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RESEARCH ARTICLE

MICROBIOLOGY

DETECTION OF CERTAIN VIRULENCE ATTRIBUTES AND ANTIMICROBIAL RESISTANCE PATTERN AMONG CLINICAL ISOLATES OF ACINETOBACTER BAUMANNII

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ABSTRACT

Acinetobacter species are ubiquitous aerobic gram negative coccobacilli, emerging as an inevitable potential pathogen to establish its survival in the host environment and among debilitated patients by producing various extracellular virulence factors. A total of 46 clinical isolates of *Acinetobacter baumannii* were collected from different clinical specimens such as blood (14), pus (6), urine (4), sputum (4), endotracheal aspirate (12) and broncho alveolar lavage (6) and screened for the presence of invasive enzymes such as gelatinase, lecithinase and lipase. 21/46 isolates were positive for gelatinase, whereas 28 isolates showed strong positivity and 15 showed weak positivity for lipase. On the other hand, 40/46 isolates showed lecithinase activity by plate method while 10 were strong positive and 32 were weak positive by tube method. A higher percentage of clinical isolates was resistant to most of the routinely used antibiotics. 6.5% of isolates were found to be sensitive to ceftazidime, 10.8% to piperacillin-tazobactam , 23.9% to meropenem, 26% to gentamicin, 19.5% to ciprofloxacin and 39.1% to tetracycline.

KEYWORDS

Acinetobacter baumannii, gelatinase, lecithinase, lipase, antibiotic susceptibility testing.

INTRODUCTION

Aerobic non-fermentative gram-negative bacilli have increasingly become important as human pathogens over the last 20 years and serve as potential reservoir for human infection. *Acinetobacter species* are opportunistic pathogens that readily colonize in patients with compromised host defenses, thereby penetrating deep into the host tissue which serves as a prerequisite for an infection[1].

Acinetobacter spp. have been reported to cause a number of outbreaks of nosocomial infections in hospitalized patients like septicemia, pneumonia, sepsis, endocarditis, meningitis and urinary tract infections. Although acknowledged to be an opportunistic pathogen in hospitalized patients, community acquired infections are reported and cause suppurative infections in virtually every organ system[2].

They are the second most common gram negative nosocomial bacilli next to Pseudomonas encountered in clinical specimens. These organisms can occur frequently as components of commensal flora of man and animals which are regular contaminants of the hospital environment[1].

The presence of virulence determinants may play a cardinal role in expressing its resistance pattern[3]. The existence of some of the virulence factors poses a deleterious effect within the host tissue and its ability to adhere to inanimate objects such as medical devices within the hospital environment renders its persistence as a successful pathogen[4].

Our study aims to elucidate the prevalence of these factors, which still remain to be enigmatic. In order to reveal how Acinetobacter makes its eternal survival within the host tissue which acts as a prerequisite for infection. The recent trend of higher resistance patterns among Acinetobacter brings down the choices of antibiotics for its treatment. Different workers document data regarding the drug susceptibility pattern differently. However, it would be appropriate to document the pattern of resistance for each area for better management strategies. Hence, we have also analyzed current resistance patterns of our isolates.

MATERIALS AND METHODS

(i) Isolation and identification:

A total of 46 clinical isolates of *A. baumannii* were collected from various clinical samples such as blood (n=14), pus (n=6), urine (n=4), sputum (n=4), endotracheal aspirates (n=12) and broncho alveolar lavage (n=6) for the period of September 2009 to May 2010. For this purpose, different city hospitals in Chennai, Tamilnadu were included in the study. Preliminary identification was performed by gram staining, capsule staining and were characterized by standard biochemical test and screened for the presence of various enzymes such as gelatinase[5], lecithinase[6], lipase[7]. Moreover, their antibiotic susceptibility pattern was analyzed by disc diffusion test as per CLSI guidelines.

Table 1
Number of A. baumannii isolates from different clinical specimens

Clinical samples	Number of isolates (n=46)
Blood	14
Pus	6
Urine	4
Sputum	4
Endotracheal aspirate	12
Broncho alveolar lavage	6

(ii) Test for gelatinase:

In brief, overnight grown cultures of *A. baumannii* from Brain Heart Infusion Agar were spot inoculated on Luria Bertani Agar medium containing 3% gelatin. After satisfactory growth, the cultures were flooded with mercuric chloride solution, which denatures and renderes opacity of any unhydrolysed gelatin. Hence, the development of opacity in the medium and zone of clearing around the colonies were considered as positive[5].

(iii)Test for lecithinase:
Plate method:

Trypticase Soy Agar with the addition of 0.11 % calcium chloride and 5% egg yolk were used for the demonstration of lecithinase activity at *p*H 7. Spot inoculation of the organism from a 0.5 Mc Farland standard broth to egg yolk agar was carried out and incubated at 37°C for 24 hours. Lecithinase positive colonies on egg yolk agar were clearly marked by an opaque zone extending from the edge of the colony[6].

Tube method for choline crystals detection:

Trypticase Soy Broth with the addition of 0.11 % calcium chloride and 5% egg yolk were used for the demonstration of lecithinase activity at *p*H 7. Egg yolk broth was inoculated using 0.5 ml of a 24 hour broth culture and incubated at 37°C for 24 hours. Lecithinase positivity was determined by a curdy appearance on the top of the broth and confirmed by the presence of choline periodide crystals by Florence reagent method[6].

(iv)Test for lipase:

A plate assay in a medium containing trioleoylglycerol and fluorescent dye Rhodamine B was used to detect lipase. The growth medium contained (per liter) TSB 8g; Sodium chloride 4g; Agar 10g. The medium was adjusted to *p*H7, autoclaved, and cooled. Then 31.25 ml of trioleoylglycerol and 10 ml of rhodamine B solution were added with vigorous stirring and it was poured over the plates. Spot inoculation was made and incubated overnight. Substrate hydrolysis causes the formation of orange fluorescent colonies visible upon UV irradiation [7].

(v) Anti microbial susceptibility testing:

Invitro activity of the routinely used antibiotics against 46 strains of *Acinetobacter baumannii* was tested on Mueller Hinton Agar by disk diffusion test, Hi Media (Mumbai). The antibiotic agents from each class were selected according to the CLSI guidelines. The antibiotics include piperacillin (100mcg), cefotaxime (30mcg), ceftazidime (30mcg), ceftriaxone (30mcg), cefipime (30mcg), meropenem (10mcg), piperacillin-tazobactam (10mcg), ticarcillin-clavulanic acid (10mcg), gentamicin (10mcg), ciprofloxacin (5mcg), and tetracycline (30mcg).

RESULTS

Amongst 46 clinical isolates of *A. baumannii* obtained from different clinical specimens, 21(45.6%) shows positivity for gelatinase activity, 28(60.8%) is strong positive and 15(32.6%) is weak positive for lipase.

40(87%) was positive for lecithinase by agar plate method; and 10(21.7%), 32(62.6%) were strong and weak producers of lecithinase by tube method respectively.

Table 2
Number of positive isolates of Acinetobacter baumannii from different clinical specimens

S. No	Virulence determinants		Number of positive isolates	Blood (n=15)	Urine (n=4)	Pus (n=6)	Endo tracheal aspirate (n=11)	Sputum (n=4)	Broncho alveolar lavage (n=6)
1	Gelatinase		21 (45.6%)	7	2	1	6	2	3
2	Lipase	Strong	28 (60.8%)	8	2	2	10	2	4
		Weak	15 (32.6%)	4	3	3	1	2	2
3	Lecithinase	Plate	40 (87%)	12	3	5	11	4	5
		Tube Strong	10 (21.7%)	4	1	2	1	1	1
		Tube Weak	32 (69.6%)	10	3	3	9	3	4

Results of antibiotic susceptibility testing were shown in Table 3. High level of resistance was observed for most of the antibiotics tested. Increased resistance were observed with modified penicillin and with β lactamase inhibitors (80-97%) followed by cephalosporin (89-97%), fluoroquinolones (80.4%), co-trimoxazole (76%) aminoglycosides (71.7%), carbapenem (63%) and tetracycline (54.3%).

Table 3
Antibiotic susceptibility pattern of 46 clinical isolates of A. baumannii

S. No	Antibiotics	Resistance	Intermediate	Sensitive
1	Piperacillin (10 mcg)	44 (95.6%)	2 (4.3%)	0
2	Ceftazidime (30 mcg)	41 (89.1%)	2 (4.3%)	3 (6.5%)
3	Ceftriaxone (30 mcg)	45 (97.8%)	1(2%)	0
4	Cefipime (30 mcg)	44 (95.6%)	2 (4.3%)	0
5	Meropenem (10 mcg)	29 (63%)	6 (13%)	11 (23.9%)
6	Ticarcillin-Clavulanic acid (10 mcg)	45 (97.8%)	1 (2%)	0
7	Piperacillin-Tazobactam (10 mcg)	37 (80.4%)	4 (8.6%)	5 (10.8%)
8	Gentamicin (10 mcg)	33 (71.7%)	1 (2%)	12 (26%)
9	Ciprofloxacin (5 mcg)	37 (80.4%)	0	9 (19.5%)
10	Co-trimoxazole (1.25/23.75 mcg)	35 (76%)	11 (23.9%)	0
11	Tetracycline (30 mcg)	25 (54.3%)	3 (6.3%)	18 (39.1%)

DISCUSSION

Until 1970, *Acinetobacter species* were considered as a rare cause of nosocomial infections in the ICU. However, the incidence of Acinetobacter infection has reached a point of concern and poses a threat to hospitalized populations around the

world[8]. In the past decade, Multi Drug Resistance (MDR) *Acinetobacter baumannii* has emerged as a major nosocomial pathogen in many parts of the world, resulting in devastating outcomes in terms of morbidity and mortality[9].

It is evident from many studies that *A. baumannii* holds a repertoire of robust virulence characters which might contribute to its pathogenic potential[5]. Versatility in its ability to grow on a variety of medical devices enables it to establish itself in hospital environment. The increased frequency of isolation from ICU setup and the resistance shown by these organisms to the routinely used antibiotics emphasizes its clinical significance.

We have analyzed the major virulence attributes of *A. baumannii* such as gelatinase, lipase and lecithinase. Out of 46 isolates tested, 45.6% of them produced gelatinase. 60.8% of *A. baumannii* isolates showed strong positivity for lipase production and 87% of isolates produced lecithinase by plate method. When subjected to tube method 21.7% of *A. baumannii* isolates showed strong positivity for lecithinase. Thus, lecithinase was the most secreting extracellular virulence factor by *A. baumannii* followed by lipase and gelatinase.

Studies from different places showed a different percentage of virulence factors. Nural Cevahir *et al.,* (2008) showed 14% of gelatinase producers in their isolates[5].

Gelatinase is a proteolytic enzyme that hydrolyse gelatin[10], which can cross cell membrane and hydrolyze collagen in subcutaneous tissues during wound infections[5]. Its ability to hydrolyze collagen and certain bioactive peptides suggests its participation in the initiation and propagation of inflammatory process[10]

Lecithinase or phospholipase are released by the bacterium to destroy tissues that splits lipoprotein present in human serum. The strong cytolytic action of lecithinase was also known to cause massive intravascular hydrolysis of erythrocytes[11]. Most of the gram positive bacteria are known to produce lecithinase, and only a few gram negative bacteria were reported to have this enzyme productivity[6].

Previous study by Yavankar *et al.,* (2007) showed very lesser percentage of lecithinase in *Acinetobacter species.* However, we could demonstrate 87% of lecithinase production in our isolates[12].

Lipase hydrolyses lipids to form fatty acids and glycerols. It plays a significant role in the digestion of host cellular lipids for nutrients acquisition. Hence, adhesion of lipase to host tissue imparts its indispensible role in infection[13].

We have observed maximum lipase activity with our isolates which were in accordance with the report of Shweta Jagtap *et al.,* (2010) from Pune. They have analyzed the *Acinetobacter haemolyticus* in chemically defined medium[14].

The present study revealed the following pattern of resistance for most of the routinely used antibiotics: piperacillin (95.6%), ceftazidime (89.1%), ceftriaxone (97.8%), cefipime (95.6%), gentamicin (71.7%), ciprofloxacin (80.4%), co-trimoxazole (76%) and tetracycline (54.3%). Even the combinational drugs with a β-lactamase inhibitors were shown to be highly resistant to these isolates such as ticarcillin-clavullanic acid (97.8%), piperacillin-tazobactum (80.4%), Carbapenems were considered as the last resort to treat infections in ICU setup. However, there are recent reports of increasing resistance to these important drugs, documented all over the world. The present study showed 63% of resistance to meropenem. Several other studies have also observed similar results from different regions.

Study conducted by Kaul *et al.,* (2007) from Vellore has reported decreased ceftazidime resistance and increased carbapenem resistance in non lactose fermenting gram negative bacilli[15]. Similarly, Srinivasa Rao *et al.,* (2008) has reported high

levels of resistance (>75%) to both carbapenem and other antibiotics routinely used for the treatment of non lactose fermenting gram negative bacilli[16].

To conclude, the present study demonstrated certain virulence factors in the clinical isolates of *A. baumannii* from Chennai. These isolates were also found to be resistant to many of the important antibiotics used in treatment of non lactose fermenting gram negative bacilli. Further studies might throw more light in understanding the relationship between the presence of virulence attributes and its role in establishment of an infection by *A. baumannii.* Though, there are many reports on this important nosocomial pathogen, data on the relationship between its virulence attributes and disease conditions are important.

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