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Original Research Article

Antibiotic susceptibility profile of Acinetobacter isolates isolated from various clinical specimens received at a tertiary care hospital in North western region of Gujarat

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ABSTRACT

Introduction: Acinetobacter species has now been identified as a prime and notorious cause of nosocomial pneumonia and bloodstream infections, which is been observed especially among immunocompromised patients with underlying co morbidities or deliberating illnesses. Globally, there has been a significant increase in resistance of this organism to most of the antimicrobials in recent period**.**

Aim: The aim of our study is to evaluate Acinetobacter isolates obtained from various clinical samples., their antimicrobial resistance pattern and changes in resistance pattern of the organism from the samples which they have been isolated from our tertiary care hospital.

Materials and Methods: In this study, Acinetobacter isolates were obtained from both the in-patients or out-patients during the period between 01.07.2021-01.07.2022 were used. The isolation and identification were done by using conventional methods. Antimicrobial susceptibility testing was done by Disc diffusion method as per CLSI guideline.

Results: More than 50% of isolates were found to be resistant to carbapenems such as Meropenem (60%) and Imipenem (55%) respectively; these antibiotics were used in our test battery. The isolates had exhibited a high level of resistance to the group of β-lactam combination antibiotics namely Piperacillin-Tazobactam (90%) and almost complete resistance to cephalosporins which were used in our testing battery at our laboratory. Moreover, they showed low level of resistance to ciprofloxacin among the fluoroquinolones group**.**

Conclusion: Our study showed high level of resistance to all available Antibiotics excluding colistin among Acinetobacter species. There has been an increased rate of complete drug-resistant isolates. In order to prevent development of multiple resistances, and produce maximum utility of the available limited antibiotic resources, each laboratory should determine the percentage of resistance to various Antibiotics among Acinetobacter species in different geographical region.

Keywords: Antibiotic susceptibility, Acinetobacter isolates

INTRODUCTION

The name of the bacteria “Acinetobacter” has been evolved from the Greek word “akinetos” which means “unable to move”, as these bacteria are non-motile. Bacteria of the genus Acinetobacter at present had increasingly obtained attention in recent years, because of their ability and their potential to cause high levels of nosocomial infections.[1]

Studies suggest the most common species of this bacterium causing hospital acquired infections are A. baumannii, followed by A. calcoaceticus and A. lwoffii [2]. We have classified the species of Acinetobacter with similar phenotypic and biochemical properties grouped into a single unit called as the A. calcoaceticus-baumannii complex which firstly had only four species namely: Acinetobacter calcoaceticus, Acinetobacter baumannii, Acinetobacter pittii and Acinetobacter nosocomialis. Then two new species, Acinetobacter seifertii and Acinetobacter dighoorniae, have also been incorporated into the A. calcoaceticus-A. baumannii complex in recent times. [3]

Acinetobacter species are one of the most resistant pathogenic organisms that are encountered in clinical practice. They are a great hindrance during the initiating of effective empirical treatment and result in poor clinical recovery due to its high-level resistance. A. baumannii which is intrinsically resistant to all the available penicillins and has acquired its resistant genes against almost all antibiotics that can treat Gram-negative organisms both the rods and cocci, which includes the fluoroquinolones, the aminoglycosides, and the cephalosporins group of drugs [4].

In recent times, multidrug-resistant and pan drug resistant strains of A. baumannii causing infections have been associated with increased severity of illness and death [5]. Thus, observing and notification of the antimicrobial resistance of Acinetobacter species consistently every now and then is a strategically important move for the selection of appropriate empirical antibiotic treatment for seriously ill patients.

The main aim of our study was to evaluate the incidence of Acinetobacter spp., Note down their antibiotic resistance pattern and the %of resistance to all the antibiotics used in the test battery.

Age (Years)	Total isolates (N)	Percentage (%)
0 - 9	36	46.1
10 - 19	5	6.4
20 - 29	12	15.4
30 - 39	5	6.4
40 - 49	7	8.9
50 - 59	3	3.9
≥ 60	10	12.9

Table-1: Age wise distribution of isolates

MATERIAL AND METHODS

This is a retrospective study conducted at Gujarat Adani Institute of Medical Sciences, Bhuj, between July 2021 and July 2022. Acinetobacter isolates from various suspected samples like Urine, blood, Sputum, pus, BAL & endotracheal aspirates sent from various departments were collected. Conventional culture techniques using blood agar, Mac- conkey agar were used. CLED agar was used for isolation of urinary isolates. BD BACTEC was used for blood culture.

These samples were inoculated and incubated for 16-18 hours in an incubator. Genus Acinetobacter was identified by Gram staining, cell and colony morphology, positive catalase test, negative oxidase test and absence of motility.[6] Speciation of Acinetobacter was performed on the basis of glucose oxidation, beta hemolysis, growth at 37°C and 42°C .[7]

Antimicrobial susceptibility testing for these isolates was done on Muller-Hinton Agar by Modified Kirby Bauer method (Disk diffusion method) and antimicrobial susceptibility testing of colistin and polymixin B were performed by using E test. [8]

ABST results were accurately analyzed as in accordance with the Clinical and Laboratory Standards Institute (CLSI) 31st edition 2021 [8].

This is the list of antibiotics which were used in our test battery:

(Ampicillin (AMP), Cefoperazone (CPZ), Cefotaxime (CTX), Ceftriaxone (CTR), Cefepime (CPM), Ceftazidime (CAZ), Piperacillin /Tazobctam (PIT) , Amikacin (AK), Gentamicin (GEN) , Tobramycin (TOB), Levofloxacin (LE), Ciprofloxacin (CIP),Imipenem (IMP), Meropenem (MRP), Tetracycline (TE), Tigecycline (TGC), Cotrimoxazole (COT), Polymixin (PB), Colistin (CL), from Hi- media was preferred in our study).

Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 were used as quality control strains.

Inclusion criteria:

Only isolates obtained from diagnostic testing were included

Exclusion criteria:

Repeat isolates from same patient were not included.

RESULTS

A total of 78 isolates Acinetobacter strains were isolated from the patients admitted for various illnesses and attending the OPD at Gujarat Adani Institute of Medical Sciences, Bhuj, during the study period (1st July 2021 to 1st July 2022).

Demographic profile

Acinetobacter spp. were isolated maximum from the age group of 0 - 9 years (46.1%), followed by individuals of 20 - 29 years (15.4%) age group; and was least isolated in the age group 50-59 (3.9 %) respectively (Table-1).

The present study had 3201 Males (60.3%) and 2108 Females (39.7%) patients whose samples were sent for culture. (Table-1.1).

Table-1.1: Gender wise distribution of isolates.

		MALE	FEMALE
Total samples for culture	5309	3201 (60.29%)	2108 (39.71%)
Total positive	1426 (26.86 %)	742 (52.03%)	684 (47.97%)
Total positive for Acinetobacter	78 (5.4%)	52 (66.67%)	26 (33.33%)

Maximum Acinetobacter strains were recovered from blood 24/78 (30.7%), followed by pus14/78 (17.9%); Endotracheal aspirate 20/78 (25.7%); Urine 06/78 (7.7%); sputum 12/78 (15.4%); BAL, 02 (2.6%) each (Table-2). Most of the bacterial isolates were isolated from neonates admitted in the Neonatal intensive care unit (NICU) 36 (46.2 %). General surgery 16 (20.6 %) were second in frequency followed by General Medicine 10 (12.8 %); TB ward and pediatrics 08 (10.2 %). The least number of isolates were obtained from TB ward and pediatrics 08 (10.2) (Table-3).

Table-2: Sample wise distribution of isolates

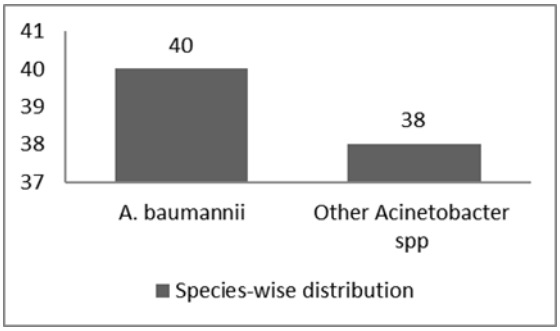
Sample	No. of isolates (N)	Percentage (%)
Blood	24	30.70
ET	20	25.70
Pus	14	17.90
Sputum	12	15.40
Urine	06	7.70
BAL	02	2.60

Table-3: Ward wise distribution of isolates

Ward	No. of isolates (N)	Percentage (%)
General Medicine including MICU	10	12.8
General Surgery including SICU	16	20.6
Pediatric ward including PICU	08	10.2
NICU	36	46.2
TB Ward	08	10.2
TOTAL	78	100

Among the total isolates we have 40 isolates identified as Acinetobacter *baumannii* complex, followed by 38 isolates belonging to other Acinetobacter spp which could not be further identified due to lack of resources (Figure-1).

Figure-1: Species-wise distribution



The ABST results of the various isolates are shown in the (Table-4).

More than 50 % of isolates were resistant to carbapenems like Meropenem (60%) and Imipenem (55%) which was used in the test battery in our study.

A high degree of resistance to β-lactam combination antibiotics such as Piperacillin-Tazobactam (90%) and almost complete resistance to cephalosporins were shown by the isolates. Out of 78 isolates, all were resistant to cefotaxime and 85 % to ceftazidime and 97.5 % to Cefepime and (100 %) resistance to Ceftriaxone was seen.

Among the fluoroquinolones Ciprofloxacin was found to be relatively sensitive showing only 15 % resistance. In case of aminoglycosides, resistance in Gentamicin was seen in 83%isolates; with Amikacin resistance was seen in more than 72% isolates, and in Tobramycin 79 %of the isolates showed resistance.

Table-4: Antimicrobial susceptibility profile of the Acinetobacter spp.

Drugs	Sensitive (%)
Ampicillin (AMP)	0%
Cefoperazone (CPZ)	2.56%
Cefotaxime (CTX)	0%
Ceftriaxone (CTR)	0%
Cefepime (CPM)	5.13%
Ceftazidime (CAZ)	14.29%
Piperacillin/Tazobactam (PIT)	10.26%
Amikacin (AK)	28.21%
Gentamicin (GEN)	17.95%
Tobramycin (TOB)	20.51%
Ciprofloxacin (CIP)	84.62%
Imipenem (IMP)	40.0 %
Meropenem (MRP)	45.0%
Tetracycline (TE)	15.39%
Tigecycline (TGC)	18.75%
Co-trimoxazole (COT)	10.26%
Polymyxin-B (PB)	100%
Colistin (CL)	100%

DISCUSSION

The Acinetobacter species has emerged and become as a significant pathogen which cause nosocomial infections in patients who are being admitted in Intensive Care units [9]. Infections caused by multidrug resistant species of Acinetobacter are very difficult to treat and obtain clinical cure and hence a major threat leading to increased morbidity and mortality in patients harbor this notorious organism [10].

The rate and frequency of antibiotic resistance that has been observed in Acinetobacter pathogens are worrisome because there are any antibiotics hardly in development against these multi-resistant strains of organism [11].

Until now, carbapenems were the drug of choice against Acinetobacter pathogens. However, the development of high level of resistance against carbapenems by this pathogen caused changes in the entire treatment protocols, making the pathogen nearly to impossible to treat the spread of such strains of Acinetobacter in the hospital environment reduces the therapeutic options available and also a serious challenge in the HIC management as it demands high level measures and causes loss of human life and resources.[12].

Our study shows the prevalence of Acinetobacter spp. infection in the hospital and to obtain their ABST profiles and resistance patterns in real- time.

A total of 5309 samples were received in which 1426 samples flagged positive for various bacterial pathogens. When we consider Acinetobacter spp we had 78 (5.4%) among the total isolates. Similar prevalence of 3% and 3.36% of Acinetobacter isolates was reported by studies conducted by Dash., et al. in Odissa and Gupta., et al. in Pune [13,14].

Prevalence rate of 14% and 9.6% was reported in the studies conducted by Mostofi.et al. from Tehran, Iran and Joshi., et al. from Pune which was much higher than that obtained from our current study [15,16].

In this study the age group 0 - 9 years (46.1%) had the highest incidence which was in similar with the study report of Mera RM., et al. in the U.S [17].

In our study we have obtained maximum number of Acinetobacter strains from blood (24/30.7%), followed by ET secretions20/25.7%), study conducted by Shivaranjani V., et al. in the year 2013in Southern peninsula India showed 39% isolated from pus, followed by 20 % isolated from endotracheal secretions, but in our current study we have maximal isolation from blood samples and nearly similar isolation frequency in ET secretion. There is some similarity in isolation in endotracheal secretions which seems relevant to our study [18].

The most prevalent species Acinetobacter spp., in human clinical specimens is Acinetobacter baumannii complex [17].

In our present study we have also 40 (51.28 %) isolates of A. Baumannii among the total 78 isolates which is also related and shows true when compared with the study conducted by Gupta N., et al. in 2015 [14] there was around 72% A. baumannii complex isolation,

We are all aware that Acinetobacter is ubiquitously present all over the hospital environment due to its high capacity to survive in hard conditions for prolonged periods and contains antimicrobial resistance genes which have made Acinetobacter a successful hospital pathogen. [13].

In our study we have concluded that Acinetobacter was extremely resistant to piperacillin (89.2%) which is fairly a high level of resistance. Our study is in concordance to a study of Shivaranjani V., et al. [18].

More than 80% of isolates were also resistant to cephalosporins from our present study correlating with results of Guckan R., et al. and Shivaranjani V., et al. [18,19,20].

We have also found in our study that resistance as high as 60% and 55%to¬ Imipenem and Meropenem were seen. Data of the susceptibility patterns of Acinetobacter from the different regions around the world had shown that of Acinetobacter spp shows high resistance patterns. Imipenem's resistance had increased to a very high level from no resistance to 40% among the isolates which were obtained in culture [21]. Imipenem resistance in A. baumannii in burns patients from USA was 8.0% earlier which shows the severe increase in resistance which has emerged in this species [22].

Major antimicrobial drugs as well as disinfectants which are being used in the hospital environment are resistant to this

organism and this is the major factor that makes it a successful and persistent hospital pathogen causing severe infections in the immune suppressed patients who are mainly admitted to the intensive care units in the hospital facility [23]. We have observed that there is no resistance to the drug colistin in our study which is having the results similar to that published by Dash., et al, Sharee., et al. in their research studies [13,24].

Neonatal intensive Care Unit (NICU) 46.2%which is considered to be a high-risk area showed the highest number of isolates of Acinetobacter which is similar to the results published in high-risk areas by Mera RM., et al. and Gupta., et al., where an increased number of Acinetobacter isolates were recovered from Intensive Care settings. Thus, such data are considerably validation our study [17,14]. The emergence of antibiotic resistant strains in ICUs is due to the higher use of anti-microbial agents per patient per surface area. The use of blanket therapy by using broad spectrum antibiotics is also an important reason for the emergence of resistance [14].

Conclusion

The resistance rates of Acinetobacter have rapidly increased to routinely used antibiotics in the current scenario because of blanket therapy using broad spectrum antibiotics. More than half of the isolates were resistant to carbapenems and fluoroquinolones which is been clearly seen from our study.

Multidrug resistant strains of Acinetobacter remained sensitive to colistin and polymyxin-B, which is to be used as the last treatment option for treating Acinetobacter infections.

MDR Acinetobacter which was widely spread in the hospital environment and in high-risk facilities. Patients admitted to intensive care are vulnerable to acquire the MDR Acinetobacter infection. This alarming situation needs to be analyzed and taken care of at regular intervals. This helps in reducing morbidity, mortality and preserving resources.

It is compulsory to do routine monitoring of the resistance pattern of isolates and inform to higher centers if any new resistance strains are being reported in any specific geographical location. Surveillance of multidrug resistant strains of Acinetobacter is critical for guiding the rational use of antibiotics and decreasing the incidence of Hospital acquired Infection.

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