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Molecular Analysis of Oxacillinase Genes in MDR Strain of Acinetobacter baumannii Isolated in Clinical Samples from North India

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ABSTRACT

Background: *Acinetobacter baumannii* is a ubiquitous coccobacillus which is currently a topic of great concern due to its high level of drug resistance to almost all classes of antimicrobials. Due to this it results in high level of morbidity and mortality in hospitalizedpatients especially in critical care areas. This opportunistic pathogen in short span of time has acquired high level of resistance against carbapenem which are one of the last resorts in our antimicrobial arsenal.

Aim and Objective: To study the molecular analysis of oxacillinase genes in MDR strain of *Acinetobacter baumannii* isolated in clinical samples from North India.

Material and Methods: Clinical samples received for bacterial culture and antimicrobial sensitivity testing were inoculated and incubated for 24-48 hours at 37°C. For blood culture incubation was done for 5 days in blood culture bottle. Bacterial identification and antimicrobial sensitivity were done by Vitek®2, bioMérieux. Antimicrobial Sensitivity of *A. baumannii*isolated was recorded and multi drug resistant isolates were preserved for Polymerase chain reaction(PCR) to detect the presence ofbeta lactam OxacillinaseblaOXA 51 and blaOXA58 genes.

Results: A total of 2644 samples were processed during the study period from 30th April,2022 to 20th October,2024. 920 samples were culture positive. Out of 920 bacterial isolates, 88 were *A. baumannii* and 84 out of these were Carbapenem Resistant *A. baumannii*(CRAB). 30 CRAB isolates were selected for PCR and all showed presence of blaOXA 51 gene while none of the isolates harbored blaOXA 58 gene.

Conclusions: There is an increased isolation rate of *A. baumannii* from clinical samples and more than 95% isolates of *A. baumannii* are CRAB. All the CRAB isolates tested by PCR showed presence of blaOXA 51 gene and none had the presence of blaOXA 58 gene. Management of *A. baumannii* infections is a challenge. In the current scenario proper implementation of Hospital Infection Control policies and adherence to antimicrobial stewardship policies as preventive strategies should be our priority.

Keywords: *Molecular, Oxacillinase, Genes, MDR, Acinetobacter baumannii*

1. INTRODUCTION

Genus Acinetobacter comprises of around 54recognized bacterial species and these areGram-negative non-fastidious, non-motile, catalase-positive, and oxidase negative coccobacilli These are ubiquitous in nature and thrive well in moist environments.[1]Clinically significant members of this genus are grouped in the *Acinetobacter calcoaceticus–baumannii* (*Acb*) complex comprising of 05 species and *A. baumannii* is the most notable pathogen of this complex and currently remains one of the most antimicrobial resistant bacteria on the planet. [2]*A. baumannii* is emerging as a prominent pathogen in critical care areas in hospitals and is frequently isolated from patients of ventilator associated pneumonia (VAP), followed by catheter associated urinary tract infections (CAUTI), bloodstream infections (BSIs), and surgical site infections (SSIs)and results in high morbidity and mortality.[2]*A. baumannii* apart from being a common multi drug resistance organism (MDRO)is

also a biofilm producer and has also adapted itself to various stresses like desiccation and common disinfectants in hospital settingand survives on hospital surfaces for long time.[3] Coupled with this property of long term survival in hospital environment and indiscriminate use of antimicrobials along with poor infection prevention control(IPC) practices these pathogen gain entry in the debilitated patientsespecially in critical care areas.[3]For resistance against betam lactam antibiotics *A. baumannii*harboursAmpC genes which are mostly non-inducible along with blaOXA genes. *A. baumannii*has become resistant to almost all carbapenemsdue to its large variety of OXA genes predominantlyblaOXA-23, blaOXA-24, blaOXA-51 and blaOXA-58 along with a few novel blaOXA genes.[4] The OXA-51-like subgroup is intrinsic to *A. baumannii* occurring in almost all the strains.[6, 7]blaOXA 23 is the most common carbapenemase.[8]On itself blaOXA-51 enzymes act as weak carbapenemase but in presence of Insertion Sequence Acinetobacter baumannii 1 (ISAba1)) its production increases manifold making it an effective carbapenemase.[8]These Carbapenem resistant Acinetobacter baumannii(CRAB) are generally resistant to various other antimicrobials also and are being isolated frequently thereby World Health Organization(WHO) has placed CRAB in the critical group in the list of bacteria that possess unprecedented risk to human health.[8]In thepresent study, a collection of well-characterizedCRABisolates obtained from various clinical specimens of infected or colonized patients were tested for the presence of different blaOXA genes.

2. MATERIAL AND METHODS

The study was conducted in the Department of Microbiology, Integral Institute of Medical Sciences and Research and Cadmus Clinical Laboratory located in Lucknow. Samples for the study comprised of Broncholaveolar lavage(BAL), mini BAL, Endotracheal aspirate, Transtracheal aspirate, Sputum, Pleural Fluid, Blood, Pus, Urine and Throat swab. Properly collected and well labelled samples timely transported to the laboratory and stored under proper condition were included in the study. Improperly labelled, leaking, improperly transported and improperly stored samples were excluded from the study.

Samples were processed on 5% Sheep Blood agar and MacConkey agar of HimediaBiosciences™ and incubated aerobically at 37°C for 24 - 48 hours. Blood samples were collected in Bactec®blood culture bottles and incubated at 37°C in BacT/Alert 3D®automated blood culture system. Blood samples having bacterial growth were detected by the machine and material from positive flagged bottles were inoculated on 5% Sheep Blood agar and MacConkey agar of HimediaBiosciences™. Blood culture bottles were incubated for 05 days before labelling the blood sample sterile.

Bacterial colonies of samples showing growth were picked from Blood agar and were re-inoculated inID-GN cards, Vitek®2 for bacterial identification through biochemical reactions[5]and AST N-406 card for antimicrobial sensitivity as per the manufacturer's instructionsand incubated in the Vitek®2 semiautomatic system by bioMérieux.

Sensitivity of *A. baumannii* isolates was recorded. Isolates of *A. baumannii* resistant to both Imipenem and Meropenem were further stored in glycerol nutrient broth for PCR to detect the blaOXA genes. Consent was taken from patients or their attendants whose sample yielded CRAB isolates during the dispersal of their report.

For PCR, bacterial DNA was extracted using Xploregen Bacterial gDNA Column filter Based Extraction Kit®. Extraction process was performed as per the manufacturer protocol. PCR mastermix constituted of 1) High-Fidelity Taq DNA Polymerase, 2) 2.5mM dNTPs. 3) 3.2mM MgCl2, 4) PCR Enzyme Buffer, 5) Forward Primer, 6) Reverse Primer, 7) 10X Taq DNA polymerase Assay Buffer, 8) RNAse Free water. Separate PCR was performed for blaOXA 58 and blaOXA 51. The blaOXA 51 was 641 base pair(bp) and blaOXA 58 was 453 bp long.

Primer Details

Oligo Name	Sequence (5`à 3`)	Tm (°C)	GC- Content
blaOXA 58Forward	5' AGTATTGGGGCTTGTGCT 3'	43	50.00%
blaOXA 58Reverse	5' AACTTCCGTGCCTATTTG 3'	41	44.44%
blaOXA 51 Forward	5'AACAAGCGCTATTTTTTATTTCAG 3'	44	29.17%
blaOXA 51 Reverse	5' CCCATCCCCAACCACTTTT 3'	46	52.63%

The PCR product was used for gel electrophoresis using agarose gel.ethidium bromide dye was mixed with the PCR product and gel electrophoresis was done. Base pair Ladder, positive control for 600 bp and 400 bp and also a negative control were used along with PCR product as internal control. Bio Rad gel documentation system™ was used to get the X Ray picture of the agarose gel after gel electrophoresis.

3. RESULTS

In total 2644 samples were processed, 920 samples were culture positive. Out of 920 bacterial isolates, 88 were *A. baumannii*

and 84of these were CRAB.Sputum sample yielded the maximum 28 isolates of *A. baumannii* followed by Endotracheal aspirate(27) and Tracheal aspirate(12). From blood culture 6 *A. baumannii* isolates were recovered, 5 from BAL, 4 from Pus and 2 from tracheostomy aspirate. Pleural fluid, Urine, semen and Throat Swab yielded 1 isolate each. Semen sample which yielded *A. baumannii*. was collected from a patient of chronic prostatitis. All the patients from whose sample *A. baumannii* were isolated were inpatients. 49 patients were admitted in Intensive care unit, 25 were in Medicine ward, 10 patients belonged to surgery ward and 04 patients were from Obstetrics & gynecology ward.In our study *A. baumannii* was more frequently isolated from male patients (64.7%) in comparison to female patients(35.3%).

Table 1:Clinical samples harboring *A. baumannii*

S. No.	Specimen	Total isolates	Male	Female
1	Sputum	28	21	7
2	Endotracheal Aspirate	27	17	10
3	Tracheal aspirate	12	10	2
4	Blood	6	3	3
5	Broncho Alveolar Lavage	5	1	4
6	Pus	4	3	1
7	Tracheostomy aspirate	2	0	2
8	Pleural Fluid	1	0	1
9	Urine	1	0	1
10	Semen	1	1	0
11	Throat Swab	1	1	0
Total		**88**	**57**	**31**

Table 2: Distribution of *A. baumannii* in patients of various ward

Unit/ Ward	Number of isolates (n=88)	Percentage
Intensive Care Unit	49 Isolates	55.00%
Medicine Ward	25 Isolates	28.40%
Surgery Ward	10 Isolates	11.36%
Obstetrics and Gynecology	4 Isolates	04.54%

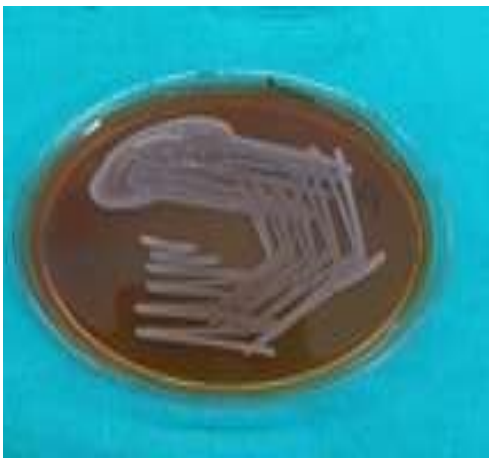


Figure 1a: Colony characteristics of *A. baumannii on* MacConkey Agar

Figure 1b: Colony characteristics of *A. baumannii on* 5% Sheep Blood Agar

Figure 2: A Bactec™ Blood Culture Bottle after positive growth. Bacteria produce Carbon dioxide as a byproduct of metabolism. The sensor at the base of the bottle changes colour in presence this gas which in turn is detected by the BacT/Alert3D machine.

Figure 3: Antimicribial Sensitivity Card of Vitek®2 system. A semiautomatic diagnostic modality, it is based on the principle of colorimetry. The machine matches the colour change in each of the 64 blocks on the card with the database and gives the sensitivity result in Minimum inhibitory concentration values

Antimicrobial **Susceptibility:**

AntimcrobialSusceptibility tests were performed on the Vitek®2 system. 95% (84 of 88) isolates of *A.baumannii*isolates were MDRO.Amiakcinwas sensitive in 6.81 % cases,Gentamicin in only 4.54% cases, Cefepime was sensitive in only 3.40% cases. All the beta lactams including carbapenem were only around 5% sensitive or even less. Fluoroquinolone sensitivity was just around 3%,Trimethoprim/sulfamethoxazole was 12.50% sensitive. Minocycline was 50% sensitive.Colistin sensitivity was86.37%.

Molecular characterization of oxacillinase genes

Of the 84 MDRO *A.baumannii*, 30 were tested for the OXA51 and OXA58 genes by the PCR. All the isolates had presence of OXA51 and all of them tested negative for OXA58 gene.

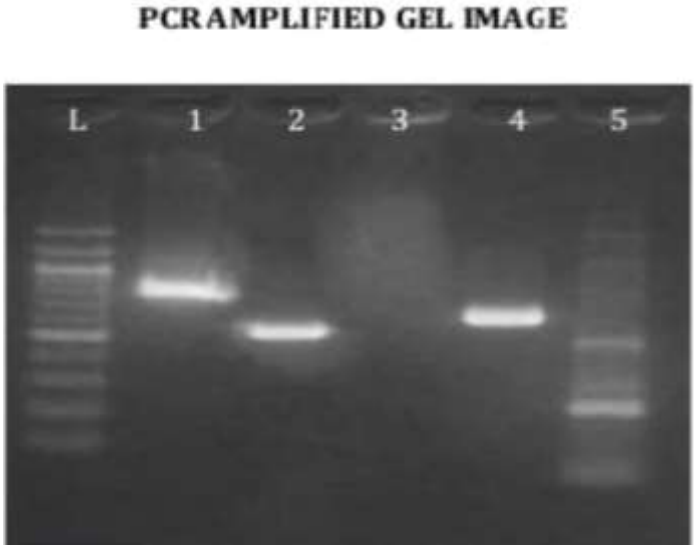
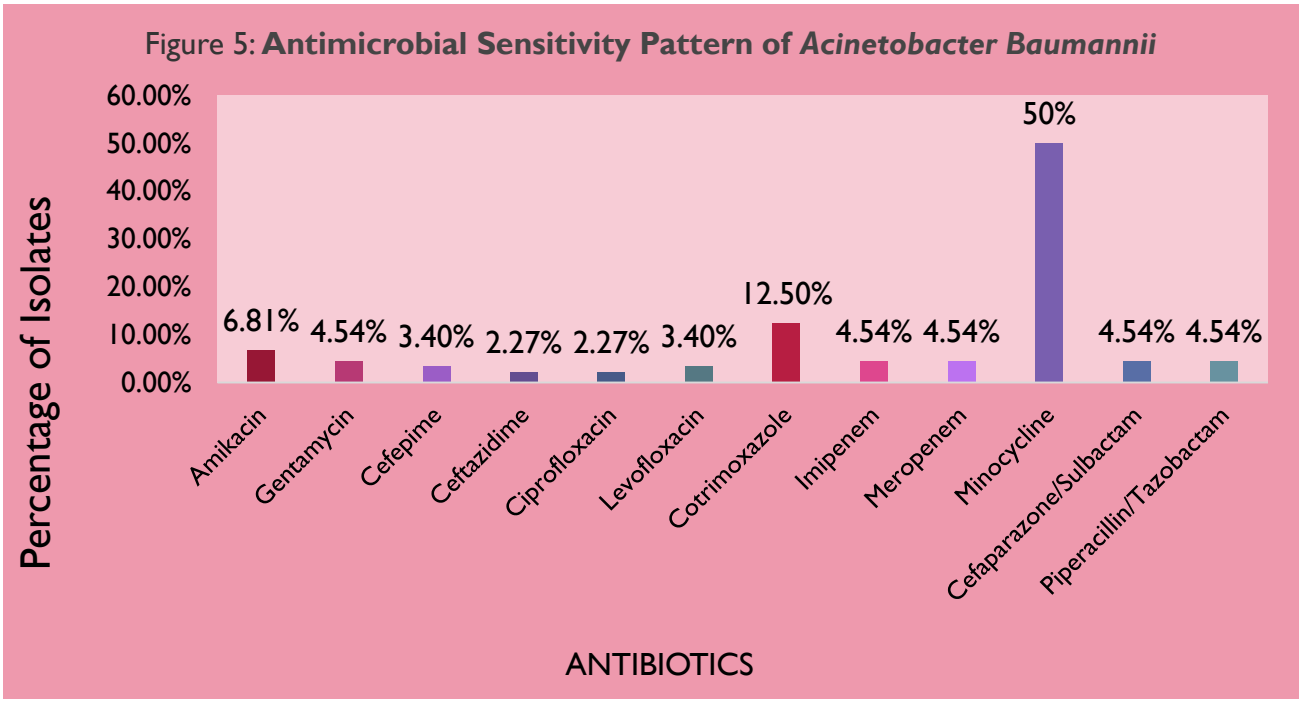


Figure 4: L-Base pair Ladder,
1- positive control for 600 bp,
2- positive control for 400 bp
3- Negative Control
4- Sample PCR product of blaOXA51
5- Sample PCR product of blaOXA58



4. DISCUSSION

The present study shows the alarming resistance rate of *A.baumannii* against all the available antimicrobials. In our study 95% of the isolates were MDRO. Carbapenems which formed the mainstray of treatment against MDROs so far stood at a mere 5% sensitivity. Colistin (86% sensitive), Minocycline(50% sensitive) and Trimethorim-Sulphamethoxazole(12.5% sensitive) were the only drugs which had sensitivity in double digits. Combined with its resistance to dessication, dsinfectants

this MDRO is a real challenge for treatment of patients in critical care.[3]Often considered the last resort Colistin had the maximum activity against *A.baumannii*in our study also but the Vitek[2] machine results for colistin sensitivity have never been satisfactory as showed by various studies.[4,10,11, 12]. Although tigecycline had shown promising results against *A.baumannii* with sensitivity of around 80-90% but it was not tested in our study[13].The almost non-existent sensitivity against Beta lactams in our study is like a prophecy come true for studies since 1999 which showeda progressive decline in the susceptibility to β-lactams antibiotics against*A. baumannii* and it had been documented several times over the past few decades [13,14]. Carbapenems resistance was mainly attributed to the spread of genes encoding for oxacillinase (blaOXA-like). In the early 2000s, OXA-58 oxacillinase were the most widespread in Italy and neighboring countries, but their prevalence has drastically decreased over the years, with the onset of OXA-23, which gradually replaced blaOXA-58.[15]In our study also none of the isolates tested showed the presence ofOXA-58 and all the isolates had presence blaOXA-51. Although blaOXA-51 is not a major carbapenemase alone on itself but in presence of ISAba1upstream in the DNA it carbapenemase activity upscales tremendously.[8]In our study we could not test ISAba1 but more studies should be done to see whether it is also playing some role in increasing the resistance against carbapenems.Managing Acinetobacter infections is challenging due to the pathogen's MDR nature. Varaiya et al., was found an increasing trend in resistance to carbapenems by Acinetobacter, Pseudomonas, and Klebsiella spp.[17]. Although future of newer antimicrobials seems promising with the advent of artificial intelligence and various other modalities but for the present, preventive strategies should be our main stray.[18, 19]

5. CONCLUSION

The main aim of this study was to achieve a baseline background to determine the resistant pattern of oxacillinase producing *A. baumannii* in clinical samples. Result from this study affords valuable knowledge to perform further large prospective study to identify other mechanisms involved in Carbapenem resistance in *A. baumannii*.

The high level of resistance of *A. baumanniiagainst all available antimicrobials is an unprecedented challenge*. As the saying goes "prevention is better than cure" so we all are required to emphasis as much as possible on the infection prevention and control practices in all healthcare centers across the globe. In the current scenario it seems like prevention is all we can do because cure is really a challenge provided all our antimicrobial options are losing their potency against this pathogen at an alarming rate. With the development of artificial intelligence and other modalities, the future of newer antimicrobials appears bright, but for the time being, our primary focus should be on prevention measures.

Declaration:

Conflicts of Interest: There is no any conflict of interest associated with this study.

Consent to participate: There is consent to participate.

Consent for publication: There is consent for the publication of this paper.

Authors & contributions: Author equally contributed the work.

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