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Isolation, Characterization and Antimicrobial Susceptibility Pattern of *Acinetobacter* Species in Various Clinical Samples

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Abstract

Introducion: Acinetobacter is a heterogeneous group of gram negative, oxidase negative, non-motile, non-fermenters emerged as an important nosocomial pathogen causing outbreaks of hospital infections. The high prevalence of multidrug resistant isolates makes initiation of effective empiric treatment challenging. *Objective:* This study was undertaken to isolate and characterise Acinetobacter species in various clinical specimens and to analyse the antibiotic susceptibility pattern. *Materials and Methods:* A total of 5395 clinical specimens were processed in the department of microbiology of a tertiary care hospital over the period of 2 years. Out of which 147 isolates were *Acinetobacter* species. Speciation and antibiotic susceptibility was determined by the standard conventional method. ESBL and MBL production was detected by disc potentiation test method and imipenem EDTA combined disc test, and MBL E-test respectively. *Results:* Prevalence was 2.72%. Most predominant species was *Acinetobacter baumannii* 128 (87.07%). Maximum isolataion was seen among ICU patients (31.97%). Most of strains were resistant to ciprofloxacin (87.5%), ceftazidime (85.94%). All strains were resistant to piperacillin (100%) and sensitive to colistin and polymyxin B. ESBL, MBL production and MDR was detected in 34.01%, 21.77% and 53.06% of the isolates respectively. *Discussion and Conclusion:* A high level of antibiotic resistance was observed in our study and maximum isolation rate of Acinetobacter was in the ICUs associated with respiratory tract infection. *Acineobacter baumani* was the most predominant species. Other species of *Acinetobacter* are also isolated and encountered in hospital acquried infection, though they are sensitive to presently used antimicrobials but in future have potential to acquire resistance. The analysis of susceptibility pattern will be useful in understanding the epidemiology of this organism in our hospital setup, which will help in treating individual cases and controlling the spread of resistant isolates to other individuals.

Keywords: *Acinetobacter* Species; Antimicrobial Susceptibility Pattern.

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Introduction

Acinetobacter species are Gram-negative, non-fermentative coccobacilli, these are saprophytic, ubiquitous and have emerged as an important nosocomial pathogen. Among non fermentative organism, it is second most common nosocomial bacteria encountered in clinical specimens [1]. Primary pathogenic role of these bacteria is undoubtedly to cause hospital-acquired infections; mainly among patients at intensive

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care units (ICUs). Even cases of community-acquired infections caused by *Acinetobacter* spp. have been reported [2]. *Acinetobacter* causes epidemic outbreaks or endemic occurrence with documented high mortality rates, which is about 25 to 30 % for bacteremia and 40–80% for pneumonia [3,4]. *Acinetobacter spp*. have been implicated in ventilator-associated pneumonia, catheter related blood stream infections, urinary tract infections, cerebrospinal-shunt-related meningitis and wound infections [2].

The most common species to cause infections are *A. baumannii*, followed by *A. calcoaceticus, A. haemolyticus* and *A. lwoffii* [2]. During the last three decades *A. baumannii* isolates have become resistant to more and more classes of antibiotics due to both intrinsic and acquired resistance mechanisms [5,6]. For a long time carbapenems was the most reliable treatment option for infections caused by *Acinetobacter* spp., but carbapenemase-producing isolates are emerging globally [5,7]. Emergence of metallo –β- lactamases (MBL) producing multidrug resistant (MDR) isolates is a matter of concern in an intensive care unit (ICU)[5].

The present study was conducted to find out prevalence of *Acinetobacter* species infection and its antimicrobial susceptibility pattern in various clinical specimens at our hospital, so as to guide the clinicians of our hospital to select appropriate antimicrobial agents and infection control protocol in order to control *Acinetobacter* infection and ultimately for the holistic healthcare.

Materials and Methods

The study was conducted in the Department of Microbiology, Government Medical College and tertiary care hospital, from Dec 2016 to Nov. 2018. A total 5395 specimens like blood, sputum, pus, CSF and other body fluids were subjected to simplified phenotypic identification scheme.

All the samples were subjected to Grams stain except blood and urine and inoculated on blood agar and MacConkey agar medium and incubated at 37^0C for 24 hours. All non-lactose fermenter colonies on MacConkey agar were subjected to gram staining, catalase, oxidase test and motility. *Acinetobacter* are Gram negative bacilli or coccobacilli, oxidase negative, nonmotile and catalase positive. Speciation was done on the basis of citrate utilization test, urea hydrolysis test, arginine hydrolysis, glucose oxidation by oxidation fermentation test, gelatin liquefaction, hemolysis, malonate assimilation and growth at 37^0C and 42^0C. Antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion method on Mueller-Hinton agar (MHA) as per CLSI guidelines.

ESBL production was detected by using the disc potentiation test method. Ceftazidime, ceftazidime -clavulanic acid and ceftriaxone, ceftriaxone-clavulanic acid discs were used. Production of enzyme Metallobetalactamases (MBL) was detected by using imipenem EDTA combined disc test and MBL E– test.

Results

During the period of study from Dec 2016 to Nov 2018, a total of 5395 specimens were examined from patients of different age group admitted in various medical wards, surgical wards and ICU at Government medical college and tertiary care hospital. A total of 147 isolates were *Acinetobacter* species. Prevalence of *Acinetobacter* species in our study was 2.72%.

Table 1: Ward wise distribution of patients with *Acinetobacter* infection

Ward/ICU	No of patients	Percentage (%)
ICU	47	31.97
Burn ward	36	24.49
Surgery ward	28	19.05
OBGY ward	16	10.89
Medicine ward	11	7.48
Paediatric ward	05	3.40
Ortho ward	04	2.72
Total	147	100

Maximum number of isolates were obtained from patients admitted in ICU (31.97%) followed by burn ward (24.49%). Maximum number of *Acinetobacter* species were obtained from respiratory tract infection 47(31.97%) followed by burn wound (24.49%). In our study, *Acinetobacter baumannii* 128 (87.07%) was predominant species isolated followed by *Acinetobacter lwoffii* 16 (10.89%), *Acinetobacter haemolyticus* 3 (2.04%) (Table 1).

Table 2: Antimicrobial susceptibility pattern of *Acinetobacter baumannii* (N=128)

Drug name	Sensitive	Resistance
Piperacillin	0	128 (100)
Piperacillin-tazobactum	32 (25)	96 (75)
Ampicillin–sulbactum	98 (76.56)	30 (23.44)
Ciprofloxacin	16 (12.5)	112 (87.5)
Levofloxacin	38 (29.69)	90 (70.31)

Tetracycline	20 (15.62)	108 (84.38)
Cotrimoxazole	46 (35.94)	82 (64.06)
Ceftazidime	18 (14.06)	110 (85.94)
Cefotaxime	18 (14.06)	110 (85.94)
Cefepime	27 (21.09)	101 (78.91)
Gentamicin	48 (37.5)	80 (62.5)
Amikacin	78 (60.94)	50 (39.06)
Tobramycin	78 (60.94)	50 (39.06)
Imipenem	87 (67.97)	41 (32.03)
Meropenem	87 (67.97)	41 (32.03)
Colistin	128 (100)	0
Polymyxin	128 (100)	0

Figures in parenthesis shows percentage

All isolates of *Acinetobacter baumannii* were resistant to piperacillin. Most of the isolates were resistant to ciprofloxacin (87.5%), ceftazidime (85.94%) and cefotaxime (85.94%), tetracycline (84.38%), cefepime (78.91%). All strains were sensitive to colistin and polymyxin B (Table 2).

Table 3: Distribution of *Acinetobacter* isolates according to ESBL, MBL production and multidrug resistance

Acinetobacter spp.	ESBL positive	MBL positive	MDR strains
Present	50 (34.01)	32 (21.77)	78 (53.06)
Absent	97 (65.99)	115 (78.23)	69 (46.94)
Total	147	147	147

In our study, 34.01% of *Acinetobacter* isolates were ESBL producer and 21.77% MBL producer and 53.06% were multidrug resistant isolates (Table 3).

Discussion

Acinetobacter species are emerging as an important organism causing hospital acquired infections [8]. These organisms cause serious health care associated infections as well community acquired infections [9,5].

Acinetobacter baumannii is the most common clinically important bacteria belonging to this genus [9,5] which causes epidemic outbreaks or endemic occurrence with documented high mortality rates [2,11] and outbreaks have also been reported from India [4]. The mortality rate of nosocomial infections caused by *A.baumannii* is relatively high, i.e. 25 to 30% for bacteremia and 40–80% for pneumonia [3]. In our study, a total 147 isolates of *Acinetobacter* species were isolated. Prevalence of *Acinetobacter* species in our study was 2.72% which is lower as compared to various studies (4 to 9%) [12,13,14]

In the present study, maximum number of isolate were from ICU (31.97%) followed by Burn ward (24.49%) and Surgery ward (19.05%) (Table no.1) which is similar to the study of Gupta N et al. [15] (2015). ICU infections more because of opportunities for cross transmission, immune-compromised patients who are colonized and having indwelling devices, heavy use of broad spectrum antibiotics and frequent contamination of the hands of health care workers during patient care. The development of ICU-acquired infections is strongly related to prolonged ICU stay.

Isolation of *Acinetobacter* species was maximally from respiratory tract infection (31.97%) followed by burn wound infection (24.49%) which is similar to study done by Singla et al. [16]. (2013) Amandeep Kaur et al. [17] (2016), Jaggi et al. [18] (2012).

In our study, *Acinetobacter baumannii* (87.07%) was predominant species isolated which is similar to studies done by Dash et al. [19] (2013) (79.6%). Somewhat lower isolation rate was seen in study done by Tripathi et al. [20] (2014) (74.50%), Mostofi et al. [14] (2011)(71%) and Singla et al. [16] (2013) (74.6%) as compared to our study.

One of the most striking feature of genus *Acinetobacter* is the ability to develop antibiotic resistance extremely rapid in response to challenge with new antibiotics. In our study, all isolates *of A.baumannii* were resistant to piperacillin, 87.5% resistant to ciprofloxacin. Resistance to cefotaxime and ceftazidime was 85.94% and carbapenam resistance was 32.03%. All isolates were sensitive to colistin and polymixin B (Table 2). As compared to our study, Gupta N et al. [15] (2015) found lower resistance pattern to piperacillin (55%). resistant to ciprofloxacin (23%) lower resistance to ceftazidime (46%) and cefotaxime (43%). carbapenam resistance (22%).

As per table 3, ESBL production was seen in 34.01% isolates which is comparable to study done by Gupta N et al. [15] (2015) (31.5%) while Kansal et al. [21] (2009) found maximal ESBL producing isolates in their study (75%). MBL production was 21.77% which is similar to Kumar et al. [22] (2011) (21%) while somewhat lower incidence was seen in a study done by Gupta N et al. [15] (2015) (14.4%). Multidrug resistance in our study was 53.06% which is comparable to study done by Mostofi et al. [14] (2011) and Dash M et al. [19] (2013) who found 54% and 54.7% of strains as multidrug resistant

respectively

The resistance patterns detected in *Acinetobacter* could reflect the antibiotic misuse and lack of regulations on drug use. Resistance to various antimicrobials agent limits the selection of appropriate drugs for the effective management making difficult to control and treat. However, as the resistance against colistin and polymyxin is not very high in our country, it can still be used as the drug of choice against multidrug resistant strains of *A.baumannii*.

Conclusion

The high prevalence of multidrug resistant *Acinetobacter* species in our hospital only underscores the urgent need for instituting control measures to limit the spread of this troublesome nosocomial pathogen in hospital areas. Definitive identification and characterization of *Acinetobacter* species by simple phenotypic methods can be used. Rationale use of antibiotics is important and necessary to prevent microbial resistance catastrophe. A continued awareness of the need to maintain good housekeeping and control of the environment, including equipment decontamination, strict attention to hand washing should undertake to control the spread of *Acinetobacter* in hospitals.

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