Isolation And Identification Of Acinetobacter SPP From Clinical Samples

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ABSTRACT: *Acinetobacter baumannii* has emerged over the last decade as a significant opportunistics pathogen. Although it is generally associated with benign colonization of hospitalized patients. The study is undertaken to isolate and identify of *Acinetobacter baumannii* from clinical infections using different laboratory methods. A total of Hundred clinical samples (wound, pus, urine, respiratory secretion, blood) were collected from February to june 2016. *Acinetobacter baumannii* isolates were preliminary identified according to the morphological characteristics and standard biochemical test.

KEYWORDS : Acinetobacter, Biochemical test, Antibiotics, Kirby Bauer, Vitek 2 compact, MALTI TOFF, ICU Patient.

INTRODUCTION

Acinetobacter baumannii is an aerobic Gram-negative coccobacillus that has emerged as an important opportunistic pathogen, especially among debilitated patients, and a common cause of hospital acquired infections (1). such as bacteremia, pneumonia, meningitis, urinary tract infection, and wound infection, especially in intensive care units (2). According to most recent scientific literature, *Acinetobacter* spp. are the second most common non-fermenting Gram negative pathogen isolated from clinical samples after *Pseudomonas aeruginosa* (3).One of the main reasonsfor the present increased in this genus is the emergence of the multiresistant strains, some of which are Pan-Resistant to antibiotics, that suddenly cause an outbreakof infection involving several patients in a clinical unit (4) .Now a days Acinetobacter infections have increased and more attention because of its prolonged environmental survival and tendency to develop drug resistance.

MATERIALS AND METHODS

SAMPLES COLLECTION

The different types of samples were collected from various hospital in Coimbatore city. Samples were taken from Immunocompromised patients from ICU. Hundred samples were isolated from Urine (6), Blood (22), Sputum (58), pus (12) and Body fluid (2). Acinetobacter spp were isolated and identified. (Table 1)

CULTURE

The samples were cultured on Macconkey agar and Blood agar. After overnight incubation at $_{37}{}^{0}{}_{C}$, the suspected colonies were processed for the further identification.

PHENOTYPIC CHARACTERIZATION

All isolates were subjected to simplified phenotypic test The phenotypic characterization was done by Gram's staining, Plating on Mac conkey agar, Blood agar and biochemical tests. The isolates were identified by using vitek 2 system.

ANTIBIOGRAM OF ACINETOBACTER

Antibiogram was done by two methods

a) Disc Diffusion
b) Vitek 2

a) DISC DIFFUSION METHOD

After identification by phenotypic methods, antibiotis susceptibility was performed for each isolates by Kirby – Bauer disc diffusion method. (6). The young culture was swabbed onto Muller Hinton Agar plates and antibiotic disc were placed on the plates and incubated at 37^0 C for 24 hours under aerobic condition. The strains were tested against PC(100µg), TC(100µg), PT(85µg), CFS(105µg), MIN(30µg), DOX(30µg) AU(30µg), CZ(30µg), CE(75µg), CL(30µg), CPM(30µg), IM(10mg) MPM(10µg), CN(30µg), G(10µg), TB(10µg), AK(30µg), CF(5µg), LEN(5µg), CT(25µg), CLN(10µg), ETP(10µg), TGC(15µg).The bacterial growth inhibition zones around the discs were measured based on CLSI guidelines.(7).

b) VITEK 2 COMPACT

Matrix Assited Laser Desorption/ Ionization used by vitek 2. MALDI TOF spectra are used for the identification of organisms. It is standard method for species identification in medical microbiological laboratory (11). The strains were processed for MALDI TOF identification after three days the positive samples were prepared according to the manufacturer's instruction. (12). Antibiotics sensitive identify testing was done using AZT 281 vitek card. Fluorescence is measured every 15 mins and the results of identification are determined after 3 hours.

RESULTS

SAMPLES COLLECTION

Table 1 *The distribution of Acinetobacter baumannii according to infection*

S. No	Type of infection	No of samples	No of positive
1	UTI	6	2
2	Septicemia	22	6
3	Tracheostomy	58	14
4	Wound infection	12	3
5	Body fluid	2	1

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CULTURE

The well mature and isolated colonies were used for this studies. The strains appear in different types of colony morphology on blood agar and macconkey agar plates. In Macconkey agar it produces partically lactose fermenting colonies. In blood agar it produce non-haemolytic, white and smooth colonies.

Table 2 *Colony morphology of different media*

S. No	Media	Morphology
1	Blood Agar	Heamolytic colonies
2	Macconkey Agar	ParticallyLactoseFermenting Colonies.

PHENOTYPIC IDENTIFICATION

In biochemical identification positive for citrate,catalase and negative for oxidase, indole, trible sugar iron test , mannitol .The strains utilized glucose, mannose, sucrose, galactose.(8).

Table 3 *Biochemical Test*

S. No	Test name	Results
1	Oxidase	Negative
2	Catalase	Positive
3	Indole	Negative
4	TSI	Negative
5	Mannitol	Negative
6	Citrate	Positive
7	Urease	Negative
8	Glucose	Positive
9	Mannose	Positive
10	Sucrose	Positive
11	Galactose	Positive
12	Maltose	Negative
13	Lactose	Negative

ANTIBIOGRAM

A) DISC DIFFUSION METHOD

Most of the strains were resistance to most of the antibiotics groups (Beta lactum, Beta lactuminhibitor, Cephalosporins, Carbapenam) except Polypetide group (Colistin, Tigecycline) (9)The disc diffusion method according to CLSI guidelines. guidelines. (7).

Table: 4 *Concentration of antibiotics disc*

S. No	Antibiotics	Concentraction
1	Piperacillin(PC)	100µg
2	Titracyclin(TC)	100µg
3	Piperacillin Tazobactam(PT)	85 µg
4	Cefoperazone Sulbactam (CFS)	105 µg
5	Minocycline (MIN)	30 µg
6	Doxycline (DOX)	30 µg
7	Amoxicillin Clavulanic acid (AU)	30 µg
8	Cefazolin (CZ)	30 µg
9	Cefotoxime(CE)	75 µg
10	Cetriaxone(CL)	30 µg
11	Cefepime (CPM)	30 µg
12	Imipenem (IM)	10mg
13	Meropenem (MPM)	10 µg
14	Cefoxtine(CN)	30 µg
15	Getamycin (G)	10 µg
16	Tobaramycin (TB)	10µg
17	Amikacin (AK)	30 µg
18	Ciprofloxion (CF)	5 µg
19	Levofloxacin (LEN)	5 µg
20	Trimrthoprim Sulfamethoxazole (CT)	25 µg
21	Clostin (CLN)	s10 µg
22	Etrapenam (ETP)	10 µg
23	Tigecycline (TGC)	15 µg

B) VITEK 2

The vitek 2 system detects metabolic changes by fluorescence-based methods which facilitate the identification of gram negative bacteria within 3 hours. This system monitors the kinetics of bacterial growth and calculates MIC using unique algorithm. (10).

DISCUSSION

Sreenivasan et al carried out an investigation to find out the Antibiogram of *Acinetobacter baumannii* by disc diffusion method and Vitek 2 method. They observed all groups of antibiotic resistant to acinetobacter expect polypeptide groups. In this study Hundread isolates were collected from hospital especially in critical care unit. All the isolate were subjected to primary screening .All the isolate were resistant to all antibiotics classes expect Colistin and Tigecycline. However use both drugs are associated with significant organ toxicity. Hence alternative therapeutic options are urgently needed to treat a patient with *Acinetobacter baumannii* infection.

CONCLUSION

In conclusion *Acinetobacter baumannii* is an important opportunistic and emerging pathogen that can lead to serious nosocomial infections. Furthermore, new experimental approaches are warranted to develop and evaluate novel therapeutic strategies for dealing with *Acinetobacter baumannii* infection.

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