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Original Article

Prevalence of Drug Resistance in Nonfermenter Gram-Negative Bacilli

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ABSTRACT

Background and Objectives: **Non-fementer gram-negative bacilli (NFGB) are ubiquitous pathogen that has emerged as a major cause of health care associated infections. The aim of this study was to determine the prevalence and antimicrobial susceptibility of NFGB in an Iranian hospital.**

Materials and Methods: **From July 2005 to November 2006 a total of 257 strains of NFGB including 109 (42.41%) strains of *Pseudomonas aeruoginosa*, 88 (34.24%) strains of *Acinetobacter baumannii*, 48 (18.67%) stains of *Stenotrophomonas maltophilia* and 12 (4.66%) strains of *Burkholderia cepacia* were isolated from clinical specimens taken from patients hospitalized in Milad Hospitsl, Tehran, Iran. Conventional bacteriological methods were used for identification and susceptibility testing of NFGB. Susceptibility testing was performed by method as recommended by Clinical Laboratory Standard Institute (CLSI). Data were analyzed using SPSS 11.5 for Windows (SPSS Inc., Chicago, IL)**

Results: **A total of 257 non-duplicating of NFGB strains were isolated from 234 hospitalized patients. The most effective antibiotic against *P. aeruginosa* and *A. baumannii* was imipenem followed by tobramycin. Fluoroquinolones had moderate activity against *P. aerugunosa*. Most isolates of *A.baumannii* were multi-drug resistant. Susceptibility of *S. maltophila* to ticarcillin-clavuanic, ofloxacin and ceftazidim was 96%, 94% and 81%, respectively. Thirty three percent of this bacterium isolates were resistant to co-trimoxazole.**

Conclusion: **In our study, imipenem was the most effective antibiotic against *P. aeruginosa* and *A. baummannii* isolates. Previous history use of antibiotics, longer duration of hospital stay and mechanical ventilation were the major risk factors for resistance acquisition in NFGB especially in *P. aeruginosa* and *A. baumannii*.**

Keywords: Gram negative bacteria, Antibacterial drug resistance, Prevalence, Iran

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Introduction

Nonfermenters are gram-negative bacilli (NFGB) without ability of fermentation sugars to generate energy for cell functions. They fail to acidify oxidative- fermentative (O-F) media overload with mineral oil or triple sugar iron agar (TSI) butts. They prefer and grow much better in an aerobic environment. NFGB account for about 15% of all gram-negative bacilli isolated from clinical specimens (1, 2). Important members of the group in non-fermenters included *Pseudomonas aeruginosa, Acinetobacter baumannii, Stenotrophomonas maltophilia* and *Burkholderia cepacia* (1, 2). These organisms are ubiquitous in nature particularly in soil and water, and on the surface in contact with soil or water. In the hospital environment, they may be isolated from instruments such as ventilator machine humidifiers, accessories, mattresses and other equipments as well as from the skin of health care workers. All of these organisms have the potential to be spread horizontally on fomites or the hands of health care workers (1-3).

These organisms are primarily opportunistic, causing infection in seriously ill-hospitalized patients, such as AIDS and patients with cystic fibrosis. For each of these organisms, underlying host factors are strongly associated with outcome (4). Multidrug resistance common and increasing among NFGB, and a number of strains are resistant nearly to all commonly used antibiotics. Multidrug resistance NFGB make treatment of infections caused by these organisms both difficult and expensive (5-7).

Data in Iran regarding prevalence and drug resistance in NFGB is limited to *P. aeruginosa*. Difficulty for identifying NFGB other than *P. aeruginosa* leading to the publication of data that is of questionable value. In a previous study, we evaluated the performance of microbiology laboratories in Tehran and districts in an External Quality Assurance Scheme (EQAS) for detection of *A. baumannii* as unknown organism. It revealed that, of 487 laboratories, only 29.8% correctly identified this organism (8). Other EQAS program showed that of 640 microbiology laboratory in our country only 11% were able for identification of *S. maltophilia* (9). However, available data suggest that NFGB are remarkable microorganisms because of their antimicrobial resistance, propensity to cause outbreaks of infection and the complexity of their epidemiology (8, 10-13).

In this study we decided to determine the frequency of isolation, prevalence of drug resistance in NFGB and predisposing factors for acquisition of infection caused by these organisms in an Iranian large 1000-bed tertiary care hospital.

Material and Methods

This study was conducted in Milad Hospital, Tehran, Iran a tertiary care hospital with 1000-beds, affiliated by social security organization, located in Tehran, Iran from July 2005 to November 2006. Consecutive non-duplicating NFGB strains were collected from different specimens of patients who were hospitalized for ≥48 hours. Samples included respiratory tract, urine, blood, wound and other clinical specimens.

Medical and demographic data of the hospitalized patients were obtained using a questionnaire. Data received included : Demographic information(sex, gender and age), underlying disease (diabetes mellitus, chronic renal failure , cancer, hepatitis, and heart condition), presence of intravascular or urinary tract catheters , admission ward, history and length of stay in intensive care units (ICU) ,being of mechanical ventilation, history of previous antibiotic therapy, history of recent hospitalization and recent surgery.

To identify the presence of NFGB, specimens were obtained from hospitalized patients. All specimens were transported to Microbiology Laboratory of Milad Hospital. Specimens were plated on blood agar MacConkey agar. Blood specimens were inoculated in trypticase soy broth (TSB) and then subcultured on chocolate agar. Specimens other than urine were inoculated also on chocolate agar. NFGB isolates were identified using by standard laboratory methods including Gram-stain appearance, colonial morphology, oxidize reaction, pigmentation and other biochemical reactions such as: oxidation of different sugars, bile esculin (CONDA Pronadisa Madrid Spain) hydrolysis, lysine and ornithin decarboxylase, arginin dihydrolase, DNase and susceptibility to polymixin B. Growth on 42°C and on MacConkey agar also were studied (2, 5).

Antimicrobial susceptibility testing of all isolates was performed by disk diffusion method as recommended by CLSI (14). All antibiotic disks were obtained from MAST Company (Mast Diagnostics Group Ltd, Merseyside UK .) Briefly a suspension of each isolates was made so that the turbidity was equal to 0.5 McFarland standard and then plated

onto Muller-Hinton agar .After incubation plates at 35ºC for 18-24 hours zone size of inhibition was measured and reported as resistant, intermediate and susceptible reference strains including E. coli ATCC 25922, Staphylococcus aurous ATCC 25923 and *P. aeruginosae* ATCC 27853 were used for quality control of susceptibility testing and equally of antibiotic disks.

Data were analyzed using SPSS 11.5 for Windows (SPSS Inc., Chicago, IL). Regarding bivariate comparisons, the Pearson $\chi 2$ test was used for categorical and binary logistic regression for quantitative variables. Those interactions with P-values <0.05 were then considered for inclusion in the multivariate model. In the final step, a multivariate logistic regression analysis was performed using a backward stepwise selection process. A two-tailed P-value <0.05 was considered statistically significant.

Results

During our study, a total of 257 non-duplicating of NFGB strains were isolated from 234 hospitalized patients. Of 257 isolates 109 (42.41%) were P.aeruginosa, 88 (34.24%) *A.baumannii*, 48(18.67%) S. maltophilia and 12(4.66%) were B. cepacia. The main clinical specimens included tracheal aspirate 34%, blood 20%, urine 19%, wound 19% .Fifty seven percent of patients were male, and 43% were female. The majority of patients (70%) were adults. Fifty one percent of patients were hospitalized in medical wards and 38% in intensive care units (ICU). 56.9% of patients had history of hospitalization, 51.7% ICU admission, 69% had history of invasive procedures and 39.3% were under mechanical ventilation. Connection to ventilator, history of invasive procedures and underlying disease accounted for significant decrease of antibiotics activity (Table 1).

Table 1: Demographic data and medical history of 234 hospitalized patients infected by NFGB

Variable	*Strain isolated*				*Total*
	P. aeruginosa (n=109)	*A. baumannii* (n=88)	*S. maltophilia* (n=48)	*B. cepacia* (n=12)	(n=234)
Age in years (median, Iq Range)	46.00 (7.00-62.50)	60.00 (43.00-75.00)	4.00 (0.88-49.75)	18.50 (1.00-57.75)	46.50 (6.75-66.25)
Gender Male	57 (52.3)	57 (64.8)	28 (58.3)	5 (41.7)	133 (56.8)
Female	52 (47.7)	31 (35.2)	20 (41.7)	7 (58.3)	101 (43.2)
Days of hospital stay	7 (4-16)	9 (5-14)	2 (2-5)	4 (2-10)	-
ICU admission	62 (56.9)	55 (62.5)	14 (29.2)	4 (33.3)	121 (51.7)
Days of ICU stay	10 (5-22)	8 (4-13)	8 (4-26)	9 (6-15)	-
Invasive procedure	80 (73.4)	83 (94.3)	17 (35.4)	5 (41.7)	163 (69.7)
Mechanical ventilation	42 (38.5)	50 (56.8)	9 (18.8)	4 (33.3)	92 (39.3)
Days on ventilator	7 (5-13)	6 (3-10)	8 (3-28)	7 (2-10)	-
Surgery in the preceding year	36 (33.0)	34 (38.6)	8 (16.7)	4 (33.3)	73 (31.2)
Hospitalization in the preceding year	59 (54.1)	49 (55.7)	27 (56.3)	4 (33.3)	126 (53.8)
Underlying disease	50 (45.9)	49 (55.7)	14 (29.2)	4 (33.3)	107 (45.7)
Transplant recipient	1 (0.9)	1 (1.1)	0 (0.0)	0 (0.0)	2 (0.9)
Previous antibiotic therapy	36 (33.0)	21 (23.9)	20 (41.7)	2 (16.7)	73 (31.2)

The results of susceptibility testing are shown in table 2, 3, and 4. The most active antibiotics against the strains of *P. aeruginosa* were imipenem, piperacillin-tazobactam with rate 79% and 68% susceptibility. The most active antibiotic against *A. baumannii* was also imipenem and 95% of strains were susceptible to imipenem. Susceptibility of *A. baumannii* to tobramycin and piperacillin-tazobactam was 53% and 18%, respectively.

Table 2: Frequency of drug resistance in *P. aeruginosa* isolates (n=109)

Antibiotic	Susceptibility pattern (%)		
	Susceptible	Intermediate	Resistant
Ceftazidime	61.5	3.7	34.9
Cefotaxime	14.7	28.4	56.9
Ceftriaxone	34	11	55
Cefepime	58.7	6.4	34.9
Aztreonam	44	12	44
Imipenem	78.9	4.6	16.5
Piperacillin	57.8	-	42.2
Piperacillin/tazobactam	67.8	-	32.1
Ticarcillin/clavulanic acid	54.1	-	45.9
Ciprofloxacin	65.1	3.7	31.2
Ofloxacin	50.5	3.7	45.9
Amikacin	61.5	0.8	36.7
Gentamycin	56.9	0.8	41.3
Tobramycin	61.5	-	38.5
Trimethoprim/sulfamethoxazole	7.3	15.6	77.1

Table 3: Frequency of drug resistance in *A. baumannii* isolates (n=88).

Antibiotic	Susceptibility pattern (%)		
	Susceptible	Intermediate	Resistant
Ceftazidime	12.5	3.4	84.1
Cefotaxime	3.4	5.7	90.9
Ceftriaxone	2.3	9.1	88.6
Cefepime	13.6	21.6	64.8
Aztreonam	3.5	4.5	92
Imipenem	95.5	3.4	1.1
Piperacillin	5.7	3.4	90.9
Piperacillin/tazobactam	18.2	39.8	42
Ticarcillin/clavulanic acid	15.9	19.3	64.8
Ciprofloxacin	9.1	0	90.9
Ofloxacin	9.1	1.1	89.8
Amikacin	11.4	3.4	85.2
Gentamycin	13.6	1.1	85.2
Tobramycin	53.4	2.3	44.3
Trimethoprim/sulfamethoxazole	25	6.8	68.2

Table 4: Frequency of drug resistance in *S.maltophilia* isolates (n=48).

Antibiotic	Susceptibility pattern (%)		
	Susceptible	Intermediate	Resistant
Ceftazidime	81.3	2.1	16.7
Cefotaxime	2.1	12.5	85.4
Ceftriaxone	2.1	4.2	93.8
Cefepime	16.7	43.8	39.6
Aztreonam	4.2	-	95.8
Imipenem	2.1	-	97.9
Piperacillin	12.5	-	87.5
Piperacillin/tazobactam	43.8		56.2
Ticarcillin/clavulanic acid	95.8	-	4.2
Ciprofloxacin	79.2	12.5	8.3
Ofloxacin	93.8	-	6.2
Amikacin	56.3	16.7	27.1
Gentamycin	14.6	-	85.4
Tobramycin	14.6		85.4
Trimethoprim/sulfamethoxazole	62.5	4.2	33.3

According to statistical analysis (Chi –square test) gender, stay in ICU, being under mechanical ventilation and surgical procedures were significant predisposing factors in acquisition of resistant in strains of *A. baumannii*. Isolates of *A.baumannii* from tracheal aspirate had higher rates of resistance to tested antibiotics.

Susceptibility of *S. maltophila* to ticarcillin-clavuanic, ofloxacin and ceftazidim was 96%, 94% and 81%, respectively. All isolates of *B. cepacia* were susceptible to imipenem. Co-trimoxazole and tetracycline were two other active antibiotics against of *B. cepacia.*

Clinical active antibiotics, i.e., ciprofloxacin, and ceftazidim for *B. cepacia*, co-trimoxazole for *S. maltophilia*, cefepime for *A.baumannii* and tobramycin, ciprofloxacin and ceftazidim for *P. aeruginosa* did not show desirable activities. Multivariable logistic regression analysis showed that isolated strains from respiratory tract specimens of patients recently connected to ventilator were a risk factor for acquisition imipenem resistance in *P. aeruginosa*. [OR (95% CI):2.90 (1.585.310-] Imipenem showed the least cross resistance with first line antibiotics against *P. aeruginisa*, *A. baumannii* and *B. cepacia* isolates. Among co-trimoxazol resistant isolates of *S. maltophilia* 38.8% were co-resistant to ceftazidim, and 11.1% to ticarcillin-clauvanic acid. 11 % of *P. aeruginosa* isolates were multi-drug resistant to pipercillin-tazobactam, ceftazidim, tobramycin and cefepime. 2.3% of *A. baumannii* isolates were multi drug resistant to piperacillin-tazobactam, cefepime, tobramycin and imipenem. 4.2% isolates of *S. maltophilia* were multidrug resistant to co-trimoxazole, ceftazidim and ticarcillin-clavulanic acid.

Discussion

In our study, imipenem was highly active against *P. aeruginosa* and *A .baumannii*. 78.9% and 59.5% isolates of these organisms were susceptible to imipenem respectively. This antibiotic is highly β-lactamase stable and has an unusual property of

causing a post antibiotic effect on gram-negative bacilli (15). The lower resistance rate of NFGB to imipenem may be due to that imipenem came in to use in 2001 in our hospital and this antibiotic is frequently used to treat infections caused by multidrug-resistant strains of Gram-negative bacteria especially *P. aeuroginosa* and *A. baumannii*. However, in other studies in Tehran hospitals the rate of susceptibility of *A. baumannii* to imipenem, piperacillin-tazobactam and amikacin was 50.7%, 42.1% and 38.2%, respectively (13).

The fluoroquinolones, primarily ciprofloxacin and ofloxacin have been reported to be active against *P. aeruginosa* and *A. baumannii* (3). Nearly 92% isolates of *P. aeruginosa* are susceptible to ciprofloxacin (16). However our study showed that 65 % of *P.aeruginosa* isolates were susceptible to ciprofloxacin .The pattern of susceptibility testing of *P. aeruginosa* in our study was similar the result of Turkey (17).

In countries such as USA and Canada, more than 90% of *P. aeruginosa* isolates are susceptible to aminoglycosides and piperacillin-tazobactam (18). Among aminoglycosides; tobramycin was moderate active against *P. aeuginosa* and *A. baumannii* isolates .In our study, 61.5% and 53.4% of these organisms' isolates were susceptible to tobramycin respectively. In our study ceftazidime was the most active antibiotic in comparison with other third generation of cephalosporins. Cefepime as a sole fourth generation of cephalosporins was active only against 58.7% of *P. aeruginisa* isolates.

The majority of *A. baumannii* isolates was multidrug resistant. Resistance in isolates of *A. bauannii* in comparison with *P. aeruginosa* was prevalent. The most effective antibiotics against *A. baumannii* were imipenem and tobramycin. Nearly 90% of *A. baumannii* isolates in our study were resistant to cephalosporin such as ceftazidim, cefotaxim and ceftriaxone. It also showed a high resistance of *A.baumannii* in our country (13).

Like other non-fermenters, S. *maltophilia* is intrinsically resistant to many common antibiotics. Nearly all strains produce an unusual chromosomally encoded zinc depended β-lactamase that confers broad resistance to carbapenems and other β-lactames (4). In our study, nearly 98% of *S. maltophilia* isolates were resistant to imipenem. Among cephalosporins, the most effective antibiotic against *S. maltophilia* was ceftazidim. Our study also showed the fluoroquinolones such as ciprofloxacin and ofloxacin are most effective antibiotics against of *S. maltophilia*. Co-trimoxazole is drug of choice for treatment infections caused by *S. maltophilia*, however, 33% of isolates were resistant to this antibiotic. In our country, we do not have any data regarding prevalence of drug resistance in *S. maltophilia* and for this reason, comparison of our study did not carried out with other findings.

Regional variation in resistance of NFGB to commonly used antibiotics is related to policy of antimicrobial use and risk factors. The important risk factors for acquisition of drug resistance in strains of *P. aeruginosa* and *A. baumannii* included previous antibiotic use, longer duration of hospital stay, ICU stay, emergent surgical operation, total parenteral nutrition, having a central venous catheter, endotracheal tube and urinary catheter or nasogastric tube (19, 20).

Conclusion

The present study showed data on the rates of antimicrobial resistance observed in NFGB in Milad Hospital. Imipenem was the most effective antibiotic against *P. aeruginosa* and *A. baummanni*i isolates. In total *A. baumannii* isolate had a high frequency of drug resistance to commonly used antibiotics. Previous use of antibiotics, longer duration of hospital stay and mechanical ventilation were the major risk factors for resistance acquisition in *P. aeruginosa* and *A. baumannii.*

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