



## Prevalence and Molecular Characteristics of Carbapenem Resistant *Acinetobacter baumannii* Isolates from A Regional Tertiary Care Hospital

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**Abstract:** *Acinetobacter* spp., is an emerging opportunistic nosocomial Gram negative bacterial pathogen with increasing prevalence in particular the species *Acinetobacter baumannii*. It infects the most vulnerable immunocompromised hospitalized patients who are critically ill. Significant levels of morbidity and mortality have been reported with outbreaks and the carbapenem hydrolyzing beta lactamases that includes MBLs and oxacillinases are recognized as important contributors of carbapenem resistance in *Acinetobacter* spp., Enzymatic degradation of drugs, target modifications, multidrug efflux pumps and permeability defects are some of the important resistance mechanisms present in *A. baumannii*. Accumulation of various resistance mechanisms made treatment of *A. baumannii* infection very difficult. The major objective of the study was to identify the pattern of antibiotic resistance and its regional prevalence. Hence this study was aimed and conducted to isolate, identify and distinguish the antibiogram of *A. baumannii* from clinical specimens and to study a molecular level identification of resistance mechanisms of the isolates from a tertiary care hospital. Various clinical specimens like blood, urine, abscess, vaginal swab were analyzed and evaluated for the presence of *Acinetobacter*. Four members of *Acinetobacter* species; *A. junii*, *A. lowffii*, *A. ursingii* and *A. baumannii*, were isolated from clinical specimens. *A. baumannii* was the predominant species and 15% of the *A. baumannii* isolates were confirmed to be resistant to carbapenems. A molecular typing was done to identify the genes conferring antibiotic resistance and five major genes were identified in the isolates. The predominant genes present in the isolates were OXA-58, OXA-23 and GIM. Presence of IMP & VIM were also identified.

**Keywords:** *Acinetobacter baumannii*, Multidrug resistance, Carbapenems, Molecular typing, OXA-23, OXA-58, GIM

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## I. INTRODUCTION

*Acinetobacter* spp., is an emerging opportunistic nosocomial Gram negative bacterial pathogen with increasing prevalence in particular the species *A. baumannii*. *Acinetobacter* genus has undergone significant taxonomic modifications over the last 30 years and the species *A. baumannii* is identified as one of the most troublesome pathogen. It infects the most vulnerable immunocompromised hospitalized patients who are critically ill. Medically relevant species, such as *A. calcoaceticus*, *A. lowffii*, *A. nosocomialis*, and *A. pittii*, have been found on vegetables, meat, dairy products, and human skin<sup>2</sup>. *A. baumannii* strains harbouring extensive antibiotic resistance have contaminated commercial food, including meat, vegetables, and various types of livestock, suggesting multiple environmental routes of transmission into human populations<sup>3</sup>. *A. baumannii* has emerged to a major nosocomial pathogen from a relatively low virulent commensal bacteria and it is a causative agent for severe infections like bacteraemia, pneumonia, urinary tract infections and wound infections<sup>4,5</sup>. Significant levels of morbidity and mortality have been reported<sup>6</sup> with outbreaks and common infections include ventilator associated pneumonia and bacteremia; less frequently burn wounds and urinary tract<sup>7</sup>. *A. baumannii* is also a common cause of bloodstream infections in the intensive care setting<sup>8</sup> and the lower respiratory tract infections and intravascular devices<sup>9-12</sup> are reported to be the common sources. The risk factors of the infection with multidrug resistant *Acinetobacter* spp., include prolonged hospital stay, exposure to an intensive care unit, receipt of mechanical ventilation, colonization pressure, exposure to antimicrobial agents, etc.<sup>13</sup> As the multidrug resistant *Acinetobacter* spp., infection usually occurs in severely ill patients in the ICU, the mortality rate is high up to 68%<sup>14</sup>. In recent years, a substantial increase in *A. baumannii* associated with nosocomial pneumonia cases are reported<sup>15</sup>. 'Peleg et al., 2008 reported that the *A. baumannii* ranks with 10<sup>th</sup> among the organisms causing monomicrobial blood stream infections. There is a need for novel therapeutic options owing to the emergence of isolates resistant to drug choice like carbapenems. In recent studies done by Chang et al., 2015 also revealed high prevalence of CRAB<sup>16</sup>, upto 60% of total isolates. Other researchers also found a high prevalence rate of CRAB in nosocomial infections<sup>17,18</sup>. In a study conducted by Henwood et al., 2002<sup>19</sup>, among consecutive *A. baumannii* isolates collected, more than 85% were resistant to cephalosporins, 43% were resistant to gentamicin and 46% were resistant to quinolones, leaving carbapenems as the only drug active against more than 90% of isolates. Carbapenems are the drugs of choice for the treatment of nosocomial infections. But resistance to this class of antibiotic is emerging, and leading to the evolution of pan resistance strains and to the need of new therapeutic options<sup>20,21</sup>. Carbapenem resistant *Acinetobacter* are becoming widespread in several regions of the world<sup>22</sup>. Mechanistically, resistance to these potential beta lactams may be due to impaired permeability resulting from altered outer membrane proteins or to alterations in the penicillin binding proteins (PBP)<sup>23</sup>. However, the carbapenem hydrolyzing beta lactamases that include MBLs and oxacillinases are recognized as important contributors of carbapenem resistance in *Acinetobacter*. Resistance offered by oxacillinases is more often than MBLs<sup>24</sup>. There are four major OXA subgroups (OXA-51, OXA-23, OXA-40 and OXA-58) associated with *A. baumanii*. In order to control the spread of *Acinetobacter baumannii* in the hospital, it is necessary to distinguish the outbreak strain and its characteristics. The major objective of the study was to

identify the pattern of antibiotic resistance and its regional prevalence. Hence this study was aimed and conducted to isolate, identify and to distinguish the antibiogram of *A. baumannii* from clinical specimens and to study a molecular level identification of resistance mechanisms.

## 2. MATERIALS AND METHODS

### 2.1 Study Design and Area

The study was conducted at Sunrise Institute of Medical Sciences (SIMS), Kerala, India. This study was reviewed and approved by the institutional ethical committee of Sunrise Institute of Medical Sciences (SIMS/IEC/03/2022).

### 2.2 Sample Collection and Isolation

The samples were collected from patients admitted in the hospital in various departments as well as from Out patients. Various clinical specimens like urine, blood, pus, abscess and endo-tracheal aspirations were screened for the presence of *Acinetobacter* spp., All the samples were collected by aseptic methods<sup>25</sup>.

### 2.3 Selective Culture and Biochemical Identification

All samples were inoculated on to two enriched and selective agar media, 5% sheep blood agar (Biomerieux) and on Mac Conkey agar and incubated at 37° C for 24 to 48 hours. All colonies resembling *Acinetobacter* were initially identified by standard morphological, cultural and biochemical characteristics. And further identification was done by Vitek 2 compact system from Biomerieux India pvt Ltd.

### 2.4 Antimicrobial Susceptibility Testing

Antibiotic susceptibility testing was done by disc diffusion on Mueller Hinton agar (Himedia) plates according to the guidelines of Clinical Laboratory Standards Institute<sup>26</sup>. All the isolates were inoculated in peptone broth and adjusted to McFarland standard and swabbed on Muller Hinton agar. The readymade antibiotic discs from Himedia were placed on the inoculated plates and incubated overnight at 37°C. The diameter of the zone of inhibition was measured and interpreted using CLSI<sup>26</sup> guideline. Along with the disc diffusion method susceptibility was also analysed using AST N280 cards on Vitek 2 compact system. The antibiotics tested include Ampicillin sulbactam(10µg), Ceftazidime (30µg), Ciprofloxacin (5µg), Levofloxacin (5µg), Gentamycin (10µg), Amikacin (30µg), Tobramycin (10µg), Imipenem (10µg), Meropenem (10µg), Piperacillin tazobactam (110µg), Cefepime (30µg), Cefotaxim (30µg) Ceftriaxone (30µg), Tetracyclin (30µg) and Collistin. The quality control of the antibiotic sensitivity was done with *Escherichia coli* ATCC25922.

### 2.5 Isolation of Genomic DNA

Four Carbapenem resistant *Acinetobacter baumannii* isolates were subjected to screening for various genes. The extraction of total genomic DNA using Mag Genome DNA isolation kit procedure as per the manufacturer instructions. Quality of the genomic DNA was assessed using 0.7 % agarose gel along with 1kb DNA ladder as size standard and the quantity of the genomic DNA was assessed in UV-Vis Spectrometer.

## 2.6 Screening for Genes Associated With Antibiotic Resistance

Amplification of genes encodes for antibiotic resistance were carried out for the sample using primers. The details of the genes and primers used are mentioned in table I. Presence or absence of expected band was considered as + ve or - ve

genotype of the strains. PCR-generated amplicon was confirmed and purified using GeneJET PCR purification kit (Thermo Scientific, EU-Lithuania) to remove the primer dimer and other carryover contaminations. The quality of the product was assessed using 2% agarose gel along with 100bp DNA ladder as size standard. The primer sequence used and corresponding genes are listed in table I.

**Table I: Primers used for Antibiotic resistance gene extraction<sup>27</sup>**

Antibiotic Resistance Mechanism	Gene	Primer	Expected Amplicon Size
Beta Lactamase – CLASS D	OXA23	F – GATGTGTACATAGTATTCGTCG R - TCACAACAACTAAAAGCACTG	1065 bp
Novel Class D Beta Lactamase	OXA58	F – CGATCAGAACATGTTCAAGCGC R - ACGATTCTCCCTCTGCGC	528 bp
Metallo beta lactamases (class B)	IMP	F – CTACCGCAGAGTCTTTG R - AACAGTTTGCCTTACCAT	587 bp
Metallo beta lactamases (class B)	VIM	F – AGTGGTGAGTATCCGACAG R - ATGAAAGTGCCTGGAGAC	261 bp
Metallo beta lactamases (class B)	GIM	F – GATTCCAATGAAAAATGTA R - TTAATCAGCCGACGCTTC	762 bp

## 3 RESULTS

### 3.1 Sample Collection and Isolation

A total of 500 clinical samples were collected aseptically from various patients from Sunrise Institute of Medical Sciences, Kochi and a total of 39 isolates of *Acinetobacter* species were identified. *A. baumannii* was confirmed to be the most abundant species (84 %), followed by *A. lowfii*, *A. junii* and *A. ursingii*. (fig 1)

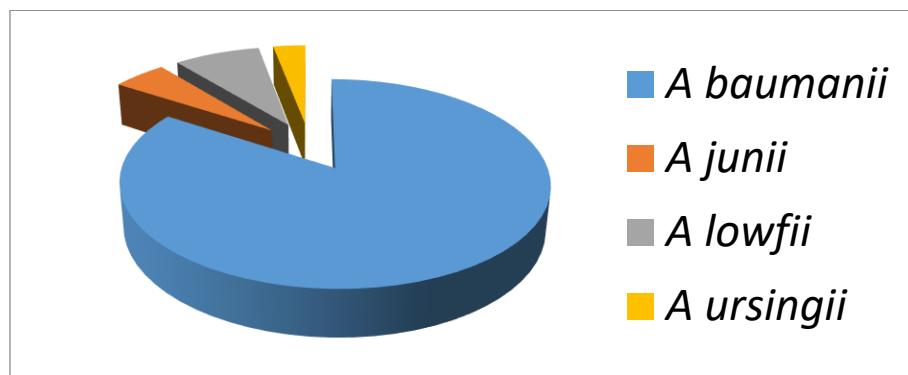


Fig 1: Distribution of various genus of *Acinetobacter* among the isolates

### 3.2 Antibiotic Sensitivity

Out of the 39 isolates, 6 isolates were observed to be multidrug resistant and carbapenem resistant strains. The most susceptible antibiotic was colistin; i.e., 83% of the Carbapenem resistant *Acinetobacter baumannii* (CRAB) isolates

were susceptible to colistin. The observed susceptibility pattern of the CRAB isolated showed that the tobramycin has second highest percentage (77%). Most of the other tested antibiotics were found to be resistant and the pattern of resistance of the isolates were shown in fig 2

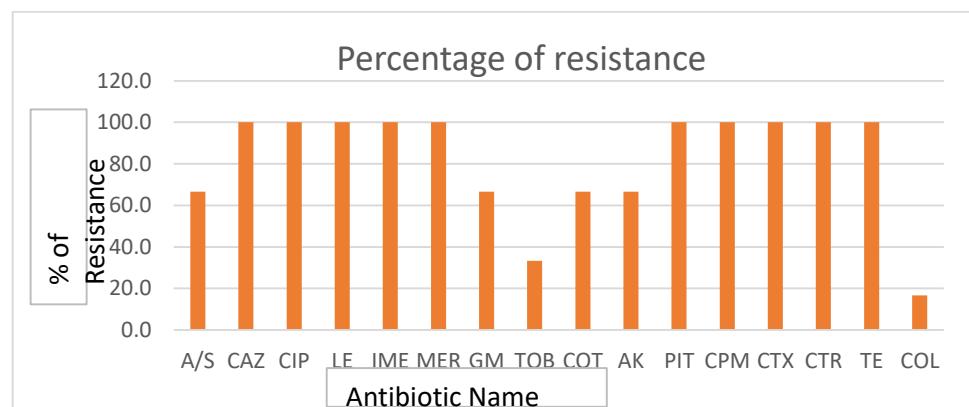


Fig 2: Antibiogram of the CRAB isolates. COL - Colistin; TE - Tetracyclin; A/S - Ampicillin sulbactam; CAZ – Ceftazidime; CIP - Ciprofloxacin; LE- Levofloxacin; GM – Gentamycin; AK –Amikacin; TOB - Tobramycin; IME – Imepenem; MER – Meropenem; PIT - Piperacillin tazobactam; CPM – Cefepime; CTX - Cefotaxim, CTR – Ceftriaxone.

### 3.3 Screening for Genes Associated with Antibiotic Resistance

Four Carbapenem resistant isolates (FEA1, FEA2, FEA3 and FEA4) of *A. baumannii* were subjected to molecular level analysis for the presence of antibiotic resistance associated genes. Screening for the presence of five genes (OXA23,

OXA58, IMP, VIM, GIM) were observed in all four CRAB isolates. (Table 2 & Fig 3). All the four isolates were screened with positive results in the presence of OXA28 and GIM genes. Among these isolates, OXA-23 was also observed as a predominant gene followed by other isolates. IMP and VIM were only present in one isolate.

**Table 2: Antibiotic Resistance genes screened from the CRAB isolates**

Gene	FEA 1	FEA2	FEA3	FEA4
OXA23	-	+	+	+
OXA58	+	+	+	+
IMP	+	-	-	-
VIM	+	-	-	-
GIM	+	+	+	+

Table 2: Four CRAB isolates (FEA1, FEA2, FEA3 and FEA4) and the presence (+) or absence (-) of particular gene



Fig 3. a: Gel image of OXA23 gene presence of 1065 band revealed that the stains/isolates are +ve for OXA23 genotype lane 1 to 4: isolates 1 to 4; lane 5: -ve control; lane 6 :100 bp ladder.

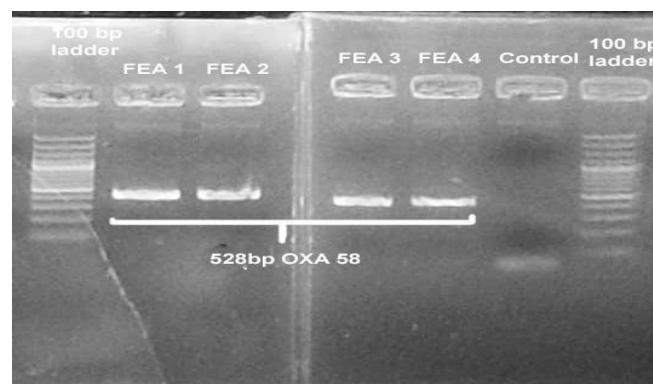


Fig 3. b: Gel image of OXA58 gene presence of 528 band revealed that the stains/isolates are +ve for OXA58 genotype lane 2 to 5: isolates 1 to 4; lane 6: -ve control; lane 1 & 6:100 bp ladder.

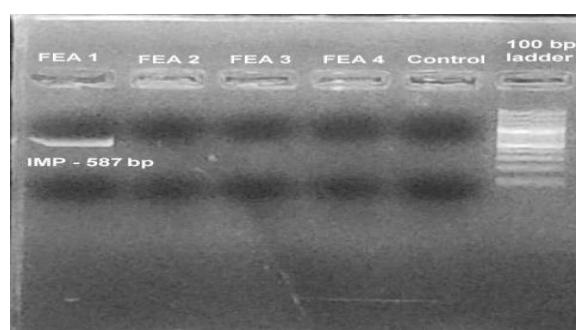


Fig 3. c: Gel image of IMP gene presence of 587 band revealed that the stains/isolates are +ve for IMP genotype lane 1 to 4: isolates 1 to 4; lane 5: -ve control; lane 6 :100 bp ladder.



Fig 3. d: Gel image of *VIM* gene presence of 261 band revealed that the stains/isolates are +ve for *VIM* genotype lane 1 to 4: isolates 1 to 4; lane 5: -ve control; lane 6 :100 bp ladder



Fig 3. e:Gel image of *GIM* gene presence of 762 band revealed that the stains/isolates are +ve for *GIM* genotype lane 1 to 4: isolates 1 to 4; lane 5: -ve control; lane 6 :100 bp ladder.

#### 4 DISCUSSION

This organism has been reported as the most frequent cause of respiratory tract infections and the strains were isolated from 3 to 5% of patients with nosocomial pneumonia. In our study, about 5% of the isolates of *A. baumannii* were from tracheal tube aspiration, and mechanical ventilation was the most important risk factor for these infections. Our present study was confirmed with the work done by Rit and his colleagues in 2012<sup>28</sup>, they observed *A.baumannii* as the prevalent species. Nearly 75% of the isolates were *A. baumannii* and only 25% of isolates were other types of *Acinetobacter*. About 74% of the isolates of this study was *A baumannii* and which is in accordance with Rit et al., 2012<sup>28</sup>. Momtaz et al. (2015)<sup>29</sup> reported that the *A.baumannii* strains were detected in 121 out of 500 human clinical samples (24.2%) which was lower than our results. It is reported<sup>30</sup> in 2012 that the prevalence of *A. baumannii* in various types of clinical infections of 9.4% which was very lower than our results. Siau et al. (1996)<sup>31</sup> reported that the prevalence of *A. baumannii* in the cases of infections in the Korean hospitals was 11% which was lower than our results. We observed the presence of *A. junii* and a total of 5% of the isolates were *A junii*. Previous reports also showed the presence of *A.junii* in clinical specimens<sup>32</sup>. *Acinetobacter* spp., are rapidly spreading with emergence of extended resistance to even newer antimicrobials. In this study we observed a high rate (15% of the isolates) of carbapenem resistance among the isolates and this finding is comparable with previous studies conducted by Ayenew et al., 2021 in Ethiopia<sup>33</sup>. Our results suggest that the carrying *OXA-23* genes is one of the main causes of the carbapenem resistance

phenotype, which is consistent with the previous reports<sup>34,36</sup>. In a study conducted<sup>37</sup> in 2018, it was reported that *OXA-23* is a prevalent mechanism for sulbactam resistance in *A. baumannii*. In our present results were also in accordance with their results. A high rate (67%) of sulbactam resistance was observed among the isolates with *OXA 23* genes. Previous reports showed that *OXA-58* gene as a major reason for carbapenem resistance<sup>38, 39, 40</sup> and it is similar to our results. All the screened carbapenem resistant isolates were positive for the presence of *OXA-58* gene. In this study *GIM* gene was also a predominant one in CRAB isolates and presence of *VIM* gene is comparatively low in the screened isolates, only one isolate was positive for *VIM* gene. But this is in contrast with the previous study<sup>41</sup>. In their study *VIM* (27.45%) was the predominant gene than *GIM* (11.76%) gene.

#### 5 CONCLUSION

The spread of *Acinetobacter* infections are related to several factors like duration of antibiotic usage, patient co-morbidities, virulence of bacteria, etc. The genomic studies revealed important factors of *Acinetobacter* which are contributing to the survival on the hospital settings and pathogenicity of *Acinetobacter baumannii*. Knowledge regarding such factors are important in preparing proper prevention protocols. A local antibiogram pattern database preparation and implementation of antibiotic stewardship based on the antibiogram might help in prevention of development of antibiotic resistance. Proper disinfection protocol development based on the virulence mechanism of the bacteria can help to restrict the *Acinetobacter* infection spread. Hence this study strongly recommends

implementation of proper sterilization protocol and antibiotic stewardship along with the continuous surveillance of spread.

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## 7 AUTHOR CONTRIBUTION STATEMENT

Ms. Fiji E conceived and planned the experiments. Fiji E and Mr. Jijo G Vaghese carried out the experiments with the support from Dr. B. Anandharaj. All authors discussed the results and contributed to the final manuscript. Dr. B. Anandharaj supervised the entire project.

## 8 CONFLICT OF INTEREST

Conflict of interest declared none.

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