



Original Research Article

Speciation and Antimicrobial Susceptibility Pattern of *Acinetobacter* from Clinical Isolates in a Tertiary Care Centre

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ABSTRACT

Keywords

Acb complex, Nosocomial, Nonfermenter, Antimicrobial susceptibility testing

Acinetobacter has emerged as an important nosocomial pathogen. Although ubiquitous in nature, it is commonly seen in hospital environment causing many outbreaks of diseases. A study was conducted in which a total of 2348 clinical samples were processed, out of which 268 were nonfermenters, among these 101 *Acinetobacter* isolates were isolated. Speciation was done which showed Acb complex 78 (77.2%), *A. lwoffii* 13 (12.9%), *A. hemolyticus* 06 (5.9%), *A. junii* 03 (03%) and *A. radioresistens* 01 (1.0%). Antimicrobial susceptibility pattern of *Acinetobacter* species: meropenem (9.5%), piperacillin+tazobactam (9.5%), netilmicin (30.5%), amikacin (37.5%), ceftazidime (38.5%), gentamicin (47.5%), ofloxacin (73.5%) and chloramphenicol (87.5%). Identification and knowing the antibiotic sensitivity pattern of *Acinetobacter* helps in formulating antibiotic policy against hospital acquired infections.

Introduction

Acinetobacter species are Gram negative nonfermentative bacteria commonly present in soil and water as free living saprophytes. They are isolated as commensals from skin and throat. There have been frequent changes in their taxonomy so that their pathogenic role is understood only recently.

Acinetobacter has emerged as an important nosocomial pathogen involved in outbreaks of hospital infections. The ubiquitous organism has been recovered from hospital environment, from colonized or infected

patients or from staff (Hand carriage). Despite the increasing significance and frequency of multidrug resistant *Acinetobacter* infections, many clinicians and microbiologists still lack an appreciation of importance of these organisms because of their confused taxonomic status. In India very few studies on *Acinetobacter* species have been reported and in view of their increasing importance in nosocomial infections further study is warranted in this part of world. In the present study attempt was made to type the *Acinetobacter*

isolates obtained from various sources by a simplified phenotypic identification scheme and also to determine their antimicrobial susceptibility.

Materials and Methods

The study was conducted in the Department of Microbiology, Shri B.M. Patil Medical College, H&RC, Vijaypur from September 2014 to August 2015. A total 2348 specimens like blood, sputum, pus, CSF and other body fluids from patients of different age group admitted in various medical wards, surgical wards and ICU were collected. These specimens were subjected to simplified phenotypic identification scheme and antimicrobial susceptibility testing was done.

Presumptive identification of *Acinetobacter* was made by inoculation on MacConkey agar medium and incubated at 37°C for 24 hours. Urine samples were inoculated on CLED. All non-lactose fermenters were subjected to Gram staining, oxidase test, hanging drop preparation and catalase test. *Acinetobacter* are Gram negative bacilli or coccobacilli, oxidase negative, nonmotile and catalase positive.

Speciation was done on the basis of glucose oxidation, gelatin liquefaction, hemolysis, growth at 37°C and 42°C, malonate assimilation and susceptibility to chloramphenicol. Antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion method for meropenem, piperacillin+tazobactam, netilmicin, amikacin, ceftazidime, gentamicin, ofloxacin and chloramphenicol.

Results and Discussion

Nonfermenter isolates accounted for 11.4% (268) and *Acinetobacter* isolates accounted

for 4.3% (101) of total number of organisms isolated during study period (Table 1).

Pseudomonas was the most common nonfermenter (57% of total nonfermenters) isolated. Male to Female ratio was 1.6:1. *Acinetobacter* infection was more common in patients of age more than 45 years. Most of these patients had respiratory problems like chronic obstructive pulmonary disease (COPD), bronchial asthma and respiratory failure. Infection in neonates was common in preterm babies. In 87.5% (175 isolates) samples, growth was monomicrobial and 12.5% (25 isolates) samples, growth were polymicrobial. *E. coli* was the most common associated organism with *Acinetobacter*. *Staphylococcus aureus* was associated organism in case of wound infection, cellulites and abscess. In our study *Acinetobacter* was isolated more commonly from surgical wards 61(30.5%) followed by ICU 54 (27%), pediatric ward 38 (19%) medical ward 33(16.5%), burn unit 10 (5%) and 2 isolates were isolated from humidifier ventilator and 2 isolates from OT table.

The present study shows more strains belonging to Acb complex (77.2% of total *Acinetobacter* isolates) than non-Acb complex. Other species include *A. lwoffii* 25 isolates (12.9%), *A. hemolyticus* 12 isolates (5.9%), *A. junii* 06 (03%). A single *A. radioresistens* was isolated from patient admitted in burn ward which was multidrug resistant (Table 2). Study conducted by Prashanth et al in 2004 showed isolation of Acb complex in 71%, *A. lwoffii* in 20.3%, *A. johnsonii* 1.6%, *A. hemolyticus* 3.38%, *A. junii* 1.6% and DNA group 1.6% (14).

Isolation rate was higher from pus, majority of them were from cellulitis and wound infections. Isolation rate from blood was 14% which is slightly higher compared to those from USA, France, Belgium (7–9.3%).

Table.1 Number of non-fermenters and *Acinetobacter* isolated from various samples

Specimen	Total number	Nonfermenters	<i>Acinetobacter</i>
Pus/swab	678	83	29
Urine	600	66	28
Sputum	477	50	25
Blood	475	40	14
CSF	44	04	01
Others (TA, ET tip)	74	26	04
Total	2348	268	101

TA-Tracheal aspirate; ET-Endotracheal tube

Table.2 Identification scheme of *Acinetobacter* species

Species (Total number)	Hemolysis on B/A	Growth		OF test	Arg	Mal	Gelatin Liquefaction	C-sensitivity
		37°C	42°C					
Acb complex (156)	-	+	+	S	+	+	-	R
<i>A. lwoffii</i> (25)	-	+	-	NS	-	+	-	S
<i>A. hemolyticus</i> (12)	+	+	-	S	+	-	+	R
<i>A. junii</i> (06)	-	+	-	NS	+	-	--	R
<i>A. radioresistens</i> (01)	-	+	-	NS	+	+		R

Arg - Arginine; Mal-Malonate; C - Chloramphenicol; S - Saccharolytic; NS - Nonsaccharolytic; S - Sensitive; R * Resistant.

Most of them were from preterm and septicaemic patients. Studies from various countries have shown predominance of isolation from urine (21–27%), tracheobronchial secretions (24.8–48.8%). *Acinetobacter* were also isolated from urine (28%) and sputum (25%). A single isolate of Acb complex was isolated from CSF in an adult female patient of 32 years suffering from meningitis. The male to female ratio is 1.6:1 which is similar to the study done in Hong Kong by Ng *et al.* (1996). In 87.5% cases infection was due to monomicrobial *Acinetobacter* infection and in 12.5% cases it was due to polymicrobial. *E. coli* 09 (36%) was the most common associated organism. In the study conducted by Joshi *et*

al. (2006), monomicrobial infection accounted for 71.2% and 28.8% was polymicrobial infection. These polymicrobial infections were more resistant to treatment and morbidity was high in these patients. Most of the isolates were from surgical wards (30.5%), ICU (27%) and pediatric ward 38 (19%). Most of them had undergone invasive procedure like intravascular catheterization, mechanical ventilation and prior surgery. In a study conducted by Anupurba and Sen in 2005, 20.8% of *Acinetobacter* were isolated from ICU, whereas in present study it is 27%. This shows increasing trend of *Acinetobacter* to cause nosocomial infections. One of the most striking features

of genus *Acinetobacter* is the ability to develop antibiotic resistance extremely rapidly in response to challenge with new antibiotics. In the present study, strains were resistant to meropenem (9.5%), piperacillin+tazobactam (9.5%), netilmicin (30.5%), amikacin (37%), ceftazidime (38.5%), gentamicin (47.5%), ofloxacin (73.5%) and chloramphenicol (88.5%). This is similar to study conducted by Capoor *et al.* (2005) and Prashanth and Badrinath (2000, 2004). The difference in the sensitivity pattern was due to environmental factors and different pattern of antimicrobial usage.

In conclusion, during routine microbiological work nonfermentative Gram negative bacilli other than *P. aeruginosa* are not taken seriously and are dismissed as contaminants. But the rate of isolation of *Acinetobacter* in various studies indicates its role as a nosocomial pathogen and also community acquired infection. Traditional typing methods like phenotyping and antibiogram typing have advantage over genotyping as they are readily available and cost effective. So, identification and knowing the antibiotic sensitivity pattern of *Acinetobacter* helps in formulating antibiotic policy against hospital acquired infections.

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