ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Isolation and study of Antimicrobial susceptibility and resistance patterns of *Acinetobacter* spp. from Intensive Care Units of a tertiary care hospital in Bengaluru

Dr. SMEETA HUIDROM MD[1], Dr. GIRISH. N, M.D.[2], Dr. RAJENDRAN R MD.[3]

1. Demonstrator, Department of Microbiology, Jawaharlal Nehru Institute of Medical Sciences
2. Associate Professor, Department of Microbiology, Vydehi Institute of Medical Sciences and Research Centre, Bengaluru.
3. Professor and Head, Department of Microbiology, Vydehi Institute of Medical Sciences and Research Centre, Bengaluru.

Manuscript Info

Manuscript History:

Received: 15 June 2015
Final Accepted: 22 July 2015
Published Online: August 2015

Key words:

Acinetobacter, nosocomial, MDR, carbapenems

Corresponding Author

Dr. SMEETA HUIDROM MD

Abstract

BACKGROUND:
Acinetobacter spp. is a ubiquitous gram-negative non-motile, encapsulated, and non-fermentative coccobacillus that has emerged as an important nosocomial pathogen. The incidence of *Acinetobacter* pneumonia (especially LRTIs) in the Intensive care Units (ICU) has increased significantly, accounting for 6-30% of all ICU infections. Emerging infections due to multi drug resistant (MDR) strains of *Acinetobacter* sp. in the ICU is a therapeutic concern for clinicians worldwide.

Materials and Methods:
A retrospective study was undertaken in the Department of Microbiology, VIMS & RC, Bengaluru, to isolate *Acinetobacter* spp and study antibiotic resistance patterns from various clinical samples in ICUs, including pus, sputum, tracheal aspirates, catheter tips from June 2013 to May 2014. Samples were processed and identified by standard protocol. *Acinetobacter* isolates were tested for antibiotic resistance by as per CLSI guidelines.

Results:
A total of 62(12.6%) cases of *Acinetobacter* spp were isolated from 491 samples. Most of the *Acinetobacter* spp. were highly resistant to Ampicillin (83.9%), Amikacin (77.4%), Gentamicin (77.4%), Ceftazidime (85.5%), Ceftriaxone (62.9%), Cefotaxime (77.4%), Imipenem (40.3%), Meropenem (50.0%), Cotrimoxazole (83.9%), Piperacillin-Tazobactam (46.8%). Out of the 62 isolates, 56 (90.3%) were MDR. Carbepenems were seen to be relatively still effective on the *Acinetobacter* isolates.

Conclusion:
This study showed that *Acinetobacter* spp. isolated showed multi drug resistance to antibiotics particularly to the Penicillins, the third generation Cephalosporins, Aminoglycosides and the Carbapenems. To avoid resistance, antibiotics should be used judiciously and urgent steps should be taken to identify and prevent spread of such resistant strains of *Acinetobacter* spp in ICUs.

INTRODUCTION

The genus *Acinetobacter* has undergone significant taxonomic changes over the last 30 years. Its most significant species *Acinetobacter baumanii* has emerged as one of the most troublesome pathogens for health care institutions

globally. Its clinical significance over the past 15 years has been propelled by its ability to up-regulate or acquire resistance determinants, making it one of the organisms threatening the current antibiotic era. *Acinetobacter baumanii* strains resistant to all known antibiotics have been reported, signifying a sentinel event that should be acted upon promptly by the International health care community. Acting in synergy with this emerging resistance profile is the uncanny ability of *Acinetobacter baumanii* to survive for prolonged periods throughout the hospital environment, thus potentiating its ability for nosocomial spread.[1]

Acinetobacter are gram negative, non-motile, non-sporing encapsulated coccobacilli belonging *Neisseriacae*. It is an opportunistic pathogen found to be associated with a wide spectrum of infections including nosocomial pneumonia, meningitides, endocarditis, skin and soft tissue infections , UTI, conjunctivitis, burn wound infections, bacteraemia.[2]

Acinetobacter baumanii is one of the commonest isolates in immunocompromised patients, posing risk for high mortality. During recent years, *Acinetobacter baumanii* has emerged as a significant pathogen especially in Intensive Care Units (ICU). MDR *Acinetobacter baumanii* is a rapidly emerging pathogen in the healthcare setting.[2]

MDR *Acinetobacter* isolates is a growing problem and has been widely reported. Resistance of *Acinetobacter* to a majority of commercially available anti-microbials (Aminoglycosides, Cephalosporins, Quinolones including Carbapenems) raises an important therapeutic problem.[2]

The presence of Resistance plasmids is a significant feature of the organism. Also, *Acinetobacter* has the capacity to serve as a potential reservoir of transmissible drug resistance genes especially in nosocomial environment.[2]

This study was thus undertaken to isolate *Acinetobacter* and analyse the resistance patterns form ICUs in a tertiary hospital in a south Indian setting at VIMS & RC, Bangalore.

MATERIALS AND METHODS

Study area, population, and methodology

A retrospective, hospital record–based, study was carried out from June 2013 to May 2014 in the Department of Microbiology at a tertiary care hospital, Vydehi Institute of Medical Sciences and Research Centre, in Bangalore. Isolates of *Acinetobacter* species were recovered from various clinical specimens from the ICU, including pus, throat swabs, CSF, vaginal swabs, tracheal aspirates and other unspecified swab samples. Only the isolates grown from the samples of patients developing various symptoms after admission in different wards of the hospital for more than 48 hours (nosocomial isolates) and subsequently transferred to the ICU were selected for the study.

An infection was considered as nosocomial if all the elements of a site-specific infection criterion of Center of Disease Control and Prevention (CDC) were first present together on or after the 3rd hospital day (day of hospital admission is day 1).[3]

Sample processing and antibiogram

Isolates of *Acinetobacter species* were recovered from various clinical specimens from the ICU, including pus, throat swabs, sputum, CSF, vaginal swabs, tracheal aspirates and other unspecified swab samples.

The samples received in the laboratory were inoculated on 5% sheep blood agar and MacConkey agar and incubated aerobically at 37^{o}C. Thereafter, species identification and antimicrobial resistance patterns were studied were performed according to CLSI guidelines.

Acinetobacter spp. were identified by characteristic colonies (non Lactose fermenting, glistening small mucoid colonies), Gram staining pattern and standard biochemical reactions – Catalase, Hugh Leifson's oxidative fermentative test, Indole production, Citrate utilization, Motility, Urease test, TSI medium) as described in the table (Table 1).

The biochemical characteristics of *Acinetobacter* sp. Strains[4]

Test, substrate	*A. baumanii*	*A. lwoffii*
Morphology	coccobacilli	Coccobacilli
Motility	Non-motile	Non-motile
Fermentative or oxidative	O	O
Catalase	+	+
Oxidase	-	-
Growth on		
MacConkey agar	+	+
SS agar	V	V
Acid from:		
Glucose	+	-

Xylose	-	-
Mannitol	-	-
Sucrose	+	-
Galactose	+	-
Manose	+	-
Rhamnose	+	-
Lactose	V	--
Maltose	-	-
Esculin hydrolysis	+	-
TSI acid:		
Slant	-	-
Butt	-	-
H2S: on TSI	-	-
Simmons citrate	+	V
Urea,Christensen	V	-
Nitrate reduction	-	-
Methyl red	-	-
Voges-Proskauer	-	-

Key reactions: O = oxidative; NO = non-oxidiser; + = positive reaction; - = negative reactions; V = variable reactions.

After identification was done by phenotypic methods, AST was performed for each isolate by Kirby Bauer disc diffusion method on Mueller Hinton Agar using 0.5 Mc Farland turbidity standard and comparing zone sizes with control strain against the reference strains of *Escherichia coli ATCC 25922* as the negative control and *A. baumannii ATCC 19606* as the positive control. The following antibiotic discs were used: Ampicillin (Amp), Amikacin (Ak), Gentamicin, Ceftazidime, Ceftriaxone, Cefotaxime, Imipenem, Meropenem, Cotrimoxazole, Piperacillin-Tazobactam and Colistin.

Susceptibility results were interpreted by measuring the zone diameters produced and correlating those with the CLSI standards.

Multi drug resistant (MDR) *Acinetobacter* spp. was defined as those isolates resistant to more than three classes of antibiotics.[3]

RESULTS

During the study period from June 2013 to May 2014, of all the samples (491) received from the ICU, a total of 62 (12.6%) *Acinetobacter* spp. were isolated and studied.

Of all the isolates, 41 (66.1%) were males and 21 (33.9%) were females (Table 1). The patients were seen displaying a wide range in age, youngest in the study being 23 years and the oldest being 86 years of age. The maximum number of patients, 24 (38.7%) were observed in the elderly in the 60-70 years age group (Table 2).

Majority of the *Acinetobacter* spp. were isolated from sputum (14/22.6%), and pus/swab samples (14/22.6%), followed by pleural fluid (09/14.5%), BAL/tracheal aspirate (07/11.3%), Catheter tip (07/11.3%), blood (6/9.7%), endotracheal tube tip (03/4.8%), CVP tip (01/01.6%) and CSF (01/01.6%). (Table 3)

In the present study, most of the *Acinetobacter* spp. isolated was highly resistant to Ampicillin (83.9%), Amikacin (77.4%), Gentamicin (77.4%), Ceftazidime (85.5%), Ceftriaxone (62.9%), Cefotaxime (77.4%), Imipenem (40.3%), Meropenem (50.0%), Cotrimoxazole (83.9%), Piperacillin-Tazobactam (46.8%).

Out of the 62 isolates, 56 (90.3%) were MDR.

All (62/100%) the isolates were sensitive to Colistin.

DISCUSSION

In our study, from 491 clinical isolates from the ICU, 62 (12.6%) *Acinetobacter* spp. were obtained. In comparison, similar prevalence rates of 14% and 9.6% among hospital isolates were observed by Mostofi *et al*.[5] in Tehran, Iran and Joshi *et al*.[6] in Pune, India, respectively; and lower rates by Rit *et al*.[7] (4.5%) in West Bengal, India and Dash *et al*.[3] (3%) in Odisha, India.

Acinetobacter spp. were isolated from various clinical samples including blood, body fluids, tracheal aspirate, endotracheal tubes, intravenous catheters, and other samples, but most commonly from pus/swab (22.6%). Similar findings were obtained by Chakraborty *et al*.[8] in West Bengal and Dash *et al*.[3] (3%) in Odisha.

Our study revealed that majority (54.7%) of the isolates were MDR *Acinetobacter* spp. (90.3%), which was much higher than the other studies conducted by Bhattacharyya *et al*.[9] (29%) in West Bengal, Mostofi *et al*.[5] (54%) in Tehran and Dash *et al.*[3] (54.7%) in Odisha.

This means MDR isolates are increasing day by day, probably due to indiscriminate use of these antibiotics in healthcare settings.

It was however observed that Imipenem, Meropenem, and Piperacillin/Tazobactam were still relatively more potent against this pathogen as compared to the commonly used antibiotics. The resistance pattern observed was similar to the ones in previous studies. Differences observed between the studies could be due to the methods and the resistance patterns that are influenced by environmental factors and the antimicrobial patterns used.

All the isolates were still however seen to be sensitive to Colistin, highlighting it as new alternative in the treatment of *Acinetobacter* spp. This observation has also been reported by Dash *et al.*[3] and Taneja *et al.*[10] in Chandigarh.

CONCLUSION

This hospital based epidemiological data has showed that a very high number (90.3%) of *Acinetobacter* isolates were MDR but still relatively more sensitive to Imipenem, Meropenem, and Piperacillin/Tazobactam. This information is alarming and highlights the problem of the emerging infection of MDR *Acinetobacter spp.* especially in an ICU setting. This will help to implement better infection control strategies and improve the knowledge of antibiotic resistance patterns in our region.

Table 1. Distribution of isolates in relation to Patient's Sex

Sex	Number	Percentage
Males	41	66.1%
Females	21	33.9%
Total	62	

Table 2. Distribution of patient's age

Age group (years)	Number	Percentage
20-30	4	06.5%
30-40	9	14.5%
40-50	14	22.6%
50-60	4	06.5%
60-70	24	38.7%
70-80	3	04.8%
90-100	4	06.5%

Table 3. Distribution of *Acinetobacter* spp. in various clinical samples (*N* = 62)

Type of sample	Total	Percentage
Sputum	14	22.6%
Catheter tip	07	11.3%
Pus/ Wound swab	14	22.6%
Blood	06	09.7%
Endotracheal tube tip	03	04.8%
CVP tip	01	01.6%
Pleural fluid	09	14.5%
BAL/tracheal aspirate	07	11.3%
CSF	01	01.6%

Table 4. Antibiotic susceptibility test of *Acinetobacter spp*. (*N* = 62)

Antibiotic	Disk content (mcg)	Sensitivity patterns in percentage			
		Sensitive		Resistant	
		Number	Percentage	Number	Percentage
Ampicillin		10	16.1%	52	83.9%
Amikacin		14	22.6%	48	77.4%
Gentamicin		14	22.6%	48	77.4%
Ceftazidime		9	14.5%	53	85.5%
Ceftriaxone		23	37.1%	39	62.9%
Cefotaxime		14	22.6%	48	77.4%
Imipenem		37	59.7%	25	40.3%
Meropenem		31	50.0%	31	50.0%
Cotrimxazole		10	16.1%	52	83.9%
Piperacillin-Tazobactam		33	53.2%	29	46.8%
Colistin		62	100.0%	00	00.0%

Table 5. Frequency of multidrug resistance in *Acinetobacter* spp. (*N* = 62)

Parameter	Resistance to one or several classes of Antibiotics (n/%)					
Number of classes of antibiotics	0	1	2	3	4	>4
No. of isolates of *Acinetobacter sp.*	2(3.2%)	1(1.6%)	3(4.8%)	7(11.3%)	13(21%)	36(58.1%)

ACKNOWLEDGEMENTS: None

REFERENCES:

1. Peleg AP, Seifert H, Paterson DL. *Acinetobacter baumannii* Emergence of a successful Pathogen. Clin Microbiol Rev 2008;21:538-82.
2. R.B. Patwardhan, P.K. Dhakephalkar, K.B. Niphadkar, B.A. Chopade. A study on nosocomial pathogens in ICU with special reference to multiresistant *Acinetobacter baumannii* harbouring multiple plasmids. Indian J Med Res 128, August 2008,178-87.
3. Dash M, Padhi S, Pattnaik S, Mohanty I, Misra P. Frequency, risk factors, and antibiogram of *Acinetobacter* species isolated from various clinical samples in a tertiary care hospital in Odisha, India. Avicenna J Med 2013;3:97-102.
4. S Constantiniu, A Romaniuc, LS Iancu, R Filimon, I Taraşi. Cultural and biochemical characteristics of *Acenitobacter* spp. strains isolated from hospital units. The J of Prev Med 2004;12(3-4):35-42.
5. Mostofi S, Mirnejad R, Masjedian F. Multi- drug resistance in Acinetobacter baumannii strains isolated from clinical specimens fromthree hospitals in Tehran- Iran. Afr J Microbiol Res 2011;5:3579-82.
6. Joshi SG, Litake GM, Satpute MG, Telang NV, Ghole VS, Niphadkar KB. Clinical and demographic features of infection caused by *Acinetobacter* species. Indian J Med Sci 2006;60:351-60.
7. Rit K, Saha R. Multidrug- resistant Acinetobacter infection and their susceptibility patterns in a tertiary care hospital. Niger Med J 2012;53:126-8.
8. Chakraborty B, Banerjee D, Chakraborty B. Acinetobacter baumannii: No more a choosy intruder? Indian J Med Sci 2011;65:344-8.
9. Bhattacharyya S, Bhattacharyya I, Rit K, Mukhopadhyay PK, Dey JB, Ganguly U, Ray R. Antibiogram of *Acinetobacter* spp. isolated from various clinical specimens in a Tertiary care hospital in West Bengal, India. Biomedical Research 2013; 24 (1): 43-46.
10. Taneja N, Singh G, Singh M, Sharma M. Emergence of tigecycline and colistin resistant *Acinetobacter baumannii* in patients with complicated urinary tract infections in north India. Indian J Med Res 2011;133:681-4.