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Detection of gelatinase and lipase among clinical isolates of *Acinetobacter baumannii*

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ABSTRACT

The role of *Acinetobacter* in causing nosocomial infections has been increasingly reported worldwide. Moreover, it gains an attention towards severe cases of infections associated with intensive care unit (ICU) patient. A sum of 20 clinical isolates of *A. baumanni* isolates were studied for antibiotic sensitivity pattern to routinely used antibiotics followed by the detection of gelatinase and lipase by plate methods. We have observed increased percentage of resistance to all of the antibiotics tested. 14% and 70% of our isolates were producing gelatinase and lipase respectively. The results of our study showed the presence of many enzymes necessary for the establishment of the infection are present in *Acinetobacter baumannii.*

KEY WORDS: *Acinetobacter baumannii*, antibiotic sensitivity pattern, virulence, gelatinase, lipase.

1. INTRODUCTION

Over the past 3 decades, the role of *Acinetobacter* in causing nosocomial infections has been increasingly reported worldwide. Moreover, it gains an attention towards severe cases of infections associated with intensive care unit (ICU) patient. These infections manifest as serious diseases in immune compromised human hosts, particularly in cases of ventilator acquired pneumonia, urinary tract infections, septicaemia, wound infection, sporadic cases of peritoneal dialysis peritonitis, endocarditis, meningitis, osteomyelitis, arthritis, pancreatic and liver abscesses and eye infections have also reported very rarely.

Increasing frequency of blood stream infections due to *A. baumannii* were also noted, resulting in significant morbidity and mortality rates up to 75%. The capacity of *A. baumannii* to cause disease in debilitated patients and persist in the medical environment/devices could be attributed to its resistance to major antimicrobial drugs and desiccation. The emergence of multi drug resistant (MDR) *Acinetobacter spp*. to several routinely used antibiotics, such as aminoglycosides, fluoroquinolones, cephalosporins, β-lactams and carbapenems have been increasingly worldwide. The existence of some of the virulence factors of *A.baumannii* such as enzymes (gelatinase, lipase, lecithinase, protease and esterase) possess a deleterious effect within the host tissue.

2. MATERIALS AND METHODS

Bacterial isolates: A total of 20 non repetitive clinical isolates of *A. baumannii* were collected from Saveetha Medical College, Thandalam. They were processed for a battery of standard bio chemical tests and confirmed. Isolates were preserved in semi-solid trypticasesoy broth stock and stored at 4^0C until for that use.

Antibiotic susceptibility testing: Antibiotic resistance pattern was detected on Mueller Hinton agar by standard disk diffusion method. The antibiotics from each class were selected according to the CLSI guidelines. Then the zone of inhibition was measured and compared as per the CLSI interpretatitive chart.

Table.1. Antibiotics used in this study

Name of the antibiotics	Disc potency(mcg/disc)	Name of the antibiotics	Disc potency(mcg/disc)
Piperacillin (P)	**100**	Imipenem (I)	**10**
Piperacillin-Tazobactam (PIT)	**100/10**	Meropenem (MRP)	**10**
Ticarcillin-Clavulanic acid (TC)	**75/10**	Gentamycin (G)	**10**
Ceftazidime (CF)	**30**	Tetracycline (TE)	**30**
Cefipime (CPM)	**30**	Ciprofloxacin (CIP)	**5**
Ceftriaxone (CTR)	**30**	Co-trimoxazole (Co)	**25**

Gelatinase detection: Gelatin medium was prepared by adding 3% of gelatin to the Luria Bertanimedium. Overnight grown cultures of *A. baumannii* from Brain Heart Infusion Agar were spot inoculated on Luria Bertani Agar medium containing 3% gelatin. The inoculated plates were incubated at 37˚C for 24 hours. After satisfactory growth, the cultures were flooded with 1%mercuric chloride solution. The development of opacity in the medium and zone of clearing around the growth were considered as positive. No clearing of zone around the growth were considered as negative.

Lipase detection: A plate assay to detect lipase in a medium containing trioleoylglycerol and fluorescent dye Rhodamine B was used. The growth medium contained (per liter) TSB 8g; Sodium chloride 4g; Agar 10g. The medium was adjusted to pH7, autoclaved, and cooled. Then 31.25 ml of trioleoylglycerol and 10 ml of rhodamine B solution was added with vigorous stirring and poured over the plates. Spot inoculation were made and incubated at

37˚C for 24 hours. Substrate hydrolysis causes the formation of orange fluorescent colonies visible upon UV irradiation.

3. RESULTS

Sample wise distribution of clinical isolates of *A. baumannii*: Of the 20 isolates of clinical isolates of *A. baumannii,* 10/20 (50%) isolates were from sputum, 5/20 (25%) from blood, 3/20 (15%) from urine, 2/20 (10%) from pus.

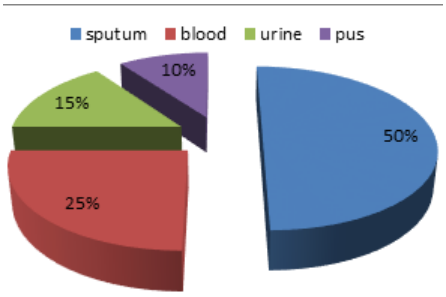

Figure.1. Sample wise distribution of clinical isolates of *A. baumannii*

Results of antibiotic sensitivity pattern of *A. baumannaii*: We have observed increased percentage of resistance to all routinely used antibiotics in this study. Complete resistance 100% was found in piperacillin, piperacillin – tazobactam, ticarcillin - clavulanic acid, ciprofloxacin, gentamycin, followed by 95% of isolates were showed resistance to cefipime, co-trimoxazole, tetracycline and to ceftriaxone it showed 85% of resistance rate. Imipenem alone showed 40% of sensitivity by disc diffusion method.

Table.2. Antibiotic sensitivity pattern in *A.baumannii* isolates

Antibiotics	Sensitive (%)	Intermediate (%)	Resistance (%)
Piperacillin	0	0	20 (100)
Piperacillin - Tazobactam	0	0	20 (100)
Ticarcillin - Clavulanic acid	0	0	20 (100)
Cefipime	0	1 (5)	19 (95)
Ceftazidime	2 (10)	0	18 (90)
Ceftriaxone	2 (10)	1(5)	17(85)
Ciprofloxacin	0	0	20(100)
Co-Trimoxazole	0	1(5)	19(95)
Gentamicin	0	0	20(100)
Tetracycline	0	1(5)	19(95)
Imipenem	8(40)	2(10)	10(50)

Results of gelatinase and lipase by *A. baumannii*: Of the 20 clinical isolates of *A. baumannii*, 12/20 (60%) of isolates were found to be positive for gelatinase production. 8/20 (40%) were shown to be strong positive, while 6/20 (30%) were considered to be weak positive producers of lipase production by *A. baumannii* isolates.

Figure.2. Picture showing gelatinase production by *A. baumannii* isolates

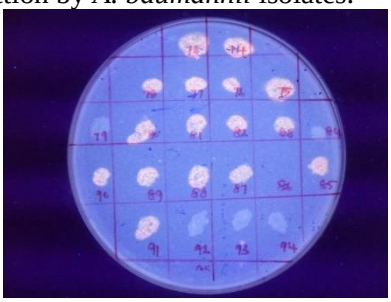

Figure.3. Picture showing gelatinase production by *A. baumannii* isolates

DISCUSSION

Acinetobacter, once considered to be a low grade pathogen indeed has many putative virulence factors. It causes a major problem in ICU, particularly in patients receiving artificial ventilations. Despite the significance of increasing antibiotics resistance exerted by *A.baumannii*, an impact of the potential importance of this pathogen are identified inturn is associated with virulence determinants.

We have observed the isolates showed multiple resistance to antibiotics were correlated with the rate of biofilm formation. Studies conducted by Rodriguez (2008) from Spain showed high biofilm producers showed less resistance, wherein highly drug resistant strains produced negligible amount of biofilm. These results are inconsistent with our findings. However, Rao (2008), from Puducherry reported similar results as that of our study, demonstrates

a high propensity of *A,baumannii* to form biofilm and a significant association of biofilms with multiple drug resistance.

Studies from different places showed a different percentage of virulence factors. But in our study we observed 60% of positivity for gelatinase production.

4. CONCLUSION

As the pathogenicity of *Acinetobacter baumannii* is not well defined that made its consideration as an emerging pathogen. Thus the presence of various virulence factors was assessed which contributes for its successful pavement in host tissues. The results of our study showed the presence of many enzymes necessary for the establishment of the infection are present in *Acineto bacterbaumannii.*

5. ACKNOWLEDGEMENT

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