Prevalence and antibiogram of *Acinetobacter* species isolated from various clinical samples in a Tertiary Care Hospital, Haryana, India

Dr. Pushkar*, Senior Resident
Dr. Aashana**, Postgraduate Student,
Dr. Priyanka Jain, Senior Resident
Dr. Madhu Sharma, Professor,
Dr. AparnaYadav, Senior Professor and Head
DEPARTMENT OF MICROBIOLOGY AND BIOCHEMISTRY**
PT. B.D. SHARMA PGIMS, ROHTAK-124001, HARYANA (INDIA)
***Corresponding Author:**
MCF-46, Kundan Colony
Ballabhgarh, Distt. Faridabad, Haryana-121004
E-mail: pushkarvasisht742@gmail.com

ABSTRACT

Background:*Acinetobacter* species has emerged as a significant pathogen in health care setting, and are becoming increasingly drug resistance. They cause outbreaks in intensive care units, health care units and hospital wards.

Aim: A retrospective study was conducted in Department of Microbiology, Pt. B.D Sharma Institute of Medical Sciences, Rohtak, Haryana to determine the prevalence and antibiotic susceptibility pattern of *Acinetobacter*spp isolated from various clinical samples collected from patients admitted in various intensive care units and wards of the hospital over a period of six month (1st January2021 to 30th June 2021).

Methods: Study included all the patients who had been admitted in various wards, ICUs and whose various clinical samples were sent to the microbiology laboratory for routine culture and antibiotic susceptibility tests.The samples were inoculated onto Blood Agar and MacConkey Agar plates. Urine Samples were inoculated on Cysteine Lactose Electrolyte Deficient (CLED) agar. All isolates obtained were further processed and identified by standard routine microbiological processes. Antibiotic susceptibility testing was performed by standard Kirby Bauer disc diffusion method for the following antimicrobial agents- Ceftazidime (30µg), Ciprofloxacin(5µg), Cotrimoxazole (25µg), Amikacin (30µg), Gentamicin (10µg), Ciprofloxacin (5µg), Polymyxin B (300 units), Meropenem (10µg), Colistin (10µg), Ceftriaxone (30µg), Piperacillin-Tazobactam (100/10µg), and Norfloxacin (10µg) for urine samples. The zones of inhibition were measured and interpreted as per Clinical and Laboratory Standards Institute guidelines (CLSI).

Results: Out of samples, 2306 (40%) yielded significant growth and out of these positive cultures, 305 (13.2%) *Acinetobacter*spp were isolated. Majority of isolates 58 (19.01%) were isolated from General intensive care unit (GICU). Maximum sensitivity of *Acinetobacter*spp was seen towards polymyxin B and colistin i.e. 305 (100%). Two hundred forty-nine(81.6%) isolates were found to be multidrug resistant.

Conclusions:*Acinetobacter* isolates showed multidrug resistant pattern mostly in inpatients. To avoid resistance, rationale use of antibiotics should be done. There is also an urgent need for emphasizing the importance of hand washing and use of disinfectants in prevention of transmission of infection in health care setup.

Keywords: *Acinetobacter*spp; antibiotic resistance; intensive care units; multidrug resistance.
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INTRODUCTION

The genus *Acinetobacter* are Gram-negative, strictly aerobic non-fermenting, non-fastidious, non-motile, catalase-positive and oxidase negative coccobacillary bacteria.[1] They prefer moist environment and can easily obtained from soil, water, food and sewage.[2] They are usually considered to be opportunistic pathogens, and of recent have been reported to cause a number of outbreaks of nosocomial infections in hospitalized patients like septicaemia, pneumonia, wound sepsis, endocarditis, meningitis and urinary tract infections (UTI).[3,4] Such infections are often extremely difficult for the clinician to treat because of the widespread resistance of these bacteria to the major group of antibiotics. More than two third of *Acinetobacter* infections are due to *Acinetobacter baumanii*. *Acinetobacter baumanii*causes health care associated infections.[5-8]*Acinetobacter baumanii*also has the ability to form biofilms, which may play a role in the process of colonization. Biofilms help the bacteria resist disinfection while also allowing the participating cells to trade resistance genes, further facilitating the persistence of the pathogen.[9]*Acinetobacter* associated infections represent a tough challenge to control in severely ill patients especially those in ICU. *Acinetobacter* species have the capacity to acquire resistance to almost all presently existing antimicrobial agents.[10]Despite the increasing significance and frequency of multidrug resistant *Acinetobacter* infections, many clinicians and microbiologists still lack an appreciation of importance of these organisms because of their confused taxonomic status.[11] Because of their increasing importance of nosocomial infections and multidrug resistant pattern, further study is warranted.

METHODS

The present study was conducted in the Department of Microbiology, Pt. B.D Sharma Institute of Medical Sciences, Rohtak, Haryana during a period of 6 months (1stJanuary 2021 to 30thJune 2021). Study included all the patients who had been admitted in various wards, ICUs and whose various clinical samples were sent to the microbiology laboratory for routine culture and antibiotic susceptibility tests. The samples were inoculated onto Blood Agar and MacConkey Agar plates. Urine Samples were inoculated on Cysteine Lactose Electrolyte Deficient (CLED) agar. All isolates obtained were further processed and identified by standard routine microbiological processes. Genus *Acinetobacter* was identified by Gram staining as Gram negative coccobacilli, colony morphology, non-motile, oxidase negative, catalase positive, TSI reaction (K/ K) and citrate utilization test positive. Speciation of *Acinetobacter* was done on the basis of glucose oxidation (OF test), hemolysis on blood agar, growth at 37°C and 44°C, citrate utilization, Arginine decarboxylation, Glucose utilization[12,13] as shown in Table 1. Antibiotic susceptibility testing was performed by standard Kirby Bauer disc diffusion method for the following antimicrobial agents- Ceftazidime (30µg), Ciprofloxacin(5µg), Cotrimoxazole (25µg), Amikacin (30µg), Gentamicin (10µg), Ciprofloxacin (5µg), Polymyxin B (300 units), Meropenem (10µg), Colistin (10µg), Ceftriaxone (30µg), Piperacillin-Tazobactam (100/10µg), and Norfloxacin (10µg) for urine samples. The zones of inhibition were measured and interpreted as per Clinical and Laboratory Standards Institute guidelines (CLSI).[14]

Table 1. Speciation scheme of *Acinetobacter* species.							
Species	**Hemolysis on Blood agar**	**Growth at 37°C**	**Growth at 44°C**	**Citrate utilization**	**Glucose oxidation fermentation**	**Arginine decarboxylation**	**Glucose utilization**
Acinetobacter baumannii		+	+	+	+	+	+
Acinetobacter lwoffi		+					+
Acinetobacter calcoaceticus	+	+			+	+/-	+
Acinetobacter junii		+				+	+
Acinetobacter radioresistens		+				+	+

RESULTS

Out of the total 5765 samples, 2306 (40 %) were culture positive, and out of the total 2306 culture positive samples, 305 (13.2%) were *Acinetobacter* (Table 2).

Table 2. Distribution of culture of total sample.		
Cultured Sample	**Number of isolates**	**Percentage**
Culture positive sample	2306	40%
Acinetobacter organism	305	13.20%
Other organism	2001	34.70%
Culture negative	3459	60%

Acinetobacter isolates were obtained from various specimens. The isolation rate of *Acinetobacter* spp. was maximum in General ICU 58 (19.01%) followed by General medical ward 45 (14.70%) (Table 3). There was higher incidence of *Acinetobacter* infection in males (59.0%) then females (41.0%). *Acinetobacter* spp. was more common in patient with age group of >55years with an incidence of (74.0 %). The isolation rate of *Acinetobacter* spp was maximum from sputum sample 98 (32.10%), followed by endotracheal tip/aspirates 52 (17.04%), followed by pus/swab 49 (16.06%), other tips 40

(13.11%) which include drain tip, catheter tip, suction tip, CVP tip (Table 4). The most predominant species of *Acinetobacter* isolated was *A. baumanii*232 (76.06%) followed by *A. lwoffi*35 (11.40 %). *A. baumanii*was also the predominant species isolated from general medicine ward i.e.38.18% (Table 5).

Table 3. Distribution of isolates in various wards/ICUs.

Unit	Number of isolates	Percentage
General ICU	58	19.01%
General medical wards	45	14.70%
Neurosurgical ward	34	11.14%
CTVS	30	9.80%
Surgical ward	25	8.19%
Neurosurgical ICU	22	7.21%
Surgical ICU	20	6.55%
Orthopedic ward	18	5.90%
Post-Op ward	16	5.24%
Gynaecology	15	4.91%
Neonatal ICU	12	3.93%
Paediatric ward	10	3.27%

Table 4. Specimen distribution of *Acinetobacter* isolates.

Sample	Number	Percentage
Sputum	98	32.10%
Endo tracheal tip/aspirate	52	17.04%
Pus/swab	49	16.06%
Tips	40	13.11%
Urine	29	9.50%
Body fluids	17	5.57%
Blood	14	4.59%
High vaginal swab	6	1.90%

Table 5. Species distribution of *Acinetobacter* isolates.

Acinetobacter species	Number	Percentage
Acinetobacter baumannii	232	76.06%
Acinetobacter lwoffi	35	11.40%
Acinetobacter calcoaceti-cus	25	8.19%
Acinetobacter junii	8	2.60%
Acinetobacter radioresis-tens	5	1.60%

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Among 305 isolates of *Acinetobacter*, all showed 100% sensitivity to colistin and polymyxin B. High levels of resistance were seen for Ampicillin (94.09%), ceftazidime (92.10%) and ceftriaxone (84.20%). The p-value was found to be statistically significant for these resistant antibiotics (Table 6).Norfloxacin was tested only in urine isolates and (67.80 %) of isolates were resistant to this antibiotic. The percentage of drug resistant *Acinetobacter* isolates which were multi drug resistant (MDR) was 249 (81.63%). All MDR isolates were resistant to at least one agent in three or more antimicrobial categories; penicillins, cephalosporins, aminoglycosides, fluoroquinolones and carbapenems.[15,16] Out of 81.63% extensively drug resistant isolates, 35.3%were isolated from the sputum sample followed by ET tips (23.29%) and pus/swab (19.2%).

Table 6. Antibiotic resistance pattern of *Acinetobacter* isolates in various wards and ICUs.

Antibiotic	Sensitive No.	Sensitive %	Resistance No.	Resistance %
Ceftriaxone	48	15.77%	257	84.20%
Gentamicin	80	26.20%	225	73.70%
Meropenem	178	58.30%	127	41.60%
Amikacin	122	40%	183	60%
Cotrimoxazole	77	25.20%	228	74.70%
Ciprofloxacin	55	18%	250	81.90%
Ceftazidime	24	7.80%	281	92.10%
Colistin	305	100%		
Polymixin B	305	100%		
Piperacillin-Tazobactum	146	47.80%	159	52.10%
Ampicillin	18	5.90%	287	94.09%
Norfloxacin	98	32.10%	207	67.80%
Cefepime	136	44.50%	169	55.40%

DISCUSSION

Acinetobacter is a nosocomial pathogen. Its ability to infect healthy hosts and its propensity to develop antimicrobial drug resistance is a cause for concern among infectious disease specialty. *Acinetobacter* isolated from normal skin and mucous membranes are reported to cause serious and sometimes fatal infections.[17]

Acinetobacter spp has emerged as a cause of ICUs infection. Multiresistant*Acinetobacter* spp including the leading species, *A. baumanii*, are developing as real infectious threat mainly in the intensive care units (ICUs).[18] Their ubiquitous nature in the ICU environment and inadequate infection control practice has continuously raised the incidence of *Acinetobacter* infection over the past two decades.[19] All the isolates of *Acinetobacter* spps were isolated from different ICUs and wards, which indicate all isolates were nosocomial. The same observations have been reported by Raina et aland Lahiri et alwherein nosocomial isolates of *Acinetobacter* spps from hospital patients were 98.1% and 82.9%.[11,20] A number of risk factors enhances the spread and persistence of *Acinetobacter* spp like mechanical ventilation, admission to ICUs, underlying chronic debilitating conditions and prolonged hospital stay have been found to be significant risk factors for the spread of this organism in the hospital environment.

Majority of the isolates were recovered from the General ICU patients (19.01%) followed by patients admitted in general medicine wards (14.70%) while lower percentage of isolation were observed from other wards in the current study. Various other studies have reported the rate of isolation varying from 4.25% to (20.1 %).[21,22] This variation can be attributed to the varying prevalence rates of different *Acinetobacter* spp in the hospital environment and the community in different geographical areas.

Maximum number of *Acinetobacter* isolates were from sputum (31.88%) followed by endotracheal tip/aspirate (16.66%), pus (15.21%), tips (12.31%) and urine (10.14%) in the present study. This is in variance with other studies as by Lahiri et al and Raina et al in which the isolates were maximum from tips (43.4%), Oberoi et al found maximum isolates from pus samples (86.2%).[23]Apoorva et al found maximum number of *Acinetobacter* isolates from respiratory samples (35.78%) followed by pus (32.84%).[24]Singla et al also isolated 25.6% of the *Acinetobacter* isolates from respiratory tract. This indicates that *Acinetobacter* infections were most frequently involved in the respiratory tract of intubated patients.[25]

In this study, the most common *Acinetobacter* species isolated from the clinical samples of our institute was *Acinetobacter baumanii*(76.06%), followed by *Acinetobacter lwofii*(11.40%). Almost similar results were observed in studies conducted by Apoorva et al, who found 74.50% and 24.50% of *Acinetobacter baumanii*and *Acinetobacter lwofii*respectively. Predominance of *A. baumanii*isolated from various samples were observed by Raina et al. and also by Lone et al.[11,26]There are three major factors possibly contributing to the persistence of *A. baumanii*in the hospital environment, i.e., resistance to major antimicrobial drugs, resistance to desiccation, and resistance to disinfectants. Resistance to antibiotics may provide certain *A. baumanii*strains with a selective advantage in an environment, such as the modern ICU, when microorganisms are confronted with extensive exposure to antimicrobials.[27] Therefore in ICUs, where the pathogen is endemic, empirical antibiotic therapy should include drugs that are effective according to microbiological ecology.[28]

High levels of resistance were seen for ampicillin (94.09%), ceftazidime (92.01%) and ceftriaxone(84.20%). Significant levels of resistance were also recorded for ciprofloxacin (81.90%), gentamicin (73.70%), cotrimoxazole (74.70%). Norfloxacin was tested only in urinary isolates and (67.80%) isolates were resistant to this antibiotic.Taneja et al in their study reported that the resistance of *Acinetobacter* to gentamicin and ciprofloxacin was 79.5% and 72.8% respectively and is in accordance with our study.[29]Resistance towards meropenem was recorded to be 41.60%. In a study by Kaur et al, resistance towards imipenem and meropenem were recorded to be 42.6% and 55.4% respectively and is also in accordance with our study.[30] However, Shareek et al and Raina et al reported that 75% and 74.1% of the strains were resistant to carbapenems, which is higher than our findings.[31,11]

In our study, 100% sensitivity was recorded for colistin and polymixin B. Raina et al also recorded 100% sensitivity for colistin.[11]Among a total of 305*Acinetobacter* isolates, 249 (81.6%) isolates were MDR. All MDR isolates belonged to *A. baumanii*except for oneisolate which belonged to *A. lwofii*. Similar studies by Uwingabiye et al and Kaur et al reported MDR *Acinetobacter* of 77.4% and 76.6% respectively and the figure is near about similar with our studies.[32,30]

This wide variation can be due to various factors like patient's co-morbid conditions, compliance of infection control programs, type of strains, their survival in the environment and further colonization of the

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patients. According to our study, highest MDR *Acinetobacter* were isolated from sputum (35.30%), followed by endotracheal tip (23.29%) and pus (19.20%). These findings were comparable with the studies done by Sivaranjanet al.[33] Whereas, in the study done by Uwingabiye et al,highest percentage of MDR *Acinetobacter* was demonstrated in vascular catheter.[32]

CONCLUSION

Among non-fermenters, *Acinetobacter* species is highly prevalent in hospital settings.As indicated by various studies, the rate of isolation of *Acinetobacter* spp. as nosocomial pathogen, especially in critically ill patients admitted in ICUs is alarming.It is a great challenge for the physicians to treat MDR *Acinetobacter* spp. In our study, *Acinetobacter* spp.were resistant to most commonly used antibiotics and emergence of carbapenem resistance is a point of great concern. In our study, colistin and polymyxin B was the most sensitive antibiotic (100%). However, colistin resistant is gradually emerging.Rationale use of antibiotics is important and necessary to prevent microbial resistance catastrophe. A strict control of the hospital environment, hand hygiene and optimizing/judicious use of antibiotics is the need of hour.

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