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Detection of Carbapenamase Resistance in Non-Fermenter Gram Negative Bacilli by Phenotypic Methods in A Tertiary Care Hospital

R.P.R. Suyambu Meenakshi*, S. Gowri, G. Sucilathangam and C. Revathy

Department of Microbiology, Tirunelveli Medical College, Tirunelveli - 627 011, Tamil Nadu, India

**Corresponding author*

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A B S T R A C T

Non fermenter Gram negative bacilli were considered as contaminants or commensals of little significance1. However, recent literature review shows that these organisms are now associated with life-threatening infections such as Septicemia, Pneumonia, Urinary Tract Infection, Meningitis, Surgical Site Infection, Ventilator Associated Pneumonia, Wound Infection, Osteomyelitis etc. Carbapenemases are diverse enzymes that vary in their ability to hydrolyze Carbapenems and other beta lactams. Detection of Carbapenemase is a crucial infection control issue because they are often associated with extensive antibiotic resistance, treatment failures and infection- associated mortality. A total of 100 isolates of Non Fermenter Gram Negative Bacilli were isolated from specimens of Respiratory Tract Infection, Wound Infection, Urinary Tract Infections, Septicemia, Post Operative Wound Infections, Ear Infections. The isolated NFGNB were subjected to Meropenem resistance by disc diffusion test and those strains which show resistance to Meropenem were subjected to Carbapenamase detection method by using, Double disc synergy test, combination disc method and MBL E test. Among the 100 Non fermenter isolates, 87 were *Pseudomonas aeruginosa* and 13 were *Acinetobacter baumanii*. Screening with Meropenem disk showed 32% of isolates were positive for Carbapenem resistance. Among them CDT detected 59.3%, DDST 50% and E test 46.8% of the MBL producer. 13 MDR NFGNB were detected among the 32 isolates. The proper identification of NFGNB up to the species level together with monitoring their susceptibility patterns are mandatory for proper management of infections caused by these pathogens.

Introduction

Non fermentative Gram negative bacteria are a group of aerobic, non-spore forming, gram negative bacilli or coccobacilli that either do not use carbohydrate as a source of energy or degrade them through oxidative pathway rather than fermentation (Washington *et al.*, 2006). Gram negative non fermentative bacteria are ubiquitous in nature and widely distributed in soil, water, sewage and plants or as harmless bacteria on mucous membrane of humans or animals. These bacteria can cause disease by colonizing or subsequently infecting immune compromised patient or gaining access to normally sterile body site through trauma (Murray Patrick *et al.*, 2003; McGowan, 2006).

However, recent literature review shows that these organisms are now associated with life-threatening infections such as septicemia, pneumonia, urinary tract infection, meningitis, surgical site infection, ventilator associated pneumonia (VAP), wound infection, osteomyelitis etc. They account for around 15% of all bacterial isolates from clinical samples (Gladstone *et al.*, 2005).

Infections due to NFGNB are very difficult to treat as they show intrinsic resistance and resistance by other mechanisms, to multiple classes of drugs. Regular monitoring of carbapenem resistance is essential in developing strategies to control infection. Hence the present study was undertaken to detect the production of carbapenemase by non-fermenting gram negative bacilli using phenotypic methods.

Materials and Methods

After getting clearance from the Institutional ethical committee, this prospective cross sectional study was undertaken at the Department of Microbiology, Tirunelveli Medical College during the period of 2016 - 2017.

Sample collection and processing

A total of 100 non fermenter Gram negative bacilli were collected from various clinical samples such as pus, urine, burn, wound, sputum, pleural fluid and CSF were taken in the study.

The specimens were inoculated on to Nutrient agar MacConkey agar and Blood agar. The media were incubated at 37°C overnight. Plates were examined for growth.

Organisms that failed to acidify the butts of triple sugar iron media were considered Nonfermenters and were subjected to a battery of tests. All the isolates of NFGNB were tested for the susceptibility for Meropenem by Disc diffusion method as per the CLSI guidelines.

The isolates which were found to be resistant to Meropenem were selected and subjected to various phenotypic methods like CDT, DDST, MBL E Test to detect carbapenem resistance.

Screening by disc diffusion method with Meropenem

According to the CLSI guidelines the zone of inhibition by disc diffusion test was measured and interpreted. For Meropenem disc, zone size of ≤ 15mm was taken as resistant for pseudomonas and other species and zone size of ≤ 13mm was taken as resistant for Acinetobacter.

Phenotypic methods to detect carbapenemases

The isolates which were found to be resistant to Meropenem were selected and subjected to various phenotypic methods like CDT, DDST, MBL - E Test.

Combined Disc Test procedure:

The 24 hr young culture isolate of the test strain was inoculated on to the Muller Hinton agar plate. Two Meropenem discs 10 µg were placed on the dried agar plate at a distance of 50 mm. To one of the disc 10µl (750µg) of 0.5 M EDTA solution was added. The plate was incubated at 37˚C aerobically for 16-18 hours.

The zone of inhibition was measured for the Meropenem and the Meropenem EDTA combined disc. The test is considered positive for the detection of MBL enzyme production when there is increase in the diameter of the zone by more than or equal to 7mm (≥7mm) for the Meropenem EDTA combined disc

Meropenem-EDTA Double Disc Synergy Test (DDST)

The test strain was inoculated as lawn culture on to the Muller Hinton agar plate as per the CLSI guidelines. The Meropenem (10 µg) disc was placed 10 mm apart from the sterile blank disc. The sterile blank disc was added with 10 µl of EDTA.The plate was incubated at 37˚C for 16-18 hours. The enhancement of the zone of inhibition towards EDTA disc was considered positive for MBL production of the test strain.

MBL E test

Make 0.5 McFarland suspension of the test strain in normal saline. With the help of sterile swab, test strain is inoculated as a lawn on the MHA plates. Place the E test strips (mixture of Meropenem + EDTA and Meropenem in a concentration gradient manner) on the plate. The strip was then taken with a sterile forceps or E test applicator and applied to the dried agar surface with the MIC scale facing upwards. Incubate the plate at 37°C for 24 h. The MIC for MP/MPI ≥ 8 or deformation of ellipse or phantom zone was considered positive for MBL production.

Results and Discussion

From the 100 NFGNB, the different types of NFGNB isolated were as follows. *Pseudomonas aeruginosa* was the most common isolate (87%) followed by *Acinetobacter baumanii.* (13%). Meropenem resistance in NFGNB by disc diffusion test showed that 32% of the isolates were resistant to carbapenem. Among them, 25 isolates were *pseudomonas aeruginosa* and 7 isolates were for *Acinetobacter baumanii* (Figure 1).

Among the CR NFGNB, Cephalosporins showed 65-70% resistance and ciprofloxacin showed 65% resistance. This is due to the wide usage of these antibiotics in the community in the treatment of suspected Gram negative infections.

Out of 100 isolates, 32 showed Meropenem resistance. They were subjected to phenotypic test for carbapenamase detection. CDT detected 19 isolates producing Carbapenamse, DDST detected 16 isolates and E test detected 15 isolates producing carbapenamase (Table 1).

Non fermenter Gram negative bacilli are once considered as commensal and contaminant, but nowadays their pathological role is well established.[71] The outbreaks of nosocomial infections, emerging antimicrobial resistance and epidemiological complexity have made NFGNB the remarkable organism. They are now considered as important nosocomial pathogen. (Rajendra *et al.,* 2012; Noyal *et al.,* 2009; Brooks Geo *et al.,* 2001) Resistance pattern among the nosocomial bacterial pathogen may vary widely from place to place even within the same country over time (Mandell Gerald and Bennet John, 2010). In the present study, out of 100 NFGNB, the most common organism isolated was *Pseudomonas aeruginosa* (87%) followed by *Acinetobacter baumanii* (13A study conducted by Kamalraj *et al.,* also showed that among the non-fermenters, Pseudomonas spp.,(83.5%) were the maximum isolate followed by *Acinetobacter* spp (12.7). A study conducted by Gokhale and Metgud *et al.,* at 2013 at Bangalore also showed the similar result with isolation rate of *Pseudomonas aerugin*osa 82.3% followed by 16% of *Acinetobacter baumanii* (Vijaya *et al.,* 2000; Juyal *et al.,* 2013).

Meropenem is used to detect carbapenam resistance in the current study according to CLSI guidelines. Screening test for carbapenem resistance was done with Meropenem disc by disc diffusion method.

Fig.1 Meropenem resistance *in Pseudomonas aeruginosa* and *Acinetobacter baumanii* by disc diffusion test

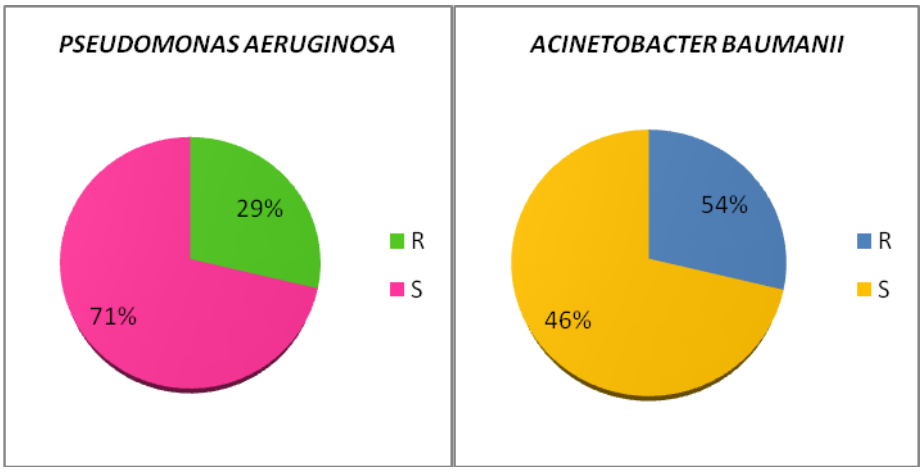

Table.1 Detection of MBL by phenotypic methods

METHOD	CR NFGNB	
	NO	%
CDT	19	59.3
DDST	16	50
E TEST	15	46.8

In the present study 32% of the isolates showed Meropenem resistance. Among them *A. bauamnii* is more resistant than *Pseudomonas aeruginosa*.

Ruta S Patwardhan's study also agrees with the present study and showed 31.46 %resistance to Meropenem. A study by Manoharan *et al.,* has shown 42.7 % resistance to Meropenem. (Gokhale and Metgud, 2012; Benachinmardi, *et al.,* 2014)

In India, resistance to carbapenems in *A. baumannii* ranges from 14% to 59%. Most resistant isolates were recovered from respiratory samples of patients in the ICU. (Sidhu *et al.,* 2010) In a multicentric study including centres all over India conducted during 2005-07, 42.6 % of *P. aeruginosa* were resistant to Imipenem/Meropenem. (Manoharan *et al.,* 2010). Meropenem resistant strains were screened for MBL production by CDT, IMP-EDTA disc synergy test and MBL E test. The present study detected 59.8% and 50% MBL producers by CDT and DDST among Meropenem resistant NFGNB respectively. Studies across India report rates of MBL production ranging from 72%-100% among carbapenem resistant NFGNB. (Walsh *et al.,* 2002)

A number of studies were conducted in various parts of the world for MBL detection which showed increased sensitivity to DDST compared to CDT. But in this study CDT was superior to DDST in MBL detection. This discrepancy may be due to the difference in geographical regions, difference in kind of infections, the vast usage of antimicrobials, or difference in antibiotic policy in the particular hospitals. (Shivaprasad *et al.,* 2014; Mlynarczyk *et al.,* 2009)

There was 48.3% positivity for MBL E test in the present study among the Meropenem resistant isolates. Manoharan *et al.,* (2010) in their study reported 42.6% positive MBL producers by E test (Maria Renata *et al.,* 2010) which is in agreement with the present study results.

Not all Meropenem resistant isolates were found to be MBL producers in the present study. This is due to Mechanisms of such as mutation in the outer membrane permeability, loss of porins or the up regulation of efflux systems attributed to the resistance. (Fereshteh *et al.,* 2010; Sevitha *et al.,* 2013)

Among the CR NFGNB, Cephalosporins showed 65-70% resistance and ciprofloxacin showed 65% resistance. This is due to the wide usage of these antibiotics in the community in the treatment of suspected Gram negative infections.

Among the 32 CR NFGNB, 13 were multidrug resistant non fermenters. MBL producers were 5 among them. All of them were susceptible to aztreonam.

Drug resistance also leads to epidemics, and consequently there is a greater risk of infection to others. Improved infection control measures and judicious antibiotic usage are necessary to contain the emergence and spread of multiply drug resistant non-fermenters in health care setting. Moreover, timely dissemination of the local antibiogram will aid the clinician in choosing the appropriate antibiotic.

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References

Benachinmardi, *et al.,* Prevalence of NFGNB and their in vitro susceptibility pattern, Journal of the Scientific Society, Vol 41 / Issue 3 / September-December 2014.

Brooks Geo F, Carroll Karen C, Butel Janet S, Morse Stephen A, Mietzner Timothy A. Pseudomonads, *Acinetobacters* and uncommon Gram negative bacilli" Jawetz, Melnick and Adelberg's Medical Microbiology, 25th edition, 2001 McGraw Hill; 227-233

Fereshteh S, Vajiheh SN *et al.,* Identification and genetic characterization of metallo-beta-lactamase-producing strains of *Pseudomonas aeruginosa* in Tehran, Iran. New Microbiologica, 33, 243-248, 2010.

Gladstone P, Rajendran P, Brahmadathan KN. Incidence of carbapenem resistant non-fermenting gram negative bacilli from patients with respiratory infections in the intensive care units. Indian J of Med Microbiol 2005; 23(3): 189-191

Gokhale S, and Metgud SC. Characterization and antibiotic sensitivity pattern of non-fermenting Gram negative bacilli from various clinical samples in a tertiary care hospital. Belgaum J Pharm Biomed Sci 2012; 17:1-5.

Juyal D, Prakash R, Shankarnarayan S A, Sharma M, Negi V, *et al.,* Prevalence of non-fermenting Gram negative bacilli and their in vitro susceptibility pattern in a tertiary care hospital of Utterakhand: A study from foothills of Himalayas. Saudi J Health Sci 2013; 2:108-12.

Mandell Gerald L, and Bennet John E, Dolin Raphael Principles and Practice of Infectious Diseases, 7th edition, 2010, Churchill Livingstone Elsevier; 2835-2964

Manoharan, A., S. chatterji *et al.,* detection and characterization of

metallobetalactamse producing *Pseudomonas aeruginosa*. Indian Journal of Medical Microbiology. 2010; (28) (3): 241-244.

Maria Renata GF, Helio Hehl Caiaffa-Filho, Marcelo NB *et al.*, Metallo-beta-lactamases among imipenem-resistant *Pseudomonas aeruginosa* in a Brazilian university hospital. Clinics 2010; 65(9): 825-829.

McGowan JE Jr. "Resistance in non-fermenting gram-negative bacteria: multidrug resistance to the maximum." Am J Med. 2006 Jun; 119(6 Suppl 1): S29-36

Mlynarczyk G, Sawicka-Grzelak A, Szymanek K, Buczkowska T *et al.*, Resistance to carbapenems among *Pseudomonas aeruginosa* isolated from patients of transplant wards. Transplant Proc. 2009 Oct; 41(8): 3258-60.

Murray Patrick R, Baron Ellen Jo, Jorgensen James H, Landry Marie Louise, Manual of clinical Microbiology, Vol I, 8th edition, ASM Press, 2003; 734-834

Noyal M.JC, Menezes GA, Harish BN, Sujatha S, Parija SC. Simple screening tests for detection of carbapenemases in clinical isolates of Non-fermentative Gram-negative bacteria" Indian J Med Res 129, 2009; 129: 707- 712

Rajendra D, Ramana BV, Chaudhury A. Spectrum of non-fermenting gram negative bacilli infection (excluding pseudomonads) in a tertiary care hospital. Int J Biol Med Res. 2012; 3(3):1892-1894

Sevitha B, Renu S, Zenith E. Carbapenem resistance in clinically significant non-fermenting gram negative bacilli. Journal of Evolution of Medical and Dental Sciences 2013; 2(47): e9131-9134

Shivaprasad A, Beena A, Poornima S. Comparative Evaluation of Four Phenotypic Tests for Detection of Metallo-B-Lactamase and *Carbapenemase* Production in *Acinetobacter Baumannii*. *JCDR* 8.5 (2014): DC05–DC08. *PMC*. Web. 4 Oct. 2017.

Sidhu S, Arora U, Devi P. Prevalence of non-fermentative Gram negative bacilli in seriously ill patients with bacteraemia. JK Sci 2010; 12:168-71.

Vijaya D, Kamala, Bavani S, Veena M. Prevalence of non-fermenters in clinical specimens. Indian J Med Sci 2000; 54:87-91.

Walsh TR, Bolmstrom A, Qwarnstrom A, Gales A. Evaluation of a new E-test for detecting Metallo-β-lactamases in Routine Clinical Testing. J Cli Micribiol. 2002; 40(8):2755–9

Washington W, Jr., Allen S, Janda W, Koneman E, Procop G, Screckenberger P. *et al.*, The non-fermentative Gram negative bacilli. Koneman's Colour atlas and textbook of diagnostic Microbiology, 6th edition, 2006, Lippincott William & Wilkins; 303-392

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