 385 clinical samples were obtained from catheter urine (100), wound drain (68), wound sores (83), respiratory fluids (57), skin swabs (45), and bed rail swabs (32) of patients admitted in orthopedic ward, surgical ward, medical ward and ICU of Alex Ekwueme-Federal University Teaching Hospital, Abakaliki during six months study periods (May 2019 until November 2019). All the samples were

immediately inoculated aseptically onto nutrient agar prior to inoculation onto 5% sheep blood agar and MacConkey agar, respectively (Merck Co., Germany), except for urine samples which were first streaked on Cysteine Lactose Electrolyte Deficient (CLED), (Merck Co., Germany) prior to inoculation onto 5% sheep blood agar and MacConkey agar (Merck Co., Germany). *Acinetobacter* spp that grew on MacConkey agar appeared as non-lactose fermenters (slightly beige and colorless). All the culture media were prepared according to the manufacturer's instructions. The inoculated culture plates were then incubated overnight at 37°C and 42°C under aerobic conditions before carrying out Gram staining on all the samples. All coccobacillus, Gram-negative bacterial isolates, were subjected to conventional microbiological and biochemical tests such as oxidase, catalase, coagulase, indole, citrate utilization, urease, methyl red, and motility tests. *A. baumannii* isolates were identified using standard microbiological procedures and biochemical tests [20-22]. The isolated *A. baumannii* were further confirmed molecularly using 16S rRNA PCR.

2.6 Antimicrobial Resistance Testing (ART)

The antimicrobial resistance tests of all isolated *A. baumannii bacterial* isolates were determined using the Kirby-Bauer disc diffusion method. Briefly, the colony suspension from each overnight culture was prepared using nutrient broth and compared with 0.5 MacFarland turbidity standards. Using a sterile swab stick, Mueller-Hinton agar (MHA) plates were inoculated with 0.5 McFarland standard equivalent of the test organism and then allowed for 30 minutes for pre–diffusion. Antibiotic impregnated discs containing meropenem (10µg), ertapenem (10µg), imipenem (10µg), aztreonam (30µg), ceftriaxone (30µg), cefuroxime (30µg), cefepime (30µg), ceftazidime (30µg), cefotaxime (30µg), ciprofloxacin (5µg), colistin sulfate (25µg), ofloxacine (5µg), tetracycline (30µg), amikacin (30µg), sulfamethoxazole/ trimethoprim (25µg), amoxicillin/clavulanic acid (30µg), and ampicillin/sulbactam (20µg) obtained from Oxoid Ltd, Basingstroke, UK were carefully placed on the surfaces of the MHA plates and incubated at 37ºC for 24h. The inhibition zone diameters were measured using a meter rule and interpreted according to clinical and laboratory standard institute (CLSI) guidelines [23].

2.7 Phenotypic Detection of ESBLs

Phenotypic detection of ESBL production in *A. baumannii* isolates was determined by the double disc synergy test (DDST) method. All the *A. baumannii* isolates exhibiting resistance to more than two different classes of antibiotics were selected and screened with five discs of aztreonam (30µg), cefotaxime (30µg), ceftriaxone (30µg), ceftazidime (30µg) and cefotetan (30µg). Subsequently, all *A. baumannii* isolates that resisted the five antibiotics mentioned above were considered ESBL-positive. Finally, the double disc synergy test (DDST) method using cefotaxime (30 µg) and ceftazidime (30 µg) antibiotic discs was used for the confirmation of ESBL production by placing the two antibiotics 15 mm away from amoxicillin-clavulanate disc (20/10 µg) on Mueller-Hinton agar as recommended by CLSI [23].

2.8 Molecular Detection of β-lactamase Genes

2.8.1 DNA Extraction

The DNA extraction of all ten isolates confirmed as ESBL-producing *A. baumannii* using DDST was carried out using the genomic DNA purification kits supplemented by the Manufacturer Company (Promega, USA).

2.8.2 Amplification of Extended Spectrum β-lactamase Genes

Polymerase chain reaction (PCR) was used to detect the genes encoding resistance to the extended-spectrum β-lactams: bla_{TEM}, $bla_{SHV,}$ and bla_{OXA}. Accordingly, the PCR sequencing preparation cocktail consisted of 10 µl of 5x GoTaqcolourless reaction, three µl of 25mM $MgCl_2$, 1µl of 10 mM of dNTPs mix, 1µl of 10 pmol each of the 16S rRNA gene with forward primer (16SF: GTGCCAGCAGCCGCGCTAA) and reverse primer (16SR: AGACCCGGGAACGTATTCAC) and 0.3 units of Taq DNA polymerase (Promega, USA) made up to 42 µl with sterile distilled water, 8 µl DNA template to form a final reaction master mix volume of 50µl. PCR was carried out in a GeneAmp 9700 PCR system thermal cycler (Applied Biosystem Inc., USA) with a PCR profile consisting of an initial denaturation at 94 °C for 5 min, followed by 30 cycles consisting of 94 °C for 30 s, 30 seconds annealing of primer at 56 °C and 72 °C for 1 minute 30 seconds; and a final termination at 72 °C for 10 mins and chilled at 4°C. The integrity of the DNA and PCR amplification were checked on 1% and 1.5% agarose gel, respectively.

2.8.3 Purification of Amplified Product

The amplified product was purified according to the method described by Essan *et al*. [1]. After gel integrity, the amplified fragments were purified with ethanol to remove the PCR reagents. The purified fragment was then checked on a 1.5 % agarose gel run on a voltage of 110 V for about 1 hr to confirm the presence of the purified product and quantified using a Nanodrop spectrophotometer. After electrophoresis, the gel was photographed under UV light.

2.9 Statistical Analysis

The statistical analysis used an independent samples t-test to compare the percentage resistance and susceptibility frequencies of multidrug-resistant *A. baumannii* isolated from different clinical samples in AE-FUTHA. $P<0.05$ was considered statistically significant.

3. Results and Discussion

3.1. Isolation and Identification of A. baumannii from Clinical Samples

A. baumannii is a significant opportunistic pathogen that is responsible for various nosocomial infections that result in notable morbidity and mortality, thus presenting an important threat to those with underlying illnesses, especially among ICU patients [24-26]. In this study, *A. baumannii* was isolated from clinical samples obtained from critically ill patients with underlying illnesses who have spent at least ten days in various hospital wards. A total of 23 (6%) *A. baumannii* isolates were recovered from 385 clinical samples collected from debilitated/clinically ill patients admitted to Alex Ekwueme-Federal University Teaching Hospital Abakaliki (AE-FUTHA). Among the clinical samples analyzed, the highest number of *A. baumannii* isolates were recovered from catheter urine

8(8%) and wound sores 7(8 %), while none (0%) was recovered from samples collected from skin swabs respectively (**Table 1**).

Table 1: Frequency and percentage of *A. baumannii* isolation from clinical samples.

Samples Source	TNSA	TNABI (n (%))
Catheter urine	100	8 (8)
Wound Sores	83	7 (8)
Wound Drain	68	3 (4)
Respiratory Fluid	57	5 (9)
Bed Rails	32	0 (0)
Skin swab	45	0 (0)
Total	**385**	**23 (6%)**

TNSA: Total Number of Samples Analyzed, TNABI: Total Number of *A. baumannii* Isolated.

The 23 (6%) prevalence rate of *A. baumannii* infection reported in this study (Table 1) is in line with the 8.5 % prevalence rate of *A. baumannii* reported in a previous study conducted at Ladoke Akintola University Teaching Hospital, Nigeria [27]. Similarly, *A. baumannii* prevalence of 4.5% was also reported in a tertiary care hospital in West Bengal, India [28]. However, a higher % prevalence rate of 14% was reported in Nigeria at the University College Hospital (UCH) Ibadan [29]. Another 14% and 9.6% prevalence among hospital isolates was also reported in Tehran, Iran, and Pune, India, respectively [30, 31]. These reports and so many other reports of *A. baumannii* isolation in various healthcare institutions all over the world further established *A. baumannii* as a global threat and a troublesome Gram-negative pathogenic organism with serious health implications on patients, especially those with lower immunity and those admitted in the intensive care unit (ICU).

Infections due to *A. baumannii* may result from cross-contamination from environmental sources to health facilities, medical devices to patients, and/or adherence to infection control principles by patients, caregivers, or other health personnel. Also, congestion and improper bed spacing due to inadequate hospital wards and beds in tertiary care systems, especially in developing countries, may increase *A. baumannii* infection and resistance. The report of *A. baumannii* infection in this study and other reports in Nigeria indicates that this pathogen is gradually gaining more ground in Nigeria and should be considered a threat in various healthcare institutions in Nigeria and beyond.

3.2. Antimicrobial Resistance Testing (ART)

The result of antimicrobial resistance testing of all the isolated *A. baumannii* is shown in Table 2. The result revealed that most of the isolated *A. baumannii* were resistant to cefuroxime 22 (96%), tetracycline 22 (96%), sulfamethoxazole/trimethoprim 22 (96%), and ofloxacin 21(91%). Also, resistance frequencies of *A. baumannii* to ceftriaxone 20 (87%), ampicillin/sulbactam 20 (87%), aztreonam 19 (83%), ciprofloxacin 19 (83%), amoxicillin/clavulanic acid 18(78%), and cefepime 18 (78%) were also observed. Amikacin, cefotaxime, and ertapenem exhibited higher activity against the isolated *A. baumannii*. The results also showed that meropenem and imipenem were the most active antibiotics against the isolated *A. baumannii*, with 2(9%) and 5(22%) resistance, respectively (**Table 2**).

Table 2: Frequency of antimicrobial resistance patterns of *A. baumannii* (n = 23).

Antimicrobial agent (symbol and disk content µg/ml)	*A. baumannii* isolatesn (% Resistant)
Meropenem (MEM –10)	2 (9)
Ertapenem (ETP - 10)	15 (65)
Imipenem (IMP - 10)	5 (22)
Aztreonam (ATM - 30)	19 (83)
Ceftriaxone (CRO - 30)	20 (87)
Cefuroxime (CXM – 30)	22 (96)
Cefepime (FEB – 30)	18 (78)
Ceftazidime (CAZ – 30)	15 (65)
cefotaxime (CTX – 30)	14 (61)
Ciprofloxacin (CIP – 5)	19 (83)
Tetracycline (TE –30)	22 (96)
Colistin sulfate (CT – 25)	18 (78)
Ofloxacin (OFX –5)	21 (91)
Amikacin (AMK –30)	13 (57)
Sulfamethoxazole/trimethoprim (SXT –25)	22 (96)
Amoxicillin/Clavulanic acid (AMC –30)	18 (78)
Ampicillin/sulbactam (SAM – 20)	20 (87)

Antimicrobial resistance due to Gram-negative organisms isolated from healthcare facilities has increased worldwide, and *A. baumannii* is one of the most essential antibiotic-resistant pathogens encountered in medical practice in recent times. In this study, the isolated *A. baumannii* showed high resistance frequency to most of the commonly used antibiotics; cefuroxime 22 (96%), ceftriaxone 20 (87%), cefotaxime 14(61%), ceftazidime 15 (65%), cefepime 18 (78%), amikacin 13 (57%), ciprofloxacin 19 (83%), ofloxacin 21(91%), tetracycline 22(96%), aztreonam 19(83%), amoxicillin/clavulanic acid 18(78%), ampicillin/sulbactam 18(87%), colistin sulfate 18 (78%), ertapenem 15 (65%), and sulfamethoxazole/trimethoprim 22 (96%). In a previous study, high antimicrobial resistance frequencies to ciprofloxacin (100%), amikacin (100%), ceftriaxone (90.9%), ceftazidime (90.9%), imipenem (72.7%) and meropenem (63.6%) have been observed [27]. However, their reported resistance to imipenem (72.7%) and meropenem (63.6%) is in contrast to the high susceptibility frequency observed in meropenem 21 (91%) and imipenem 18 (78%) in this study. The high susceptibility of *A. baumannii* to meropenem and imipenem observed in this study may be due to the infrequent use of carbapenems in this hospital and, extension, South-Eastern Nigeria.

Furthermore, there are several reports of resistance of *A. baumannii* isolates to several antibiotics such as ceftazidime (93%), cefepime (89%), amikacin (61%), ciprofloxacin (86%), ofloxacin (81%), and ampicillin/sulbactam (79%) as reported by Dash *et al*. [32]. Also, in another study, *A. baumannii* isolates showed resistance to cefepime (73.1%), cefotaxime (93.3%), and amikacin (53%) [33]. These reports of antibiotic resistance are concordant with this study's report and further established *A. baumannii* as a worrisome pathogen confronting the health system and overall healthcare of patients.

Following the high antibiotic resistance frequency of *A. baumannii* observed in this study and several other reports of resistance in healthcare facilities in Nigeria, Africa, and across the globe, it is imperative to speculate that multidrug-resistant (MDR) *A. baumannii*is gradually increasing, gaining more attention globally and call for urgent attention as it is gradually spreading from one health facility to another. The high frequency of antibiotic resistance by *A. baumannii* in our study may not be unconnected with the indiscriminate use of these antibiotics in Nigeria and other African countries.

An independent samples t-test carried out to compare the percentage resistance frequencies of multidrug-resistant *A. baumannii* isolated from different clinical samples in AE-FUTHA to their susceptibility frequencies showed that there was a statistical difference in the resistance frequencies (mean=72.5, SD=24.8) and susceptibility frequencies (mean=27.8, SD=24.5); t (32)=5.289, and P=0.000 ($p<0.05$).

3.3 Phenotypic Screening/Detection of ESBLs

ESBL-producing bacteria, especially pathogenic Gram-negative organisms such as *A. baumannii*, are distributed worldwide, and their prevalence is increasing geometrically from region to region and from one healthcare to another. The result showed that out of the 23 *A. baumannii* isolates, 10 of the *A. baumannii* isolates showed complete resistance to five antibiotics (aztreonam 30µg, cefotaxime 30µg, ceftriaxone 30µg, ceftazidime 30µg and cefotetan 30µg) and were also confirmed as ESBL-producing *A. baumannii* using DDST methods (**Figure 1** and **2**).

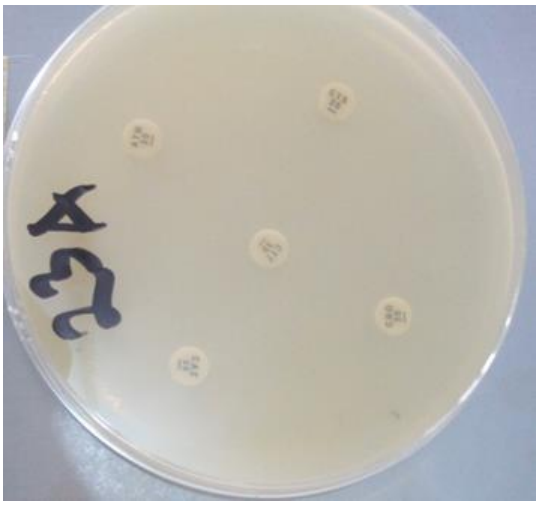

Figure 1. Plate of screening test of *A. baumannii* isolate resistant to aztreonam (30µg), cefotaxime (30µg), ceftriaxone (30µg), ceftazidime (30µg) and cefotetan (30µg).

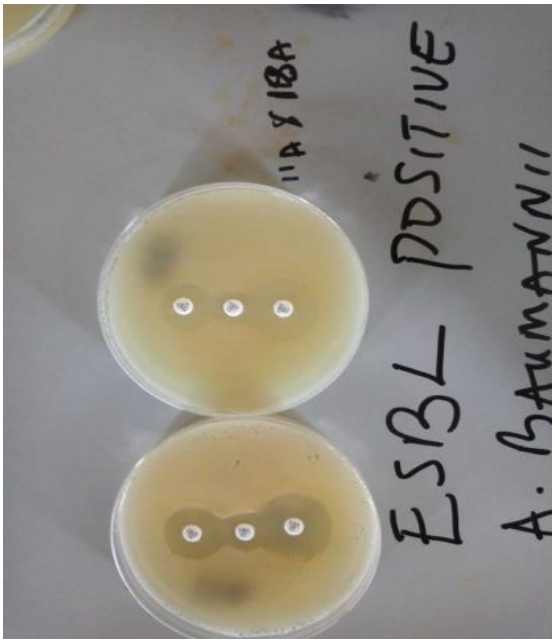

Figure 2. Plate of ESBL-positive *A. baumannii* isolates using DDST methods.

The percentage of ESBL-producing *A. baumannii* was 43.5%, as shown in **Table 3**. A previous study also reported a similar ESBLs prevalence of 23% *A. baumannii* [34]. However, the 23% ESBLs producing *A. baumannii* reported in the above study were lower than the 43.5% ESBLs producing *A. baumannii* as observed in this study. It is necessary to state that *A. baumannii* isolates can easily harbor resistance genes, as observed in this study and the results of other studies [34].

Table 3: Number and percentage of ESBL-Producing *A. baumannii*.

TNABI	TNABR5DC	T	P
23	10	10	23 (43.5)

TNABI: Total Number of *A. baumanni* Isolated, **TNABR5DC**: Total Number of *A. baumannii* Resistant to Five Antibiotic Discs of aztreonam (30µg), cefotaxime (30µg), ceftriaxone (30µg), ceftazidime (30µg) and cefotetan (30µg), n = Number Isolated/ Tested, **T**: Total No of ESBL-producing *A. baumannii* confirmed by DDST methods, **P**: Percentage (%) of ESBL-Producing *A. baumannii* in AE-FUTHA

3.4. Molecular Detection of ESBL Genes by PCR Methods

The agarose gel electrophoresis results of ESBL genes *bla*$_{TEM}$, *bla*$_{SHV,}$ and *bla*$_{OXA}$ are shown in **Figures 3-5,** respectively.

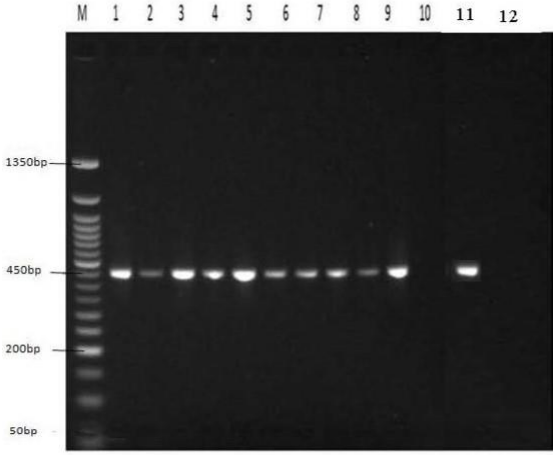

Figure 3. Agarose Gel Electrophoregresis of PCR Products (band size 450bp) of ESBL *bla*$_{TEM}$ Gene.

Key: Lane M=50 bp ladder; Lane 1-9=Positive for bla*$_{TEM}$*; Lane 10=Negative for *bla*$_{TEM}$ gene; Lane 11= Positive control for *bla*$_{TEM}$ and Lane 12= Negative control for *bla*$_{TEM}$.

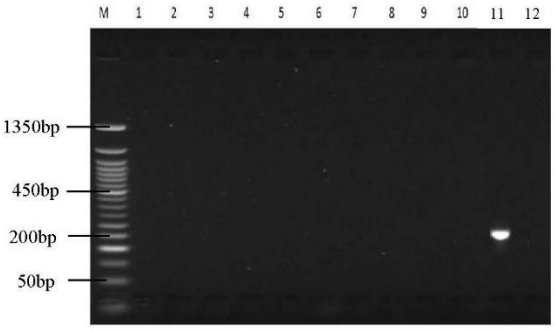

Figure 4. Agarose Gel Electrophoresis of PCR Products of ESBL *bla*$_{SHV}$ Gene.

Key: Lane M = 50 bp ladder, Lane 1-10 = Negative for *bla*SHV gene; Lane 11 = Positive control for *bla*SHV and Lane 12 = Negative control for *bla*SHV.

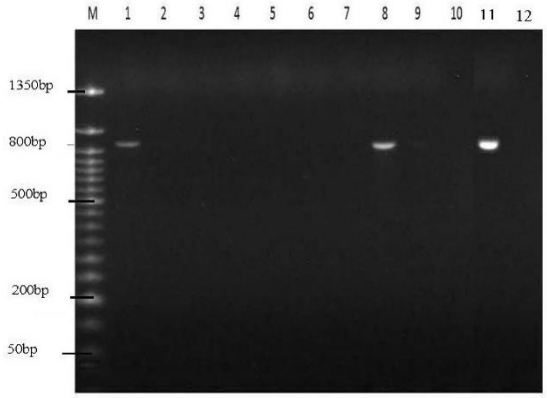

Figure 5. Agarose Gel Electrophoresis of PCR Products (band size = 800 bp) of ESBL *bla*$_{OXA}$ Gene.

Key: Lane M = 50 bp ladder; Lane 1, 8, and 9 = Positive for *bla*$_{OXA}$ gene; Lane 2 – 7 and 10 = Negative for *bla*$_{OXA}$ genes; Lane 11= Positive control for *bla*$_{OXA}$ and Lane 12 = Negative control for *bla*$_{OXA}$.

The ESBL phenotype of *A. baumannii* isolates correlates with the genotypic results, showing that 9(90%) and 3(30%) *A. baumanni* isolates harbored *bla*$_{TEM}$ and *bla*$_{OXA}$ genes, respectively. Results also revealed that *bla*$_{SHV}$ genes were not harbored by the *A. baumannii* isolates. A 1.5 kb molecular size marker was used to estimate the band size of the genes. The 90% and 0% prevalence of *bla*$_{TEM}$ and *bla*$_{SHV}$ genes observed in this study is similar to 71% and 0% for *bla*$_{TEM}$ and *bla*$_{SHV}$ genes, respectively, among *A. baumannii* isolates reported by Essam *et al.* [1] in another study. The presence of *bla*$_{TEM}$ and *bla*$_{OXA}$ genes in most strains of *A. baumannii* isolates in this study is a confirmation that the isolated *A. baumannii* harbored extended-spectrum β-lactamases (ESBLs) and the presence of more than one of these genes in an isolate further established the fact that the isolated *A. baumannii* is a multiple drug-resistant

(MDR- *A. baumannii*) strain. We therefore speculate that if more drug-resistant genes were screened, we would have found pan-drug-resistant *A. baumannii* isolates.

4. Conclusion

ESBLs Producing-*A. baumannii* is known to be very problematic in medical practice as they are responsible for increased antibiotic resistance in tertiary healthcare institutions. The isolated *A. baumannii* showed high resistance to most of the commonly used antibiotics. However, meropenem (9%) and imipenem (22%) were the most potent antibiotics for treating *A. baumannii* infections. This study's most predominant ESBL genes are bla_{TEM} genes and bla_{OXA} genes, with 9 (90%) and 3 (30%), respectively. None of the isolated *A. baumannii* were found to possess the bla_{SHV}gene. The presence of these resistance genes in this study highlights the importance of ESBL screening among clinical isolates in tertiary healthcare institutions and the adoption of strict antibiotic usage policy to reduce the impact of bacteria resistance. This research is the first report of ESBL-producing *A. baumannii* in Ebonyi State, Nigeria.

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Conflict of interest

The authors declare to have no conflict of interest.

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