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Research Article

PREVALENCE AND ANTIMICROBIAL RESISTANCE OF CLINICAL ACINETOBACTER BAUMANNII FROM A MAJOR DIAGNOSTIC LABORATORY IN COIMBATORE CITY-SOUTH INDIA

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ABSTRACT

Aim: Isolation and identification of *Acinetobacter baumannii* in all the clinical samples and to determine antibiotic susceptibility pattern of the isolated. **Materials and Methods:** This study has been conducted in the Microtech Diagnostic Centre over a period of 24 months from Jan 2013 to Dec 2015. *Acinetobacter baumannii* and other gram negative were isolated and identified from clinical specimens by standard procedure and antibiotic sensitivity test was performed. **Results:** The gram negative and *Acinetobacter baumannii* isolates rate in the present study were 2063 and 116 respectively. Among the clinical samples, *Acinetobacter baumannii* was highly predominant in respiratory samples. In this current study, different antimicrobials showed higher resistance i.e. 74.1% to ceftazidime, 71.5% to piperacillin, 58.6% to cefepime and lowest resistance was observed to tigecycline (18.1%). All isolates were sensitive to colistin and polymyxin B. **Conclusion:** *Acinetobacter baumannii* are now emerging as organisms of nosocomial infections. Hence, antibiotic sensitivity testing and infection control measures are needed to prevent the emergence and spread of multi drug resistant isolates in health care settings.

INTRODUCTION

Healthcare-associated infections are caused by a wide variety of common and unusual bacteria, among them, Acinetobacter causing infection are raised over the past decades, especially, *Acinetobacter baumannii* causing infection are at high risk of morbidity and mortality to patients (Cisneros *et al*., 2002). Infections caused by *A. baumannii* may also be highly resistant to antimicrobials, particularly those strains isolated from critically ill patients in intensive care settings.

In the hospital environment, *A.baumannii* can colonize the respiratory, urinary, gastrointestinal tract and wounds of the patients and can cause infections in burn, trauma, mechanically ventilated and immunocompromised patients. This isolate was easily transferable even at dry or moisture condition in the hospital environment. Because, *A. baumannii* does not have fastidious growth requirements and is able to grow in various temperatures and pH conditions (Jaggi *et al*., 2012).

The epidemiological, clinical, prognostic, and therapeutic characteristics of *A.baumannii* isolated from infected patients have been studied widely in the last decade. During the infection, most of the isolates escape from the antibiotic action and became resistant to commercially available antibiotics (Sinha *et al*., 2013). This has resulted in a limited choice of antimicrobial for treatment of MDR isolates of *A. baumannii*. The most active agents in vitro against the multidrug resistant *A.baumannii* are the polymixins-A, polymixin B and polymixin E (Colistin) and tigecycline (Jaggi *et al*., 2012).

Among the nosocomial infection, MDR isolates of *A. baumannii* causing infection were insecurity to hospital admitted patients (Bhattacharyya *et al.,* 2013). Hence it is important to look for novel classes of antibiotics, which are effective in treating infection due to *Acinetobacter* spp. The aim of this study was to isolate *Acinetobacter* species from clinical specimens and to study the antimicrobial susceptibility pattern of *Acinetobacter* isolates.

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MATERIALS AND METHODS

This study was conducted from Jan 2013 to Dec 2015. Totally 29,588 samples of blood, respiratory Specimens, urine, Pus wound and fluids were collected from patients of different hospitals of Coimbatore area and transferred to the Microtech Diagnostic Centre by BHI broth medium.

In the laboratory each sample was cultured on blood agar and MacConkey agar (Merck Co., Germany) and incubated for 24 h in 37°C. Blood specimens were cultured in Trypticase Soy Broth (TSB) (Merck, Germany) and sub-cultured on chocolate agar. After 24 h, with direct examination (Gram staining) presence of gram negative coccobacillus was confirmed by the microscopic approach. In order to recognize different species of Acinetobacter, all suspected colonies were identified by colonial morphology, Gram-staining, positive catalase, negative oxidase, growth in 37 and 42°C and other biochemical reactions. Then *Acinetobacter* was characterized and confirmed by vitek 2 compact (Jaggi *et al.,* 2012).

After identification of Acinetobacter species, in order to determine the drug resistance phenotype, disk diffusion method as recommended by clinical laboratory and standards institute (CLSI) was performed. In this study 19 different antibiotics were utilized. In addition, the antibiotic potency of the disks was standardized against the reference strains of *A. boumanii* (ATCC 19606) was used.

RESULTS

During the study, a total of 29,588 clinical samples were aerobically cultured, out of which 3142 (11%) yielded significant growth. From the 3142 growths-positive samples, a total of 2063 gram negative isolates were observed, among them 116 of (6%) *Acinetobacter baumannii* were isolated. Of the 116 isolates, majority 64 (55.1%) were detected from respiratory specimen, followed by 26 (22.41%) from pus and wound samples and lowest from fluids samples 1 (0.86%). Among the 116 isolates, predominant isolates were observed from male patients 85 (73.2%) and 31(27%) were from female patients. In this study other gram negative isolates were also observed. This were highly observed in Urine samples (44%) and second most in Pus and wound samples (21%). (Table.1). In case of age wise occurrence, the highest were in >55 (56%) age group of patients and followed by <15 yrs children (20%) and 24.13% of from middle age group (15-55yrs).

Table 1 Prevalence of *Acinetobacter baumanii* and other gram negative isolates from various clinical Specimens

S.No	Samples	*Acinetobacter baumanii*		Other gram negative isolates	
		Number	%	Number	%
1.	Urine	3	2.5	912	43.9
2.	Blood	22	18.9	303	14.6
3.	Respiratory	64	55.1	413	19.9
4.	Pus/wound	26	22.4	431	20.7
5.	Fluids	1	0.8	16	0.7
		Total 116		**2075**	

Antibacterial susceptible

Sensitivity pattern of Acinetobacter species to different antimicrobials showed higher resistance i.e. 74.1% to ceftazidime, 71.5% piperacillin, 58.6% cefepime and lowest resistance was observed to tigecycline (18.1%). In this study, 100% of sensitive was observed against to colistin and polymixin-B (Table.2).

Table 2 Percentage of antibiotic resistance on *Acinetobacter baumanii*

S.NO	Antimicrobial agents	*Acinetobacter baumanii* n=116			Other Gram negative bacilli n=2075
		S	R	%	Resistant %
1.	Amikacin	58	58	50.0	14.9
2.	Gentamicin	58	58	50.0	39.1
3.	Ampicillin/Sulbactum	55	61	52.5	62
4.	Tobramycin	61	55	47.4	54.2
5.	Piperacillin	33	83	71.5	-
6.	Cefipime	48	68	58.6	58.5
7.	Ceftazidime	30	86	74.1	59.6
8.	Cefaperazone /Sulbactum,	58	58	50	27.5
9.	Piperacillin/tazobactum,	57	59	50.8	23.6
10.	Ciprofloxin	50	66	56.8	49.8
11.	Levofloxin	67	49	42.2	46.9
12.	Ofloxacin	54	62	53.4	49.8
13.	Imepenem	55	61	52.5	9.5
14.	Meropenem	55	61	52.5	9.5
15.	Tigecycline	95	21	18.1	4.0
16.	Colistin	116	0	0	3.6
17.	Polymixin-B	116	0	0	3.1
18.	Doxycyclin	76	40	34.4	55.7
19.	Cotrimoxazole	52	64	55.1	69.1

In this study, three or more antibiotic resistance isolates were multidrug resistance, the worrying aspect of the current study is that 42 (66%) of the isolates as multidrug resistance. Among the 5 types of samples, highest drug were observed in respiratory samples (55.3%) and second most in the blood samples (36.5%) and lowest in urine samples (28%), but same time single isolate of fluid had 79% of resistance. Fig.1 illustrates the distribution of MDR isolates on different clinical samples.

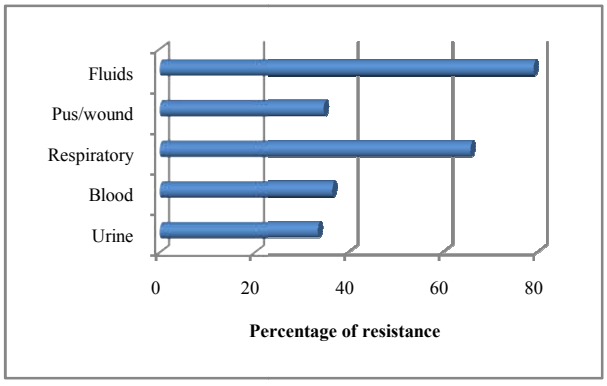

Fig 1 Percentage of MDR isolates on clinical samples

Table 3 revealed that antibiotic resistance patterns of *A. baumannii*. Totally 33 types of resistance patterns were observed, among them, AS,TB,PC,FOR,CPM,AMK,OF, LEV, CIF,DO,CO,G,ZO,MGX,IM,MR pattern was observed in 16 (14%) of isolates. In this current study, 65 (56%) of MDR isolates were observed, which were highest in Respiratory samples.

Table 3 Antibiotic resistance patterns of *Acinetobacter baumanii*

S.No	Resistance patterns	No.of isolates
1.	FOR	6
2.	PC,FOR	9
3.	FOR,CO	2
4.	PC	4
5.	PC,FOR,CPM.CIF	1
6.	AS,TB,PC,FOR,CPM,AMK,OF,LEV,CIF,DO,CO,G,ZO,MGM,IM,MR,TGC	14
7.	AS,TB,PC,FOR,CPM,AMK,OF,LEV,CIF,CO,G,ZO,MGX,IM,MR	9
8.	AS,PC,FOR,CPM,AMK,OF,LEV,CIF,DO,CO,G	1
9.	AS,TB,PC,FOR,CPM,AMK,OF,CIF,CO,G,ZO,MAX,IM,MR	2
10.	AS,TB,PC,FOR,CPM,AMK,OF,CIF,G,ZO,IM,MR	4
11.	AS,TB,PC,FOR,CPM,AMK,OF,LEV,CIF,CO,G,ZO,MGX,IM,MR,TGC	2
12.	AS,TB,PC,FOR,CPM,AMK,OF,LEV,CIF,DO,CO,G,ZO,MGX,IM,MR	16
13.	AS,TB,PC,FOR,CPM,AMK,OF,CIF,DO,CO,G,ZO,MGX,IM,MR	3
14.	PC, FOR,OF,G	1
15.	PC, CO	1
16.	CIF,CO	1
17.	CO	1
18.	PC,FOR,CPM	1
19.	FOR,CPM	2
20.	CPM	1
21.	PC,FOR,CIF,DO,CO	1
22.	PC,FOR,CIF	1
23.	PC,CPM	2
24.	NF	2
25.	AS,TB,PC,FOR,AMK,OF,LEV,CIF,G,ZO,MGX,IM,MR,TGC,NF,NOR	1
26.	AS,PC,FOR,CPM,AMK,OF,LEV,CIF,DO,CO,ZO,MGX,IM,MR	1
27.	AS,TB,PC,FOR,CPM,AMK,OF,CIF,G,ZO,MGX,IM,MR	3
28.	AS, PC,FOR,CPM,OF,CIF,DO,CO,ZO,MGX,IM,MR	1
29.	AS,PC,FOR,CPM,AMK,OF,LEV,CIF,CO,ZO,MGX,IM,MR	1
30.	AS,PC,FOR,CPM,OF,CO,IM,MR	1
31.	AS,TB,PC,FOR,CPM,AMK,OF,CIF,DO,CO,G,ZO,MGX,IM,MR	2
32.	AS,PC,FOR,CPM,OF,CIF,CO,G,ZO,MGX,IM,MR,TGC	1
33.	CIF	1

Antibiotic resistance in other gram negative isolates

In this study, 35.5% of antibiotic resistance was observed from other gram negative isolates, among the 19 antibiotics, Co trimoxazole was highly resistance to other gram negative isolates and second most was Ampicillin/Sulbactum, the results were depicted in table 2. In case of sample wise, the highest MDR was observed in Respiratory samples.

DISCUSSION

Acinetobacter spp., are fast emerging as agents of hospital acquired infection, which are strongly the viable and rapid development of drug resistance ability and has raised an important challenge for treatments. *A. baumannii* is known for its propensity to cause outbreaks in healthcare settings. Transmission occurs mainly via the hands of healthcare workers, imperfect nature of the hospital environment and inadequate infection control practice has continuously raised the incidence of *Acinetobacter* infections over the past two decades. This leads to prolonged hospital stay and increases the mortality and morbidity to infections by Acinetobacter spp.

In our study, a total number of 116 (6%) *Acinetobacter* strains were isolated from processed clinical specimens. This corresponds to a similar study carried out by Jaggi *et al*., (2012) where figures of *A.baumannii* isolates were 9.4% of the total gram negative isolates. This result was in accordance with the prevalence studies by Oberoi *et al.,* (2009), Banerjee *et al.,* (2011) and Wankhede *et al*., (2016). They reported the prevalence of 8.4%, 11% and 9% respectively. The wide variations of the prevalence of Acinetobacter species may be due to variations in geographical distribution as well as the difference in antibiotic policy used by different institutes. More of the *A. baumannii* isolates identified in our study were from male (73.2%) than female patients, which are in disagreement with observations in a previous study by Batarseh *et al.,* (2015). They observed 62.9% of isolates from male samples.

Among the gram-negative isolates from the respiratory secretions, *A.baumannii* was the most common (55.1%). In most institutions, the majority of *A.baumannii* isolates were from the respiratory tracts of hospitalized patients. Studies similar to this, were carried out by Jaggi *et al* (2012) observed 57.4% of occurrence from respiratory tract samples. Mayasari (2014) has also reported a predominance of *A.baumannii* in tracheo-bronchial secretions as 59%.

In this current study, second most predominant in pus and wound samples and followed by blood, urine and fluid. The similar line of the observation was recorded by Sivaranjani *et al.,* (2013). In another study by Bhattacharyya *et al*., (2013) recorded that pathogen was mostly isolated from urine (54%) followed by pus (23%) and CSF (12%). In case of age wise prevalence, the highest percentage of isolates were observed in >55 (56%) age group of patients. This was in agreement with earlier study of Gupta *et al*., (2015).

In the present study, all isolates of *A.baumannii,* showed 46.2% of resistance to different generations of the antibiotics. Among the 19 antibiotics, highest antibiotic resistance was observed in ceftazidime (74.1%) and second most in piperacillin (71.5%). A similar study was done by Wankhede *et al* (2016), who reported 77% of ceftriaxone resistance. In another study by Mathew *et al.,*(2013) observed 86% of ceftriaxone resistance by the isolates. Tazobactam combined with piperacillin

increased the level of sensitivity from 15% to 20%, 24 strains did not record a readable against piperacillin-tazobactam. This occurrence was low than earlier report of Necati *et al*., (2013). They were observed 89.2% of isolates against piperacillin-tazobactam. Among the 5 types of samples, highest drug were observed in respiratory samples (55.3%). This result was in agreement with previous studies of Kaur *et al.,* (2016).

In a study by Hoe Koo *et al* (2010), determined amikacin as the most effective drug among nine antimicrobial agents, but in this study, 50% of amikacin resistance was observed and Colistin and Polymixin-B were the most effective agents among 19 antimicrobial agents which were used. A study in New Delhi, India showed 96.4% sensitivity to polymyxin B in *A.baumannii* (Behera *et al.,* 2009). Another Indian study showed 100% sensitivity to both colistin and polymyxin B (Bhose *et al.,* 2013). So, on the basis of our results and other studies, colistin and polymyxin B may be used as the drug of choice for treatment of infections caused by gram negative bacilli.

A. baumannii infections are more difficult to treat when it has been multidrug resistance. In recent decades, carbapenem resistance isolates are increased in hospital sources and difficult to manage the hospital associated infection (Looveren *et al*., 2004). In this current study, 52.5% of Imepenem and Meropenem resistance isolates were observed. The previous studies of Gaur *et al.,* (2008) observed lowest occurrence of carbapenem resistance by the isolates which showed 9.8-18.5%. Our result clearly indicated that increasing resistance in *A.baumannii* towards carbapenems. Most of the patients were treated with carbapenems for long term which might have contributed to resistance to these drugs.

In case of gram negative isolates, highly resistance to Co trimoxazole and second most Ampicillin/Sulbactum. In this study, most of the isolates were resistance to more than 3 groups of antibiotics. On that account, this study highlights the trends of multidrug resistance among gram negative isolates, therefore indicating an alarm of threat of emergence of drug resistant pathogens.

CONCLUSION

In this study number of resistance patterns were observed, this may due to selective pressure of extensive usage of antibiotics. It has also been observed that Acinetobacter can develop resistance when the patient is on treatment. So strictly follow the antibiotic policy and prevent the emergence of such strains and infections caused by them.

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