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Plasmid profile of multidrug resistant *Acinetobacter baumannii* strains from wounds of patients attending Federal Medical Centre, Abeokuta, Southwest, Nigeria

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ABSTRACT

Acinetobacter baumannii which was once considered non-pathogenic Gram-negative bacteria has emerged as a stubborn nosocomial pathogen. The study was conducted to investigate prevalence, antimicrobial resistance and profile plasmids carried by *Acinetobacter baumannii* from wounds of patients attending Federal Medical Centre, Abeokuta, Nigeria. Wound swabs of 140 patients were collected from the Bones and Plastic Surgery Department from November 2018 to March 2019. Swabs collected were cultured on Leeds Acinetobacter media. Isolates were identified by biochemical method using Oxoid Microbact™ Gram-negative identification system. Polymerase chain reaction was used to investigate the presence of $bla_{\text{-OXA-51-}}$like gene intrinsic to *A. baumannii* in isolates observed for authentication. Antimicrobial susceptibility test was performed by the agar disc diffusion method and inhibition zones were interpreted following Clinical and Laboratory Standard Institute guidelines. Isolates were cured using Sodium Dodecyl Sulphate and subjected to second antimicrobial susceptibility test. A total of 18 (12.8%) isolates were identified as *Acinetobacter baumannii* from 140 patients. All the isolates (100%) were resistant to at least three antimicrobial agents. Cured *A. baumannii* isolates showed that 11 (61%) of the multi-drug resistant isolates were plasmid-mediated, with plasmid sizes ranging from 60-1,333 bp. This study revealed high rate of resistance to multiplicity of antimicrobial agents by plasmids carried on strains of *Acinetobacter bauamannii*.

Key words: Multidrug resistant, *Acinetobacter baumannii*, wounds, Plasmid, Nigeria.

INTRODUCTION:

Acinetobacter baumannii has been delineated to be one of the most important opportunistic pathogens that cause nosocomial infections in hospitals of the 21st century. This is because of its multi-resistant genetic determinants, ability to tolerate a wide range of humidity, pH, salinity, and its survival on many natural sources (Muhammad *et al*., 2018). The aforementioned traits make this pathogen ubiquitous in the hospital environment. They are frequent colonizers of the throat, skin, respiratory tract, and the digestive tracts which commonly infect patients with impaired host defences. The most common clinical presentation of *A. baumannii* is pneumonia in mechanically ventilated patients in the intensive care units (ICUs) (Demirdal *et al*., 2016) leading to high mortality rates. Other infections caused by this pathogen are bacteraemia, wound infections, secondary meningitis, urinary tract infections, peritonitis, osteomyelitis, keratitis, and native-valve endocarditis. The first list of antibiotic-resistant "priority pathogens" published by the World Health Organization to secure and guide research and development related to new antibiotics, enlisted *A. baumannii* as priority 1 (critical) pathogen. This highlights its serious threats to public health, being particularly problematic due to the frequency of multi-drug resistance (MDR) and the high epidemic potential (Ruiqiang *et al*., 2018). Currently, *A. baumannii* has developed resistance to almost all known antibiotics, and the MDR has been widely documented (Potron *et al*., 2015). On the other hand, the emergence and wide spread of antibiotic resistance have diminished the options of effective therapeutic drug for *A. baumannii* infection; a clinician has to choose the previously abandoned antibiotic colistin, which is generally associated with more serious adverse effect. Most importantly, it was reported that clinical isolates resistant to colistin have emerged in certain geographical areas (Ruiqiang *et al*., 2018), making the last resort of antibiotics in human medicine ineffective. *A. baumannii* strains that are resistant to all major antibiotic classes normally used to treat infections with the bacterium, including β-lactams, aminoglycosides, fluoroquinolones, macrolides, tetracyclines, and carpabenem, are now emerging, and the prevalence of these multidrug-resistant *A. baumannii* strains leaves limited clinical options for treatment (Odewale *et al*., 2016). Hence, there is dire need to develop novel antibiotics for bacterial pathogens in general as well as harmful gram-negative organisms in particular. Although nosocomial infections caused by *A.*

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baumannii have been reported worldwide (WHO, 2017), information on the emergence and antibiotic susceptibility *A. baumannii* isolates in Nigeria is sparse and rudimentary. The aim of this work was, therefore, to assess the prevalence, the current level of antimicrobial resistance pattern as well as the plasmid profiles of *A. baumannii* isolates in Federal Medical Centre (FMC) Abeokuta, Southwest Nigeria.

MATERIALS AND METHODS

Ethical approval

Clearance of research involving human subjects was granted by the Ethics and Research Committee of the Federal Medical Centre, Abeokuta Ogun State, Nigeria for this study.

Collection of clinical specimens

Wound swabs of 140 patients (86 male and 54 female) were collected aseptically and transported immediately to the research laboratory for microbiological analysis. The study population comprised of patients who have had surgery, prior history of antibiotic use and patients with new injury cases.

Identification of Acinetobacter baumannii from clinical specimen Wound swabs of patients were streaked on Leeds Acinetobacter media and incubated for 24 to 48 hours at 37ºC aerobically. Acinetobacter species were primarily identified on the basis of their phenotypic characteristics on the media and further biochemical tests were done with the aid of Microbat (Oxoid) gram negative identification system (UK) to identify the isolates to species level.

Amplification of bla$_{-OXA-51-}$like gene Polymerase chain reaction (PCR) was used to detect gene encoding *bla$_{-OXA-51-}$*like gene intrinsic to *A. baumannii* on the genes of the bacteria isolated according to the method described by Lopes et al. (2012). The detail of primer that was used in this study is shown below.

Table 1: Sequence and base pair of Bla$_{-OXA-51-}$like primer

Bla **like** ***-OXA-51-*** **Forward**	**TAA TGC TTT GAT CGG CCT TG**	**353Bp**
Reverse	TGG ATT GCA CTT CAT CTT GG	

Antimicrobial susceptibility testing

Antimicrobial susceptibility test of the isolates to meropenem, ceftazidime, gentamicin, ciprofloxacin, cefuroxime, erythromycin, ofloxacin, augumentin and tetracycline obtained from distributor of Oxoid Company in Lagos, Nigeria was carried out using the disc diffusion method. The results were interpreted according to the manufacturer's instructions and CLSI guidelines (2019).

Plasmid Analysis

Plasmid analysis was done for the extraction and elimination of plasmid DNA using miniprep plasmid DNA extraction method. A 1.5mL of an overnight culture containing suspected plasmid was transferred to an eppendorf tube and spun at 5000 revolution per minute for 5 min in a tabletop centrifuge to pellet the cells. The supernatants were discarded and pellets were resuspended in 50 µL of lysogeny broth. 300 µL TENS buffer was added to the solution and shaken five times by inverting it. After which 100 µL of 3M NaAc at pH 5.2 was added to the previous solution and mixed thoroughly. The solution was spun the second time for five times at top speed. The supernatant was transferred to a new tube and 1 mL of 100% absolute ethanol was added. The resulting precipitate was spun again at top speed for five times after which it was washed with 0.5 mL 70% ethanol. The supernatants were dispensed into a separate container while the precipitates were air dried for 10 minutes. Thereafter, the dried pellets were resuspended into 30 µL of water. The extracted plasmid DNA of the isolates was run on gel electrophoresis and viewed in gel doc system to view the plasmids bands. The whole procedure was carried out again, and thereafter, 20% Sodium dodecyl sulphate and 5mL 1Mole of Tris-hydrogen chloride at pH 8.0 was added after which 5ml of 10N NaOH was added to the solution and kept at 46ºC for 48 hours to eliminate the plasmids present. The cured isolates DNA were re-run on gel electrophoresis for confirmation. All the test isolates were cured to identify and profile the plasmids present. After the test organisms had been cured of their plasmid DNA, they were subjected to test antibiotics again so as to phenotypically detect if the genes conferring resistance to antibiotics were plasmid borne on *A. baumannii* isolates using Kirby Bauer disc method.

Data analysis

Data are presented as frequencies and percentages.

RESULT

Of one hundred and forty wound swabs analysed in this study, eighteen (18) bacterial isolates were molecularly authenticated as *A. baumannii* with the amplification of 353base pair on gel electrophoresis with the *bla$_{-OXA-51-}$*like primer (Fig 1). High rate of (88.9%) resistant was recorded for Tetracycline, Erythromycin, Meropenem, Cefuroxime, Augumentin, Gentamicin and Ceftazidime. High rate of resistance (77.8%) was equally recorded for Ofloxacin while Ciprofloxacin showed a resistance rate of 66.7%. The second sensitivity test carried out after curing showed the following resistant patterns: Tetracycline (16.1 %), Meropenem (27.8 %), Erythromycin (44.4 %),

Ciprofloxacin (27.8 %), Ceftazidime (22.2 %), Cefuroxime (50 %), Ofloxacin (5.6 %), Augmentin (38.9 %) and Gentamicin (33.3 %) as illustrated in Table 2 and 3. Profile of extracted plasmid on agarose gel electrophoresis revealed all but one isolate carried a single plasmid while one of the eleven isolates (9.1%) carried three plasmids with different base pairs sizes (Table 2).

Fig 1: PCR results to *bla*$_{OXA-51}$like gene amplified from *A. baumannii* strains from FMC, Abeokuta Southwest Nigeria.

Table 2: Resistant patterns of cured and uncured *A. baumannii* strains to different antibiotics.

S/N	Isolate Id	Antimicrobial susceptibility test				Plasmid size (Base pair)
		Before curing		After curing		
		S	R	S	R	
1.	A0016	OF, CP	TE, ME, AU, CX, ER, GT,CZ	TE, ME, ER, CP, OF, GET	CZ, CX, AU	60bp
2	A0443	NN	TE, CP, CX, AU, GT, ER, OF, ME,CZ	TE, ER, CZ, OF, AU, GT	ME, CX, CP	0
3	A0040	NN	TE, ME, AU, CX, ER, CP, OF, GT, CZ	TE, ME, CZ, CX, OF	ER, CP, AU, GT	160bp
4	A0035	TE	ME, CX, ER, GT, CP, CZ, OF, AU	ER, CP, CZ, OF, GT, TE	ME, CX, AU	200bp
5	A0037	OF	TE, ME, ER, CP, CX, CZ, GT, AU	TE, ME, ER, CP, CZ, CX, OF,	AU, GT	190bp
6	A0887	CP, OF	TE, ME, ER, CX, AU, GT,CZ	TE, ME, ER, CP, CZ, OF, GT	CX, AU	180bp
7	A1527	NN	TE, ME, ER, CP, CZ, CX, GT, AU, OF	TE, ME, CZ, OF	ER, CP, AU, GT, CX	100bp
8	A0486	AU, GT	TE, ME, ER, CP, CZ, CX, OF	ME, CP, CZ, GT, AU	CX, TE, ER, OF	0
9	A0265	NN	TE, ME, ER, CP, CZ, CX, GT, AU, OF	CP, OF, AU	TE, ME, ER, CZ, CX, GT	0
10	A1945	NN	TE, ME, ER, GT, AU, OF, CP, CZ, CX	TE, ME, CP, CZ, OF, AU, GT	ER, CX	370bp, 500bp,1,333bp
11	A7669	OF	TE, ER, CZ, GT, ME, CP, CX, GT	ME, CP, CZ, CX, OF, AU	GT, TE, ER	0
12	A0038	CP	TE, ME, ER, OF, AU, CX,GT, CZ	TE, ME, CP, OF, ER	CZ, CX, AU, GT	0
13	A0041	CZ, OF	TE, ME, ER, AU, GT, CX, CP	TE, ER, OF, CX, CZ	ME, CP, AU, GT	350bp
14	A0042	CP, ME, CX	TE, ER, AU, GT, CZ, OF	TE, ME, ER, CP, CZ, OF, AU, GT	ER	0
15	A1747	CP	TE, ME, ER, CZ, AU, GT, CX, OF	TE, CZ, OF, GT, CP	ME, ER, CX, AU	0
16	A1393	CP, AU, GT	TE, ME, ER, CZ, OF, CX	ME, CP, CZ, CX, OF, AU, GT	TE, ER	100bp
17	A0130	TE, ME, CX	ER, AU, GT, OF, CP,CZ	TE, ME, CP, CZ, CX, OF, AU	ER, GT	120bp
18	A0181	AU	TE, OF, CP, CX, ME, GT, CZ, ER	TE, ME, CZ, AU	ER, CP, CX, OF, GT	100bp

NOTE: S means Susceptible, R means Resistance, NN means None; TE-Tetracycline, ME-Meropenem, ER-Erythromicin, CP-Ciprofloxacin, CX-Cefuroxime, CZ-Ceftazidime, OF-Ofloxacin, AU-Augumentin, GT-Gentamicin. Intermediates were considered as resistance in this study.

Table 3: Percentage of Resistance patterns of *Acinetobacter baumannii* strains

Antibiotics (µg)	Resistance rate before curing(%)	Resistance rate after curing (%)
Tetracycline (30µg)	88.9	16.1
Meropenem (10µg)	88.9	27.8
Erythromycin (15µg)	88.9	44.4
Ceftazidime (30µg)	88.9	22.2
Ciprofloxacin (10µg)	66.7	27.8
Cefuroxime (30µg)	88.9	50.0
Ofloxacin (30µg)	77.8	5.6
Augumentin (30µg)	88.9	38.9
Gentamicin (10µg)	88.9	33.3

DISCUSSION

From wound swabs of 140 patients analysed, 18 patients were positive for *Acinetobacter baumannii* infection of which 11 were male and 7 were female and all the isolates were authenticated as *Acinetobacter baumannii* by the presence of bla_{OXA-51} like gene. This explains the fact that they are emerging nosocomial pathogens in this part of the country, also the infection has been revealed to be more associated with the male sex (Odewale *et al.*, 2016). The antimicrobial susceptibility test carried out revealed that all 18 molecularly identified isolates were MDR in nature. Antimicrobial resistance among *Acinetobacter* species has increased significantly in the past decades. The ability of *Acinetobacter* species to extensively resist antimicrobial agents may be explained in part by the organism's relatively impermeable outer membrane, selective pressure, and environmental exposure to a large reservoir of resistance genes (Ike *et al*., 2014). Different definitions have been proposed for multi-drug resistant Acinetobater species. One of the most common definitions is the Carbapenem resistance (Ike *et al*., 2014). The presence of bla_{OXA-51} like gene in some of the bacteria isolates was a confirmation that the isolates were *Acinetobacter baumannii* because it is an intrinsic gene that is specific for only this species and also a confirmation that they are extended spectrum β-lactamase producers (Lopes *et al*., 2012) These genes are chromosomally-borne, this further explains why these organisms have firmly established themselves as multiple drug resistant nosocomial pathogens whose infections no longer respond to treatment by commonly used antibiotics. In this study, all eighteen (100%) strains isolated exhibited resistance to ≥3 antimicrobial agents of nine antimicrobial agents employed. In 1970s, *Acinetobacter* infections were treated with ampicillin, second generation cephalosporins, carbapenem, colistin, and gentamicin (Iregbu *et al*., 2002; Ike *et al*., 2014; Odewale *et al*., 2016). Today, most strains are resistant to these agents, with reports of 72.2% resistant to gentamicin and 77.8% resistant to Meropenem. This is consistent with the observation made in this study. More than 80% of the strains were susceptible to antibiotics after curing. It could be said that most of the genes of the isolates conferring resistance to the antimicrobial agents are plasmid mediated. This is in accordance with the study by Hadis et al. (2015). Survival of this bacterium in the hospital, especially in the ICU and surgical wards seems to be related to its ability to acquire resistant genes (Hadis *et al*., 2015). All but one strain of eleven isolates with plasmid DNA harboured single plasmid while one strain harboured three plasmids DNA with varying sizes. This might be as a result of acquisition of the plasmid from the environment either by contact with other bacterium cell wall or by conjugative transfer. The presence of resistant markers in bacterial chromosome, plasmids, integration and transposons are the reason for transferring genes among bacteria (Chopade *et al*., 1985; Montefour *et al*., 2008). In the hospital, this phenomenon will happen often because of the existence of drug resistant bacteria. Hence, microorganisms that are opportunistic with low virulence can cause severe disease in the hospital on acquisition of these resistant markers. Among all species of Acinetobacter, *A. baumannii* has become a significant pathogen, especially in the hospital. Survival of this bacterium in the hospital, especially in the ICU and surgical wards seems to be related to their ability of acquiring resistant genes.

CONCLUSION

Acinetobacter baumannii is usually a healthcare-associated pathogen affecting the very ill patients and is emerging as a cause of numerous global outbreaks hence should be greatly controlled as quickly as possible. This study revealed high rate of resistance to multiplicity of antimicrobial agents. Carbapenem and Cephalosporin resistant *A. baumannii* is now emerging in this part of the country as revealed in this study and other related studies. Genes coding for resistance can be carried either on chromosomal DNA or plasmid DNA and Plasmidal genes can easily be transferred within different bacterial cells. It has been revealed from this study that *A. baumannii* is a multi-drug resistant organism and the genes conferring resistance to the antimicrobial agents used in this study are more plasmid-mediated. Federal Medical Centre, Abeokuta Ogun state (Southwest) Nigeria showed unacceptably high rates of resistance to multiplicity of antimicrobial agents. We, therefore, propose routine surveillance of MDR *A. baumannii* in hospitals, improvement on existing antibiotics as well as development of novel antibiotics that target plasmids, judicious prescription, completion of antibiotics regime by patients and early detection of resistance to commonly used antibiotics in order to forestall the menace of multiple drug resistant *A. baumannii* isolates in FMC, Abeokuta and beyond.

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