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# ANTIBIOTIC SENSITIVITY PATTERN AND GENETIC VARIABILITY STUDIES OF CLINICAL ISOLATES OF LOWER RESPIRATORY PATHOGEN *Acinetobacter baumannii*

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## ABSTRACT

*Acinetobacter* is one of the important opportunistic pathogens involved in hospital acquired infection. They cause various types of human infections leading to morbidity and mortality. Of these *Acinetobacter* species, *Acinetobacter baumannii* is the most prevalent in clinical specimens causing pneumonia. It is very common among inpatients admitted in ICUs for various ailments. Around hundred sputum samples were collected from patients visiting various hospitals of Kanyakumari District, Tamil Nadu. From this clinical specimen, 16 were identified preliminarily as *Acinetobacter baumannii by* various morphological and biochemical tests. The identities of the bacterial isolates were confirmed by 16S rRNA sequence analyses. Antibiotic susceptibility testing was performed by the agar disc diffusion method. Variations were observed in the resistance pattern of the bacterial isolates towards various commercial antibiotics. The genetic variability of *Acinetobacter baumannii* isolates were detected using RAPD assay. The RAPD assay using different primers showed distinct molecular variation among the 16 isolates suggesting the emergence of new resistant strains among the clinical isolates.

**KEYWORDS:** ***A. baumannii,*** **RAPD, Genetic variability, Antibiotic susceptibility.**

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# INTRODUCTION

*Acinetobacter baumannii* has emerged as one of the most difficult nosocomial pathogens globally[1]. These pathogens were found to be ubiquitous in hospital environments. They are frequent colonizers of respiratory and digestive tracts, skin and throat[2, 3]. It has a significant ability to up-regulate or acquire resistance determinants. Infections due to this organism are often very difficult to treat because of the widespread resistance to the major antibiotic groups[4]. The various forms of infections by *A. baumannii* include bacteremia, urinary tract infection (UTI), meningitis, wound and burn infections, and most importantly nosocomial pneumonia, particularly in ventilated patients[5,6,7,8,9]. During the last two decades, *A. baumannii* has become a pathogen of increased clinical importance due to its remarkable ability to cause outbreaks and its ability to acquire resistance to almost all available antibiotics, including the carbapenems (such as imipenem and meropenem)[10, 11]. Different molecular typing methods have been used for epidemiological investigation of outbreaks caused by *Acinetobacter spp*. RAPD-PCR is one of the most rapid and simple methods that generate fingerprints and it can be applied to detect polymorphism in a wide variety of organisms. In RAPD-PCR, random primer sequences may be used in organisms where a specific genome sequence is not known. Random parts of the organism genome are produced, which are expected to be identical among related species, and so similar banding patterns should be produced in gel electrophoresis. This technology is proving to be quite useful in typing strains of bacteria involved in respiratory tract diseases[12]. The aim of the present study was isolation, biochemical characterization and identifying antibiotic sensitivity pattern of the clinical isolates of *A. baumannii* and to study its genetic variability patterns.

# MATERIALS AND METHODS

### *Sample collection*

A total of 100 throat swab samples was aseptically collected from different patients visiting various multispecialty hospitals in Tamil Nadu using sterile cotton swabs. Immediately after collection the samples were inoculated into nutrient broth. These were then transferred to the Microbiology laboratory, Noorul Islam University, Kumaracoil, Tamil Nadu.

### *Isolation and Identification of Acinetobacter baumannii*

In the laboratory under aseptic conditions, the collected specimens were streaked directly on blood agar and MacConkey agar and incubated for 24 hrs at 37ºC. Characteristic colonies from the plates were isolated and then sub cultured to obtain a pure culture. The isolated organisms were identified based on colonial morphology, and various biochemical tests according to standard laboratory methods[13]. Stock cultures were maintained in both agar slant and 20% sterile buffered glycerin. The non hemolytic opaque creamy colonies on blood agar and non lactose fermenting colonies on MacConkey agar were sub cultured on MacConkey agar and incubated for another 24 hours at 37ºC[14].

### *Antibiotic sensitivity tests*

Antibiotic sensitivity tests were performed using disc diffusion method [15]. Commercially available antimicrobial discs of Ampicillin, amikacin, meropenem, imipenem and Cefipime were used for the determination of drug sensitivity. Inhibition zones developed around the discs were measured in millimeter (mm) using a metric ruler according to Clinical Laboratories Standards Institute[16].

### *DNA extraction and PCR amplification*

Amplification of genomic DNA was made on an Agilent cycler 2200 (Germany), using the arbitrary decamers[17]. RAPD primers were purchased from Eurofins, Germany (Table 1.0); these primers included OPA-1, OPA-2, OPA-3 and OPA-4. Amplifications of genomic DNA were performed in 25-µl reaction volumes containing 1.2 units of Taq polymerase (Sangon, Shanghai, China), 10 mM Tris-HCl (pH 9.0), 25 mM KCl, 2 mM MgCl 2, 0.2 mM of each dNTP, 24 ng each of random primer and 40 ng of template DNA.

The cycle program included an initial 75 sec denaturation at 94°C, followed by 45 cycles of 15 sec at 94°C, 30 sec at 42°C and 75 sec at 72°C, with a final extension at 72°C for 7 min. RAPD fragments were separated electrophoretically on 1.5% agarose gels in 1X TBE buffer, stained with ethidium bromide, and photographed on a UV transilluminator using a digital camera. DNA from each band was amplified with the same primer more than once, and the banding patterns were compared[18].

**Table 1**
***RAPD primers***

| S. No | Primer name | Sequence |
|---|---|---|
| 1 | OPA-01 | CAGGCCCTTC |
| 2 | OPA-02 | TGCCGAGCTG |
| 3 | OPA-03 | AGTCAGCCAC |
| 4 | OPA-04 | AATCGGGCTG |

***Cluster Analysis and Construction of Phylogenetic tree***

The presence or absence of each individual band of the DNA from RAPD analysis was recorded for each lane on the gel representing a given sample. The data thus obtained was analyzed by Neighbour - joining method and the binary output was used to generate a phylogenetic tree for the sixteen bacterial isolates[19].

## RESULTS AND DISCUSSION

***Isolation and Identification of Acinetobacter baumannii***

*Acinetobacter baumannii* is a dreadful pathogen and spread easily in infected *or* colonized patients and persist in hospital environments for many days[18]. Of the 100 bacterial isolates collected from patients only 16 strains that were identified as *A. baumannii* in the preliminary biochemical analyses were taken for further studies. *Acinetobacter baumannii* strains grew well on usual culture media and produce a pale yellow to white greyish pigment on the solid medium. The colonies were not pigmented when they grew on blood agar. All the strains were gram negative and non motile. These strains had the capacity to produce acid from glucose and lactose. All strains were positive to Simmons citrate, catalase and oxidative fermentation. The negative reactions were: the acid production from sucrose, $H_2S$ on TSI and gas production, mannitol, indole and oxidase[20]. The results biochemical tests are presented in table 2.

**Table 2**
***Biochemical tests for the strains of Acinetobacter baumannii***

| S.No | Biochemical tests | Result |
|---|---|---|
| 1 | Glucose | + |
| 2 | Lactose | + |
| 3 | Sucrose | - |
| 4 | $H_2S$ Production | - |
| 5 | Gas Production | - |
| 6 | Mannitol | - |
| 7 | Motility | - |
| 8 | Citrate | + |
| 9 | Indole | - |
| 10 | Catalase | + |
| 11 | Oxidase | - |

***Antibiotic sensitivity tests***

Antimicrobial agents that were tested against the pathogen include the Carbapenems (Imipenem and Meropenem), Amikacin, Cefexime and Ampicillin. Combination therapy can be considered, but is controversial due to no proven improvement in mortality and increased toxicity[21]. The

antibiotic sensitivity test revealed a definite pattern. The isolates 14, 4 and 12 were resistant to ampicillin, amikacin and cefexime whereas, they were moderately sensitive to meropenem and imipenem. The isolates 11, 15, 10 and 5 were moderately sensitive to ampicillin, amikacin and cefexime whereas they are highly sensitive to carbopenems. The other isolates were sensitive and highly sensitive to the above antibiotics. In general all the isolates were either resistant or slightly sensitive to ampicillin, amikacin and cefexime whereas they were moderately or highly sensitive to carbopenems. According to an earlier study of 113 blood culture isolates of *Acinetobacter* sp., only one isolate was found to be resistant to carbopenems[22]. The antibiotic susceptibility of *Acinetobacter baumannii* isolates are listed in table 3 & Fig.1.

**Table: 3**
***Antibiotic Susceptibility of Acinetobacter baumannii***

| Isolate | Zone of inhibition (mm) | | | | |
|---|---|---|---|---|---|
| | Ampicillin | Amikacin | Cefixime | Imipenem | Meropenem |
| A1 | 8.0 | 9.0 | 8.0 | 22.0 | 17.0 |
| A2 | 9.0 | 12.0 | 12.0 | 25.0 | 20.0 |
| A3 | 7.0 | 11.0 | 11.0 | 23.0 | 19.0 |
| A4 | 7.0 | 7.0 | 10.0 | 25.0 | 16.0 |
| A5 | 11.0 | 13.0 | 15.0 | 23.0 | 21.0 |
| A6 | 7.0 | 13.0 | 13.0 | 26.0 | 19.0 |
| A7 | 9.0 | 9.0 | 9.0 | 22.0 | 10.0 |
| A8 | 10.0 | 12.0 | 12.0 | 22.0 | 20.0 |
| A9 | 6.0 | 10.0 | 10.0 | 20.0 | 19.0 |
| A10 | 12.0 | 14.0 | 14.0 | 25.0 | 21.0 |
| A11 | 12.0 | 15.0 | 15.0 | 23.0 | 24.0 |
| A12 | 7.0 | 10.0 | 10.0 | 22.0 | 18.0 |
| A13 | 8.0 | 11.0 | 10.0 | 20.0 | 18.0 |
| A14 | 7.0 | 7.0 | 9.0 | 24.0 | 15.0 |
| A15 | 13.0 | 14.0 | 14.0 | 24.0 | 20.0 |
| A16 | 10.0 | 10.0 | 9.0 | 25.0 | 17.0 |

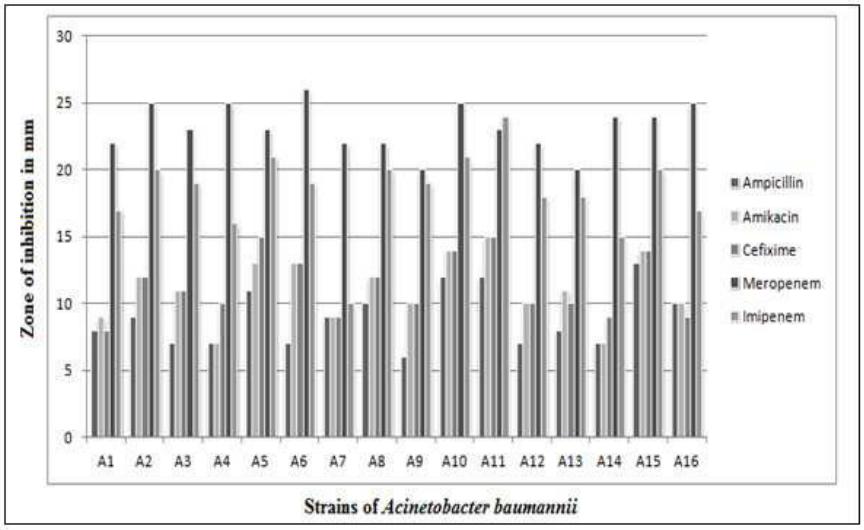


**Figure: 1**
***Antibiotic sensitivity pattern of Acinetobacter baumannii***

***RAPD Analysis***

Genomic DNA was isolated from sixteen clinical isolates of *Acinetobacter baumannii* and the DNA was separated in Gel electrophoresis to assess their purity. The isolated DNA samples of the sixteen clinical

isolates of *Acinetobacter baumannii* were amplified in PCR and the PCR amplified products of the DNA of the sixteen isolates were subjected to RAPD analysis using the four different primers (Fig. 2).

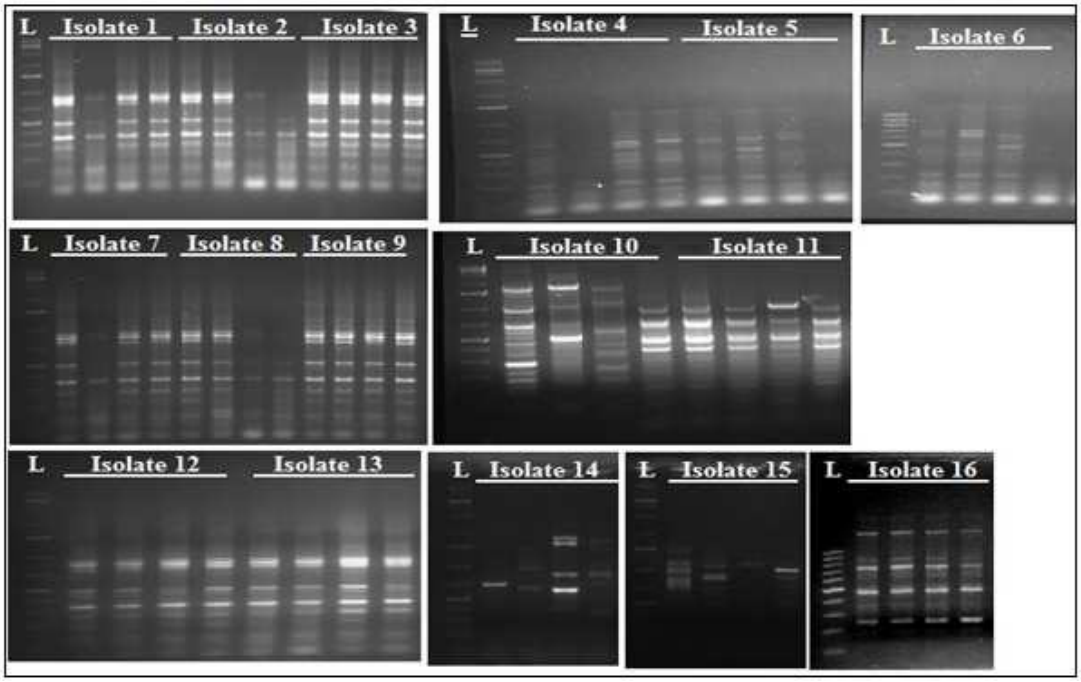


**Figure 2**
***RAPD profile of clinical isolates of Acinetobacter baumannii***

***Cluster Analysis***

Phylogenetic diversity between the sixteen clinical isolates of *Acinetobacter baumannii* were determined by converting RAPD data into similar matrix and analyzed by Neighbour-Joining method to produce a phylogenetic tree (Fig. 3). The cluster analysis revealed four primary clusters. The isolates 14 and 4 were distantly related to isolate 11 whereas isolates 7, 1 and 16 and 5, 10 and 15 showed definite patterns. The RAPD PCR profile of *Acinetobacter baumannii* isolated from a multiprofile hospital revealed seven discrete clusters[23]. Similar genetic polymorphism in the RAPD analysis of other pathogenic bacteria was reported by many authors[19, 24]. The variation in genetic pattern was also evident in the antibiotic sensitivity pattern among the sixteen clinical isolates of *Acinetobacter baumannii.* The isolate 11 was moderately resistant to gentamycin and amikacin whereas the isolate 14 was highly sensitive to the above antibiotics. Similar distinct variations in sensitivity pattern towards various antibiotics were witnessed among the isolates. The variation in antibiotic sensitivity pattern among the *Acinetobacter baumannii* isolates as supported by PCR assays were reported[22].

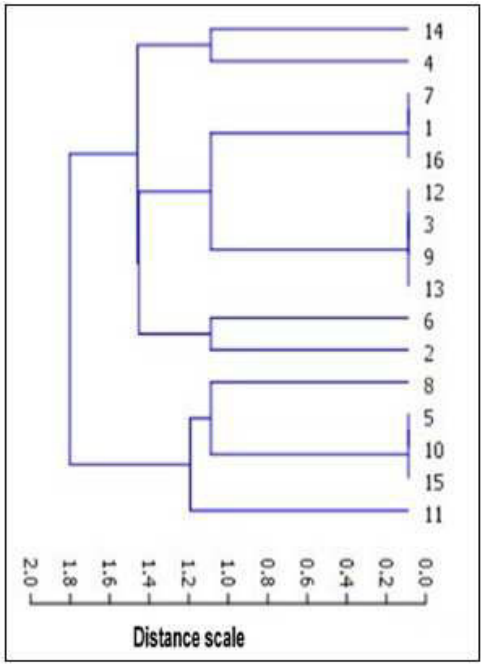


**Figure 3**
***Dendrogram showing the phylogenetic diversity of sixteen clinical isolates of Acinetobacter baumannii***

## CONCLUSION

*A.baumanii* is one of the most serious lower respiratory and nosocomial pathogen and it is resistant to many of the existing antibiotics.Emergence of drug resistant strains has posed serious concerns to the civil society.Hence ascertaining such drug resistant strains and desigining new drugs is need of the hour. Hence in the present investigation ,an attempt has been made to isolate and to study the antibiotic sensitivity pattern of *A.baumanii.* Further the gentic variation among these isolates and correlation these variation with the antibiotic variation has been made.The study concludes that there exixts genetic polymorphism among the isolates and the existing carbomenam group of antibiotics should not be used indiscriminately and be used judiciously and also there is a need to identify conserved regions in the virulent genes of the pathogen and desigin a drug which can contol the existing and new strains of this dreadful pathogen.

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