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Antimicrobial Susceptibility Pattern and Molecular Identification of *Acinetobacter baumannii* in Alex Ekwueme-Federal University Teaching Hospital Abakaliki, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background and Objectives: *Acinetobacter baumannii,* a notorious opportunistic pathogen known to seriously affect debilitated individuals especially intensive care unit (ICU) patients and others with underlying illness, have consistently jeopardized many antibiotics. This study was therefore

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aimed to ascertain the antimicrobial susceptibility profile and molecularly identify *A. baumannii* pathogens in Alex Ekwueme-Federal University Teaching Hospital, Abakaliki, Nigeria.
Methodology: A total of 385 clinical samples were collected aseptically from debilitated patients and analyzed following standard microbiological procedures. *Acinetobacter* species was confirmed by Gram staining reaction and biochemical tests. All bacterial isolates were phenotypically screened for multidrug resistance using the Kirby–Bauer disc diffusion technique and results interpreted as per CLSI criteria. *A. baumannii* isolates were finally confirmed using 16S rRNA sequencing.
Results: A total of 23(6%) *A. baumannii* isolates were recovered from 385 clinical samples collected from 87 patients comprising 48 males and 39 females admitted in various hospital wards of AE-FETHA. The age of the patients varied from 20–79 years. The commonest sites for isolation of *A. baumannii* pathogen were catheter urine (8/8%) and wound sores (7/8%). The highest percentage resistance was observed with cefuroxime (96%), tetracycline (96%), sulfamethoxazole/trimethoprim (96 %), and ofloxacin (91%) while meropenem (91%) and imipenem (78%) were the most effective antibiotics against *A. baumannii*. The isolated *A. baumannii* was re-confirmed genotypically by 16S rRNA gene amplification. Variations were observed in the gene sequence of all the isolated *A. baumannii.*
Conclusion: Catheter urine, wound sores, and respiratory fluids were the more easily colonized samples. Also, high frequency of multidrug resistance observed in this study further established *A. baumannii* as a notorious opportunistic pathogen.

Keywords: Acinetobacter baumannii; antibiotic susceptibility profiles; multidrug resistance; 16S rRNA sequencing.

1. INTRODUCTION

Acinetobacter baumannii is a Gram-negative bacterium, strictly aerobic, non-fermenting, non-fastidious, non-motile, catalase positive, and oxidase negative bacteria with a DNA G + C content of 39 to 47 (%) [1,2,3]. *A. baumannii* is a notorious opportunistic pathogen known to seriously affect debilitated individuals especially intensive care unit (ICU) patients. Its capability to up- regulate and/ acquire resistance determinants increases its significance in medical practice, thus making them one of the most trouble organisms facing clinicians in every healthcare system. The importance of *A. baumannii* over the last 15 years has been increased as the current antibiotic era globally has been consistently jeopardized by these life-threatening pathogens [4,5]. Acting synergistically with this emerging trait of high antibiotic resistance profile is the inexplicable ability of *A. baumannii* to thrive for prolonged periods within and around hospital environment, thus potentiating its capacity for nosocomial spread [1,6]. The helpless, critically ill, and vulnerable hospitalized patients especially those with breaches in airways protections and breaches in the skin integrity are most often the targets of *A. baumannii* infections [7]. Infections as a result of *A. baumannii* are generally very severe with high morbidity and mortality index [2,8,9] especially amongst patients with underlying illness in medical and surgical wards.

Presently, *A. baumannii* is a significant agent of infections in the hospital, especially among ICU patients for which effective therapy is critically and constantly [10]. The commonest clinical presentations of *A. baumannii* include, but are not limited to, pneumonia, meningitis, septicaemia, and urinary tract infections (UTIs) [11]. Other important infections caused by *A. baumannii* pathogen are bacteraemia, wound infections, peritonitis, osteomyelitis, keratitis, and native-valve endocarditis [5]. The most common risk factors associated with *A. baumannii* infection includes environmental contamination, prolonged hospital stay, high exposure to antibiotics, admission to emergency units and other high risk areas, exposure to patients colonized with *A. baumannii*, poor adherence of patients and staff to hand hygiene, immunosuppression, respiratory failure or use of mechanical ventilators, lumbar puncture and use of other invasive procedures such as catheterization, endotracheal tube, and myelography [12,13].

Infections due to *A. baumannii* pathogen were known to be susceptible to many antibiotics over the years [14]. However, recent reports have described this pathogen as a multidrug-resistant opportunistic human pathogen that is frequently

responsible for many nosocomial outbreaks worldwide [13,15,16]. Strains of *A. baumannii* which are resistant to all major classes of antibiotics that where frequently used for the treatment of infection caused by them, including β-lactams, aminoglycosides, fluoroquinolones, chloramphenicol, tetracyclines, and rifampin. They have now emerged and their prevalence considerably differs from country to country, hospitals, and even departments [13].

There are several reports of nosocomial infections due to *A. baumannii* pathogen globally. However, there are very few reports on the prevalence, antimicrobial susceptibility profiles, and molecular identification of *A. baumannii* in Nigeria. This study was therefore aimed to ascertain the prevalence, determine the antimicrobial susceptibility profile, and molecularly identify *A. baumannii* pathogens in Alex Ekwueme-Federal University Teaching Hospital, Abakaliki, Nigeria.

2. MATERIALS AND METHODS

2.1 Collection of Clinical Samples

This study area was at Alex Ekwueme-Federal University Teaching Hospital, Abakaliki, Nigeria- a tertiary care teaching hospital that serves as a reference healthcare hospital to other General and Mission's hospitals in the area. During a study period of 6 months, a total of 385 clinical samples comprising of catheter urine, wound drain, wound sores, respiratory fluids, skin swab, and bed rails swab of patients were collected aseptically immediately transported to the research laboratory of Applied Microbiology Department, Ebonyi State University, Abakaliki, Nigeria, for analysis.

2.2 Inclusion and Exclusion Criteria of Sample Population

A random sampling of all critically ill patients who consented to participate in this study; aged 20 yrs and above, and have spent at least 10 days in medical wards, surgical wards and orthopaedic wards of the hospital was carried out. While those sampled in the ICU were patients who have spent at least 72 h in the ICU.

2.3 *A. baumannii* Identification from Clinical Samples

All the collected samples were cultivated onto MacConkey agar and 5% blood agar (Merck, Germany) while urine samples were first streaked on Cysteine Lactose Electrolyte Deficient (CLED), (Merck Co., Germany) prior to inoculation onto MacConkey agar and 5% blood agar. The inoculated samples on the MacConkey agar and 5% sheep blood agar were incubated for 24 h at 37°C under aerobic conditions. *Acinetobacter* species that grew on MacConkey agar appeared as non-lactose fermenters [17,18,19]. A colony of non-lactose fermenting bacteria (colourless or slightly beige) were further sub-cultured on MacConkey agar and incubated for additional 24 h at 37° C and 42° C. In order to confirm the growth of *Acinetobacter*, Gram staining was carried out and coccobacilli organisms were subjected to oxidase test, catalase test, coagulase test, indole test, citrate utilization test, urease test, methyl red test, and motility test. In the next step, using the above standard biochemical tests and growth at both 37°C and 42°C, the identification *Acinetobacter* species was concluded [20].

2.4 Determination of Antibiogram of the Isolated *A. baumannii*

The responses of all the isolates to meropenem (10 µg), ertapenem (10 µg), imipenem (10 µg), aztreonam (30 µg), ceftriaxone (30 µg), cefuroxime (30 µg), cefepime (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), tetracycline (10 µg), colistin sulphate (25 µg), ofloxacin (5 µg), amikacin (30 µg), sulfamethoxazole/ trimethoprim (25 µg), amoxicillin/clavulanic acid (30 µg), and ampicillin/sulbactam (20 µg) which were obtained from Oxoid Company, UK was determined using the Kirby-Bauer´s disc diffusion method on Mueller-Hinton (MH) agar incubated at 37°C for 24 h. Previously, the overnight cultures of the tested isolates were suspended in a freshly prepared nutrient broth and turbidity were adjusted to a 0.5 McFarland standard. The 0.5 McFarland standard equivalent of the test isolates was then streaked on the molten MH agar before antibiotic discs were carefully placed. The results were interpreted according to the Clinical Laboratory Standards Institute (CLSI) guidelines [21].

2.5 Genotypic Identification of *A. baumannii* by 16S rRNA Sequencing

2.5.1 DNA extraction and purification

The DNA extraction was carried out by adding 100 µg (wet weight) of bacterial cells that have

been re-suspended to 200 µl of isotonic buffer to a ZR Bashing™ lysis tube before about 750 µl lysis solution was added to the tube. The ZR Bashing Bead™ lysis tube was then centrifuged in a micro-centrifuge at 10,000 x g for 1 minute. All genomic DNA was however extracted and purified using ZR bacterial DNA Mini prep following manufactures' specifications.

2.5.2 PCR amplification and gel electrophoresis

PCR sequencing preparation cocktail consisted of 10 µl of 5x GoTaq colourless reaction, 3 µl of 25 mM $MgCl_2$, 1 µl of 10 mM of dNTPs mix, 1 µl 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). PCR was carried out in a Gene Amp 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 secs 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. PCR products (amplicon) were run 1.5% agarose gel. The buffer (1XTBE buffer) was prepared and subsequently used to prepare agarose gel. The suspension was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool to 60°C and stained with 3 µl of 0.5 g/ml ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). The gel was electrophoresed at 120 V for 45 minutes visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a hyper ladder one that ran alongside the experimental samples in the gel. All the above protocols were implemented following the methods previously described by [22,23,24].

2.5.3 Sequencing

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using manufacturers' manual while the sequencing kit used was that of Big Dye terminator V3.1 cycle sequencing kit. Bio- Edit software and MEGA 6 were used for all genetic analysis.

3. RESULTS AND DISCUSSION

3.1 Socio-demographic Data and Frequency of *A. baumannii* Isolation

A total of 87 patients comprising 48 males and 39 females admitted into the orthopaedic wards, surgical wards, medical wards, and ICU of the hospital were recruited in this study and a total of 385 clinical samples were obtained. Out of the 385 clinical samples, 23 *A. baumannii* isolates were recovered and identified phenotypically. The age of the patients recruited in this study ranged from 20-79 years while the majority of samples collected (130) were from patients between the age group of 30 - 39 years and the least samples (3) were collected from patients within the age group of 70 – 79 years. Patients within the age group of 50 - 59 years were mostly infected with *A. baumannii* pathogen (10), while the least infected patients (1) were between 20-29 and 60-69 (years) respectively. However, no (0) *A. baumannii* was isolated from patients between the ages of 70- 79 years (Table 1). Out of the 385 clinical samples analyzed in this study, 201 samples were from 48 male patients and the percentage of *A. baumannii* isolation was (14/6.9%) while 184 clinical samples were from 39 female patients and the percentage *A. baumannii* isolation was (9/4.8%). Patients who are married were more infected with *A. baumannii* pathogen (21/7.3%) when compared to 2/2.1% obtained from patients who are still single. The occupation of the patients sampled and percentage isolation of *A. baumannii* included; farmers (116/8.6%), civil servants (130/6.2%), students (34/0%), and others which included artisans and patients into various businesses (105/4.8%). The results further revealed that the prevalence of *A. baumannii* pathogen in AE-FUTHA was 23 (6%) while the prevalence of *A. baumannii* pathogen from each hospital wards/units were; orthopaedic wards (4/25%), surgical wards (4/34%), Medical wards (15/32%) and ICU (nil) respectively (Table 1).

The mean age for isolation of *A. baumannii* was ≥ 20 to 69 years and patients within 50-59 years of age were mostly infected with *A. baumannii* infection **(**14.5%). This result further established the fact that older age is a risk factor in *A. baumannii* infection. Odewale *et al*. [13] also reported that patients within the age bracket of 41-70 years are more susceptible to *A. baumannii* infection. Several studies have reported age as one of the major factors of *A.*

baumannii infection [25,26]. Patient's occupation was also an important contributing factor for increased infection by *A. baumannii* pathogen as farmers 116(8.6%) and civil servants 130(6.2%) were the most infected patients. The high risk of *A. baumannii* infection observed in this study amongst farmers and civil servants may be attributed to constant rotation and shifting of locations by farmers and civil servants due to the nature of their jobs and work which demands constant movement from one area to another, thus exposing them to accidental injuries, trauma, and other numerous pathogenic bacterial infections. Male patients 14 (6.9 %) are more susceptible to *A. baumannii* infection than their female counterparts 9 (4.8%). The high isolation of *A. baumannii* among male patients to female patients in this study, may not be unconnected with the number of samples collected from both patients; male (201 clinical samples) against females (184 clinical samples). However, we speculate that males are generally more predisposed to infections due to the nature of their jobs than their female counterparts. In another study, a higher percentage of 182 (76.2%) and 57 (23.8%) isolation of *A. baumannii* among male and female patients respectively was reported by Al Samawi *et al*. [27] in Hamad General Hospital, Qatar. This report is also suggestive that males are more predisposed to pathogenic organisms compared to female counterparts.

The highest isolation of *A. baumannii* was observed among patients admitted in medical ward 15(32%) and surgical ward 4(34%) respectively. However, a higher prevalence of 18.4 % was reported in another study by Natalia *et al*. [28] among patients admitted in surgical ICU at the University of Maryland Medical Center, USA. In this study, no *A. baumannii* pathogen was isolated in the ICU which is in contrast with the report of other studies which reported high isolation frequency of *A. baumannii* pathogen amongst ICU patients [2,13]. The zero (0) isolation frequency of *A. baumannii* pathogen amongst ICU patients in this study may be attributed to the low number of patients sampled (1 patient) and number of clinical samples (3) collected which corresponds to number of patients admitted in the ICU during the time of the sample collection. We therefore speculates that, if more patients were sampled in the ICU and probably more samples collected, there might be a tendency of isolating higher frequency of *A. baumannii* pathogens in our study area.

3.2 Prevalence of *A. baumannii* Pathogen

Out of the 385 clinical samples analyzed, the highest number of samples collected was from catheter urine (100); followed by wound sores (83), wound drains (68), respiratory fluids (57), bed rails (32), and skin swabs (45). Results showed that the most common sites of isolation of *A. baumannii* pathogen was catheter urine (8/8%) and wound sores (7/8%). Other common sites of *A. baumannii* infection include respiratory fluids (5/9%) and wound drain (3/4%). The result further revealed that no (nil) *A. baumannii* isolate was recovered from bed rails and skin swabs of the patients respectively (Table 2).

The most common sites of *A. baumannii* pathogen isolation are catheter urine (8/8%) and wound sores (7/8%) respectively. Patients' urine samples (39.6%) and wound exudates/pus (29.5%) have also been reported as the commonest sites of *A. baumannii* pathogen in the result of other studies by Chakraborty *et al*. [29] in West Bengal and Lone *et al*. [30] in Srinagar, India respectively.

3.3 Antimicrobial Susceptibility Profiles of Isolated *A. baumannii*

The result of antibiograms showed that the isolated *A. baumannii* were multidrug-resistant (MDR) strains as they exhibited resistance to at least 3 classes of antibiotics tested. The highest percentage of resistance of the isolated *A. baumannii* was observed with cefuroxime (96%), tetracycline (96%), sulfamethoxazole/ trimethoprim (96%), and ofloxacin (91%). Other notable high resistance to commonly used antibiotics was also observed with ceftriaxone (87%), ampicillin/sulbactam (87%), aztreonam (83%), ciprofloxacin (83%), cefepime (78%), colistin sulphate (78%), amoxacillin/clavulanic acid (78%) ertapenem (65%), ceftazidime (65%), cefotaxime (61%), and amikacin (57%). However, the most effective antibiotics against *A. baumannii* were meropenem (91%) and imipenem (78%) respectively.

Antibiotic resistance is a global threat in the treatment of infectious diseases and several local and international studies have reported the ability of *Acinetobacter* spp. to develop resistance to commonly used antibiotics [23,31]. In this study, most of the patients who were admitted in the hospital in AE-FUTHA had previously at the course of their treatment, received different

antibiotics including combination of β-lactam antibiotics such as second-, third- and fourth-generation cephalosporins (cefuroxime, ceftazidime, ceftriaxone, cefoxitin, and cefepime) along with aminoglycosides (amikacin) or fluoroquinolones (ciprofloxacin and ofloxacin) and tetracyclines (tetracycline) either in tablets, injection or infusion.

The isolated *A. baumannii* in this study showed high resistance to most of the antibiotics tested such as cefuroxime (96%), ceftriaxone (87%), cefoxitin (61%) ceftazidime (65%), cefepime (78%), amikacin (57%), ciprofloxacin (83%), ofloxacin (91%), tetracycline (96%), aztreonam (83%), amoxicillin/clavulanic acid (78%), ampicillin/sulbactam (87%), colistinsulfate (78%), ertapenem (65%), and sulfamethoxazole/ trimethoprim (96%). The high resistance pattern observed in this study is similar to those reported in different studies (25,32]. However, meropenem (91%) and imipenem (78%) respectively are the most potent antibiotics for the treatment of *A. baumannii* infections. This result is in agreement with the results of another study where meropenem (31%) was the least resistant antibiotics [32,33], although the *A. baumannii* screened showed high resistance to imipenem (76%) in their study as against the result of our study. We however speculate that the high susceptibility of *A. baumannii* to meropenem and imipenem as recorded in this study may be due to the fact that these antibiotics are not commonly used or abused in AE-FUTHA, while other antibiotics that are constantly used for treatment of infectious diseases in AE-FUTHA, developed various degrees of resistance.

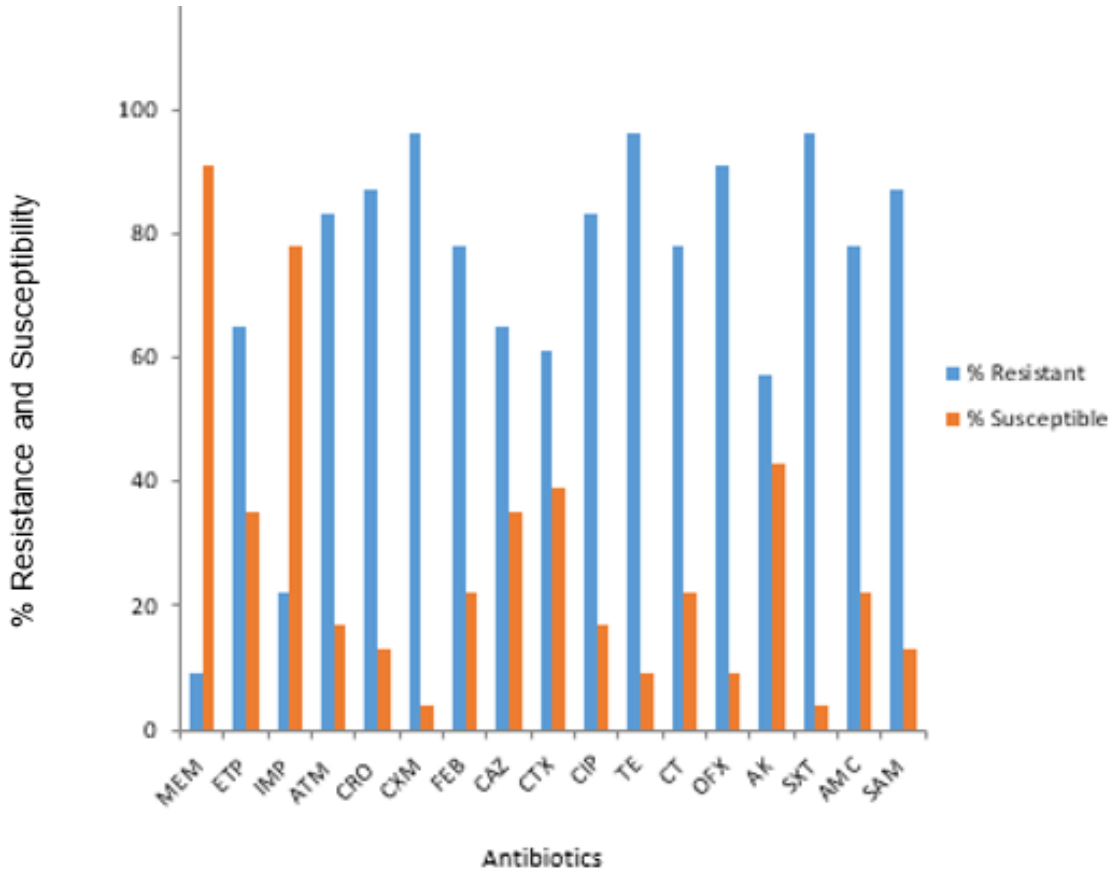

Fig. 1. Percentage Susceptibility Profile of Isolated *A. baumannii* in AE-FUTHA

Key: MEM = Meropenem (10 µg), ETP = Ertapenem (10 µg), IMP = Imipenem (10 µg), ATM = Aztreonam (30 µg), CRO= Ceftriaxone (30), CXM = Cefuroxime (30 µg), FEB = Cefepime (30 µg), CAZ = Ceftazidime (30 µg), CTX= cefotaxime (30 µg), CIP = Ciprofloxacin (5 µg), TET=Tetracycline (10 µg), CT= Colistin sulphate (25 µg), OFX= Ofloxacin (5 µg), AMK = Amikacin (30 µg), SXT =Sulfamethoxazole/trimethoprim (25 µg), AMC = Amoxacillin/Clavulanic acid (30 µg),SAM = Ampicillin/sulbactam (20 µg)

Table 1. Socio-Demographic Data of Patients and Frequency of *A. baumannii* Isolated from Various Hospital Wards in AE- FUTHA

	TNSA				F(PIAB)				
Age	**MFOW**	**MFSW**	**MFMW**	**ICU**	**MFOW (%)**	**MFSW (%)**	**MFMW (%)**	**ICU (%)**	**TNSC (PIAB)**
≥ 20 – 29	18	12	26	0	0 (0)	0 (0)	01 (4)	0 (0)	56(1.8%)
30 – 39	49	32	49	0	1(2)	0 (0)	2 (4)	0 (0)	130(2.3%)
40 – 49	17	24	63	0	2 (12)	1(4)	5 (8)	0 (0)	104(7.7%)
50 – 59	09	16	44	0	1 (11)	2 (13)	7(16)	0 (0)	69(14.5%)
60 – 69	03	06	11	3	0 (0)	1(17)	0 (0)	0 (0)	23(4.5%)
70 – 79	0	0	03	0	0(0)	0 (0)	0 (0)	0 (0)	3(0%)
Total	96	90	196	3	04(25%)	4(34%)	15(32%)	0(0%)	385(6%)
Sex									
Male (48)	52	41	105	3	3 (6)	2 (5)	9 (9)	0 (0)	201(6.9%)
Female (39)	44	49	91	0	1 (2)	2 (4)	6 (7)	0 (0)	184(4.8%)
Marital status									
Married	77	59	149	3	04 (5)	3 (5)	14 (9)	0 (0)	288(7.3%)
Single	19	31	47	0	0 (0)	1 (3)	1 (2)	0 (0)	97(2.1%)
Occupation									
Farmers	28	25	60	3	2 (7)	1 (4)	7 (12)	0 (0)	116(8.6%)
Civil servants	23	32	75	0	1 (4)	2 (6)	5 (7)	0 (0)	130(6.2%)
Students	08	13	13	0	0 (0)	0 (0)	0 (0)	0 (0)	34(0%)
Others	37	20	48	0	1 (3)	01 (5)	3 (6)	0 (0)	105(4.8%)

Keys: TNSA- Total Number of Samples Analyzed; MFOW- Male and Female Orthopaedic Wards; FPIAB – Frequency and Percentage Isolation of A. baumannii; MFSW- Male and Female Surgical Wards; ICU- Intensive Care Unit (Adult); MFMW- Male and Female Medical Wards; TNSC (PIAB)- Total Number of Samples Collected (percentage isolation of A. baumannii)

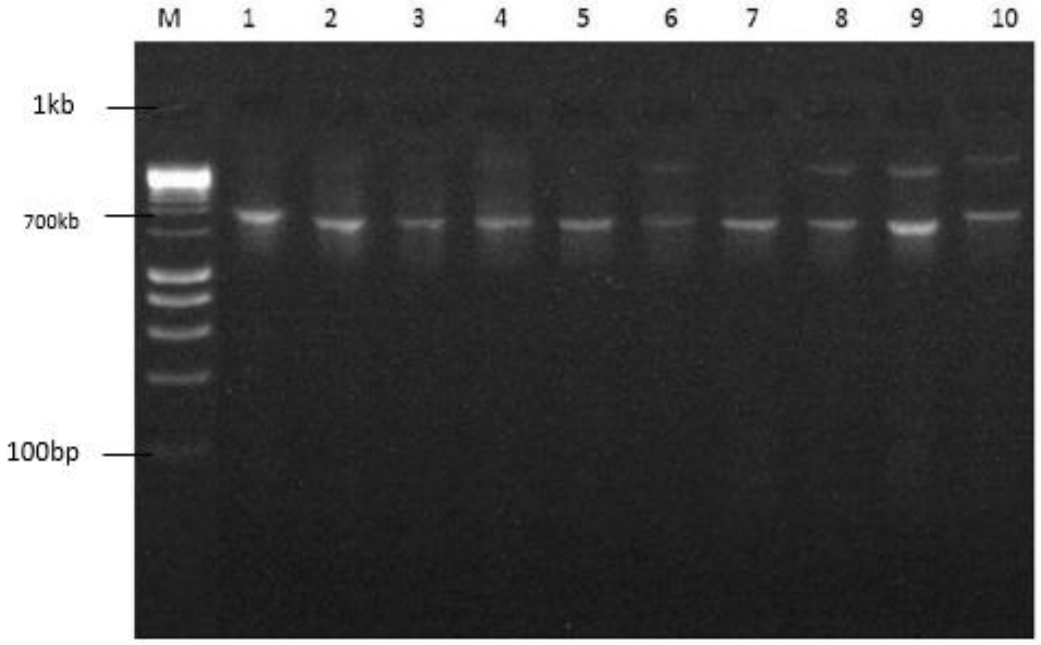

Plate 1. Agarose Gel Electrophoregram of 16S rRNA Gene Amplification of *A. baumannii* Isolates (band size = 700 kb)

Key: Lane M= molecular size markers (100 bp); Lanes 1-10= Number of isolates

Table 2. Frequency and Percentage Isolation of *A. baumannii* pathogen from Clinical Samples

Samples Source	TNSA	TNABI (%)
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%)

Keys: TNSA= Total Number of Samples Analyzed; TNABI= Total Number of A. baumannii Isolated; PABI= Percentage of A. baumannii Isolated; TNNABS= Total Number of Non A. baumannii Samples; PNABS= Percentage of Non A. baumannii Samples

Table 3. The Percentage pairwise Identity According to the Gene Sequences of the Isolated *A. baumannii*

Isolates code	Organism Description	% Pair wise identity	NCBI Accession Number	E-value
1	*A. baumannii* strain IRZHN7	83.1 %	MG554737	0
2	No similarity with *Acinetobacter* found			
3	*A. baumannii* strain IRZHN7	89.41 %	MG554737.1	0
4	*A. baumannii* strain FMHLN5	90.66%	MH542624	0
5	*A. baumannii* strain MDPSBR172c	84.82 %	JF513192	0
6	*A. baumannii* strain JM58	85.67%	KC461191	0
7	*A. baumannii* strain VB35435	83.04 %	CP040056	0
8	*A. baumannii* strain IRZHN7	81.45 %	MG554737.17	0
9	*A. baumannii* strain IRZHN7	86.08 %	MG554737.06	0
10	*A. baumannii* strain L9	83.79 %	KU922258	0

3.4 Genotypic Identification by 16S rRNA Sequencing

Result revealed that the 10 isolates tested were *A. baumannii* as all the isolates showed clear bands at 700 bp in the gel electrophoregram of the 16S rRNA gene amplification (plate 1). Out of the 10 *A. baumannii* isolates confirmed using 16S rRNA gene amplification, 9 isolates were further re-confirmed and identified as *A. baumannii* by sequencing as compared with the Gene Bank and their respective accession numbers as obtained were deposited in the NCBI database. The percentage pair-wise identity numbers of the 9 *A. baumannii* isolates ranged from 81.45; 83.04; 83.1; 83.79; 84.82; 85.67; 86.08; 89.41 and 90.66 (%). The result of the sequencing further revealed the strains of the isolated *A. baumannii* isolates. The isolated *A. baumannii* with code numbers 1, 3, 8 and 9 were of the same strain 'IRZHN7' whereas other 5 *A. baumannii* isolates with code numbers 4, 5, 6, 7 and 10 were *A. baumannii* strains EMHLN5, MDPSBRI72c, JM58, VB35435, and L9 respectively. There was also a clear difference/variation in the gene sequence of all the isolated *A. baumannii*. (Table 3).

Out of the 10(43%) suspected *A. baumannii* isolates previously identified by phenotypic methods, 16S rRNA gene amplification confirmed the 10 (100%) to be *A. baumannii*, as all the 10 isolates showed clear bands with molecular size of 700 bp in the agarose gel electrophoregram. This result further confirmed and authenticated the result of phenotypic tests for identification of *A. baumannii* isolates. This result is in agreement with the report of Essam *et*

al. [34] in which they used 16S rRNA gene amplification to confirm 100 out of 107 *A. baumannii* isolates among ICU patients in their study. Also, this study is in line with the result of Tuwaij [23] who used 16S-23S rRNA gene amplification to identify *A. baumannii* which yielded 100 % accuracy. Several reports have shown that genome sequencing and 16S rRNA gene amplification are very crucial in bacterial investigation and identification [24,35,36]. To authenticate the result of 16S rRNA gene amplification and identify genetic variations existing between the strains, the 10 isolates of *A. baumannii* were further sequenced and the result of the sequencing confirmed 9 (90%) out of the 10 (100%) isolates to be *A. baumannii.* While 1 (10%) of the isolates was negative as compared in the Gene Bank. The genomes of the isolates were compared with those in the Gene Bank and NCBI accession numbers were assigned to them. However, variation exists among the genomes of the 9 isolates of *A. baumannii* sequenced, indicating that the isolated *A. baumannii* were not of the same spp.

4. CONCLUSION

Infections due to *A. baumannii* is frequently increasing in the hospitals due to its ability to persist and colonize hospital environment for a prolonged period of time. In this study, we observed that catheter urine, wound sores, and respiratory fluids were the most easily colonized samples and older patients were more susceptible to *A. baumannii* infection than patients who are young in age. Also, due to high frequency of multidrug resistance exhibited by *A. baumannii* in our study area, imipenem, meropenem, and amikacin have shown to be the most potent antibiotics for the treatment of *A. baumannii* infections. To identify and detect the molecular diversity of *A. baumannii* at all times and in a particular area, 16S rRNA gene amplification and gene sequencing methods has proved to be more reliable.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

All authors declare that written informed consent was obtained from the patient or care-giver of the patient before collection of sample.

ETHICAL APPROVAL

This study was approved by the Research and Ethics Committee (REC) of Federal University Teaching Hospital Abakaliki (FETHA) now known as Alex Ekwueme-Federal University Teaching Hospital, Abakaliki (AEFUTHA) with REC approval number: FETHA/REC/VOL1/2017/581.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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