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# **Molecular characterization of *Acinetobacter baumannii* from patients with prolonged hospital stays in three tertiary hospitals of Kano Metropolis, Northwestern Nigeria**

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Received 6 August, 2019; Accepted 10 September, 2019

*Acinetobacter baumannii* is one of the most important opportunistic bacterial pathogens that cause serious health care associated complications in hospitalized patients. This leads to prolong hospital stay which increase cost to both healthcare provider and family of the patients. The study aimed at molecular characterisation of *A. baumannii* from patients with prolonged hospital stays in three tertiary hospitals of Kano Metropolis, Northwestern Nigeria. A total of 401 samples were collected from orthopedic and post-surgical wound infections, urine, urine catheters and nasal intubation. *Acinetobacter* spp was isolated using standard microbiological methods. Identification of *A. baumannii* isolates were done using Phynotypic methods such as culture on Leed *Acinetobacter* medium, conventional biochemical tests and API 20NE. Suspect *Acinetobacter* species were further identified using polymerase chain reaction (PCR) and Sanger sequence typing methods. Out of 401 samples collected 138 (34.4%) were positives by yield suspect bacterial isolates 14 (10.1%) of which were suspect *A. baumannii*. The results of confirmatory sequence typing of isolates showed that 9 (6.5%) of suspect *Acinetobacter* spp were *A. baumannii*. The result of susceptibility test showed that *A. baumannii* isolates were highly resistance to Ampicillin\ salbactam 13 (92.8%) and least resistance to Ciprofloxacin 2 (14.3%) and Amikacin 3 (21.4). The results of this finding showed presence of *A. baumannii* species resistant to conventional antibiotics and associated with prolonged duration of patients admission in the three studied hospitals. There is need for improved sanitary working condition and proper patients management to reduce the spread of this health care associated infection agent.

**Key words:** Molecular, characterization, *Acinetobacter baumannii*, patients, prolonged hospitals, Kano, Northwestern Nigeria.

## INTRODUCTION

*Acinetobacter baumannii* is a Gram negative coccobacillus, aerobic, non fermentative and non-motile bacterium that belong to the genus *Acinetobacter*. Current taxonomic classification of this bacterium put it in  $\gamma$ -proteobacteria, family Moraxellaceae and order Pseudomonadales (Nemec et al., 2016). It belongs to *Acinetobacter calcoaceticus-baumannii* complex group which comprises four different *Acinetobacter*: *A. baumannii*, *Acinetobacter pittii*, *Acinetobacter nosocomialis*, and *Acinetobacter calcoaceticus* (Pourabbas et al., 2016; Nemec et al., 2016; Muhammad et al., 2018). *A. baumannii* repels complete decolorization and can mislead as Gram-positive cocci. It is non-fastidious, and does not produce cytochrome oxidase, urease, citrate, and indole; however, it produces catalase enzyme. Furthermore, *A. baumannii* grow well at 35-37°C; however, some environmental isolates grow well in the temperature range of 20-30°C. *A. baumannii* is the only bacterium in the genus that can grow at 44°C (Bouvet and Grimont, 1987; Muhammad et al., 2018). *A. baumannii* grow well on culture media such as blood agar, chocolate agar, and MacConkey agar. On blood agar, it forms colorless, non-hemolytic, shiny mucoid colonies, smooth in contexture with a diameter of 1-2 mm after 18-24 h of incubation at 37°C. It produces colorless colonies on MacConkey agar which are shiny mucoid and tomb shaped, indicating its non-lactose fermenting ability. On selective media, Leeds *Acinetobacter* Medium, it gives pink color colonies when grown in the presence of supplement (Almasaudi, 2018; Muhammad et al., 2018).

*A. baumannii* was reported in health care environments and recently considered one of the important opportunistic bacterial pathogens that cause health care associated infections worldwide (Muhammad et al., 2018; Mirnejad et al., 2018). This leads to prolong hospital stay (>14 days) which increase cost to both healthcare provider and family of the patients. *A. baumannii* was the most dangerous among *Acinetobacter calcoaceticus-baumannii* complex, however, *A. pittii*, *A. nosocomialis* was also reported to cause infections (Muhammad et al., 2018).

In the hospital environments, *A. baumannii* can survive on beds, curtains, walls, roofs, medical devices, equipment, belongings of medical personnel, tap water sinks, telephones, door handles, hand sanitizers, dispensers, trolleys, bins, and even on computers (Muhammad et al., 2018). Furthermore, *A. baumannii* was isolated from different parts of healthy person such as nose, ear, throat, forehead, trachea, conjunctiva, vagina and perineum, axillae, groin, hands, and toe webs. *A. baumannii* was reported to be involved in bloodstream

infections and account for about 15% cases due to invasive procedures such as intravascular or respiratory catheters, tubes, or cannulas among others (Muhammad et al., 2018; Mirnejad et al., 2018). The origin of infections (20-70%) caused by *A. baumannii* still remain unknown and the ability of *A. baumannii* to cause infections in hospitals was linked to its ability to survive in desiccants, resistance to vital antimicrobial drugs and disinfectants (Muhammad et al., 2018).

Literature have shown that global burden of infections cause by *A. baumannii* still remain unknown due the lack of comprehensive data especially from African countries (Egwuenu et al., 2018) but the burden can be up to 35% (Xie et al., 2018) with mortality rate of 26% and this can increase up to 45% in intensive care unit (ICU) (Muhammad et al., 2018). Nigeria like other African countries, the story remain the same, but Egwuenu et al. (2018) reported that, *A. baumannii* was associated with blood stream catheter associated infection from different parts of the country including carbapenem resistant *Acinetobacter* spp (Aibinu et al., 2003; Taiwo et al., 2005; Ngwa et al., 2007; Jido and Garba, 2012; Nwadike et al., 2014; Bashir et al., 2019).

This leads to delay of patients in the hospitals due to treatment failure. More than 90% of the researches done on *A. baumannii* in Nigerian health care settings focused widely on phenotypic identification and antimicrobial resistant profile neglecting molecular aspect which gives more insight on the different types of strains involved in health care associated infections and antimicrobial resistance within a particular community. In our recent study (Bashir et al., 2019) reported superbugs-related prolonged admissions in three tertiary hospitals, Kano State, Nigeria including *Acinetobacter* spp which we lack information about their genetics relatedness with other known *Acinetobacter* spp sequences stored in global genebanks. Therefore this study aimed at molecular characterization of *A. baumannii* from patients with prolonged hospital stays in three tertiary hospitals of Kano Metropolis, Northwestern Nigeria.

## MATERIALS AND METHODS

### Study area

The study was conducted in Kano State metropolis located in Northwest geopolitical zone of Nigeria. The state is made up of 44 Local Governments with an estimated population of over 13 million people (NBS, 2018). The study was carried out at 3 tertiary hospitals within the state and these were; Aminu Kano Teaching Hospital (AKTH), Murtala Muhammad Specialist Hospital (MMSH) and Muhammad Abdullahi Wase Specialist Hospital (MAWSH). All hospitals were strategically located for access to both rural and

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urban populations throughout Kano State.

### Study design

This was a cross sectional descriptive hospital based study which involved molecular characterization of *A. baumannii* isolated from urine, orthopedic and post-surgical wound infections, urine catheter and nasal feed tube from patients who were eighteen years and above of ages and both sexes with prolonged hospital admission admitted in AKTH, MMSH and MAWSH. The isolated *A. baumannii* were characterised using both phynotipic, polymerase chain reaction (PCR) and Sanger sequencing methods.

### Sample collection

A total of 401 sample were collected from patients who were admitted for  $\geq 14$  days and aged  $\geq 18$  years from three study hospitals. The samples collected included orthopedic and post-surgical wound infections, urine, urine catheters and nasal intubation. The swab samples were collected after cleaning the wound with physiological saline (0.85%) as described by Ibrahim et al. (2018) all swabbed samples were transported to Microbiology laboratory in a Stuart media. Urine samples were collected in sterile clean leak proof bottles from each patient according to the method described by Odoki et al. (2019).

### Isolation and identification of *A. baumannii*

All samples (both urine and swabs) were inoculated on freshly prepared MacConkey agar (HiMedia Laboratories Pvt Ltd, Mumbai, India, M173) media and incubated for 24 h at 37°C. After incubation, isolates that were non lactose fermenting (shiny mucoid and tomb shaped) on MacConkey agar, Gram negative coccobacilli and oxidase negative were subcultured on Leed *Acinetobacter* Media (HiMedia Laboratories Pvt Ltd, Mumbai, India, M1839) and incubated at 37°C for additional 18-24 h. Suspected *Acinetobacter* spp from Lead *Acinetobacter* Media (that is, pink color) colonies were further identified using biochemical tests such as, catalase, coagulase, indole, citrate utilization, urea, methyl red, Voges-proskauer, motility and Triple sugar Iron tests (Cheesbrough, 2010).

### API 20 Multi test systems

The suspected *Acinetobacter* spp were further subjected to API 20 NE multi test system (Biomeniueux, France) test. These tests were used according to manufactures protocol for identification of non enteric bacteria. Well of the biochemical test were inoculated with bacterial suspension (0.5 McFarland) made from fresh bacterial colony. The inoculated try was incubated at 37°C for 18-24h. The result was read after addition of appropriate reagents as 7-digit number that identify API 20 NE analytical index (API 20 Biomeriux France, 2010).

### Molecular identification of *A. baumannii*

#### DNA extraction

Extraction of DNA was done by ethanol precipitation after phenol:chloroform:isoamyl alcohol (24:25:1 v/v) treatment as previously described (Gumińska et al., 2018). Briefly, 200  $\mu$ l of 24 h

suspected *Acinetobacter* spp culture was transferred into 2 ml sterile tube, to which 500  $\mu$ l of lysis buffer (eBioscience™ Thermo Fisher Scientific, USA) and 20  $\mu$ l of proteinase K (200  $\mu$ g/ml) (Thermo Fisher Scientific, USA) were added. This was then vortexed and incubated at 65°C for 1 h. The lysate was extracted twice with 500  $\mu$ l of phenol:chloroform:isoamyl alcohol (24:25:1, v/v). The aqueous fraction was transferred to a clean 1.5 ml tube and DNA precipitated at -20°C for 3 h after the addition of 0.1 volume of 3 M sodium acetate (pH 5.2) and 2.5 volumes of 100% ethanol. The DNA was recovered after centrifugation at 15,000 g for 20 min, supernatant discarded and the DNA pellet washed with 70% ethanol. After a final centrifugation step, the supernatant was carefully removed, DNA pellet was allowed to air-dry and finally re-suspended in 100  $\mu$ l of sterile distilled water. The extracted DNA was stored at -20°C until required for further study.

#### Polymerase chain reaction

A PCR was performed using the extracted DNA as template. A set of two primers, 16S Fw (5'-GTG CCA GCA GCC GCG CTA-3') and 16S Rev (5'-AGA CCC GGG AAC GTA TTC AC-3'), amplifying a 850 base pair (bp) 16S rRNA genomic region were used. Amplification reactions were run in a 10  $\mu$ l final volume containing; 2  $\mu$ l of extracted DNA, 1  $\mu$ l of 10x standard Taq reaction buffer (Thermo Fisher Scientific, USA), 1  $\mu$ l of 2.5 mM MgCl<sub>2</sub> (Thermo Fisher Scientific, USA), 0.5  $\mu$ l of 0.25 pMol of each primer (Thermo Fisher Scientific, USA), 1  $\mu$ l of 10% DMSO (Thermo Fisher Scientific, USA), 0.8  $\mu$ l of 200  $\mu$ M dNTPs (Thermo Fisher Scientific, USA) and 0.1  $\mu$ l of 0.5 units of Taq polymerase (NEB, UK) and 3.1  $\mu$ l of nuclease free water. The PCR conditions included an initial denaturation step at 94°C (5 min), followed by 36 cycles of 94°C (30 s), 56°C (30 s) and 72°C (45 s). Following amplification, a final extension at 72°C (7 min) was done. The amplified products were analyzed on a 1.5% agarose gel containing 0.5% ethidium bromide and visualized under U.V. illumination. A band corresponding to 850 bp was gel purified using the QIAquick gel extraction kit (QIAGEN, Thermo Fisher Scientific, USA).

#### Sequencing

The purified PCR product was subjected to cycle sequencing using the BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems, USA). Sequencing reactions were prepared as a 6  $\mu$ l reaction mix containing; BigDye Direct Sequencing Master mix (2  $\mu$ l), sequencing primer (MP13Forward/ Reverseprimer) (1  $\mu$ l) and PCR product (3  $\mu$ l), and loaded 3  $\mu$ l of the reaction mix to the appropriate well in the respective forward or reverse reaction plate. Sequencing was performed in a thermocycler using the following conditions; at 96°C (1 min), followed by 25 cycles of 96°C (10 s), 50°C (5 s) and 60°C (75 s). At the end of the reaction, the tubes were briefly centrifuged and samples loaded onto the ABI 3700 gene sequencer. The results obtained were analyzed using MEGA software (version 6.0) and blastn (NCBI). A phylogenetic tree was constructed using Neighbor-Joining method and bootstrapping performed by creating 1000 trials. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The optimal tree with the sum of branch length = 1.11983619 is shown. The confidence probability (multiplied by 100) that the interior branch length is greater than 0, as estimated using the bootstrap test (200 replicates is shown next to the branches (Rzhetsky and Nei, 1992; Dopazo, 1994). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004) and are in the

**Table 1.** Socio demographic characteristics distribution of *A. baumannii* in the 3 studied hospitals.

	AKTH	MAWSH	MMSH	
Variable	<i>A. baumannii</i> n (%)	<i>A. baumannii</i> n (%)	<i>A. baumannii</i> n (%)	Total (%)
Ages (years)				
18 – 28	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
29 – 38	0 (0.0)	0 (0.0)	1 (16.7)	1 (7.1)
39 – 48	0 (0.0)	1 (33.3)	0 (0.0)	1 (7.1)
49 – 58	3 (60.0)	1 (33.3)	1 (16.7)	5 (35.7)
59 – 68	1 (20.0)	1 (33.3)	2 (33.3)	4 (28.6)
69 – 78	1 (20.0)	0 (0.0)	2 (33.3)	3 (21.4)
Total	5 (35.7)	3 (21.4)	6 (42.9)	14 (100.0)
Sex				
Male	4 (80.0)	2 (66.7)	2 (33.3)	8 (57.1)
Female	1 (20.0)	1 (33.3)	4 (66.7)	6 (42.9)
Total	5 (35.7)	3 (21.4)	6 (42.9)	14 (100.0)

units of the number of base substitutions per site. This analysis involved 20 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1610 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar et al., 2018).

#### Antimicrobial susceptibility testing

The susceptibility testing of isolates to various antibiotics was carried out by the disk diffusion method using a modified form of the Kirby Bauer method (CLSI, 2015, 2016, 2018). Briefly, freshly prepared Mueller Hinton agar (Oxoid, UK) plates were inoculated with 0.5 McFarland standard of *Acinetobacter* spp suspension and placed the following single antibiotic discs on the inoculated plates: Amoxicillin (AM, 10 µg), Gentamycin (CN, 10 µg), Ceftriaxone (CRO, 30 µg), Ciprofloxacin (CIP, 5 µg), Ceftazidime (CAZ 30 µg), Imipenem (IMP, 10 µg), Tetracycline (TET, 30 µg), Amoxicillin/Clavulanic (20 µg), Ampicillin/Subactam (SAL, 20 µg), Nitrofurantion (NIT, 300 µg), Amikacin (AK, 30 µg) and Sulfamethoxazole/trimethoprim (SXT, 1-25/23.75µg). The plates were allowed to stand for 5-10 min at room temperature and then incubated at 37°C for 24 h, after which the zone of inhibition was measured and interpreted according to the method described by (CLSI, 2015, 2016). *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC was used as quality reference strains.

#### Ethical permission

Ethical approval for this study was obtained from the Medical advisory committee of Aminu Kano Teaching Hospital and the health service management board of Kano State with numbers NHREC/21/08/2008a/ AKTH/EC/1780 and HMB/GEN/488/VOL respectively.

## RESULTS

Out of 401 samples collected, 138/401 (34.4%) bacterial isolates were obtained in which 14/138 (10.1%) were *A.*

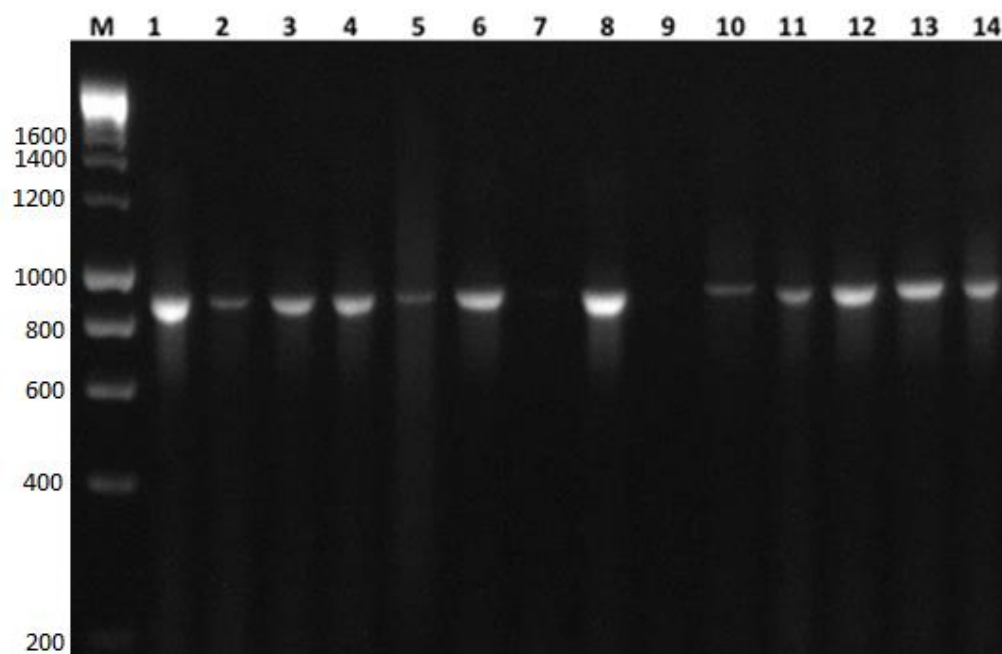
*baumannii* using phenotypic methods. The results of prevalence of *A. baumannii* according to studied hospitals showed that, MMSH has the highest prevalence 6/14 (42.9%) of *A. baumannii* followed by AKTH 5/14 (35.7%) and MAWSH had the least prevalence 3/14 (21.4%). The prevalence of *A. baumannii* according to the age of the studied participants showed that, age groups 49-58 has the highest prevalence 5/14 (35.7%). Prevalence of *A. baumannii* according to gender showed that male had the high prevalence 8/14 (57.1%) (Table 1). Furthermore, the prevalence of *A. baumannii* according to the sites of infection showed that, urine samples had the highest prevalence 5/14 (35.7%) while nasal intubation has the least 1/14 (7.1%) (Table 2).

All 14 suspected *A. baumannii* identified using phenotypic methods were subjected to molecular characterization and results showed that, 12 isolates were confirmed to be *A. baumannii* using PCR method (Figure 1). The PCR results showed that a band of about 850 base pairs (bp) of ribosomal RNA gene were obtained. However, the gel electrophoresis result showed that isolates 7 and 9 were not successfully amplified during PCR, this could be due to quality of the extracted DNA of those isolates or the concentration of extracted DNA was very low. Furthermore, sequencing results using Sanger sequencing method were blasted in NCBI database using BLASTn search to confirmed their identities. The blasted results showed that 9 isolates (16SF 1, 16SF 3, 16SF 4, 16SF 6, 16SF 8, 16SF 10, 16SF 12, 16SF 13, and 16SF 14) were found to have 99.5, 99.5, 97.66, 99.01, 99.14, 90.15, 99.75, 99.26 and 98.11% identities respectively with *A. baumannii* strain DSM 30007, which represents 9/138 (6.5%) of the isolates (Figure 2).

The antimicrobial susceptibility profile of the isolated *A.*

**Table 2.** Distribution of *A. baumannii* according to sampling sites based on three study site.

Samples	Hospitals			Total n (%)
	AKTH n (%)	MAWSH n (%)	MMSH n (%)	
Urine	2 (14.3%)	1 (7.1)	2 ((14.3%))	5 (35.7)
Urine Catheter	2 (14.3%)	1 (7.1)	1 (7.1)	4 (28.5)
Nasal Intubation	0 (0.0)	0 (0.0)	1 (7.1)	1 (7.1)
Wound Swab	1 (7.1)	1 (7.1)	2 (14.3%)	4 (28.5)
<b>Total</b>	<b>5 (35.7)</b>	<b>3 (21.3)</b>	<b>6 (42.8)</b>	<b>14 (100.0)</b>

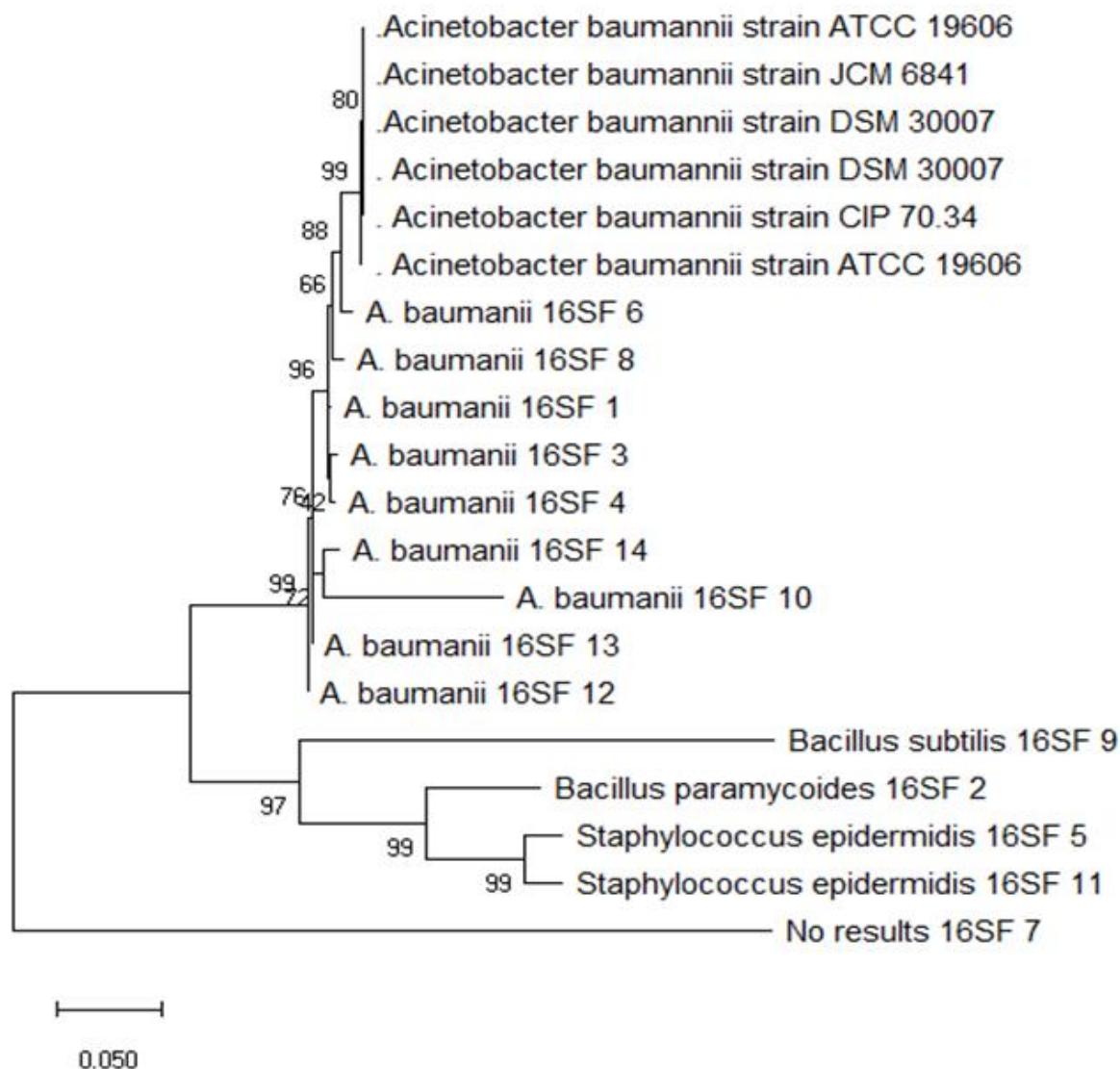
**Figure 1.** PCR results 1.5% agarose gel showing results of 16S rRNA PCR amplified products from extracted DNA of *A. baumannii* isolates. The electrophoresis was performed for 1.5 h. Lane M: DNA molecular size marker (2000bp ladder) (manufacturer, city, country); Lanes 1-14: the 14 *A. baumannii* isolates

*baumannii* in this study showed that, the isolates were highly resistant to Ampicillin salbactam and Perfloxacin 13 (92.9%) each. The least resistant were observed from Ciprofloxacin and Amikacin 2 (14.3%) and 3 (21.4%) respectively (Table 3).

## DISCUSSION

*A. baumannii* is emerging as a cause of health care associated outbreaks world wide (Villegas and Hartstein, 2003; Kais et al., 2016; Muhammad et al., 2018). Molecular characterization of any bacterial pathogens is important in ruling out the sources of infection, understanding the relationships and distribution patterns

of that pathogens (Mirnejad et al., 2018). From a total of 138 isolates obtained in this study, 14/138 (10.1%) were *A. baumannii* which was inline with the findings of Nwadike et al. (2014) who reported prevalence of *Acinetobacter* spp (9.0%) from ICU deparment University College Hospital, Ibadan, Nigeria. However, the prevalence reported in this study was higher compared to the prevalence 1 (0.7%) reported by Heydarpour et al. (2017) from open-heart surgery patients at Imam Ali Hospital in Kermanshah, Iran. The mix in sites swabed including the inherent microbial sub population must be more contaminated compared to theatre in specialized open heart surgical attention in the place. Prevalence of *A. baumannii* according to the age groups of the studied participant showed that age group 49-58 years had the



**Figure 2.** Phylogenetic tree showing evolutionary relationships isolated *A. baumannii* with other known *A. baumannii* sequences from NCBI.

**Table 3.** Antimicrobial susceptibility profile of the isolated *A. baumannii* from all the three study site.

Antibiotics (µg)	Sensitivity n (%)	Intermediate n (%)	Resistance n (%)
Amikacin (30)	11 (78.6)	0 (0.0)	3 (21.4)
Amoxicillin (10)	1 (7.1)	1 (7.1)	12 (85.7)
Ampicillin sulbactam (10)	1 (7.1)	0 (0.0)	13 (92.8)
Amoxicillin/Cluvanic (20)	3 (21.4)	0 (0.0)	11 (78.4)
Ceftazidime (30)	3 (21.4)	1 (7.1)	10 (71.4)
Ceftriaxone (30)	2 (14.3)	0 (0.0)	12 (85.7)
Ciprofloxacin (5)	11 (78.6)	1 (7.1)	2 (14.3)
Gentamycin (10)	1 (7.1)	1 (7.1)	11 (78.6)
Imipenem (10)	2 (14.3)	0 (0.0)	12 (85.7)
Nitrofurtoin (300)	6 (42.9)	2 (14.3)	6 (42.9)
Tetracycline (30)	3 (21.4)	1 (7.1)	10 (71.4)
Trimetoprim sulfamethoxazole (1.25/23.75)	2 (14.3)	0 (0.0)	12 (85.7)



highest prevalence (35.7%). This could be due to immunity issues, long time hospitalization and use of invasive procedures which can raise the risk of infections by opportunistic pathogens such *A. baumannii*. This was in agreement with Odewale et al. (2016) who reported in their study that age 41- 70 years are more susceptible to *A. baumannii* infection. Prevalence of *A. baumannii* according to the gender showed that, male had the highest prevalence (57.1%) this was in line with report of Pal et al. (2017) who reported higher prevalence in male patients (76.4%). This could be as a result of male patients constantly shifting their locations due to their job placements which make them more prone to accidental traumas since they are the most frequent patients using invasive devices. Prevalence of *A. baumannii* according to the site of infections showed that urine samples had the highest prevalence (35.7%) which correspond with the finding of Zuhair (2011) who reported high prevalence of *A. baumannii* from urine samples. However, this was contrary to the findings of Pal et al. (2017) who reported that frequently isolated *A. baumannii* were from secretions 54.6% and Suction tip 23.5%.

Out of the 14 phenotypically isolated *A. baumannii* obtained in this study, 12 were confirmed to be *A. baumannii* using PCR which represented 12/138 (8.7%) of the isolates. However, the sequencing results and blast search in the NCBI database confirmed only 9 isolates (isolates 16SF 1, 16SF 3, 16SF 4, 16SF 6, 16SF 8, 16SF 10, 16SF 12, 16SF 13, and 16SF 14) to be *A. baumannii*. The prevalence of *A. baumannii* using molecular method reported in this study was inline with finding of Nabil et al. (2001); Namita et al. (2012) and Odewale et al. (2016) who reported the prevalence of 8.8, 9.4 and 8.5% respectively using molecular method. However, these results indicate that we can not completely rely only on biochemical tests for identification of *A. baumannii*, but there is need to also use molecular techniques such as PCR and sequencing for accurate diagnosis.

The unique character of *A. baumannii* in resistance to most antibiotics makes it an organism of high importance especially in hospital setting as a nosocomial pathogen among immune compromised and patients with prolonged hospital stay. Majority of the isolates in our study were resistant to commonly used antibiotics such as ceftazidime, gentamicin, ceftriaxone, amoxicillin/Clavanic, cotrimoxole, amoxicillin, imipenem and ampicillin/sulbactam. Sensitivity was only found to be in presence of amikacin, ciprofloxacin, and nitrofurantion. This means Multi drugs resistant (MDR) isolates are increasing, probably due to indiscriminate use of these antibiotics in healthcare settings. *A. baumannii* showed high resistant of 92.8% to Ampicillin salbactam (10 µg) followed by Amoxicillin (85.7%) and Ceftriaxone (85.7%).

Carbapenems have been the drug of choice for treating *Acinetobacter* infections, but unfortunately, carbapenem resistant *A. baumannii* is becoming common worldwide (Towner, 2009). Of the  $\beta$ -lactamases, those with

carbapenemase activity are the most concern for drug resistance and include the serine oxacillinase (belonging to Ambler class D OXA type) and the metallo- $\beta$ -lactamases (Ambler class B) (Walsh et al., 2005). The present study observed Imipenim (85.7%) lower than 92.2% as reported by Anil and Nirav (2015) but higher than Mostofi et al. (2011) who showed low resistance of 76% (Mostofi et al., 2011). Trimetoprim sulfamethoxole showed 85.7% resistance, the least resistant of *A. baumannii* were obtained in Ciprofloxacin and Amikacin 14.3 and 21.4% respectively. In another study done by Pal et al. (2017) reported high resistance of *A. baumannii* to penicillin and cephalosporin antibiotics while Odewale et al. (2016) reported 100% resistance of Ciprofloxacin and Amikacin which was contrary to the present study.

## Conclusion

The result of this finding showed the presence of *A. baumannii* associated with health care associated infection among prolonged hospitalized patients from the studied hospitals. There is needs for the concern management of the studied hospitals to improved sanitary working condition and proper patients management that can reduce the spread of health care associated bacterial pathogens especially *A. baumannii*.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGMENT

The authors appreciate the management of the three studied hospitals for granting us permission to carry out this study. Study participants for participating in this study. Finally the staff of the microbiology laboratories for their kind support during this study.

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