ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 10, Issue 6, 2017

Online - 2455-3891
Print - 0974-2441

Research Article

MULTIPLE ANTIBIOTIC RESISTANCE INDEXING OF NON-FERMENTING GRAM-NEGATIVE BACILLI

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Received: 14 February 2017, Revised and Accepted: 07 March 2017

ABSTRACT

Objective: The multiple antibiotic resistance (MAR) indexing and finding multidrug-resistant (MDR) bacteria will help to indicate the origin from the high risk of contamination where the antibiotics are often used. Hence, this study was carried out to give the MAR index of non-fermenting Gram-negative bacilli in a tertiary care hospital which would help our infection control team also.

Methods: Drug resistance was tested using Kirby-Bauer disc diffusion method. MAR index was calculated using the formula, a/b (where, a=number of antibiotics to which the organism was resistant and b=total number of antibiotics to which the organism was tested).

Results: Of 240 Gram-negative non-fermenters isolated, 117 (49%) strains were >0.2 of MAR index, 95 (81%) was from inpatient department. 73 (62%) were hospitalized for more than 3 days, 44 (38%) was from surgery department. 49 (42%) was wound specimen. Out of 117 multiple antibiotic resistant isolates, 99 (85%) were MDR isolates.

Conclusion: Nearly 51% prevalence of isolates >0.2 MAR index shows that the source of contamination can still be brought up down by proper surveillance and management with proper usage of surface and skin disinfectants, especially in surgery ward where the MAR index has indicated more usage of antibiotics.

Keywords: Multi-drug resistance, Multiple antibiotic resistance, Non-fermenters, Antibiotic susceptibility testing, Hospital acquired the infection.

 DOI: http://dx.doi.org/10.22159/ajpcr.2017.v10i6.17717

INTRODUCTION

Non-fermenting Gram-negative bacilli (NFGNB) are ubiquitous in nature. They are primarily opportunistic pathogens [1]. It has an ability to survive in varying temperatures, pH, and humidity which acts as a major factor for being nosocomial pathogens. The infection posed by them is now a worldwide problem because of their multiple, intrinsic or acquired drug resistance that can lead to increased morbidity and mortality. They are capable of producing biofilms. Hence, there is a risk of colonization during the presence of underlying severe illnesses, long-term hospitalization, stays in intensive care units, selective antimicrobial pressure, and invasive interventions [2]. The relationship between antibiotic use and resistance is complex; a major driving factor for antibiotic resistance is antibiotic use/abuse [3]. In developing countries like India, where the infectious disease burden in high, these antimicrobial resistance is particularly pressing because of cost constraints also [4]. Only a few studies from India provide the antimicrobial susceptibility data of NFGNBs [5]. The emergence of multiresistant strains and pan-resistant strains of these organisms can even cause a sudden outbreak of infection in a clinical unit. High prevalence of multidrug resistance indicates a serious need for surveillance and planning of effective interventions to reduce multidrug resistance in such pathogens [6]. Multiple antibiotic resistance (MAR) indexing has been shown to be a cost-effective and valid method of bacteria source tracking. MAR in bacteria is most commonly associated with the presence of plasmids which contain one or more resistance genes, each encoding a single antibiotic resistance phenotype [5]. MAR index is calculated as the ratio of a number of antibiotics to which organism is resistant to total number of antibiotics to which organism is exposed. MAR index values >0.2 indicate high-risk source of contamination where antibiotics are often used [7]. The emergence of MAR pathogenic strains of NFGNB will indicate the possible nosocomial infection in the hospital environment [8]. Hence, it is important for the clinicians to remain updated with the current susceptibility profile and MAR index of the NFGNB, which will help in proper usage of antibiotics and even in preventing nosocomial infections. Thus, this study was carried out to evaluate the MAR indices of NFGNB in our set up.

METHODS

The descriptive study to analyze the MAR indices of NFGNB was carried out in the Department of Microbiology, Saveetha Medical College and Hospital after getting approval from the Institutional Human Ethical Committee and Scientific Review Board during June 2016 - September 2016. NFGNB was isolated from the clinical samples received in microbiology laboratory and identified by conventional phenotypic methods such as pigment production, oxidase, triple sugar iron agar, and oxidative-fermentative testing. Antimicrobial susceptibility testing was performed using Kirby Bauer disc diffusion method and compared with Central Laboratory Standard Institutes 2016 guidelines [9]. Multidrug-resistant (MDR) strains (i.e. strains showing resistance to at least two of the following group of antibiotics fluoroquinolone, aminoglycosides, and cephalosporins) were identified. MAR index was calculated using the formula a/b, in which, a is the total number of antibiotics to which the organism was resistant, and b is the total number of antibiotics to which the organism was tested [9]. Here, the antibiotics used were amikacin (AK), gentamicin (G), ceftazidime, cefepime, ciprofloxacin, ofloxacin, piperacillin tazobactum, imipenem (IMP), and meropenem (MR). *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as the control strains.

RESULTS

Out of 3500 clinical samples, 240 (7%) yielded NFGNB. Of which, 102 (42%) were identified as *P. aeruginosa,* 45 (19%) were

Table 1: Distribution of MAR index among NFGNB

S.No.	Name of the organism	Total number of patient	MAR index									
			0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
1.	*Pseudomonas aeruginosa*	102	54	2	11	3	7	Nil	4	6	7	1	7
2.	*Acinetobacter* species	45	20	1	1	1	1	Nil	1	4	3	1	6
3.	*Pseudomonas* species	93	40	Nil	18	5	5	Nil	2	7	7	3	6

MAR: Multiple antibiotic resistance, NFGNB: Non-fermenting Gram-negative bacilli

Table 2: Percentage of NFGNB with MAR index >0.2

S.No.	Name of the organism	MAR index									Total	Percentage
		0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1		
1.	*Pseudomonas aeruginosa*	11	3	7	Nil	4	6	7	1	7	46	39
2.	*Acinetobacter* species	1	1	1	Nil	1	4	3	1	6	18	16
3.	*Pseudomonas* species	18	5	5	Nil	2	7	7	3	6	53	45
Total											117	100

MAR: Multiple antibiotic resistance, NFGNB: Non-fermenting Gram-negative bacilli

Acinetobacter spp. and 93 (39%) were identified as *Pseudomonas* species. Species-level identification of these 137 organisms was not done. 117 (49%) of 240 NFGNB were found to have MAR index >0.2. MAR index distribution among NFGNB is shown in Table 1. Among these non-fermenters, *P. aeruginosa* exhibits 39%, *Acinetobacter* species 16%, and *Pseudomonas* species 45% of MAR index >0.2. This is explained in Table 2.

95 (81%) was from inpatient department. 73 (62%) were hospitalized for more than 3 days, 44 (38%) was from surgery department. 49 (42%) was wound specimen. The department-wise and sample-wise distribution is shown in Tables 3 and 4, respectively.

Out of 117 multiple antibiotic resistant isolates, 99 (85%) were MDR isolates. The anti-microbial susceptibility pattern of NFGNB with MAR index >0.2 is shown in Table 5.

The isolates were highly susceptible to IMP (70%) and AK (69%). The antimicrobial susceptibility profile of those 99 MDR isolates is shown in Table 6.

Table 3: Department wise distribution of NFGNB with MAR index >0.2

Department	Inpatient	Out patient	Total	Percentage
Medicine	20	5	25	22
Surgery	39	5	44	38
OG	6	1	7	6
Orthopedics	3	1	4	3
Urology	2	2	4	3
Pediatrics	4	3	7	6
ICU	18	3	21	18
ENT	3	2	5	4
Total	95	22	117	100

MAR: Multiple antibiotic resistance, NFGNB: Non-fermenting Gram negative bacilli, OG: Obstetrics gynecology, ENT: Ears nose throat, ICU: Intensive care unit

Table 4: Sample-wise distribution of NFGNB with MAR index >0.2

Sample	Inpatient	Out patient	Total	Percentage
Urine	26	4	30	25
Wound	37	12	49	42
Pus	16	3	19	16
Sputum	9	3	12	10
Tracheal aspirate	2	0	2	2
Tissue	1	0	1	1
Ascitic fluid	1	0	1	1
Blood	3	0	3	3
Total	95	22	117	100

MAR: Multiple antibiotic resistance, NFGNB: Non-fermenting Gram negative bacilli

Table 5: Antimicrobial susceptibility pattern of NFGNB with MAR index >0.2

Antibiotics	Sensitive (%)	Resistant (%)
AK	69 (59)	48 (41)
G	41 (35)	76 (65)
CAZ	13 (11)	104 (89)
CPM	13 (11)	104 (89)
CIP	21 (18)	96 (82)
OF	27 (23)	90 (77)
PIT	51 (44)	63 (56)
IMP	70 (60)	47 (40)
MR	68 (58)	49 (42)

MAR: Multiple antibiotic resistance, NFGNB: Non-fermenting Gram-negative bacilli, AK: Amikacin, G: Gentamicin, CAZ: Ceftazidime, CPM: Cefepime, CIP: Ciprofloxacin, OF: Ofloxacin, PIT: Piperacillin tazobactam, IMP: Imipenem, MR: Meropenem

DISCUSSION

Although the NFGNB's being the life-threatening pathogens, the proper surveillance and treatment will help to manage those organisms. In this study, the overall prevalence rate of NFGNB was only 7%. Which correlates with the prevalence rate of 10.5% with Olayinka and Onile [8]. NFGNB was of 18% with arora which was seen to be increased. This may be due to the difference in the study population. In this study, *P. aeruginosa* (42%) was the predominant organism followed by 39% of *Pseudomonas* species and 19% of *Acinetobacter* species. However in a study carried out by arora, *Acinetobacter* spp. (62%) was the most common followed by *P. aeruginosa* (18%), *Burkholderia cepacia* complex (5%) and *S. maltophilia* (3%). 12% (221/1781) of the NFGNBs could not be identified. The MAR index of >0.2 was seen more with *Pseudomonas* species, that is, 45%, followed by *P. aeruginosa* 39% then with *Acinetobacter* species 16%. In this study, the NFGNB with >0.2 MAR index was isolated more from the surgical unit (38%) and sample-wise they were isolated more from wound samples (42%) followed by urinary samples (25%). This was correlated with the study done by Stark and Maki, 1984 [10], where majority strains were isolated from wound swabs. However in the study conduction by Olayinka and Onile [8], *P. aeruginosa* strains were isolated more from urinary specimens. In this study, 70% were sensitive to IMP, 69% were sensitive to AK and 68% were sensitive to MR. However, 98.1% were sensitive to AK in the study done by Olayinka and Onile [8] being the first-line drug, in our study AK exhibited only 69% susceptibility. Compared with the study done at the Lagos University teaching hospital, only 12.5% exhibited resistance to IMP. However in our study, 30% had shown resistance to IMP. Similarly, Olayinka and Onile [8] also had reported

Table 6: The antimicrobial susceptibility profile of MDR isolates

Susceptibility profile	Number of isolates
G, CAZ, CPM, CIP, OF, PIT, MR	7
G, CAZ, CPM, PIT, MR	2
G, CAZ, CPM, CIP, OF, PIT	2
G, CAZ, CPM, CIP, OF, PIT, IMP, MR	7
AK, G, CAZ, CPM	5
AK, G, CAZ, CPM, CIP, OF	19
AK, G, CAZ, CPM, CIP, OF, PIT, IMP, MR	19
AK, G, CAZ, CPM, CIP, OF, MR	1
AK, G, CAZ, CPM, CIP, OF, PIT	3
AK, G, CIP, OF	3
CAZ, CPM, CIP, OF	13
CAZ, CPM, CIP, OF, PIT, IMP, MR	10
CAZ, CPM, PIT, IMP, MR	1
CAZ, CPM, CIP	2
CAZ, CPM, PIT, MR	1
CAZ, CPM, PIT	3
CAZ, CPM, CIP, OF, PIT	1
Total	99

MDR: Multi-drug resistant, AK: Amikacin, G: Gentamicin, CAZ: Ceftazidime, CPM: Cefepime, CIP: Ciprofloxacin, OF: Ofloxacin, PIT: Piperacillin-tazobactam, IMP: Imipenem, MR: Meropenem

more than 80% sensitivity to IMP in her study. In this study, 99 isolates exhibited multidrug resistance mechanism. Of which, 19 were pan-drug resistant. Susceptibility profile of MDR isolates revealed that the resistance pattern is equally distributed and none of the isolate is 100% susceptible to a drug. Whereas in a study conducted by Olayinka and Onile [8], 100% MDR strains were sensitive to IMP and 16 out of 18 were resistant to gentamicin. It has been said that there is generally an excess of resistance among isolates from hospitalized patients compared with those from outpatients [6]. This has been correlated well with this study, were 81% (98) were inpatient and 62% of which have been hospitalized for more than 3 days. MAR index higher than 0.2 has been said to be an indication of isolates originating from an environment where antibiotics were often used [7,11]. Analysis of the MAR index of the *Pseudomonas* strains in a study done by Olayinka and Onile [8], showed that 60.9% had MAR index of 0.3 and above. In this study, 49% exhibited >0.2 of MAR index. It shows that the use of antibiotics is still under control.

CONCLUSION

From this study, it is clear that the prevalence rate of NFGNB is less, that is, 7% in our set up. Of which only 49% exhibited MAR of >0.2. This can be controlled by proper management and surveillance. The isolates were more from surgery department. This shows that the chance of contamination of MDR isolates will be more in these units, which has to be taken into consideration. In this study, 85% were MDR isolates. None were 100% sensitive to any of the drugs because the resistance profile is equally distributed. Nearly 62% were hospitalized for more than 3 days which indicates that these organisms may be the probable source for nosocomial infection in future that need to be treated immediately. When strains have multiple antibiotic resistance, the choice of therapy is limited and difficult. Thus, it is important to have antibiotic policies and surveillance programs. Moreover, it is desirable to periodically monitor the susceptibility pattern of NFGNB as they were the common pathogens causing nosocomial infections worldwide. This will help to administer an effective therapeutic agent whenever there is a need to do so.

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