AJMS
(An US National Library of Medicine enlisted journal)
Al Ameen J Med Sci (2010)3(4):345-349
ISSN 0974-1143

SHORT COMMUNICATION

Identification And Speciation Of Acinetobacter And Their Antimicrobial Susceptibility Testing

Preeti B. Mindolli[1]*, Manjunath P. Salmani[2], Vishwanath G[3] and Hanumanthappa A.R[3]

[1]Department of Microbiology, Al-Ameen Medical College, Athani Road, Bijapur - 586108, Karnataka, India, [2]Department of Microbiology, Shri BM Patil Medical College, Solapur Road, Bijapur-586103, Karnataka, India and [3]Department of Microbiology, JJM Medical College, Davangere – 577004, Karnataka, India

Abstract: Acinetobacter has emerged as an important nosocomial pathogen. Although obvious in nature, it is commonly seen in hospital environment causing many outbreaks of diseases. A study was conducted in which a total of 4696samples, out of which 536 were nonfermenters, among these 200 Acinetobacter isolates were isolated. Speciation was done which showed Acb complex 156 (78%), A. lwoffii 25 (12.5%), A. hemolyticus 12 (06%), A. junii 06 (03%), A. radioresistens 01 (0.5%). Resistance pattern to various drugs were Meropenem 19 (9.5%), Piperacillin-tazobactum 19 (9.5%), Netilmicin 61 (30.5%), Amikacin 75 (37.5%), Ceftazidime 77 (38.5%), Gentamicin 95 (47.5%), Ofloxacin 147 (73.5%), Chloramphenicol 175 (87.5%).

Key words: Acb complex; Nosocomial; Nonfermenter

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) [1]. 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 [2]. 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 [2]. 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 [2].

Materials and Methods

The study was conducted in the Department of Microbiology, JJM Medical College, Davangere from June 2007 to May 2008. A total 4696 specimens like Blood, Sputum, Pus, CSF and other body fluids 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 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^{o}C and 42^{o}C, malonate assimilation and susceptibility to Chloramphenicol. Antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion method for Meropenem, Piperacillin-tazobactum, Netilimicin, Amikacin, Ceftazidime, Gentamicin, Ofloxacin and Chloramphenicol.

Results

During the period of study from June 2007 to May 2008, a total of 4696 specimens were examined from patients of different age group admitted in various medical wards, surgical wards and ICU in Bapuji Hospital and Chigateri General Hospital. Nonfermenter isolates accounted for 11.4% and Acinetobacter isolates accounted for 4.25% of total number of organisms isolated during study period. Pseudomonas was the most common nonfermenter (57% of total nonfermenters) isolated (Table -1).

Table1: Number of Nonfermenters and Acinetobacter isolated from various samples

Specimen	Total number	Nonfermenters	Acinetobacter
Pus/swab	1356	165	58
Urine	1200	132	56
Sputum	954	100	50
Blood	950	79	28
CSF	89	08	01
Others (TA, ET tip)	147	52	07
Total	4696	536	200

Male to Female ratio was 1.6:1. Acinetbacter 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 unit10 (5%) and 2 isolates were isolated from humidifier ventilator and 2 isolates from OT table.

Discussion

A total of 4696 samples were studied, out of which 536 were non-fermenters which accounted for 11.4%. Pseudomonas was the most common non-fermenter isolated

(57% of total non-fermenters). Acinetobacter accounted for 37.3% of total non-fermenters and 4.25% of total positive culture. The present study shows more strains belonging to Acb complex (78% of total Acinetobacter isolates) than non-Acb complex. Other species include A. lwoffii 25 isolates (12.5%), A. hemolyticus 12 isolates (06%), 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 K. Prashant 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) [3].

Table 2: Identification scheme of Acinetobacter species

Species (Total number)	Hemo lysis on B/A	Growth		OF test	Arg	Mal	Gelatin Liquefactn	C sensiti vity
		37°c	42°c					
Acb complex (156)	-	+	+ (50%)	Saccharolytic (S)	+	+ (79.4 %)	-	R
A. lwoffii (25)	-	+	-	NS	-	+ (02 %)	-	S
A. hemolyticus (12)	+	+	-	S (75%)	+	-	+	R
A. junii (06)	-	+	-	NS	+	-	-	R
A. radioresistans (01)	-	+	-	NS	+	+	-	R

Arg - Arginine; Mal-Malonate; C – Chloramphenicol; NS-Nonsaccharolytic; S – Sensitive; R – Resistant

Isolation rate was higher from pus, majority of them were from cellulites and wound infections. Isolation rate from blood in this study was 14% which is slightly higher compared to those from USA, France, Belgium (7-9.3%). 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%) [4]. In the present study, Acinetobacter was isolated from urine (28%) and sputum (25%). A single isolate was isolated from CSF it was Acb complex in an adult female patient of 32 years suffering from meningitis. The male to female ratio is 1.6:1 in this study. This is similar to the study done in Hong Kong by TK et al in 1993-94 [5]. 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 SG et al in 2006 [6]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 61 isolates (30.5%), ICU 54 isolates (27%) and pediatric ward 38 isolates (19%). Most of them had undergone invasive procedure like intravascular catheterization, mechanical ventilation and prior surgery. In a study conducted by Anupurba S et al

[7] 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 rapid in response to challenge with new antibiotics. In the present study, strains were resistant to Meropenem in (9.5%), Piperacillin tazobactum (9.5%), Netilmicin (30.5%), Amikacin (37%), Ceftazidime (38.5%), Gentamicin (47.5%), Ofloxacin (73.5%) and Chloramphenicol (88.5%) (Table-3). This is similar to study conducted by Capoor et al [8] and K Prashant et al [3]. The difference in the sensitivity pattern was due to environmental factors and different pattern of antimicrobial usage.

Table 3: Sensitivity pattern of Acinetobacter isolated to different antibiotics

Antibiotics	Sensitivity (S)	Moderatively sensitive (MS)	Resistant (R)
Meropenem (Mr) 10mcg/disc	169 (84.5%)	12 (06%)	19 (9.5%)
Piperacillin-tazobactum (Pt) 100/10 mcg/disc	166 (83%)	15 (7.5%)	19 (9.5%)
Netilmicin (Nt) 30 mcg/disc	116 (58%)	23 (11.5%)	61 (30.5%)
Amikacin (Ak) 30 mcg/disc	113 (56.5%)	12 (06%)	75 (37.5%)
Ceftazidime (Ca) 30 mcg/disc	102 (51%)	21 (10.5%)	77 (38.5%)
Gentamicin (G) 10 mcg/disc	87 (43.5%)	18 (09%)	95 (47.5%)
Ofloxacin (Of) 5mcg/disc	44 (22%)	09 (4.5%)	147 (73.5%)
Chloramphenicol (C) 30 mcg/disc	18 (09%)	06 (03%)	176 (88.5%)

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 has 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.

References

1. Bergogne-Berezin E. Acinetobacter species, saprophytic organisms of increasing pathogenic importance. *Ind J Med Microbial Virol Parasitol Infect Dis* 1994; 281 (4): 389-405.
2. Prashanth K, Badrinath S. Simplified phenotypic tests for identification of Acinetobacter species and their antimicrobial susceptibility status. *J Med Microbiol* 2000; 49: 773-8.

3. Prashanth K, Badrinath S. Invitro susceptibility pattern of Acinetobacter species to commonly used cephalosporins, quinolones and amino glycosides. *Indian J med Microbiol* 2004; 22 (2): 97-103
4. Lahiri K, Mani NS, Purai SS. Acinetobacter species as nosocomial pathogen: Clinical significance and antimicrobial sensitivity. *Med J Armed Forces India* 2004; 60: 7-10.
5. Ng TK, Ling JM, Cheng AF, Norrby SR. A retrospective study of clinical characteristics of Acinetobacter bacteremia. *Scandinavian J of Infect Dis* Suppl 1996; 101: 26-32.
6. Joshi SG, Litake GM, Satpute MG, Telang NV, Ghole VS, Niphadkar KB. Clinical and demographic features of infections caused by Acinetobacter species. *Indian J Med Sci* 2006 ; 60 (9): 357-360.
7. Anupurba S, Sen MR. Antimicrobial resistance profile of bacterial isolates from ICU: Changing trends. *J Commun Dis* 2005; 37 (1): 58-65.
8. Capoor MR, Nair D, Srivastava L, Gupta B, Aggarwal P. Characterization and changing minimum inhibitory concentration of Acinetobacter species from a tertiary care set up. *J Commun Dis* 2005 ; 37 (2): 99-107.

*All Correspondence: Dr. Preeti B. Mindolli, Asst. Professor, Department of Microbiology, Al-Ameen Medical College, Athani Road, Bijapur -586108, Karnataka, India. E-mail: drmindolli@rediffmail.com

