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Evaluation of clinical and prognostic factors associated with nosocomial Acinetobacter infections

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ABSTRACT

Acinetobacter species are opportunistic pathogens with increasing relevance in nosocomial infections. Such infections are often extremely difficult to treat because of the widespread resistance of these bacteria to the major groups of antibiotics. Despite the increasing significance and frequency of multiple resistant *Acinetobacter* infections, many clinicians still lack an appreciation of the potential importance. Epidemic outbreaks of multi-drug resistant (MDR) Acinetobacter baumannii (AB) are increasing through widespread dissemination of the pathogen.

The aims of the study were to identify the *Acinetobacter* species isolated from clinical samples taken from hospitalized patients by a simplified phenotypic identification, to evaluate various risk and clinical factors predisposing to MDR Acinetobacter infections in a hospital setting and investigate the antibiotic sensitivity of these bacteria.

Identification, speciation and antibiotyping were performed for the isolates of Acinetobacter recovered from infective samples. Risk factors and clinical characteristics were studied retrospectively. Of 2633 culture positive samples, 186 (7.064%) were found to be due to Acinetobacter. Respiratory and urine samples were significant contributors towards infection with A. baumannii and was most prevalent in the intensive care unit . *A. baumannii* was the most predominant species with the isolation rate of 74%. Prolong hospital stay, Mechanical ventilation and Intensive Care Units were found to be potential risk factors. Resistance to antibiotics such as ceftazidime, cefepime, piperacillin/tazobactam, ticarcillin/clavulanic acid, fluoroquinolones, amikacin and gentamicin was seen more in the case of *Acinetobacter baumannii* as compared with other species.High prevalence of MDR acinetobacter spp in a hospital setup where the selective pressure of antibiotics is already high, showing a need for rational use of antimicrobials. Strict infection-control measures may prevent nosocomial infection and reduce mortality. The analysis of risk factors and susceptibility pattern will be useful in understanding epidemiology of this organism in a health care setup.

INTRODUCTION

Acinetobacter are aerobic gram negative coccobacillary rods with a natural reservoir in soil and water sources around the world and have emerged as health care associated pathogens, in part because they are resilient bacteria with a diverse natural habitat. The organism commonly targets the most vulnerable hospitalized patients, those who are critically ill with breaches in skin integrity and airway protection. However, in more recent times, infections involving the central nervous system, skin and soft tissue, and bone have emerged as highly problematic for certain institutions.[1] Interest in Acinetobacter, from both the scientific and public community, has risen sharply over recent years and significant advances have been made in our understanding of this fascinating organism. As a consequence of its immense ability to acquire or up-regulate antibiotic drug resistance determinants, it causes outbreaks in healthcare institutions and has justifiably been propelled into the forefront of scientific attention. Although *A. baumannii* appears

to be the *Acinetobacter* genomic species of greatest clinical importance, repeated isolation of other genomic species from patients, should be considered as a cause for suspicion of infection, especially if clinical symptoms are also present. From a research and clinical perspective, studies using proper methods for species identification of acinetobacters, are mandatory to increase our knowledge of the epidemiology, pathogenicity, and clinical impact of the various species of this diverse genus. [2]

This prospective study aimed to identify the *Acinetobacter* species by a simplified phenotypic identification scheme isolated from clinical samples taken from hospitalized patients, to analyse various clinical factors predisposing to Acinetobacter infection and investigate the antibiotic sensitivity of these bacteria.

MATERIAL & METHODS

SAMPLE COLLECTION AND ANALYSIS

Clinical samples of different types were taken between JUNE 2010 and FEB 2012 from patients admitted to various departments within our hospital who had hospital-acquired infections. Various types of specimens like blood, sputum, pus, CSF and other body fluids were taken. The Ethics Committee approved the study and all patients gave written informed consent. All clinical specimens were analyzed at the Bacteriology Laboratory, Department of Microbiology, Punjab institute of Medical sciences Jalandhar, Punjab. The specimens were subjected to simplified phenotypic identification scheme after they were cultured on 5% blood agar and MacConkey agar and incubated at 35 °C for24 48 h. All non-lactose fermenters were subjected to Gram staining, oxidase test, motility and catalase test. Acinetobacter are Gram negative bacilli or coccobacilli, oxidase negative, nonmotile and catalase positive. Speciation was done by the method previously published using a battery of series of biochemical tests.[3]

IDENTIFICATION OF NOSOCOMIAL INFECTIONS

Various clinical characteristics were recorded like age, sex, duration of hospitalization, presence of underlying disease(s) and risk factors, days on previous antibiotic therapy, possible source of infection and antibiogram [Table - 1]. Standard definitions as given by Centre for Disease Control and Prevention were used to differentiate categories of infection versus colonization[4] The infection acquired upon hospitalization for 72 h or more was hospital-acquired; and it was community-acquired if the patient had not recently been in a health care facility or been in contact with someone who had been recently in a health care facility. Patients from whom *Acinetobacter* was isolated in absence of clinical disease suggesting colonization were not included in this study.

ANTIBIOTIC SENSITIVITY

Antimicrobial susceptibility was done by disc diffusion method as per the clinical and laboratory standards institute (CLSI) guidelines using the Muller-Hinton agar and antimicrobial discs (Hi-Media, Mumbai). The following antimicrobial agents (µg) were used - cefotaxime (30), cefoperazone (75), gentamicin (10), amikacin (30), ciprofloxacin (5), nalidixic acid (30), norfloxacin (10), co-trimoxazole (25), nitrofurantoin (30), imipenem (10), piperacillin and tazobactam (75+10) and cefoperazone and sulbactam (75+30), tigecycline (15) and colistin (10). The isolates were labeled as susceptible, intermediate sensitive and resistant based on inhibition zones as per standard guidelines.

Antibiotic sensitivity was tested using the disc diffusion method in accordance with the Clinical Laboratory Standards guidelines.[5]

STATISTICAL ANALYSIS

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, total 13,280 samples were cultured, of which 2633 (19.82%) were found to be infected. Out of these infected samples, 186 (7.064%) were found to be due to Acinetobacter. A. baumannii was found to be the predominant species responsible for 74% of the infection, followed by A. lwoffii. Respiratory and urine samples were significant contributors towards infection with A. baumannii. The variables such as age, sex, seasonal incidence, hospital-versus community-acquired infection, possible source of infection and risk factor distribution analyzed are shown in [Table - 1]. The patients ranged in age from new born to 89 years .

Acinetobacter was isolated from various types of samples among these, respiratory samples were extremely significant ($p<0.0001$), followed by urine, pus and wound exudates. Two bacteremia cases confirmed were due to intravenous catheters, as evident from cultures of the catheter tips. Distribution of specimens from which Acinetobacter species were isolated is shown in (Table 2)

Statistical analysis showed that ICU stay, previous ICU stay and its length are significantly associated with the acquisition of *A. baumannii* isolates ($p<0.05$).

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, season and surgery.

Antimicrobial susceptibilities of Acinetobacterspp

The results of antimicrobial susceptibilities against acinetobacters are presented in [Table - 3]. High resistance rate was observed in cephalosporins and flouroquinolones. *A. baumannii* was found to be significantly more resistant to all groups of antimicrobials as compared to other acinetobacter spp. Carbapenems considered as the drugs of choice for Acinetobacter are also losing their efficacy. 21% and 29% isolates were resistant to imipenem and meropenem respectively. The isolates were fairly susceptible to cefoperazone + sulbactam. Polymyxin B and tigecycline showed promising results. Multi drug resistance in the isolates, demonstrates a rising concern for infection control in hospitals. The higher resistance was observed in respiratory and ICU isolates where *A. baumannii* was most prevalent. Majority of the *A. baumannii* isolates were multi-drug resistant, showing resistance to two or more antimicrobial agents. Five isolates showed pan drug resistance and were resistant to all antimicrobials including polymyxin B and tigecycline.

DISCUSSION

A. baumannii is well equipped with several characteristics to emerge as a significant nosocomial pathogen. First, it can survive on a multitude of environmental surfaces, which facilitates transmission. Second, broad-spectrum antimicrobial resistance

TABLE 1: Various prognostic and clinical factors associated with Acinetobacter infections

Factor	Infecting strains	%
Age		
17 years	20	10.75
18-35years	42	22.58
36-60 years	78	41.93
60 years	46	24.72
SEX		
Male	104	55.91
Female	82	44.09
Hospital stay		
8days	112	70.43
8days	74	29.56
Hospital ward		
ICU	96	51.61
Surgical	40	21.5
Paediatric	19	10.2
Ortho	14	7.5
Dialysis	7	3.76
Medical	10	5.7
Source of isolation		
Respiratory samples	73	39.2
Urine	51	27.4
Pus	26	13.97
Wound swabs	16	8.60
Blood	9	4.83
CSF	2	1.07
Body fluids	6	3.22
central line tip	3	1.61
Associated risk factors		
Mehanical ventilation	32	22.04
Urinary and I.V catheters	19	10.21
Endotracheal intubation	10	5.37
Others	125	17.2

can be due to a variety of intrinsic and acquired mechanisms[6] The overall incidence of *Acinetobacter* isolation from all infective samples was 7.06% (186 of 2633), indicating its importance as a nosocomial pathogen. *Acinetobacter* species accounted for 1.4% of all nosocomial infections during 19711981 in a university hospital in the United States[7] . An other study in a university hospital found that hospitalization in an intensive care unit and previous administration of antibiotics were associated with *Acinetobacter* colonization at various sites of the body in 3.210.8 per 1,000 patients [8]. Molecular epidemiologic studies have revealed the presence of heterogeneous strains in a given hospital or service center where they have become both endemic and epidemic[9] especially the members of *Acb* complex. Our study showed high rate of infection in males as compared to females as indicated by many other authors and do not have statistical significance[10,11]

These bacteria have been reported as the most frequent cause of respiratory tract infections. The respiratory system was also the most common site for *Acinetobacter* infection in our study. Mechanical ventilators are the most important risk factor for these infections[12,13].High frequency of isolation from respiratory tract specimens was suspected due to the use of pre- and post-operative antibiotics. In addition to reports that *Acinetobacter* is the most frequent cause of respiratory tract infections, some studies have also reported that these micro-organisms were most frequently isolated from surgical wound infections.[14] In our study, wound infections were ranked third, after respiratory and urinary system infections, for *Acinetobacter* species isolation. Total isolates from all urine samples were 30.6%, which is comparable to the studies from various countries have shown predominance of isolation from urine (21-27%), tracheobronchial secretions (24.8- 48.8%) [15]. The strains showing multi-drug resistance were highest from the respiratory isolates (mainly because of *A. baumannii,* which were resistant).

Isolation rate from blood in this study was 4.83%, most of them were from preterm and septicaemic patients and 2 cases of bacteremia cases were associated with intravenous catheterization. This was in concordance with the results of other studies[15] . A single isolate was isolated from CSF, it was Acb complex in an adult male patient of 58 years suffering from meningitis.

Despite many intensive efforts, the nosocomial acquisition of *Acinetobacter* remains problematic, especially in ICUs. Most of the positive specimens for acinetobacter in our study came from patients in ICUs; several studies conducted world wide have shown that hospital-acquired *Acinetobacter* infections are frequently observed, especially in ICUs., [10,16].The literature search demonstrates that *A. baumannii*, is predominantly involved in hospital acquired infection. This holds true in our study too. About 74% of the isolates belonged to *Acb* - complex group. *A. baumannii* , a major species isolated from most of the clinical specimens of this hospital, is reportedly a main species responsible for nosocomial infection in other parts of the world also.[17]

As resistance patterns among nosocomial bacterial pathogens vary widely from country to country at any given point and within the same country over time,therefore a surveillance of nosocomial pathogens for resistograms is needed for every country for appropriate selection for empiric therapy. MDR *A. baumannii* has been implicated in several nosocomial outbreaks more often than other *Acinetobacter* species.[1,18] The antibiotic sensitivity analysis showed imipenem , cefoperazone + sulbactam to be the most effective antibiotic agents. In a similar study by Gülseren *et al* imipenem was reported to be the most effective agent against *Acinetobacter[19]* Low resistance rates of *A. baumannii* to imipenem (about 3%) were reported from Saudi Arabia and Japan.[19,20] and Turkey [21]. Carbapenems have been the drug of choice for the treatment of infections caused by *A. baumannii* ,however, bacterial resistance to imipenem is increasing[22]. The important risk factors for the acquisition of carbapenem-resistant *A. baumannii* include previous carbapenem use, longer duration of hospital stay until infection, ICU stay, urgent surgery, total parenteral nutrition, having a central venous catheter, endotracheal tube and urinary catheter or nasogastric tube.[23]

By the disc diffusion method it was clear that aminoglycosides were active against less than half of our A. baumannii isolates as they showed least resistance to amikacin and netilmicin (56% and 51% resistance-respectively). Increasing resistance for cephalosporins was observed mainly in strains belonging to *Acb*-complex. All other antibiotics tested showed resistance ranging from 68.8 to 92.2% suggesting that most of the first generation drugs were ineffective. Same results have been reported by various authors.[24,25] In the present study, five strains of A.baumannii were found to be PDR *i.e.*,they

TABLE 2: Department wise distribution of specimens from which Acinetobacter species were isolated

SPECIMEN		ICU	SURGERY	MEDICINE	PAEDS	ORTHO	DIALYSIS	TOTAL
Central line tip		2	1	0	0	0	0	3
Sputum	RESPIRATORY SAMPLES	24	5	8	2	0	1	40
Tracheal aspirate		11	3	1	0	0	0	15
Endotracheal tube secretion		9	1	0	0	0	0	10
Chest tube drains		3	1	0	0	0	0	4
Broncho alveolar lavage		3	1	0	0	0	0	4
Urine		22	11	8	2	0	8	51
Pus		12	6	4	0	4	0	26
Wound swabs		6	5	1		3	0	16
Blood		6	0	0	3	0	0	9
CSF		1	0	0	1	0	0	2
Body fluids		3	1	2	0	0	0	6

were resistant to even tigecycline and colistin. This finding is significant as it heralds the beginning of the post-antibiotic era where only a few therapeutic options would be available for treatment.

Through regular surveillance studies, clinicians should be kept informed about the predominant bacteria and their resistance pattern in their hospital. This will help them choose the most suitable agents.

CONCLUSION

Hospital-acquired infections involving multiresistant *A. baumannii* isolates have been reported, often in association with contamination of the hospital equipment or cross-contamination by the colonized hands of patient- attending personnel. Risk factor analyses will be useful for further hospital epidemiology studies of *Acinetobacter species* . The increasing recovery in the clinic of multi-drug resistant (MDR) *Acinetobacter baumannii* is becoming a frightening reality. Strict infection-control measures may prevent nosocomial infection and reduce mortality. The analysis of risk factors and susceptibility pattern will be useful in understanding epidemiology of this organism in a health care setup.

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TABLE 3: Antimicrobial sensitivity pattern of Acinetobacter spp. to different groups of antibiotics

GROUP	ANTIBIOTIC	RESISTANT ISOLATES (Acb complex) n=138 (74%)	RESISTANT ISOLATES (A.lwoffii + Others) n=48 (26%)
β lactams	Piperacillin	83	60
	Cefotaxime	77	62
	Ceftazidime	78	60
	Cefoperazone	83	57
	Ceftriaxone	81	63
	Cefepime	70	42
β lactams+ Inhibitors	Piperacillin – Tazobactam	31	18
	Cefoperazone – Sulbactam	29	15
Carbapenems	Imipenem	21	14
	Meropenem	29	15
Aminoglycosides	Gentamicin	60	33
	Amikacin	56	27
	Netilimicin	51	27
Flouroquinolones	Ciprofloxacin	86	57
	Pazufloxacin	73	42
Others	Polymixin B	4	0
	Tigecycline	8.27	0

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