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Original article:

STUDY OF CARBAPENEMASE PRODUCING NON-GLUCOSE FERMENTERS IN TERITIARY CARE HOSPITAL

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Abstract

Carbapenems have been used as the last resort antimicrobials in the treatment of serious infections caused by gram negative bacteria. The rate of carbapenem resistance in non-glucose fermenting gram negative bacilli has been gradually increasing worldwide over last 10 years. These organisms can also cause life threating infections which are difficult to treat.

Aim: This study was conducted to identify phenotypically for the presence of Carbapenemase production in glucose non-fermenting bacilli.

Materials and methods: Among 200 Imipenem resistant isolates, 62 imipenem resistant samples showed glucose non-fermenting bacilli. All the isolates were tested for anti-microbial susceptibility (HI-Media Mumbai) by Kirby-Bauer disk diffusion method on Muller-Hinton agar. Carbapenemase production was detected by Modified-Hodge test, Combined Disc test, and AmpC Disc test phenotypically.

Results. Out of 62 imipenem resistant isolates of glucose non-fermenting bacilli, 49 isolates were *Pseudomonas spp.* and 13 isolates were *Acinetobacter spp..* Out of 62 , 13 were MHT positive, 36 were CDT positive and 14 were AmpC positive, 16 were Multiple enzyme producers, 17 were Non-Enzyme producers.

Conclusion: Carbapenem resistance rate was more in *Acinetobacter spp.* than *Pseudomas spp..* Our study has implicated the severity of carbapenem resistant non-fermenters, which are effecting the infection control activities among hospital setup.

Key words: MHT=Modified Hodge test, CDT=Combined Disc test, MBL=Metallo betalactamase

Introduction

Non-fermenting gram negative bacilli are a taxonomic diverse group of aerobic non sporing bacilli that either do not utilize glucose as a source of energy or utilize it oxidatively (1, 2)

Carbapenems have been used as the last resort antimicrobials in the treatment of serious infections caused by gram negative bacteria (3, 4). The increased rate of carbapenem resistance in gram negative bacteria is a serious global health threat.

The rate of carbapenem resistance in non-glucose fermenting gram negative bacilli has been gradually increasing worldwide over last 10 years. These organisms can also cause life threating infections which are difficult to treat. This mini review will focus on carbapenem resistant non – glucose fermenting gram negative bacilli particularly *Pseudomonas spp.* and *Acinetobacter spp..*

Carbepenem resistance among carbapenem resistance non-glucose fermenting bacilli can be mediated by multiple different mechanisms including carbapenemase production, decreased permeability due to porin mutation, over expression of efflux pumps (5, 6,7).

The Ambler classification scheme is a method of differentiating carbapenemase based on their Amino acid sequences (8). The most commonly acquired carbapenemase among *Acinetobacter spp.* are from Ambler class D, in particular OXA-23, OXA-40, OXA-58 and OXA-143.

In contrast, the most commonly encountered carbapenemase among *Pseudomonas spp.* are class B MBLs, such as VIM and IMPS (8, 9). Other carbapenemases such as GES, NDM, and KPC are now also being detected and reported in *Pseudomonas and Acinetobater spp.* (10, 11).

The present study was to determine the antimicrobial susceptibility pattern and detection of carbapenemase resistance in glucose non-fermenters.

Materials and Methods:

The present prospective study was carried in AMC MET Medical College, Ahmedabad. It was approved by the institutional review board. A total of 200 isolates which are imipenem resistant are included. Samples like endotracheal aspirate, pus, urine, blood, sputum, CSF were received.

Direct gram stain was done from all specimens except blood. The specimens were inoculated onto Mac-Conkey agar, blood agar medium and incubated at 37 °C for overnight. Those organisms which showed non-lactose fermenting colonies on Mac-Conkey's agar and not acidify the butts of Triple sugar iron (TSI) agar were presumptively identified as non-fermenters and confirmed by standard microbiological techniques(2,12). All the isolates were tested for anti-microbial susceptibility (Hi-Media Mumbai) by Kirby-Bauer disk diffusion method on Muller-Hinton agar (13). Imipenem resistant isolates were proceed to Modified-Hodge test, combined disc test and AmpC disc test for detection of Carbapenemase, Metallo-β-Lactamase and AmpC-β-Lactamase.

Quality control strains were *Escherichia coli* ATCC 25922, for Modified Hodge test-Positive control-*Klebsiella pneumonia* ATCC BAA-1705.

Modified-Hodge test

Lawn culture of *E.coli* ATCC 25922 was made from overnight culture suspension adjusted to 0.5 McFarland standards on Muller-Hinton agar. After drying the plate, a 10µg Ertapenem disc was placed at the centre and test strain was streaked from edge of the disc to periphery of plate. The plate was incubated at 37 °C for overnight. The presence of distorted zone of inhibition (clover leaf pattern) was interpreted as positive result (14).

Combined Disc test

Test organism was inoculated on Muller-Hinton agar and two 10µg imipenem disc were placed. 10µl solutions (750 µg) of Ethylene Diamine Tetra Acetic Acid (EDTA) were added to one of them & incubated the plate at 37 °C for 16-18 hours. Metallo-β-lactamase positive result considered, if zone of inhibition of imipenem+ EDTA disc was >7mm than that of imipenem disc alone (15) .

AmpC Disc test

Lawn culture of *E.coli* ATCC 25922 was made from an overnight culture suspension adjusted to 0.5 McFarland standards on Muller-Hinton agar plate. A 30 µg cefoxitin disc placed in centre and a blank disc (6mm diameter) which was moistened with sterile normal saline and inoculated with few colonies of test organism, was placed beside the cefoxitin disc **almost touching it**. The plate was incubated at 37 °C for overnight. A flattening or indentation of zone of inhibition of cefoxitin in the vicinity of the disc containing test organism was interpreted as positive (16).

Result

Among 200 patients, 62 showed non fermenting gram negative bacilli, therefore, isolation rate was 31.0 %. Majority of them are isolated in surgical ward. The isolates are *Pseudomonas spp.* and *Acinetobacter spp.*. All the isolates showed low sensitivity to piperacillin, ceftazidime, amikacin, however, most effective antibiotic in the present study was colistin, for *Acinetobacter spp.* colistin and tigecycline. Out of 62 isolates Metallo-β-lactamase was positive in 36 isolates, MHT was positive in 13 isolates and AmpC β lactamase was positive in 14 isolates respectively, 16 were Multiple enzyme producers, 17 were Non-Enzyme producers.

Table 1

Non fermenters isolated from different specimens

Specimen	*Pseudomonas spp.*	*Acinetobacter spp.*
Pus	27	3
Sputum	9	4
Urine	5	0
Blood	2	0
Tissue	1	0
ET	4	5
Fluid(CSF)	1	1
Total	49	13

Table 2

Phenotypic tests in imipenem resistant isolates

Bacteria	No. of isolates	No. of positive test			Multiple enzyme producer			
		MHT	CDT	AmpC disc test	MBL+AmpC	MBL+MHT	AmpC+ MHT	MBL +AmpC+ MHT
Pseudomonas spp.	49	9	29	10	06	04	02	00
Acinetobacter spp.	13	4	7	4	02	01	00	01
Total	62	13	36	14				

17 were non-enzyme producers

MHT=Modified Hodge test, CDT=Combined Disc test

Discussion

The development of antibiotics was definitely one of the greatest achievements in modern medicine. Unfortunately, the rising of antibiotic resistance in nonfermenter infections is threatening which minimizes the use of antibiotics which may be due to survival of organisms in health care setup, disruption of normal flora, increasing duration of hospital stay, use of steroids and immunosuppressive therapy (17, 18).

Isolation rate of non-fermenters was 31.0%, higher than the reports by Benachinmardi et al., and Bruno et al.(17,18). A case-controlled study from Japan showed that patients infected with MBL-producing *Pseudomonas spp.* were more likely to receive multiple antibiotics and also infection related deaths due to MBL producing *Pseudomonas spp.* were more frequent than deaths caused by MBL negative *Pseudomonas spp.* 27% isolates which are non-enzyme producers.

According to the sensitivity pattern, antibiotics such as colistin, tigecycline and amikacin are available for treatment of non-fermenters. In the present study amikacin sensitivity was 25.8% which is also reported by Kumar R et al., (14). A total of 58.0 % Metallo beta lactamase were detected which is also reported by Hariom Sharan et al.,(12) . 22.5% MHT and AmpC beta lactamase was detected.

Conclusion

Carbapenem resistance rate was more in *Acinetobacter spp.* than *Pseudomas spp..* Our study has implicated the severity of carbapenem resistant non-fermenters, which are effecting the infection control activities among hospital setup.

Implication & Future research:

Carbapenemase resistance can be confirmed further with Gene expression.

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