Multidrug and co-resistance patterns of non-fermenting Gram-negative bacilli involved in ventilator-associated pneumonia carrying class 1 integron in the North of Iran

Masoumeh Bagheri-Nesami[1], Mohammad Sadegh Rezai[2,], Fatemeh Ahangarkani[3], Alireza Rafiei[4], Attieh Nikkhah[5], Gohar Eslami[6], Kheironesa Shafahi[7], Azin Hajalibeig[8], Rezvan Khajavi[9]*

Abstract

Introduction **Ventilator-associated pneumonia (VAP) due to non-fermenting Gram-negative bacilli (NFGNB), especially *Pseudomonas aeruginosa* and *Acinetobacter spp.*, is one of the main hospital-acquired infections leading to mortality and morbidity, especially in intensive care units (ICUs). This study seeks to determine the multidrug and co-resistance (MDR) patterns of NFGNB that are agents of VAP, and assess the presence of class 1 integron in these bacteria.**

Methods **This cross-sectional study involved VAP patients admitted in the ICUs of 18 hospitals in the Mazandaran province, located in the North of Iran. The antibiotic susceptibility pattern was determined by the minimum inhibitory concentration (MIC) test by using broth microdilution method. Presence of class 1 integron was evaluated by the polymerase chain reaction (PCR) assay.**

Results **Out of a total of 83 patients who were microbiologically diagnosed as VAP, 52 non-duplicated NFGNBs (24 *P. aeruginosa* and 28 *A. baumannii*) were causative of VAP, out of which MDR NFGNBs were responsible for 48 (57.83%) cases. The frequencies of MDR NFGNBs were as follows: 27 (56.25%) *A. baumannii* and 21 (43.75%) *P. aeruginosa*. *P. aeruginosa* isolates were resistant to all aminoglycoside antibiotics (50%), ciprofloxacin (45.8%), ceftazidime (70.8%), cefepime (87.5%), colistin (62.5%), and imipenem (29.2%). *A. baumannii* isolates were resistant to aminoglycosides (53.6%), ciprofloxacin (85.7%), ceftazidime (92. 9%), cefepime (92.9%), colistin (35.7%), and imipenem (57.1%). Twelve isolates were resistant to all 10 tested antibiotics. The number of rates of class 1 integron, positive for MDR *P. aeruginosa* and MDR *A. baumannii*, were 20 (95.23%) and 21 (77.78%), respectively.**

Conclusion **The high prevalence of multidrug resistance and incidence of class 1 integron is a therapeutic concern. Employing antibiotic stewardship in hospitals could prevent the dissemination of MDR bacteria.**

Keywords **VAP, *A. baumannii, P. aeruginosa*, MDR, class 1 integron, co-resistance**

Introduction

Ventilator-associated pneumonia (VAP) is one of the main hospital-acquired infections (HAI) leading to mortality and morbidity, especially in

Received: 10 January 2017; revised: 21 January 2017 and 01 May 2017; accepted: 04 May 2017.

[1]PhD, Department of Medical-Surgical Nursing, Nasibeh Faculty of Nursing and Midwifery, Infectious Diseases Research Center with Focus on Nosocomial Infection, Mazandaran University of Medical Sciences, Bou Ali Sina Hospital, Pasdaran Boulevard, Sari, Iran; [2]MD, Department of Pediatrics, Infectious Diseases Research Center with Focus on Nosocomial Infection, Mazandaran University of Medical Sciences, Bou Ali Sina Hospital, Pasdaran Boulevard, Sari, Iran; [3]PhD student, Department of Infectious Diseases, Antimicrobial Resistant Research Center, Student Research Committee, Mazandaran University of Medical Sciences, Razi Teaching Hospital, Yosuf Reza Street, Sari, Iran; [4]PhD, Molecular and Cell Biology Research Center, Faculty of Medicine, Mazandaran, University of Medical Sciences, Km 18 Khazarabad Road, Sari, Iran; [5]MSc, Infectious Diseases Research Center with Focus on Nosocomial Infection, Mazandaran University of Medical Sciences, Km 18 Khazarabad Road, Sari, Iran; [6]PhD, Department of Clinical Pharmacy, Faculty of Pharmacy, Mazandaran, University of Medical Sciences, Km 18 Khazarabad Road, Sari, Iran; [7]PhD student, Fatemeh Zahra Hospital, Mazandaran University of Medical Sciences, Shahrdari Square, Artesh Street, Sari, Iran; [8]MD, Department of Pediatrics, Infectious Diseases Research Center with Focus on Nosocomial Infection, Mazandaran University of Medical Sciences, Bou Ali Sina Hospital, Pasdaran Boulevard, Sari, Iran; [9]MSc, Molecular and Cell Biology Research Center, Faculty of Medicine, Mazandaran, University of Medical Sciences, Km 18 Khazarabad road, Sari, Iran.

intensive care units (ICU). VAP refers to pneumonia which occurs in patients who need mechanical ventilation through tracheostomy or endotracheal tubes for at least 48 hours.[1] Potential multidrug-resistant (MDR) pathogens, including non-fermenting Gram-negative bacilli (NFGNB), mainly *Pseudomonas aeruginosa* and *Acinetobacter* spp., are associated with VAP. Antibiotic therapy of VAP caused by MDR *P. aeruginosa* and *Acinetobacter* spp., and co-resistance of these pathogens to routine antibiotics, represent real challenges, leading to a deficit of treatment options.[2,3] Due to the mentioned resistance, the efficacy of routinely-prescribed antibiotics is becoming increasingly compromised worldwide as reported before, and this resistance is mostly attributed to the production of extended-spectrum beta-lactamases and carbapenem hydrolyzing enzymes.[4,5] The increasing emergence of MDR NFGNB isolates may be due to the acquisition or horizontal transfer of antibiotic-resistance genes. Integrons as mobile genetic elements can capture and spread antimicrobial resistance genes. As of now, five classes of integrons have been described, among which class 1 is most frequently identified in clinical isolates, and the strains carrying these genes are resistant to multiple classes of antimicrobial agents.[6] Hitherto, many studies have reported high rates of VAP due to MDR NFGNB. On the other hand, being aware of local epidemiology of the agents is of great importance. The aim of this study was to determine the multidrug and co-resistance patterns of NFGNB agents isolated from VAP patients, and to identify the presence of class 1 integron in the ICU wards of 18 different hospitals of the Mazandaran province, located in the North of Iran.

***Corresponding author:** Mohammad Sadegh Rezai, MD, Associated Professor, Department of Pediatrics, Infectious Diseases Research Center with Focus on Nosocomial Infection, Mazandaran University of Medical Sciences, Bou Ali Sina Hospital, Pasdaran Boulevard, Sari, Iran. drmsrezaii49@gmail.com

Article downloaded from www.germs.ro
Published September 2017

ISSN 2248 – 2997
ISSN – L = 2248 – 2997

Methods

Study population and ethics

This descriptive cross-sectional study was conducted during a period between 2014 and 2015, in the ICU wards of 18 different hospitals of the Mazandaran province, Iran This study was approved by the Ethics Committee of Mazandaran University of Medical Sciences (code no: 879, date: 09 July 2014).

A VAP case was diagnosed in "a mechanically ventilated patient with a chest radiograph that showed new or progressive infiltrates, cavitation, consolidation, or pleural effusion 48 h after hospitalization. The patient must have had at least one of the following criteria: new onset of purulent sputum or change in character of sputum; organism cultured from blood or from a specimen obtained by tracheal aspirate, bronchoalveolar lavage or bronchial brushing, or biopsy".[6]

All NFGNB that were confirmed as causing VAP were evaluated. Deep tracheal aspirates from endotracheal tubes were obtained by head nurses and samples were immediately transported in transport sterile container tubes to the microbiology laboratory. Samples were cultured on blood agar, chocolate agar, and Mac Conkey's agar, and were incubated at 37°C for 24 hours. Identification of NFGNB was performed according to standard microbiological procedures.[7]

Antibiotic susceptibility test, isolation of MDR NFGNB and determination of co-resistance profile

Antibiotic susceptibility tests were performed by the standard broth microdilution technique, according to the standard CLSI 2010 protocol. The bacterial suspensions equivalent to 0.5 McFarland standard were prepared in Müller-Hinton Broth (Merck, Darmstadt, Germany). The final standard bacterial concentrations were increased to 5×10^5 CFU/ mL. Serial antibiotics concentrations were prepared ranging from 512 µgr/mL to 1 µg/mL. The tested antibiotics were amikacin (AN), ciprofloxacin (CP), imipenem (IPM), gentamicin (GM), ceftazidime (CAZ), tobramycin (TOB), piperacillin-tazobactam (TZP), cefepime (CPM), colistin (CST), and co-

trimoxazole (TMP/SMX). Minimum inhibitory concentration (MIC) was defined as the lowest concentration of an antibiotic that inhibited the visible growth of bacteria after overnight culture.

MDR pathogens were defined as the isolates that were resistant to at least three and possibly more different classes of antimicrobial agents such as extended-spectrum cephalosporins, antipseudomonal fluoroquinolones, aminoglycosides, anti-pseudomonal carbapenems, antipseudomonal penicillins plus β-lactamase inhibitors, antipseudomonal cephalosporins and polymyxins.[8] Co-resistance was determined by the crossover table of resistant isolates.

DNA extraction and detection of integron class 1

To obtain the best possible results, a single colony from each isolate was selected for DNA extraction and genotyping. Bacterial DNA was extracted with a commercial gene-extraction kit (Takapou Zist, Tehran, Iran) according to the manufacturer's instructions. Then, MDR strains were screened for integron class 1 genes by PCR. To amplify the int1 region, a set of primers were used, and their sequences were: [F:5' CAGTGGACATAAGCCTGTTC3' R:5' CCCGAGGCATAGACTGTA3']. Temperature conditions for the PCR were: primary denaturation at 1 cycle of 2 min at 94°C, followed by 35 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 55°C, extension for 30 sec at 72°C, one cycle for the final extension for 3 min at 72°C. PCR amplification was performed in the final volume of 20 µL containing 100 ng of the template DNA, 10 pM of each primer, and 10 µL of 2x master mix (Ampliqon, Odense, Denmark). Amplified products were visualized by 2% (w/v) agarose gel electrophoresis in Tris/Borate/EDTA (TBE) buffer, stained with Cyber green (0.5 µg.mL-1), and photographed under UV transilluminator. *E. coli* 96K062 was used as positive control for class 1 integron.

Statistical analysis

Data were analyzed using SPSS 16 software (SPSS Inc., Chicago, IL, USA). Descriptive statistics and Chi-square test were used; p values <0.05 were considered as significant.

Results

Out of 83 patients who were microbiologically diagnosed as VAP in the ICU wards of the 18 hospitals of Mazandaran province, 52 non-duplicated NFGNBs (24 *P. aeruginosa* and 28 *A. baumannii*) were causative of VAP, among which MDR NFGNBs were responsible for 48 (57.83%) cases. The most frequently found MDR NFGNBs were: *Acinetobacter baumannii* 27 (56.25%) and *Pseudomonas aeruginosa* 21 (43.75%). Other agents of VAP belonged to the Enterobacteriaceae family.

The demographic features of patients with MDR *P. aeruginosa* were as follows: 21 patients (11, 52.38%, male and 10, 47.61%, female) with an average age of 41.01±24.05 years and an average duration of hospitalization in the ICU of 17.69±14.51 days; 27 patients (17, 62.96% male and 10, 37.03% female) with an average age of 62.17±18.31 years and an average duration of hospitalization in the ICU of 12.43±4.23 days.

The resistance patterns of MDR *P. aeruginosa* and *A. baumannii* are shown in Table 1. Overall, twelve isolates were resistant to all 10 tested antibiotics.

About 50% of isolated *P. aeruginosa* were resistant to all aminoglycoside antibiotics (amikacin, gentamicin, and tobramycin), 45.85% were resistant to a fluoroquinolone antibiotic (ciprofloxacin), 70% were resistant against a third generation cephalosporin (ceftazidime), and 87.5% were resistant to a fourth generation cephalosporin (cefepime). Resistance to colistin and imipenem among *P. aeruginosa* were 62.5% and 29.2% respectively.

Table 1. Multidrug resistance patterns of non-fermenting Gram-negative bacilli

Bacteria	Number of isolates	Pattern of resistance to different antibiotics
Pseudomonas aeruginosa N=21	6	AN, CP,IPM, GM, CAZ, TOB, TZP, CPM, CST, TMP/SMX
	1	AN, CP,IPM, GM, CAZ, TZP, CPM, CST, TMP/SMX
	1	AN, IPM, GM, CAZ, TOB, TZP, CPM, CST, TMP/SMX
	2	AN, GM, CAZ, TOB, TZP, CPM, CST, TMP/SMX
	1	CP, GM, CAZ, TOB, TZP, CPM, CST, TMP/SMX
	1	AN, CP, GM, CAZ, TZP, CPM, CST, TMP/SMX
	1	AN, GM, CAZ, TOB, CPM, CST, TMP/SMX
	1	AN,CP,GM, CAZ, TZP, CPM, TMP/SMX
	1	AN, GM, TOB, TZP, CPM, TMP/SMX
	1	AN,GM,CAZ,TOB,CPM
	1	CP, GM, TZP, CPM, TMP/SMX
	1	GM,CAZ, TZP, CPM, TMP/SMX
	1	AN, CAZ, CPM, TMP/SMX
	1	GM, CPM, CST, TMP/SMX
	1	GM, TOB, CST, TMP/SMX
Acinetobacter baumannii N=27	6	AN, CP, IPM, GM, CAZ, TOB, TZP, CPM, CST, TMP/SMX
	2	AN, CP, IPM, GM, CAZ, TOB, TZP, CPM, TMP/SMX
	1	AN, CP, GM, CAZ, TOB, TZP, CPM, CST, TMP/SMX
	2	AN, IPM, GM, CAZ, TOB, TZP, CPM, TMP/SMX
	1	CP, IPM, GM, CAZ, TOB, TZP, CPM, TMP/SMX
	1	AN, CP, GM, CAZ, TOB, TZP, CPM, TMP/SMX
	1	AN, CP, IPM, GM, CAZ, TZP, CPM, TMP/SMX
	1	CP, GM, CAZ, TOB, TZP, CPM, CST, TMP/SMX
	1	AN, CP, GM, CAZ, TOB, TZP, CST, TMP/SMX
	1	AN, CP, IPM, CAZ, TOB, TZP, CPM, TMP/SMX
	1	AN, CP, GM,CAZ, TZP, CPM,CST, TMP/SMX
	2	AN, CP, GM,CAZ, TZP, CPM, TMP/SMX
	1	AN, IPM, GM, CAZ, TOB, TZP, TMP/SMX
	1	AN, CP,IPM, GM, CAZ, TZP, TMP/SMX
	1	CP, GM, CAZ, TOB, TZP, CPM, TMP/SMX
	1	AN, CP, IPM, GM, TZP, CPM
	1	AN, CP, GM, TZP, CPM, TMP/SMX
	1	IPM, GM, CAZ, TZP, CPM
	1	CP, CAZ,TZP, CPM, TMP/SMX
	1	CP, CAZ,TZP, CPM, TMP/SMX

AN – amikacin; **CAZ** – ceftazidime; **CP** – ciprofloxacin; **CPM** – cefepime; **CST** – colistin; **IPM** – imipenem; **GM** – gentamicin; **TOB** – tobramycin; **TZP** – piperacillin-tazobactam; **TMP/SMX** – co-trimoxazole

About 53.6% of *A. baumannii* isolates were resistant to all aminoglycoside antibiotics (amikacin, gentamicin, and tobramycin), 86% were resistant to a fluoroquinolone antibiotic (ciprofloxacin), 92.9% were resistant to both a third generation cephalosporin (ceftazidime), and a fourth generation cephalosporin (cefepime). Resistances to colistin and imipenem were 35.7% and 57.1% respectively.

The co-resistance patterns of *P. aeruginosa* and *A. baumannii* are shown in Tables 2 and 3 respectively. The rates of class 1 integron being positive in MDR *P. aeruginosa* and MDR *A. baumannii* isolates were 20 (95.23%) and 21 (77.78%) respectively.

The relationship between antibiotic resistance and the presence of class 1 integron in NFGNBs is shown in Table 4. About 61.9-100%

of antibiotic-resistant isolates contained the integron class 1 gene. In addition, there was a significant relationship between the integron and resistance to them ($p<0.05$) – Table 4.

The results of this study revealed that MDR NFGNB were responsible for 57.83% of VAP infections in the ICUs of the 18 hospitals that were included in this study. In addition, twelve isolates were resistant to all the ten tested

Table 2. Co-resistance pattern of *Pseudomonas aeruginosa* to the tested antibiotics

		Number of isolates (percentage in parenthesis) resistant to antibiotics									
Antibiotics	Number	AN N (%)	CP N (%)	IPM N (%)	GM N (%)	CAZ N (%)	TOB N (%)	TZP N (%)	CPM N (%)	CST N (%)	TMP/SMX N (%)
AN	18		9 (50)	7 (38.9)	16 (88.9)	15 (83.3)	11 (61.1)	15 (83.3)	16 (88.8)	16 (88.9)	16 (88.9)
CP	11	9 (81.8)		7 (63.6)	11 (100)	10 (90.9)	7 (63.6)	11 (100)	11 (100)	9 (81.8)	11 (100)
IPM	7	7 (100)	7 (100)		7 (100)	7 (100)	6 (85.7)	7 (100)	7 (100)	7 (100)	7 (100)
GM	21	16 (76.2)	11 (52.4)	7 (33.3)		16 (76.2)	13 (61.9)	18 (85.7)	18 (85.7)	14 (66.7)	19 (90.5)
CAZ	17	15 (88.2)	10 (58.8)	7 (41.2)	16 (94.1)		12 (70.6)	15 (88.2)	17 (100)	12 (70.6)	16 (94.1)
TOB	13	11 (84.6)	7 (53.8)	6 (46.2)	13 (100)	12 (92.3)		12 (92.3)	12 (92.3)	11 (84.6)	12 (92.3)
TZP	20	15 (75)	11 (55)	7 (35)	18 (90)	15 (75)	12 (60)		18 (90)	14 (70)	19 (95)
CPM	21	16 (76.2)	11 (52.4)	7 (33.3)	18 (85.7)	17 (81)	12 (75.1)	18 (85.7)		14 (66.7)	19 (90.5)
CST	15	12 (80)	9 (60)	7 (46.7)	14 (93.3)	12 (80)	11 (73.3)	14 (93.3)	15 (100)		15 (100)
TMP/SMX	21	16 (76.2)	11 (52.4)	7 (33.3)	19 (90.5)	16 (76.2)	12 (75.1)	19 (90.5)	19 (90.5)	15 (71.4)	

AN – amikacin; **CP** – ciprofloxacin; **IPM** – imipenem; **GM** – gentamicin; **CAZ** – ceftazidime; **TOB** – tobramycin; **TZP** – piperacillin-tazobactam; **CPM** – cefepime; **CST** – colistin; **TMP/SMX** – co-trimoxazole

Discussion

In recent years, Gram-negative bacteria causative of HAI, although not necessarily increasing in number, are becoming increasingly resistant to the existing antibiotics.[4] One of the most alarming facts however, is the evolution of VAP caused by NFGNB, and particularly those that are MDR, and are associated with significant mortality and morbidity, as well as economic problems.[9]

antibiotics. The rates of VAP caused by NFGNB vary geographically. For example, in the study by Dellit et al., the prevalence of NFGNB was about 42%.[10] The rates of VAP due to *A. baumannii*, were 11.6, 35, and 34.5% in the studies by Shete et al., Ebrahimi et al., and Japoni et al., respectively, compared to the rates of VAP due to *P. aeruginosa*, which were 15.5 and 9% in the studies by Japoni et al. and Erahimi et al., respectively.[1,11,12]

Table 3. Co-resistance pattern of *Acinetobacter baumannii* to the tested antibiotics

		Number of isolates (percentage in parenthesis) resistant to antibiotics									
Antibiotics	Number	AN N (%)	CP N (%)	IPM N (%)	GM N (%)	CAZ N (%)	TOB N (%)	TZP N (%)	CPM N (%)	CST N (%)	TMP/SMX N (%)
AN	23	■	20 (87)	15 (65.2)	22 (95.7)	21 (91.3)	15 (65.2)	23 (100)	21 (91.3)	9 (39.1)	22 (95.7)
CP	24	20 (83.3)	■	13 (54.2)	22 (91.7)	22 (91.7)	15 (62.5)	24 (100)	23 (95.8)	10 (41.7)	23 (95.8)
IPM	16	15 (93.8)	13 (81.3)	■	15 (93.8)	15 (93.8)	13 (81.3)	16 (100)	15 (93.8)	6 (37.5)	15 (93.8)
GM	27	22 (81.5)	22 (81.5)	15 (55.6)	■	24 (88.9)	17 (63)	26 (96.3)	24 (88.9)	10 (37)	24 (88.9)
CAZ	26	21 (80.8)	22 (84.6)	15 (57.7)	24 (92.3)	■	18 (69.2)	26 (100)	24 (92.3)	10 (38.5)	25 (96.2)
TOB	17	15 (88.2)	15 (88.2)	13 (76.4)	17 (100)	17 (100)	■	17 (100)	16 (94.11)	9 (52.9)	17 (100)
TZP	28	23 (82.1)	24 (85.7)	16 (57.1)	26 (92.9)	26 (92.9)	18 (64.3)	■	26 (92.9)	10 (35.7)	26 (92.9)
CPM	26	21 (80.8)	23 (88.5)	15 (57.7)	24 (92.3)	24 (92.3)	16 (61.5)	26 (100)	■	9 (34.6)	24 (92.3)
CST	10	9 (90)	10 (100)	6 (60)	10 (100)	10 (100)	9 (90)	10 (100)	9 (90)	■	10 (100)
TMP/SMX	26	22 (84.6)	23 (88.5)	15 (57.7)	24 (92.3)	25 (96.2)	18 (69.2)	26 (100)	24 (92.3)	26 (100)	■

AN – amikacin; **CP** – ciprofloxacin; **IPM** – imipenem; **GM** – gentamicin; **CAZ** – ceftazidime; **TOB** – tobramycin; **TZP** – piperacillin-tazobactam; **CPM** – cefepime; **CST** – colistin; **TMP/SMX** – co-trimoxazole

Luna et al. demonstrated that effective early therapy for VAP associated a reduced mortality while inadequate therapy during the first 48 hours associated a mortality rate of 91%.[13] The antimicrobial resistance in NFGNB is associated with production of extended-spectrum β-lactamase enzymes, carbapenem hydrolyzing enzymes, efflux system overexpression, and other mechanisms.

Although carbapenems are among the most effective options for the NFGNB that cause VAP, the high resistance rate to imipenem (about 30-60%) in the findings of this study, is in fact concerning, and one should practice more caution in using this antibiotic for empiric therapy. On the other hand, imipenem-resistant NFGNB in this study had co-resistance with cefepime (93-100%). Colistin is not commonly used in clinical practice because of its neurotoxicity and nephrotoxicity, and this antibiotic has been considered as a therapeutic option for treatment of VAP caused by MDR NFGNB. In the present study, the rates of colistin resistance in NFGNB were high. A great variability exists regarding the occurrence of colistin resistance in different geographical areas. Shaheer Ahmed et al. in a global report on colistin-resistant *A. baumannii*, reported that the highest resistance rates were from Asia-Pacific followed by Europe, the Americas and Africa.[14]

Overall, the majority of the NFGNB exhibited co-resistance to the antibiotics that are used conventionally, which limits the application of suitable antibiotics whenever empiric or an alternative therapy needs to be considered.

Resistance genes are acquired mostly through transferable plasmids and in most cases, integrons are responsible.[15] MDR strains can

emerge through the transfer of integrons into other bacteria or the insertion of gene cassettes which encode resistance genes.[6] Therefore in this study, the high rates of class 1 integron among *P. aeruginosa* and *A. baumannii* were to be expected due to the high rates of resistance among these isolates. In addition, association of antibiotic resistance and presence of class 1 integron in most antibiotic-resistant isolates was significant ($p<0.05$) – Table 4.

There are several mechanisms by which bacteria may acquire co-resistance to aminoglycosides, and plasmid-mediated enzymatic inactivation appears to be the most important one. The presence of the aminoglycoside-resistance gene cassettes located in the integrons has been proven in several studies.[15,16]

Fortunately, the presence of class 1 integron in imipenem-resistant *P. aeruginosa*, ciprofloxacin-resistant *P. aeruginosa*, and colistin-resistant *A.*

Table 4. Association between antibiotic resistance and the presence of integron class 1 in NFGNBs

Antibiotics	Bacteria	Resistant isolates (number)	Incidence of class 1 integrons in resistant isolates N (%)	Chi square value	P value	OR	CI 95%	
							lower	upper
AN	*P. aeruginosa*	18	17 (94.4)	12.250	<0.0001	17	15.9	18.02
	A. baumannii	23	17 (73.9)	5.261	0.022	2.83	2.36	3.3
CP	*P. aeruginosa*	11	9 (81.8)	4.455	0.350	4.5	3.72	5.28
	A. baumannii	24	19 (79.16)	348	0.007	3.6	3.1	4.1
IPM	*P. aeruginosa*	7	6 (85.7)	3.571	0.060	6	4.92	7.08
	A. baumannii	16	10 (62. 5)	1.667	0.190	2	1.5	2.5
GM	*P. aeruginosa*	21	19 (90.5)	11.842	0.001	9.5	8.76	10.24
	A. baumannii	27	21 (77.77)	6.760	0.009	3.3	2.84	3.76
CAZ	*P. aeruginosa*	17	16 (94.1)	13.235	<0.0001	16	14.97	17.03
	A. baumannii	26	20 (76.9)	6.760	0.009	3.16	2.7	3.62
TOB	*P. aeruginosa*	13	13 (100)	-	-		-	
	A. baumannii	17	13 (76.4)	2.882	0.090	2.4	1.87	2.93
TZP	*P. aeruginosa*	20	13 (61.9)	10.889	0.001	5.64	5.02	6.26
	A. baumannii	28	22 (78.6)	8.333	0.004	3.5	3.04	3.96
CPM	*P. aeruginosa*	21	18 (85.7)	12.800	<0.0001	5.66	5.04	6.28
	A. baumannii	26	20 (76.9)	6.760	0.009	3.5	3.04	3.96
CST	*P. aeruginosa*	15	14 (66.7)	11.267	0.001	14	12.97	15.03
	A. baumannii	10	8 (80)	3.600	0.610	4	3.21	4.79
TMP/SMX	*P. aeruginosa*	21	19 (90.5)	12.800	<0.0001	9.5	8.76	10.24
	A. baumannii	26	20 (76.9)	6.760	0.009	3.16	2.7	3.62

AN – amikacin; **CAZ** – ceftazidime; **CP** – ciprofloxacin; **CPM** – cefepime; **CST** – colistin; **IPM** – imipenem; **GM** – gentamicin; **TOB** – tobramycin; **TZP** – piperacillin-tazobactam; **TMP/SMX** – co-trimoxazole.

The high presence of class 1 integron in the present study shows its association in dissemination of antibiotic-resistance genes in MDR NFGNB. The incidence of class 1 integron among the isolates resistant to aminoglycosides was considerable. Co-resistances among aminoglycosides in *P. aeruginosa* and *A. baumannii* were about 61-88.9% and 61-95% respectively. *baumannii* was not very high and there is hope that class 1 integron will not play a role in the widespread dissemination of the resistance genes for these critical options for treatment of MDR NFGNB.

The presence of integrons in *A. baumannii* causing VAP in the study by Mohammadi-Barzelighi et al. was 8%, which is the opposite of

this study's findings, in the way that a high number of MDR isolates in their study lack integrons.[17] Consistent with the findings of this study, in the report by Farshzadeh et al., 84% of carbapenem-resistant isolates had class 1 integron.[18] The rates of class 1 integron in imipenem-resistant isolates from this study were 85.7-68.75%. Peymani *et al.* reported that all imipenem-resistant *P. aeruginosa* contained class 1 integron during a 17-month evaluation in Tabriz.[19]

The prevalence of class 1 integron in MDR isolates was notably higher than that in non-MDR bacteria. One could even claim that this gene has a higher rate in the bacteria isolated from patients in high risk wards in comparison with patients admitted to non-high-risk wards. For example, the rates of class 1 integron in the studies by Shahcheraghi et al. (100% integron positive in MDR *P. aeruginosa*), Nikokar et al. (69. 2% integron positive in MDR *P. aeruginosa*), Doosti et al. (70.5% class 1 integron positive in *P. aeruginosa* in ICU patients), as well as in this study, were higher in comparison with the findings of Goudarzi et al. (22.8% class 1 integron positive in *P. aeruginosa* isolated from all clinical samples), and Gu et al. (40.82% class 1 integron positive in *P. aeruginosa* and 52.8% class 1 integron positive in *A. baumannii* isolated from a variety of clinical specimens from diverse wards of four different hospitals).[20-24] Horizontal transfer of antibiotic-resistance genes by integrons is considered to be a major driver facilitating rapid spread of antibiotic resistance in bacteria.

For starting empiric therapy in patients in high risk wards such as the ICU, local microbiology patterns should be considered and specialists should avoid prescribing non-helpful antibiotics such as aminoglycosides for MDR NFGNB. Regardless of whether the resistance genes are included within the integrons, the results shown here demonstrate the association between integron carriage and an increased drug-resistance rate.[25] The selection and dissemination of class 1 integron carrying these resistance genes may be amplified in the clinical settings due to the indiscriminate use of antibiotics.

Conclusions

The results shown here indicate the association that exists between integron carriage and increased drug-resistance rates. The presence of class 1 integron can be contributed to the increasing multidrug resistance that is occurring in hospital settings. Employing antibiotic stewardship could prevent the dissemination of MDR bacteria.

Authors' contributions statement: MSR and MBN designed the project, collected data and wrote the manuscript. FA, GE, AH, KS, RK and AN collected data. AR carried out laboratory examinations. All authors read and approved the final manuscript.

Conflicts of interest: All authors – none to declare.

Funding: No funding declared

References

1. Shete VB, Ghadage DP, Muley VA, Bhore AV. Multi-drug resistant *Acinetobacter* ventilator-associated pneumonia. Lung India 2010;27:217-20. [Crossref] [PubMed] [FullText]
2. Malini A, Deepa E, Gokul B, Prasad S. Nonfermenting Gram-negative bacilli infections in a tertiary care hospital in Kolar, Karnataka. J Lab Physicians 2009;1:62-6. [Crossref] [PubMed] [FullText]
3. Garnacho-Montero J, Corcia-Palomo Y, Amaya-Villar R, Martin-Villen L. How to treat VAP due to MDR pathogens in ICU patients. BMC Infect Dis 2014;14:135. [Crossref] [PubMed] [FullText]
4. Behzadnia S, Davoudi A, Rezai MS, Ahangarkani F. Nosocomial infections in pediatric population and antibiotic resistance of the causative organisms in north of Iran. Iran Red Crescent Med J 2014;16:e14562. [PubMed] [FullText]
5. Rezai MS, Pourmousa R, Dadashzadeh R, Ahangarkani F. Multidrug resistance pattern of bacterial agents isolated from patient with chronic sinusitis. Caspian J Intern Med 2016;7:114-9. [PubMed] [FullText]
6. Bagheri-Nesami M, Rafiei A, Eslami G, et al. Assessment of extended-spectrum β-lactamases and integrons among Enterobacteriaceae in device-associated infections: multicenter study in north of Iran. Antimicrob Resist Infect Control 2016;5:52. [Crossref] [PubMed] [FullText]
7. Koneman E, Allen S, Janda W, Schreckenberger R, Winn W. Introduction to microbiology. In: Koneman EW, Alien SD, Janda WM, Schreckenberger RC, Winn W, editors. Part II, Guidelines for collection, transport, processing, analysis, and reporting of cultures from specific specimen sources, Color Atlas and Textbook of Diagnostic Microbiology. 5th ed. Philadelphia: Lippincott; 1997, p. 121-70.
8. Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug- resistant, extensively drug- resistant and

pandrug- resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012;18:268-81. [Crossref] [PubMed]
9. Ramírez-Estrada S, Borgatta B, Rello J. *Pseudomonas aeruginosa* ventilator-associated pneumonia management. Infect Drug Resist 2016;9:7-18. [Crossref] [PubMed] [FullText]
10. Dellit TH. Development of ventilator-associated pneumonia guidelines based on local microbiology and resistance patterns. Presented at: 42nd Annual Meeting of IDSA, Boston, 1 Oct 2004.
11. Ebrahimi M, Khansari-nejad B, Ghaznavi-Rad E. High frequency of ventilator associated pneumonia nosocomial co-infection caused by methicillin resistant *Staphylococcus aureus* and carbapenem resistant *Acinetobacter baumannii* in intensive care unit. J Iran Clin Res 2015;1:67-71.
12. Japoni A, Vazin A, Davarpanah MA, et al. Ventilator-associated pneumonia in Iranian intensive care units. J Infect Dev Ctries 2011;5:286-93. [Crossref] [PubMed]
13. Luna CM, Vujacich P, Niederman MS, et al. Impact of BAL data on the therapy and outcome of ventilator-associated pneumonia. Chest 1997;111:676-85. [Crossref] [PubMed]
14. Ahmed SS, Alp E, Hopman J, Voss A. Global epidemiology on colistin resistant *Acinetobacter baumannii*. J Infect Dis Ther 2016;4:4.
15. Reyes A, Bello H, Domínguez M, Mella S, Zemelman R, González G. Prevalence and types of class 1 integrons in aminoglycoside-resistant Enterobacteriaceae from several Chilean hospitals. J Antimicrob Chemother 2003;51:317-21. [Crossref] [PubMed]
16. Lee MD, Sanchez S, Zimmer M, Idris U, Berrang ME, McDermott PF. Class 1 integron-associated tobramycin-gentamicin resistance in *Campylobacter jejuni* isolated from the broiler chicken house environment. Antimicrob Agents Chemother 2002;46:3660-4. [Crossref] [PubMed] [FullText]
17. Mohammadi-Barzelighi H, Talebi-Taher M, Adabi M, Javad-Moosavai S, Jabbari M, Rastegar-Lari A. Investigation of class I, II and III integrons among *Acinetobacter* strains isolated from ventilator-associated pneumonia patients in intensive care unit of Rasoul Akram Hospital in Tehran, Iran. J Med Bacteriol 2012;1:1-9.
18. Farshadzadeh Z, Hashemi FB, Rahimi S, et al. Wide distribution of carbapenem resistant *Acinetobacter baumannii* in burns patients in Iran. Front Microbiol 2015;6:1146. [Crossref] [PubMed] [FullText]
19. Peymani A, Higgins PG, Nahaei MR, Farajnia S, Seifert H. Characterisation and clonal dissemination of OXA-23-producing *Acinetobacter baumannii* in Tabriz, northwest Iran. Int J Antimicrob Agents 2012;39:526-8. [Crossref] [PubMed]
20. Shahcheraghi F, Badmasti F, Feizabadi MM. Molecular characterization of class 1 integrons in MDR *Pseudomonas aeruginosa* isolated from clinical settings in Iran, Tehran. FEMS Immunol Med Microbiol 2010;58:421-5. [Crossref] [PubMed]
21. Nikokar I, Tishayar A, Flakiyan Z, et al. Antibiotic resistance and frequency of class 1 integrons among *Pseudomonas aeruginosa*, isolated from burn patients in Guilan, Iran. Iran J Microbiol 2013;5:36-41. [PubMed] [FullText]
22. Doosti M, Ramazani A, Garshasbi M. Identification and characterization of metallo-β-lactamases producing *Pseudomonas aeruginosa* clinical isolates in University Hospital from Zanjan Province, Iran. Iran Biomed J 2013;17:129-33. [PubMed] [FullText]
23. Moazami Goudarzi S, Eftekhar F. Multidrug resistance and integron carriage in clinical isolates of *Pseudomonas aeruginosa* in Tehran, Iran. Turk J Med Sci 2015;45:789-93. [Crossref] [PubMed]
24. Gu B, Tong M, Zhao W, et al. Prevalence and characterization of class I integrons among *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates from patients in Nanjing, China. J Clin Microbiol 2007;45:241-3. [Crossref] [PubMed] [FullText]
25. Rezai MS, Salehifar E, Rafiei A, et al. Characterization of multidrug resistant extended-spectrum beta-lactamase-producing *Escherichia coli* among uropathogens of pediatrics in North of Iran. Biomed Res Int 2015;2015:309478. [PubMed] [FullText]

Please cite this article as:
Bagheri-Nesami M, Rezai MS, Ahangarkani F, Rafiei A, Nikkhah A, Eslami G, Shafahi K, Hajalibeig A, Khajavi R. Multidrug and co-resistance patterns of non-fermenting Gram-negative bacilli involved in ventilator-associated pneumonia carrying class 1 integron in the North of Iran. GERMS 2017;7(3):123-131. doi: 10.18683/germs.2017.1117