Original Article

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Microbiology Section

Phenotypic Methods for the Detection of Various Betalactamases in Carbapenem Resistant Isolates of Acinetobacter baumanii at a Tertiary Care Hospital in South India

DHEEPA MUTHUSAMY, APPALARAJU BOPPE

ABSTRACT

Purpose of the Study: The purpose of this study was to evaluate the production of various beta lactamases among multi-drug resistant *Acinetobacter baumanii* isolates from clinical specimens.

Methods Used: A total of 100 *Acinetobacter baumannii* meropenem resistant isolates were used for the detection of carbapenemases (Modified Hodge test), AmpC beta lactamase (the AmpC disc test) and metallo beta lactamase (the Disc potentiation test).

Results and Conclusion: Among 100 meropenem resistant isolates, 20% were carbapenemase producers, 73% were AmpC producers, and 10% were metallo beta lactamase producers. MBL production, AmpC beta lactamase production and carbapenemase production is becoming common in the multi drug resistant isolates of *A. baumannii*. Our study suggests that the disc potentiation test, the Amp C disc test, and the Modified Hodge test are simple tests that can be done routinely in the lab for all the multi drug resistant isolates of *A. baumannii*.

Key Words: *Acinetobacter baumanii*, Metallo beta lactamase, Amp C, Carbapenem resistance, Phenotypic methods

INTRODUCTION

Acinetobacter baumannii (A. baumanii) has recently emerged as a major cause of hospital acquired infections, because of the extent of its anti-microbial resistance and its propensity to often cause large and multi-facility nosocomial outbreaks. It causes a wide range of clinical infections such as pneumonia, septicaemia, urinary tract infections, wound infections and meningitis [1]. The mortality in patients who suffer from the *A. baumannii* infections can be as high as 75%.

Multi-drug resistant isolates of *Acinetobacter baumannii* have reportedly been increasing during the last decade, probably as a consequence of the extensive use of anti-microbial agents. Multi-drug resistance is witnessed only in *A.baumannii* and not in any other species of *Acinetobacter.* Multi-drug resistant (MDR) *A.baumannii* is not a new or emerging phenomenon, as *A. baumannii* has always been an organism which was inherently resistant to multiple antibiotics [2]. Thus, the infections which are caused by *A. baumannii* are frequently found in intensive care units and thus, it is implicated as a cause of ventilator associated pneumonia, urinary tract infections and bacteraemia. Less frequently, *A.baumannii* also causes complicated skin and soft tissue, abdominal, and central nervous system infections [3].

Carbapenem resistance in *Acinetobacter* is now being observed increasingly worldwide and it constitutes a sentinel event for the emerging anti-microbial resistance. The purpose of this study was to look at the screening tests which are used for the detection of various β-lactamase productions among the multi-drug resistant *Acinetobacter baumanii* isolates from clinical specimens.

MATERIALS AND METHODS

Sample Collection: A total of 100 *Acinetobacter baumannii* isolates from samples of sputum, urine, wound swabs, tracheal tips, tracheal aspirates, central line tips, ear swabs, blood, pus, bronchial lavage and endotracheal tubes, which were received in the microbiology laboratory of PSG hospitals, Coimbatore, South India, from July 2009 to January 2010, were included in the study. This was a prospective study where convenience sampling was done for selecting the isolates. The anti-microbial susceptibility testing was performed by using 13 different therapeutically relevant antibiotics, by the Kirby Bauer disk diffusion method, according to the norms of the Clinical Laboratory Standards institute (CLSI). The antibiotic discs tested were obtained from Hi-Media, Mumbai.

DETECTION OF VARIOUS BETALACTAMASES:

1. The Modified Hodge Test

The meropenem resistant strains were subjected to the modified Hodge test for the detection of carbapenemases. An overnight culture suspension of *Escherichia coli* ATCC 25922 which was adjusted to 0.5 Mcfarland's standard, was inoculated by using sterile cotton swabs, on the surface of Muller-Hinton agar plates. After drying, 10µg meropenem discs were placed at the center of the agar plates. The test strain was streaked from the edges of the discs to the peripheries of the plates in four different directions .The plates were then incubated overnight at 37°C. The presence of a clover leaf shaped zone of inhibition in the test strain growth was considered as positive for carbapenemase production [4].

2. The Disc Potentiation Test

This test was done for the detection of metallo β-lactamase production. A 750µg EDTA solution (pH 8.0) was prepared and it was sterilized by auto claving. The test organisms were inoculated onto plates of Muller-Hinton agar (the opacity of the organisms were adjusted to 0.5 McFarland's opacity standards). Two 10µg imipenem discs were placed on the inoculated plates and 10µl of EDTA solution was added to one imipenem disc. The zone of inhibition around the imipenem alone disc and the one around the imipenem-EDTA disc were recorded and they were compared after 16-18 hours incubation at 35ºC. An increase in the zone size of at least 7mm around the imipenem–EDTA disc was recorded as a positive result [5].

3. The Amp C Disc Test

This disc test was done for the meropenem resistant strains to test for the production of Amp C β-lactamases. On a Muller-Hinton agar plate, a lawn culture of *Escherichia coli* ATCC 25922 was made from an overnight culture suspension which was adjusted to 0.5 Mcfarland's standard. A 30µg cefoxitin disc was kept on the surface of the agar. A blank disc was moistened with sterile saline and it was inoculated with few colonies of the test strain. The inoculated disc was then placed beside the cefoxitin disc, in such a way that it was almost touching it. Then, the plate was incubated overnight at 37°C. A flattening or indentation of the cefoxitin inhibition zone in the vicinity of the disc with the test strain was interpreted as positive for the production of AmpC beta lactamase. An undistorted zone was considered as negative.

RESULTS

42 *Acinetobacter baumannii* (42%) strains were isolated from the tracheal aspirates, 11% were isolated from tracheal tips, 9% were isolated from endotracheal tips, 8% were isolated from wound swabs, 5% were isolated from bronchoalveolar lavage, pus and ear swabs, 4% were isolated from sputum and blood, 2% were isolated from endotracheal aspirates, and 1% were isolated from bed sore swabs, Foley's tips, femoral tips and central line tips. The sources of isolation for the 100 isolates which were used in our study are shown in [Table/Fig-1].

Culture	Number (%)
Tracheal aspirate	42
Tracheal tip	11
Endotracheal tip	9
Wound swab	8
Bronchioalveolar lavage (BAL)	5
Pus	5
Ear swab	5
Sputum	4
Blood	4
Endotracheal aspiration	2
Bed sore swab	1
Foley's catheter tip	1
Femoral catheter tip	1
Central line tip	1
Total	100

[Table/Fig-1]: Source of Acinetobacter baumannii isolates are shown in the following table.

Bacteria	Number of Meropenem resistant isolates	Number of imepenem resistant isolates	Number of positives (%)		
			Modified Hodge test.	Disc potentiation test.	AmpC disc test
Acinetobacter baumannii	100	100	20	10	73

[Table/Fig-2]: Results of Modified Hodge test, disc potentiation test and Amp C disc test

S. No.	Ward	Number of patients
1.	ICU	35
2.	Neurosurgery	19
3.	Neurology	12
4.	Paediatric surgery	8
5.	Surgery	6
6.	Nephrology	5
7.	Gastroenterology	4
8.	Pulmonology	4
9.	Medicine	4
10.	Plastic surgery	1
11.	Psychiatry	1
12.	ENT	1

[Table/Fig-3]: Wardwise distribution of 100 meropenem resistant isolates

S. No.	Antibiotic	Number [% Resistant]
1.	Imepenem 10 µg	100
2.	Meropenem 10 µg	100
3.	Cefipime 30 µg	100
4.	Ceftazidime 30 µg	99
5.	Co- trimoxazole 1.25µg +23.75 µg	99
6.	Ciprofloxacin 5 µg	95
7.	Amikacin 30 µg	92
8.	Gentamicin 10 µg	84
9.	Doxycycline 30 µg	73
10.	Ampicillin + sulbactum 10 µg +10µg	83
11.	Piperacillin + tazobactum 100 µg + 10 µg	69
12.	Levofloxacin 10 µg	77
13.	Tobramycin 10 µg	55

[Table/Fig-4]: Antibiogram of 100 Acinetobacter baumannii isolates to different antibiotics

Among the 100 meropenem resistant *A. baumannii* isolates, 20% were positive for carbapenemase production, 10% were metallo beta lactamase producers, and 73% were AmpC producers, which are been shown in [Table/Fig-2]. The distribution of the meropenem resistant isolates which were obtained from different wards in the hospital are shown in [Table/Fig-3]. The antibiotic susceptibility pattern of the 100 isolates of *A. baumanii* for thirteen different antibiotics has been depicted in [Table/Fig-4].

DISCUSSION

Acinetobacter baumannii infections present a global medical challenge. The interest in this organism has been growing rapidly because of the emergence of multi-drug resistant strains, some of which are pan resistant to antimicrobial agents. Multidrug-resistant

Acinetobacter baumannii has been reported worldwide and it has now been recognized as one of the most difficult healthcare-associated infections to control and treat.

In our study, *A. baumannii* was frequently isolated from tracheal aspirates, which was about 42%, from tracheal tips, 11% strains were isolated and from wound swabs, 8% strains were isolated. In another study from India, 59.8% *A. baumannii* isolates were reported from respiratory secretions, followed by 18.6% from blood [6].

In a study which was done by Sinha et al., which involved 150 clinical isolates of *Acinetobacter*, 14% of the isolates were found to be resistant to meropenem, when they were tested by the disc diffusion method [7]. Another Indian study by Taneja et al., in 2003, reported an incidence of about 18.5% imepenem resistance among *Acinetobacter* [8] isolates, while Manikal et al., observed a high rate of 50% carbapenem resistance among *Acinetobacter* isolates in New York [9]. Corbella and coworkers from Spain found the carbapenem resistance among the *Acinetobacter spp.* from patients in ICUs to be as high as 20% [10]. A study which was conducted in Bangalore, India, reported that 38% Acinetobacter isolates were obtained from patients who were admitted in intensive care units [11]. In our study, about 35% of the patients with carbapenem resistance were admitted in intensive care units.

In our study, metallo beta lactamase was detected in 10% of the meropenem resistant *Acinetobacter baumannii* isolates. Studies from the Indian subcontinent on the occurrence of metallo beta lactamase production by resistant *Acinetobacter* isolates are minimal. An Indian study on the *Acinetobacter baumanii* species stated that 70.9% of these isolates produced metallo beta lactamase [12], while another study reported from Kerala, India, states that 21% of the *Acinetobacter baumannii isolates were* found to be metallo-β-lacatmase producers [13].

AmpC β -lactamases have been found to be an important factor in carbapenem resistance in porin deficient isolates . In our study, AmpC β-lactamase production was detected in 73% of the isolates. In a recent study from Pondicherry, India, 67.4 % isolates were found to be AmpC β-lactamase producers, which are comparable with the results of our study [4]. Carbapenemase resistance in *A.baumannii* has been increasingly detected worldwide. Carbapenems (e.g. Imipenem and Meropenem) have become the drug of choice for the treatment of *Acinetobacter* infections in most of the medical centres. An indiscriminate use of carbapenems could have resulted in the increase in the carbapenem resistance.

A study which was done in Estonia showed that 60% *A.baumannii* isolates were sensitive to ampicillin- sulbactam, 95% were sensitive to imipenem and meropenem and that 70% were sensitive to amikacin [5]. Another study which was done in USA, detected 79.5% multi- drug resistant *A.baumannii* isolates. Among these, 62% were resistant to ceftazidime and 66 % were resistant to imipenem. The imipenem resistant isolates were also resistant to kanamycin, gentamicin, streptomycin, tetracycline, ciprofloxacin and nalidixic acid [14]. Our study showed 100% resistance to imipenem, meropenem and cefipime, 99% resistance to ceftazidime and cotrimoxazole, 95% resistance to ciprofloxacin, 73% resistance to doxycycline, 83% resistance to ampicillin- sulbactum, 69% resistance to piperacillin-tazobactam and 55% resistance to tobramycin.

In conclusion, carbapenem resistance in *Acinetobacter baumannii* is an emerging problem and it is a cause of concern, as many nosocomial *Acinetobacter* strains are detected to be resistant to most of the antibiotics, especially in the ICUs. There is a need to do surveillance for detecting the different beta lactamases routinely in the microbiology laboratory. Several phenotypic and molecular typing methods are used to investigate the origin of the infection, the route of its spread and the prevalence of the isolates in a bacterial population. However, simple tests like the Modified Hodge test for carbapenemase production, the disc potentiation test for metallo beta lactamase production and the disc test for AmpC production may be performed in most of the laboratories to determine a few common mechanisms of resistance and appropriate infection control measures can be implemented to control the spread of such infections in hospitals.

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AUTHOR(S):

1. Dr. Dheepa M.
2. Dr. Appalaraju B.

PARTICULARS OF CONTRIBUTORS:

1. Associate Professor, Department of Microbiology, PSG Institute of Medical Sciences & Research Centre, Peelamedu, Coimbatore - 641004, Tamil Nadu, India.
2. Professor and Head, Department of Microbiology, PSG Institute of Medical Sciences & Research Centre, Peelamedu, Coimbatore - 641004, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. M. Dheepa M.D., (Microbiology),
Associate Professor, Department of Microbiology,
PSG Institute of Medical Sciences & Research Centre,
Peelamedu, Coimbatore - 641004, Tamil Nadu, India.
Phone: 9843009211
E-mail: dheepamicro@gmail.com

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