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Detection of *bla*$_{OXA-23}$geneamong carbapenem-resistant*Acinetobacter species* isolated from various clinical samples

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Abstract: Invasive infections due to Acinetobacter baumannii are among the leading nosocomial infections in patients hospitalized in the intensive care unit (ICU). An increase in multidrug-resistant has been observed in recent years. A hospital-based prospective study was carried out on 287 laboratory-confirmed *Acinetobacter species* from various clinical samples out of which 232 (81%) were found to be Acinetobacter baumannii and other species of Acinetobacter were 55 (19%). Most of the isolates were obtained from pus samples (30.31%) followed by Urine (25.43%), ET Tip (14.28%) ET Aspirate (12.19%) blood (4.18%). The number of Isolates was more in IPD (91.99%) and less in OPD (8.01%). Among IPD patients, the highest number of isolates were obtained from ICU (52.65%), surgery (21.97%), and Obstetrics and gynecology (13.63%). Isolates showed the highest resistance towards cephalosporins Ampicillin (89%) followed by Cefotaxime (87%), Ceftazidime (85%), and ceftriaxone (84%). Among the carbapenem group, Meropenem was found more resistant in comparison to Imipenem with a resistance rate of 61% and 56% respectively. Colistin was found to be the most effective drug. Carbapenem resistance among other species of Acinetobacter was 172 (61.82%) by the Modified carbapenem inactivation method (mCIM). Out of 172 carbapenem-resistant isolates screened, 144 (83.72%) gave MHT positive that confirms the production of carbapenemase by the isolates. A total of 140 (97.23%) out of 144 MHT positive isolates showed the presence of the target gene i.e., blaOXA-23.

Keywords: AcinetobacterBaumannii, Metallo-β lactamases (MBLs), Carbapenemase, Modified carbapenem inactivation methods (mCIM), Modified Hodge Test (MHT).

1. INTRODUCTION:

The genus Acinetobacter includes opportunistic pathogens that can be causing both community- and healthcare-associated infections. It has recently emerged as a major cause of infection due to its predisposition to accumulate resistance to multiple antimicrobial drugs.(1)It causes nosocomial infection worldwide, including skin and soft tissue infections, wound and bloodstream infections, urinary tract infections, meningitis, and ventilator-associated pneumonia, the most common and fatal infection caused by A. baumannii.(2)Acinetobacter baumannii isthe most common and clinically significant among the Acinetobacter species.(3)In recent years, the multidrug resistance of A. baumannii against the routine drugs of choice has become a major concerndue to their alarmingly rise in the

resistance pattern of carbapenems and are considered as sentinels of drug resistance with the designation as carbapenem-resistant A. baumannii (CRAB).[4]Resistance to carbapenems is the most concerning, as carbapenems have potent activity against Acinetobacter species and are often used as a last resort for the treatment of infections caused bymultidrug-resistant A. baumannii isolates.[5]Antibiotic resistance in A. baumannii is mediated by enzymatic degradation of antibiotics, mutations/modification of target sites, reduced expression of porins, andoverexpression of multidrug efflux pumps.[6]Infections caused by CR-AB pathogens are difficult to treat due to the acquisition of various resistant genes such as class D oxacillinase (OXA) and Metallo-β-lactamase (MBL).[7]The production of acquired OXA-type carbapenemase is the main mechanism of resistance to carbapenems in A.baumanniiwhich have been frequently identified worldwide. These are clustered in three major subfamilies (blaOXA-23, blaOXA-24, andblaOXA-58), and more rarely Metallo-β-lactamases (VIM, IMP,and SIM types), which have been infrequently reported in someparts of the world.[8]
OXA type carbapenemase, which canhydrolyse carbapenem and be first studied from a clinical isolate of A. baumannii, isplasmid-encoded and transferable. It was named blaOXA−23 and is now studied extensively because it is the most common mechanism that contributes to carbapenem resistance in A. baumannii. The blaOXA−23 gene clusterhas two other enzymes that are closely related, blaOXA−27 and blaOXA−49. In addition, two more gene clusters contributing to resistance that include blaOXA−24-like and blaOXA−58-like have been reported.[3][9] The upstream of OXA-type class D carbapenemases in Acinetobacter is often associated with insertion sequence (IS), of which ISAba1 is the most commonly detected. ISAba1 and other IS may modulate the expression and transfer of OXA-type carbapenemase genes.[10] Since, hospital acquired infection caused by Acinetobacter species has been increased and causing obstacles in treatment of hospitalised patients, it is important to know the prevalence of antibiotic resistance pattern and resistance genes of the bacteria to initiate antibiotic and to control, prevent and cure infections caused by Acinetobacter species. Therefore, the aim of this study was to detect the antibiotic susceptibility profile of the isolated organism and to see the carbapenemase production by the isolates and to detect the frequency of carbapenemase encoding gene blaOXA-23 in Acinetobacter species isolates obtained from various clinical samples.

2. MATERIAL AND METHODS:

A hospital-based prospective study was carried outbetween January 2019 and December 2020 in the Department of Microbiology, Index Medical College, Hospital & Research center, Indore. A total of 287 laboratory-confirmed *Acinetobacter species* isolated from various clinical samples. Ethical clearance was taken from the institutional ethical committee.

Sample collection and processing: Samples were collected under aseptic precautions by standard procedures and processed according to standard guidelines. Direct smears with Gram stain were screened for the presence of inflammatory cells and type of microbial flora. Followed by growth characterisation on blood agar and MacConkey's agar plate. Brain Heart Infusion broth was used for blood culture. Isolates were confirmed by putting a battery of biochemical tests.

The antibiotic susceptibility test was performed by the Kirby Bauer Disc diffusion method on Muller Hinton agar using Ampicillin, Amoxiclav, gentamicin, amikacin, cotrimoxazole, piperacillin-tazobactam, ceftriaxone, ceftazidime, Cefepime, cefotaxime, ampicillin-sulbactam, Tetracycline, imipenem meropenem, Colistin and Polymyxin B recommended by the CLSI guidelines 2018.

Screening method for detection of carbapenemase production: Modified carbapenem inactivation methods (mCIM) for suspected carbapenemase production in Acinetobacter sp. mCIM was used for detecting carbapenemase in Acinetobacter sp.Modified Hodge Test (MHT)was performed for the confirmation of carbapenemase production by the isolates as described by **Amjad A. et.al.**[11]

PCR screening for the detection of OXA-type carbapenemases blaOXA-23:

DNA extraction: DNA extraction was done as described by **M. Smiljanic et.al**. [12]

PCR Analysis of carbapenemase-encoding gene:

Identification of the oxacillinase (blaOXA-23) gene was done by Multiplex Real-time PCR assays as described by **QiuYanget. et.al**.[13] and **BadrulHasan**[14]**et.al.** using the primers OXA-23- (F: GATCGGATTGGAGAACCAGA and R: ATTTCTGACCGCATTTCCAT). The details of the reference genes used in the assays were obtained from the NCBI homepage (http://www.ncbi.nlm.nih.gov/).

In multiplex real-time PCR, primers specific for the blaOXA-23-positive strains were combined. The real-time PCR assays were performed on an QuantStuio-3 (Applied Biosystems) using the HotStarTaq polymerase µl-1 (Qiagen) as recommended by the manufacturers.

Standard curve and sensitivity test on recombinant plasmids:

To determine the efficiency of the multiplex real-time PCR assays, the Ct values obtained from a series of template DNA dilutions were graphed on the y axis versus the log of the dilution on the x axis.

3. RESULTS & DISCUSSION:

A total of 287Acinetobacter species were isolated and processed of which, 232 (81%) infections were found to be due to *Acinetobacter baumannii*. This was concordance with the study of **Safa Hasan Radhi** [15] **et. al.** which shows 85.72% i.e., 30 Acinetobacter baumannii out of 35 Acinetobacter species isolated in their study. A study done by **AnushaKarunasagar**[9] **et.al.**at Mangalore India found 77.42% of Acinetobacter baumannii. Another study by **Neetu Gupta** [16] **et.al.**revealed a lower rate (72%) of *Acinetobacter baumannii* isolates in their study in comparison to our study.

Most of the isolates were found in male 161 (56%) in comparison to female 126(44%). A total of 137 Acinetobacter baumannii were isolated in male and 95 (33.1%) in female. Other species of Acinetobacter species were found mostly in female 31 (10.8%) in comparison to male 24 (8.36%). **(Table-I)**

Table-I: **Gender wise distribution of Acinetobacter sp. Isolates**

Gender	Acinetobacter baumannii	Acinetobacter Sp.	P Value
Male	137 (47.73%)	24(8.36%)	0.0383
Female	95 (33.1%)	31 (10.8%)	

This is in line with the study of **Baris Boral** [17] **et.al.**that showed a positivity rate of 53.6% in male and 46.3% in female. **BhuvaneshSukhlalKalal**[18] **et.al.**in their study found almost a

similar result with the present study with a distribution of 55% isolates in male and 45 % in female that supports present study.

Highest number of isolates were obtained from pus samples 87 (30.31%) followed by urine 73(25.43%), ET Tip 41 (14.23 %), ET aspirate 35 (12.19%), Sputum 22(7.67%), Blood 12 (4.18), Umbilical Tip 7(2.44%), only 2 (0.69%) isolates were from Pleural Fluid, semen, Ascitic fluid, Catheter Tip, and Foley's tip and High Vaginal swab showed growth in 1 (0.34%) isolate each **(Table-II).** The highest number of isolates 30.31% were obtained from pus samples which are supported by a study done by **BhuvaneshSukhlalKalal**[18] **et.al.**where they revealed 28.1% of positivity rate in pus samples. **Dr. N. Rathnapriya**[19] **et.al.**in their study revealed the isolation rate from various samples and the result was Urine (50%), blood (20%), and (10%) from pus samples. A study was done by **Zahra Moulana**[20] **et. al**. revealed that 4% of isolates were from blood samples which are almost similar to our study but they found the highest rate (60%) of isolates from respiratory samples and endotracheal aspirates that's again much higher than our study which shows 37.17% of growth rate combine.

Table-II Clinical specimens showing isolation rates of Acinetobacter species.

Sample Type	**No. Of Isolates**	**Percentage**
Pus	87	30.31
Urine	73	25.43
ET Tip	41	14.28
ET Aspirate	35	12.19
Sputum	22	7.67
Blood	12	4.18
Umbilical Tip	7	2.44
Pleural Fluid	2	0.69
Semen	2	0.69
Ascitic fluid	2	0.69
Catheter Tip	2	0.69
Foley's tube	1	0.34
High Vaginal Swab	1	0.34

The majority of Acinetobacter species were isolated from IPD patients (91.99) predominantly from ICU possessing 52.65 % of total IPD samples followed by Surgery ward 58 (21.97%), Obstetrics and gynaecology ward 36 (13.63%), and General Medicine ward 14 (5.30%) **(Table-III)**.

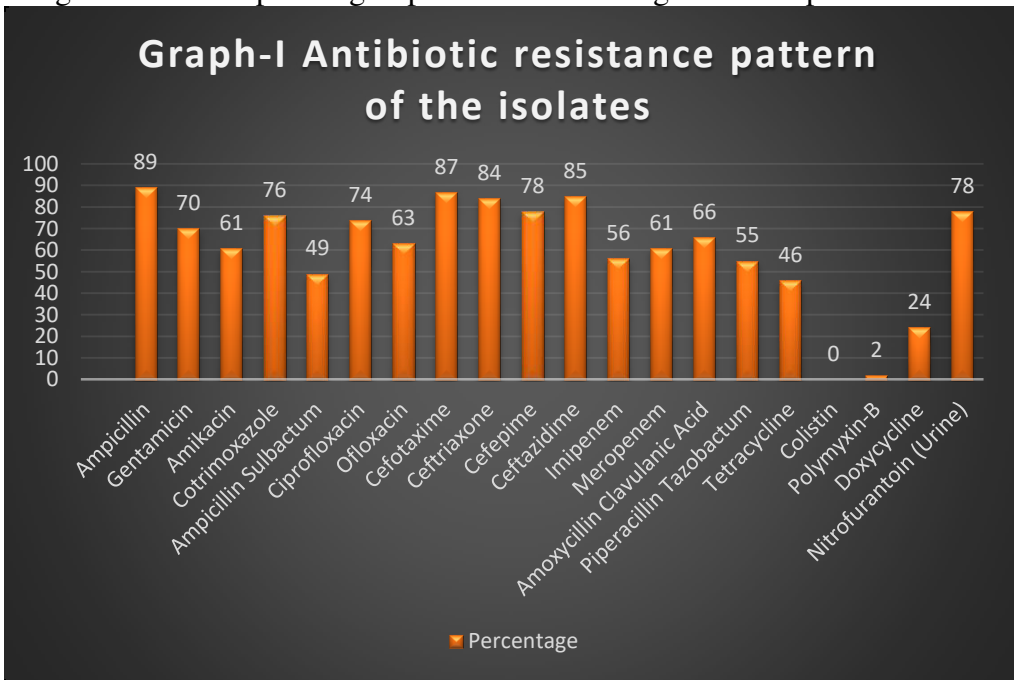
Table- III: Inpatient Distribution of Isolation of *Acinetobacter* species.

Ward	**No. of Isolates (264)**	**Percentage (100)**
ICU	139	52.65
Surgery	58	21.97
OBG	36	13.63
Medicine	14	5.30
Ortho	8	3.03
Labor Room and Nursery	5	1.89
Pediatrics	2	0.75
TB & Chest	2	0.75

This is supported by the studies of **Amrita Talukdar** [21] **et. al.** in which 51% of isolates were obtained from ICU wards, 23% from combined neurosurgery and general surgery department but reported a higher rate of isolates from the Medicine department. Also, this result is in line with the study of certain international studies. **Faten M. Elabd**[5] **et.al.**in their study showed 61.1 % isolates from ICU, 17.6% from General Surgery and 12% of isolates were obtained from the Medicine ward. **Hasan Ejaz** [7] **et. al.** in their study in Pakistan showed a higher number of isolates from ICU patients in comparison to the present study. A rate of 75.2% of isolates was obtained from ICU, 10.2 % from surgery, and 9.7 % were from the Medicine ward. **Neelam Taneja** [22] **et.al**. in their study conducted at PGIMER Chandigarh found 22.8% of isolates from ICU, 9% from Medicine, and only 6% were from the Surgery ward.

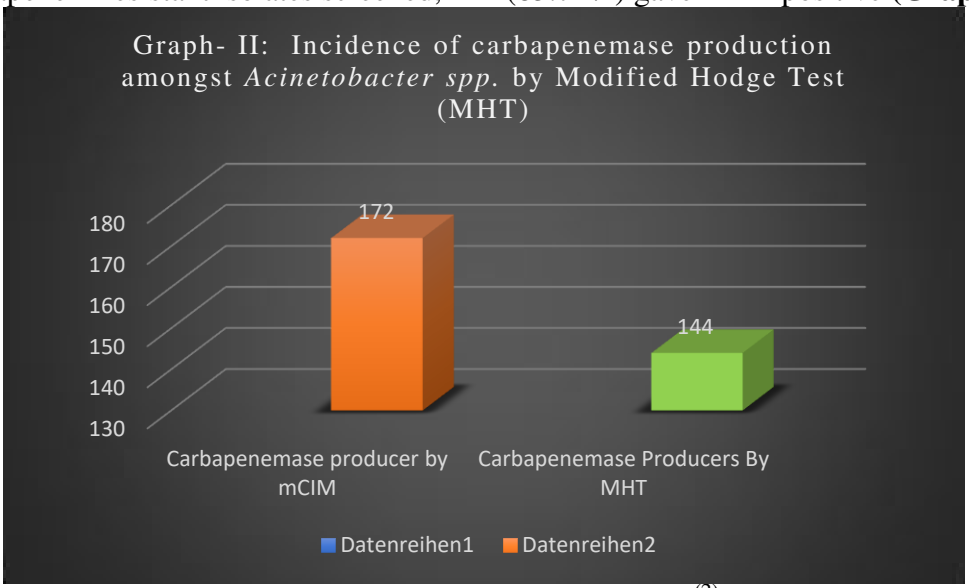
Acinetobacter infection was higher among the age group of 60 years and above i.e., 63 out of 287 which is 21.95% followed by 56 (19.51%) of isolates were found in 21-30 years, 48 in 51-60 years, 35 in 41-50 years, 33 in 31-40 years, 25 (8.71%) were from children below 1 Year and 6 (2.09%) isolates from the age group of 2- 10 years. Antibiotic susceptibility profile revealed the highest resistance towards Ampicillin (89%) followed by Cefotaxime (87%), Ceftazidime (85%), and ceftriaxone (84%). The resistance pattern of quinolones i.e., Ciprofloxacin and Ofloxacin showed a rate of 74% and 63% respectively. Among aminoglycosides, Amikacin (61%), and Gentamicin (70%) isolates were found resistant. Nitrofurantoin used only in isolates from urine samples showed 78% resistance. Among the carbapenem group, Meropenem was found more resistant in comparison to Imipenem with a resistance rate of 61% and 56% respectively.

Resistance pattern of other antibiotics Cotrimoxazole (75%), Ampicillin Sulbactam (49%), Piperacillin Tazobactam (55%), Amoxycillin Clavulanic Acid (66%). Colistin was found to be the most effective drug against all the isolates with a zero percent resistance followed by polymyxin B against which only 2% of resistance was shown **(Graph-I)**. This was in concordance with the several studies within India and other countries. **DabetRynga**[23] **et.al.**in their study showed a 100 % resistance pattern against the cephalosporin group and resistance against the carbapenem group was also much higher in comparison with our study.



They reported that 85 % of isolates were resistant to Imipenem whereas in this study it was only 56%. Another study by **Neelam Taneja** (22) **et.al**. showed that only 25.4% were resistant to Imipenem and 74.1% against Cefotaxime. Also they revealed 79.5% and 73.2 % of resistance towards Gentamicin and Amikacin respectively. **BadrulHasan**(14) **et.al.**in their study revealed most of the cephalosporins were resistant, (98.8%) to ceftriaxone and (93%) to cefepime. In our study, we got 100% susceptibility towards Colistin which is supported by the study of **Yisheng Chen** [24] **et. al.**their study found 100% susceptibility towards Colistin. In other studies a variable results in colistin resistance. **KritThirapanmethee**[25] **et. al.** showed that 87.98% of isolates were sensitive toward colistin.

Modified carbapenem inactivation methods (mCIM) for suspected carbapenemase production in Acinetobacter sp. and the result revealed that 59.93% of isolates were carbapenemase producer, which was further processed for confirmation by Modified Hodge Test and out of 172 carbapenem-resistant isolates screened, 144 (83.72%) gave MHT positive **(Graph- II)**.



This is in concordance with the study of **AlaaAbouelfetouh**[2] **et. al.** where they found a 78.4% positivity rate by MHT. Another study by Amrita Talukdar et.al. showed 84%, Another study by **Zahra Moulana**[20] **et.al.** showed an 84% of positivity rate by the Modified Hodge Test. **Laxmi Neupane** [26] **et.al.**in their study reported a 69.8% of positivity rate by MHT which is lower than our study. **Mohammed Gohar Mohammed Elsherbeny et.al.**[27]in their study also found a 70% of positivity rate by this method. All the 144 MHT positive isolates were further assessed for the detection of blaOXA-23 gene by multiplex PCR and revealed that 140 (97.23%) of the isolates possess the target gene. The result of the current study is in agreement with several studies that showed almost similar results. **Baris Boral**[17] **et.al.**reported 96% of blaOXA-23 gene detection in their study. **BhuvaneshSukhlalKalal**[18] **et.al**. in their study in South India showed 98% of gene detection rate. Another study by **AlaaAbouelfetouh**[2] **et.al.**showed 100% of blaOXA-23 gene detection in their study.

4. CONCLUSION:

In Conclusion, our study revealed a higher rate of carbapenem producing organisms and their resistance towards commonly used antibiotics including carbapenems due to the presence of blaOXA-23 gene. So, this is a serious threat and also the possible cause of recurrences so,

early detection, identification of the organism, and knowing the mechanism of drug resistance of these isolates in a diagnostic laboratory could help to avoid treatment failure.

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