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ORIGINAL RESEARCH ARTICLE

Pattern of Antimicrobial Resistance in Clinical Isolates of Acinetobacter Species Isolated from Intensive Care Unit Patient's Sample at a Tertiary Care Hospital in North India

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ABSTRACT

Introduction: *Acinetobacter* species are one of the most common pathogens causing hospital-acquired infections (HAIs) and multi-drug resistant *Acinetobacter* isolates are a rapidly emerging pathogen in healthcare settings and have limited options for effective treatment. It is increasingly reported as the cause of outbreaks and nosocomial infections such as blood-stream infections, ventilator-associated pneumonia, urinary tract infections and wound infections. To determine the antimicrobial susceptibility/resistant pattern among isolated *Acinetobacter* species.

Material and Methods: A total of 140 *Acinetobacter* species were isolated from various clinical specimens. The isolated *Acinetobacter* species were further processed for antibiotic susceptibility testing (AST) using the Kirby-Bauer disc diffusion method. All Imipenem-resistant cases were further evaluated for Metallo-β-lactamase (MBL) production using Imipenem Ethylenediamine tetraacetic acid (EDTA) combined disc test and modified Hodge test, two phenotypic methods. Statistical analysis was done using Microsoft office excel 2010.

Results: The present study was carried out for one and a half years from 1st January 2013 to June 2014 in the microbiology department on the samples received from the intensive care unit (ICU) of SRMS-IMS Bareilly. During this period total of 140 *Acinetobacter* species were isolated. The majority were isolated from respiratory samples, followed by urine, pus and blood. *Acinetobacter* isolates were found to be resistant to most of the commonly used antibiotics. Antimicrobial susceptibility testing showed the highest resistance to cefepime 5/140 (96.43%), ceftazidime 6/140 (95.72%), levofloxacin 8/140 (94.29%), amikacin 9/140 (93.58%) and highest sensitivity 135/140 (96.42%) to polymyxin and colistin 132/140 (94.28%). 72/83 (86.7%) and 62/83 (74.7%) *Acinetobacter* species show metallo-β-lactamase (MBL) production by Imipenem-EDTA combined disc test and modified Hodge test, respectively.

Conclusion: The increasing trends towards antibiotic resistance reflect the extensive use of antibiotics in hospitals which in turn exerts selective pressure on *Acinetobacter* in the hospital environment. Therefore, surveillance is needed to detect multi-drug resistant (MDR) *Acinetobacter* species, judicious use of antibiotics and implement appropriate infection control measures to control the spread of these MDR strains in hospitals.

Keywords: *Acinetobacter* species, Antibiotic susceptibility testing, Hospital acquired infection, Multi drug resistance.

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INTRODUCTION

Antibiotic resistance is a primary concern of contemporary medicine. The continuing emergence of resistant strains that cause nosocomial infections contributes to substantially high morbidity and mortality among hospitalized patients. Of the nosocomial pathogens, *Acinetobacter* species are of most significant concern for hospitalized patients, particularly those in intensive care units (ICU), where these pathogens are capable of causing life-threatening severe invasive infections in critically ill and immunocompromised patients. Antimicrobial resistance among nosocomial isolates of *Acinetobacter* species, most importantly *Acinetobacter* baumanni, complicates the treatment of infections and adversely affects clinical outcomes and patient treatment costs.[1,2]

The advent of carbapenems in the 1980s heralded a new treatment option for serious bacterial infections. However, resistance to carbapenems has been frequently observed in gram-negative bacilli, specially non-lactose fermenters e.g., *Acinetobacter* species. The common form of resistance is mediated by lack of drug penetration (i.e., porin mutations and efflux pumps) and/or carbapenem hydrolysing-beta-lactamase enzymes including the metallo beta lactamases (MBL). MBLs are enzymes requiring divalent cations, usually zinc as metal cofactors for enzyme activity, being inhibited by the action of metal ion chelators such as ethylene diamine tetra acetic acid (EDTA). The MBLs efficiently hydrolyze all beta-lactams, except aztreonam, *in-vitro*.[3,4] Acquired MBLs are encoded

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mobile gene cassettes of the organism and such strains are often resistant to different groups of antimicrobial agents with transferable properties to various types of bacteria.[5] The rapid detection of MBL-producing gram-negative bacilli is therefore necessary to aid in infection control measures and to prevent their dissemination.[6]

Because of the prevalence of multiple resistance, MBL-producing isolates are often refractory to all the other treatment options, signaling the need to develop new, potent therapeutic agents with novel modes of action. Many hospitals are forced to resort to older and more toxic drugs such as colistin and polymyxin B.[7] Many risk factors are known to influence the mortality and survival of patients infected with MDR organisms.[8,9,10,11]

With this background, the present study was undertaken to study antimicrobial susceptibility patterns among *Acinetobacter* species and detect MBL production in carbapenem-resistant isolates.

MATERIALS AND METHODS

A descriptive study was conducted for one and a half year from 1st January 2013 to June 2014 in the Department of Microbiology and ICU of SRMS-IMS, Bareilly. ethical clearance was taken from the Ethical clearance committee. Clinical specimens were obtained from ICU patients aged 15 years and above.

The samples were processed following standard laboratory protocols. Samples were inoculated on blood agar, MacConkey agar and CLED agar. After overnight incubation growth was further identified by gram stain and standard biochemical methods. Finally, antibiotic susceptibility testing (AST) of the isolates was performed using Kirby-Bauer disc diffusion method as per Clinical and Laboratory Standard Institute (CLSI) guidelines and following most commonly used antibiotics (cefepime, ceftazidime, levofloxacin, amikacin, meropenem, imipenem, cefoperazone-sulbactam, piperacillin-tazobactam, colistin and polymyxin) were tested in the study.

Carbapenem (Imipenem and Meropenem) resistant *Acinetobacter isolates* were further processed for MBL production by using Imipenem-EDTA combined disc test and modified Hodge test. *Acinetobacter baumannii* species ATCC 19606 was used as quality control strains. Data analysis was done by graphs, charts and *p-value* was calculated with less than 0.05 considered as significant.

Imipenem-edta Combined Disc Test

Mueller Hinton agar (MHA) plate is prepared by inoculating with standard inoculum (0.5 McFarland) of the test organism to form a lawn culture. Two 10 mcg imipenem discs are placed on the plate. 10 mcl of 0.5M EDTA solution is added to one of the Imipenem discs to obtain a concentration of 750 mcg and was incubated overnight at 37°C. If an increase in inhibition zone with IMP + EDTA disc was ≥ 7 mm than IMP disc alone, it is considered an MBL producer.[8,9]

Table-1: Distribution of *Acinetobacter* isolates according to clinical samples (n = 140)

Sample	No. of samples	Percentage
Respiratory samples	104	74.3%
Urine	21	15%
Pus	10	7.1%
Blood	5	3.5%
Total	140	100%

Modified Hodge Test

The surface of the MHA plate was lawned evenly with an overnight culture suspension of *Escherichia coli* ATCC 25922, which was adjusted to one-tenth turbidity of the 0.5 McFarland standards. After allowing the lawn to dry, an imipenem disc was placed in the Centre of the plate and imipenem-resistant isolates from the overnight culture plates were streaked heavily from the edge of the disc to the periphery of the plate. The presence of a distorted inhibition zone (Cloverleaf shape) after overnight incubation was interpreted as modified Hodge test positive.[8]

RESULTS

In the present study 140 *Acinetobacter* species were isolated from ICU samples and most were obtained from the respiratory tract 104 (74.3%) followed by urinary tract 21 (15%), pus 10 (7.1%) and blood 5 (3.57%). (Table 1). These isolates were found resistant to most commonly used antibiotics (Table 2). The highest resistance was seen against cephalosporins cefepime; (96.43%), ceftazidime (95.72%), fluoroquinolone, levofloxacin (94.29%), aminoglycoside amikacin (93.58%). Resistance to carbapenems (Imipenem and Meropenem) was recorded at 59.29 and 82.15%, respectively.

The present study showed an increased prevalence of resistance of the *Acinetobacter* isolates against piperacillin/tazobactam and cefoperazone/sulbactam combination, which was found at 88.58% and 82.15%, respectively. In this study *Acinetobacter* species showed maximum sensitivity to colistin (94.28%) and polymyxin B (96.42%) In the present study, 83 isolates were found resistant to most drugs, including carbapenems such as imipenem and meropenem. They were also resistant to fluoroquinolones, aminoglycosides, and β lactam–β lactamase inhibitor combination drugs, and these isolates were further processed for MBL detection (Table 3) by two phenotypic methods. 83/72 (86.7%) and 83/62 (74.7%) isolates were found MBL positive by imipenem-EDTA combined disc test and modified Hodge test, respectively.

DISCUSSION

Resistance to antimicrobial agents is an increasing public health threat. It limits the therapeutic options and leads to increased mortality and morbidity.[1,11] Resistance to carbapenem in *Acinetobacter* species is often due to loss of outer membrane proteins and upregulation of active efflux pumps or production of MBL. MBLs have been identified from clinical isolates worldwide with increasing frequency over the past few years and strains producing these enzymes have been responsible for prolonged nosocomial outbreaks that were accompanied by serious infections.[5] The development of a simple and inexpensive screening method is necessary to detect MBL production in a microbiology laboratory.

Among 140 *Acinetobacter* species isolated from samples of ICU patients, most of the isolates were obtained from the respiratory tract 104 (74.3%), followed by urinary tract 21 (15%), pus 10 (7.1%) and blood 5 (3.57%). A similar result was seen in a study done by Shanti M *et al.* in Chennai, India, which showed 41.8% from respiratory samples followed by urine (25.5), wound (20%) and blood (12.7%) (Table 1).

These isolates were found resistant to most commonly used antibiotics (Table 2). The highest resistance was seen against cefepime; (96.43%), ceftazidime (95.72%), levofloxacin (94.29%), amikacin (93.58%) which was similar to the finding of other studies such as Patel *et al.* showed 100% resistance against Cefepime and ceftazidime, guvan *et al.* showed around 97% resistance against cefepime and ceftazidime between 2008 to 2011. Jaggi *et al.* showed 90.3, 92.1, 87.6 and 84.2% resistance against cefepime, ceftazidime, levofloxacin and amikacin, respectively. In the present study, resistance to Carbapenems recorded was 59.29 and 82.15% against imipenem and meropenem, respectively. Increasing resistance against imipenem (54% in 2008 and 99.9% in 2011) and meropenem (73.5% in 2008 and 99.9% in 2011) also shown by Guvan *et al.* In another study, Jaggi *et al.* showed relatively high resistance against Imipenem(89.6%) and Meropenem (89.6%).

Table 2: Antibiotic susceptibility pattern of *Acinetobacter* species against different antibiotics (n=140)

Drugs	Total no. of isolates	Sensitivity (%)	Resistant (%)
Cefepime	5	3.57	96.43
Ceftazidime	6	4.28	95.72
Levofloxacin	8	5.71	94.29
Amikacin	9	6.42	93.58
Meropenem	25	17.85	82.15
Imipenem	57	40.71	59.29
Cefoperazone sulbactam	25	17.85	82.15
Piperacillin-tazobactam	16	11.42	88.58
Colistin	132	94.28	5.72
Polymyxin	135	96.42	3.58

The present study showed an increased prevalence of resistance of the *Acinetobacter* isolates against Piperacillin/tazobactam and cefoperazone/sulbactam combination, which was found at 88.58% and 82.15%, respectively. This is in accordance with the studies done by Jaggi *et al.* which showed 89.7% resistance against piperacillin/tazobactam and 92.3% against cefoperazone/sulbactam. Another study by Jamshidi *et al.* showed 88.58% resistance against piperacillin/tazobactam and 82.15% against cefoperazone/sulbactam.

In this study *Acinetobacter* species showed maximum sensitivity to colistin (94.28%) and polymyxin B (96.42%), similar to many other studies done by Jamshidi *et al.* showed 94.28% and 96.42% sensitivity against colistin and polymyxin B, respectively, Bose *et al.* showed 100% sensitivity against both colistin and polymyxin B, and Jaggi *et al.* showed 98.8% sensitivity against colistin and 98.1% against polymyxin B.

In the present study, out of a total of 140 isolates of *Acinetobacter* species, 83 isolates were found carbapenem-resistant. These isolates were further processed for MBL detection and found 72 (86.7%) MBL positive by imipenem-EDTA combined disc test and 62 (74.7%) by modified Hodge test. The study done by Muthusamy *et al.*, shows 20% MBL production by the Modified Hodge test, while a study done by Prakash *et al.* and Singla *et al.* shows 32% and 55.7% MBL production, respectively by imipenem-EDTA combined disc test. Another study by Hasan R *et al.* and Irfan S *et al.* found high 96.6% and 97% MBL production, respectively.

Several studies have reported screening methods using metal chelators such as EDTA.[3,5,6] We screened for MBL production by two phenotypic methods and a majority of the study isolates showed MBL production. Though

Table 3: Detection of metallo-β-Lactamase by 2 methods in *Acinetobacter* species (n = 140)

Method	Total no.	Imipenem resistant isolates	MBL positive isolates	Percentage (%)
Imipenem-EDTA Combined Disc Test	140	83	72	86.7
Modefied Hodge Test	140	83	62	74.7
Inference	Both methods are significantly correlated with detection of MBL with p-value = 0.036			

molecular methods for the detection of MBL-producing genes is the confirmatory test, easy and simple phenotypic tests are required for early identification. EDTA disk screening test is a useful tool for clinical laboratories to notify the treating physicians and also devise methods to contain their spread

CONCLUSION

The increasing trends towards antibiotic resistance reflect the extensive use of antibiotics in hospitals, which exerts selective pressure on *Acinetobacter* in a hospital environment. Therefore, there is a need for systematic surveillance to detect MDR and MBL-producing *Acinetobacter* species, judicious use of antibiotics, and implementation of appropriate infection control measures to control the spread of these MDR and MBL-producing strains in the hospitals. The combined-disk test, which is simple and susceptible method, can be used for the routine phenotypic detection of MBL production in any laboratory settings.

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