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MOLECULAR CHARACTERIZATION OF CARBAPENEM RESISTANT *Acinetobacter baumannii* ISOLATED FROM CLINICAL SAMPLES OBTAINED FROM IN-PATIENTS OF TWO SELECTED HOSPITALS IN MINNA, NIGERIA

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ABSTRACT
Carbapenem resistant *Acinetobacter baumannii* (CRAB) are fast rising threat to the global medical health care system. This study determined the presence of carbapenem resistant *A. baumannii* among in-patients of two selected hospitals in Minna, Niger State. One-hundred and ten samples comprising of urine, sputum and wound swab were collected from in-patients of each hospital. The samples were inoculated on MacConkey agar for the isolation of Gram negative bacteria. *Acinectobacter baumanii* isolates were identified using biochemical tests and polymerase chain reaction (PCR), the resistance profile of the isolates were determined using the Kirby Bauer's disc diffusion method and interpreted according to the Clinical and Laboratory Standards Institute guidelines. Carbapenemase production was detected using simplified carbapenem inactivation method while the presence of betalactamase encoding genes (blaOXA-23 and blaOXA-51) were detected using PCR. The results indicated that 7(5.65%) *A. baumanii* were isolated from all the samples. The isolates were identified as strain Ab7 (42.86%), NCTC_7364 (28.57%), Ab21 (14.29%) and NCTC_7412 (14.29%). About 71.5% and 85.7% of the isolates showed resistance against meropenem and imipenem respectively. Total (100%) resistance to Trimethoprim-sulphamethaxole, amoxicillin clavulanic acid, fosfomycin and tetracycline was also observed. Whereas, they were highly susceptible to ciprofloxacin (85.7%), gentamycin (85.7%), colistin (71.4%) and ceftriaxone (71.4%). The result also indicated that 100% and 42.86% of the isolates were positive for blaOXA-51 and blaOXA-23 genes respectively. High prevalence of CRAB among patients was recorded. This is of concern, routine surveillance and comprehensive infection control measures are needed to minimize the spread of this pathogen.

Keywords: *Acinetobacter baumannii, betalactamase,* Carbapenem, Carbapenemase, Resistance

INTRODUCTION

Acinetobacter baumannii is undoubtedly one of the most successful pathogens responsible for hospital acquired nosocomial infections in the modern healthcare system. Due to the prevalence of infections and out breaks caused by carbapenem resistant *A. baumannii* which are also usually multidrug- resistant, very few antibiotics are effective for treating infections caused by this pathogen (Lee *et al.,* 2017).

The hospital environment is one in which continuous close contact and interactions between in-patients, health workers and the environment is almost unavoidable and as such, poses a major challenge directly responsible for the rising rates of infections from carbapenem resistant *Acinetobacter baumannii* worldwide. Studies by Zuhair *et al.,* (2014) indicated that most carbapenem resistant isolates were obtained from patients with urinary tract infection (9.5%), then wound (6.2%) and lower respiratory tract (5.1%) infections.

Nigeria as a developing country is seriously challenged in the area of accurate data collection and records keeping which is an essential step in the fight against diseases, especially the management of highly resistant strains with limited therapeutic options.

Without proper management, the spread of carbapenem resistant *A. baumannii* is not only limited to hospital environments but can be transmitted through community infections which is usually expressed as community-acquired pneumonia which is fatal.

Acinetobacter baumannii in addition to building resistance against the carbepenem class of antibiotics is an important opportunistic pathogen capable of adhering to both abiotic and biotic surfaces, forming biofilms and acquiring genetic material from unrelated genera thus leading to serious nosocomial infections that are extremely hard to treat with current medications. Therefore, *the proper documentation of areas confirmed to be reservoirs of carbapenem resistant Acinetobacter baumannii* is the first step in managing the spread of this important pathogen. Although currently, treatment for carbepenem resistant *Acinetobacter baumannii* is almost impossible, knowledge from this study can contribute in the proper management of the spread of this organism.

MATERIALS AND METHODS

Study Area

The study was carried out in Minna, Niger state. Minna is the capital of Niger State which is the twelfth largest state in Nigeria in terms of population and the largest by land mass.

Study Population

The study was conducted among in-patients of General Hospital Minna and IBB Specialist Hospital, both within Minna, Niger state, Nigeria.

Ethical Approval

Ethical approval was obtained from the Research, Ethics and Publication Committee of both hospitals (General Hospital, Minna and IBB Specialist Hospital, Minna) while individual patient consent was obtained before inclusion in this study.

Inclusion and Exclusion Criteria

Only patients who had been admitted for 14 days or more were included in this study while out-patients and other in-patients who had not stayed for more than 14 days were excluded from this study.

Consent Form

An informed consent form was given to the patients involved in this study to allow their samples collected to be used for the purpose of this research.

Questionnaire

A brief questionnaire for descriptive data was issued to the patients involved in this study.

Collection of Samples

Urine, sputum and wound swabs were collected according to methods described by (Karah *et al.,* 2020) and (Cooper, 2010). Samples collected were then transported to the laboratory for analysis.

Sample inoculation and incubation

(i) MacConkey Agar

MacConkey agar was prepared according to manufacturer's instructions for the selective isolation, cultivation, and differentiation of gram negative bacteria based on their ability to ferment lactose. Lactose-fermenting organisms appeared as red to pink colonies while *Acinetobacter* which is a non-lactose fermenting organism appeared as colorless or transparent colonies.

Identification of Isolates

Gram staining, oxidase, motility, methyl red, urease, indole and catalase tests were used to further identify the isolates as described by Cheesebrough (2010).

Sub-Culturing

The suspected isolates obtained from the above techniques were sub-cultured on Leeds *Acinetobacter* selective medium using a sterile wire loop and incubated at 37^0C for 24 hours according to the manufacturer's instructions. Distinct colonies which were pink, circular, smooth and mucoid were isolated and finally subjected to molecular analysis for final confirmation as *A. baumannii*.

Molecular Identification

To perform PCR, the blaOXA-51-like gene which is intrinsic to *A. baumannii* with 5' AATGATCTTGCTCGTGCTTC 3′ (forward), 5′ CATGTCCTTTTCCCATTCTG 3′ (reverse) primers was used (Saber and Alireza, 2021). PCR was performed according to methods described by Xiaopeng *et al.,* 2018.

25 µL of PCR Master Mix (CWBio, Beijing, China) was mixed with 4 µL of forward and reverse primers and water to a final volume of 45 µL. Then, 5 µL of sample lysate was added to the mix after which the PCR program consisting of an initial denaturation step at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 57°C for 45 s, elongation at 72°C for 1 min, and a final extension at 72°C for 5 min. The resulting PCR products was examined by electrophoresis and visualization by gel documentation system on 1.5% agarose gel containing ethidium bromide. PCR products were selected for sequencing and sequences were aligned using the BLAST software tool from the GENE bank to determine identities and accession numbers of isolates.

Antibiotic susceptibility testing

0.5 Macfarland standard of the test isolates were prepared and spread onto Mueller-Hinton agar using a sterile cotton swab. Single antimicrobial disc of Imipenem (10 µg), Meropenem (10 µg), Colistin sulphate (10 µg), Trimethoprim-sulfamethoxazole (25 µg), Amoxicillin-clavulanic acid (30 µg), Fosfomycin (50 µg), Gentamicin (10 µg) Ciprofloxacin (10 µg), Ceftriaxone (30 µg) and Tetracycline (10 µg) were applied aseptically on the surface of the inoculated plates. The plates were allowed to sit for a while at room temperature and then incubated at 37°C for about 18-24 hours. The diameter of the zone of inhibition around the antibiotic discs was measured and interpreted in accordance with the guidelines of the Clinical Laboratory Standards Institute (CLSI, 2017).

Phenotypic Test for Carbapenemase Production

Detection of carbapenemase enzyme production was carried out using the simplified Carbapenem Inactivation Method as described by Xiaopeng *et al.,* (2018).

0.5 McFarland standard suspension of *A. baumannii* was diluted 1:10 in saline (using the direct colony suspension method) and inoculated onto Mueller Hinton Agar (MHA) plate, following the routine disk diffusion procedure. Plates were allowed to dry for 3–10 min. Then, an overnight colony of the test organism grown on blood agar was

smeared onto a Meropenem disk (10 μg; Oxoid, Hampshire, United Kingdom) to allow one side of the disk be evenly coated with the test bacteria; immediately afterward, the side of the disk having bacteria was placed on the MHA plate previously inoculated with *A. baumannii*. All plates were incubated at 35°C for 16–18 h in ambient air.

Detection and Molecular Characterization of Genes Responsible for Carbapenem Resistance

The PCR procedure used for the detection and molecular characterization of genes responsible for carbapenem resistance were the same as those of molecular identification of *A. baumannii* isolates. Primers used for the identification of blaOXA-23-like gene, forward (5'-GATCGGATTGGAGAACCAGA-3') and reverse (5'-ATTTCTGACCGCATTTCCAT-3') primers designed to detect the blaOXA−23−like gene were used (Li *et al.,* 2012). PCR was performed according to methods described by Xiaopeng *et al.,* 2018.
25 μL of PCR Master Mix (CWBio, Beijing, China) was mixed with 4 μL of forward and reverse primers and water to a final volume of 45 μL. Then, 5 μL of sample lysate was added to the mix after which the PCR program consisting of an initial denaturation step at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 25 s, annealing at 52°C for 40 s, elongation at 72°C for 50s, and a final extension at 72°C for 6 min. The resulting PCR products were examined by electrophoresis and visualization by gel documentation system on 1.5% agarose gel containing ethidium bromide.

Data Analysis

Data analysis was carried out using Microsoft excel and quantitative data were represented as percentages in this study.

RESULTS

Distribution and prevalence of Bacterial Isolates in Clinical Specimens

Table 4.1 below shows the distribution of isolates obtained from both hospitals. *E. coli* had the highest prevalence with 47 (37.9%) followed by *Pseudomonas sp.* 34 (27.4%), *Klebsiella sp.* 21 (16.9%), *Enterobacter sp.* 15 (12.1%) while *A. baumannii* had the least distribution 7 (5.7%). Highest prevalence of *A. baumannii* was observed in wound swabs 4 (57.1%), followed by sputum 2 (28.6%) while urine had the least 1 (14.3%).

Table 1: Distribution of Bacterial Isolates in Different Clinical Specimens (n=124)

Specimen	*Klebsiella sp.*	*Pseudomonas sp.*	*E. coli*	*Acinetobacter baumannii*	*Enterobacter sp*	Total
Urine	7 (5.65 %)	8 (6.45%)	31(25.00 %)	1(0.81 %)	8(6.45 %)	55(44.36%)
Wound swab	9(7.26 %)	21(16.94%)	10(8.06 %)	4(3.23 %)	5(4.03 %)	49(39.52%)
Sputum	5(4.03 %)	5(4.03 %)	6(4.84 %)	2(1.61 %)	2(1.61 %)	20(16.12%)

Total	21(16.94%)	34(27.42%)	47(37.90 %)	7(5.65 %)	15(12.09 %)	124(100%)

Molecular identification of A. baumannii isolates

Identification of *A. baumannii* isolates was carried using the 16S rRNA sequence analysis. Plate 1 shows the agarose results of 16S rRNA PCR amplified products from extracted DNA of *A. baumannii* isolates. The blaOXA-51-like gene primer has a band size of 353 base pairs. This gene is intrinsic to the *A. baumannii* species and is vital for its molecular identification. All 7 suspected isolates of *A. baumannii* were confirmed as shown in the gel image with horizontal lines across all lanes. The electrophoresis was performed for 90 minutes at 70 volts. Lane (L), DNA molecular size marker (l000 bp ladder), Lane (U17b-S9b) show positive results with positive bands of 353 bp. Lane (N) is the negative control with no DNA template.

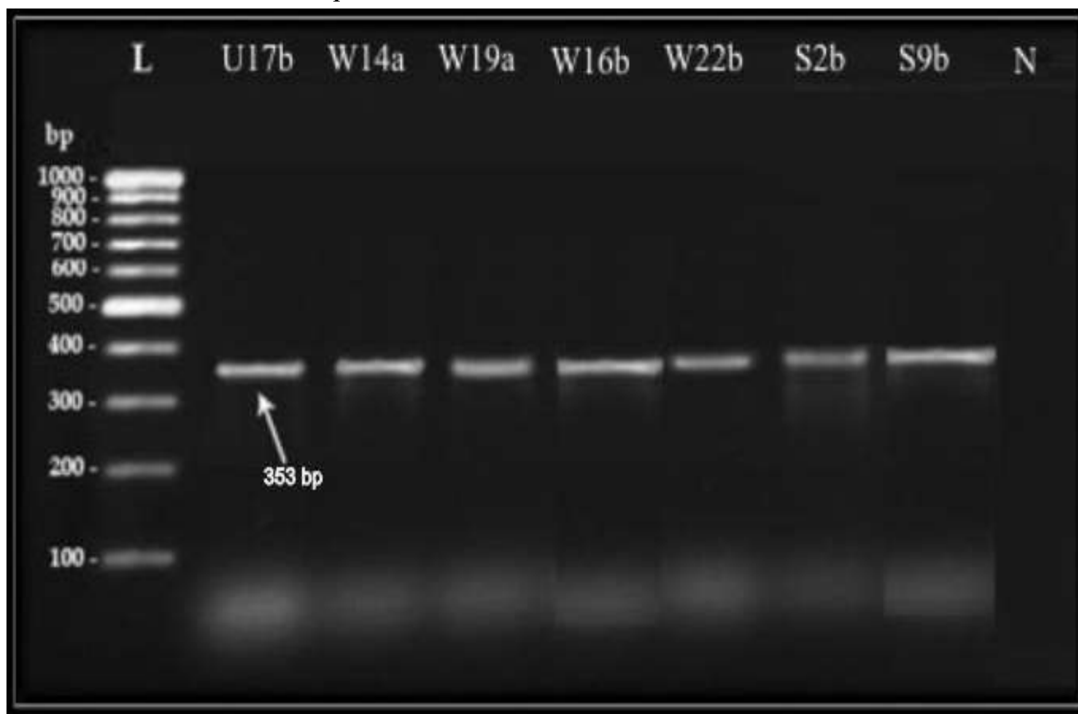

Plate 1: Agarose gel electrophoresis of blaOXA-51-like gene

The dendogram below (figure 1) shows the relative similarities between strains of *A. baumannii* isolated in this research. The seven isolates were identified as four different strains with isolates W14a, W19a and W22b showing varying degrees of 99% similarity to *Acinetobacter baumannii* strainAb7 while isolate W16b showed a 99.2% similarity index to *Acinetobacter baumannii* strainAb421. Isolates S9b and U17b showed varying degrees of

99% similarity to *Acinetobacter baumannii* strain NCTC_7364 while isolate S2b showed a 99.9% similarity index to *Acinetobacter baumannii* strain NCTC_7412.

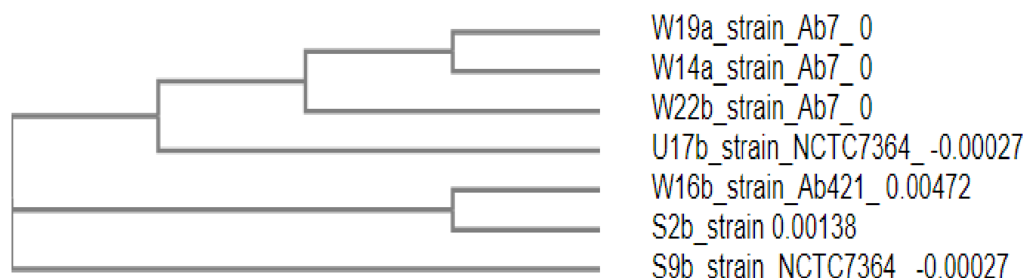

Figure 1: Dendogram of molecular similarity between *A. baumannii* isolates obtained from this study.

Distribution and prevalence of *A. baumannii* isolates among age groups, gender and hospital

The distribution of *A. baumannii* isolates according to age, gender and hospital is showed in table 4.2. IBB specialist Hospital had the highest distribution of *A. baumannii* with 5 isolates representing 71.4%. Of these 5 isolates, 3(60%) were in the age bracket of 36-50 years while the remaining 2 (40%) isolates were in the 51-75 years age group. 3 isolates were obtained from males patients while 2 were from female patients. In General Hospital only 2 isolates representing 28.6% were obtained, both isolates were from male patients and within the 51 -75 years age group. From this study, the highest prevalence of *A. baumannii* isolates by gender with a total prevalence of 71.4% was from the male patients while the 51-75 age bracket had the highest prevalence of *A. baumannii* with 57.1%.

Table 2: Age and gender specific distribution of *A. baumannii* among patients of Hospitals involved in this research (n=7)

	General Hospital		IBB Specialist Hospital		Total
Age	Male 2 (28.57%)	Female 0 (0.00%)	Male 3 (42.86%)	Female 2 (28.57)	7 (100%)
0-17	0 (0.00%)	0(0.00%)	0 (0.00%)	0(0.00 %)	0(0.00%)
18-35	0(0.00%)	0(0.00%)	0(0.00%)	0 (0.00%)	0(0.00%)
36-50	0(0.00%)	0(0.00%)	**2(28.57%)**	**1**(14.29%)	**3(42.86%)**

51-75	**2** (28.57%)	0(0.00%)	**1**(14.29%)	**1**(14.29%)	**4(57.14%)**
>75	0(0.00%)	0(0.00%)	0(0.00%)	0(0.00%)	0(0.0%)
Total	2(28.57%)	0(0.00%)	3(42.86%)	2(28.57%)	7(100%)

Antibiotic susceptibility profile of *A. baumannii* isolates

Using CSLI as a standard reference, the distribution of resistance of *A. baumannii* isolates to selected antibiotics was established.

A. baumannii isolates showed high level of resistance to Trimetoprim/sulfamethoxazole 7 (100 %), Amoxicillin/Clavulanic Acid (Augmentin) 6(85.7 %) and Tetracycline 4 (57.1 %). The isolates were highly susceptible 6 (**85.7** %) to Ciprofloxacin and Gentamycin followed by Ceftriaxone and Colistin 5 (71.4 %). *A. baumannii* isolates mostly showed intermediate susceptibility to carbapenems, Meropenem 3(42.9 %) and Imipenem 6 (85.7%). The Antibiotic susceptibility profile of the *A. baumannii* isolates is presented in Table 3.

Table 3: Antimicrobial susceptibility profile of *A. baumannii* isolates

Antibiotics	Susceptible (%)	Intermediate (%)	Resistant (%)
Ciprofloxacin	**6 (85.7)**	1 (14.3)	-
Ceftriaxone	**5 (71.4)**	2 (28.6)	-
Gentamycin	**6(85.7)**	1 (14.3)	-
Tetracycline	-	3 (42.9)	**4 (57.1)**
Meropenem	2 (28.6)	**3(42.9)**	2 (28.6)
Imipenem	1 (14.3)	**6 (85.7)**	-
Fosfomycin	-	**5 (71.4)**	2 (28.6)
Amoxicillin/Clavulanic Acid	-	1 (14.3)	**6 (85.7)**
Trimethoprim/sulfamethoxazole	-	-	**7 (100)**
Colistin sulphate	**5 (71.4)**	2 (28.6)	-

Highest carbapenem resistance was recorded in 2 (28.6%) isolates (W14a and W22b) which showed full resistance to Meropenem but were intermediate to Imipenem. 3 isolates representing 42.9% showed susceptibility to carbapenem (W19a and W16b to Meropenem while S9b to Imipenem). The remaining isolates showed intermediate response to the antibiotic. This data is represented in table 4.

Table 4: Total % efficacy of Carbapenem (Meropenem and Imipenem) among *A. baumannii* isolates

		Meropenem	
Sample code	Susceptible	Intermediate	Resistant
U17b		+	
W14a			+
W19a	+		
W16b	+		
W22b			+
S2b		+	
S9b		+	

		Imipenem	
Sample code	Susceptible	Intermediate	Resistant
U17b		+	
W14a		+	
W19a		+	
W16b		+	
W22b		+	
S2b		+	
S9b	+		

The Multiple Antibiotic Resistance index was also determined by following the procedure described by Krumperman (1983). The formula used to calculate the MAR index for each isolate is given below.

$$\text{MAR index} = \frac{\text{Number of antibiotics to which isolate is resistant}}{\text{Total number of antibiotics against which isolate was tested}}$$

Four *A. baumannii* isolates had a MARI >0.2. These were the W14a and W22b which had a MARI of 0.5 and W17b and S2b which both had a 0.3 MARI. The remaining isolates had a 0.2 MARI except S9b which had a 0.1 MARI. The summary of The MAR index result is presented as table 5.

Table 5: Result showing the Multiple Antibiotic Resistant Index (MARI) of *A. baumannii* isolates

Sample code	MAR index
U17b	0.3
W14a	0.5
W19a	0.2
W16b	0.2
W22b	0.5
S2b	0.3
S9b	0.1

Simplified Carbapenem Inactivation Method Two isolates (W14a and W22b) were positive for the production of carbapenemase during the simplified Carbapenemase Inactivation Method (sCIM) assay which was observed using the absence of a zone of clearance after incubation. The result for the sCIM assay is given below as table 6.

Table 6: Result for the sCIM test for A. baumannii isolates.

Sample code	Carbapenemase Producer
U17b	-
W14a	+
W19a	-
W16b	-
W22b	+
S2b	-
S9b	-

Key:
+ means isolate is positive for carbapenemase production
- means isolate is negative for carbapenemase production

Molecular identification of resistant gene in *A. baumannii* isolates

Plate 2 shows the agarose results of 16S rRNA PCR amplified products from extracted DNA of *A. baumannii* isolates. The blaOXA-23-like gene primer has a band size of approximately 501 base pairs. The electrophoresis was performed for 90 minutes at 70

volts. . Lane (L), DNA molecular size marker (l100 bp ladder), Lane W14a, W22b and S2b show positive results with positive bands of 501 bp.

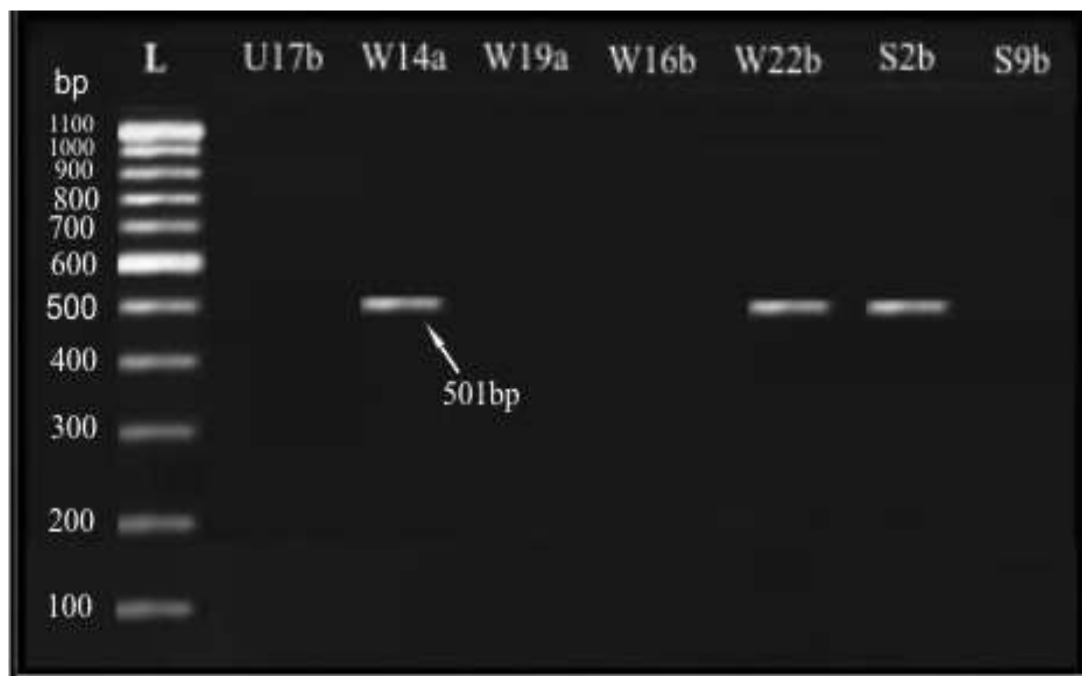

Plate 2: Agarose gel electrophoresis of blaOXA-23-like gene

DISCUSSION

Distribtion and prevalence of bacterial isolates in clinical specimen

A prevalence of 5.6% obtained in this study for *A. baumannii* corresponds to the findings of similar studies carried out by Bashir *et al.,* 2019, Odewale *et al*., 2016 and Nwadike *et al.,* 2014 with their percentage prevalence being 6.5%, 8.5% and 7.1% respectively. Seven *A. baumannii* isolates were obtained during the course of this study and four isolates were resistant to three or more classes of antibiotics (multidrug resistant). Two (W14a and W22b) out of the five isolates showed resistance to 5 different classes of antibiotics (carbapenem inclusive).

Antibiotic susceptibility profile of *A. baumannii* isolates

A total of 10 antibiotics representing 9 classes were used for this study with the focus being on the carbapenem class of antibiotics which was represented by imipenem and meropenem. A 100% resistance was recorded for Trimethoprim/sulfamethoxazole,

followed by a resistance of 85.7% to Amoxicillin/Clavulanic Acid (Augmentin). Tetracycline had a resistance of 57.1% while Fosfomycin and Meropenem both had resistant values of 28.6%. Meanwhile, highest susceptibility of 85.7% was recorded against both gentamycin and ciprofloxacin, followed by ceftriaxone and colistin which both showed susceptibilities of 71.4%.

The high levels of resistance to Trimethoprim/sulfamethoxazole and Amoxicillin/ Clavulanic Acid are in line with results by Rana and Asmaa, 2020 which showed 75.9% and 89.7% and Bashir *et al.,* 2019 which also showed 85.7% and 78.4% levels of resistance respectively.

High susceptibility levels to Ciprofloxacin and gentamycin in this study were in disagreement with previous studies by Nwadike *et al.,* 2014 and this difference may be due to varying prior exposure levels of patients in both studies to antibiotics.

The multiple antibiotic resistance index suggest that the four isolates (W14a, W17b, W22b and S2b) having MARI values greater than 0.2 are likely to originate from a high risk sources which are patients who have been exposed to several antibiotics while MARI values of ≤0.2 as seen in three isolates (U19a, W16b and S9b) suggest that the isolates originated from sources where antibiotic exposure was low.

Resistance to meropenem in this study was at 28.6% which was similar to the result carried out by Nwadike *et al.,* 2014 that revealed a 35.7% resistance to the antibiotic. Meanwhile, a 28.6% susceptibility to meropenem was in agreement with Odewale *et al.,* 2016 which had a similar result of 27.3% susceptibility to the drug. Intermediate susceptibity of 42.9% was neither in agreement or disagreement with previous studies consulted during the course of this study.

A lack of complete resistance to imipenem in this study was not recorded in previous studies and this may be due to a lack of full expression of resistant genes or even the absence of selected genes that could provide for antibiotic resistance in isolates involved in this study. A high percentage (85.7%) of intermediate resistance to imipenem was recorded in this study but even though it was not in agreement with Bashir *et al.,* 2019, who recorded 0%, a susceptibility level of 14.3% was identical in both studies.

Phenotypic and molecular assays for carbapenem resistance genes

In this study, the phenotypic assay for the detection of carbapenem resistance was based on antibiotic susceptibility which was carried out via disc diffusion method after which

the isolates were subjected to the simplified carbapenemase inactivation method to determine if the production of carbapenemase was reseponsible for resistance. Isolates that showed intermediate and full resistance were then subjected to molecular tests to detect the presence of the most likely gene responsible for their given resistance.
70.5% and 85.7% of the isolates in this study showed cumulative (intermediate and full) resistance to meropenem and imipenem respectively during the disc diffusion test.
To determine carbapenemases production in *A. baumannii*, the phenotypic confirmatory test was performed and interpreted as previously described by CLSI guidelines. Two isolates (W14a and W22b) were positive for the production of carbapenemase and from plate 2, it can be observed that these isolates contained the blaOXA-23-like gene thus showing a 66.6% resistance to one of the carbapenems while those lacking the gene showed both susceptible and intermediate response to the carbepenem class of antibiotics. The isolate, S2b even though it had the blaOXA-23-like gene was not a carbapenemase producer but was intermediate to both imipenem and meropenem. The wide zone of clearance in plate 3 here represented by S9b showed the absence of carbapenemase production whereas the lack of a zone of clearance in plate 4 here represented by W14a indicated that carbapenemase was produced. Both plates are available as appendix 3 and 4 respectively.
These results go on to show that even though the oxacillinase gene type 23 (blaOXA-23-like gene) which is an acquired gene is a key factor in conferring resistance to *A. baumannii* via carbapenemase production for the hydrolysis of these penems, other mechanisms were also in play which most likely came from the blaOXA-51-like gene which is fundamental to *A. baumannii* and because it is not acquired, it confers a level of decreased susceptibility of the isolates to carbepenem. These other mechanisms have been researched in other studies carried out by **Patrice and Laurent, (2014) and** also by Limansky *et al.,* (2012*)* **where they noted that non-carbapenemase producing carbapenem resistant A. baumannii are less of a threat to the health care system than carbapenemase producing isolates due to the fact that non-carbapenemase mechanisms of resistance are not transferrable thus making such isolates easier to treat. These other mechanisms as mentioned earlier can include the overexpression of** efflux pumps, formation of biofilms and

mutations that alter the expression and/or function of porins and Penicillin Binding Proteins (PBPs).

CONCLUSION

Seven *A. baumannii* isolates representing a 5.6% prevalence were obtained in this study. Four *A. baumannii* isolates were multidrug resistant (MDR) with two showing resistance to five different classes of antibiotics including carbapenem.

Phenotypically, 70.5% and 85.7% of the *A. baumannii* isolates in this study showed cumulative (intermediate and full) resistance to meropenem and imipenem respectively during the disc diffusion test while 28.6% were positive for the assay on carbapenemase production.

Molecular characterization of the *A. baumannii* isolates using primers for the blaOXA-51-like gene which is intrinsic to the species and blaOXA-23-like gene which is an acquired gene showed that isolates with the blaOXA-23-like gene showed a 66.6% higher resistance to meropenem while those lacking the gene showed both susceptible and intermediate responses to the carbapenem class of antibiotics. Also of importance was the isolate S2b which had the blaOXA-23-like gene but was not a carbapenemase producer and was intermediate to both meropenem and imipenem.

All *A. baumannii* isolates involved in this study (with the exception of W19a, W16b and S9b) showed a general decreased level of susceptibility to carbapenem due to the blaOXA-51-like gene which is known for conferring resistance to the species while the W14a and W22b isolates combined resistance mechanisms using the blaOXA-23-like gene for carbapenemase production to achieve a much stronger resistance level to the carbapenem.

Recommendations

Based on the findings of this research, the following recommendations are made:

a. strict measures should be put in place to control the prescription and use of antibiotics for the treatment of illness.
b. routine surveillance should be carried out on in-patients to detect early resistance to antibiotics used for treatment.

c. proper sanitary measures should be put in place to prevent the spread of carbapenem resistant *A. baumannii.*
d. further studies should be done to observe the interactions between other acquired genes and how they relate to resistance mechanisms in CRAB.

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