

# MULTIDRUG RESISTANCE OF *ACINETOBACTER BAUMANNII* IN LADOKE AKINTOLA UNIVERSITY TEACHING HOSPITAL, OSOGBO, NIGERIA

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*Acinetobacter baumannii* is a ubiquitous pathogen that has emerged as a major cause of healthcare-associated infections at Ladoke Akintola University Teaching Hospital. Isolates were assayed according to standard protocol. The isolates were subjected to molecular techniques to detect *blaOXA*, *blaTEM*, *blaCTX-M*, and *blaSHV* genes in strains of the *A. baumannii* isolates.

The prevalence of *A. baumannii* was 8.5% and was most prevalent among patients in the age group 51–60 (36%); the male patients (63.6%) were more infected than their female counterparts. Patients (72.7%) in the intensive care unit (ICU) were most infected with this organism. The isolates showed 100% resistance to both amikacin and ciprofloxacin and 90.9% to both ceftriaxone and ceftazidime, while resistance to the other antibiotics used in this study were: piperacillin (81.8%), imipenem (72.7%), gentamycin (72.2%), and meropenem (63.6%). None of the isolates was, however, resistant to colistin. PCR results showed that *blaOXA*, *blaTEM*, and *blaCTX-M* genes were positive in some isolates, while *blaSHV* was not detected in any of the isolates.

This study has revealed that the strains of *A. baumannii* isolated are multiple drug resistant. Regular monitoring, judicious prescription, and early detection of resistance to these antibiotics are, therefore, necessary to check further dissemination of the organism.

**Keywords:** *Acinetobacter baumannii*, antibiotic-resistant genes, Nigeria

## Introduction

*Acinetobacter baumannii* has been described as one of the most important opportunistic pathogens that cause nosocomial infections in hospitals of the 21st century. This is because of its multiresistant genetic determinants, ability to tolerate a wide range of humidity, pH, salinity, and its survival on many natural sources [1]. The organism's ability to survive under a wide range of environmental conditions and to persist for extended periods of time on surfaces make it a frequent cause of outbreaks of infections and an endemic health-care-associated pathogen. The aforementioned traits make this pathogen to be ubiquitous in the hospital environment. Outbreaks of *A. baumannii* colonization and infection in intensive

care units have been described as a common problem [2, 3]. *Acinetobacter* infections typically occur in very ill patients and can either cause or contribute to death in these patients. They are frequent colonizers of the throat, skin, respiratory tract, and the digestive tract [4] and infect mostly patients with impaired host defenses. The most common clinical presentation of *A. baumannii* is pneumonia in mechanically ventilated patients in the intensive care units (ICUs) [5] with high mortality rates. Mortality rate for central nervous system (CNS) infection has been revealed to be 20–27% in patients, in whom the organism was isolated from cerebrospinal fluid (CSF) [6]. Other infections caused by this pathogen are bacteraemia, wound infections, secondary meningitis, urinary tract infections, peritonitis, osteomyelitis,

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keratitis, and native-valve endocarditis [7]. Invasive procedures involving endotracheal tube, urinary catheter insertions, central venous catheter, ventriculography, myelography, lumbar puncture, exposure to antibiotics, increased length of hospital stay, exposure to patients colonized with *A. baumannii*, environmental contamination, immunosuppression, emergency admission, respiratory failure or mechanical ventilation, and poor adherence of staff to hand hygiene are the risk factors for the infections caused by *A. baumannii* [8].

*A. baumannii*, which was susceptible to antibiotics many years ago [1], is now a multidrug-resistant opportunistic human pathogen that is a frequent cause of nosocomial outbreaks worldwide. *A. baumannii* strains that are resistant to all major antibiotic classes normally used to treat infections with them, including  $\beta$ -lactams, aminoglycosides, fluoroquinolones, chloramphenicol, tetracycline, and rifampin, are now emerging, and the prevalence of these multidrug-resistant *A. baumannii* strains leaves limited clinical options for treatment [1], underscoring the need to develop novel antibiotics for bacterial pathogens in general and gram-negative pathogens in particular.

Although nosocomial infections caused by *A. baumannii* have been reported worldwide [9], very little has been reported on the prevalence and antibiotic susceptibility of *A. baumannii* isolates in Nigeria. The aim of this work was, therefore, to assess the prevalence and the current level of antimicrobial resistance pattern of the isolates in Ladoke Akintola University of Technology (LAUTECH) Teaching Hospital.

## Materials and methods

### Collection of clinical specimens

Tracheal aspirate, blood, urine, sputum, CSF, and wound swabs of 150 patients (89 male and 61 female) were collected aseptically and transported immediately to the research laboratory for microbiological analysis. The study population comprised of patients who have had surgery, on ventilators or intubation, and prior history of antibiotic use.

### Identification of *Acinetobacter baumannii* from clinical specimens

Samples from the patients were cultivated onto MacConkey agar and incubated at 37 °C for 24–48 h under aerobic conditions. *Acinetobacter species* were primarily identified on the basis of their Gram's reaction and cultural characteristics, further biochemical tests were done with the aid of the API 20NE (Bio Merieux, France) to identify their different strains.

### Antimicrobial susceptibility testing

Antimicrobial susceptibility of the isolates to imipenem, meropenem, ceftazidime, piperacillin, amikacin, gentamicin, ciprofloxacin, colistin, and ceftriazone which were obtained from Oxoid Company, UK was determined by using the disc diffusion method. The results were interpreted according to the manufacturer's instructions and CLSI guidelines [10].

### Amplification of extended spectrum $\beta$ -lactamase genes

Polymerase chain reaction (PCR) was used to detect genes encoding resistance to the extended spectrum  $\beta$ -lactams: *blaOXA*, *blaTEM*, *blaCTX-M*, and *blaSHV* according to the method described by Olowe et al. [11]. The list of primers that were used is shown in Table 1.

### Data analysis

Data are presented as frequencies and percentages. Statistical analysis was performed using the chi-square ( $\chi^2$ ) test.

### Ethical approval

Clearance of research involving human subjects was granted by the Ethics and Research Committee of the Ladoke Akintola University Teaching Hospital, Osogbo, Nigeria for this study.

**Table 1.** Primers used for the amplification of genes

Primer	Sequence 5 <sup>1</sup> –3 <sup>1</sup>	Gene	Product size (bp)	Annealing temp. (°C)
OXA-F	GCGTGGTTAAGGATGAACAC	<i>blaOXA</i>	620	56
OXA-R	CATCAAGTTCAACCAACCG			
CTX-M F	CGATGTGCAGTACCAGTAA	<i>blaCTX-M</i>	585	60
CTX-M R	TTAGTGACCAGAATAAGCGG			
TEM F	CCCCGAAGAACGTTTTTC	<i>blaTEM</i>	517	51
TEM R	ATCAGCAATAAACCAGC			
SHV F	AGGATTGACTGCCTTTTTT	<i>blaSHV</i>	393	56
SHV R	ATTTGCTGATTCGCTCG			

## Results

Majority of the *A. baumannii*-positive patients, 10 (90.9%), were in the age bracket 41–70 (Table 2). The blood sample of a 1-year-old patient who had malaria sepsis was also positive for this organism. The male patients, 7 (63.6%), were more positive for *A. baumannii* than their female counterparts, 4 (36.4%) (Table 2). A larger percentage of the isolates, 8 (72.7%), were isolated from the ICU, 2 (18.2%) from the surgical wards, and 1 (9.1%) from the paediatric wards (Table 2). The risk factors for the acquisition of *A. baumannii* infection were age >40 years and <10 years, mechanical ventilation and comorbidities, es-

pecially malaria sepsis (Table 3). *A. baumannii* was mostly isolated from the blood samples of patients followed by tracheal aspirate (Table 3).

A high resistance of *A. baumannii* isolates was seen for ciprofloxacin (100%), amikacin (100%), ceftriaxone (90.9%), ceftazidime (90.9%), piperacillin (81.8%), imipenem (72.7%), gentamycin (72.2%), and meropenem (63.6%) while they were all susceptible to colistin (100%) (Table 4).

The *blaOXA* gene was present in two isolates (18.2%), *blaCTX-M* carriage was found in five strains (45.5%), and also *blaTEM* gene in five (45.5%). The *blaSHV* gene was not detected in any of the isolates (Table 5).

**Table 2.** Frequency of isolation of *A. baumannii* from the patients distributed by age, sex, and hospital wards

Variable	Number of patients	No. of <i>A. baumannii</i>
Age (years)		
<10	10 (6.7%)	1 (9.1%)
10–20	12 (8%)	0
21–30	29 (19.3%)	0
31–40	44 (29.3%)	0
41–50	30 (20%)	3 (27.3%)
51–60	11 (7.3%)	4 (36.4%)
61–70	8 (5.3%)	3 (27.3%)
>70	6 (4%)	0
Sex		
Male	89 (59.3%)	7 (63.6%)
Female	61 (40.7%)	4 (36.4%)
Ward		
Surgical	78 (52%)	2 (18.2%)
Pediatric	27 (18%)	1 (9.1%)
Accident and emergency	26 (17.3%)	0
Intensive care unit	19 (12.7%)	8 (72.7%)

**Table 3.** Clinical characteristics of patients that were *A. baumannii* positive

Patient no.	Sex/age	Underlying condition	Sample
P 1	F/1	Malaria sepsis	Blood
P 2	M/56	Hepatoencephalopathy	Sputum
P 3	M/61	Chronic obstructive pulmonary disease with mechanical ventilation	Tracheal aspirate
P 4	F/64	Sepsis	Blood
P 5	F/49	Chronically ill sepsis with mechanical ventilation	Tracheal aspirate
P 6	M/48	Road traffic accident, with mechanical ventilation	Blood
P 7	M/60	Upper GIT bleeding	Blood
P 8	M/55	Sepsis	Urine
P 9	F/63	Sepsis	Blood
P 10	M/51	Malaria sepsis	Blood
P 11	M/47	Head injury with mechanical ventilation	Tracheal aspirate

P = patient, F = female, M = male, GIT = gastrointestinal tract

**Table 4.** The antibiotic resistance patterns of *A. baumannii*

Antibiotics	Number sensitive (%)	Number intermediate (%)	Number resistant (%)
Meropenem	3 (27.3)	1 (9.1)	7 (63.6)
Imipenem	1 (9.1)	2 (18.2)	8 (72.7)
Ceftazidime	0	1 (9.1)	10 (90.9)
Ceftriazone	0	1 (9.1)	10 (90.9)
Colistin	11 (100)	0	0
Ciprofloxacin	0	0	11 (100)
Piperacillin	2 (18.2)	0	9 (81.8)
Gentamicin	2 (18.2)	1 (9.1)	8 (72.7)
Amikacin	0	0	11 (100)

**Table 5.** Extended spectrum  $\beta$ -lactamase (ESBL) genotypes in *A. baumannii*

ESBL genes	Number amplified (%) primers used
Single ESBL genes	
<i>blaTEM</i> only	5 (45.5%)
<i>blaSHV</i> only	0
<i>blaCTX-M</i> only	5 (45.5%)
<i>blaOXA</i> only	2 (18.2%)
Two ESBL genes	
<i>blaOXA</i> + <i>blaCTX-M</i>	2 (18.2%)

## Discussion

*A. baumannii* is usually a healthcare-associated pathogen affecting the very ill patients and is emerging as a cause of numerous global outbreaks [12]. Out of the 150 patients that were screened in this study, 8.5% of them were *A. baumannii* positive which corresponds to the findings of similar studies carried out by Siau et al. [13], Nabil et al. [14], and Namita et al. [15] with their percentage prevalence being 11%, 8.8%, and 9.4%, respectively.

Age has been discussed as a major factor associated with the acquisition of *Acinetobacter* [16]. Although the sample size used in this study was smaller, patients in the age bracket 41–70 years were more susceptible to *A. baumannii* infections. This is similar to the report of Mera et al. [17], that is, people of the working age have a higher risk of developing *A. baumannii* than do elderly people. This may be attributed to the fact that this category of people is constantly shifting their locations due to their job placements and as such is more prone to malaria attacks and accidental traumas. In this study, seven (63.6%) of the affected patients were male. Similar result was reported in a study conducted in a tertiary care hospital in South India in which 58% of the patients were male [18].

About 72.7% of the *A. baumannii* isolates were isolated from the ICU. This suggests that these strains are opportunistic pathogens infecting mostly the immunocom-

promised patients and may be circulating in the hospital setting. This observation is consistent with other studies where *Acinetobacter* infections were mostly prevalent in the ICU [19, 20].

It was found in this study that the risk factors for the acquisition of *A. baumannii* infections were age, mechanical ventilation, comorbidities, especially malaria sepsis, neurologic impairment, hepatoencephalopathy, upper GIT bleeding, chronic obstructive pulmonary disease, and major trauma which is in concomitance with earlier findings by Baran et al. [21].

*A. baumannii* strains that were isolated in the current study were resistant to multiple classes of antibiotics but were all sensitive to colistin which is in line with the results of Wang et al. [22] whose isolates were also multiply resistant but susceptible to colistin. Contrary to the detection of a high rate of resistance to the carbapenems used in this study, Farahani et al. [23] recorded a very low rate of resistance to this class of antibiotics by their isolates. It is quite possible that the discrepancies associated with this finding were due to the differences in the time of the studies [10]. These differences can also be traced to the type of infections and the kind of antibiotic sensitivity discs used and may also be attributed to the excessive use of this class of antibiotics in the hospital setting where this study was carried out. This assumption is supported by the fact that patients with chronic lung disease are at increased risk of airway colonization and pneumonia, especially when they require intubation. Additionally, intubated patients with chronic pulmonary disease are often treated with prophylactic antibiotics which increase the risk of resistance [24]. Numerous Indian studies from different regions have also documented a low (9%) to a very high (90%) carbapenem resistance in *Acinetobacter* isolates [25]. Carbapenem resistance in *A. baumannii* is a growing concern since it limits the therapeutic options that are left for the treatment of infections caused especially by the strains of this organism.

The presence of *blaTEM*, *blaCTX-M*, and *blaOXA* in most strains of the *A. baumannii* isolates was a confirmation that these bacteria are extended spectrum  $\beta$ -lactamase producers and the presence of more than one of these

genes in an isolate emphasizes the fact that *A. baumannii* is multiply resistant. These genes are plasmid-borne and are easily transmitted from one bacterium to another; this 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.

Owing to the fact that the strains of *A. baumannii* that have been found prevalent within our hospital setup are resistant to virtually all the classes of antibiotics tested against them, the infection caused by these organisms is becoming difficult to treat day by day. Effective management and control of *Acinetobacter* infections do not appear to have a simple approach. We suggest the use of colistin to treat infections caused by them. Colistin, however, has a high toxicity to humans, so then, what options do we have left to treat infections caused by these superbugs? We, therefore, suggest routine surveillance, judicious prescription, and early detection of resistance to commonly used antibiotics in order to forestall the menace of multiple drug resistant *A. baumannii* isolates.

### Conflict of interest

There are no conflicts of interest to declare.

### Funding sources

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