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**Phenotypic Profiling and Prevalence of MDR *Acinetobacter baumannii* in Comparison with Molecular Characterization in University of Ilorin Teaching Hospital, Ilorin, Kwara State, Nigeria.**Mohammed, T.O.<sup>1</sup>, Omosigho, P.O.<sup>2</sup>, Baba, R.T.<sup>2</sup>, Mohammed-Nasir Ramat, O.<sup>2</sup>, Isah, S.B.<sup>2</sup>Department of Medical Laboratory Science, Faculty of Allied Health Science, Elizade University, Ilara-Mokin Ondo State<sup>1</sup>, Department of Medical Laboratory Science, Faculty of Applied Health Science, Edo State University Iyamho, Edo State, Nigeria<sup>2</sup>

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toyeebmohammed8@gmail.com/toyeeb.mohammed@elizadeuniversity.edu.ng/+234-703-628-4843/+234-803-067-6973. <https://dx.doi.org/10.4314/sokjmls.v10i4.5>**Abstract**

*Acinetobacter baumannii* is a major opportunistic pathogen responsible for hospital-acquired infections, often associated with multidrug resistance (MDR). Timely and accurate detection of MDR strains is critical for infection control and antimicrobial stewardship. This study aimed to determine the phenotypic prevalence and molecular characteristics of *A. baumannii* isolates in a tertiary hospital in Nigeria. A total of 36 clinical isolates presumptively identified as *A. baumannii* were obtained from various wards and specimen types at the University of Ilorin Teaching Hospital. Phenotypic and biochemical testing was done for identification. MDR status was defined as resistance to at least one agent in three or more antimicrobial categories. All 36 isolates underwent molecular confirmation through PCR targeting the 23S rRNA gene. Further screening for resistance genes (e.g., blaOXA, KPC, IMP) was performed on selected MDR isolates. Out of the 36 isolates, 34 (94.4%) were phenotypically confirmed as multidrug-resistant. The highest resistance was observed against gentamicin, ceftazidime, and imipenem. PCR confirmed all 36 isolates as *A. baumannii* with successful amplification of the 23S rRNA gene (~580 bp). Molecular screening revealed the presence of key resistance genes in several MDR isolates, confirming the genetic basis of their resistance. There is a high prevalence of MDR *A. baumannii* in the study setting, with strong concordance between phenotypic and molecular findings. Routine molecular diagnostics alongside phenotypic methods is recommended for early detection and containment. The study

underscores the urgent need for enhanced infection control and antimicrobial resistance surveillance programs.

**Keywords:** *Acinetobacter baumannii*, multidrug resistance, PCR, antimicrobial susceptibility, Nigeria, molecular characterization.

**Introduction**

*Acinetobacter* is a genus of strictly aerobic, Gram-negative, non-motile, catalase-positive, oxidase-negative bacteria commonly found in soil, water, and hospital environments (Towner, 2020). They are coccobacilli, often occurring in pairs, with variable morphology and can survive on dry surfaces for weeks, contributing to their persistence in healthcare settings. *Acinetobacter* species are generally low in virulence but can cause serious infections, especially in immunocompromised or critically ill patients, including ventilator-associated pneumonia, bloodstream infections, and soft tissue infections (Kumar *et al.*, 2021).

*Acinetobacter* also colonizes human skin and mucous membranes, with some evidence suggesting a protective role against allergies in healthy individuals. The bacteria's environmental resilience, metabolic versatility, and virulence factors like fimbriae and capsular polysaccharides contribute to its ability to cause nosocomial infections and spread in hospital settings (Guryanova, 2019).

Carbapenem susceptibility shows considerable geographic variation. According to the SENTRY Antimicrobial Surveillance Program

(2013–2016), the lowest meropenem susceptibility rates were observed in Latin America (13.7%), followed by the Asia-Pacific region (21.0%), Europe (22.2%), and the United States (54.9%) (Gales *et al.*, 2019). A later study (2016–2018) by Seifert *et al.* (2022) showed similar trends, with the lowest rates in Africa and the Middle East (17.2%), followed by Latin America (19.6%), Asia–South Pacific (31.4%), Europe (33.8%), and North America (63.6%). The WHO and European Centre for Disease Prevention and Control (ECDC) reported that in 2020, among 38 countries in Europe, 35 had carbapenem resistance rates 50%, with only three countries (Ireland, the Netherlands, and Norway) reporting rates below 1%.

Colistin resistance was rare until the late 1990s, with the first reported case from the Czech Republic in 1999. However, data from the SENTRY Program indicated a marked decline in colistin susceptibility from 2005–2008 to 2013–2016. Europe saw a drop from 99.2% to 89.6%, Asia-Pacific from 99.1% to 93.7%, and North America from 98.4% to 93.6% (Castanheira *et al.*, 2023).

In Africa, multidrug-resistant *A. baumannii* (MDRAB) has been implicated in approximately 4.7% of all ICU infections. Reported antibiotic resistance rates for *A. baumannii* in Africa are highly variable, ranging from 31.8% to 92.1% for cephalosporins, 8.8% to 88.8% for carbapenems, 28.8% to 91.6% for fluoroquinolones, 30% to 90.3% for aminoglycosides, and 12.2% to 89.9% for  $\beta$ -lactam/ $\beta$ -lactamase inhibitor combinations such as piperacillin/tazobactam (Agyepong *et al.*, 2023).

Despite this, several studies have shed light on regional patterns, particularly in East and West Africa. In Nigeria, for example, hospital-based studies have reported *A. baumannii* prevalence rates ranging from 8.5% to 9.0% (Odewale *et al.*, 2016). Alarming high resistance rates were observed in one study: 100% resistance to amikacin and ciprofloxacin, 90.9% to ceftriaxone and ceftazidime, and 81.8%, 72.2%, 72.7%, and 63.6% to piperacillin, gentamicin, imipenem, and meropenem, respectively (Nwadike *et al.*, 2014). These findings highlight the consequences of suboptimal antibiotic stewardship protocols in many Nigerian hospitals.

## METHODOLOGY

### STUDY AREA:

This study was conducted in the University of Ilorin Teaching Hospital which is located in Oke-Ose, Ilorin East local government area of Kwara State. The University of Ilorin Teaching Hospital (UITH) belongs to the second (2nd) generation of Teaching Hospitals in the country

### STUDY POPULATION

The study populations were patients admitted into Medical wards, Surgical wards and Intensive care unit (ICU) of University of Ilorin Teaching Hospital, Ilorin Kwara State from March, 2024 to February 2025 whose clinical samples were sent to the Medical Microbiology Laboratory to isolate *Acinetobacter spp.*

### SPECIMEN COLLECTION

Various clinical samples were collected through aseptic techniques by medical practitioners to prevent contamination. For optimal results, specimen was collected into a clean sterile, wide-bore containers. The clinical samples were samples already sent to the laboratory for investigation which included urine, pus, blood, fluid (pleural, pericardial, synovial and peritoneal), wound swab, cerebrospinal fluid (CSF), sputum, central venous pressure (CVP), catheter tip, stool, throat swab, ear swab, nasal swab from the tip of Foley catheter, tissue (wound and soft tissues), and bronchial washing samples. The sent samples to the Medical Microbiology and Parasitology Laboratory of the University of Ilorin Teaching Hospital were processed for microbiological analysis and isolates of *Acinetobacter baumannii* were isolated from the clinical samples.

### STUDY PERIOD

The study was conducted between March, 2024 and February, 2025

### SAMPLE SIZE DETERMINATION

Sample size was determined using the sample size formula for estimating single proportion

$$n = \frac{z^2 pq}{d^2}$$

Where,  
N=Minimum Sample Size

Z= Standard normal deviation, usually set at 1.96 which corresponds to 95% confidence level

P= The best estimated prevalence of target MDR *Acinetobacter*; 8% from a study, = 0.5% (Niang *et al.*, 2006).

Q= 1-p; 1-0.5=0.5

d= Minimum statistically significant difference to be measured at 0.15 precision

Hence  $N = \frac{1.96 \times 1.96 \times 0.05 \times (1-0.5)}{0.15 \times 0.15}$

N=43.

However, 36 clinical isolates were isolated and used due to the fact that multiple isolates (from different sites) were not isolated from some patients.

### CHARACTERIZATION OF BACTERIAL ISOLATES

Colonial morphology of suspected colonies from both Blood agar and MacConkey agar (Hi Media Laboratories Pvt Ltd, Mumbai, India, M173) media plate was characterized based on their appearances. Gram staining was done on the isolates and biochemical testing such as oxidase, catalase and triple sugar iron was further done on the gram negative, coccobacillus isolates for characterization. The isolated *Acinetobacter spp* was sent to Clinical Virology Laboratory, College of Medicine University of Ibadan, Oyo State, Nigeria for molecular studies.

### ETHICAL CLEARANCE

Ethical approval was obtained from the Ethical Review Committee of the University of Ilorin Teaching Hospital, Ilorin.

### STATISTICAL ANALYSIS

Data obtained was fed into a computer and analyzed using statistical package for the social sciences (SPSS) version 20.0. Data was presented in frequency tables, charts and prose. Cross tabulation of important table was also be done. Continuous data was presented as means. The distribution of categorical data was compared with chi-square ( $X^2$ ) tests and presented as absolute count and percentage. Relationship between multidrug resistant *Acinetobacter species* shown by the sequencing parameters and the antibiotics was ascertained. Similarly, their correlation was examined.

### INCLUSION CRITERIA

All isolates that are *Acinetobacter species* characterized by the biochemical test was further examined molecularly

### EXCLUSION CRITERIA

All isolates that are not *Acinetobacter species* was left out of the study

### ISOLATION AND IDENTIFICATION OF *ACINETOBACTER. SPP*

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

### MOLECULAR IDENTIFICATION OF *ACINETOBACTER. SPECIES*:

#### DNA EXTRACTION

Extraction of DNA was done by using ethanol precipitation after phenol: chloroform: isoamyl alcohol (24:25:1 v/v) treatment as previously described (Gumińska *et al.*, 2018). Briefly, 200 µl of 24 hours suspected *Acinetobacter spp* culture will be transferred into 2 ml sterile tube, to which 500 µl of lysis buffer (eBioscience™ Thermo Fisher Scientific, USA) and 20 µl of proteinase K (200 µg/ml) (Thermo Fisher Scientific, USA) will be added. This will then be vortexed and incubated at 65°C for 1hour. The lysate will be extracted twice with 500 µl 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 hours 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 was washed with 70% ethanol. After a final centrifugation step, the supernatant was carefully removed, DNA pellet was allowed to air-dry and finally resuspended in 100  $\mu$ l of sterile distilled water. The extracted DNA was stored at -20°C until required for further study.

### POLYMERASE CHAIN REACTION

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 will be used. Amplification reactions will be 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 was analyzed on a 1.5% agarose gel containing 0.5% ethidium bromide and visualized under U.V. illumination. A band corresponding to 850 bp will be gel purified using the QIA quick gel extraction kit (QIAGEN, Thermo Fisher Scientific, USA).

### SEQUENCING

The purified PCR product was subjected to cycle sequencing using the Big Dye terminator v3.1 cycle sequencing kit (Applied Bio systems, USA). Sequencing reactions was prepared as a 6  $\mu$ l reaction mix containing; Big Dye Direct Sequencing Master mix (2  $\mu$ l), sequencing

primer (MP13Forward/ Reverse primer) (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 was 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 was shown. The confidence probability (multiplied by 100) that the interior branch length is greater than 0, s 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 was computed using the Maximum Composite Likelihood method (Tamura *et al.*, 2004) and are in the units of the number of base substitutions per site. This analysis involved 20 nucleotide sequences. All ambiguous positions was removed for each sequence pair (pairwise deletion option). The total positions in the final dataset was determined. Evolutionary analyses will also be conducted in MEGAX (Kumar *et al.*, 2018)

### RESULTS

During the period of this study, Blood, wound swab, Endocervical swab, Urine, Pleural fluid, Exudate of 36 isolates were isolated over a period of a year.

**Table 1: Prevalence of *Acinetobacter baumannii* by Age group**

Age Group	Number of Patients	Percentage
0–20	7	19.44%
21–40	15	41.66%
41–60	9	26.00%
60+	5	13.88%

**Age (Mean ± SD) = 29.75 ± 21.60**

Table 1 shows the demographic distribution of patients with *Acinetobacter baumannii*. The highest number of patients is in the 21-40 age group, followed by 41-60 years and 0-20 years. There is a significant prevalence in adults aged 21-60, with a slightly higher proportion of patients in the 21-40 range.

**Table 2: Demographics – Gender**

Gender	Frequency	Percentage
Female	20	55.6
Male	16	44.4

Descriptive statistics showed a nearly equal distribution between genders with 16 males (44.4%) and 20 females (56%). The mean age of patients was 29.75 years, indicating that *Acinetobacter* infections affect a wide age range, from neonates to elderly.

**Table 3: Ward- Frequency of Isolates**

Ward	Frequency	Percentage
NICU	6	16.9
FMW	5	13.9
MMW	5	13.9
EPU	4	11.1
MSW	4	11.1
FSW	4	11.1
GOPD	3	8.3
SOPD	2	5.6
GYNAE	2	5.6
EMOG	1	2.8

The highest number of isolates were from clinical samples obtained from Neonatal Intensive Care Unit (NICU), Female Medical Ward (FMW), and the Male Medical ward (MMW). These wards were likely high-burden zones for infection, possibly due to underlying patient vulnerability and invasive procedures.

**Table 4: Specimen- Distribution of Isolates**

Specimen	Frequency	Percentage
Wound Swab	18	50.0
Blood	11	30.6
Urine	4	11.1
Pleural Fluid	2	5.6
Endocervical Swab	1	2.8

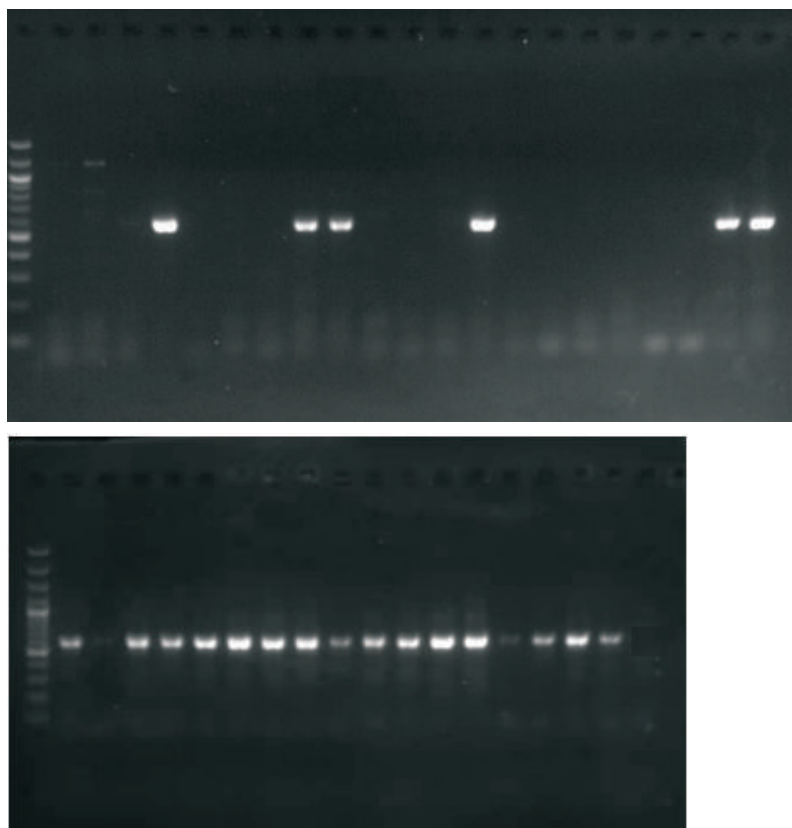
Wound swab and blood samples constituted the majority of isolates, suggesting that surgical site infections and bloodstream are the major presentations of most isolates.

**Table 5: MDR VS Non-MDR**

Group	Frequency	Percentage
MDR (R)	34	94.4
Non-MDR (S)	2	5.6

As shown in Figure 5, the most commonly resisted antibiotics were Gentamycin (GN), Ceftazidime (CAZ), Imipenem (IMI), and Colistin-related compounds (CLP).

**Figure 1: PCR Amplified Images of *Acinetobacter baumannii***

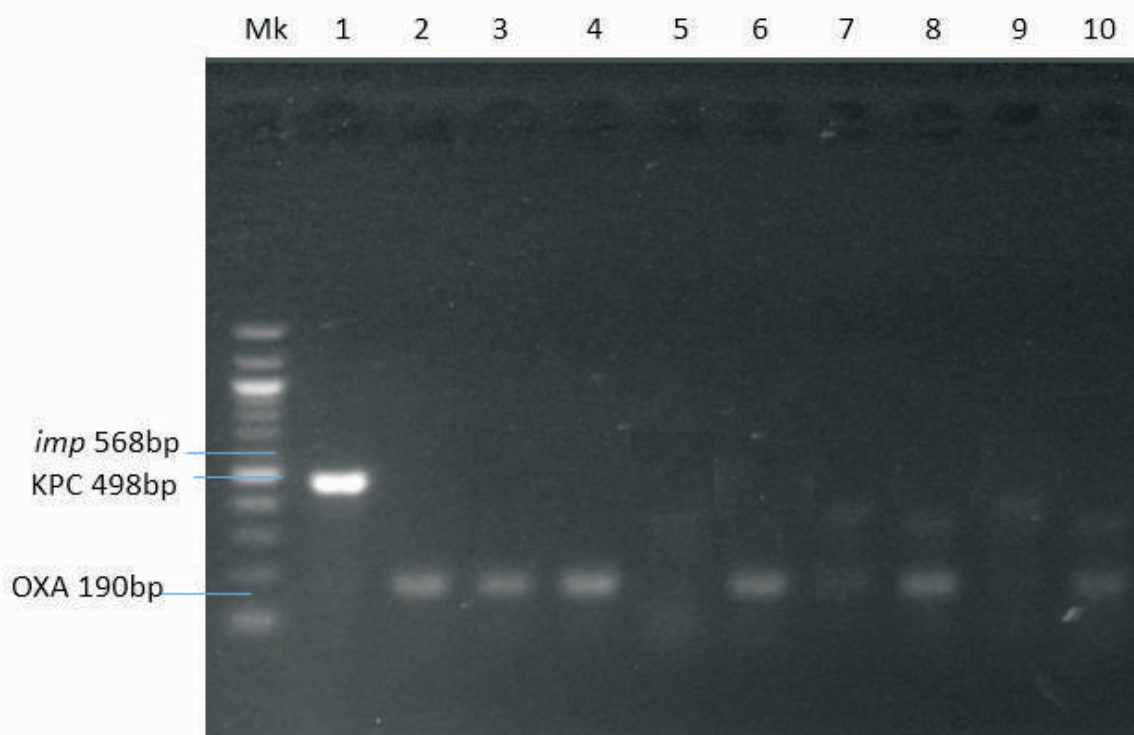


Agarose gel electrophoresis of the PCR products for the identification of *Acinetobacter baumannii*. (Band size approximately 580bp). Gel images show positive *Acinetobacter baumannii*

Gel electrophoresis confirmed the presence of *Acinetobacter baumannii* through amplification of the 23S rRNA gene (~580bp). Multiple samples tested positive with distinct visible bands

Agarose gel electrophoresis of the PCR products of ESBL KPC IMP and OXA gene amplified from selected bacteria isolates. (Band size approximately 498bp 568BP and 109bp indicating KPC IMP and OXA respectively).

**Figure 2: PCR Amplified Images of *Acinetobacter baumannii***



Agarose gel electrophoresis of the PCR products of ESBL KPC IMP and OXA gene amplified from selected bacteria isolates. (Band size approximately 498bp 568BP and 109bp indicating KPC IMP and OXA respectively).

## DISCUSSION

The phenotypic isolation of *Acinetobacter* species typically begins with culture on selective and differential media such as MacConkey agar and blood agar. Colonies usually appear as smooth, opaque, and non-pigmented. Unlike fermenters like *E. coli*, *A. baumannii* does not ferment lactose, hence appears as colorless colonies on MacConkey agar (Peleg *et al.*, 2008).

Biochemical tests are traditionally used to identify non-fermenting Gram-negative bacilli. *A. baumannii* is oxidase-negative, catalase-positive, non-motile, and capable of growing at 44°C. Commercial systems like API 20NE or automated systems such as VITEK 2 have been widely used; however, they may misidentify closely related *Acinetobacter* species (Howard *et al.*, 2012).

**Molecular Confirmation:** Given the limitations of phenotypic methods, molecular tools such as PCR have become standard for confirming *A. baumannii* identity. In this study, isolates were confirmed via amplification of the 23S rRNA

gene, a conserved genetic marker that produces a band of approximately 580 base pairs (bp) upon electrophoresis. The 23S rRNA gene is preferred due to its stability, universality among bacteria, and sufficient species-level variation for differentiation (Turton *et al.*, 2006). PCR primers targeting species-specific regions of the 16S-23S rRNA spacer or housekeeping genes like *gyrB*, *recA*, and *blaOXA-51*-like are also commonly used in literature to differentiate *A. baumannii* from other *Acinetobacter* species (Zander *et al.*, 2013).

In the context of this research, the consistent amplification of a ~580 bp band across multiple isolates on agarose gel confirmed the presence of *A. baumannii*, thus validating culture-based presumptive identification.

The phenotypic and molecular data converge to demonstrate a high burden of MDR *A. baumannii* in University of Ilorin Teaching Hospital. The prevalence (94.4%) is consistent with other Nigerian reports such as Nwadike *et*

al. (2014), who documented high resistance in ICU isolates. The dominance of blaOXA-51 and blaOXA-23 mirrors global trends, as these genes often mediate carbapenem resistance (Evans & Amyes, 2014). The detection of blaNDM in some isolates is particularly concerning, since metallo- $\beta$ -lactamases degrade a wide spectrum of  $\beta$ -lactams.

Molecular confirmation via gene detection adds rigor to phenotypic findings and helps clarify the genetic underpinning of resistance. Phylogenetic clustering suggests potential nosocomial spread within wards, emphasizing the importance of robust infection control

In Conclusion, Combining culture, biochemical, and molecular identification ensures high accuracy in diagnosing *A. baumannii*. The application of PCR targeting 23S rRNA is a reliable method for confirming the identity of this clinically significant pathogen, especially in resource-limited settings where misidentification can lead to treatment failure.

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