ORIGINAL RESEARCH PAPER

Volume - 11 | Issue - 06 | June - 2022 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

EMERGENCE OF CARBAPENEM RESISTANT ACINETOBACTER SPECIES ISOLATED FROM VARIOUS CLINICAL SPECIMENS IN TEACHING HOSPITAL FROM CENTRAL INDIA

Microbiology

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ABSTRACT

Acinetobacter Species is one of the most challenging life-threatening pathogens. Increase in multidrug resistant has been observed in recent years. A hospital based prospective study was carried out. A total of 287 laboratory confirmed Acinetobacter species isolated 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 sample (30.31%) followed by Urine (25.43%), ET Tip (14.28%) ET Aspirate (12.19%) blood (4.18%). Number of Isolates was more in IPD (91.99%) and less in OPD (8.01%). Among IPD patients, highest number of isolates were obtained from ICU (52.65%), surgery (21.97%), Obstetrics and gynecology (13.63%). Isolates showed highest resistance towards Ampicillin (89%) followed by Cefotaxime (87%), Ceftazidime (85%) and ceftriaxone (84%). Among carbapenem group, Meropenem was found more resistance in comparison to Imipenem with a resistance rate of 61% and 56% respectively. Colistin found to be the most effective drug. Carbapenem resistance among other species of Acinetobacter was 172 (61.82%) by 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.

KEYWORDS

Acinetobacter Baumannii, metallo-β lactamases (MBLs), Carbapenemase, Modified carbapenem inactivation methods (mCIM), Modified Hodge Test (MHT).

INTRODUCTION: *Acinetobacter* species are aerobic gram-negative bacilli. These are opportunistic pathogen that causes a range of infections, including bacteraemia, pneumonia, meningitis, urinary tract infection and wound infection (1). They also causes healthcare-associated infections and can survive for longer duration in the environment and on the hands of healthcare workers(2). Among the Acinetobacter species, the most common and clinically significant species is Acinetobacter baumannii. (1) Acinetobacter baumannii has become one of the most challenging life threatening Pathogens (3). It is an important nosocomial pathogen and is considered as a frequent cause of infections among patients in intensive care units (ICUs) —(4). It has become a challenging pathogens for global healthcare because of rapid acquisition of antibiotic-resistance biomarkers(5). Infections are often difficult to eradicate due to acquisition of intrinsic and acquired mechanisms by the pathogens leading to a high-level resistance to many antibiotics (6). Various resistance mechanisms of Acinetobacter strains include efflux pumps, β -lactamases, and modifications in porin proteins. Enzymatic degradation by β -lactamases is the leading Prevalent mechanism of β -lactam resistance in A. baumannii. Serine oxacillinases and metallo-β lactamases (MBLs) are β-lactamases with carbapenemase activity (7). Carbapenems are the antibiotics of choice, for the treatment of infections caused by A. baumannii when these bacteria are resistant to other β-lactam antibiotics. (8). But infections due to carbapenem-resistant gram-negative bacteria, especially bacteraemia, are increasingly challenging and have become a major concern for clinicians worldwide (9). Often, there are very limited or no remaining options to treat A. baumannii infections (10). Due to unpredictable multidrug resistance patterns of Acinetobacter, it is important to know the institutional prevalent susceptibility profiles (11) . Hence, this study was conducted to isolate and identify the Acinetobacter species from various clinical samples by phenotypic method and to determine the antibiotic susceptibility pattern and to investigate the characterization of carbapenemase producing isolates.

MATERIALS AND METHODS: A hospital based prospective study was carried out in the Department of Microbiology, Index Medical College, Hospital & Research centre, Indore. A total of 287 laboratory confirmed *Acinetobacter species* isolated from various clinical samples over a period of 2 years between January 2019 and December 2020 were included in the study. Ethical clearance was taken from institutional ethical committee.

Sample collection and processing: All 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. Specimens were inoculated 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. Antibiotic susceptibility test was performed by the Kirby Bauer Disc diffusion method on Muller Hinton agar. Antimicrobial agents tested were 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): MHT was performed for the confirmation of carbapenemase production by the isolates as described by Amjad A. et.al.(12). A 0.5 McFarland dilution of the Escherichia coli ATCC 25922 in 5 ml of broth or saline was prepared. A 1:10 dilution was streaked as lawn on to a Mueller Hinton agar plate. A 10 µg meropenem or ertapenem susceptibility disk was placed in the centre of the test area. Test organism was streaked in a straight line from the edge of the disk to the edge of the plate. The plate was incubated overnight at 35 ± 2oC in ambient air for 16-24 hours.

Interpretation of result: After 24 hrs, MHT Positive test showed a clover leaf-like indentation of the Escherichia coli 25922 growing along the test organism growth streak within the disk diffusion zone. MHT Negative test showed no growth of the Escherichia coli 25922 along the test organism growth streak within the disk diffusion.

RESULTS & DISCUSSION:

A total of 287Acinetobacter species were isolated and processed of which, 232 (81%) infections were found to be due to *Acinetobacter baumannii* (Table-I).

Table-I: Distribution of isolated Acinetobacter species

Acinetobacter isolated	Acinetobacter Baumannii	Acinetobacter species
287	232 (81%)	55 (19%)

This was concordance to the study of Safa Hasan Radhi (7) et. al. which shows 85.72% i.e., 30 Acinetobacter baumannii out of 35 Acinetobacter species isolated in their study. Study done by Anusha Karunasagar (13) et.al. at Mangalore India found 77.42% of Acinetobacter baumannii. Another study by Neetu Gupta (11) et.al. revealed a lower rate (72%) of Acinetobacter baumannii isolates in their study in comparison to our study.

Maximum isolated species were from male 161(56%) in comparison to female 126 (44%). Most of the 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). Highest number of isolates 30.31% were obtained from pus samples which is supported by a study done by Bhuvanesh Sukhlal Kalal (14) et.al. where they revealed a 28.1% of positivity rate in pus samples. Dr. N. Rathnapriya (15) et.al. in their study revealed the isolation rate from various sample and the result was Urine (50%), blood (20%) and (10%) from pus samples. Study done by Zahra Moulana (16) et. al. revealed 4% of isolates were from blood samples which is almost similar to our study but they found 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

Majority of Acinetobacter species were isolated from IPD patients (91.99) predominantly from ICU that possess 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).

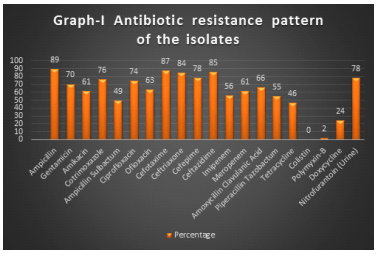
Table- III: Inpatient Distribution of Isolation of Acinetobacter species.

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 (6) 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 Medicine department. Also, this result is in line with the study of certain international studies. Faten M. Elabd (17) et.al. in their study showed 61.1 % isolates from ICU, 17.6% from General Surgery and 12% of isolates were obtained from Medicine ward. Hasan Ejaz (18) et. al. in their study in Pakistan showed a higher number of isolates from ICU patients in comparison to present study. A rate of 75.2% of isolates were obtained from ICU, 10.2 % from surgery and 9.7 % were from Medicine ward. Neelam Taneja (19) et.al. in their study conducted at PGIMER Chandigarh found 22.8% of isolates from ICU, 9% from Medicine and only 6% were from Surgery ward.

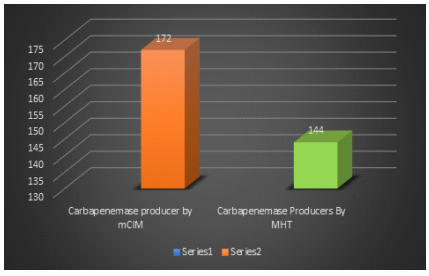
There was higher incidence of Acinetobacter infection among the age group of 60 years and above i.e., 63 out of 287 which is 21.95% followed by 56 (19.51%) of isolates were found in 21-30 years, 48 in 51-60 years, 35 in 41-50 years, 33 in 31-40 years, 25 (8.71%) were from children below 1 Year and 6 (2.09%) isolates from the age group of 2- 10 years. Antibiotic susceptibility profile revealed highest resistance towards Ampicillin (89%) followed by Cefotaxime (87%), Ceftazidime (85%) and ceftriaxone (84%). Resistance pattern of quinolones i.e., Ciprofloxacin and Ofloxacin showed a rate of 74% and 63% respectively. Among aminoglycosides, Amikacin (61%), Gentamicin (70%) isolates were found resistant. Nitrofurantoin used only in organisms isolated from urine sample showed 78% resistance. Among carbapenem group, Meropenem was found more resistance 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 of 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 in other countries. Dabet Rynga (20) et.al. in their study showed 100 % resistance pattern against cephalosporin group and resistance against carbapenem group was also much higher in comparison with our study.



They reported 85 % of isolates were resistant towards Imipenem whereas in this study it was only 56%. Another study by Neelam Taneja (19) et.al. showed that only 25.4% were resistant against Imipenem and 74.1% against Cefotaxime. In their study resistance pattern of Gentamicin and Amikacin were 79.5% and 73.2 % in line with that we found 70% and 61% of resistance respectively. Badrul Hasan (5) et.al. in their study showed that most of the antibiotics of cephalosporin group were found 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 (21) et. al. where they found 100% susceptibility towards Colistin. Other studies a variable result in colistin resistance. Krit Thirapanmethee (22) et. al. showed 87.98% of isolates were sensitive towards colistin.

Modified carbapenem inactivation methods (mCIM) for suspected carbapenemase production in Acinetobacter sp. mCIM was used for screening of carbapenemase among Acinetobacter species and the result revealed 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).



Graph- II: Incidence of carbapenemase production amongst Acinetobacter spp. by Modified Hodge Test (MHT).

This is in concordance with the study of Alaa Abouel fetouh (3) et. al. where they found 78.4% positivity rate by MHT. Another study by

Amrita Talukdar er.al. showed 84%, Another study by Zahra Moulana (16) et.al. showed 84% of positivity rate by Modified Hodge Test. Laxmi Neupane (23) et.al. in their study reported 69.8% of positivity rate by MHT which is lower than our study. Mohammed Gohar Mohammed Elsherbeny et.al. (24) in their study also found 70% of positivity rate by this method.

CONCLUSION:

Increased in carbapenemase production by the isolates had showed a rise. So, drug resistance is a serious threat also the possible cause of recurrences so, early detection, identification of organism and knowing the mechanism of drug resistance of these isolates in a diagnostic laboratory could help to avoid treatment failure, as often the isolates producing this enzyme show a susceptible phenotype in routine susceptibility test. Aggressive infection control measures should be applied to limit the emergence and spread of these pathogens.

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