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ORIGINAL ARTICLE

Antimicrobial susceptibility pattern of carbapenemase-producing Gram-negative nosocomial bacteria at Al Zahra hospital, Isfahan, Iran

Sima Bahrami[1], Fatemeh Shafiee[2], Atousa Hakamifard[3], Hossein Fazeli[4], Rasool Soltani[5*]

[1]Students Research Committee, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran
[2]Department of Pharmaceutical Biotechnology, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran
[3]Department of Infectious Diseases, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
[4]Department of Microbiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
[5]Department of Clinical Pharmacy and Pharmacy Practice, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran

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ABSTRACT

Background and Objectives: Bacterial antibiotic resistance is one of the most important threats for public health around the world. Carbapenemase-producing Gram-negative bacteria have resistance to most antibiotics including carbapenems complicating the treatment of infections. The aim of this study was to determine the antimicrobial susceptibility pattern of carbapenemase-producing nosocomial Gram-negative pathogens at a referral teaching hospital to reveal the best options for treatment of related infections.

Materials and Methods: Gram-negative bacteria, isolated from hospitalized patients with nosocomial infections, underwent meropenem susceptibility test by disk diffusion method. Meropenem-resistant strains were evaluated for the presence of carbapenemase using Modified Hodge test (MHT). Finally, the antibiotic susceptibility test was performed to determine the sensitivity of each carbapenemase-positive strain against various antimicrobial agents according to the guidelines of Clinical and Laboratory Standards Institute (CLSI).

Results: Over the study period, 155 carbapenemase-positive isolates were detected. Pneumonia was the most frequent related nosocomial infection (67.1%) followed by UTI (23.2%). *Acinetobacter baumannii* (53.5%) and *Klebsiella pneumoniae* (40%) were the most frequently isolated pathogens. The pathogens had high rate of resistance to all antibiotics. Colistin had the most *in vitro* effect against all pathogens. Also, *K. pneumoniae* had a co-trimoxazole sensitivity rate equal to colistin (30.6%).

Conclusion: Carbapenemase-positive Gram-negative bacteria causing nosocomial infections are common in our hospital and have high rate of resistance to most antibiotics. Improvement in the pattern of antibiotic use and infection control measures are necessary to overcome this resistance.

Keywords: Nosocomial infections; Carbapenemase; Antibiotic; Susceptibility

*Corresponding author: Rasool Soltani, Ph.D, Department of Clinical Pharmacy and Pharmacy Practice, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran.
Tel: +98-313-7927067
Fax: +98-313-6680011
Email: soltani@pharm.mui.ac.ir

INTRODUCTION

Nosocomial infections occur during hospital care for other illnesses or even after the patient is discharged (1). Nosocomial infections lead to increased duration of hospital stay, physical disability, antibiotic resistance, socioeconomic disturbances, and finally, increased mortality (2). According to the Center for Disease Control and Prevention (CDC), nearly 1.7 million hospitalized patients annually acquire health care-associated infections (HCAIs) while being treated for other disorders and more than 98,000 of these patients (one in 17) die because of these infections (3). Generally, the prevalence of this type of infections is 3-20 times higher in low-income countries compared to the high-income ones (4). So, it is important to provide proper guidelines for the effective prevention of these infections. On the other hand, given the high prevalence of antibiotic resistance, choosing the right treatment for this type of infection is another challenge (5).

Bacterial antibiotic resistance is one of the most important threats of public health around the world based on the opinion of World Health Organization, and this needs to be addressed (6). The Centers for Disease Control and Prevention (CDC) estimates the additional cost of health care related to microbial resistance as 20 billion dollars (6).

Carbapenems are important therapeutic antibiotics against Gram-negative pathogens causing nosocomial infections (7). However, carbapenemase-producing strains develop resistance against these antibacterial agents (8).

In recent years, there has been a significant increase in infections caused by multidrug-resistant bacteria in Gram-negative bacteria, especially carbapenemase-positive pathogens, worldwide (9-11). In a European survey, endemic spread of carbapenemase-producing Enterobacteriaceae was reported in 13 of 38 European countries in 2015, compared with 6 of 38 in 2013 (12). Furthermore, a higher spread was reported for *Acinetobacter baumannii*, with 12 of 27 European countries having more than 50% carbapenem resistance among the isolates (12). The vital problem is that carbapenemase-producing bacteria show resistance to other antibiotics, including fluoroquinolones and aminoglycosides, in addition to beta-lactam agents (8).

Given the growing trend of microbial resistance among carbapenemase-producing Gram-negative bacteria, which has led to limited treatment options as well as the irrational prescribing of antibacterial agents, the aim of the present study was to investigate the prevalence of Gram-negative carbapenemase-producing bacteria and their antibiotic resistance patterns at a referral teaching hospital to guide better selection of antimicrobial drugs for the treatment of these infections.

MATERIALS AND METHODS

Patients and setting. This was a prospective cross-sectional study performed at Al-Zahra hospital affiliated to Isfahan University of Medical Sciences (IUMS), Isfahan, Iran from January to June 2019.

The inclusion criteria for eligible patients were: 1) age ≥ 18 years; 2) hospitalization for at least 48 hours; 3) having clinical signs and symptoms of any infection with initiation after at least 48 hours of hospitalization; and 4) Growth of Gram-negative pathogen from the sample culture. Biological samples, including tracheal secretions, sputum, urine, wound secretions, cerebrospinal fluid, and blood were collected from the patients with signs and symptoms of related infections in various clinical wards of the hospital.

After transferring to the microbiology department of the hospital laboratory, the samples were cultured and grown microorganisms underwent differential tests in order to diagnose various bacterial species. Gram-negative pathogens were included in the study and the patients' information including demographic data, hospitalization ward, the biological sample, the type of infection and its causative pathogen were recorded.

Carbapenem susceptibility test. The yielded Gram-negative pathogens were screened for carbapenem resistance using disk diffusion (Kirby-Bauer) method according to the Clinical and Laboratory Standards Institute (CLSI) instructions (13). Briefly, microbial suspension with 0.5 McFarland standard turbidity was cultured on Mueller-Hinton agar (Hi-Media, India) with meropenem disk (10 µg, Padtan Teb, Iran) being placed on it. The plates were incubated for 24 hours at 37°C. Following incubation, if the inhibition zone diameter around the meropenem disk showed resistance according to CLSI breakpoints (13), the pathogen underwent the test of carbapenemase presence at the next step.

Modified Hodge test. To determine the presence of carbapenemase in each bacterial sample, modified Hodge test was used (14). Accordingly, 0.5 McFarland of microbial suspension containing *E. coli* (ATCC 25922), as a standard microorganism, was cultured on the surface of Mueller-Hinton agar with 1/10 dilution rate and allowed to dry for 3-5 minutes. After placing a meropenem disk (10 µg) in the middle of the plate, each microbial sample was drawn by a sterile swap as a straight line to the edge of the plate. For each sample, the test was repeated for *Klebsiella pneumoniae* (ATCC 1705) (containing carbapenemase enzyme) and the *Klebsiella pneumoniae* (ATCC 1706) without carbapenemase, as the positive and negative controls, respectively. After incubation for 24 hours at 37°C, the formation of a clover-like growth inhibition zone around the line confirmed the presence of carbapenemase in each microbial sample, while a negative MHT indicated no production of carbapenemase by the isolate. The quality control of meropenem disk was performed according to CLSI guidelines (13).

Antibiotic susceptibility test. Carbapenemase-positive bacterial isolates underwent antibiotic susceptibility test by disk diffusion method. After the inoculation of Mueller-Hinton agar with 0.5-McFarland microbial suspension, antibiotic disks including cefepime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), ampicillin (10 µg), piperacillin/tazobactam (100/10 µg), ampicillin/sulbactam (10/10 µg), trimethoprim-sulfamethoxazole (co-trimoxazole, 1.25/23.75 µg), gentamicin (10 µg), amikacin (30 µg), tetracycline (30 µg), azithromycin (15 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), and colistin (10 µg) were placed on the surface at a distance of not less than 24 mm (center to center). After 24 hours of incubation at 37°C, the non-growth halo diameter around each disk was determined and recorded with the results being reported as susceptible (S), intermediately resistant (I), or resistant (R) according to the CLSI breakpoints (13). Due to the lack of zone diameter breakpoints for colistin against *A. baumannii* and Enterobacteriaceae, the following values were used based on a previous study (15): (i) *A. baumannii*: R≤12, 12<I<14, and S≥14 mm; (ii) Enterobacteriaceae: R≤11, 11<I<14, and S≥14 mm.

RESULTS

Over the study period, 300 patients were diagnosed with nosocomial infection that 261 of cases (87%) were recognized as Gram-negative infections including 168 and 93 cases in males and females, respectively. Furthermore, pneumonia was recorded as the most frequent infection (n = 172, 65.9%). followed by urinary tract infection (UTI; n = 61, 23.3%), central nervous system (CNS) infections (n = 15, 5.7%), wound infection (n = 9, 3.4%), and bacteremia (n = 4, 1.5%).

Regarding the frequency of the causative Gram-negative pathogens, *A. baumannii* (n = 130, 49.8%) and *K. pneumoniae* (n = 107, 41%) were the most frequently isolated microorganisms followed by *P. aeruginosa* (n = 23, 8.8%) and *E. coli* (n = 1, 0.38%).

Regarding the results of Hudge test, 155 cases (59.4%) was positive for carbapenemase with *A. baumannii* being the most frequent pathogen (n = 83, 53.5%) followed by *K. pneumoniae* (n = 62, 40%), *P. aeruginosa* (n = 9, 5.8%), and *E. coli* (n = 1, 0.7%). In addition, Pneumonia was the most frequent nosocomial infection due to these pathogens (n = 104, 67.1%; 57.7% in males), followed by UTI (n = 36, 23.2%; 50% in each gender), CNS infection (n = 11, 7.1%; 63.6% in males), and wound infection (n = 4, 2.6%; 50% in each gender). Table 1 shows the frequency of various carbapenemase-positive pathogens for each nosocomial infection. As shown, carbapenemase-positive *A. baumannii* and *K. pneumoniae* were the most frequent cause of pneumonia and UTI, respectively, while they had similar frequencies in CNS and wound infections.

Table 2 shows the antibiotic susceptibility of isolated carbapenemase-positive pathogens. As seen, there was high resistance rate amongst the isolated microorganisms to the tested antibiotics. Furthermore, colistin had the most *in vitro* effect against all pathogens. Also, *K. pneumoniae* had a co-trimoxazole sensitivity rate equal to colistin (30.6%).

Table 3 represents the antibiotic susceptibility of detected carbapenemase-positive nosocomial infections. As shown, pneumonia, CNS infections, and wound infections had the most susceptibility to colistin, while UTI had the most sensitivity to colistin (36%) and co-trimoxazole (26%).

DISCUSSION

In the present study, the prevalence of Gram-negative carbapenemase-producing bacteria and their

Table 1. The frequency of carbapenemase-positive Gram-negative pathogens for each detected nosocomial infection.

Pathogen	n (%)	Infection n (%)			
		Pneumonia	UTI	CNS infection	Wound infection
A. baumannii	83 (53.5)	67 (64.4)	9 (25)	5 (45.45)	2 (50)
K. pneumoniae	62 (40)	33 (31.7)	22 (61.1)	5 (45.45)	2 (50)
P. aeruginosa	9 (5.8)	3 (2.9)	5 (13.9)	1 (9.9)	0
E. coli	1 (0.7)	1 (1)	0	0	0
Total	155	104	36	11	4

Table 2. Antibiotic susceptibility of isolated carbapenemase-positive Gram-negative pathogens

Pathogen	Antibiotic	n	Susceptibility n (%)		
			Sensitive	Intermediate	Resistant
Acinetobacter baumannii	Ceftazidime	83	0	0	83 (100)
	Cefepime	83	0	3 (3.7)	78 (96.3)
	Ampicillin/sulbactam	83	5 (6.2)	2 (2.5)	78 (91.3)
	Levofloxacin	83	1 (1.3)	0	80 (98.7)
	Amikacin	83	5 (6.2)	1 (1.2)	75 (92.6)
	Co-trimoxazole	83	3 (3.7)	0	78 (96.3)
	Colistin	83	25 (30.9)	0	56 (69.1)
Klebsiella pneumoniae	Ceftazidime	62	1 (1.6)	1 (1.6)	60 (96.8)
	Cefepime	62	0	0	62 (100)
	Piperacillin/tazobactam	62	1 (1.6)	0	61 (98.4)
	Ciprofloxacin	62	2 (3.3)	0	60 (96.7)
	Amikacin	62	7 (11.3)	1 (1.7)	54 (87)
	Co-trimoxazole	62	19 (30.6)	2 (3.2)	41 (66.1)
	Colistin	62	19 (30.6)	0	43 (69.4)
Pseudomonas aeruginosa	Ceftazidime	9	2 (22.2)	0	7 (77.8)
	Cefepime	9	2 (22.2)	0	7 (77.8)
	Piperacillin/tazobactam	9	1 (21.2)	0	8 (88.8)
	Ciprofloxacin	9	1 (11.1)	1 (11.1)	7 (77.8)
	Amikacin	9	2 (22.2)	0	7 (77.8)
	Co-trimoxazole	9	0	0	9 (100)
	Colistin	9	5 (55.6)	0	4 (44.4)
Escherichia coli	Ceftazidime	1	0	0	1 (100)
	Cefepime	1	0	0	1 (100)
	Piperacillin/tazobactam	1	1 (100)	0	0
	Ciprofloxacin	1	1 (100)	0	0
	Amikacin	1	1 (100)	0	0
	Co-trimoxazole	1	0	0	1 (100)
	Colistin	1	0	0	1 (100)

antibiotic resistance patterns at a referral university hospital was determined. Our results showed that 59.4% of isolated Gram-negative organisms were carbapenemase-positive. Furthermore, *A. baumannii* and *K. pneumoniae* were responsible for about 50% and 40% of detected hospital infections with 64% and 58% of the isolates being carbapenemase-positive, respectively. Various rates of carbapenemase resistance have been reported in the studies. In Zhang et al study in China, among 664 cases with resistance to carbapenems, 73.9% of infections were caused by *K. pneumoniae*, and 16.6% by *E. coli* (16). Howev-

Table 3. Antibiotic susceptibility of detected carbapenemase-positive nosocomial infections.

Antibiotic	Susceptibility	Infection n (%)			
		Pneumonia	UTI	CNS infection	Wound infection
Ceftazidime	S	1 (1)	1 (2.9)	1 (10)	0
	I	0	1 (2.9)	0	0
	R	100 (99)	33 (94.2)	9 (90)	2 (100)
Cefepime	S	1 (0.9)	1 (3)	0	0
	I	3 (2.7)	0	0	0
	R	108 (96.4)	35 (97)	10 (100)	2 (100)
Piperacillin/tazobactam	S	0	1 (3.6)	0	1 (100)
	I	0	0	0	0
	R	39 (100)	27 (96.4)	5 (100)	0
Ampicillin/sulbactam	S	6 (9.5)	0	0	0
	I	1 (1.6)	0	1 (20)	0
	R	56 (88.9)	8 (100)	4 (80)	1 (100)
Ciprofloxacin	S	0	2 (7.4)	0	1 (100)
	I	0	1 (3.7)	0	0
	R	35 (100)	24 (88.9)	5 (100)	0
Levofloxacin	S	0	0	0	0
	I	0	0	0	0
	R	65 (100)	9 (100)	5 (100)	1 (100)
Amikacin	S	9 (8.8)	4 (11.1)	2 (20)	0
	I	0	1 (2.8)	0	1 (50)
	R	93 (91.2)	31 (86.1)	8 (80)	1 (50)
Co-trimoxazole	S	12 (12.1)	8 (25.8)	1 (11.1)	0
	I	1 (1)	1 (3.2)	0	0
	R	86 (86.9)	22 (71)	8 (88.9)	2 (100)
Colistin	S	23 (22.5)	13 (36.1)	3 (27.3)	2 (50)
	I	0	0	0	0
	R	79 (77.5)	23 (63.9)	8 (72.7)	2 (50)

er, *A. baumannii* was not included in the evaluated pathogens. In another study performed in Uganda, the prevalence of carbapenemase-resistant Enterobacteriaceae was 22.4% (44/196) among the isolated pathogens and *K. pneumoniae* had the most frequency (52.2%) (17). In another study conducted in South Africa, it was shown that 68% of investigated microorganisms contained a carbapenemase-producing gene with 71% of the isolates being *Klebsiella* species (18). Similarly, in these two later studies, *A. baumannii* was not evaluated. Furthermore, in contrast to our work, the source of the isolates was not limited to the nosocomial ones. The results of Amjad et al. study in Pakistan were somewhat similar to our results as 138 out of 200 Gram-negative isolates (69%) were positive for carbapenemase in Modified Hodge test; however, the frequency of pathogens was very different so that *E. coli* (38%), and *P. aeruginosa* (30%) were the most frequent carbapenemase-positive bacteria followed by *K. pneumoniae* (17%), *A. baumannii* (12%), *Citrobacter diversus* (2%) and *Enterobacter agglomerans* (1.4%) (14). The differences in the type of evaluated microorganisms (Enterobacteriaceae vs. all Gram-negative pathogens), the region, and the source of the isolates (hospital-acquired vs. community-acquired) are responsible for these various results.

Our results showed that the most prevalent carbapenemase-positive hospital infections were pneumonia caused mostly by *A. baumannii* and UTIs mostly due to *K. pneumoniae*. Consistently, in a cohort study published in 2012, among 21 patients with UTIs, in 20 cases, the causative pathogen was *K. pneumoniae* with positive Hodge test (19). In the study of Gheitani

and Fazeli on carbapenem-resistant *K. pneumoniae*, UTI was the most frequent infection (52.5%) caused by this pathogen (20). Therefore, it seems that carbapenem-resistant *K. pneumoniae* is a major cause of nosocomial UTIs and this should be considered in the empiric treatment of this type of infections. Furthermore, resistant strains of *A. baumannii* have become increasingly common cause of nosocomial infections globally since 1980 (21, 22). The most frequent clinical manifestations of *A. baumannii* infection are pneumonia, as observed in our study, and BSIs (23) *A. baumannii* was among the most common pathogens causing nosocomial pneumonia in a prospective observational study from 27 ICUs in nine European countries (23).

According to our results, carbapenemase-resistant CNS infections were caused equally by *A. baumannii* and *K. pneumoniae*. Gram-negative bacilli are common etiology of nosocomial meningitis, often occurring as a complication of head trauma and craniotomy (24). In a study performed in Turkey, among Gram-negative pathogens, *A. baumannii* was the most frequently isolated pathogen (59.8%) from post-operative meningitis followed by *P. aeruginosa* (13.4%) and *K. pneumoniae* (12.2%) (25). Therefore, it seems that the type of causing pathogen is different between the centers.

Another aspect in our study was determining the susceptibility of isolated carbapenemase-positive Gram-negative pathogens to various antibacterial agents. Our results showed that colistin was the most effective antibiotic against all isolated pathogens. However, yet a high rate of resistance was seen against this antibiotic (about 70% for *A. baumannii* and *K. pneumoniae* and 44% for *P. aerugonosa*). Different rates of colistin-resistance among *A. baumannii* strains have been reported in several reports, mainly as a result of increased use of this agent for MDR isolates (26-28); In the study of Azimi et al. on carbapenem-resistant *A. baumannii* isolated from burn patients in Terhran, only 55% strains with resistance to all investigated antibiotics were susceptible to colistin (29). Therefore, considering that colistin has been used as a first-line agent for the treatment of infections caused by carbapenem-resistant Gram-negative pathogens, implementing the strategies for saving this antibiotic is necessary. Furthermore, based on our results, none of other evaluated antibiotics was effective against *A. baumannii*. Similarly, in the study of Paranandi et al. all of 19 isolates of carbapenemase-producing *A. baumannii* were resistant to cefepime, ceftazidime, and levofloxacin and only 14% were sensitive to tobramycin; however, in contrast to our results, all isolates (100%) were susceptible to colistin (30). Generally, it seems that carbapenemase-producing strains of *A. baumannii* convert to XDR (extensively drug resistant) strains with resistance to most antibiotics used for the treatment of infections due to this pathogen (29).

In the present study, co-trimoxazole had a sensitivity rate similar to colistin (30.6%) against *K. pneumoniae*. However, we did not find any other report of co-trimoxazole sensitivity among these strains. Therefore, this agent could be an option for treatment of carbapenemase-producing *K. pneumoniae* if susceptibility testing confirms its effectiveness. Of note, in contrast to our results, the sensitivity of carbapenem-resistant *A. baumannii* to co-trimoxazole, determined by microbroth dilution method, has been shown in the study of Nepka et al. (31). The difference could be due to various methods of susceptibility testing as we used disk diffusion method. However, the low susceptibility rates in several other studies, as reported in the review of Falagas et al. (32), are consistent with our result. Nevertheless, as polymyxin-resistant *A. baumannii* might still be susceptible to co-trimoxazole, the use of this agent should be carefully considered if it is still active.

One study showed that the most effective antibiotic groups for KPC-producing *K. pneumoniae* are polymyxins and aminoglycosides (33), while 87% of amikacin (aminoglycoside) resistance was observed in our study. This difference could be due to the fact that we included the pathogens with all carbapenemase types, not only KPC type. Of note, according to other reports, KPC-positive isolates of *K. pneumoniae* are commonly resistant to many antibiotics such as colistin (34, 35).

Overall, according to our results, colistin, co-trimoxazole, and an aminoglycoside (e.g., amikacin) could be considered for treatment of carbapenemase-producing nosocomial Gram-negative pathogens in our hospital based on the results of antibiotic susceptibility testing. Although tygecycline could also be an option in this regard (36), we did not apply this agent in our investigation because of its unavailability in Iran.

The main limitation of this study was use of disk diffusion method for susceptibility testing of colistin in spite of CLSI statement recommending only broth

microdilution method for polymyxin susceptibility testing (13), as the poor agar diffusion characteristics of polymyxins limit the predictive accuracy of the disk diffusion test. However, broth microdilution is impractical for most clinical microbiology laboratories.

CONCLUSION

Carbapenemase-producing Gram-negative bacteria causing nosocomial infections are common in our hospital and have high rate of resistance to most antibiotics. *Acinetobacter baumannii* and *Klebsiella pneumoniae* are the most common pathogens with the most sensitivity to colistin. Improvement in the pattern of antibiotic use and infection control measures are necessary to overcome this resistance.

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