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"STUDY OF RISK FACTORS ASSOCIATED WITH THE EMERGENCE OF DRUG RESISTANT ACINETOBACTER BAUMANNII FROM A REFERRAL HOSPITAL IN SIKKIM, INDIA."

Microbiology

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ABSTRACT

Background: *Acinetobacter baumannii* is an important pathogen in health care associated infections. Objectives: To determine the risk factors and drug resistance from the various samples. Material and Methods: Pro**spective review based study conducted in referral hospital in** Sikkim, India for one year. The samples were identified and their sensitivity determined by VITEK 2. **A retrospective chart review was performed to determine the potential risk factors.**

Results: *From 917 isolates, A.baumannii* accounted for 158 (17.2%) and was predominantly isolated from sputum 53(18.9%) and pus 73(9.91%) and 109 (68.9%) isolates were from ICU and 49(31%) from ward. The risk factors associated were invasive devices, antibiotics and multiple hospitalizations in past and prolonged ICU stay. *A.baumannii* were 100% resistant to amoxyclav, ampicillin, and cefuroxime. Higher degrees of resistance were recorded for aminoglycosides, cephalosporins and carbapenems while colistin (5%) and tigecycline (12%) showed lower resistance. Conclusion: Rationalizing the use of antimicrobials should be done, which can be achieved by an effective antimicrobial stewardship program and close monitoring.

KEYWORDS

Acinetobacter baumannii, antibiotics, drug resistance, risk factors.

INTRODUCTION

Acinetobacter baumannii is an important pathogen known as a major agent in health care and nosocomial associated infections. The incidence of *Acinetobacter baumannii* infections has raised over the past decade[1,2] which could be related to a rise in the proportions of susceptible populations as a result of advancements in medical support of critically ill and frail patients[3]. Though, Acinetobacter is freely found in the environment and can be obtained from various sources of nature. It is opportunistic in nature and has the ability to survive in the hospital environment for a considerable period of time and cause health care associated infections and multiple outbreaks[4,5]. These organisms are harmless to healthy individuals but are often highly resistant to commonly used antibiotics. Infections by such organisms are difficult to treat. Furthermore, their eradication from the hospital environment can require targeted means such as isolation of patients and temporary closure or even reconstruction of wards, therefore poses both a medical and an organizational burden to health-care facilities[6,7]. Risk factors for multidrug-resistant *A. baumannii* infection include prolonged length of hospital stay, exposure to the ICUs, mechanical ventilation, urinary catheterization, prior exposure to antimicrobials, greater severity of illness and surgery[8]. The therapeutic options available for the infected patients are severely limited and the other drawback is the difficulty in interpreting the significance of isolates from the clinical specimens and the ability of the organism to accumulate diverse mechanisms of resistance[9,10]. The information on this organisms and antibiotic susceptibility pattern among the hospitalized patients in Sikkim, India is very hard to find and so the aim of the study was to determine the prevalence of drug resistance in *Acinetobacter baumannii* and predisposing factors for acquisition of infection caused by it.

MATERIALS AND METHODS

Study design and settings: Prospective review based study conducted in Department of Microbiology, Central Referral Hospital, (CRH) Sikkim Manipal Institute of Medical Sciences (SMIMS), Sikkim, India.

Study period: January - December 2016

Study population: Clinical samples were collected from patients admitted in ICUs and wards. Patients admitted at least for 48 hours in the hospital were taken as an inclusion criterion. Previous institutional/ hospitalization were defined as an admission in any hospital during the last year. Antibiotic therapy was noticed if any patient was on any antibiotic for at least 3 days within the last 3 months before isolating the organism.

Sample collection: Various samples like blood, urine, pus, sputum, endotracheal tubes and central line tips were collected.

All the samples were processed on routine culture media using blood agar and MacConkey agar. The blood cultures bottles were processed in the Bact/ALERT 3D system (bioMerieux, France), an automated culture system which continuously monitors for any growth in every 10 min in each bottle independently. Gram staining was performed from the positive blood culture and subculture on routine medias and incubated at 37°C±2°C. All the suspected colonies were then processed and identification and sensitivity on by VITEK 2 Compact (bioMerieux, France). VITEK 2 Compact is an automated microbial identification and antibiotic susceptibility testing system. It has advanced expert system (AES) software where the interpretation of the antibiotic is given on the Clinical Laboratory Standards Institute (CLSI) guidelines[11]. A retrospective chart review was performed on selected patients, identified through the hospital infection control data base. Documented patient demographics and potential risk factors included age, sex, patient's location, source, department, use of catheter, ICU stay, mechanical ventilation or tracheotomy use, previous antibiotic use, previous institutional/ hospitalization, length of stay, underlying diseases, current diagnosis and polymicrobial growth.

RESULTS

During the period of study,a total of 917 isolates were obtained from patients admitted in various ICUs and wards. Out of 917 positive growth of various organism, Acinetobacter baumannii accounted for 158 (17.2%) of total organism. The male: female ratio was 1.7: 1. *Acinetobacter baumannii* infection was significantly observed among patients of age-group of 41-60 years (35.4%), followed by 21-40 years (29.1%) ($p<0.000$). Out of 138 samples, monomicrobial growth was observed in 135(85.4%) samples and 23(14.5%) sample growth was polymicrobial. *Klebsiella spp.* 7(30.4%) and *Pseudomonas aeruginosa* 6(26%) was the most common organism associated with *Acinetobacter baumannii*. Most of the patients had existing diseases like diabetes, liver disease, GI infections, malignancy, tuberculosis and hypertension which was found to be insignificant but respiratory problems like COPD etc ($p<0.036$) was significant. In the current disease category, factors like accident, psychiatric cases, respiratory diseases and sepsis were found to be significant with *Acinetobacter baumannii infection* (Table 1). *Acinetobacter baumannii* was predominantly isolated from sputum sample 53(18.9%), followed by pus 73(9.91%) and endotracheal tube 16(7.8%).About 109 (68.9%) isolates of *Acinetobacter baumannii* were isolated from Intensive Care

Unit (ICU) and 49(31%) from ward (Table 2). The significant risk factors associated with *Acinetobacter baumannii* was ICU stay, prolonged hospitalization, use of invasive devices like catheter and ventilator and antibiotics in the past. (Table 3). All the isolates were totally resistant to Amoxyclav, Ampicillin, and Cefuroxime. Higher degrees of resistance were recorded for Amikacin, Cefoperazone-Sulbactam, Ceftriaxone, Ciprofloxacin, Cotrimoxazole, Gentamicin, Imipenem, Meropenem, Piperacillin/Tazobactam and Tetracycline. Colistin and Tigecycline showed lower resistance in *Acinetobacter baumannii* isolates (Table 4).

Table1: General demographic and clinical characteristics of the patients with bacteriological isolation of *Acinetobacter baumannii* in various departments of CRH

Characteristics		zNICU n(%)	MICU n(%)	SICU n(%)	WARD n(%)	Total n(%)	X2 value	p -value
Age	0-20	10(100)	2(3.8)	2(4.3)	8(16.3)	22 (13.9%)	79.58	0.000
	21-40	0(0)	15(28.3)	16(34.8)	15(30.6)	46 (29.1%)		
	41-60	0(0)	17(32.1)	18(39.1)	21(42.9)	56 (35.4%)		
	61-80	0(0)	18(34)	10(21.7)	5(10.2)	33 (20.8%)		
	81-100	0(0)	1(1.9)	0(0)	0(0)	01(0.6%)		
Sex	Male	7(70)	30(56.6)	30(65.2)	33(67.3)	100 63.2%)	1.634	0.651
	Female	3(30)	23(43.4)	16(34.8)	16(32.7)	58 (36.7%)		
Underlying disease:	Respiratory	0(0)	10(18.9)	2(4.3)	3(6.1)	15	8.535	0.036
Current disease:	Accident	0(0)	4(7.5)	12(26.1)	9(18.4)	25	8.481	0.037
	Psychiatric	0(0)	9(17)	3(6.5)	0(0)	12	11.57	0.009
	Respiratory	7(70)	19(35.8)	6(13)	7(14.3)	39	20.80	0.000
	Sepsis	3(30)	2(3.8)	1(2.2)	1(2)	07	16.70	0.000

Table 2: *Acinetobacter baumannii* isolated from different samples in various departments of CRH

Source (Total)	*Positive for Acinetobacter baumannii n (%)*	NICU n(%)	MICU n(%)	SICU n(%)	WARD n(%)	X^2 value	p-value
Blood (1881)	10 (0.53)	5(50)	5(9.4)	0(0)	0(0)	135.29	0.000
CT (56)	02 (3.57)	1(10)	0(0)	0(0)	1(2)		
ET (205)	16 (7.8)	0(0)	4(7.5)	8(17.4)	4(8.2)		
Pus (736)	73 (9.91)	3(30)	42(79.2)	25(54.3)	3(6.1)		
Sputum (279)	53 (18.9)	0(0)	1(1.9)	12(26.1)	40(81.6)		
Urine (1884)	04 (0.21)	1(10)	1(1.9)	1(2.2)	1(2)		

Table 3: Risk factors of health care-associated infection caused by antimicrobial resistant *Acinetobacter baumannii* in CRH.

Characteristics		NICU n(%)	MICU n(%)	SICU n(%)	WARD n(%)	Total n (%)	X^2 value	p -value
Catheter use		2(20)	52(98.1)	46(100)	33(67.3)	133(84.1%)	57.71	0.00
Ventilator use		6(60)	40(75.5)	27(58.7)	1(2)	74 (46.8%)	60.23	0.00
Antibiotic use in past		0(0)	22(41.5)	13(28.3)	10(20.4)	45 (28.4%)	9.96	0.01
Recent hospitalization / Operation		0(0)	8(15.1)	5(10.9)	2(4.1)	15 (9.4%)	4.75	0.19
ICU stay								
Length of stay in ICU	0-20 days	4(40)	27(55)	22(47.8)	0(0)	53 (33.5%)	40.8	0.00
	21-40 days	5(50)	15(30.6)	16(34.8)	0(0)	36 (22.7%)		
	41-60 days	1(10)	6(12.2)	8(17.4)	0(0)	15 (9.4%)		
	61-80 days	0(0)	1(2)	0(0)	0(0)	01 (0.6%)		

Table 4: Antibiotic resistance profile of the *Acinetobacter baumannii* isolates

Antibiotic	NICU n(%)	MICU n(%)	SICU n(%)	WARD n(%)
Amikacin	8(80%)	53(100%)	43(93.5%)	47(95.5%)
Amoxyclav	10(100%)	53(100%)	46(100%)	49(100%)
Ampicillin	10(100%)	53(100%)	46(100%)	49(100%)
Cefepime	10(100%)	53(100%)	43(93.5%)	49(100%)
Cefoperazone/Sulbactam	8(80%)	46(86.8%)	39(84.8%)	28(57.1%)
Cefotaxime	10(100%)	53(100%)	45(97.8%)	48(98%)
Ceftazidime	10(100%)	52(98.1%)	46(100%)	47(95.5%)
Ceftriaxone	9(90%)	53(100%)	39(84.8%)	42(85.7%)
Cefuroxime	10(100%)	53(100%)	46(100%)	49(100%)
Ciprofloxacin	7(70%)	51(96.2%)	39(84.8%)	40(81.6%)
Colistin	0(0%)	3(5.7%)	4(8.7%)	1(2%)
Cotrimoxazole	9(90%)	50(94.3%)	40(87%)	40(81.6%)
Gentamicin	8(80%)	50(94.3%)	41(89.1%)	40(81.6%)
Imipenem	8(80%)	50(94.3%)	39(84.8%)	35(71.4%)
Meropenem	8(80%)	51(96.2%)	42(91.3%)	40(81.6%)
Piperacillin/Tazobactam	9(90%)	52(98.1%)	42(91.3%)	40(81.6%)
Tetracycline	9(90%)	51(96.2%)	45(97.8%)	47(95.5%)
Tigecycline	1(10%)	7(13.2%)	10(21.7%)	1(2%)

DISCUSSION

In our study out of 917 growths of various organisms, *Acinetobacter baumannii* accounted for 158(17.2%). Various studies has isolated higher rate by H. Siau et al[12]. (11%), Jaggi N et al[13]. (9.4%), and Joshi et al[14]. (9.6%). Out of 158 isolates of *Acinetobacter baumannii,* 109(69%) was from ICUs and 49(31%) was from different wards. Jaggi N et al[13] also showed similar findings of 76.6% of *Acinetobacter baumannii* from ICU and 18.7% from IPD and 4.5% from OPD. Though, Seifert et al. isolated Acinetobacter sp. as high as 75% in patients hospitalized in non-ICU and up to 43% of healthy adults[15]. It is known that clinically ill patients have more tendencies to acquire an infection during their stay in an ICU though the frequency of these infections varies considerably in different populations and clinical settings[16-18]. Our study shows the isolation of *Acinetobacter baumannii* from various clinical samples but maximum isolates was observed from sputum 53 (18.9%) but isolation from ET secretion was found to be in average rate of 16(7.8%). Various studies have reported different rates of *Acinetobacter baumannii* from different sites like Villers et al. have isolated *Acinetobacter baumannii* in trachea-bronchial secretions (24.8%)[19], Suri et al[20]. as 45.6% in their studies, Cucunawangsih et al[21] as 28.5% from sputum, 41.7% from ET secretions and 2.4% from bronchial lavage. All in all these are specimens of respiratory tract infections. Acinetobacter infections usually involve organ systems with high fluid content (e.g, respiratory tract, blood, CSF, peritoneal fluid, urinary tract)[22-24]. Our study identified the significant risk factors associated with antimicrobial resistance *Acinetobacter baumannii*, these includes ICU stay, length of stay in hospital, use of catheter and ventilators and long term use of antibiotics. Various risk factors for infection with drug resistant *Acinetobacter baumannii* have been identified which includes prior exposure to antimicrobial therapy, mechanical ventilation, a longer hospital stay in a high risk unit, length of ICU stay, severity of illness, underlying co-morbid conditions, recent surgery and invasive procedures[25-30]. This indicates the hardy nature of Acinetobacter sp., allowing it to survive in the environment

for several days, even in dry conditions on particles and dust, thereby probably contributing to the development and persistence of outbreaks. Compounding to the problem of the ease to survive in a hospital environment and increasing antibiotic resistance, is the ability of this organism to form biofilms. It has been shown that Acinetobacter sp. can form biofilms on the surface of various implants and also in the environment[31,32]. The antibiogram details of our study showed *Acinetobacter baumannii* were 100% resistant to ampicillin, amoxyclav and cefuroxime. Higher level of resistance was also recorded for 3rd and 4th generation cephalosporins, aminoglycosides, fluoroquinolones and carbapenems. Similar findings were reported by surveillance studies[33] from Europe, the Asia Pacific region, Latin America and North America over the last 3-5 years revealing increasing rates of *A.baumannii*, however lower rates of carbapenem resistance have been reported in studies carried out by Knam Soo Koo et al[33]. (8.3%) and Gaur et a[34]l. (9.8-18.5%). The resistance pattern observed by us was in contrast to those described in previous studies[35,36]. Differences observed between the studies could be due to the methods and the resistance patterns that are influenced by the environmental factors and the antimicrobial patterns used. The higher degree of resistance among this group of antibiotics could be attributed to the fact that most of our cases had already been exposed to commonly used antibiotics like cefepime, ceftazidime, gentamicin etc, which indicates that multidrug resistance is increasing due to the selective pressure exerted by the use of broad-spectrum antimicrobials and the transmission of strains among the patients[7]. Colistin and tigecycline are new but last alternative in the treatment of Acinetobacter species. In our study, *Acinetobacter baumannii* showed lower resistance to colistin (5%) and tigecycline (12%). Various authors have reported the resistance rate to colistin between 1.8% and 2%[37,38] while resistance to tigecycline varies from being non-existent to 66%[39,40]. A study from the Western Pacific region showed 3.3% resistance of *A.baumannii* to colistin[41]but in contrast, a study in Korea[42], there was high resistance to colistin (30.6%). In comparision to our study, some studies have demonstrated a higher rate of resistance to tigecycline like Navon et al[40]. (66%), Behara et al[43]. (42%). Resistance to our only available drugs like colistin and tigecycline is slowly emerging and will soon be on the higher side leaving us all in unfavourable territory and ultimately with higher mortality rate as seen in our study, the mortality rate being 10.75% ,which is a grave matter of concern. However, the true frequency of nosocomial infection caused by Acinetobacter spp. is difficult to assess because its isolation in clinical specimens may reflect colonization rather than infection and also the overuse of antibiotics reflects the tendency to treat *A.baumannii* infections based on bacteriological reports alone and not the patient in entirety[13]. The rapid emergence of resistance patterns detected in *A.baumannii* reflects the antibiotic misuse and lack of regulations. Therefore, an attempt is to be made by rationalizing the use of antimicrobials in order to delay the emergence of XDR *A.baumannii* , which can be achieved by using an effective antimicrobial stewardship program and monitoring of the program.

CONCLUSION

Acinetobacter baumannii are rapidly spreading with emergence of extended resistance to even newer antimicrobials. The findings in our study highlights several risk factors like stay in ICU, length of stay, invasive procedures, prior use of antimicrobials etc for the spread of drug resistant *Acinetobacter baumannii* . The study also reveals the resistance pattern to almost all the available and common drugs being currently in use and slow and emerging resistance to colistin and tigecycline. Therefore to avoid resistance, well judged use to antibiotics is to be considered and empirical antibiotic therapy should be given based on local antibiotic sensitivity pattern of the given organisms. Various infection control measures can also be adopted and involvement of healthcare personnel at all levels.

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