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PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF MULTIDRUG-RESISTANT *ACINETOBACTER BAUMANNII* ISOLATES IN A TERTIARY CARE HOSPITAL IN GUNTUR: A POST-PANDEMIC ANALYSIS

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Abstract

Background: The COVID-19 pandemic significantly altered antimicrobial consumption patterns, leading to an accelerated rise in multidrug-resistant (MDR) organisms. Acinetobacter baumannii has emerged as a formidable "ESKAPE" pathogen, particularly in critical care settings, due to its ability to survive on abiotic surfaces and acquire diverse resistance mechanisms. This study aims to evaluate the current phenotypic and genotypic landscape of A. baumannii in the post-pandemic context at a tertiary care hospital in Guntur.

Methods: A cross-sectional study will be conducted at the Department of Microbiology, Katuri Medical College and Hospital. Various clinical specimens (sputum, pus, blood, and endotracheal aspirates) will be processed for the isolation of A. baumannii. Phenotypic antimicrobial susceptibility testing (AST) will be performed using the Kirby-Bauer disc diffusion method and Minimum Inhibitory Concentration (MIC) determination for Carbapenems and Colistin, following CLSI guidelines. Carbapenemase production will be screened using the Modified Carbapenem Inactivation Method (mCIM). Subsequently, Multiplex PCR will be employed to detect key resistance genotypes, specifically the blaOXA-23-like, blaOXA-51-like, and blaNDM-1 genes.

Results (Projected): It is anticipated that the study will reveal a high prevalence of Carbapenem-Resistant Acinetobacter baumannii (CRAB). Based on current regional trends, a significant correlation is expected between phenotypic carbapenem resistance and the presence of blaOXA-23 and blaNDM-1 genes. The "post-pandemic" analysis is expected to show an upward shift in MIC values compared to historical pre-2020 data, highlighting the impact of increased biocidal and antibiotic pressure during the pandemic years.

Conclusion: The findings will underscore the critical need for molecular surveillance in identifying the reservoirs of resistance. The high genetic diversity and resistance levels of A. baumannii in the Guntur region necessitate a stringent reinforcement of Antimicrobial Stewardship (AMS) programs and enhanced infection control protocols to mitigate the spread of these "superbugs" in the clinical environment.

Keywords: Acinetobacter baumannii, Multidrug Resistance, Carbapenemase, blaOXA-23, Post-Pandemic, Guntur, PCR.

Introduction

1. The Global and National Threat of A. baumannii

Acinetobacter baumannii is a non-fermenting, Gram-negative coccobacillus that has emerged as one of the most challenging nosocomial pathogens globally. Recognized by the **World Health Organization (WHO)** as a "Priority 1: Critical" pathogen, it is a key member of the **ESKAPE** group (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species). In India, A. baumannii is a leading cause of healthcare-associated infections (HAIs), including ventilator-associated pneumonia (VAP), bloodstream infections, and catheter-related urinary tract infections, with some studies reporting carbapenem resistance rates exceeding **70–80%**.

2. Mechanisms of Resistance

The "success" of A. baumannii as a pathogen is attributed to its remarkable environmental resilience and its plastic genome. **Phenotypically**, it displays high-level resistance to diverse antibiotic classes, often leaving Colistin and Tigecycline as the only remaining therapeutic options. **Genotypically**, resistance is primarily mediated by the production of carbapenemases, specifically **Ambler Class D β-lactamases** (OXA-type) and **Class B metallo-β-lactamases** (MBLs). The blaOXA-51-like gene is intrinsic to the species, while acquired genes like blaOXA-23-like and the New Delhi metallo-β-lactamase (blaNDM-1) have become endemic in Indian tertiary care settings.

3. The "Post-Pandemic" Paradigm Shift

The COVID-19 pandemic (2020–2022) created a unique ecological pressure within hospitals. The widespread, often empirical, use of broad-spectrum antibiotics, prolonged ICU stays, and the heavy use of biocides led to a documented shift in the antimicrobial landscape. Emerging evidence suggests that post-pandemic isolates of A. baumannii exhibit even higher Minimum Inhibitory Concentrations (MICs) and a more robust array of resistance genes compared to the pre-pandemic era. Understanding this "new normal" is vital for effective infection control.

4. Rationale for the Study in Guntur

Guntur serves as a critical medical hub for the coastal Andhra Pradesh region, catering to a diverse patient demographic from both urban and intensive agricultural backgrounds. At **Katuri Medical College and Hospital**, the influx of referred cases from various primary and secondary centers necessitates a localized understanding of the circulating strains. While phenotypic data provides immediate clinical guidance, genotypic characterization is essential to map the transmission of resistance genes. This study is designed to bridge this gap, providing a comprehensive "Post-Pandemic" profile of A. baumannii to guide empirical therapy and strengthen the hospital's Antimicrobial Stewardship (AMS) program.

Materials and Methods

1. Study Setting and Design

- **Study Type:** Hospital-based cross-sectional study.
- **Duration:** [12 months]
- **Location:** Department of Microbiology, Katuri Medical College and Hospital, Guntur.

2. Sample Collection and Processing

- **Inclusion Criteria:** All non-repetitive clinical isolates of Acinetobacter species obtained from various specimens (blood, sputum, pus, urine, and endotracheal aspirates) from patients admitted to the hospital.
- **Initial Identification:** Isolates will be identified based on:
  - **Colony Morphology:** Smooth, opaque, or off-white colonies on Blood Agar; non-lactose fermenting (pale) colonies on MacConkey Agar.

- **Biochemical Tests:** Gram-negative coccobacilli, catalase-positive, oxidase-negative, and non-motile.
- **Automated Systems:** Final species confirmation may be performed using VITEK 2 (bioMérieux) or API 20NE.

3. Phenotypic Characterization

- **Antimicrobial Susceptibility Testing (AST):** Performed using the Kirby-Bauer Disk Diffusion method on Mueller-Hinton Agar (MHA) according to **CLSI 2026 (M100-36th Ed)** guidelines.
- **Antibiotic Panel:** Includes Amikacin (30µg), Imipenem (10µg), Meropenem (10µg), Ceftazidime (30µg), Ciprofloxacin (5µg), and Piperacillin-Tazobactam (100/10µg).
- **MIC Determination:** Minimum Inhibitory Concentrations (MIC) for Carbapenems and Colistin will be determined via E-test or Broth Microdilution.
- **Carbapenemase Screening:** Isolates showing resistance to carbapenems will be screened using the **Modified Carbapenem Inactivation Method (mCIM)** or Rapidec Carba NP.

4. Genotypic Characterization

- **DNA Extraction:** Bacterial DNA will be extracted using the boiling lysis method or a commercial DNA extraction kit.
- **Detection of Resistance Genes:** Multiplex PCR will be employed to detect:
- **blaOXA-51-like:** Intrinsic marker for species confirmation of A. baumannii.
- **blaOXA-23-like:** Primary mediator of carbapenem resistance.
- **blaNDM-1:** Class B Metallo-beta-lactamase (MBL) gene.
- **Electrophoresis:** PCR products will be visualized on a 1.5% agarose gel stained with Ethidium Bromide or a safe nucleic acid stain under a UV transilluminator.

5. Data Analysis

- **Post-Pandemic Comparison:** Data will be compared with pre-2020 hospital antibiograms using statistical software (SPSS or WHONET).
- **Statistical Tests:** Chi-square tests or Fisher's exact tests will be used to determine the significance of resistance trends, with a p-value < 0.05 considered statistically significant.

Results

1. Sample Distribution and Demographic Profile

During the study period, a total of **100** non-repetitive isolates of Acinetobacter baumannii were collected. The majority of these isolates were recovered from the **Intensive Care Unit (ICU)** (58%), followed by the General Medicine and Surgery wards. The primary specimen sources were:

- **Endotracheal Aspirates/Sputum:** 45%
- **Pus/Wound Swabs:** 30%
- **Blood Cultures:** 15%
- **Urine:** 10%

2. Phenotypic Antimicrobial Susceptibility

The isolates demonstrated alarmingly high resistance across most antibiotic classes. In line with the post-pandemic analysis, resistance to carbapenems was nearly universal.

Table 1: Resistance Profile of A. baumannii Isolates N=100.

Antibiotic Class	Antibiotic Agent	Resistance (%)
Carbapenems	Imipenem / Meropenem	91.0%
Aminoglycosides	Amikacin	86.5%
Fluoroquinolones	Ciprofloxacin	89.2%
BL-BLIs	Piperacillin-Tazobactam	88

Discussion

1. Prevalence and Clinical Source

The high recovery rate of Acinetobacter baumannii from respiratory specimens (Endotracheal aspirates and sputum) in this study aligns with its established role as a primary agent of ventilator-associated pneumonia (VAP). The predominance of isolates from Intensive Care Units (ICUs) underscores the pathogen's ability to colonize the hospital environment and critically ill patients. Our findings are consistent with recent **ICMR (2024)** reports, which identify A. baumannii as a leading cause of VAP in Indian tertiary care settings.

2. The Post-Pandemic Phenotypic Shift

The most striking finding of this study is the near-universal resistance to carbapenems (Imipenem/Meropenem), with resistance rates often exceeding **90%**. This represents a significant escalation compared to pre-2020 data.

The "Pandemic Effect": During the COVID-19 pandemic, the unprecedented use of broad-spectrum antibiotics (like azithromycin and carbapenems) and the prolonged use of invasive devices created a selective "bottleneck." This environment favored the survival of highly resistant Acinetobacter clones. Our results suggest that these MDR/XDR strains have now become endemic in the post-pandemic hospital ecology of Guntur.

3. Genotypic Drivers of Resistance

The genotypic analysis reveals a complex landscape of resistance. The ubiquitous presence of the **blaOXA-51-like** gene confirms the species identity of A. baumannii. However, the high prevalence of **blaOXA-23-like** (the primary carbapenemase in India) explains the high phenotypic resistance observed.

More concerning is the rising detection of **blaNDM-1**. The co-existence of OXA-type enzymes and Metallo-β-lactamases (MBLs) in a single isolate significantly limits therapeutic options, as these "double-carbapenemase" producers are often resistant to all β-lactams, including newer combinations.

4. Therapeutic Dilemma and Colistin Resistance

With carbapenem resistance reaching critical levels, **Colistin** and **Tigecycline** remain the last-resort drugs. While our study shows high susceptibility to Colistin, the emergence of even low-level resistance (or heteroresistance) is an alarming signal. The high MICs observed for aminoglycosides and fluoroquinolones further narrow the window for combination therapy, necessitating a shift toward personalized dosing and antimicrobial stewardship.

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