World Journal of Pharmaceutical ReseaRch
SJIF Impact Factor 5.045
Volume 3, Issue 8, 372-379. Research Article ISSN 2277 – 7105

DRUG RESISTANCE AND BIOFILM FORMATION OF ACINETOBACTER BAUMANII ISOLATED FROM INTENSIVE CARE UNITS

Mamatha. C* and Dr. J. Vimalin Hena**

*Research Scholar, Department of Microbiology, Hindusthan College of Arts and Science, Coimbatore.

**Assistant Professor, Department of Microbiology, Hindusthan College of Arts and Science, Coimbatore.

Article Received on
30 July 2014,

Revised on 24 August 2014,
Accepted on 19 Sept 2014

***Correspondence for Author**
Dr. J. Vimalin Hena
Assistant Professor, Department of Microbiology, Hindustan College of Arts and Science, Coimbatore.

ABSTRACT

A. baumanii was not given much importance until recently when it started emerging as a serious threat in hospitals all over the world. Development of multiple drug resistance and ability to form extensive biofilms is considered as the major reasons behind this emergence. Hence it is the need of the hour to understand more about this ability exhibited by this pathogen. This study was designed with this aim in focus. Here the organism was isolated from the patients admitted in ICU and was identified by its phenotypic characterization and the antibiotic sensitivity testing was done by Kirby Bauer method. Further the biofilm forming ability of the organism was checked by growth on Congo red agar and by tube method and then the extend of bio film formation was checked on both plastic and glass tube. All the strains Isolated had the ability to form biofilms and were resistant to almost all the antibiotics tested. Plastic tube supported the formation of biofilms more than glass test tubes.

KEYWORDS: Kirby Bauer method, red agar, plastic and glass tube.

INTRODUCTION

Acinetobacter sp. are gram negative aerobic coccobacilli and are generally harmless organisms with the ability to persist in the hospital environment for prolonged periods. Although colonization of patients is common, this organism is a major cause of clinical infection, especially in the immunocompromised patients and those in intensive care units.

Acinetobacter baumannii is the major species responsible for the infections in humans, causing pneumonia, endocarditis, meningitis, wound and urinary tract infection (Ayan *et al*, 2002). *Acinetobacter* spp. are commonly found as major contaminants of the environment. The recent development of drug resistance among these organisms has rendered the current antibiotics ineffective (Meritxell *et al*, 2012).

Biofilm formation is an ability possessed by many bacteria and such organisms are generally highly resistant to environmental stresses. *Acinetobacter baumanii* has also been found to produce biofilms and cause infections associated with medical devices, e.g., vascular catheters, cerebrospinal fluid shunts, foley catheters etc. (Dheepa *et* al, 2011). The drug resistance exhibited by the organism has been shown to be due to the ability of the organism to produce biofilms (Srinivasa Rao *et al*, 2008).

Multiple drug resistance is the ability of microorganisms to resist certain antibiotics and *A. baumanii* possess this ability to a high extend. This is one another reason why this organism is feared in hospitals (Enoch *et al*, 2008). As such Colistin and Tigercycline remains the only two antibiotics mainly used against this pathogen and resistance is slowly developing against Tigercycline (Dizbay *et al*, 2008). This study is designed to find out the extend of biofilm formation and the multiple drug resistance in the organism. Also we aim to find if this extracellular polysaccharide adheres more on plastic or glass.

MATERIALS AND METHODS

Sample Collection

The samples were collected from KMCH, Coimbatore. Swabs taken from ICU patients in various departments was used to isolate the bacteria. Twenty *Acinetobacter baumanii* samples were isolated.

Culture

The samples were cultured on Brain Heart Infusion agar (Dror Marchaim *et al*, 2007) and MacConkey agar plates and incubated for 24 hours at 37^{O}C.

Phenotypic Characterisation

The phenotypic characterisation was done by Gram's staining and biochemicals testing. IMViC, Catalase, Oxidase, Glucose fermentation and Lactose fermentation was done and incubated for 24 hours at 37^{O}C (Prashanth *et al*, 2000).

Antibiogram

Twenty four hour culture of *Acinetobacter baumanii* was swabbed on Muller Hinton agar plates and antibiotic discs were placed on the plates and incubated for 24 hours at 37 ^{O}C. The discs used were Ticarcillin, Ceftazidime, Imipenem, Gentamicin, Amikacin and Ciprofloxacin (Imane M'hamedi *et al*, 2014).

Biofilm Detection

Biofilm formation is the ability of certain bacteria to produce an extracellular polysaccharide, thus facilitating attachment and matrix formation (Dheepa *et al*., 2001).

I) Congo Red Assay

Congo red agar plates prepared using 50gm/l sucrose, 37gm/l Brain Heart Infusion agar and 20gm/l agar. These were sterilised and 0.8gm/l sterilized Congo red stain was added to the media after it has cooled down to 55^{O}C (Yasmeen Taj, 2012) and the samples isolated on MacConkey or Brain Heart Infusion agar were inoculated onto the media and incubated.

II) Tube Assay

Acinetobacter baumanii culture was inoculated into sterile Brain Heart Infusion broth tubes and incubated for 48 hours at 37^{O}C. The tubes were then drained and washed with Phosphate Buffer Saline. Further the tubes were kept for drying at room temperature and 4% Crystal Violet solution was added through the sides of the tube and decanted. The tubes were dried and biofilm formation studied (Mathur *et al*, 2006).

Comparing the Biofilm Production on Different Materials

A. Baumanii was inoculated into test tubes made of glass and tubes made of plastic containing 5 ml of sterilized Brain Heart Infusion broth. The tubes and incubated at 37^{O}C for 48 hours. After incubation, the tubes were decanted, washed with Phosphate Buffer Saline and air dried. Staining was done using 4% Crystal Violet solution and the biofilm formation was observed in the tubes (Nadia Kazemi Pour *et al*, 2011).

RESULT AND DISCUSSION

Culture

Well isolated colonies of the organism were obtained as the organism grew well on all the media used. It showed faint pinkish taint on Mac Conkey agar (Lahiri *et al*, 2004) and creamy colour colonies on Brain Heart Infusion agar.

Phenotypic Characterisation

Strain No.	Indole	Methyl Red	Voges Proskauer	Citrate	Catalase	Oxidase	Glucose Utilisation	Lactose Utilisation
Ab1	-	-	-	+	+	-	+	+
Ab2	-	-	-	+	+	-	+	+
Ab3	-	-	-	+	+	-	+	+
Ab4	-	+	--	+	+	-	+	+
Ab5	-	-	-	+	+	-	+	+
Ab6	-	-	-	+	+	-	+	+
Ab7	-	-	-	+	+	-	+	+
Ab8	-	-	-	+	+	-	+	+
Ab9	-	-	-	+	+	-	+	+
Ab10	-	-	-	+	+	-	+	+
Ab11	-	-	-	+	+	-	+	+
Ab12	-	-	-	+	+	-	+	+
Ab13	-	-	-	+	+	-	+	+
Ab14	-	-	-	+	+	-	+	+
Ab15	-	-	-	+	+		+	+
Ab16	-	-	-	+	+	-	+	+
Ab17	-	-	-	+	+	-	+	+
Ab18	-	-	-	+	+	-	+	+
Ab19	-	-	-	+	+	-	+	+
Ab20	-	-	-	+	+	-	+	+

The isolated strains were negative for Indole,VP but positive for Citrate. Except one strain all others gave negative result for MR. They showed negative for oxidase and positive for catalase. The samples utilized glucose and lactose but did not ferment them.

Antibiogram

Most of the strains were resistant to most of the antibiotics used except Imipenem or amikacin.

STRAIN NO.	Ticarcillin Zone dia. (in mm)			Imipenem Zone dia. (in mm)			Gentamicin Zone dia. (in mm)			Amikacin Zone dia. (in mm)			Ciprofloxacin Zone dia. (in mm)		
	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
Ab1	-	-	-	10	-	-	-	-	-	-	-	-	-	-	-
Ab2	-	-	-	9	-	-	--	-	-	-	-	-	-	-	-
Ab3	-	-	--	10	-	-	-	-		-		-	-	-	-
Ab4	-	-	21	9	-	-	-	-	13	-	-	18	-	-	23
Ab5	-	-	-	9	-	-	-	-	-	-	-	-	-	-	-
Ab6	-	-	-	-	-	-	-	-	-	-	-	15	-	-	-
Ab7	-	-	-	10	-	-	-	-	-	14	-	-	-	-	-
Ab8	-	-	-	12	-	-	-	-	-	-	-	18	9	-	-
Ab9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ab10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Ab11	-	-	-	10	-	-	-	-	-	-	-	-	-	-	-
Ab12	-	-	-	8	-	-	-	-	-	-	-	-	-	-	-
Ab13	-	-	-	12	-	-	-	-	-	13	-	-	-	-	-
Ab14	-	-	-	10	-	-	-	-	-	10	-	-	-	-	-
Ab15	-	-	-	10	-	-	-	-	-	-	-	-	-	-	-
Ab16	-	-	-	10	-	-	-	-	-	10	-	-	-	-	-
Ab17	-	-	-	12	-	-	-	-	14	-	-	-	14	-	-
Ab18	-	-	-	11	-	-	-	-	13	-	-	15	-	-	-
Ab19	-	-	-	-	-	14	-	-	13	13	-	-	14	-	-
Ab20	-	-	-	11	-	-	-	-	8	10	-	-	-	-	-

The organism was found to be Multiple Drug Resistant. Most of the strains showed resistance to all the antibiotics except Gentamicin and Amikacin. Only some showed sensitivity to Imipenem and Ciprofloxacin. Only one strain was sensitive to Ticarcillin.

Biofilm Detection Using Tube Assay

All the samples tested produced biofilm which, after staining was visible as a layer on the walls and bottom of the tube (Anagha Kinikar *et al*, 2014) and not as a ring in the middle. Though all the samples produced biofilms, the strength of the biofilm varied between the various samples.

STRAIN NO.	BIOFILM STRENGTH
Ab1	Very High
Ab2	Very High
Ab3	Very High
Ab4	Very High
Ab5	High
Ab6	Moderate
Ab7	Moderate
Ab8	Low
Ab9	Very High
Ab10	Very High
Ab11	High
Ab12	Very High
Ab13	Low
Ab14	Moderate
Ab15	Very High
Ab16	High
Ab17	Very High
Ab18	Very high
Ab19	High
Ab20	Moderate

Most of the strains produce very strong biofilms. Only Ab8 and Ab 13 produced weak biofilms. Ab6, Ab7, Ab14 and Ab 20 produced moderate biofilms whereas all the other

strains produced strong biofilms. This proves that all *A.baumanii* possess the ability to produce biofilms.

Comparing the Biofilm Production on Different Materials

The biofilm formation abilities of all the 20 isolates were determined on glass test tubes and also tubes made of plastic and it was seen that the strains produced biofilms more heavily on plastic than on glass (Nadia Kazemi Pour *et al*, 2011).

CONCLUSION

Acinetobacter species are among the most common causes of device related nosocomial infection that results when the organism is able to resist physical and chemical disinfection. Due to this focus has been shifted to the study of this organism worldwide. This study gives significant insight to the nature and threat of *Acinetobacter baumanii* infections. The emergence of Multiple Drug resistance is a global phenomenon (Anthony *et al*, 2008). This poses danger to human health as the spread of drug resistance has risen uncontrollably. The extensive nature of biofilm formation has been seen as one of the reasons for the emergence of Multiple Drug Resistance (Rao *et al*, 2008). Hence in this study, the extend of Multiple Drug Resistance in *A.baumanii* was checked. It was found that the pathogen was resistant to almost all the antibiotics used. Similar results were obtained in case of biofilm production as well. Different strains produced this extracellular polysaccharide in varying degrees of strength. However the most significant result obtained was that biofilm formation is higher on plastic surfaces than on glass. This finding is crucial as this shows that medical devices made of plastic (eg. Catheters) have more chance to get colonized by the organism and thus spread infection to the patient. This way it has also been proved that the material used for manufacturing clinical equipments also plays a major role in determining the extend of infection it can cause to the patient. Such analysis helps in controlling the spread of the contaminant by taking extra care for those equipments which are more vulnerable.

REFERENCES

1. M. Ayan, R. Durmaz, E. Aktas, B. Durmaz, Bacteriological, clinical and epidemiological characteristics of hospital-acquired *Acinetobacter baumannii* infection in a teaching hospital, Journal of Hospital Infection, 2002; 54: 39–45
2. Meritxell Garcı´a-Quintanilla*, Marina R. Pulido, Rafael Lo´ pez-Rojas, Jero nimo Pacho´ n, and Michael J. McConnell, Emerging therapies for multidrug resistant Acinetobacter baumannii, Trends in Microbiology, 2013; 21(3): 157-163

3. M. Dheepa, Vinitha L Rashme, B. Appalaraju, Comparision of biofilm production and multiple drug resistance in clinical isolates of Acinetobacter baumanii from a tertiary care hospital in South India, *Int J Pharm Biomed Sci*, 2011; 2(4): 103-107
4. R Srinivasa Rao, R Uma Karthika, SP Singh, P Shashikala, R Kanungo, S Jayachandran, K Prashanth, Correlation Between Biofilm Production And Multiple Drug Resistance In Imipenem Resistant Clinical Isolates Of *Acinetobacter baumannii*, *Indian Journal of Medical Microbiology, 2008; 26(4): 333-7*
5. D.A. Enoch , C. Summers , N.M. Brown, L. Moore, M.I. Gillham, R.M. Burnstein, R. Thaxter, L.M. Enoch, B. Matta, O. Sule, Investigation and management of an outbreak of multidrug-carbapenem-resistant *Acinetobacter baumannii* in Cambridge, UK, Journal of Hospital Infection, 2008; 70: 109-118
6. M. Dizbay, A. Altuncekic, B. Ergut Sezer, K. Ozdemir, D. Arman, Colistin and tigecycline susceptibility among multidrug-resistant *Acinetobacter baumannii* isolated from ventilator-associated pneumonia, International Journal of Antimicrobial Agents, 2008; 32: 29–32
7. Dror Marchaim, Shiri Navon-Venezia, David Schwartz, Jalal Tarabeia, Iris Fefer, Mitchell J. Schwaber and Yehuda Carmeli, Surveillance Cultures and Duration of Carriage of Multidrug-Resistant *Acinetobacter baumannii*, Journal of Clinical Microbiology, 2007; 45(5): 1551–1555
8. Prashanth. K and S. Badrinath, simplified Phenotypic tests for the detection of *Acinetobacter* sp. and their antimicrobial susceptibility tests, Journal of Medical Microbiology, 2000; 49: 773-778
9. Imane M'hamedi, Hafida Hassaine, Samia Bellifa, Meriem Lachachi, Ibtissem Kara Terki and ryad Djeribi, Biofilm formation by *Acinetobacter baumannii* isolated from medical devices at the intensive care unit of the University Hospital of Tlemcen (Algeria), African Journal of Microbiology Research, 2014; 8(3): 270-276
10. Yasmeen Taj, Farhan Essa, Faisal Aziz, Shahana U. Kazmi, J Infect Dev Ctries, Study on biofilm-forming properties of clinical isolates of *Staphylococcus aureus*, 2012; 5(6): 403-409.
11. T Mathur, S Singhal, S Khan, DJ Upadhyay, T Fatma, A Rattan, Detection of Biofilm formation among the Clinical Isolates of *Staphylococci:* An Evaluation of three different screening methods, Indian Journal of Medical Microbiology, 2006; 24(1): 25-9
12. Lt Col KK Lahiri, Lt Col NS Mani, Lt Col SS Purai, *Acinetobacter* spp as Nosocomial Pathogen : Clinical Significance and Antimicrobial Sensitivity,

13. Nadia Kazemi Pour, Devendra H. Dusane, Prashant K. Dhakephalkar, Farokh Rokhbakhsh Zamin, Smita S. Zinjarde & Balu A. Chopade, Biofilmformation byAcinetobacter baumannii strains isolated fromurinary tract infectionand urinary catheters, Federation of European Microbiological Societies, 2011; 62: 328–338
14. Anagha Kinikar, S. Saini, Seema Bose, Sachin Deorukhkar, Acinetobacter Species an Emerging Nosocomial Pathogen: It's Isolation Pattern, Biofilm Formation and Antibiotic Susceptibility Profile, International Journal of Health Sciences & Research, 2014; 79(4): 79-84
15. Anthony. M. Nicasio, Joseph. L. Kuti, David Nicolau, The Curent State of Multidrug Resistant Gram Negative Bacilli in North America,Pharmactherapy, 2008; 28(2): 235-249
16. Rao RS, Karthika RU, Singh SP, Shashikala P, Kanungo R, Jayachandran S, Prashanth K, Correlation between biofilm production and multiple drug resistance in imipenem esistant clinical isolates of Acinetobacter baumannii, Indian Journal of Medical Microbiology, 2008; 26(4): 333-7