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IN VITRO EVALUATION OF MDR IN *ACINETOBACTER BAUMANNII MJ121*: AN EMERGING HEALTH THREAT

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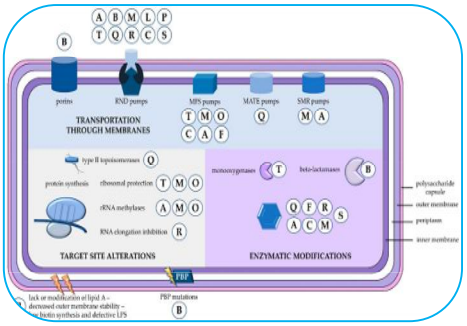
ABSTRACTS



In the present study, the antibiotics susceptibility of multi-drug resistant species of Acinetobacter baumannii MJ121 from clinical samples was done. The strain was identified on the basis of cultural, morphological characterizations, by 16s rRNA gene sequencing analysis. With the intention of determining the sensitivity of this isolate of Acinetobacter to 13 antibiotics, standard methods according to CLSI guidelines were performed. In the present study, resistance to three or more of three classes of antibiotics was regarded as multidrug resistance. Multidrug resistance (MDR) is a common occurrence among A. baumannii and it complicates the eradication and treatment of serious infections caused by them. Acinetobacter baumannii MJ121 has now emerged as one of the most troublesome pathogens for health care institutions globally. In the present study, the antibiotic susceptibility of Acinetobacter baumannii MJ-121 was shown. The present isolate 121 of Acinetobacter showed the highest resistance to almost all the antibiotics used Viz. Meropenem (10µg), Cefepime (30µg), Amoxycillin(10µg), Gentamicin(10µg), Nitrofurantoin(300µg), Nalidixic acid(30µg), Tigecycline(15µg), Ampicillin(10µg), Imipenem(10µg), Amikacin(30µg), Ertapenem(10µg), Colistin(10µg) and Piperacillin(100µg). The present study was done to find out the resistant pattern in this geographical area which can help to formulate an antibiotic policy in the hospital.

KEY WORDS : *Acinetobacter, In Vitro, multi-drug resistance (MDR), nosocomial infection.*

INTRODUCTION

In the general parlance, infections among inpatients acquired from hospital milieu, termed nosocomial infections are the major concern for the clinicians globally (Peleg *et al*., 2008). Nosocomial infections are more challenging to treat as they are usually caused by the drug resistant bacteria (Peleg *et al*., 2008). Further, most common nosocomial bacteria have been categorized as ESKAPE pathogens, which include Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter spp. encompassing both gram positive and gram negative bacteria.

Existing antimicrobials are losing their effect. At the same time there is a decline in the development of new antimicrobials. Similarly, there is insufficient new research into new diagnostics to detect resistant microorganisms; and vaccines for preventing and controlling infections. If this trend continues, the arsenals of tools to combat resistant microorganisms will soon be depleted. The threat from drug resistance is increasing. There is a need for urgent action; everyone can play a part. The emergence of multidrug resistant strains of Gram-negative bacteria (Pseudomonas, Klebsiella, Enterobacter, Acinetobacter, Salmonella species) and Gram-positive organisms (Staphylococcus, Enterococcus, Streptococcus species) is the more worrisome in the present therapeutic scenario. It is urgently required to ban sale of such types of antibiotics without any prescription, to employ antibiotics more judiciously in the different hospitals by the intensive teaching of the principles of the employment of antibiotics and to establish better control measures for nosocomial infections.

Frequent use of broad spectrum antibiotics raises the risk of development of resistance in the nosocomial pathogens. In the past few decades, the organism exerts the resistance to nearly all classes of antibiotics which is defined as Multi Drug Resistant pathogens.

Acinetobacter is an opportunistic human pathogen and its infections are raising alarm in the hospitals (Bouvet and Grimont, 1987), accountable for a variety of nosocomial infections; consisting of ventilator-associated pneumonia, surgical site infections, bacteremia, secondary meningitis, urinary tract infections and secondary infections of burn patients in the Middle East region (Ranjbar R. *et al*., 2007) and other parts of the world (Bergogne-Bérézin E., Towner K.J.1996; Khoshnood S, Eslami., *et al*., 2017).

The present investigation is carried out to know the antibiotic resistance in *Acinetobacter baumannii* MJ*121* isolated from the clinical samples causing the nosocomial infections which can help to formulate an antibiotic policy in the hospital.

MATERIALS AND METHODS

Identification of human clinical *Acinetobacter baumannii MJ121*

A series of morphological, physiological and biochemical tests were performed to identify the suspected *Acinetobacter baumannii* MJ121 isolates. The test included Gram staining, motility, Catalase production, Oxidase activity, Oxidation-Fermentation (OF) test, Glucose, Xylose, Mannitol, Sucrose, Galactose, Mannose and Rhamnose fermentation, Indole Production test (I), Voges-Proskauer (VP) test, methyl red (MR) reaction, hydrogen sulfide (H_2S) production. All the tests were conducted according to the Bergey's Manual of Determinative Bacteriology.

Colony characteristics of *Acinetobacter baumannii MJ121*

Colony characterizations of *Acinetobacter baumannii MJ121* is shown in **Table 1**. The specific types of colony characterizations shown by the bacteria will help for their identification in addition to focus on the effective treatment against pathogens.

Table 1: Colony Characterizations of *Acinetobacter baumannii MJ121* on the MacConkey's Agar incubated at 37^{0}C for 24 hours

Culture Code	Size in mm	Shape	Color	Margin	Elevation	Opacity	Consistency	Gram nature
MJ121	1.8	Circular	Pink	Entire	Raised	Opaque	Moist	Gram negative rod shaped bacteria

Biochemical characterizations of *Acinetobacter baumannii MJ121*

In the present study, the conventional biochemical characterizations were executed in the Microbiology laboratory, bacterial isolate was identified up to Genus level and species level by referring

Bergey's Manual of Determinative Bacteriology and 16s rRNA gene sequencing respectively. The results of biochemical characterizations of the present isolates is shown in the **Table 2.**

Table 2: Biochemical Characterizations of *Acinetobacter baumannii* 121 incubated at 37^{0}C for 24 hours

Characterizations	MJ121
Morphology	Coccobacilli
Motility	Non-motile
Fermentative or Oxidative	**O**
Catalase	**+**
Oxidase	**-**
Glucose	**+**
Xylose	**+**
Mannitol	**-**
Sucrose	**-**
Galactose	**+**
Mannose	**+**
Rhamnose	**+**
Citrate Utilization	**+**
Urea, Christensen	**v**
Methyl Reel	**-**
V-P Test	**-**
Lactose	**+**

Note: + = Positive test, **-** = Negative test, **v** = variable

16s rRNA sequencing of *Acinetobacter baumannii MJ121*

The isolate *Acinetobacter baumannii MJ-121*, was resistant to Almost all antibiotics used in the study i.e. Meropenem, Cefepime, Amoxycillin, Gentamicin, Nitrofurentoin, Nalidixic acid, Tigecycline, Ampicillin, Imipenem, Amikacin, Ertapenem, Colistin, Piperacillin. These Multi-Drug Resistant Acinetobacter bacterial isolates from this study were deposited to DNA Data Bank of Japan (DDBJ). The Accession numbers were obtained.

Identification of *Acinetobacter baumannii MJ121* by 16s rRNA Analysis

Identification of the promising isolate *Viz.* MJ121 by morphological, colony and biochemical characterizations and 16s rRNA analysis (**Figure 1**).

Nucleotide Sequence Accession Numbers

The data of nucleotide sequence of MJ121 accounted in the present study have been submitted to the DNA Data bank of Japan (DDBJ) sequence database and assigned accession number **LC490700**.

Phylogenetic Analyses of the 16s rRNA sequences

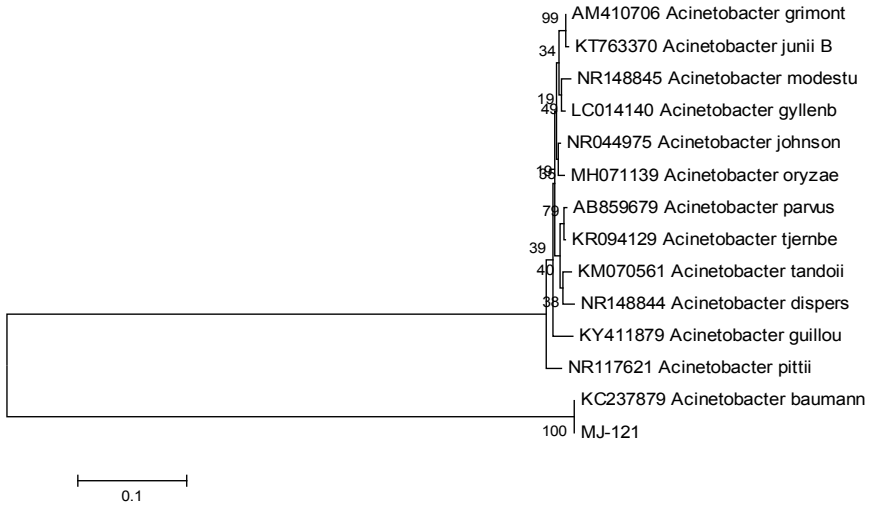


Figure 1: Phylogenetic placement of MJ121 (DDBJ Accession Number LC490700) based on 16S rRNA analysis

Phylogenic tree of ***Acinetobacter baumannii MJ-121***. Phylogenetic analysis of 16s rRNA gene sequence of *Acinetobacter baumannii MJ-121*. The percent numbers at the nodes indicate the levels of bootstrap support based on neighbor-joining analyses of 1,000 replicates. The scale bar (0.1) indicates the genetic distance.

The susceptibility to the antimicrobial agents was determined by using the Disc Diffusion method. The antimicrobial agents used were: Meropenem (10µg), Cefepime (30µg), Amoxycillin(10µg), Gentamicin(10µg), Nitrofurantoin (300µg), Nalidixic acid(30µg), Tigecycline(15µg), Ampicillin(10µg), Imipenem(10µg), Amikacin(30µg), Ertapenem(10µg), Colistin(10µg) and Piperacillin(100µg).

The rising number of nosocomial infections and rapid increase in the antibiotic resistant Acinetobacter isolates has prompted us to investigate the prevalence and antibiotic resistant Acinetobacter isolate from various clinical samples.

Muktikesh Dash *et al*., (2013) study revealed that the majority (54.7%) of the isolates was multi drug resistant (MDR) Acinetobacter spp., and amongst them, eight isolates were PDR.

The other studies conducted by Bhattacharyya *et al*., (2013) in West Bengal and Mostofi *et al*., (2011) in Tehran reported in their study that the multi drug resistant (MDR) isolates to be 29% and 54%, respectively.

There are numerous reports on outbreaks of multidrug resistant (MDR) *Acinetobacter baumannii* in an ICU (Pimentel *et al*., 2005; Jeon *et al*., 2005). In ICU critically ill patients are forever at higher risks of developing nosocomial infections with the antibiotic resistant strains. The emergence and spread of multidrug resistant *Acinetobacter baumannii* and its genetic potential to hold and transfer varied antibiotic resistant determinants pose a main threat in the hospitals (Navon *et al*., 2005).

In a study by Jain R. and Danziger L.H., (2004), all the bacterial isolates showed high frequency of resistance to the multiple antibiotics but maximum resistance was seen in *Acinetobacter* isolates. *Acinetobacter* isolates have a tendency to readily develop resistance to the second and third generation antibiotics like ciprofloxacin, cefotaxime and giving rise to therapeutic problems. Since higher generation antibiotics are being exploited to overcome problem of resistance against the available antibiotics, bacteria are developing mechanisms to resist the newer antimicrobials. In this study *Acinetobacter baumannii* isolates showed resistance to both the old and new generation antibiotics.

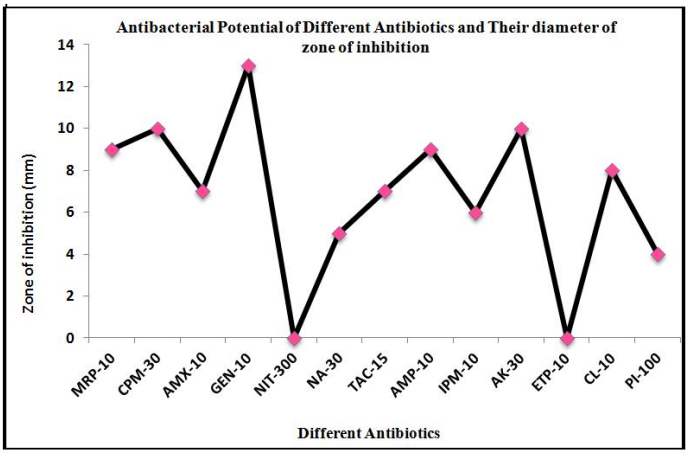
Evaluation of the Antibiotic Sensitivity Response of Clinical Isolates

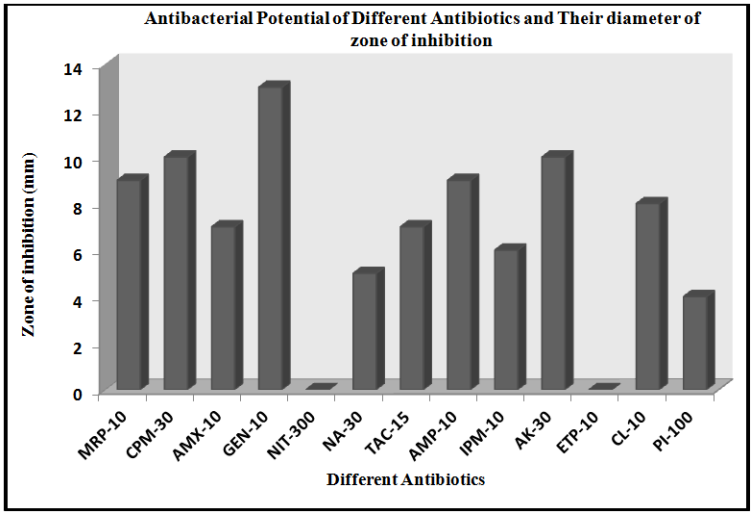
Antibiotic resistance profile for all the infectious organisms isolated from various clinical samples was established using 13 different 1st to 4th generation antibiotics belonging to 13 different groups.

Antibiotic Susceptibility Response of *Acinetobacter baumannii 121*			
Sr. No.	**Name of Antibiotics**	**Short Terms**	**Zone of Inhibition (mm) and Sensitivity**
1.	Meropenem	MRP-10	09-R
2.	Cefepime	CPM-30	10-R
3.	Amoxycillin	AMX-10	07-R
4.	Gentamicin	GEN-10	13-I
5.	Nitrofurentoin	NIT-300	00-R
6.	Nalidixic acid	NA-30	05-R
7.	Tigecycline	TAC-15	07-R
8.	Ampicillin	AMP-10	09-R
9.	Imipenem	IPM-10	06-R
10.	Amikacin	AK-30	10-R
11.	Ertapenem	ETP-10	00-R
12.	Colistin	CL-10	08-R
13.	Piperacillin	PI-100	04-R
Percentage of Resistance (%) = 92.30			

Reza Mirnejad *et al*., (2011) investigated the multi-drug resistance (MDR) in *Acinetobacter baumannii* strains which was isolated from different clinical samples of three extremely large hospitals in Tehran-Iran. The promising isolates of *Acinetobacter baumannii* showed the highest resistance to meropenem, cefepime, amikacin, norfloxacin, ceftazidime, aztreonam, Tobramycin, ofloxacine and ciprofloxacin considered as effective drugs. The percentage of Multi-drug resistance in these strains was 55.4. Multi-drug resistant Acinetobacters are mounting and considered as an important threat for the hospitalized patients. Consequently change in consumption patterns of the antibiotics and control of hospital infections appears to be necessary.

Figure 2: Antibiotic Susceptibility Response of *Acinetobacter baumannii 121* and its zone of inhibition (mm)





It is found from the **Figure 2** that the promising isolate MJ121 shows the largest inhibition zone diameter i.e. 13mm to Gentamicin followed by Cefepime (10mm), Amikacin (10mm), Meropenem (9mm), Ampicillin (9mm), Colistin (8mm), Amoxycillin (7mm), Tigecycline (7mm), Imipenem (6mm), Nalidixic acid (5mm), Piperacillin (4mm) whereas no zone of inhibition was observed to Nitrofurentoin and Ertapenem.

From the results it is found that the MJ121 is resistant to Meropenem, Cefepime, Amoxycillin, Nitrofurentoin, Nalidixic acid, Tigecycline, Ampicillin, Imipenem, Amikacin, Ertapenem**,** Colistin, Piperacillin and showed intermediate resistance to Gentamicin used in the present study.

Bergogne-Berezin E., (2001) stated in their study that the major problem met by ICU clinicians associates to readily transferable resistance to the antibiotics expressed by *Acinetobacter*. *Acinetobacter baumannii* has the ability to acquire resistance to several major classes of antibiotics.

In this study *Acinetobacter* was isolated in a noteworthy proportion from clinical samples in ICU infections and multidrug resistant (MDR) *Acinetobacter* isolates were found to be related with almost all kinds of nosocomial infections like bacteraemia, UTIs, RTIs, septicaemia, wound infections and meningitis.

Plasmid Isolation, Curing and Gel Electrophoresis

Plasmids are the extrachromosomal elements which are responsible for the development and spread of antibiotic resistance in Acinetobacter species. Isolation of plasmid DNA from *Acinetobacter baumannii MJ-121* isolate revealed the presences of single plasmid. The size of the isolated plasmid was approximately more than 22 kb (**Figure 3**).

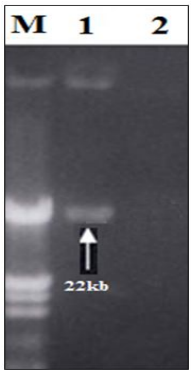


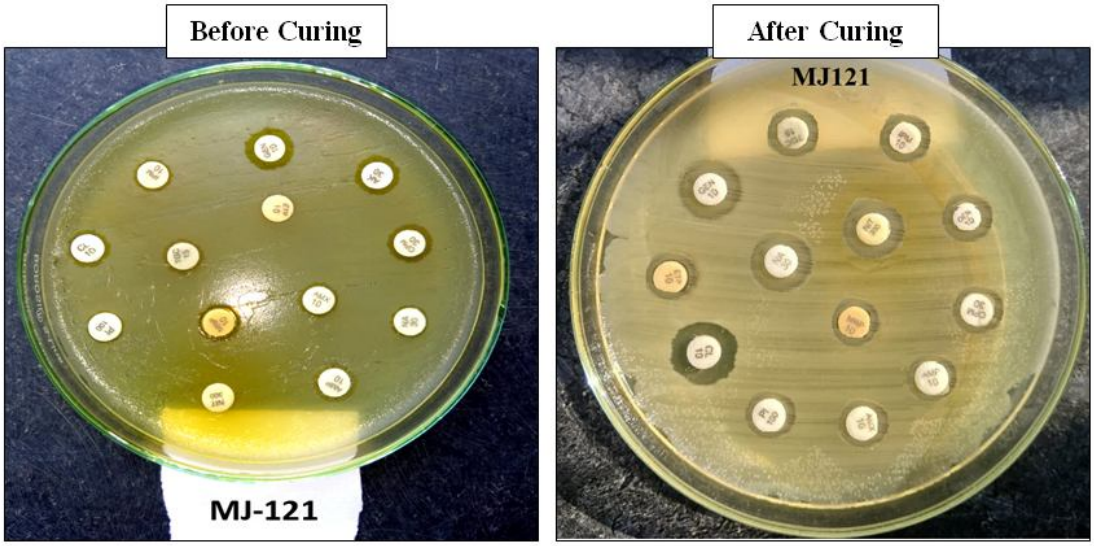
Figure 3: 1. Plasmid DNA profile of (Lane 1) *Acinetobacter baumannii MJ-121* (Lane 2) *Acinetobacter baumannii MJ-121* Cured and (Lane M) DNA lambda EcoR1

Plasmids are the extrachromosomal elements which are responsible for the development and spread of antibiotic resistance in Acinetobacter species. Isolation of plasmid DNA from *Acinetobacter baumannii MJ-121* isolates revealed the presences of single plasmid. The size of the isolated plasmid was approximately more than 22kb (**Figure 3**). The Plasmid isolation has been successfully carried out in *Acinetobacter baumannii MJ-121*.

In a study performed by Prashanth and Badrinath, (2006) reported the multidrug resistant *Acinetobacter* responsible for majority of the infections. The presence of multidrug resistant plasmid harbouring *Acinetobacter baumannii,* causing nearly all types of nosocomial infections could lead to the therapeutic problems.

Heritier *et al*., (2005) demonstrated that the clinical *Acinetobacter baumannii* isolate in addition to unrelated environmental *Acinetobacter baumannii* isolate had a similar carbapenem resistance plasmid implying spread of this genetic character. A single plasmid which acts as vector of several resistance genes which can carry a number of genes coding for multiple drug resistance (MDR).

Photoplate 1: Antibiotic Sensitivity of Promising Isolate Before and After Curing



Kapil A., (2005) reported in their study that, the transferable plasmid mediated antibiotic resistances sets a great threat as it can achieve much larger dimension owing to rapid and wide dissemination. This transferable resistance is carried on the R-plasmids.

CONCLUSION

Multidrug resistance (MDR) is a common occurrence among *A. baumannii* and it complicates the eradication and treatment of serious infections caused by them. *Acinetobacter baumannii* has now emerged as one of the most troublesome pathogens for health care institutions globally. Its clinical significance has been set in motion due to its remarkable ability to up regulate or acquire resistance determinants, making it one of the organisms threatening the current antibiotic era. *Acinetobacter baumannii MJ121* strain resistant to all known antibiotics has now been reported, signifying a sentinel event that should be acted on promptly by the international health care community. As a result, in the perspective of this study, it could be concluded that emergence of high-grade MDR *Acinetobacter baumannii MJ121* within the ICUs of Barshi, Dist: Solapur, Maharashtra, India, is the newest problem on the board. The ongoing multi drug resistant (MDR) nature of *Acinetobacter baumannii* pathogen to the multiple drugs or even to the last line antibiotics is a terrible looming threat with respect to already immunity weaned ICU inhabitants. The possible escape lies in the thorough periodic examining of the

health-care setups in order to plan out effective infection control strategies and chalking out the new treatment options for genuinely controlling such stubborn hospital-based *Acinetobacter baumannii MJ121* pathologies within the overall domain of Barshi, Maharashtra, India.

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