Original Article

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Microbiology Section

Emergence of Hospital Acquired Carbapenem Resistant Non-Fermenters in Teaching Institute

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ABSTRACT

Introduction: Non-Fermenting Gram Negative Bacilli (NFGNB) are emerging now-a-days because of their tendency to colonize various surfaces and inherent resistance to commonly used disinfectants. They are responsible for multi-drug resistant hospital acquired infections. Detection of carbapenem resistance mechanisms is essential for treatment and infection control purpose as can spread to other organisms causing hospital outbreaks.

Aim: To characterize non-fermenters from various clinical samples and to detect different carbapenem resistance mechanisms in meropenem resistant isolates.

Materials and Methods: The prospective study was conducted at Sri Aurobindo Medical College and Post Graduate Institute, Indore over a period of one and half year from December 2014 to May 2016. A total of 1310 samples were collected from Ventilator Associated Pneumonia (VAP), Surgical Site Infection (SSI), Urinary Tract Infection (UTI), septicaemia, Lower Respiratory Tract Infection (LRTI) and middle ear infected patients. Non-fermenters were identified by standard microbiological tests. Meropenem resistance was determined by Kirby-Bauer disk diffusion method and resistant isolates were further tested by Modified Hodge test, Combined disc test and AmpC disc test.

Results: Isolation rate of non-fermenters was 13.82% (181/1310). Colistin, amikacin and imipenem were the antibiotics with maximum sensitivity. Overall meropenem resistance was found to be 44.2% (80/181). Metallo-β-lactamase and AmpC-β-lactamase were produced by 56.82% (25/44) and 72.22% (26/36) of meropenem resistant *Pseudomonas* and *Acinetobacter* species respectively.

Conclusion: Detection of carbapenem resistance mechanisms and implementation of antibiotic policy are needed to prevent the emergence of non-fermenter infections.

Keywords: Combined disc test, AmpC disc test, Modified Hodge test

INTRODUCTION

Non-Fermenting Gram Negative Bacilli (NFGNB) are a group of aerobic, non-sporing bacteria that are either incapable of utilizing carbohydrates as a source of energy or degrade them oxidatively [1]. They are found as saprophytes in hospital environment and are responsible for hospital acquired infections like Ventilator Associated Pneumonia (VAP), Septicaemia, Urinary Tract Infection (UTI), meningitis, Surgical Site Infection (SSI) and osteomyelitis particularly in critically ill and immunocompromised patients [2]. Non-fermenters are difficult to treat as they exhibit many mechanisms of drug resistance, non standardization of anti-microbial susceptibility testing for many organisms and poor correlation between in vitro disc diffusion testing results and in-vivo effectiveness of the drugs [3].

There are various mechanisms of carbapenem resistance like mutation in outer membrane proteins, development of efflux pumps and lack of drug penetration [4,5]. Carbapenem resistance can spread to other species, therefore, its monitoring is essential to prevent hospital acquired infections. Combination of antibiotics has shown better response when treating non-fermenter infections [6].

Hence, the present study was to determine the antimicrobial susceptibility pattern and detection of contributing mechanisms in carbapenem resistance development.

MATERIALS AND METHODS

The present prospective study was carried out over a period of one and half year from December 2014 to May 2016. It was approved by the Institutional Ethical Committee. A total of 1310 samples like endotracheal aspirate, pus, urine, blood, sputum, catheter tip and ear swabs were collected from patients admitted in Intensive Care Unit, medicine, surgery, obstetrics gynaecology, otorhinolaryngology, paediatric wards and burn unit. A total of 925 males and 385 females between 5-75 years of age group, who developed VAP, SSI, UTI, septicaemia, Lower Respiratory Tract Infection (LRTI) and middle ear infection after 48 hours of admission were included in the study. Those who developed these infections before 48 hours of admission were excluded from study.

Direct gram stain was done from all specimens except blood. The specimens were inoculated onto Mac-Conkey's agar, blood agar and cystine lactose electrolyte deficient (in case of urine sample) medium and incubated at 37°C for 24 hours in 7-10% CO_2 concentration. 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 [1]. All the isolates were tested for anti-microbial susceptibility (Hi-Media Mumbai) by Kirby-Bauer disk diffusion method on Mueller-Hinton agar [7]. Meropenem 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 pneumoniae* ATCC BAA-1705 and Negative control- *Klebsiella pneumoniae* ATCC BAA-1706.

Modified-Hodge test

Lawn culture of *E.coli* ATCC 25922 was made from an overnight culture suspension adjusted to 0.5 McFarland standards on Mueller-

Hinton agar. After drying the plate, a 10 µg meropenem disc was placed at the centre and the 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 [Table/Fig-1] result [8].

Combined disc test

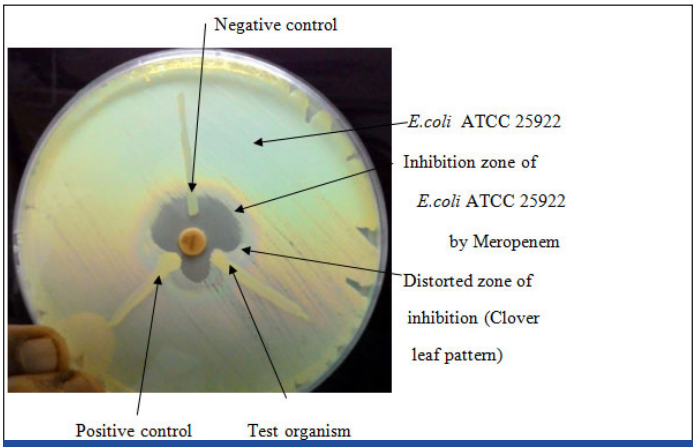
Test organism was inoculated on Mueller-Hinton agar and two 10µg imipenem discs were placed. 10µl solutions (750µg) of Ethylene Diamine Tetra Acetic Acid (EDTA) were added to one of them & incubated the plate at 35°C for 16-18 hours. Metallo-β-lactamase positive [Table/Fig-2] result considered, if zone of inhibition of imipenem + EDTA disc was >7mm than that of imipenem disc alone [9].

AmpC Disc test

Lawn culture of *E.coli* ATCC 25922 was made from an overnight culture suspension adjusted to 0.5Mc Farland standards on Mueller-Hinton agar plate. A 30µg 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 [Table/Fig-3] result [10].

RESULTS

Among 1310 patients, 181 showed NFGNB growth, therefore, isolation rate was 13.82%. Majority of non-fermenters were isolated from endotracheal aspirate, but isolation rate was maximum in surgical ward. Predominant isolates were *Pseudomonas aeruginosa* and *Acinetobacter baumannii* [Table/Fig-4]. All the isolates showed low sensitivity to piperacillin, ticarcillin and ceftazidime, however, most effective antibiotic in the present study was colistin followed by amikacin and imipenem [Table/Fig-5]. Meropenem resistance was 44.2% (80/181). Metallo-β-lactamase and AmpC β lactamase were produced by 56.82% (25/44) and 72.22% (26/36) of meropenem resistant *Pseudomonas* and *Acinetobacter* species respectively [Table/Fig-6].

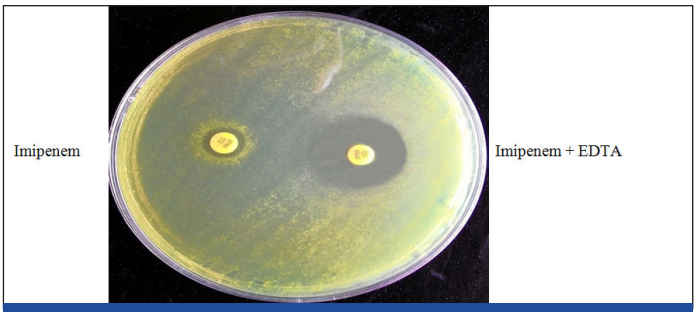


[Table/Fig-1]: Modified Hodge test.

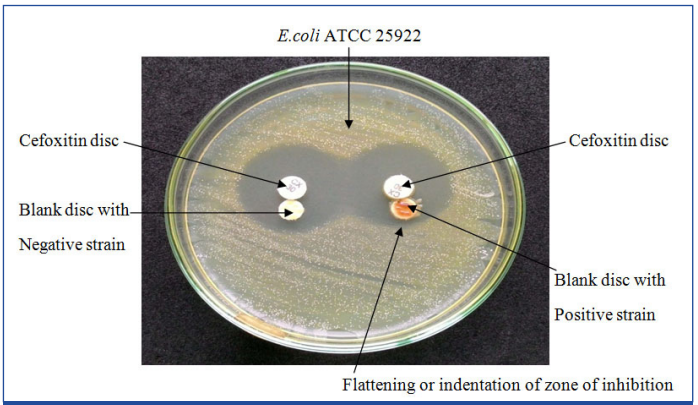
DISCUSSION

Isolation rate of non-fermenters was 13.82%, higher than the reports by Benachinmardi et al., and Bruno et al., indicating the rising trend of non-fermenter infections which may be due to survival of organisms in health care setup, disruption of normal flora by excessive use of antibiotics, therapeutic intervention, increased duration of hospital stay, use of steroids and immunosuppressive therapy [11,12]. Non-fermenters were isolated most commonly from endotracheal aspirate indicating respiratory colonization of *Pseudomonas* and *Acinetobacter* species. The commonest isolate was *Pseudomonas aeruginosa,* which is similar to some studies [13,14] and higher isolation rate of *Acinetobacter* species corresponding to other study [15].

According to sensitivity pattern, antibiotics such as colistin, amikacin and imipenem are available for treatment of non-fermenters. Sensitivity rate was more in *Pseudomonas aeruginosa* than



[Table/Fig-2]: Combined disc test.



[Table/Fig-3]: AmpC disc test.

Specimens	Sample	*Pseudomonas aeruginosa*	*Pseudomonas fluorescens*	*Pseudomonas stutzeri*	*Stenotropho--monas maltophila*	*Sphingo bacterium* species	*Acinetobacter baumannii*	*Acinetobacter lwoffii*	Total
Endotracheal aspirate	659	45	3	2	3	0	28	4	85
Pus	331	25	2	1	2	1	18	2	51
Urine	160	12	1	1	1	1	6	1	23
Blood	75	4	0	0	0	0	1	0	5
Sputum	60	5	0	0	1	0	2	2	10
Cather tip	15	2	0	0	0	0	1	0	3
Ear swab	10	2	0	0	0	0	1	1	4
Total	1310	95	6	4	7	2	57	10	181

[Table/Fig-4]: Non-fermenters isolated from different clinical specimens.

Antibiotics	Conc (µg)	*Pseudomonas aeruginosa* N (%)	*Pseudomonas fluorescens* N (%)	*Pseudomonas stutzeri* N (%)	*Stenotrophomonas maltophila* N (%)	*Sphingo bacterium* species N (%)	*Acinetobacter baumannii* N (%)	*Acinetobacter lwoffii* N (%)
Amikacin	30	76(80)	5(83.3)	4(100)	NT	2(100)	35(61.4)	5(50)
Aztreonam	30	30(31.6)	1(16.7)	0	NT	0	NT	NT
Cefepime	30	57(60)	2(33.3)	2(50)	NT	0	15(26.3)	3(30)
Ceftazidime	30	38(40)	2(33.3)	2(50)	NT	0	12(21)	4(40)
Ciprofloxacin	5	48(50.5)	2(33.3)	3(75)	NT	0	13(22.8)	4(40)
Colistin	10	95(100)	6(100)	4(100)	NT	2(100)	NT	NT
Cotrimoxazole	23.75/1.25	NT	1(16.7)	0	7(100)	1(50)	16(28)	3(30)
Gentamicin	10	47(49.5)	3(50)	3(75)	NT	1(50)	23(40.4)	5(50)
Imipenem	10	59(62.1)	4(66.7)	3(75)	NT	2(100)	40(70.2)	8(80)
Meropenem	10	57(60)	2(33.3)	2(50)	NT	0	26(45.6)	5(50)
Piperacillin	100	33(34.7)	1(16.7)	2(50)	NT	0	5(8.8)	2(20)
Piperacillin –Tazobactam	100 / 10	58(61)	2(33.3)	2(50)	NT	1(50)	16(28)	5(50)
Ticarcillin	75	38(40)	2(33.3)	2(50)	NT	0	9(15.8)	3(30)
Ticarcillin-Clavulanic acid	75/10	53(55.8)	4(66.7)	1(25)	NT	0	18(31.6)	4(40)
Tobramycin	10	49(51.6)	2(33.3)	3(75)	NT	0	26(45.6)	4(40)

[Table/Fig-5]: Antibiotic sensitivity pattern of Nonfermenters.
NT- Not Tested

Bacteria	No. of Isolates	No. of Meropenem Resistant Isolates	N. of Positive Test		
			MHT	CDT	AmpC disc test
Pseudomonas aeruginosa	95	38	12	23	18
Pseudomonas fluorescens	6	4	1	1	2
Pseudomonas stutzeri	4	2	0	1	1
Stenotrophomonas maltophila	7	0	0	0	0
Sphingobacterium species	2	0	0	0	0
Acinetobacter baumannii	57	31	3	5	23
Acinetobacter lwoffii	10	5	0	1	3
Total	181	80	16	31	47

[Table/Fig-6]: Phenotypic tests in meropenem resistant isolates.
MHT= Modified Hodge test, CDT= Combined Disc test

other species of *Pseudomonas* and resistance rate was higher in *Acinetobacter baumannii* than *Acinetobacter lwoffii* in most of the antibiotics. In the present study, amikacin sensitivity was 80% for *Pseudomonas aeruginosa* which is contrast to the finding of Kumar R et al., who have reported the same to be 32% [16]. Imipenem resistance was 62.1% in *Pseudomonas aeruginosa* whereas Malini et al., detected it 6% only [14]. In our study, high percentage of resistance has been detected against beta-lactam (piperacillin, ticarcillin) and cephalosporin (ceftazidime). This leaves us with limited therapeutic options, thereby, resulting in resistance among other group of antibiotics and such resistance have a potential for rapid spread since, they are usually plasmid mediated. The present study showed higher resistance in ciprofloxacin which can be explained by the fact of non-judicious use and easy availability even without prescription. Meropenem resistance was 40% in *Pseudomonas aeruginosa* and 54.39% in *Acinetobacter baumannii* which may be up to 42.7% [17] and 90.3% [18] indicate increasing trend of carbapenem resistance in non-fomenters. A total of 56.82% Metallo beta lactamase and 72.22% AmpC beta lactamase were detected among *Pseudomonas* and *Acinetobacter* isolates. Among carbapenem resistance acquisition, AmpC-beta-lactamase mechanism is more common than Metallo beta lactamase and carbapenemase other than Metallo beta lactamase in our health care setup. Overall our study has implicated the severity of carbapenem resistant non-fermenters, which are affecting the infection control activities among hospital setup.

LIMITATION

Numbers of samples are less. Our study was limited to the detection of carbapenemase production only among carbapenem resistant non-fermenters isolated from inpatients.

CONCLUSION

Carbapenem resistance rate was more in *Acinetobacter* than *Pseudomonas* species. Our study highlights that no single phenotypic test is sufficient for detection and differentiation of carbapenem resistance among non-fermenters. Detection of carbapenem resistance (Metallo-β-lactamase, Carbapenemases other than MBL and AmpC β lactamase) is essential because they limit treatment options and can spread to susceptible bacteria by gene transfer. Antibiotics according to susceptibility pattern and infection control measures are necessary in prevention of emergence of non-fermenters in our teaching institute. However, higher resistance to beta lactam and cephalosporin is one of the alarming sign in our region.

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