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Research Article

Antibiogram of *Acinetobacter* spp. Isolates in a Tertiary Care Hospital: A Lead towards Antibiotic Stewardship

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Abstract

Acinetobacter spp. is an important nosocomial pathogen especially in intensive care settings and is resistant to commonly available antimicrobial agents. Active surveillance is therefore necessary in order to determine appropriate antibiotic for the treatment. The purpose of this study was to determine the antibiogram of *Acinetobacter* spp. isolated in patients attending the tertiary care hospital. It is a prospective study conducted in the Department of Microbiology, Sharda Hospital.

Acinetobacter from various clinical samples were included in this study during six months period from May 2019 to October 2019. The isolates were identified using conventional and automated methods (Vitek2 COMPACT, bioMérieux) and the susceptibility was done using the Kirby-Bauer disk diffusion assay. During the study period, a total of 40 *Acinetobacter* spp. were isolated from various clinical specimens, out of which 47.5% isolation was from ICUs. Highest isolation was observed from pus samples (22.5%) followed by blood (17.5%) and endotracheal aspirate (17.5%). 77.5% isolates were MDR (Multidrug Resistant), however they remained susceptible to colistin and tetracycline. It is necessary to regularly monitor the resistance phenotypes of *Acinetobacter*. Enhanced surveillance of MDR *Acinetobacter* is critical for guiding the rational use of antibiotics and reducing the incidence of hospital acquired infections.

Keywords: *Acinetobacter* spp; Multi-Drug Resistance (MDR); ICU; Antibiotic Susceptibility

Abbreviation

ICU: Intensive Care Unit; MDR: Multi-Drug Resistant; OPD: Out Patient Department; ICCU: Intensive Coronary Care Unit; HIC: Hospital Infection Control; BAL: Bronchioalveolar Lavage; CVP: Central Venous Pressure; NICU: Neuro Intensive Care Unit; RICU: Respiratory Intensive Care Unit; MICU: Medicine Intensive Care Unit

Introduction

Acinetobacter spp. are Gram negative, strictly aerobic, non-fastidious, non-fermentative coccobacilli, occurring in diploid formation, or in chains of variable lengths. They are non-motile, oxidase negative and catalase positive [1]. *Acinetobacter spp.* are recognised as an important nosocomial pathogen, particularly in patients admitted to Intensive Care settings (ICUs) and in immunocompromised patients [2]. These remain as one of the most challenging pathogens owing to their uniqueness and multiplicity of their resistance mechanisms [3]. Some of the risk factors for acquisition of infection by *Acinetobacter* spp. include prolonged hospitalization, immunocompromised status of patients, mechanical ventilation, cardiovascular or respiratory failure, previous infection and antimicrobial therapy, and presence of indwelling catheters such as central venous or urinary catheters [1].

Acinetobacter spp. as pathogens are developing resistance at a very rapid pace to almost all antimicrobial agents that are available which includes, aminoglycosides, quinolones and broad-spectrum β lactams [4]. Almost, 60 - 70% of these bacteria have developed resistance to many antibiotics, including carbapenems. And they are associated with higher patient morbidity, attributable mortality, and few or no antimicrobials remain effective for their treatment [5].

Materials and Methods

It is a prospective study carried out in the Sharda Hospital and Department of Microbiology, School of Medical Sciences and Research, Sharda University, Greater Noida. It was conducted for a period of 6 months from 1st May 2019 - 31st October 2019.

Forty (40) isolates of *Acinetobacter spp.* were recovered from various clinical specimens, namely pus (09 samples), blood (07 samples), Endotracheal Aspirate (07 samples), urine (06 samples),

sputum (05 samples), Bronchioalveolar Lavage (BAL) (02 samples), Gluteal abscess (01 sample), throat swab (01 samples), CVP Tip (02 samples).

The isolates were speciated and their antimicrobial resistance pattern was studied. The samples received in the laboratory were inoculated on 5% Sheep Blood Agar and MacConkey Agar and incubated overnight aerobically at both 37°C (to isolate *Acinetobacter spp.*). Thereafter species identification and *in-vitro* antibiotic susceptibility tests were performed. In case of urine samples, the isolates were subjected to biochemical tests and antimicrobial susceptibility only if the colony count was significant (> 10^5 CFU/ml). *Acinetobacter spp.* were identified by colony characteristics (Non-Lactose-fermenting, glistening, small mucoid colonies), Gram staining pattern and standard biochemical reactions (Catalase, Oxidase, Indole production, Citrate utilization, Motility, Urease activity, Reaction in Triple Sugar Iron medium).

After identification by phenotypic methods, antibiotic susceptibility was performed for each isolate by the Kirby-Bauer disc diffusion method on Mueller-Hinton Agar using 0.5 MacFarland Turbidity standard. The following antibiotic discs were used: Ampicillin (100 mcg), Piperacillin-tazobactam (100/10 mcg), ceftazidime (30 mcg), cefepime (30 mcg), ceftriaxone (30 mcg), cefotaxime (30 mcg), doripenem (10 mcg), imipenem (10 mcg), meropenem (10 mcg), gentamicin (10 mcg), tobramycin (10 mcg), amikacin (30 mcg), ciprofloxacin (5 mcg), levofloxacin (5 mcg), tetracycline (30 mcg), trimethoprim sulfamethoxole (25 mcg), colistin (0.016 - 256 µg/mL).

Result

A total of forty non-duplicated *Acinetobacter* strains (belonging to various species) were isolated from patients admitted and attending the OPD at Sharda Hospital, during the study period (1st May 2019 to 31st October 2019).

Demographic profile

Most of the patients from whom *Acinetobacter spp.* were isolated were in the age group of 0 - 9 years (22.5%), followed by age group 20 - 29 years (17.5%); 50 - 59 years (15%); 40 - 49 years (12.5%); 60 - 69 years (12.5%); 30 - 39 years (10%); 70 - 79 years (7.5%); ≥ 90 years (2.5%) respectively (Table 1).

The patient's samples included in the present study had 20 Males (50%) and 20 Females (50%) (Figure 1).

Maximum number of *Acinetobacter* strains were recovered from pus 09 (22.5%), followed by blood 07 (17.5%); Endotracheal aspirate 07 (17.5%); Urine 06 (15%); sputum 05 (12.5%); BAL, swab (Gluteal abscess and throat swab) and CVP tip 02 (5%) each (Table 2).

Age in Years	No. of isolates (N)	Percentage (%)
0 - 9	09	22.5
10 - 19	0	0
20 - 29	07	17.5
30 - 39	04	10
40 - 49	05	12.5
50 - 59	06	15
60 - 69	05	12.5
70 - 79	03	7.5
80 - 89	0	0
≥ 90	01	2.5

Table 1: Age wise distribution of Acinetobacter.

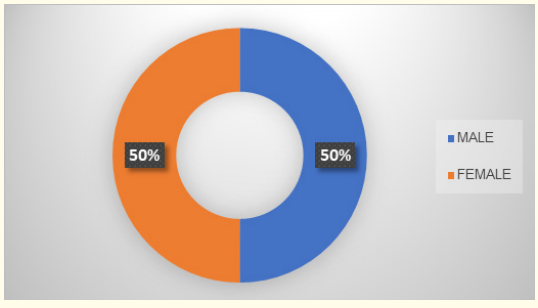

Figure 1: Gender wise distribution of Acinetobacter isolates.

Sample	No. of isolates (N)	Percentage (%)
Pus (Surgical wound)	09 (06)	22.5 (15)
Blood	07	17.5
Endotracheal Aspirate	07	17.5
Urine	06	15
Sputum	05	12.5
BAL	02	5
Swab (Gluteal abscess)	01	2.5
Throat swab	01	2.5
CVP Tip	02	5

Table 2: Sample wise distribution of Acinetobacter isolates.

Most of the isolates were obtained from patients admitted in the Intensive Coronary Care Unit (ICCU) 14 (35%). Obstetrics and gynaecology 07 (17.5%) were next in frequency followed by General Medicine 06 (15%); General Surgery 03 (7.5%) and paediatrics 03 (7.5%). The least number of isolates were obtained from Neuro Intensive Care Unit 02 (5%), Respiratory Intensive Care Unit 02 (5%), Medicine Intensive care unit 01 (2.5%), Burn unit 01 (2.5%) and Orthopaedics 01 (2.5%) (Table 3).

Ward	No. of isolates (N)	Percentage (%)
ICCU	14	35
Obs/Gyn	07	17.5
General Medicine	06	15
General Surgery	03	7.5
Pediatrics	03	7.5
NICU	02	5
RICU	02	5
MICU	01	2.5
Burn Unit	01	2.5
Orthopedics	01	2.5

Table 3: Ward wise distribution of Acinetobacter isolates.

Out of 40 isolates identified by Vitek 2, 82.5% were *Acinetobacter baumannii* complex, followed by *Acinetobacter lwoffii* and *Acinetobacter haemolyticus* 5% each and *Acinetobacter radioresistens, Acinetobacter ursingii* and *Acinetobacter junii* 2.5% respectively (Figure 2).

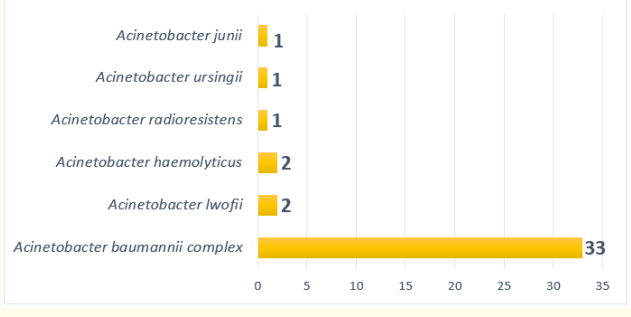

Figure 2: Species wise distribution of Acinetobacter isolates.

The Antimicrobial Susceptibility pattern of the various isolates are depicted in the table 4 and figure 3. 24/40 (60%) and 22/40 (55%) of isolates were resistant to carbapenems which is Meropenem and Imipenem respectively.

The isolates exhibited a high degree of resistance to piperacillin (92.5%), β-lactam combination antibiotics such as Piperacillin-Tazobactam 29/40 (72.5%) and cephalosporins. Out of 40 isolates, 32 (80%) were resistant to cefotaxime and ceftazidime and cefepime and 31 (77.5%) resistant to ceftriaxone.

There was a variable sensitivity to fluroquinolones. Thirty isolates (75%), were resistant to ciprofloxacin, twenty-four isolates (60%) to levofloxacin. In case of aminoglycosides, with amikacin resistance was seen in thirty-two (80%) isolates, followed by tobramycin 26/40 (65%) and in gentamicin it was seen in 22 (55%) isolates.

Antibiotic	Sensitive N (%)	Resistant N (%)
Piperacillin	03 (7.5)	37 (92.5)
Piperacillin - tazobactam	11 (27.5)	29 (72.5)
Cefotaxime	04 (10)	32 (80)
Ceftazidime	05 (12.5)	32 (80)
Cefepime	07 (17.5)	32 (80)
Ceftriaxone	05 (12.5)	31 (77.5)
Imipenem	11 (27.5)	22 (55)
Meropenem	15 (37.5)	24 (60)
Ciprofloxacin	08 (20)	30 (75)
Levofloxacin	13 (32.5)	24 (60)
Gentamicin	15 (35.5)	22 (55)
Tobramycin	14 (35)	26 (65)
Amikacin	08 (20)	32 (80)
Trimethoprim-sulfamethoxazole	09 (22.5)	27 (67.5)
Colistin	40 (100)	0 (0)
Tetracycline	06 (100)	0 (0)

Table 4: Antimicrobial susceptibility profile of Acinetobacter isolates.

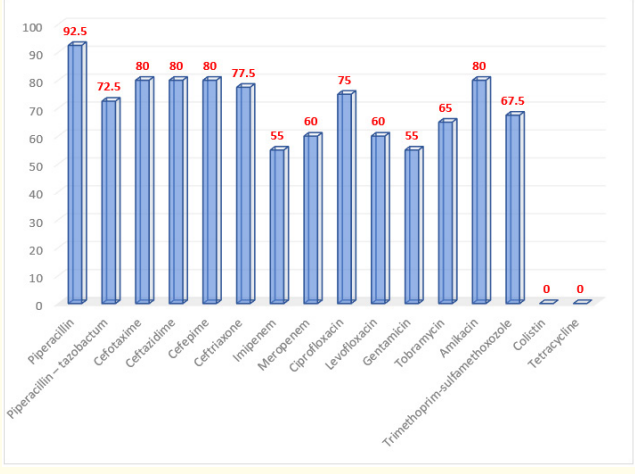

Figure 3: Antibiogram of Acinetobacter isolates (Resistant %).

Tetracycline were tested against 6 isolates recovered from urine. None of the isolates (0/6) were resistant against tetracycline. There were 100% sensitive.

All the isolates 40/40 (100%) were sensitive to colistin.

Out of 40 *Acinetobacter* isolates 31 (77.5%) were MDR *Acinetobacter,* 02 (5%) were resistant to 2 classes of antibiotics (penicillin and 3rd generation cephalosporins) and 04 (10%) were resistant to only 1 class of antibiotics (penicillin) (Table 5).

MDR *Acinetobacter* N (%)	Resistant to piperacillin only N (%)	Resistant to piperacillin and 3rd generation cephalosporins N (%)	Sensitive *Acinetobacter* N (%)
31 (77.5)	04 (10)	02 (5)	03 (7.5)
Total 40			

Table 5: Distribution of MDR Acinetobacter.

Discussion

Acinetobacter have emerged as one of the most significant pathogens causing nosocomial infections in patients admitted to Intensive Care settings [2]. Infections caused by multidrug resistant *Acinetobacter* are difficult to treat and are a major cause of increased morbidity and mortality in hospitalized patients [5]. The frequency of antibiotic resistance in *Acinetobacter* is worrisome since there are hardly any antibiotics in development process which have suitable activity against these multi-resistant strains of organism [6]. Until recently, carbapenem class of antibiotics were the drug of choice against this pathogen. However, with the development of resistance against carbapenems by *Acinetobacter spp.* the entire scenario has changed, making the pathogen difficult to treat [7].

The spread of such strains of *Acinetobacter* is a threat to the therapeutic options available and also a serious concern in the hospital infection control management. Such organisms are often difficult to treat and pose a significant risk due to their easy spread [7].

The present study was conducted to know the prevalence of *Acinetobacter spp.* infection in our hospital and to know their antibiotic susceptibility profiles and resistance patterns.

From amongst a total of 1,556 bacterial isolates cultured from various (6,687) clinical specimens over a period of 6 months 40 (2.5%) isolates were identified as *Acinetobacter spp.,* similar prevalence of 3% and 3.36% of *Acinetobacter* isolates was reported by Dash., *et al.* in Odisha and Gupta., *et al.* in Pune [8,9]. Higher prevalence rate of 14% and 9.6% was reported by Mostofi., *et al.* in Tehran, Iran and Joshi., *et al.* in Pune [10,11].

Most of the patients in this study were in the age group 0 - 9 years (22.5%) and > 60 years (22.5%) which was in concordance with the study conducted by Mera RM., *et al.* in United States, where the rise in isolation of *Acinetobacter* was seen among these age groups [12].

Maximum number of *Acinetobacter* strains in this study were isolated from pus (09/22.5%), followed by blood (07/17.5%); Endotracheal aspirate (07/17.5%); urine (06/15%); and sputum (05/12.5%). A similar observation has been reported in the study done by Shivaranjani V., *et al.* in South India (2013) which showed 38.5% isolates from pus, followed by 20.4% isolates from endotracheal aspirate [13].

According to the literature, amongst *Acinetobacter spp.,* commonest species isolated in human clinical specimens is *Acinetobacter baumannii* [14]. In this study also 33 (82.5%) isolates were *A. baumannii* complex, followed by *Acinetobacter lwoffii* and *Acinetobacter haemolyticus* 02 (5%) each and *Acinetobacter radioresistens, Acinetobacter ursingii, Acinetobacter junii* 01 (2.5%) each. This again is in concordance with the study done by Gupta N., *et al.* in 2015 [9] where 72% were *A. baumannii* complex, followed by *A. lwoffii, A. haemolyticus* and 1% *A. radioresistens, A. junii*.

In the present study, *Acinetobacter spp.* were found to be resistant to most commonly used antimicrobial agents as a routine and prevalence of 77.5% MDR was observed. Similar reports of MDR *Acinetobacter* isolates have been reported with 88.02% resistance to commonly applied antibiotics [15]. *Acinetobacter* is ubiquitous in the hospital setting. It has the ability to survive for longer periods and also demonstrates a number of antimicrobial resistance genes which has made *Acinetobacter* a successful hospital pathogen [8].

Acinetobacter were extremely resistant to piperacillin (92.5%) which correlates with the study done by Shivaranjani V., *et al.* in South India (2013) [13]. 32 (80%) of isolates were also resistant to cephalosporins correlating with studies done by Guckan R., *et al.* in 2015 and Shivaranjani V., *et al.* in 2013 [13,16]. Resistance towards imipenem and meropenem was seen to be 55% and 60% respectively. Data of the antibiotic susceptibility patterns of *Acinetobacter* from different geographical areas revealed that the resistance of *Acinetobacter* spp. to imipenem rose from no resistance to 40% (2000 - 2004) [17]. The prevalence of imipenem resistance in *A. baumannii* isolated from a burns unit of the USA was 8% earlier (2007) [18]. Resistances to major antimicrobial drugs as well as disinfectants are the major factors that make it a successful and persistent hospital pathogen [19]. No resistance to colistin was seen in this study which is similar to the studies published by Dash., *et al*, Shareek., *et al.* and Nazir A [8,19,20].

Initial concern about multidrug resistant (MDR) and carbapenem resistant *Acinetobacter baumannii* (CRAB) associated infections began when the first hospital wide outbreak occurred in New York

City in 1991 [21]. Since then, reports of CRAB from other parts of the world including India [20,22] are coming in. Out of the total isolates 26 (65%) were multidrug resistant (MDR) in this study. Other studies conducted by Dash., *et al.* (2013) and Rekha., *et al.* (2011) reported MDR isolates to be 55% and 74% respectively [8,23].

Most of our *Acinetobacter spp.* were isolated from patients admitted in the high-risk settings like Intensive Coronary Care Unit (ICCU) 35%. The results are concordant with the results seen in the study conducted by Mera RM., *et al.* (2010) and Gupta., *et al.* (2015), where an increased number of *Acinetobacter* isolates were recovered from Intensive Care settings [9,12]. The emergence of antibiotic resistant strains in ICU is because of higher use of antimicrobial agents per patient and per surface area [9].

Conclusion

Resistance rate of *Acinetobacter* to routinely used antibiotics is increasing rapidly. 57.5% and 67.5% isolates in this study were resistant to carbapenems and fluroquinolones respectively. MDR *Acinetobacter* isolates remained susceptible to colistin and tetracycline, which can be used as the treatment option for management of most of the cases of infections caused by this organism, however with caution as colistin is and should be a last resort. Patients infected with MDR *Acinetobacter* is widely spread in our hospital and occurred mostly in children below 10 years of age and in patients admitted to intensive care settings and the reason/s behind this alarming situation need to be ascertained and taken care of at regular intervals.

It is necessary to regularly monitor the resistance phenotypes of these isolates. Enhanced surveillance of MDR *Acinetobacter* is critical for guiding the rational use of antibiotics and reducing the incidence of Hospital Infection Control (HIC).

Conflict of Interest

There are no conflicts of interest.

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