ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Detection Of Carbapenamase Production Among MDR Acinetobacter Species Isolated From Clinical Samples.

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ABSTRACT

Increasing antimicrobial resistance especially carbapenem resistance worldwide is a concern as it limits the range of alternative agents. Acinetobacter species, a gram negative bacteria commonly found in hospital environment and hospitalized patients, is causing various nosocomial infections with increasing resistance by producing carbapenemase. This descriptive study was carried out at Department of Microbiology of a tertiary care hospital from Jan 2018 to June 2019. In this period, total 9475 samples were received in laboratory from wards, OPDs and ICUs and processed based on standard conventional methods and out of these samples, 300 Acinetobacter species were isolated by standard biochemical tests. The antibiotic sensitivity testing is done by Kirby Bauer Disc Diffusion method using Muller Hinton agar. Carbapenemase production is tested by using Rapidec Carba NP test. It is found that this infection is common in extremes of age groups like neonates of preterm deliveries and patients of age over 45 years associated with other conditions. Out if these isolates, 73% of Imipenem resistant *Acinetobacter* isolates were carbapenemase producers and 281 (93.66%) *Acinetobacter* isolates were from wards. This study will help to understand the emerging threat of multidrug resistance and help physicians to select most accurate and appropriate antibiotic treatment.

Keywords: Carbapenamase, MDR Acinetobacter.

https://doi.org/10.33887/rjpbcs/2023.14.1.20

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ISSN: 0975-8585

INTRODUCTION

According to the World Health Organization (WHO), worldwide over 1.4 million people at any given time suffer from infectious complication acquired from hospitals [1, 2]. An important consequence of Hospital Acquired Infections (HAIs) is the increasing antimicrobial resistance due to tremendous antimicrobial pressure, especially in ICUs [3]. A vicious cycle of 'more infections, more antimicrobial use, followed by development of multidrug resistant bacterial infections and use of still higher generation antimicrobial' is seen in many hospitals. There has been increasing incidence of Acinetobacter species causing serious nosocomial infections which are being reported worldwide. Acinetobacter species is the 2nd most frequent non fermenter encountered in clinical laboratories and is one of the common causative pathogens for late onset hospital acquired pneumonia [4]. Since, last 30 years, strains of A. baumannii have acquired resistance to newly developed antimicrobials. Carbapenems remains the treatment of choice if isolates are sensitive to this class. Unfortunately, carbapenem-resistant Acinetobacter isolates are increasingly reported throughout the world as Efflux pumps may affect Meropenem, whereas specific β-lactamases hydrolyse Imipenem [5, 6]. Genes coding for these enzymes can be transferred from cell to cell via transposons. For penicillins, cephalosporins, and carbapenems a common enzyme is β-lactamase, which hydrolyzes and confers resistance to these drugs. Mutated genes like VIM, IMP, and OXA can also be transferred from other bacteria which ultimately alter bacterial targets of antimicrobials leading to decrease in affinity for the bacteria and increasing the minimum inhibitory concentration (MIC) for the drug [6].

Thus the main objective of this study is to isolate *Acinetobacter species* from various clinical samples by phenotypic method and study their resistance pattern to 1st line and 2nd line (including carbapenem) antibiotics by Kirby-Bauer disk diffusion method and know the prevalence of Carbapenemase production in *Acinetobacter species* resistant to carbapenem by Rapidec Carba NP Test.

MATERIALS AND METHODS

The study was carried out at Department of Microbiology of a tertiary care hospital from Jan 2018 to June 2019.

Study design

Descriptive study

Material

Acinetobacter species were isolated from various clinical samples from hospitals. The various clinical samples were pus, blood, urine, body fluid, sputum, throat swab, and swab from surgical, non-surgical wound tissue. These samples were collected from patients of all age groups and both sexes admitted in outpatient and various inpatient departments of tertiary care hospital.

Sample size

The study includes 300 isolates of *Acinetobacter species* from various clinical samples during the period of Jan 2018 to June 2019.

Methods

Presumptive identification of Acinetobacter was done by phenotypic method and was put through battery of tests:

1) Hanging drop for motility
2) Study of cultural characteristics on

- Blood Agar
- MacConkey Agar

ISSN: 0975-8585

3) For production of enzymes

- Catalase
- Oxidase
- Urease

4) For substrate utilization

- Citrate test

5) For metabolism of proteins and amino acids

- Indole production test

6) Utilization of carbohydrates

- Exhibits rapid utilization of 1% Glucose and 10% Lactose with production of acid

8) Antibiotic sensitivity testing by Kirby Bauer disc diffusion test for

- **1st line drugs:** Amikacin (30µg), Cefotaxime (30µg), Gentamicin (10µg), Co-Trimoxazole (1.25/23.75µg), Doxycycline (30µg), Ciprofloxacin (5µg)

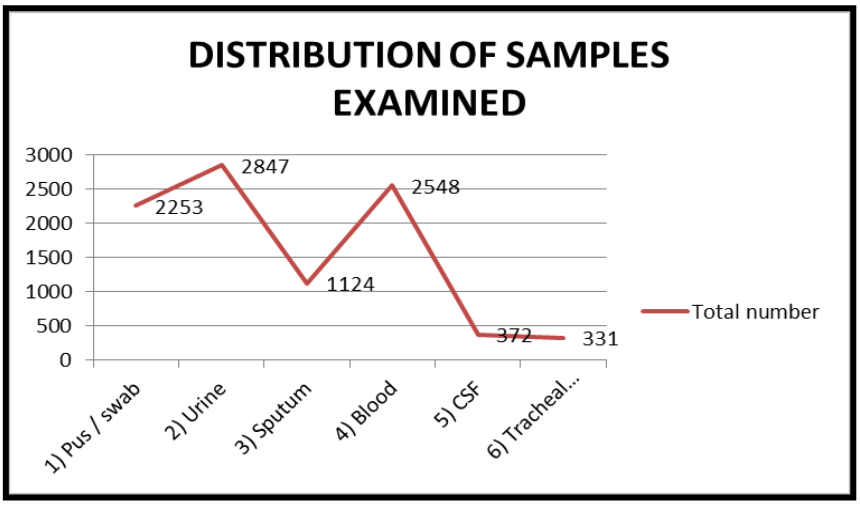
- **2nd line drugs:** Imipenem (10µg), Piperacilin- tazobactum (100/10µg).

9) Carbapenemase production in Acinetobacter species- resistant to carbapenem (Imipenem) by Rapidec Carba NP Test.

RESULTS

- During 18 months of study period from Jan 2018 to June 2019, a total of 9475 specimens were examined from patient of different age group; admitted in various medical, surgical wards, ICU and were included in this study.
- Distribution of Samples examined and number of *Acinetobacter species* isolated from various samples is illustrated in Figure 1.

Table/Figure-1



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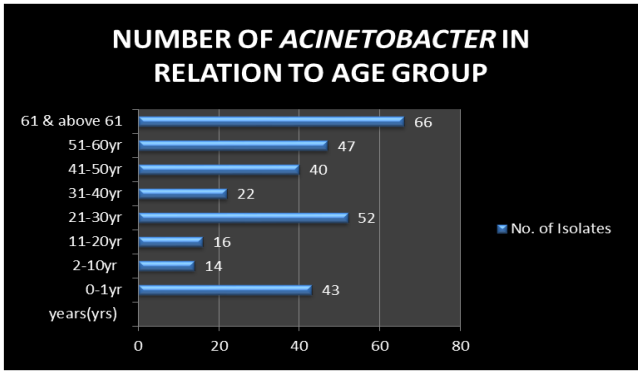
Table/Figure-2

Number Of Acinetobacter Isolated From Various Samples During Study Period

Specimen	Acinetobacter	Percentage (%)
1) Pus / swab	104	34.66%
2) Urine	61	20.33%
3) Sputum	56	18.66%
4) Blood	10	3.33%
5) CSF	2	0.66%
6) Tracheal aspirate/endotracheal tube tip	67	22.33%
TOTAL	300	100%

- A total of 300 isolates of Acinetobacter species were isolated during the study period.
- Highest isolation of Acinetobacter was from pus / swab sample followed by tracheal aspirate or Endo-tracheal tip and urine.

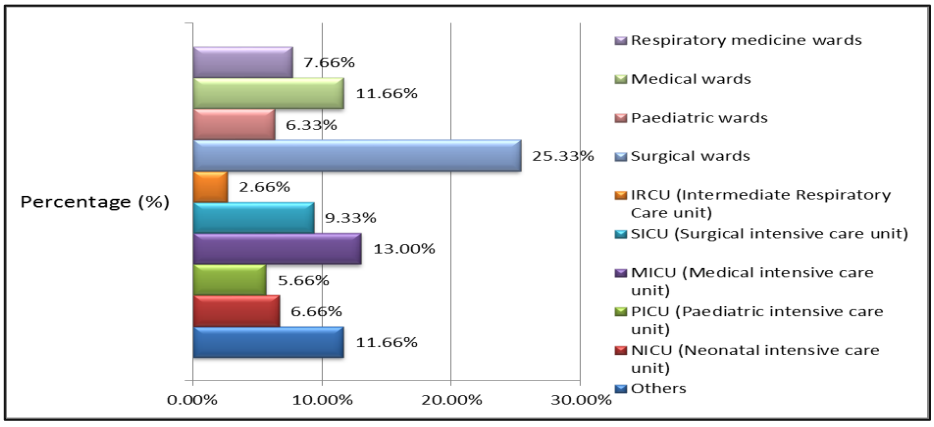
Table/Figure-3



- Male: Female ratio 1.4: 1.
- Acinetobacter infection was more common in patients over 45 years of age.
- Most of these patients had respiratory problems like chronic obstructive pulmonary disease (COPD), bronchial asthma, respiratory failure, and other predisposing factors like diabetes mellitus, cellulitis, surgical site infection, chronic renal failure, liver cirrhosis, and immunosuppression.
- Infection in neonates was common in preterm babies.

Table/Figure-4

Ward Wise Distribution Of Acinetobacter Isolates



- Number of Acinetobacter isolates was more from surgical wards followed by ICU.
- Most of the isolates from paediatric ward were from preterm babies.
- Others include samples from Orthopedic, Obstetric, Ent, Skin etc wards.

Table / Figure-5

Sensitivity Pattern Of Acinetobacter Isolates To Different Antibiotics

Antibiotics	Sensitive (S)	Intermediate Sensitive (IS)	Resistance (R)
Amikacin(Ak)	89	4	207
Cefotaxime(Ctx)	18	1	281
Gentamicin(G)	85	1	214
Co-trimoxazole(Sxt)	78	0	222
Doxycycline(Do)	100	0	200
Ciprofloxacin(Cip)	62	1	237
Imipenem(Imp)	77	4	2I9
Pipercillin-tozabactum(Ptz)	58	3	239

Table / Figure-6

Carbapenemase production in resistant *Acinetobacter* isolates

Imipenem			Carbapenemase producers (Rapidec Carba NP)
Sensitive	Intermediate	Resistant	
77(25.66%)	4(1.33%)	219(73%)	219

- 73% of Imipenem resistant *Acinetobacter* isolates were carbapenemase producers.

DISCUSSION

Acinetobacter species are emerging as agents of opportunistic nosocomial infection with evolving drug resistance very rapidly and has become a real problem in hospital set-up, particularly in the critical care units [7]. *Acinetobacter species* are now one of the most common organisms isolated from hospital environments and hospitalized patients [8]. Especially carbapenem-resistant *Acinetobacter species* are increasingly recognized as major hospital acquired pathogens, in patients with critical illnesses or in intensive care. These organisms have the capability to accumulate diverse mechanisms of resistance thus limits the available antimicrobial agents. Making the treatment difficult for infection, and is associated with increased risk of mortality [9].

Present study was conducted in a tertiary care hospital and total of 9475 samples were collected and studied [Figure1]. Out of these specimens 300 isolates were *Acinetobacter species*, which were studied for their antimicrobial susceptibility [Table 1]. *Acinetobacter species* isolated accounted for 3.16% of total culture. Similar study was conducted by Mindolli PB et al in 2010 Acinetobacter isolates accounted for 4.25% of total number of organisms isolated during study period [10]. Study done during 1971-81 in United States, *Acinetobacter species* accounted for 1.4% of all infections. Higher percentage in this study i.e. 3.16% *Acinetobacter species* may be due to better identification scheme. This also shows the role of *Acinetobacter* as nosocomial pathogen since in most cases patients were symptomatic with fever, leucocytosis, urinary tract infection (UTI). In another study conducted by Joshi et al in Mumbai in 2006, *Acinetobacter* isolation rate was 9.6% [11]. Huang CH et al observed that high prevalence can probably be related to non-compliance with the recommendations for hospital infection control policy [12]. Fishbain J et al reported that higher prevalence can be due to lack in hands hygiene and misuse of antibiotics [13]. Since hand transmission is a major factor in the spread of this pathogen [12], hand hygiene and disinfection of equipment/environment are the two most important factors to control and prevent the outbreak of an epidemic Acinetobacter

In this study 281 (93.66%) *Acinetobacter* isolates were from inpatient departments (IPD) and 19 (6.33%) were from outpatient departments (OPD). In the study conducted by Rebic V et al prevalence was more among the inpatients (98%), which clearly reflects the nosocomial origin of this pathogen [14]. Prevalence of *Acinetobacter* in the review done by Manchanda V et al [15] was 85% (ICUs), 60% (medical wards), and 59% (surgical wards) and they have summarized the sources for colonization or infection with multidrug-resistant *Acinetobacter species* in hospitalized patients which are

- Hands of the hospital staff
- Respiratory therapy equipment
- Food (including hospital food)
- Tap water
- Infusion pumps
- Mattresses, pillows, bed curtains and blankets in vicinity of infected patients
- Soap dispensers
- Fomites like bed rails, stainless steel trolleys, door handles, telephone handles, tabletops
- Hospital sink traps
- Hospital floor

All these factors could be the risk factor in this study also as majority of the isolates were from inpatient departments.

Acinetobacter infections were more common in age group 61 & above (22%), followed by age group 21-30yr (17.33%) and age group 51-60yr (15.66%) in this study [Figure 2]. In the study conducted by Rebic V et al, the proportion of isolates was more in the age group over 60(44.60%) [14]. Uwingabiye J et al observed 31.3% were above 65 years having *Acinetobacter infections* [16]. These patients had underlying predisposing factors like COPD, diabetes mellitus, renal failure, liver cirrhosis and were on prior antibiotic therapy. Turkoglu M et al in their study observed that old age of patients is as an independent risk factor of the acquiring *Acinetobacter infections* [17]. Lower immunity with predisposing chronic condition leading to longer duration of hospital stay justifies the infection rate to be common in old age. Neonates suffering from septicaemia showed 6.66% of septicaemia was due to Acinetobacter spp in the present study. Vinodkumar C S et al in Karnataka observed that 8.9% babies showed septicaemia due to *Acinetobacter species* [18]. Most of the isolates, from paediatric age group were from preterm babies and septicaemic patients. The use of antibiotics probably alters the normal flora and result in selection of microorganisms like *Acinetobacter species*.

Isolation rate was highest from pus sample 34.66%, majority of them were from cellulitis and wound infections [Table/Figure-7]. Isolation rate from blood in this study was 3.33%; most of them were from preterm and septicaemic patients. While study done by Mindolli PB et al [10] from JJM Medical College Davangere, Karnataka isolation of Acinetobacter from blood was 2.9%.

Studies of *Acinetobacter* from various countries as shown in Table given below have shown predominance of isolation from urine (21-27%), tracheobronchial secretions (24.8-48.8%). In the present study *Acinetobacter* was isolated from urine (20.33%), tracheobronchial secretions (22.33%), and sputum (18.66%).

Acinetobacter species isolation from various countries [19].

Table / Figure-6

Samples	USA (%)	France (%)	Belgium (%)	Present Study (%)
Urine	27	21	27	20.33
Pus	21.5	27.5	22.3	34.66
Blood	9.3	7.5	7.6	3.33
Tracheal aspirate/ endotracheal tube tip	28.9	27	24.8	22.33

Acinetobacter baumannii in Spain (1999-2005) in a study conducted by Asensio A et al was isolated maximum from respiratory tract (42.2%) followed by surgical wound (15.1%), urinary tract (12.9%), skin (11.7%) [20]. Another study by Joshi et al in 2006 from King Edward Memorial Hospital, Mumbai *Acinetobacter* isolated from urine was 30.6% and 27.5% were isolated from samples in case of wound infection [11].

Most of the isolates were from surgical wards 76 isolates (25.33%), followed by MICU 39 (13%), medicine ward 35 (11.66%) who had undergone invasive procedures like intravascular catheterization, urinary tract catheterization, mechanical ventilation, prior surgery etc [Figure 3]. This corresponds to the study done by Cisneros JM et al [36] and Siegman IY et al [21]. Study conducted by Cisneros JM et al reported 56 patients (71%) were in an ICU, 13 (16%) were being treated in medical wards, and 10 (13%) were in surgical wards at the time of the bacteremic episode. These findings were in concordance with the study done by Mindolli PB et al where most of the isolates were from surgical wards 61 isolates (30.5%), ICU 54 isolates (27%) and paediatric ward 38 isolates (19%). In a study conducted by Anupurba S et al in 2005, 20.8% of *Acinetobacter* were isolated from ICU, whereas in the present study it was 37.33% which shows the increasing trend of *Acinetobacter* to cause nosocomial infections.

In this study, out of the 300 *Acinetobacter* isolates, strains showed 69% resistant to Amikacin by disc diffusion method [Table 2]. Similar study by Joshi SG et al [11]. also reported resistant to Amikacin was 68.6%. Others studies by Sinha N et al from a tertiary care setting in North India [6] reported 84.8% resistance to Amikacin in Meropenem sensitive cases. Patwardhan RB et al [24] and Mohammad R et al [25] reported high level resistance to Amikacin i.e. 96.2% and 85.2% respectively.

Resistant to Cefotaxime is 93.6% in this study [Table 2]. Similarly 90.6% was reported by Mohammad R et al [25], 100% was seen by Patwardhan RB et al [24], 89.3% was observed by Sinha N et al [26], 95.4% resistance was reported by Joshi SG et al [11].

Present study shows that the strains were resistant to Gentamicin in 71.33% cases[Table 2], 85.2% by Mohammad R et al[25], 85.7% by Sinha N et al [26], 96.2% was seen by Patwardhan RB et al [24].

Resistant to Co-trimoxazole in this study is 74% [Table 2]. 68.2% was reported by Mohammad R et al [25].

Resistant to Doxycycline in this study is 66.66% [Table 2]81.3% was observed by Sinha N et al [26], 88.5% by Patwardhan RB et al [24].

In this study strains were resistant to Ciprofloxacin in 79% cases, 72.9% cases were resistant to Ciprofloxacin by Joshi SG et al [11]. High level resistance were observed by Sinha N et al [26], Mohammad R et al [25] and Patwardhan RB et al [24] i.e. 85.7%, 90.9% and 96.2% respectively.

Piperacillin-tazobactum showed 79.66% resistance in isolated cases. 42% was observed by Mohammad R et al [25]. Higher percentage 92.3% was reported by Patwardhan RB et al [24] may be due readily transferable antibiotic resistance expressed by *Acinetobacter*. They have the ability to acquire resistance to many major classes of antibiotics [27].

In the present study, Imipenem showed 73% resistant. Shakibaie MR et al from Iran reported resistance percentage of 73.3% to imipenem [28].

The above findings clearly show the emerging resistance to co-trimoxazole and ciprofloxacin followed by imipenem and increased level of resistance to piperacillin-tazobactam, and third-generation cephalosporins. These drugs remain the main stay of treatment for *Acinetobacter infections* and are usually reserved for severely ill patients. In order to prevent resistance and for antibiotic stewardship and rapid implementation of outbreak control measures, rapid detection of carbapenemase producers provides critical information.[29] The use of the Rapidec Carba NP test may allow to the identify carbapenemase producers and improve infection control [30].

Various modalities are available for the rapid identification of carbapenemase producers [31]

1.	UV spectrophotometry[32]
2.	Matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) technology [33-35]
3.	Molecular techniques [36-38]

These modalities have good sensitivities and specificities but have disadvantages like

- Require trained microbiologists
- Expensive equipment
- In addition, if carbapenemase genes are not included in the given gene panel or unknown carbapenemase genes, molecular techniques may fail to detect [39].

A rapid and biochemical detection of carbapenemase production was used in this study, namely The Rapidec Carba NP test. All carbapenem resistant isolates came out to be positive for carbapenemase production by Rapidec Carba NP test [Table 3]. It has excellent specificity and sensitivity, allowing reliable detection of known carbapenemases in *Acinetobacter species* [39].

CONCLUSIONS

Since there is lack of data on antimicrobial surveillance, study on antimicrobial resistance surveillance is the need of the hour. It will help the centres to generate local antibiogram which will helps in having a national data. It also guides the clinicians to choose appropriate empirical therapy as well as assist in escalation and de-escalation whenever possible. Hence this study can help in establishing antimicrobial stewardship and regulate the antimicrobial use.

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