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Original Article

Nosocomial Multi-Drug-Resistant Acinetobacter Infections - Clinical Findings, Risk Factors and Demographic Characteristics

Rubina Lone,[1] Azra Shah,[2] Kadri SM,[3] Shabana Lone,[4] Shah Faisal[5]

[1]Department of Microbiology, Sheri Kashmir Institute of Medical Sciences (SKIMS) Medical College, Srinagar, Kashmir, India; [2]Department of Pathology, SKIMS Medical College, Srinagar , Kashmir, India; [3]Public Health Specialist, Regional Institute of Health and Family Welfare, Directorate of Health Services, Srinagar, Kashmir, India; [4]Royal Hayat Hospital, Kuwait; [5]Resident, SKIMS Medical College, Bemina, India

Abstract

Recently, Acinetobacter emerged as an important pathogen and the rate of isolation has increased since the last two decades worldwide. Objectives of the present study were to see the incidence of Acinetobacter infection at a tertiary care hospital at Kashmir, India, demographic features of the infections, species identification and antibiotic sensitivity and resistance pattern of the isolates. The clinical samples submitted to Microbiology laboratory at SKIMS over a period of 2 years (June, 2001 to June, 2003) were investigated. Identification, speciation and antibiotyping were performed for the isolates of Acinetobacter recovered from clinical samples including urine, pus, sputum, blood, CSF and other body fluids. Clinical and demographic characteristics were studied retrospectively. Out of a total of 5352 infected samples, 258 (4.8%) were found to be due to Acinetobacter. The organism was responsible for 76 (39.64%) cases of urinary tract infection and 38 (29.45%) cases of wound infection and was most prevalent in the intensive care unit (29.84%). *A. baumannii* was the most predominant species. Prolong hospital stay, Mechanical ventilation and Intensive Care Units were found to be potential risk factors. High level of resistance was recorded for Ampicillin (86.3%), Cefazolin (93.2%) Gentamicin (61.5%), Cefotaxime (65.8%), Ceftriaxone (61.5%) and Ciprofloxacin (69.2%). Although no specific pattern during antibiotyping was observed, but most of them were multi-drug resistant. Nosocomial infections by multi-drug-resistant Acinetobacter have emerged as an increasing problem especially in the intensive care units of the hospital. The analysis of risk factors and susceptibility pattern will be useful in understanding epidemiology of this organism in a hospital setup.

Key words: Acinetobacter, Nosocomial infection, Antibiotyping, Multi-drug resistant

Introduction

Acinetobacter is one of the important nosocomial pathogens and has been known to cause different kinds of opportunistic infections.[1] These gram negative coccobacilli are ubiquitous in nature, responsible for causing intermittent outbreaks especially in regions where temperature is hot and humid. Infections caused by them are difficult to control due to multidrug resistance, which limits therapeutic options in critically ill and debilitated patients, especially from the intensive care units (ICU), where prevalence of the organism is the most noted.[2]

Acinetobacter baumannii is now recognized to be the species of great clinical importance being capable of causing life-threatening infections including pneumonia, septicemia, wound sepsis, urinary tract infection, endocarditis and

✉ ***Correspondence:***
Dr. Rubina Lone
Assistant professor
Department of Microbiology
SKIMS Medical College, Srinagar, India
E-mail: kadrism@gmail.com

meningitis.[3] Also it is currently the most common isolate from gram negative sepsis in immunocompromised patients posing risk for high mortality.[4] The organism prefers moist environment, therefore, its colonization among damaged tissues is common.[5]

It is very difficult to explain the role of Acinetobacter acquisition in the ICU, since the organism does not always act as an infecting pathogen, as it is widely distributed in nature and has tremendous colonizing potential.[1,6] Also there is a significant difference in the behaviour of this organism among isolates recovered from various geographic locations.[7] In addition, risk factor for Acinetobacter acquisition, may vary in different set-ups with epidemic outbreaks of infection or endemic colonization.[8] Although various factors predisposing to Acinetobacter infection have been analyzed in different studies, there are only few authentic reports from India that have attempted to determine the risk factors and *in vitro* susceptibility and resistance patterns of clinically significant Acinetobacter isolates.[9,10,11]

The present study describes the experiences with clinical materials and cases from which the strains of Acinetobacter were isolated and to determine the resistance pattern of Acinetobacter isolates to various antimicrobial agents by disc diffusion method and micro-broth dilution method obtained from a tertiary care hospital.

Methods

After taking approval from the hospital ethical committee, the study was carried out in a 600-bed tertiary care hospital, the Sheri Kashmir Institute of Medical Sciences, located in North India during 2 years period (June, 2001 through June, 2003). Nosocomially acquired Acinetobacter infection was defined as the case from which isolation of the organism was done repeatedly from blood cultures and other specimens, 72 hours following a patient was admitted to the hospital. Standard definitions as given by Centre for Disease Control and Prevention were used to differentiate categories of infection versus colonization.[13]

In brief, patients from whom Acinetobacter was isolated in absence of clinical disease suggested colonization and were not included in the study. Clinical specimens included were blood, CSF, endotracheal aspirate, urine, sputum, pus and other body fluids like pleural and peritoneal fluids. The following variables were analyzed: patient age, sex, and the presence of underlying diseases or conditions, admission to ICU, mechanical ventilation, urinary and intravenous (IV) catheterization, number of hospital days and surgery, if any.

All clinical specimens were initially processed by the routine microbiological and biochemical tests. Typical colonies were enumerated, picked and examined further. Acinetobacter was identified by gram-staining, cell and colony morphology, activity of oxidation/ fermentation tests, absence of motility and negative oxidase and positive catalase tests. Speciation of Acinetobacter into various genomic species (GS) was done by using a battery of bio-chemical tests.[14]

Disc diffusion susceptibility testing was performed on Mueller-Hinton agar for following anti-microbial agents with their concentrations given in parentheses: Ampicillin (10µg), Amikacin (30µg), Gentamicin (10µg), Ciprofloxacin (5µg), Ofloxacin (5µg), Cefazolin (30µg), Cefotaxime (30µg), Cefoperazone+Sulbactam (75µg) and Imipinem (10µg). Strains found resistant to various antimicrobials by disc diffusion method were tested by NCCLS broth micro-dilution method.[15] *Pseudomonas aeroginosa* ATCC 27853 was used as the control strain.

The difference in the risk-factors among patients with Acinetobacter infection and patients with other gram-negative bacterial infections were compared and investigated for significant risk factors in patients with these infections. Contingency tables were calculated with Pearson's test of Fischer's exact test by comparing the proportions, wherever necessary. The differences were considered to be significant if the p-value associated with the test was less than 0.05. For all the analysis, the SPSS software, version 10.0 was used.

Results

During the study period, 25,200 samples were cultured, of which 5352 (21.23%) were found to be infected. Out of these infected samples, 258 (4.8%) were found to be due to Acinetobacter.

The patients ranged in age from 18 days to 84 years (Mean age ± SD, 33.2± 22.8 years, median age 42 years). (Table I) Acinetobacter was isolated from various types of infections; among these, urinary tract infections were extremely

significant ($p<0.0001$), followed by pus and wound exudates ($p<0.05$). Also, 17% of the bacteremic cases were associated with catheterization, about 50% of them had undergone surgery and 24% had been intubated and ventilated.

Acinetobacter infection was significantly observed ($p<0.05$) in intensive care unit and patients on mechanical ventilation. Also, a longer stay in hospital that is beyond the first week was significantly associated with a remarkably higher rate of infection ($p<0.0001$). However, no statistically significant association was found in relation to age, sex and surgery.

A. baumanni was the main species responsible for 72% of the infections followed by *A. calcocaeticus* and *A. junii* (10.6% and 7.5 % respectively). *A. lwoffii* and *A. haemolyticus* were predominantly found in wound exudates.

Table I: Demographic and clinical characteristics of the cases infected with Acinetobacter species

Characteristics	Number of cases	Percent
Age (years):		
0 - 15	44	17.1
15- 30	43	16.6
30- 60	72	27.9
=60	99	38.4
Sex:		
Male	163	63.0
Female	95	37.0
Hospital Stay (Days):		
1 - 7	83	32.17
= 7	175	67.83
Indicated source of infection:		
Urinary	102	39.64
Pus and exudates	76	29.45
Respiratory (sputum, BAL etc.)	38	14.72
Blood	18	06.70
CSF	08	03.31
Bone	01	00.38
Peritoneal fluid	01	00.38
Unknown	14	05.42
Risk factor distribution:		
Admission to ICU	73	29.84
Mechanical Ventilation	53	20.54
Existing chronic illness	38	14.72
Urinary and IV catheterization	37	14.34
Endotrachial intubations	12	04.6
Unknown	45	15.91

The disc diffusion susceptibility testing show the percentages of resistance and sensitivity among all isolates. High level of resistance was recorded for Ampicillin (86.3%), Cefazolin (93.2%), Gentamicin (61.5%), Cefotaxime (65.8%), Ceftriaxone (61.5%) and Ciprofloxacin (69.2%). Amikacin, Cefoperazone+Sulbactam and Imipinem showed maximum activity with an overall low resistance of 17%, 11.5%, and 1.5% respectively. (Table II)

Table II: *In vitro* activity of various antimicrobial agents against 258 Acinetobacter isolates

Antimicrobial agent	Percent of isolates found-	
	Resistant	Sensitive
Ampicillin	86.3	13.7
Gentamicin	61.5	38.5
Amikacin	17.0	83.0
Ciprofloxacin	69.2	30.8
Ofloxacin	47.0	53.0
Cefazolin	93.2	6.8
Cefotaxime	65.8	34.2
Ceftriaxone	61.5	38.5
Cefoperazone+Sulbactam	11.5	88.5
Imipinem	1.5	98.5

Strains of *A. baumainnii* were found to be more resistant to all antibiotics as compared to other Acinetobacter species. The range of MIC results obtained were found highly elevated in isolates. The highest resistance was observed in ICU isolates, where *A. baumanni* was most prevalent. (Table III)

Table III: Range of MIC for multi-drug resistance strains of Acinetobacter isolates

Antibiotic	MIC (µgm/ml)		
	MIC range	MIC_{50}	MIC_{90}
Ampicillin	4 - 1024	64	≥512
Gentamicin	8 - 256	32	256
Amikacin	1 - 256	16	128
Ciprofloxacin	8- 256	64	256
Ofloxacin	0.15 - 64	4	32
Cefazolin	8 - 1024	512	ND
Cefotaxime	8 - 512	64	≥512
Ceftriaxone	8 - 512	32	≥512

Discussion

Acinetobacter has emerged as an important nosocomial pathogen, often with a rising prevalence of multidrug resistance and are associated with life-threatening infections.[15,16] The overall incidence of Acinetobacter from all infective samples was 4.8 % (258 out of 5352) indicating its importance as a nosocomial pathogen, since in most of the cases the patients were symptomatic for sepsis. There was a significantly higher incidence of infection among males which is in tandem with other studies from India.[12]

The literature search demonstrates that *A. baumannii* together with *A. calcoaceticus;* GS3, GS13 (Genomic species 3 and 13) are predominantly involved in infection and are collectively known as *A. calcoaceticus- A. baumannii* (Acb) complex group.[17] *A. baumanni* was the major species isolated from 72% of the clinical samples in the present study, is reportedly a major species in other parts of the world as well.[7] In the current study, the maximum number of isolates was from the urinary tract (39.64%) and these were the strains that showed maximum multidrug resistance. These results are comparable to some of the studies done previously.[2] About 15% of these isolates were associated with the use of indwelling catheters and 30% of the patients had serious underlying debilitating diseases. The incidence of respiratory tract infection was 14.7%. Mechanical ventilation and admission to ICU were found to be independent risk factors for these infections. Bacteremia is known to be associated with risk factors like intravenous catheterization.[19]

Overall, in the present study, the significant risk factors for Acinetobacter infection were mechanical ventilation, admission to ICU, underlying chronic debilitating condition and a prolonged hospital stay. A longer stay in a high risk unit and use of mechanical ventilation has been identified as a risk factor in previous studies as well.[17,19,20]

Despite many intensive efforts, the nosocomial acquisition of Acinetobacter remains problematic especially in the ICUs. There are difficulties in control of infections due to their high resistance to antimicrobials in the hospital environment. Exposure to certain antibiotics provides a selective advantage to a small number of resistant organisms in patients already colonized, thereby enabling them to turn into pathogens.

Susceptibilities of Acinetobacter against various antimicrobials being considerably different among countries, centres and even among different wards of the same hospital, therefore, such type of local surveillance studies are found important in deciding the most adequate therapy for Acinetobacter infection.[2] The high-level resistance of Acinetobacter to antimicrobials seems inevitable.[22] Only a few authentic data are available regarding *in vitro* susceptibility of clinical isolates of *A. baumannii* in India.[23] Increasing resistance to Cephalosporins was observed mainly in strains belonging to the Acb complex. Amikacin, Cefoperazone+Sulbactam and Imipinem showed maximum level of activity with susceptibilities of 83%, 87.5% and 98.5% respectively. This susceptibility pattern conforms to the recent introduction of these antibiotics in the hospital where the present study was carried out. The MIC range of presently isolated strains was higher than many other recent reports.[19,22] This means multi-drug resistant (MDR) isolates are increasing day by day, probably due to indiscriminate use of these antibiotics in healthcare settings. It is re-emphasized that broad-spectrum antibiotics should be used with caution. Cefotaxime, and/or Ceftriaxone should be discontinued in units where resistant strains for these two antibiotics are being reported. With revealation of Cefotaxime and/or Ceftriaxone-resistant strains from this study, the hospital ICU was advised to use other antibiotic combinations like effective beta lactams or Carbapenem along with Amikacin.

In conclusion, the MDR *A. baumannii* was the species responsible for majority of Acinetobacter infection in the hospital under study. Mechanical ventilation and admission to ICU were found to be potential independent risk factors in the setup investigated. Strict infection control measures may prevent nosocomial infections. Further research related to mechanism of resistance and extended spectrum beta-lactamases and Carbapenem is underway.

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[Conflict of Interest: none declared]