Original Article

CARBAPENEM RESISTANCE IN GRAM-NEGATIVE BACILLI ISOLATES IN AN IRANIAN 1000-BED TERTIARY HOSPITAL

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ABSTRACT

Objective: Carbapenems are beta-lactamase antibiotics, presently considered as most potent agents for treatment of infections caused by Gram-negative bacilli. The aim of this study was to determine resistance of *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumonniae* as prevalent nosocomial agents to commonly used antibiotics including carbapenems such as imipenem and meropenem.

Methodology: A total of 202 gram-negative bacilli including *K.pneumoniae, P aeruginosa* and *A.baumannii* isolated from hospitalized patients in Milad hospital of Tehran were subject for susceptibility testing. Susceptibility testing was performed by disk diffusion and MIC methods as recommended by Clinical Laboratory Standards Institute (CLSI)

Results: All isolates of *K. pneumonia* were susceptible to imipenem and meropenem. Resistance in non-fermenting gram-negative bacilli (NFGB) was prevalent. *P.aeruginosa* isolates exhibited 7.5% and 40.2% resistance to imipenem and meropenem respectively. The majority isolates of *Acinetobacter baumannii* were multi-drug resistant and resistance of this organism to imipenem and meropenem was 27.7% and 38.5% respectively.

Conclusions: Our study revealed that in spite of resistance of *K.pneumoniae* to commonly used antibiotics, all isolates were susceptible to imipenem and meropeem. More than 80% isolates of *A .bammanni* were resistant to commonly used antibiotics. About 40.2% isolates of *P.aeruginosa* and (38.5%) isolates of *A.baumannii* were resistant to meropenem respectively.

KEY WORDS: Carbapenem resistance, Non-fermenter gram- negative bacilli.

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INTRODUCTION

Carbapenems was first introduced in 1980 and are now frequently used as the last choice in treating serious infections caused by multidrug-resistant strains of gram negative bacilli. These antibiotics are stable to ß-lactamase including the extended spectrum ß -lactamase (ESBLs) and *AmpC* produced by gram –negative bacilli.[1-4]

The carbapenems are a class of beta-lactamase antibiotics that differ from the penicillins by the substitution of a carbon atom for a sulfur atom and by the addition of a double bond to the five –membered ring of the penicillin nucleus.[5] Carbapenems bind bacterial

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peptidases, the bacterial penicillin-binding proteins, which are responsible for elongation and cross –likening the peptidoglycan of the bacterial cell wall. This binding results in impairment of construction of the cell wall, inhibition of cell growth frequently, cell lysis and death. For gram-negative bacteria, it occurs in the periplasmic space between the cell wall and surrounding cell membrane.[6]

Unfortunately resistance to carbapenems started emerging from 1990 and has been reported in non-fermenter gram -negative bacilli (NFGNB) worldwide over the years with varying frequencies.[1] In the SENTRY antimicrobial surveillance programs (SASP), 10 to 30% of *P. aeruginosa* strains from various countries have been found to be resistant to imipenem. The nosocomial strains of non-fermenters exhibited a higher level of resistance.[7] The carbapenem resistance appears to be due to metallo- ß-lactamase. There is evidence of the transfer of the multiple antibiotic resistance to other species including *Escheichia coli*, *Enterobactr* spp and *Klebsiella* spp. Multi-drug resistant (including carbapenem) in gram-negative bacteria pose a serious problem due to the lack of therapeutic options and the potential transfer of antibiotic resistance to other virulent pathogens.[5] Carbapenems available to use in Islamic republic of Iran are meropenem and imipenem. Information regarding prevalence of resistance to carbapenems in clinical isolates in our country is very limited. Therefore we conducted this perspective study to evaluate antimicrobial activity of imipenem and meropenem in *Pseudomonas aerugiosa*, *Acinetobacter baumannii* and *klebsiella pneumoniae* isolated from clinical specimens in Milad Hospital of Tehran which is a tertiary 1000 bed hospital.

METHODOLOGY

Clinical isolates of various strains of *P.aeruginosa*, *A.baumannii* and *K.pneumoniae* from hospitalized patients in Milad hospital of Tehran from April 2006 to November 2006 were subject of our study. The identification of the bacteria was determined by using conventional bacteriology methods. No duplicate isolates from the same patients were included in this study. Subcultures were prepared in blood agar from the identified bacteria to produce pure colony isolates. From these pure colony isolates a bacterial suspension was prepared by inoculating 4-ml sterile normal saline and adjusted the suspension to .0.5 Mc Farland standards. The susceptibility testing was performed by disk diffusion method as recommended by CLSI.[8] The antibiotics used were imipenem 10µg, pipracillin/ tazobactam 100/ 10µg cefepime 30µg, ceftazidime 30µg, amikacin 30µg ciprofloxacin 5µg. The prepared plates were then incubated at 35°C for 24 hours. Zone of inhibition were calculated by measuring the diameter (mm) of the inhibition growth zone. Quality control was ensured by keeping weekly records of disk diffusion for *E.coli* ATCC 25922 and *Pseudomonas aeruginosa* (ATCC 27853). Minimal inhibitory concentration (MIC) values for meropenem were determined by the E-test (AB Biodisk, Solona, Sweden) as recommended by manufacture. For detection of ESBLs producing strains of *K.pnemoniae* we used both screening and confirmatory tests by methods as recommended by CLSI. *K.pneumoniae* ATCC 70603 was used as a positive ESBLs strain.[9]

RESULTS

A total of 202 gram- negative bacilli including 67 isolates of *Pseudomonas aeruginosa*, 65 isolates of *Acintobacter baumannii* and 70 isolates of *K;pneumoniae* were tested. All strains were isolated from hospitalized patients in Milad hospital during six month. Milad hospital is a 1000 bed, non-teaching and the largest hospital in Tehran. All microorganisms were isolated from clinical specimens including tracheal tube aspirates, urine, wound, blood and other sterile body fluids. Of 70 isolates of *K.pneumoniae* 56 (80%) were ESBls. All isolates of *K. pneumoniae* including ESBLs were susceptible to imipenem (Table-I). Resistance of *K.pneumoniae* isolates against pipiracllin/ tazobactam, cefepime, ciprofloxacin and amikacin were 47%, 67%, 40% and 55%

Table-I: Frequency of antibiotic resistance in gram-negative bacilli.

Gram-negative bacilli	*TZP*	*FEP*	*CIP*	*AN*	*CAZ*	*IPM*	*MEM*
K.pneumoniae	47%	67%	40%	55%	80%	0%	0
P.aeruginosae	30%	43%	30%	47%	73%	7.5%	40.2%
A.baumannii	83%	90%	94%	84%	98.5%	27.7%	38.5%

TZP=pipracillin/tazobactam, FEP=cefepime, CIP= ciprofloxacin, AN=amikacin, CAZ =ceftazidime, IPM=imipenem, MEM=meropenem.

respectively. Resistance in NFGNB was prevalent (Table-I). *P.aeruginosa* isolates exhibited 30%, 43%, 30%, 47% and 73% resistance to piptacillin / tazobactam, cefepime, ciprofloxacin and ceftazidime respectively. The most effective antibiotic against *P. aeuginosa* was imipenem and only 7.5% isolates of *P.aeruginosa* were resistant to imipenem. The most isolates of *A.baumannii* were multi-drug resistant. Resistance of *A.baumannii* to pipiracillin/tazibactam, cefepime, ciprofloxacin, amikacn and ceftazidime was 83%, 90%, 94%, 84% and 98.5% respectively. *A.baumannii* showed the lowest resistance to imipenem and 27.7% isolates of this organism were resistant to imipenem. MIC of meropenem was ranged from 0.5 -32µg/ml. All isolates of *K.pneumoniae* were susceptible to meropenem. Of 67 isolates of *P.aeruginosa*, 27 isolates (40.2%) were resistant to meropenme and of 65 isolates of *A.baumani*i, 25 (38.5%) were resistant to meropenem. The majority isolates of *P.aeruginosa* and *A.baumannii* had MIC. >32µg/ml for meropenem. Seven strains of *A.baumannii* isolates were multiple -drug resistant and all of were resistant to TZP, FEP, CIP, AN, CAZ, IMP and MEM, All strains except one had MIC >32µg/ml for meropenem. The majority of strains were isolated from tracheal tube aspirates of patient hospitalized in ICU (Table-II).

DISCUSSION

The resistance to carbapenems especially in *P.aeruginosa* results from reduced levels of drug accumulation or increased expression of pump efflux. The resistance may also be due to the production of metallo-ß-lactamase (MBL) which can be chromosomally encoded or plasmid mediated. Most of these MBL confer resistance to not only carbapenems but also to other ß-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam.[10] Mulridrug –resistant including carbapenem–resistant' pose a serious problem due to the lack of therapeutic options and the potential transfer of antibiotic resistance to more virulent pathogen. The increasing trend of carbapenem resistance in *Acinetobacter baumannii* worldwide is a concern since it limits drastically the range of therapeutic alternatives Metallo-ß-lactamases have been reported worldwide, especially in Asia and western Europe, and confer resistance to all ß-lactams except aztreonam.[11,12] In our hospital imipenem and meropenem came in to use in 2001. Both of these antibiotics are frequently used to treat infections caused by multidrug–resistanant strains of Gram-negative bacteria especially *P.aeruginosa* and *A baumannii*.

Table-II: Characteristics of 17 isolates of *A.baumannii*

Strain	*MIC for MEM*	*Resistant to other tested antibiotics*	*Ward*	*Specimen*
1	>32	+	ICU	Folly catgeter
2	>32	+	ICU	Tracheal aspirates
3	>32	+	ICU	Tracheal aspirates
4	>32	+	surgical	Wound
5	>32	+	ICU	Tracheal aspirates
6	>32	+	ICU	Tracheal aspirates
7	>32	+	surgical	Wound
8	>32	+	Internal	Ear
9	>32	+	Internal	Chest tube
10	>32	+	ICU	Folly catheter
11	>32	+	ICU	Tracheal tube
12	>32	+	ICU	Folly catgeter
13	>32	+	ICU	Tracheal tube
14	>8	+	ICU	Folly catgeter
15	>32	+	ICU	Tracheal tube
16	>32	+	ICU	Folly catgeter
17	>32	+	ICU	Tracheal tube

There is a limited literature available regarding the prevalence of resistance to carbapenems in various clinical isolates in our country. Recent study from Tehran hospitals showed that all isolates of *K.pneumonia* were susceptible to imipenem and meropenem, which is the same as our study.[13] In our study resistance to pipracillin/tazobactam, cefepime, ciprofloxacin and amikacin showed moderate action against *K.pneumoniae*. Resistance among NFGNB was prevalent. This study documented imipenem resistance of 7.5% and 27.7% among 67 isolates of *P.aeruginosa* and 65 isolates of *A. baumanni* respectively. In other study by Ahngaranzadeh-Rezaee and co-workers, 29.3% hospital isolates of *P.aeruginosa* in Tehran were resistant to imipeneme.[14] Other study by Moniri et al in a teaching hospital located in Kashan in center of Iran, they have reported a high frequency resistance of *P.aeruginosa* to imipenem. It also shows a considerably higher prevalence of resistance among *A.baumanni* which is different from other studies.[15] In a study performed in India overall 36.4% of nonfermenters were resistant to imipenem and 42% of *P.aeruginosa* and 18.5% *A, baumanni* were imipenem resistant.[10] This disturbing situation could be attributed to the increased use of antibiotics which has to be controlled by strict antibiotics policy. Regular monitoring and documentation of carbapenem resistance is therefore crucial in developing world to control infections due to these bacteria in patients admitted to hospitals.

CONCLUSIONS

In conclusion, our study highlights the increasing incidence of carbapenem resistance in Gram-negative non-fermenting bacilli. Including *P.aeruginosa* and *A,baumannii*. There is a further need for investigation and epidemiology studies in this field.

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