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Original Article

NOSOCOMIAL INFECTIONS DUE TO *ACINETOBACTER* SPECIES: CLINICAL FINDINGS, RISK AND PROGNOSTIC FACTORS

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Abstract

Purpose: Nosocomial infections caused by *Acinetobacter* species is of increasing concern in critically ill patients, and the risk factors for this infection are not well established. The present investigation was done to determine incidence of nosocomial *Acinetobacter* infections. Our study retrospectively attempts to find risk and prognostic factors for the nosocomial acquisition of *Acinetobacter* infection. **Methods:** The medical records of 43 patients with *Acinetobacter* infection during two-year period (Oct1998-Oct2000) were reviewed to find the factors involved in the nosocomial acquisition of *Acinetobacter*. *Acinetobacter* isolates that were obtained from these patients were phenotypically typed using carbon assimilation tests. Antimicrobial susceptibility testing results were noted from the microbiology records. **Results:** *Acinetobacter baumannii* accounted for 41.8% (n=18) of all the infections. By multivariate logistic regression analysis, only resistant antibiotype {(Ceftazidime- OR, 7.13 [95% CI, 1 to 46]; *P*= 0.044); (Cefotaxime- OR, 6.09 [CI, 0.87 to 30]; *P* = 0.045)} and mechanical ventilation (OR, 5.84 [CI, 0.83 to 31]; *P* = 0.05) were found to be potential independent risk factors for mortality. Overall mortality rate was 33%. **Conclusions:** Most of *A. baumannii* isolates were multidrug resistant in our set up and infections due to them were associated with high mortality. Prevention of Multiple drug resistant (MDR) *A. baumannii* infections was achieved after discontinuation of cefotaxime in ICU. Infection with resistant clones and mechanical ventilation were found to be potential independent risk factors for mortality.

Key words: *Acinetobacter species, nosocomial infection, multiple drug resistance, risk and prognostic factors*

Acinetobacter spp. is one of important nosocomial pathogens and has been known to cause different kinds of opportunistic infections.[1] *Acinetobacter baumannii* is now recognized to be the *Acinetobacter* genomic species of great clinical importance. The main sites of infection is the lower respiratory tract and urinary tract, and these distribution sites are very similar to that of other nosocomial gram-negative bacteria.[1] *A. baumannii* and DNA group13 have emerged as important organisms in ICU settings, in particular they may be related to the advanced invasive diagnostic and therapeutic procedures adopted in ICUs in the last decade.[1] *A. baumannii* is now recognized as a major pathogen involved in nosocomial infections causing epidemic outbreaks or endemic occurrence with a documented high mortality rates.[2–6] Recently a few *A. baumannii* outbreaks have also been reported from India.[2,6]

It is very difficult to explain the role of *Acinetobacter* acquisition in the ICU, since *Acinetobacter* does not always act as an infecting pathogen as it is widely distributed and has tremendous colonizing capability.[1,7,8] In addition, risk factors for *Acinetobacter* acquisition may vary in different set ups with epidemic outbreaks of infection or endemic colonization.[9] Although various factors predisposing to *Acinetobacter* infections have been analyzed in different studies, there are only few authentic reports from India that have attempted to determine the risk and prognostic factors for *Acinetobacter* infection.[2,6,10] The present study, retrospectively attempts to find possible risk and prognostic factors for the nosocomial acquisition of *Acinetobacter* infection.

Materials and Methods

The study was conducted in JIPMER hospital, Pondicherry, India. In this retrospective study, charts of 43 patients admitted to the hospital during two-year period (Oct'1998-Oct'2000) who developed *Acinetobacter* infection were analyzed. Nosocomially acquired *Acinetobacter* infection was defined as the isolation of the organism repeatedly from blood cultures or other specimens, 48 hours after a patient was admitted to the hospital. True cases of infection recorded from patient's record including the patient's history, clinical findings, microbiological results, and the number of positive cultures. Patients in whom *Acinetobacter* spp. was isolated repeatedly (more than twice) in absence of clinical disease suggesting colonization, were also included in the study. The *Acinetobacter* isolates obtained were mainly from patients admitted to 6-bedded respiratory intensive care unit (RICU). The major reasons for admission to the RICU were mechanical ventilation for respiratory failure, postoperative critical care and organ support following multiple trauma. Such admissions are usually for 4 to 6 days. Other cases of *Acinetobacter* infection were from paediatric

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and medical wards. Clinical specimens included were blood, CSF, endotracheal aspirate, pus and other body fluids. There were 152 cases of other gram negative infections recorded in the hospital during the same period. The following variables were analyzed: patient age, sex, and the presence of underlying diseases or conditions, mechanical ventilation, number of hospital days, surgery, appropriate antibiotic therapy, and infection due to multiresistant *Acinetobacter* spp.

Microbiological Investigation

The highest incidence of *Acinetobacter* spp. infection in the hospital was recorded in RICU. Fifty-five isolates were recovered from 18 patients of RICU. Forty-three isolates were from 25 patients admitted in paediatric ward and other medical wards. In cases, where multiple isolates were obtained from the same patient, a representative strain/strains were selected for further typing after initial screening by performing biochemical testing and comparing antibiograms for 10 first and second line antibiotics. Such screening helped in the short-listing of 49 isolates from overall 98 isolates obtained from 43 patients. Among 43 patients, six patients had two different strains grown. Of the 18 patients at RICU, five patients had two different strains. One patient from pediatric ward yielded two different strains. Thus, a total number of 49 isolates were characterized, which included 23 from RICU (18+5) and 26 from paediatric and other wards. All *Acinetobacter* isolates were characterized phenotypically up to biotype level using a panel of 25 carbon assimilation tests in the microbiology laboratory as described elsewhere.[11–13] The isolates belonging to *A. calcoaceticus – A. baumannii* complex (*Acb*) were further biotyped using additional five assimilation tests of 'C' sources i.e., phenylalanine levulinate, citraconate, 4-hydroxybenzoate and L-tartarate. Agar dilution method was employed to detect minimum inhibitory concentration (MIC) of important broad-spectrum antibiotics that are commonly used in the hospital[14] that included cefotaxime, ceftazidime, amikacin, ciprofloxacin and ofloxacin. Twenty-two reference strains belonging to 18 different DNA groups (assigned by DNA–DNA hybridization methods) obtained from Gerner-Smidt (Serum-institut, Copenhagen, Denmark) and Bouvet (Institut Pasteur, Paris) were also included in the investigation as controls for phenotypic assimilation tests. Since there is an overlapping in numbering of DNA groups from two research groups, the DNA groups of Bouvet and Jeanjean were referred to as BJ and that of Tjernberg and Ursing were referred to as TU.[11]

Statistical analysis

We compared the differences in the risk factors among patients with *Acinetobacter* spp. infection and the patients with other gram negative bacterial infections, patients with *Acinetobacter* spp. infection and patients with colonization with *Acinetobacter* and investigated for significant prognostic factors in patients with *Acinetobacter* spp. infection. Contingency tables were calculated with Pearson's test or Fisher's exact test by comparing the proportions, when necessary. The odds ratio (OR) and confidence intervals (CI) (95%) were calculated. Differences were significant if the p value associated with the test was < 0.05. A multivariate study was performed by using the backward stepwise logistic regression analysis for the factors influencing prognosis and nosocomial acquisition; a p value of 0.05 was the limit for entering or removing terms. For all the analysis, the SPSS software package was used.

Results

Description of Case-Patients and unit distribution

A total of 43 patients admitted to the hospital who developed *Acinetobacter* spp. infection or colonization were evaluated. The patients ranged in age from 6 months to 87 years (mean age ± SD, 32.7 ± 22.9 years; median age, 30 years). Among these 25 were male (58%) and 18 were female (42%). *Acinetobacter* spp. was isolated from various types of infections. Respiratory tract infections (n=21, 48.8%), blood stream infections (n=7, 16.27%), secondary meningitis (n=6, 14%), urinary tract infections (n=4, 9.3%), peritonitis (n=2, 4.65%), corneal infection (n=1), necrotizing fasciitis (n=1) and osteomyelitis (n=1) were the infections diagnosed. Two cases of community-acquired infections were diagnosed, one was pneumonia in 86-year male patient and the other was a corneal infection in 35-year female patient. *A. baumannii* was the main species responsible for most of the infections. However, non -*A. baumannii* species were also responsible for many infections. DNA group 13TU strains were responsible for respiratory infections in ICU. Five patients developed secondary meningitis due to *A. lwoffii*. Among these, one patient developed deep stromal keratitis. One case of peritonitis caused by a strain of DNA group 3 was observed.

Among respiratory tract infected patients, 19 patients were bacteraemic. Three patients developed blood stream infection (BSI) secondary to wound infection. A total of 29 patients (69%) developed BSI. The most common source for *Acinetobacter* BSI was the respiratory tract (65%). Seven cases from the above did not have clear cut clinical findings for supporting infection (4 - respiratory infection, 2 – UTI and 1 - meningitis) and they were regarded as colonization. Thirty-one had undergone surgery (72%); 19 patients were intubated and ventilated in RICU (44%). The mean duration of hospital stay was 19.06 ± 20.76 [median, 14 days (range, 2 to 124 days)]. Fourteen patients died during their stay at the hospital (33% mortality rate). The mortality rate was 50% in RICU admissions due to *Acinetobacter* spp. infections. Majority of the cases were from patients admitted to RICU. Unit wise distribution of cases was RICU (18 patients; 42%), medicine (16; 37%), paediatrics (10; 23%), surgery (9; 21%), orthopaedics and ophthalmology (1; 2.3%). Due to complications, 11 patients primarily admitted in medical and

pediatric wards, were later shifted to RICU. These patients were counted twice for calculation of percentage distribution in units.

Microbiological Investigations

Carbon assimilation testing

In the final study population that comprised of 49 isolates of *Acinetobacter* spp., *A. baumannii* constituted majority of isolates (35) based on assimilation test identification. One isolate belonged to DNA group 3, and 3 isolates belonged to either DNA group13TU or to *A. baumannii* biotype 9. Another isolate, which was haemolytic, belonged to DNA group 13BJ (14TU). There were five isolates of *A. lwoffii*. Other species encountered were, *A. johnsonii* (1), *A. haemolyticus* (2) and *A. junii* (1). Biotyping of *Acb*-complex strains showed biotype 10 as the most common type in the hospital constituting 10 strains followed by biotype 6 and biotype 9, which comprised of 7 and 6 strains respectively. Biotyping results have been published previously in detail.[15]

Antimicrobial susceptibility testing

Six different antibiotic susceptibility profiles were observed among the 43 *Acinetobacter* isolates when tested for five broad spectrum antibiotics. These antibiotypes were designated using Roman numerals I-VI**.** Antibiotype I was resistant to all the five broad antimicrobials such as cefotaxime, ceftazidime, amikacin, ciprofloxacin and ofloxacin. Antibiotype II showed resistance to only cefotaxime and susceptible to others. Isolates of antibiotype III were resistant to ceftazidime, amikacin, cefotaxime and susceptible to ciprofloxacin and ofloxacin. Antibiotype IV group of isolates were susceptible to ceftazidime, amikacin, cefotaxime and resistant to both the quinolones. Group V isolates were susceptible to all the antibiotics. Isolates of antibiotype VI were resistant to amikacin and ciprofloxacin only. Antibiotypes I, III, IV were the common resistant antibiotypes. Six *A. baumannii* biotype 6 strains showed the antibiotype I pattern and one belonged to antibiotype IV. All the strains of *A. baumannii* biotype 10 were of antibiotype I. Those isolates classified either as *A. baumannii* biotype 9 or as DNA group 13TU, exhibited antibiotypes I and III.

Clinical Investigation

Risk and prognostic factors for infection and mortality

Univariate analysis of the cases to assess the prognostic factors for *Acinetobacter* infection and their association with mortality, revealed some important findings. All factors significantly associated with a worse prognosis are shown in Table 1.

No statistical significance was found in relation to sex, age, hospital stay, and prior surgery or with prior use of broad spectrum antibiotics. Inappropriate antibiotic therapy was associated with death in 23% of the patients. Mechanical ventilation was associated with higher mortality (P=0.0146). The resistant antibiotype was significantly associated with mortality (P=0.007). The following variables were considered to be biologically plausible risk factors and were evaluated in a step-wise logistic regression model: having mechanical ventilation, admission to ICU, with complications, prior treatment with broad-spectrum antibiotics, inappropriate antibiotic therapy and infection with resistant antibiotype (resistant to 3rd generation cephalosporins). By multivariate analysis, resistant antibiotype {(Ceftazidime- odds ratio (OR),

Table 1: Significant prognostic factors identified in 43 patients with *Acinetobacter* spp. infection by univariate analysis

Prognostic factors	Survived	Expired	*P* value
Age			
< 40 years	19	7	ns
> 40 years	10	7	
Sex			
Male	15	10	ns
Female	14	4	
Antibiotype			
Multiple resistant type	6	9	0.007
Susceptible type	23	5	
Antibiotic treatment			
Appropriate	20	4	0.014
Inappropriate	9	10	
Antibiotic susceptibility of *Acinetobacter* spp.			
Resistant to ceftazidime	27	7	0.006
Susceptible to ceftazidime	2	6	
Resistant to ciprofloxacin	14	11	0.058
Susceptible to ciprofloxacin	15	3	
Resistant to cefotaxime	11	12	0.003
Susceptible to cefotaxime	18	2	
Resistant to amikacin	9	9	0.041
Susceptible to amikacin	20	5	
Hospital Stay			
> 8 days	22	9	ns
< 8 days	7	5	
Hospital service			
ICU	10	8	ns
Other Medical Service	19	6	
Mechanical Ventilation			
Yes	9	10	0.014
No	20	4	
Clinical findings suggesting			
Infection	22	14	0.048
Colonization	7	0	
Prior antibiotic use			
Yes	19	10	ns
No	10	4	

ns - not significant

7.13 [95% CI, 1 to 46]; *P*=0.044); (cefotaxime- OR, 6.09 [CI, 0.87 to 30]; *P*=0.045)} and mechanical ventilation [OR, 5.84 (CI, 0.83 to 31); *P*=0.05)] were independent risk factors for the mortality.

Because of the continuous occurrence and recurrence of *Acinetobacter* infections or colonizations among patients in high-risk wards and ICUs, factors associated with infection were assessed. All the risk factors that were associated with infection due to *Acinetobacter* spp are shown in table 2. Only the resistant strains isolated from the patients were significantly associated with occurrence of infection (*P*=0.0367). All patients who expired had severe infection with resistant *Acinetobacter* spp. (*P*=0.048). The following variables were considered to be biologically plausible risk factors for infection and were evaluated in a step-wise multivariate logistic regression model: prior use of broad-spectrum antibiotics, outcome and infection with resistant antibiotype. Only the resistant antibiotype [Cefotaxime-OR, 9.4 (95% CI, 1.3 to 109); *P*=0.047] was an independent risk factor for occurrence of infection.

Discussion

Acinetobacter species has emerged as an important nosocomial pathogen that is often multidrug resistant and associated with life-threatening infections.[1] *A. baumannii*, a clinically important species has a tendency toward cross-transmission, particularly in ICUs, where numerous outbreaks are encountered.[1,2,16] Respiratory infections due to *Acinetobacter* in mechanically ventilated patients in ICU were also high at JIPMER hospital (44.7%), during 1996-1997 (Dutta TK, unpublished data). One recent study revealed that *Acinetobacter* spp. was responsible for 35% of ventilator associated pneumonia (VAP), making it the most conspicuous and dominant pathogen amongst all other bacteria encountered in that study.[17] However, only few case-control studies have identified and systematically reported the various risk factors associated with *Acinetobacter* infection or colonization.[5,7]

Table 2: Comparison of risk factors identified through univariate logistic analysis and their significance for causing infection with *Acinetobacter* spp. in RICU and other medical services

Factor	Infection (n=36)	Colonization (n=7)	*P* value
Age			
< 40 years	14	3	ns
> 40 years	22	4	
Hospital Stay			
> 8 days	27	4	ns
< 8 days	9	3	
Antibiotype			
Multiple resistant type	15	0	0.0367
Susceptible type	21	7	
Hospital Service			
ICU	16	2	ns
Other Medical Service	20	5	
Mechanical			
Yes	18	1	0.0901
No	18	6	
Outcome			
Expired	14	0	0.0484
Survived	22	7	
Prior antibiotic use			
Yes	24	5	ns
No	12	2	

ns - not significant

Potential risk factors for *Acinetobacter* infection in many studies were prior surgery or prior use of broad-spectrum antibiotics.[5] The present study contradicted those findings with no such correlations found in our cases. Inappropriate therapy was associated with death in 23% of our patients as against a figure of 48% reported in one of the studies.[5] Antibiotype I resistant strains and mechanical ventilation were independent risk factors associated with high mortality in our investigation. Our study also revealed that the multidrug resistant (MDR) strains were significantly associated with infection (*P*=0.0367) and mortality was high in patients who had severe infection with MDR strains. Particularly, strains of antibiotype I were highly associated with mortality (*P* = 0.0146). Almost all the expired patients were essentially infected with MDR antibiotypes I, III and IV. High mortality rates associated with MDR strains have also been noted in other hospitals of India.[2] The most likely explanation for this phenomenon can be the extensive and indiscriminate use of antibiotics in ICUs. A progressive decrease in the effectiveness of 3rd generation cephalosporins against *Acinetobacter* has been coupled with the increased use of these antibiotics.[1,8] We re-emphasize that broad-spectrum antibiotics should be used intravenously with caution. Ceftazidime and/or cefotaxime use should be discontinued in units where resistant strains for these two antibiotics are being reported increasingly. With revelation of ceftazidime and cefotaxime resistant strains from our study, the hospital ICU was advised to use other antibiotic combinations like an effective beta-lactam or carbapenem (piperacillin, ticarcillin, or imipenem) along with amikacin.

The incidence of respiratory tract infections was 48.8% in this study. Only endemic MDR antibiotype (*P* = 0.047) and mechanical ventilation (*P* = 0.05) were independent predisposing factors for the respiratory infection. Similar observations were documented earlier.[18] However, other studies[1] also implicated many other risk factors like prior antibiotic use, ICU stay, I.V. catheters, which were found to be insignificant in our study. Mortality rates of 30 to 75% have been reported for pneumonia due to *Acinetobacter* spp. with highest rates encountered in VAP patients.[19] We documented 50% mortality rate in VAP patients, which is of major concern. The most common source for *Acinetobacter* BSI is the respiratory tract. One important study[5] implicated respiratory

infection as the most common source for BSI.[5]

A. baumannii BSIs are most common among immunocomprimised patients at our hospital, wherein majority of bacteraemia patients (31) had undergone surgery (72%); 19 patients were intubated and ventilated (44%). Other investigators have noted that *A. baumannii* BSIs been more frequent in cancer patients and in patients with severe underlying malignancies.[20]

Though *A. baumannii* was the main species responsible for most of the infections, non-*A. baumannii* species were also frequently encountered. DNA group 13TU strains were responsible for respiratory infections in ICU in the present study. DNA group 13 has been implicated in many infections earlier.[21] Five patients developed secondary meningitis due to *A. lwoffii*. Out of these, one patient developed deep keratitis caused by the same organism. *A. lwoffii*, as an opportunistic pathogen in immunosuppressed patients have been reported earlier.[22] Only one strain of DNA group 3 causing peritonitis was observed. The occurrence of DNA group 3 seems to be less frequent in this geographical region as compared to other regions wherein it is an important pathogen.[21] One particular study from Sweden[21] found DNA group 3 as a predominant species among all the clinical isolates recovered.

In the present study, one 4-year-old male patient developed secondary meningitis due to *A. johnsonii*, another rare opportunistic species, wherein CSF from the patient grew this organism twice. This infection was suspected to be iatrogenic during lumbar puncture. Iatrogenic meningitis is well documented complication of diagnostic and therapeutic lumber puncture in India.[23] *A. haemolyticus* was isolated from an 86-year old male patient suffering from recurrent bronchiectasis. Only one strain belonging to DNA group 14 was encountered. DNA group14 strains are not found in clinical materials frequently.[1,21] Corneal perforation due to *A. junii* diagnosed in this hospital was reported during the study period.[24] This observation is very important, as this infection was community acquired rather than hospital. Only few reports on *A. junii* outbreaks have been documented.[1,8]

Carbon assimilation test for biotyping appears to be the best method and it is readily available in all microbiology laboratories in India. Phenotypic identification of *Acinetobacter* to the species or DNA group level is very difficult. Bouvet *et al* used it taxonomically for typing by using large panel of 28 tests that is tedious and time consuming.[12] The reliability of this typing scheme was evaluated by another study using a numerical approach.[11] We had difficulty only in the identification of isolates that belonged to DNA groups 2 and 13 and 8/9 and 15. However, biotyping of *Acb*-complex (DNA groups 1, 2, 3, 13) was helpful in differentiating the biotypes within the complex. Identification of *Acinetobacter* species based upon growth at 44°C, 41°C and 37°C in BHI along with acid production from glucose could be very useful. In addition, biotyping of *Acb*-complex by only 5 assimilation tests seems to be best alternative method for the laboratories with fewer resources.

In conclusion, MDR *A. baumannii* was the species responsible for majority of the *Acinetobacter* infections at our hospital. MDR *A. baumannii* infections were also the cause of severe clinical disease that were associated with a high mortality rates. Resistant *A. baumannii* clones and mechanical ventilation were found to be potential independent risk factors for mortality in our set up. The use of ceftazidime and cefotaxime was associated with an increased risk of nosocomial pneumonia with resistant strains of *A. baumannii*. Prevention of recurrent MDR *A. baumannii* infections was achieved after discontinuation of cefotaxime in our RICU.

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