Acinetobacter spp as Nosocomial Pathogen : Clinical Significance and Antimicrobial Sensitivity

Lt Col KK Lahiri*, Lt Col NS Mani[†], Lt Col SS Purai[#]

Abstract

Acinetobacter spp are ubiquitous aerobic Gram negative coccobacillus, that are now increasingly responsible for a large number of nosocomial infections. In our study, over a period of six months (Jan-Jun 2000) at a tertiary care hospital, 152 (12.9%) isolates of *Acinetobacter* spp were obtained from a total of 1175 isolates grown from all clinical specimens. Most of the isolates 126 (82.9%) were from hospitalised patients in the spinal cord injury centre, intensive care units and those on prior antibiotic therapy. Community acquired infections were also seen in 26 (17.1%) out patient department (OPD) cases. Isolates were from urine, respiratory exudates, blood and pus/burn wound swabs predominantly. They were resistant to commonly used antibiotics while being sensitive to amikacin, augmentin, piperacillin, netilmicin and cefotaxime. 69.2% isolates exhibited resistance to two or more antibiotics. Clinical co-relation must be under taken to exclude commensal contaminants, before considering it to be a pathogen and prescribing antibiotics to the patient.

MJAFI 2004; 60 : 7-10

Key Words : *Acinetobacter* spp; Antibiotic resistance; Nosocomial infection

Introduction

Members of the genus *Acinetobacter* are ubiquitous, free living, small aerobic Gram negative cocco-bacilli that prefer moist environment and can be easily obtained from soil, water, food and sewage [1]. They are usually considered to be opportunistic pathogens, and of recent have been reported to cause a number of outbreaks of nosocomial infections in hospitalized patients like septicaemia, pneumonia, wound sepsis, endocarditis, meningitis and urinary tract infection (UTI) [2,3]. Although acknowledged to be an opportunist in hospitalised patients, community acquired infections are reported and they can cause suppurative infections in virtually every organ system [4]. Interpreting the significance of isolates from clinical specimens is often difficult, because of the wide distribution of *Acinetobacter* in nature and its ability to colonise healthy or damaged tissue [5]. Upto 25% of healthy ambulatory adults exhibit cutaneous colonisation and are the most common Gram negative bacilli carried on the skin of hospital personnel [6]. In our study undertaken over a period of six months (Jan-June 2000) at a tertiary care super speciality hospital, we report the significance of infections caused by *Acinetobacter* spp and their antimicrobial susceptibility pattern.

Material and Methods

The study was carried out in a 700 bedded tertiary care hospital over a six month period. All the clinical specimens were initially processed to separate the non-fermenters from the other Gram negative bacilli. There after identification was done to confirm presence of *Acinetobacter* spp.

In the protocol followed, all the clinical samples were inoculated on MacConkey agar and 5% sheep blood agar at 37°C for 24 hours. Urine samples were inoculated into CLED agar. Growth on MacConkey medium showed a pinkish tint, while no pigmentation was seen on blood agar. Gram stain of direct smear from sample showed tiny diplococci. Gram stain, oxidase, catalase and motility tests were undertaken from the growth on various media. They were Gram negative cocco bacilli, strictly aerobic, oxidase negative, nonmotile and catalase positive [7,8]. Colonies of *Acinetobacter* spp were white/cream coloured, smooth, circular with entire edges. Some of the strains showed haemolysis. Rapid utilisation of 10% glucose was seen with O-F medium. Isolates of *Acinetobacter* spp were differentiated from other oxidase negative, non-motile, non-fermenting bacilli like CDC group I and *Bordetella holmesii* by the nitrate reduction test and production of brown soluble pigments.

Species differentiation was done on basis of glucose oxidation, gelatin liquefaction, haemolysis, growth at 37°C and 44°C, susceptibility to penicillin and chloramphenicol discs. *Acinetobacter baumannii* complex (Acb complex) showed predominance amongst the isolated species. Other species identified were *A. haemolyticus* and *lowffii*.

Antimicrobial susceptibility tests using modified Stokes disc diffusion method was carried out with amikacin, gentamicin, netilmicin, ciprofloxacin, cefotaxime, piperacillin, norfloxacin, co-amoxycylav, nalidixic acid and nitrofurantoin.

Results

From a total of 1175 isolates of various bacteria obtained from different clinical specimens, 152 (12.9%) isolates belonged to the *Acinetobacter* spp. While from amongst 367 non fermentative bacilli grown during the same period, the

*Associate Professor, Department of Microbiology, Armed Forces Medical College, Pune - 411 040, [†]Classified Specialist (Pathology), Military Hospital, Jodhpur, [#]Commanding Officer, 406 Field Ambulance, C/o 56 APO.

Table 1**Identification scheme of *Acinetobacter* species**

	Glucose oxidation	Gelatin liquefaction	Haemolysis	Growth		Susceptibility		Total
				37°C	44°C	Penc.	Chlor.	
Acb-complex	+	—	—	+	—	—	—	115
<i>A. haemolyticus</i>	+	+	+	+	—	—	—	26
<i>A. lowffi</i>	—	—	—	+	—	+	+	11

total number of *Acinetobacter* species isolated was high at 41.4% (152). Of the total 152 isolates of *Acinetobacter* species, nosocomial isolates from the hospital patients were 126 (82.9%) as compared to the 26 (17.1%) community acquired isolates from the OPD, which were predominantly from urine and wound/pus swab cultures. The overall percentage of isolation in hospitalized cases is 10.7% and that of OPD cases is 2.2%. Species differentiation of the isolates showed a predominance of *A. baumannii* complex at 75.6% while *A. haemolyticus* and *lowffi* showed an isolation rate of 17.1% and 7.3% respectively (Table 1). Ward wise distribution of isolates are shown in Table 2. The highest number of isolates 28.9% (44) were from the spinal cord injury centre. From this ward in some cases isolates were grown on repeated culture from different sites of the same patient, hence we have 152 isolates from 132 patients. Other locations of isolation were intensive care unit (ICU) 11 (7.2%), surgical wards 25 (16.4%), burns unit 11 (7.2%), family wards 17 (11.1%), medical wards 7 (4.6%), orthopaedics 6 (3.9%), miscellaneous 5 (3.2%).

The distribution of the isolates from various specimens of pus, urine, blood, respiratory secretions, sputum, catheter tips etc. are as shown in Table 3. Multi drug resistant isolates showing resistance to two or more antibiotics was 69.7% (106 isolates). The highest was seen in urine specimens at 63.2% (67). Other specimens showed much less number with multidrug resistance (Table 3). Only 36 (23.6%) isolates were sensitive to all antibiotics. In this study amikacin, co-amoxycylav, netilmicin showed highest sensitivity at 73%, 67%, 88% respectively. Moderate sensitivity was seen to piperacillin (52%) and cefotaxime (57%). Poor sensitivity was seen with trimethoprim + sulphamethoxazole (23%), ampicillin (20%), nalidixic acid (25%) and nitrofurantoin (30%).

Discussion

During routine clinical microbiology work in most laboratories, non-fermentative Gram negative bacilli (NFGNB) other than *Pseudomonas aeruginosa* are not taken seriously as a pathogen [9]. They are not pursued for identification and are dismissed as contaminants. We took up this study when we regularly encountered isolates of NFGNB from various clinical samples, especially those from the spinal cord injury centre patients. These isolates were identified as *Acinetobacter* spp as per standard criteria [8]. In Table 2, of the 152 isolates of *Acinetobacter* species, 126 (87.9%) were nosocomial isolates obtained from

Table 2**Distribution of isolates in various wards and number of patients**

Ward	No. of isolates %	No. of patients
Spinal cord injury centre	44 (28.9%)	34
Surgical wards	25 (16.4%)	21
Burns unit	11 (7.2%)	07
Orthopaedic wards	06 (3.9%)	05
ICU	11 (7.2%)	11
Medical wards	07 (4.6%)	07
OPD	26 (17.1%)	25
Family wards	17 (11.1%)	17
Misc areas	05 (3.2%)	05
Total	152	132

Table 3**Clinical specimens showing isolation rates and antibiotic resistance pattern**

Specimen	Total no. of isolates %	% resistance to two or more antibiotics	Asymptomatic patients with isolates
Pus/burn wound swab	17 (11.18%)	10 (9.4%)	05 (4.7%)
Urine	79 (51.97%)	67 (63.2%)	15 (9.8%)
Blood	15 (9.87%)	05 (4.7%)	02
Resp. secretions	12 (7.90%)	06 (5.6%)	03 (1.9%)
Sputum	19 (12.5%)	11 (10.3%)	07 (4.6%)
Catheter tips	07 (4.6%)	06 (5.6%)	01
Other specimens (shunts, eye)	03 (1.97%)	01 (0.94%)	nil
	152	106 (69.7%)	33 (21.7%)

Table 4***Acinetobacter* spp isolation from various countries**

Specimen	USA (1977)	France (1991)	Belgium (1991)	Germany (1993)	Present study (2000)
Urine	27.0	21.0	27.0	8.2	51.3
Respiratory secretions	28.9	27.0	24.8	48.8	7.8
Pus	21.5	27.5	22.3	16.4	11.1
Blood	9.3	7.5	7.6	26.6	9.8
Catheter tips	0	15.5	0	0	4.6
Others	13.3	2.0	18.3	0	1.94

patients admitted to various wards, whereas only 26 (17.1%) were community acquired from the OPD cases. The overall percentage of isolation in hospitalized cases stands at 10.7% vis-a-vis 2.2% in OPD cases from amongst all bacterial isolates, thereby bringing to fore the role of *Acinetobacter* spp as an important nosocomial pathogen, since in most cases the patients were symptomatic with fever, leucocytosis, pus discharge / UTI.

Studies on *Acinetobacter* in various countries [10] have shown a predominance of isolation from urine (21-27%) and tracheo-bronchial secretions (24.8-48.8%). In this study, urine samples were predominantly received for culture as compared to respiratory secretions, hence the high figure of 51.3% (Table 4).

The 17.1% isolates of *Acinetobacter* from OPD cases as shown in Table 2, is higher than that of ward cases except spinal cord injury centre, only because all OPD cases from various specialities have been grouped together to give an overview of community acquired infection by this pathogen, while all isolates from spinal cord injury centre were from indoor patients.

Isolation rate from blood in this study at 9.8% was comparable to those from USA, France and Belgium ranging from 7-9.3%. They were high from Germany at 26.6%. Although there were no isolates from catheter tips in USA, Belgium and Germany, studies from France showed a higher growth rate at 15.5% as compared to our isolation at 4.6%. Pus / burn wound swab isolation rate in this study was lower at 11.7% as compared to the other western countries.

Previous studies [10] have identified various risk factors for *Acinetobacter* infection or colonisation, that include factors related to host like, period of hospitalisation, subject to procedures-indwelling catheters, intubation, catheter lines etc. and previous antibiotic therapy (cephalosporins/fluroquinolones). Majority of the isolates in this study (28.9%) were from patients of the spinal cord injury centre where a number of risk factors were present, including the fact that patients were hospitalised for very long periods, the moist environment of the catheters/uobags and treatment with antibiotics off and on, all giving an opportunity for the bacilli to colonise various sites and then later turn into a pathogen. Husni et al [11] found an association between cephalosporins and *Acinetobacter* infection.

The role of exposure to certain antibiotics provides a selective advantage to a small resistant sub population of organisms in patients already colonised, thereby enabling them to turn into pathogens at the opportune moment. In many of the patients *Acinetobacter* spp exhibiting two different antibiograms were isolated from

different clinical specimens of the same individual, indicating the necessity to clinically correlate the isolate as a pathogen or commensal.

Acinetobacter spp have been associated with 2-61% of nosocomial UTIs [2,4,12]. In this study 51.3% of the isolates were from urine samples, however, not all patients had evidence of UTI implying their presence as commensal. Strains showing resistance to two or more antibiotics were the highest at 63.2% from the urinary isolates. In many cases community acquired UTIs by *Acinetobacter* spp were detected in absence of known predisposing factors as reported in some studies [13]. Isolates of *Acinetobacter* from ICU, burns unit and orthopaedic centre were obtained since a number of risk factors are operational and permit their spread and persistence. Growth of this species has been documented from the ward environment like trolleys, mattresses, oxygen and suction catheters etc. [14]. Although *Acinetobacter* wound infection showed an isolation rate of 11.1%, it poses a problem to the clinician whether to treat it aggressively as a pathogen or ignore as a colonizer.

In this study, isolates were from burns patients / bed sore wounds of spinal cord injury patients, hence were treated aggressively since patients were symptomatic and in septicaemia. Tracheal aspirates and sputum sample isolates were treated only on clinical correlation of cases. Cases of pneumonia, especially ventilator associated (VAP) with fever, leucocytosis and lung infiltrates showed *Acinetobacter* in one case. Since this organism is a fast coloniser of the respiratory tract, its percentage can increase from 7% to 45% in healthy subjects to those on ventilators respectively, and all samples from such patients should be scrutinised for this bacilli [15].

Clinically *Acinetobacter* bacteraemia or sepsis does not differ from other Gram negative bacteria. In the 15 (9.8%) isolates grown from blood samples, 10 cases had features of sepsis and were debilitated due to old age, malignancy, burns and multiorgan failure. In 5 cases, the patients did not have any evidence of infection and were probably skin contaminants. Antibiotic resistance pattern of *Acinetobacter* in this study has shown multidrug resistance in 106 (69.7%) isolates from a total of 152. This follows the pattern reported by various authors [8]. Isolates of *Acinetobacter* spp from infected patients are known to be resistant to any of the relevant antibiotic and the resistance varies according to the species, site of infection and selection pressure from frequent and repeated use of antibiotics. Sensitivity to amikacin, netilmicin and co-amoxyclav as seen in this study has also been reported by Tankovic et al [16].

Overall infections caused by *Acinetobacter* spp provide an impressive demonstration of the increasing importance of this genus as a human pathogen because of the high potential of this genus to develop antibiotic resistance, leading to a considerable selective advantage in environments with widespread and heavy use of antibiotic, especially with relation to hospital environment and nosocomial infections.

References

1. Gerner-Smidt P. Taxonomy and epidemiology of *Acinetobacter* infections. Rev Med Microbiol 1995;6:186-97.
2. Towner KJ. Clinical importance and antibiotic resistance of *Acinetobacter* spp. J Med Microbiol 1997;46:721-46.
3. Levi I, Rubinstein E. *Acinetobacter* infections-overview of clinical features. In : Bergogne-Berezin I, Joly-Guilloo MI, Towner KJ, editors. *Acinetobacter : microbiology, epidemiology, infections, management*. Boca Raton, CRC Press. 1996;101-15.
4. Glew RH, Moellering RC, Kunz LJ. Infections with *Acinetobacter calcoaceticus* (*Herellea vagincola*) : Clinical and laboratory studies. Medicine 1997;56:79-97.
5. Henricksen SD. *Moraxella*, *Acinetobacter* and *Mimae*. Bacterial Rev 1973;37:522-61.
6. Mandell G, Bennett JE, Dolin R. Principles and practice of infectious diseases. Churchill Livingstone 5th ed 2000;2:239-41.
7. Colle JG, Fraser AG, Marmion BP, Simmons A. Practical Medical Microbiology. Churchill Livingstone 14th ed. 1996:294-6.
8. Koneman EW, Allen SD, Jande WM, Schreckenberger PC, Winn Jr WC. Colour atlas and text book diagnostic Microbiology. Lippincot 5th ed 1997;286-7.
9. Veenu, Rama S, Arora DR. Isolation and susceptibility pattern of non fermenting Gram negative bacilli from clinical samples. Indian J Med Microbiol 1999;17(1):14-7.
10. Villers D, Espase E, Coste-Burel M, Pharm D et al. Nosocomial *Acinetobacter baumannii* infections : Microbiological and clinical epidemiology. Ann Intern Med 1998;129:182-9.
11. Husni RN, Goldstein LS, Arroliga AC et al. Risk factors for an outbreak of multidrug resistant *Acinetobacter* nosocomial pneumonia among intubated patients. Chest 1999;115:1378-9.
12. Bergogne-Berezin E, Towner KJ. *Acinetobacter* spp as nosocomial pathogens : microbiological, clinical and epidemiological features. Clin Microbiol Rev 1996;9:148-65.
13. Rello J. *Acinetobacter baumannii* infections in the ICU. Chest 1999;115:1226-9.
14. Mayhall CG. Infection in burn patients. In : Wenzel RP, editor. CRC handbook of hospital infections. Boca Raton, CRC Press. 1981;317-39.
15. Tankovic J, Legrand P, Gatines GD et al. Characterisation of a hospital outbreak of Imipenam resistant *Acinetobacter baumannii* by phenotypic and genotypic typing methods. J Clin Microbiol 1994;32(ii):2677-81.
16. Prashanth K, Badrinath S. Simplified phenotypic tests for identification of *Acinetobacter* spp and their antimicrobial susceptibility status. J Med Microbiol 2000;49:773-8.

ATTENTION CME ORGANISERS

All service hospitals/institutions organising CME will contribute to MJAFI fund as per rates given below :-

(a) CME with less than 100 delegates	- Rs. 2500/-
(b) CME upto 300 delegates	- Rs. 5000/-
(c) CME with more than 300 delegates	- Rs. 10000/-