Original Research Article

Analysis of Resistance Pattern of *Acinetobacter* Species in a Tertiary Care Hospital: A Retrospective Study

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A B S T R A C T

Drug resistant *Acinetobacter* species increases the morbidity and mortality of hospitalized patients. This study was done with the aim of analysis of resistance pattern of *Acinetobacter* species in blood and respiratory tract sample of infected hospitalized patients. This study is a retrospective study of a tertiary care hospital and includes database of 54 patients with *Acinetobacter* species infection in blood and respiratory tract samples, isolated in six month period. An antibiogram was made for these patients along with their clinical details. Odd's ratio was calculated for each clinical condition of the patient. Rate of isolation of *Acinetobacter* species was 3.2% during six month period. 96.3% *Acinetobacter* species isolated were from ICU and 3.7% from ward patients 16.67% were MDR,72.22% were XDR, 5, 56% were PDR while only 5.56% were sensitive strains. 42.5% patients acquiring *Acinetobacter* infection had undergone some surgical procedure. Co-morbid conditions were present in 40.7% patients. An increased risk for resistance development was found only for hypertension. The mortality rate of the patients infected with *Acinetobacter* species was 55.55%. Interpretation: Drug resistant *Acinetobacter* species infection is taking foothold in intensive care settings because of pre-disposing conditions. A rigid adherence to antibiotic policies and infection control practices is essential to conquer the drug resistant *Acinetobacter* species.

Introduction

Acinetobacter species are Gram-negative coccobacilli, strictly aerobic, non-motile, catalase positive, oxidase negative. They are ubiquitous free living saprophytes and can be found throughout the natural and hospital environment on a wide range of dry and inanimate objects (Topley and Wilson, 2011). They are usually opportunistic pathogens reported to cause a number of outbreaks of nosocomial infections such as septicemia, pneumonia, wound sepsis, endocarditis, meningitis, urinary tract infections and

peritonitis but their predominant role is in ventilator associated pneumonia (VAP) in intensive care units (ICUs) (Koneman, 2006) *Acinetobacter baumanni* is the most common species implicated in hospital-acquired infections (Boo *et al.,* 2009).

Acinetobacter baumanni VAP and Blood stream infections (BSI) have been documented to be associated with high degree of morbidity and mortality (Chu *et al.,* 1999). Clinical isolates are predictably resistant to

penicillin, ampicillin, 1st generation Cephalosporins and Choramphenicol. Activity is variable against carbenicilin, tetracyclin, aminoglycosides, 2nd and 3rd generation Cephalosporin, Quinolones, Cotrimaxazole and Carbapenems. Persistence of *A.baumannii* in the hospital environment, is mainly due to resistance to major antimicrobial drug resistance to desiccation and resistance to disinfectant (Peleng *et al.,* 2008). Multidrug-resistant (MDR) *Acinetobacter* species infections are associated with increased time on mechanical ventilation, in the ICU and in the hospital. Carbapenems and Colistin are usually the agents for MDR strains (Tripathi *et al.,* 2014). However, the spectrum of antimicrobial resistance for *Acinetobacter* species has expanded and the prevalence of Carbapenem-resistant isolates appears to be increasing worldwide (Lisa *et al.,* 2008) bringing along with it definitions of Extensively drug resistance (XDR) and even Pan drug resistance (PDR) when there is loss of sensitivity to Colistin and Tigecyline. It is crucial to understand the pattern of resistance and its association with the morbidity and mortality of hospitalized patients.

This study was done with the aim of analysis of resistance pattern in of strains of *Acinetobacter* species in blood and respiratory tract samples of infected hospitalized patients.

Materials and Methods

Setting

This study was carried out in the Department of Microbiology, Gobind Ballabh Pant Institute of Post Graduate Medical Education and Research (G.I.P.M.E.R). This institution is over 700 bedded tertiary care hospital for brain, heart, gastrointestinal and psychiatric disorders. Various types of samples are received from admitted patients in bacteriology laboratory for aerobic culture and sensitivity.

Study design

This study is a retrospective review of a tertiary care hospital data base and includes 54 isolates of *Acinetobacter* species from 1646 patients.

A retrospective chart was performed consisting of demographic and clinical details of 54 patients in whom *Acinetobacter* isolates was isolated from blood and respiratory tract samples, along with the antimicrobial sensitivity profile from the period January 2015 June 2015. The identification of the isolates as *Acinetobacter* species and antibiotic susceptibility testing as per CLSI 2015 guidelines (CLSI, 2015). Additional antibiotic susceptibility testing for seven drugs, namely, Ofloxacin, Levofloxacin, Ticarcillin-clavulanic acid, Piperacillin-tazobactam, Tobramycin, Netimicin and Cefotaxime was peformed by Kirby

Bauer disc diffusion method

MDR (Multi Drug Resistant) is defined as non-susceptibility to at least one agent in three or more antimicrobial classes. XDR (Extensively Drug Resistant) is defined as non-susceptibility in all but two or fewer antimicrobial categories. PDR (Pan Drug Resistant) is defined as non-susceptibility to all agents in all antimicrobial categories.

Results and Discussion

During a period of 6 month, a total of 54(3.28%) *Acinetobacter* species isolates were obtained by processing 1646 clinical samples namely blood and respiratory tract samples. Of these 6 (11.1%) isolates were obtained from blood and 48(88.8%)were from respiratory tract samples (mucus trap and

Endotracheal tubes) Majority of *Acinetobacter* isolates obtained were from samples of ICU patients (96.29%) while only 3.7% isolates were from ward patients.

Acinetobacter species infections were more common in males [62.96% (34/54)] as compared to females [37.03% (20/54)] (Table 1).

Table 2 shows the clinical details of patients in whom *Acinetobacter* species were isolated.

Twenty three patients (42.5%) acquiring *Acinetobacter* infection had undergone some surgical procedure (major or minor), while thirty one (57.40%) were non-surgical patients. Out of twenty-three surgical patients, twenty (86.95%) were infected with resistant *Acinetobacter* species and at the same time all of the non-surgical patients were infected with resistant *Acinetobacter* species too. No association was found between surgery and resistance of the organism. (O.R=0.0930, p value>0.05) (Table 2).

Twenty-three patients (42.5%) had underlying co-morbid conditions as follows; 7/23 (30.43%) were diabetic, 4/23 (7.39%) were hypertensive and 4/23 (7.39%) had carcinoma, while 8/23 (34.78%) had more than one of the above co-morbid conditions with or without tuberculosis. None had tuberculosis alone as the co-morbid condition.

The mortality rate of the patients infected with *Acinetobacter* species was 55.55%. As shown in Table 3, resistance in *Acinetobacter* species was quite high to the important group of antibiotics.

Maximum resistance was seen to Cephalosporin group (97.22%), Ticarcillin-clavunanic acid (92.59%) and least resistance to Colistin (7.41%). Resistance to Carbapenems was seen in 81.48% isolates.

Out of 54 *Acinetobacter* isolates, 16.67% (9/54) were MDR, 72.22% (39/54) were XDR, 5, 56% (3/54) were PDR while only 5.56% (3/54) were sensitive strains (Table 4). Considering whether any associated condition of the patient acted as risk factor for acquiring infection with resistant *Acinetobacter* species, it was seen that co-morbid conditions were present in 23(40.7%) patients, the most common was diabetes mellitus (DM) [7(12.96%)], Other co-morbid conditions were tuberculosis (TB) with hypertension (1.85%), tuberculosis with diabetes mellitus (1.85%) all three condition (1.85%) and carcinoma with diabetes mellitus (1.85%). Amongst the co-morbid conditions, an increased or resistance development was found only for hypertension [Odd's Ratio (O.R) = 1.9877]. O.R for cancer, DM and TB was 0.172, 0.6842 and 0.5052 respectively.

Table 6 shows that amongst the surgical patients, 14 (60.86%) had one or more co-morbid conditions associated, 12(85.71%) were infected with resistant *Acinetobacter* species. There was a risk of association between co-morbid conditions and resistance amongst the surgical patients (O.R=4.6667)

Taking into account the mortality of the infected patients, 30(55.55%) patients with *Acinetobacter* strains died in average duration of 84.73 days of hospital admission. Amongst the *Acinetobacter* species isolated in this group of patients, 3(10%) were PDR strains, 21(63.33%) were XDR and 4(13.33%) were MDR (Table 5). There was no significant risk between resistance organism and mortality of the patients (OR=0.6087, p value>0.05). Patients who were discharged/transferred had an average length of hospital stay of 78.92 days (Table 7).

Acinetobacter species is commonly isolated from skin and throat of people. The respiratory tract is an important site of

colonisation and most the frequently infected site. *Acinetobacter* species is repeatedly isolated from nares, nasopharynx and tracheostomy site. The rate of colonisation increases during ICU stay (Nahar *et al.,* 2014). In the present study, the rate of isolation of *Acinetobacter* species was 3.2% during six month period. 96.3% and 3.7%

Acinetobacter species isolates were from ICU and ward patients respectively. 88.8% of the isolates were from respiratory tract sample (Mucus trap or Endotraceal tube). Differentiation between colonisation and actual infection could not be done as it was a retrospective study.

Following antibiotics were tested in the present study

Antimicrobial category	agent
Aminoglycoside	Gentamicin Tobramycin Amikacin Netilmicin
Carbapenems	Imepenen Meroenem
Quinolones	Levofloxacin Ciprofloxacin
Beta-lactam and beta-lactamase inhibitor combination	Piperacillin-Clavulanic acid Ticarcillin-Clavulanic acids
Extended spectrum cephalosporin	Ceftriaxone Cefipime
Polymyxin	Colistin

Statistical analysis was done isung SSPS.

Table.1 Distribution of *Acinetobacter* isolates

Clinical sample	*Acinetobacter* species	Sample			Patient		Gender	
		Blood	Mucus trap	Ward	ICU		Male	Female
1646	54(3.28%)	6 (11.1%)	48 (88.8%)	2 (3.7%)	52 (96.29%)		34 (62.97%)	20 (37.03%)

Table.2 Clinical profile of patients with *Acinetobacter* species infection

Gender	Surgical/non-surgical				Co-morbid conditions					outcome	
	surgical	non-surgical	DM	HTN	Cancer	T.B	multiple	none	death	discharge
Male(34)	14 MDR-1 XDR-10, PDR-1 Sensitive-2	20 MDR-2 PDR-1 XDR-17	6	2	2	0	5	19	18	16
Female (20)	9 MDR-4 XDR-4 Sensitive -1	11 MDR-2 XDR-8 PDR-1	1	2	2	0	3	12	12	8
Total	**23**	**31**	**7**	**4**	**4**	**0**	**8**	**31**	**30**	**24**

DM-Diabetes Mellitus, HTN-Hypertension, T.B-Tuberculosis

Table.3 Resistance pattern of the commonly prescribed antibiotics

Antibiotic	**Resistance%**
Cephalosporins	97.22
Aminoglycosides	84.26
Levofloacin	77.78
Piperacillin-tazobactum	90.74
Ticarcillin-clavulanic acid	92.59
Carbapenems	82.41
Colistin	7.41

Table.4 Resistant status of the *Acinetobacter* isolates

Acinetobacter species	Number (%)
MDR	9(16.67%)
XDR	39(72.22%)
PDR	3(5.56%)
Sensitive	3(5.56%)

Table.5 Co-morbid conditions versus resistance

Acinetobacter species	cancer	DM	HTN	Multiple	None	Total
MDR	1	0	2	2	4	
XDR	2	6	2	5	24	
PDR	0	0	0	1	2	
Sensitive	1	1	0	0	1	
Total	4	7	4	8	31	

DM-Diabetes Mellitus, HTN-Hypertension

Table.6 Co-morbidity of the operated patients versus resistance

Co-morbid condition	Number of patients	Resistant *Acinetobacter* strain	Sensitive *Acinetobacter* strain
Present	14	12	2
Absent	9	8	1
Total	23	20	3

Table.7 Resistance in *Acinetobacter* species Vs clinical outcome

Acinetobacter species	Patients who were discharged		Patients who died In hospital	
	patients	Average length of stay (days)	patients	Average length Of stay(days)
MDR	5(20.83%)	73.3	4(13.33)	89.88
XDR	18(75%)	73.5	3(10%)	101.53
PDR	0	-	2(6.66%)	60
sensitive	1(4.1%)	55	21(70%)	78.22
Total	24	71.6	30	84.73

Other studies have reported a rate of *Acinetobacter* species isolation of about 100% from endotracheal samples (Nahar *et al.,* 2014), 12% and 24 % from endotracheal aspirate and tracheal sample respectively (Deepha *et al.,* 2011) and 31% from sputum (Dent *et al.,* 2010) Joshi, *et al.,* reported that 27.50% *Acinetobacter* infections were from respiratory tract of intubated patients (Joshi *et al.,* 2006).

Acinetobacter species infection was more common in male (67.97%) as compared to female (3.03%) (Table 1). This finding was similar to several other studies (Joshi *et al.,* 2006; Prashant *et al.,* 2006; Tripathi *et al.,* 2014). This can be attributed to the fact that male visit the hospital more frequently (Tripathi *et al.,* 2014).

A high degree of resistance was seen to cephalosporins (90.22%), PIT (90.74%), TC (92.59%) in the present study (Table 3) Very high degree of resistance to commonly used antibiotics have been reported in earlier literatures too (Sinha *et al.,* 2007; Goudarzi *et*

al., 2013; Mohammed F. AL-Marjani *et al.,* 2013; Tripathi *et al.,* 2014). Carbapenem antibiotics play a crucial role in the treatment of serious nosocomial infections due to *A. baumannii*. Resistance to Carbapenems in this study was quite high. 85.19% resistance was seen to Imepenem alone and 79.63% resistance was seen to both Imepenem and Meropenem. A high degree of resistance to Imepenem have been reported earlier too ranging from 43% to 91.5% (Goudarzi *et al.,* 2013; Nahar *et al.,* 2012; Mohammed F. AL-Marjani *et al.,* 2013; Tripathi *et al.,* 2014). Resistance rate of 35.3% -52.9% to both the carapenems have been reported in a study (Mohammed F. AL-Marjani *et al.,* 2013). Carbapenems are an important class of drug for treating *Acinetobacter* species infections and loss of sensitivity to this group of antibiotic is in itself therapeutic challenge.

Colistin showed a sensitivity of 92.6% in the present study which is similar to few other studies (Nahar *et al.,* 2012; Goudarzi *et al.,* 2013). Although decreased susceptibility to Colistin among *A. baumannii* isolates has

been reported but they can be used as effective drugs for treatment of *A. baumannii* infections (Goudarzi *et al.,* 2013). Use of such reserved drugs should therefore be restricted.

Of the fifty four *Acinetobacter* species isolated, 16.67% were MDR, 72.22% were XDR and 5.56 % were XDR (Table 4) Dent, *et al.,* reported a higher number of MDR (72%), XDR (17%), PDR (46%) (Dent *et al.,* 2010), while Inchai *et al.,* (2015) reported 21.4%, 65.3%, and 3.6% MDR, XDR, and PDR respectively. 44.8% XDR have been reported earlier (Goudarzi *et al.,* 2013).

Because of high intrinsic resistance and the growing decreased susceptibility of *Acinetobacter* species to common antibiotics, it is important to understand the nature of resistance trend in an institution.

By identifying the factors responsible for increasing drug resistance, it will be possible to formulate an antibiotic policy which is crucial for management of critically ill patients as well as to curb the growing menace of drug resistance.

Occurrence of *Acinetobacter* is contributed by several factors like immunosuppressed hosts, patients with severe underlying disease, previous use of antibiotics, duration of hospital stay and more frequent use of antibiotics in ICU. The development of ICU-acquired infections is strongly related to prolonged ICU stay and is associated with worse outcomes including increased morbidity and mortality (Tripathi *et al.,* 2014). The different co-morbid conditions associated with *Acinetobacter* species infection in the present study were DM, Tuberculosis, Carcinoma, and Surgery. However, significant association for acquiring resistant was found only between hypertension (O.R=1.9877). The significant factors for multidrug resistance in other

studies were mechanical ventilation, multiple isolates, and neurologic impairment (Mahgoub *et al.,* 2003; Deepha *et al.,* 2011) debilitating chronic illness, postoperative surgical trauma, urinary catheterization (Joshi *et al.,* 2006), parental nutrition, anemia and catheterization (Tripathi *et al.,* 2104) A risk difference for sensitive and resistant isolates could not be established as sensitive strains in this study were very few (5.56%) *Acinetobacter* species infection is associated with increased morbidity and a prolonged length of hospital stay (Lisa *et al.,* 2008) No significant difference in the length of stay between patients with resistant *Acinetobacter* species was observed in this study. This finding consistent with t other studies (Garnacho *et al.,* 2003; Lisa *et al.,* 2008). The length of stay in patients with resistant organisms may be confounded by their increased mortality. A 5-day excess length of mechanical ventilator dependence and ICU stay, compared with critically ill patients without *Acinetobacter* infection was observed in a cohort study (Blot *et al.,* 2003). Duration of ICU stay and the median duration of hospitalization had seen to be prolonged MDR *Acinetobacter* infection in two other studies (The Brooklyn Antibiotic Resistance Task Force, 2002; Sunenshine, 2007). The impact on length of stay may depend on the type of infection and the extent of antimicrobial resistance.

The mortality rate in this study (55.55%) was within the range of 26% to 68 as reviewed by Lisa *et al.,* (2008). Mortality may be related to the extent of antimicrobial resistance, the effectiveness of empirical therapy, and the availability of definitive therapeutic options (Lisa *et al.,* 2008). The limitation of our study was its inability to differentiate between colonisation versus actual infection.

Our study highlighted the trend of resistance of *Acinetobacter* species over a six month

period in blood and respiratory tract of patients admitted in a tertiary care centre. The results shows the rate of *Acinetobacter* species infection was 3.28%, which were mostly from ICU settings (96.29%) with a variable degree of resistance to important group of antibiotics and a remarkably good sensitivity to colistin (92.59%) without much association with co-morbidities except hypertension. Reserved antibiotics like colistin should not be used empirically. Lastly, whether colonization or actual infection, the spread of resistant bugs should be curbed by good infection control practices and a well formulated antibiotic policy.

The further scope of the study is to analyse the annual trend of resistance in *Acinetobacter* species and like superbugs so as to help in proper guidance of antimicrobial therapy.

Acknowledgement

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