Research Article

Detection of AMPC Beta Lactamase Producing Acinetobacter Species with Their Antibiotic Resistance Profile in Tertiary Care Hospital

Manisha M. Rajguru[1], Deepashri Naik*[2], Sareena Rao[3], Amit P Khekade[4]
[1,2,3]Department of Microbiology, MGM Medical College, Kamothe, Navi Mumbai, Maharashtra, India.
[4] Department of Microbiology, Datta Meghe Medical College, Datta Meghe Institute of Higher Education & Research, Nagpur, Maharashtra, India.
***Corresponding author's E-mail:** deepashri82@gmail.com

Received: 05-03-2024; **Revised:** 22-04-2024; **Accepted:** 30-04-2024; **Published on:** 15-05-2024.

ABSTRACT

Due to the increased use of broad-spectrum antibiotics in hospitalised patients, *Acinetobacter species* has become a prominent multi drug resistant nosocomial pathogen being able to colonize and develop infections in patients. *Acinetobacter species* has numerous mechanisms for resistance, including an impermeable outer membrane, enzymes that break down antibiotics, particularly AmpC, Metallo β-lactamases, class D OXA-type and class B Metallo β- lactamases that allow the organism to resist carbapenems, porin channel alterations as well as efflux pumps, and other genetic changes. It is well known that AmpC β-lactamases confer resistance to cephalosporins in the oxyimino group and are unaffected by currently available β-lactamase inhibitors. The aim of the study was to detect AmpC β-Lactamase in *Acinetobacter species* with their Antibiotic Resistance pattern. 100 *Acinetobacter species* isolates from various clinically relevant samples were included. Methods such as the Disc Approximation test and the Modified 3-Dimensional test were employed on ceftazidime resistant isolates of Acinetobacter species to detect AmpC β-lactamase phenotypically. Highest prevalence of *Acinetobacter* was found in Blood (37%). Maximum number of *Acinetobacter* were isolated from MICU. The Modified 3-Dimensional produced the largest percentage of AmpC positive *Acinetobacter* isolates (40.67%) compared to the Disc Approximation Test method (33.89%). Early detection of these β-lactamases production is essential for planning appropriate medication in accordance with the resistance mechanisms of the MDR strains, as well as for epidemiological research and efficient infection control procedures to prevent the spread of infection.

Keywords: Acinetobacter, Antibiotic Resistance, AmpC β-lactamase, Disc approximation test (DAT), Modified 3- Dimensional test.

INTRODUCTION

Acinetobacter species are non-fastidious, strict aerobe, Gram-negative cocco-bacilli, Oxidase-negative, Catalase positive, non-fermentative and non-motile[1,2]. *Acinetobacter species* are commonly found in nature, in water and in soil and also, they have also been isolated from animals. In humans, they are found to reside on mucous membrane, skin and also sometimes the membrane of the intestinal tract[3]. They are usually plumpy, small, measuring 1.0-1.5 µm by 1.5-2.5 µm in diameter. More than 50 species in the *Acinetobacter genus*, the *Acinetobacter baumanni complex* (*Acinetobacter nosocomialis, Acinetobacter pitti* and *Acinetobacter baumanni*) are the most clinically important ones. But sometimes they develop into more coccoid form, usually present in pairs or long chains of variable duration[4]. It has led to many hospital outbreaks in the past years due to its ability to survive in the hospital environment and also to stay for long period of time on surfaces. In recent years, it has been labeled as a "red alert" human pathogen, expressing problems among medical fraternity due to its wide range of antibiotic resistance[5]. In the hospital setting, *Acinetobacter baumannii* has emerged as a major opportunistic pathogen, being able to colonize and develop infections in patients with Ventilator Associated Pneumonia, Secondary Meningitis, Urinary Tract Infection, Septicemia and other conditions in the Intensive Care Unit (ICU). It also causes infections in other immune-compromised individuals, including burn patients[6].

Infections with *Acinetobacter baumannii* in particular Carbapenem-resistant *Acinetobacter baumannii* (CRAB), because of their correlation with high care rates, mortality and morbidity, are of public health concern worldwide. According to the World Health Organization (WHO), Carbapenem-resistant *Acinetobacter baumannii* has high rates globally in the target list of antibiotic resistant bacteria, as a vital priority pathogen to drive drug research and development[7]. Owing to the widespread use of β-lactam antimicrobials, bacterial resistance has increased and is now a significant threat to the continued use of antibiotic therapy.[8]

AmpC β-lactamases may be chromosomal or plasmid-mediated. Plasmid mediated AmpC β lactamases hydrolyze all β-lactam antibiotics omitting Cefepime and Carbapenems. The genotypes that are widely mentioned are ACC, FOX, MOX, DHA, CMY, CIT and EBC. These mobilized plasmid-mediated enzymes, which also include β-lactam antibiotics, impart a resistance pattern identical to the overproduction of chromosomal AmpC β-lactamases[9]. The objective of this study is to enhance the clinical management of patients suffering from infections, diagnosis of AmpC β-lactamases which in turn will also provide us with relevant epidemiological details. Each health care center needs a local monitoring program since it's important to understand local trends of resistance when choosing the right antibiotics to treat illnesses. Therefore, the goal of the current study was to identify

Acinetobacter species that produce AmpC beta-lactamase by using phenotypic methods in tertiary care hospital.

MATERIALS AND METHODS

This cross-sectional prospective study was conducted in the Department of Microbiology, MGM Medical College & Hospital, Navi Mumbai, India during July 2019 to January 2021, after obtaining approval of Institutional Ethics Committee (N-EC/2019/SC/07/97) and the informed consent was obtained from all the patients.

Sample Size: 100 *Acinetobacter species* isolates from various clinical samples such as Blood, Cerebrospinal Fluid, Sputum, Broncho alveolar lavage, Pus, Urine, Wound swab, Body fluids will be included in the study. Samples received from age group below 18 years were excluded. Antibiotic susceptibility testing of all *Acinetobacter species* isolated was carried out by modified Kirby Bauer Disc Diffusion Technique by using Mueller Hinton Agar plates according to recent Clinical and Laboratory Standard Institute (CLSI) guidelines 2020[10].

Disk Approximation Test (D.A.T)

The surface of the Mueller Hinton Agar plate was inoculated with a 0.5 McFarland bacterial suspension that had been prepared. In the middle of the plate, a 30-µg ceftazidime disc was placed, followed by placing discs containing 10 µg imipenem, 30 µg cefoxitin, and 20/10 µg amoxicillin/clavulanate at a distance of 20 mm from the ceftazidime disc. At 37°C, the plate was incubated inverted for the entire night. A good result for AmpC production was evaluated if, after an overnight incubation, there was any visible blunting or flattening of the zone of inhibition between the ceftazidime disc and the inducing substrates (imipenem, cefoxitin, and amoxicillin/clavulanate disc) [11].

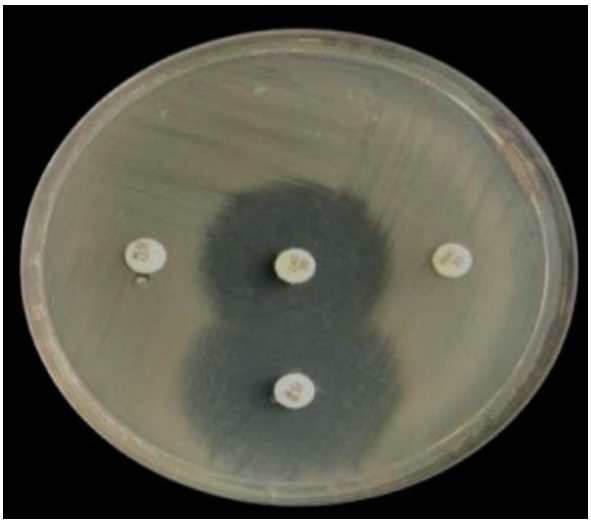

Figure 1: Showing positive test for AmpC by Disk Approximation Test

Modified 3-Dimensional Test

On Mueller Hinton agar plate, a lawn culture of *E. coli* ATCC 25922 with turbidity of 0.5 McFarland was made, and a cefoxitin 30 µg disc was placed in the center of the plate. A sterile surgical blade was used to create a 3 cm long linear incision 3 mm away from the cefoxitin disc. A little circular well was created at the other end of the slit. The extract of the enzyme AmpC lactamases was made by freezing and thawing the test organism 7-8 times and then centrifuging it at 2000 rpm for 15 minutes. The prepared well was then loaded with 20 µl to 30 µl of the supernatant containing the extract. After allowing the liquid to seep and diffuse into the slit for 5-10 minutes, the plates were incubated at 37°C for 24 hours. Isolate with clear distortion was designated as an AmpC producer, isolate with no distortion as an AmpC non-producer, and isolate with minimal distortion as intermediate[12, 13].

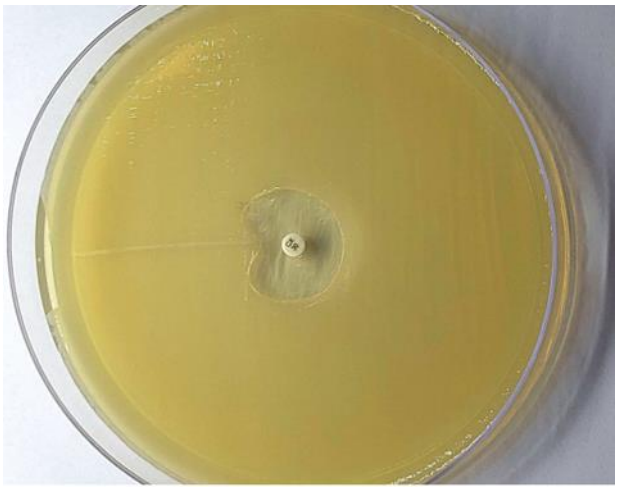

Figure 2: Showing positive test for AmpC by Modified 3-Dimensional Test

RESULTS

From July 2019 to January 2021, 100 samples of various body fluids were collected in the Microbiology Laboratory at MGM Hospital in Kamothe, Navi Mumbai. All of the samples collected were OPD and IPD from male and female patients of various ages.

Table 1 displays the age and sex distribution of 100 *Acinetobacter species* from various clinical samples. In both age groups, male patients had the highest percentage of *Acinetobacter species* isolations (79%) compared to the female population (21%). Blood had the highest prevalence of *Acinetobacter* (37%), followed by Sputum (31%), and then other bodily fluids such pus, urine, ascetic fluid, CSF, BAL, and pleural fluid.

The distribution of *Acinetobacter species* by ward is shown in Table 2, and it shows that MICU had the highest number of *Acinetobacter* isolates (21%) followed by Medicine, Surgery, EMS ICU, COVID ICU, SICU, OPD, Orthopedics, Respiratory Medicine, HDU, and Geriatrics.

Table 1: Age & Sex Wise Distribution of Samples Showing Growth of *Acinetobacter species* (n=100)

Samples	18-50 Age		51 & above		Isolates of *Acinetobacter*
	Male	Female	Male	Female	
Blood	12	10	12	3	37
Sputum	9	2	16	4	31
Pus	13	1	4	0	18
Urine	3	0	5	1	9
Ascitic Fluid	0	0	2	0	2
CSF	1	0	0	0	1
Pleural Fluid	0	0	1	0	1
Broncho alveolar Lavage	1	0	0	0	1

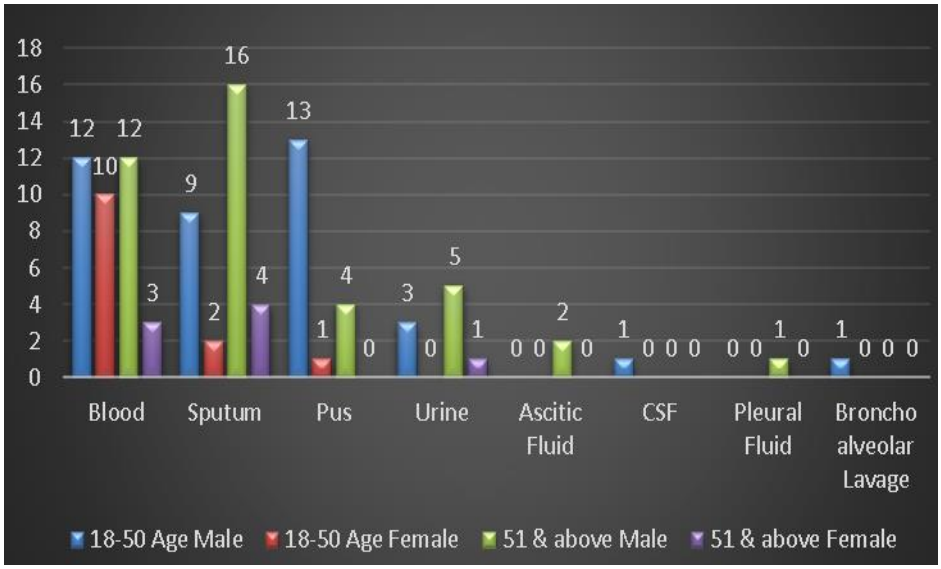

Table 2: Ward wise distribution of *Acinetobacter species* (n=100)

Ward	Isolation	Percentage (%)
MICU	21	21%
Medicine	19	19%
Surgery	18	18%
EMS ICU	8	8%
COVID ICU	8	8%
SICU	7	7%
OPD	7	7%
Orthopedics	6	6%
Respiratory Medicine	4	4%
HDU	1	1%
Geriatrics	1	1%

In order to identify the multi-drug resistant strains, all 100 isolates of *Acinetobacter species* were subjected to antimicrobial susceptibility testing (AST). Table 3 shows the resistance pattern of *Acinetobacter species,* revealing that 88% of *Acinetobacter species* were resistant to Augmentin (AMC), followed by Nitrofurantoin (82%), Tobramycin (68%), Cefotaxime, and Piperacillin (67%).

62 isolates of *Acinetobacter species* were identified as multi-drug resistant after testing resistant for three or more antibiotic groups. These isolates were then put through an AST test employing second-line antibiotics, as indicated in Table 3. *Acinetobacter species* were shown to be extremely resistant to Ticarcillin/Clavulanic acid (61, 98.38%), Piperacillin/Tazobactam (59, 95.16%), Meropenem (57, 91.19%), and Imipenem (56, 90.32%) in the second-line antibiotics.

On isolates of *Acinetobacter species* that were Ceftazidime-resistant, AmpC β-lactamase was phenotypically detected (59). For the phenotypic identification of AmpC β-lactamase, the Disc Approximation Test (DAT) and Modified 3-Dimensional Test (Mod. 3D) were employed. The percentage of AmpC β-lactamase is shown in Table No. 5 for *Acinetobacter species* strains that are ceftazidime resistant. The Modified 3-Dimensional test (Mod. 3D) (40.67%) yielded the largest proportion of AmpC β-lactamase-positive *Acinetobacter isolates* compared to the Disc Approximation test (DAT) (33.89%) method.

Table 3: Antibiotics (First Line Antibiotics)

Antibiotic	Concentration (mcg)	Sensitive	Intermediate	Resistant
Amoxyclav (AMC)	20/ 10 mcg	10	2	88
Tobramycin (TOB)	10 mcg	31	1	68
Gentamicin (GEN)	10 mcg	30	7	63
Amikacin (AK)	30 mcg	34	0	66
Cefotaxime (CTX)	30 mcg	30	3	67
Ciprofloxacin (CIP)	5 mcg	34	10	56
Ceftazidime (CAZ)	30 mcg	36	0	64
Tetracycline (TE)	30 mcg	73	3	24
Piperacillin (PI)	100 mcg	24	9	67
Trimethoprim- sulfamethoxazole (COT)	23.75/ 1.25 mcg	30	9	61
Nitrofurantoin (NIT)	300 mcg	10	8	82
Levofloxacin (LE)	5 mcg	64	0	36

Table 4: Antibiotics (Second Line Antibiotics)

Antibiotics	Concentration (mcg)	Sensitive	Intermediate	Resistant
Imipenem (IPM)	10 mcg	5	1	56
Meropenem (MRP)	10 mcg	3	2	57
Levofloxacin (LE)	5 mcg	54	2	6
Cefepime (CPM)	30 mcg	4	2	56
Piperacillin/ Tazobactam (PIT)	100/ 10 mcg	3	0	59
Ticarcillin/ Clavulanic acid (TCC)	75/10 mcg	0	1	61
Polymyxin B (PB)	300 units	60	0	2
Aztreonam (AT)	30 mcg	2	3	57
Tigecycline (TGC)	15 mcg	41	11	10
Colistin (CL)	25 mcg	58	0	4

Table 5: AmpC Production Test by Different Methods Among the Ceftazidime Resistant *Acinetobacter species*

Antibiotics	D.A. T		MOD. 3D Test	
	Positive	Negative	Positive	Negative
Ceftazidime (n=59)	20	39	24	35

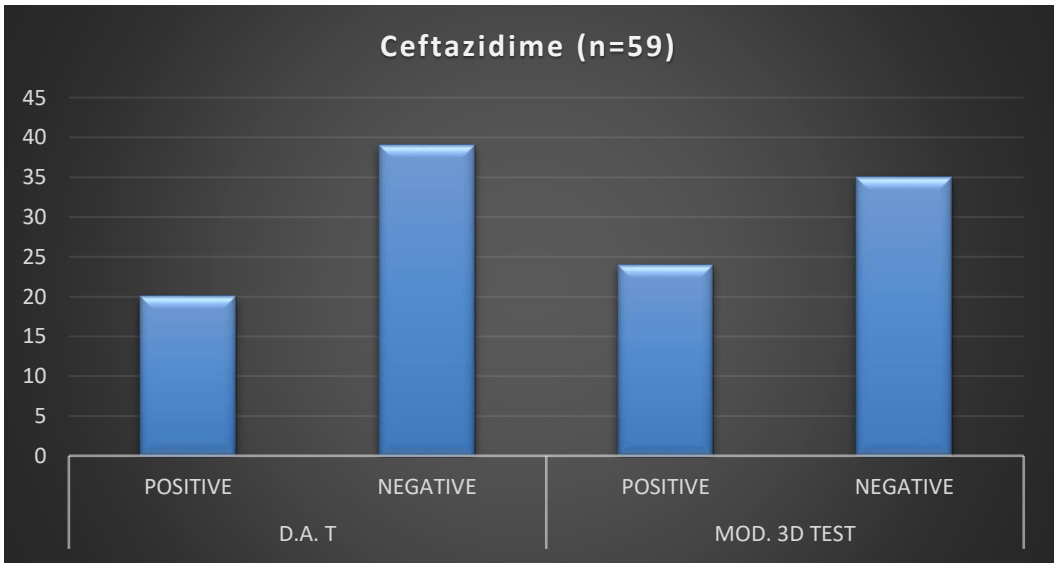

Table 6: Showing various studies and their sample wise distribution

Sample	Present Study	F Sahcheraghi, et al.[23] 2011	Dr. S Das, et.al.[16]	M Hajjar, et.al.[19] 2017	S.K. Yadav, et.al.[14] 2020	A. Rezaei. et.al. 2018 [22].
Blood	37%	47%	13.3%	5%	6.2%	3%
Sputum	31%	10%	3%	43%	-	3%
Pus	18%	12%	38.3%	21%	27.3%	9%
Urine	9%	7%	15%	10%	6.8%	-
Other Body Fluids	5%	-	-	19%	-	10%
Catheter Tips	-	-	-	2%	1.2%	2%
Medical Devices	-	-	20%	-	-	-

DISCUSSION

During the study period of July 2019 to January 2021, 100 samples of *Acinetobacter species* were isolated from all the body fluids which were included in this study. In this study, it was found that Blood had the highest prevalence of *Acinetobacter species* accounting for 37%, followed by Sputum (31%), Pus (18%), Urine (9%) and other body fluids which included Ascitic fluid (2%), Cerebrospinal Fluid, Broncho-alveolar Lavage and Pleural fluid accounting for only 1%. Some of the studies and their findings are discussed in table 6.

In the present study, two age groups were considered to analyze their distribution. By this study, it was found that the species of *Acinetobacter* were widely distributed in the age group of 18-50 (52%) as compared to 51 and above (48%). The other study done in the year 2020 by S.K. Yadav, et.al, [14]. included various age groups in their study. In their study, the highest distribution of *Acinetobacter species* was in the age group of 16-32 years (23.6%) followed by ≥ 65 years (22.4%), ≤ 15 years (19.8%), 33-48 years (18.7%) and 49-64 years (15.5%).

The other study done in the year 2018 by V. Rebic, et.al.,[15]. studied the distribution of *Acinetobacter species* in three age groups. The ratio of isolates was higher in the over 60 years age group (p= 0.763). In the present study, it was discovered that the isolates of *Acinetobacter species* were more in males (79%) as compared to females (21%). In the other study done by S.K. Yadav, et.al[14]. the distribution of species of *Acinetobacter* was higher in males with 58.3% than females comprising of 41.7% (ratio being 1:4). In another study done by V. Rebic et.al, [15]. Acinetobacter infections were more frequent in males (54.20 %) as compared to females (45.80 %). In the present study, the growth of *Acinetobacter* isolates in blood was more in the age group of 18-50 with males (12) and females (10), which was followed by sputum in which the highest number of isolates were found from the age group of 51 and above with males (16) and females (4).

In pus sample the maximum number of *Acinetobacter* was isolated from the age group of 18-50 in which males were 13 and female was 1. In the urine sample, the highest isolates were found in the age group of 51 and above with males (5) and female (1).

The current analysis found that 93% of the species of *Acinetobacter* were isolated from inpatient (IPD) and the remaining 7% of the species of *Acinetobacter* were isolated from outpatient (OPD). Amongst these 93 inpatients, the highest number of *Acinetobacter species* were isolated from MICU (22.58%) followed by Medicine (20.43%), Surgery (19.35%), EMS ICU (8.6%), COVID ICU (8.6 %), SICU (7.52%), Orthopedics (6.45%), Respiratory Medicine (4.3%), HDU (1.07%) and Geriatrics (1.07%).

The other study done by Dr. S. Das, et.al[16], presented the highest prevalence of *Acinetobacter species* from ICUs (23.3%), followed by Orthopedics (21.7%), Surgery (20%), Medicine (15%), Obstetrics and Gynecology (8.3%), Pediatrics (5%), Respiratory Medicine (3.34%) and others (3.34%). Another study done by S.K. Yadav, et.al[14]. among the inpatients the highest distribution of *Acinetobacter species* was found in the ICUs (49.6%), followed by Surgical (19.9%), Medicine (14.3%), Orthopedics (5.6%), Pediatrics (3.7%), Maternity (2.5%), Ophthalmology (2.5%) and Burnt (1.9%).

In the study done in the year 2013 by V. Sivaranjani et.al[17]. the distribution of *Acinetobacter species* isolated in the various hospital wards were divided into 2 groups based on the number of non-MDR isolates (n= 35) and number of MDR isolates (n=87). The distribution of *Acinetobacter species* was as followed: ICU (number of Non-MDR isolates = 7, number of MDR isolates= 37), General Surgery (number of Non-MDR isolates = 7, number of MDR isolates= 23), Obstetrics and Gynecology (number of Non-MDR isolates = 7, number of MDR isolates= 15), Orthopedics (number of Non-MDR isolates = 1, number of MDR isolates= 4), General Medicine (number of Non-MDR isolates = 6, number of MDR isolates= 1), Pediatrics (number of Non-MDR isolates = 1, number of MDR isolates= 0) and Urology (number of Non-MDR isolates =0, number of MDR isolates=1).

In our study, when tested the antimicrobial susceptibility Testing on the *Acinetobacter species* isolated from blood, pus, sputum and other body fluids (Cerebrospinal Fluid, Bronchoalveolar Lavage, Pleural fluid and Ascetic fluid), it was revealed that the *Acinetobacter* showed 88% resistance to Augmentin (AMC), which was followed by Tobramycin (68%). On the other hand, the *Acinetobacter species* showed highest sensitivity to Tetracycline (TE) with percentage of 68%.

In the second line antibiotics, the highest degree of resistance was found to be 98.38% for Ticarcillin/ Clavulanic acid (TCC) and Piperacillin/ Tazobactam (PIT). On the other hand, the highest sensitivity was found against Polymyxin B (PB) with 96.77% followed by 93.5% to Colistin (CL), 87.09% to Levofloxacin (LE) and 66.12% to Tigecycline (TGC). Another study done by Dr. S. Das, et.al,[16] presented the Antimicrobial Susceptibility Pattern which showed 100% sensitive to Colistin, followed by 75% sensitive to Aztreonam and 65% sensitive to Imipenem and Meropenem. Along with this it was also found that the lowest sensitivity was towards Ceftazidime (21.7%).

In the other study done by S.K. Yadav, et.al,[14] showed that according to the Antibiotic Sensitivity Profile, the majority of MDR isolates were resistant to the majority of the first-line antibiotics. The isolates were totally resistant to Piperacillin and Cefotaxime, 99.4% to Ceftazidime and Cefepime. 98.7% to Piperacillin- tazobactam and Ciprofloxacin, 93.8% to Gentamicin and 89% to Ampicillin-sulbactam and Meropenem. MDR isolates were fully immune to all antibiotics except Polymyxin B and Colistin sulphate. In another study done in the year 2018 by A. Kaur, et.al,[18] most of the antibiotics examined demonstrated high levels of resistance in *Acinetobacter baumannii isolates. Acinetobacter baumannii strains* were found to be resistant to Ceftazidime in 96.6% of cases, Cefepime in 94.8% of cases, Imipenem in 60.3% of cases and Meropenem in 68.1% of cases. However, Polymyxin B has a sensitivity of 96.5% and Colistin has a sensitivity of 97.4%.

Our present study is also comparable with the study done by M. Hajjar, et.al[19] in which according to Antibiotic Sensitivity Testing, 95% of *Acinetobacter species* were resistant to Cefoxitin, 87% were resistant to Ciprofloxacin, 86% resistant to Trimethoprim- sulfamethoxazole and Piperacillin-tazobactam, 83% were resistant to Cefotaxime, 81% to Ceftazidime and 80% to Gentamicin. Also, 78% of the isolates of *Acinetobacter species* were also resistant to Imipenem and 84% to Meropenem. There was only 1 isolate which was resistant to Colistin. The present study was conducted on 100 isolates of *Acinetobacter species*, to detect the prevalence of AmpC β-lactamase production. The production of AmpC β-lactamase was carried out on those isolates that were resistant to Ceftazidime (59). AmpC β-lactamase was detected by 2 methods- Disc Approximation test (DAT) and Modified 3-dimensional Test (Mod 3D Test). Out of 59 Ceftazidime resistant *Acinetobacter species* isolates, 33.89% was positive by DAT and the positivity by Mod 3D Test was 40.67%.

Our study is comparable with the study done in the year 2019 by Mittal N, et.al.,[20] in which just 180 (47.3%) of the isolates were confirmed AmpC β-lactamase producers, while 96.3% (366) were probable AmpC β-lactamase producers. Furthermore, 292 (76.8%) isolates were likely MBL producers with only 72 (19%) were confirmed as MBL producer. Another study done by S.K. Yadav, et.al.,[14] out of 161 isolates of MDR *Acinetobacter species*, 67.7% were MBL producers and 38.5% were AmpC β-lactamase producers.

In a study done by L. Oberoi, et.al.,[21] in the year 2013, 30 (10.98%) Metallo β-lactamase (MBL) producers and 15 (5.4%) AmpC producers were found among the 273 Gram-negative isolates. Co-production of AmpC and Metallo β-lactamase was observed in 10 (3.67 percent) of the strains, with *Escherichia coli* being the most common. Another study done in the year 2013 by V. Gupta, et.al. [6] detected the co-production of MBL and AmpC β-lactamase in *Acinetobacter baumannii* isolated from burn patients. It was seen that 16 out of 100 were the isolates of *Acinetobacter baumannii* were showing the co- production of MBL and AmpC β-lactamase. Also, just 25 out of total 100 isolates revealed only the production of MBL.

In order to identify resistance mechanisms and stop the indiscriminate use of antibiotics, it is advised that certain straightforward tests, such as the Disc approximation test and the Modified 3-dimensional test for AmpC β-lactamase production, be carried out in microbiology laboratories.

The maximum number of *Acinetobacter species* was isolated from the age group 18-50 years as compared to the age group of 50 & above. The present study shows that Males had the predominance over females (4:1) in both the age groups in all the samples. According to the present study, the *Acinetobacter species* showed highest percentage of resistance to Augmentin (AMC) (88%), which was followed by Nitrofurantoin (NIT) (82%). On the other hand, the *Acinetobacter species* showed highest susceptibility to Tetracycline (TE) (73%). In the second line antibiotics, the highest degree of resistance was found to be for both Ticarcillin/ Clavulanic acid (TCC) and Piperacillin/ Tazobactam (PIT) i.e., 98.38%. On the other hand, the highest susceptibility was found against Polymyxin B (PB) (96.77%) followed by Colistin (CL) (93.5%), Levofloxacin (LE) (87.09%) and Tigecycline (TGC) (66.12%). The highest percentage of AmpC positive *Acinetobacter* isolates were given by the Modified 3-Dimensional method (40.67%) as compared to Disc Approximation Test method (33.89%). Due to the increasing frequency of resistance, clinicians must therefore administer antibiotics sparingly and establish effective infection control strategies to stop the spread of illnesses in hospitals.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. Almaghrabi M, Joseph M, Assiry M, et.al, Multidrug-Resistant Acinetobacter baumannii: An Emerging Health Threat in Aseer Region, Kingdom of Saudi Arabia. Canadian Journal of Infectious Diseases and Medical Microbiology. 2018;18:1-4.
2. Mathai A, Oberoi A, Madhavan S, Kaur P. Acinetobacter infections in a tertiary level intensive care unit in northern India: Epidemiology, clinical profiles and outcomes. Journal of Infection and Public Health. 2012;5(2):145-152.
3. Manchanda V, Sinha S, Singh N. Multidrug resistant Acinetobacter. Journal of Global Infectious Diseases. 2010;2(3):291-7.
4. Almasaudi S. Acinetobacter spp. as nosocomial pathogens: Epidemiology and resistance features. Saudi Journal of Biological Sciences. 2018;25(3):586-596.
5. Peleg A, Seifert H, Paterson D. Acinetobacter baumannii: Emergence of a Successful Pathogen. Clinical Microbiology Reviews. 2008;21(3):538-582.
6. Gupta V. Coexistence of Extended Spectrum Beta-Lactamases, AmpC Beta-Lactamases and Metallo-Beta-Lactamases in Acinetobacter baumannii from burns patients: a report from a tertiary care centre of India. Ann Burns Fire Disasters. 2013;26(4):189-192.
7. Ayobami O, Willrich N, Harder T, Okeke I, Eckmanns T, Markwart R. The incidence and prevalence of hospital-acquired (carbapenem-resistant) Acinetobacter baumannii in Europe, Eastern Mediterranean and Africa: a systematic review and meta-analysis. Emerging Microbes & Infections. 2019;8(1):1747-1759.
8. Palzkill T. Metallo-β-lactamase structure and function. Annals of the New York Academy of Sciences. 2012;1277(1):91-104.
9. Gupta G, Tak V, Mathur P. Detection of AmpC β Lactamases in Gram-negative Bacteria. Journal of Laboratory Physicians. 2014;6(1):001-006.
10. CLSI, Performance Standards for Antimicrobial Disk Susceptibility Test. 13th ed. CLSI standard M02. Wayne, PA: Clinical and Laboratory Standards Institute, 2018:46-49.
11. Inamdar DP, Anuradha B. Phenotypic methods fordetection of Amp Cβlactamases in Gram negative clinical isolates of atertiary care hospital. Indian J Microbiol Res 2020;7(2):125-129.
12. Maraskolhe, D. L., Deotale, V. S., Mendiratta, D. K., & Narang, P. Comparison of Three Laboratory Tests for Detection of AmpC β Lactamases in Klebsiella Species and E. Coli. Journal of Clinical and Diagnostic Research: JCDR, 2014;8(6):DC05. https://doi.org/10.7860/JCDR/2014/8256.4432
13. Rudresh SM, Nagarathnamma T. Two simple modifications of modified three-dimensional extract test for detection of AmpC β-lactamases among the members of family Enterobacteriaceae. Chron Young Sci 2011;2:42-6.
14. Yadav S, Bhujel R, Hamal P, Mishra S, Sharma S, Sherchand J. Burden of Multidrug-Resistant *Acinetobacter baumannii* Infection in Hospitalized Patients in a Tertiary Care Hospital of Nepal. Infection and Drug Resistance. 2020;13:725-732. https://doi.org/10.2147/IDR.S239514
15. Rebic V, Masic N, Teskeredzic S, Aljicevic M, Abduzaimovic A, Rebic D. The Importance of *Acinetobacter Species* in the Hospital Environment. Medical Archives. 2018;72(3):330-6.
16. Das S, Basak S. Acinetobacter baumanii complex and its beta-lactamase production: are we moving towards pre antibiotic era? Int J Health Sci Res. 2018;8(3):60-69.
17. Sivaranjani V, Umadevi S, Srirangaraj S, Kali A, S Seetha K. Multi-drug resistant *acinetobacter species* from various clinical samples in a tertiary care hospital from south india. Australasian Medical Journal. 2013;06(12):18-24.
18. Kaur A, Singh S. Prevalence of Extended Spectrum Betalactamase (ESBL) and Metallobetalactamase (MBL) Producing *Pseudomonas aeruginosa* and *Acinetobacter baumannii* Isolated from Various Clinical Samples. Journal of Pathogens. 2018;12:1-7.
19. Hajjar Soudeiha M, Dahdouh E, Daoud Z, Sarkis D. Phenotypic and genotypic detection of β-lactamases in Acinetobacter spp. isolates recovered from Lebanese patients over a 1-year period. Journal of Global Antimicrobial Resistance. 2018;12:107-112.
20. Mittal N, Chhina D, Gupta U. Incidence of ESBL, AmpC ß-lactamases and Metallo ß-lactamase producing *Acinetobacter baumannii* in intensive care units of a tertiary care hospital from north India. International Journal of Research in Health Sciences. 2019;7(1):9-13.
21. Oberoi L. ESBL, MBL and Ampc Lactamases Producing Superbugs – Havoc in the Intensive Care Units of Punjab India. Journal of Clinical and Diagnostic Research. 2013;7(1):70-73.
22. Rezaei A, Fazeli H, Halaji M, Moghadampour M, Faghri J. Prevalence of metallo-beta-lactamase producing *Acinetobacter baumanii* isolated from intensive care unit in tertiary care unit in tertiary care hospitals. Department of Microbiology, School of Medicine, Isfahan University of Medical sciences, Iran. 2018;30:330-336.

    https://doi.org/10.7416/ai.2018.2224
23. Shahcheraghi F, Abbasalipour M, Akbari N, Nikbin V, Ebrahimipour G. P24.09 Study of beta-lactamases in clinical Acinetobacter isolates from Tehran, Iran. Journal of Hospital Infection. 2010;76:S70.

    https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3279807/