Annals of Medicine and Surgery 77 (2022) 103690

Contents lists available at ScienceDirect

Annals of Medicine and Surgery

journal homepage: www.elsevier.com/locate/amsu

Experimental Research

Prevalence of KPC-producing bacteria in negative gram of clinical samples obtained from patients

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ARTICLE INFO

Keywords:
Carbapenemase
Antibiotics
Infections
Klebsiella pneumonia
KPC

ABSTRACT

Objective: Carbapenems are beta-lactam antibiotics that can play an important role in infections with multiple and severe resistance. The aim of this study was to investigate the frequency of carbapenem-producing bacteria in gram-negative isolates of clinical samples obtained from patients.
Methods: 291 g-negative bacilli were isolated from the samples of hospitalized patients using gram staining method, conventional methods and biochemical tests. The antibiotic susceptibility of the isolates was determined using the agar disk diffusion method for 5 different antibiotics. Strains that were resistant to Meropenem antibiotic, KPC enzyme production was examined by the Modified Hodge test method.
Results: Out of 291 g-negative bacilli, 14 isolates showed resistance to Meropenem by a disk agar diffusion method where 12 (85.8%) strains were producing KPC enzyme. The highest frequency of Gram-negative KPC-producing bacilli was related to *Klebsiella pneumoniae* and the most positive samples were urine. The prevalence of this type of bacteria was highest in NICU and the male internal ward, respectively.
Conclusion: It was shown that carbapenem-resistant strains are considered as a growing problem in hospitals, especially in the intensive care unit for children and men.

1. Introduction

The growing prevalence of bacterial resistance to antibiotics is a major public health problem. Today, the major threat of antibiotic-resistant bacteria is from multidrug-resistant (MDR) gram-negative organisms, especially those that have developed carbapenem resistance [1,2]. Carbapenems have conventionally been used for treating infections caused by extended-spectrum β-lactamase-producing bacteria, and are still considered as last resort antibiotics to date [3]. Carbapenem resistance in gram-negative bacteria has become a global concern. In 2017, the World Health Organization (WHO) published a list of carbapenem-resistant pathogens in which Enterobacteriaceae, *Pseudomonas aeruginosa*, and Acinetobacter baumannii had the highest frequency [4]. To address this global epidemic, continuous identification and monitoring of carbapenem-resistant gram-negative bacteria is required.

Klebsiella pneumoniae is an opportunistic Enterobacteriaceae, which mainly affects patients with defective immune systems and causes various infections of the system. Over the past decade, overuse of antibiotics, including third-generation cephalosporins and carbapenems, for *Klebsiella pneumoniae* infections has led to a rapid increase in drug resistance [5]. Studies show that patients infected with carbapenem-resistant microorganisms have an increased likelihood of mortality than those infected with susceptible microorganisms [6–8], possibly due to antibiotics with ineffective activity or inactivity against these bacteria [9].

Klebsiella pneumonia carbapenemase (KPC) is a widespread Class A serine β-lactamases (SBLs) that hydrolyzes carbapenems [10]. Studies conducted by the China Antimicrobial Surveillance Network (CHINET) have shown that carbapenem resistance to Enterobacteriaceae increased from 0.7% in 2000 to 14% in 2015, and carbapenem resistance to *K. pneumoniae* increased from 3% in 2005 to 18.9% in 2016 [11,12].

Abbreviations: KPC, Klebsiella pneumonia carbapenemase; MDR, multidrug-resistant.

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https://doi.org/10.1016/j.amsu.2022.103690
Received 17 February 2022; Received in revised form 24 April 2022; Accepted 25 April 2022
Available online 29 April 2022

Given the relationship between the prevalence of KPC-producing members of the Enterobacteriaceae family and high mortality, especially among very serious patients with a long history in the hospital [13–15], take sufficient preventive measures and early detection of carbapenem resistance mechanisms to control the prevalence of these pathogens are essential. The aim of this study was to investigate the frequency of KPC-producing bacteria in gram-negative isolates in clinical samples obtained from patients.

2. Methods

Clinical samples (blood, urine and stool) of patients admitted to (XXX) were collected.

In order to separate gram-negative bacilli from other bacteria (gram-negative cocci, gram-positive cocci, etc.), we first cultured the samples on blood agar medium. After 24–48 h of incubation, we prepared smear from the grown colonies and isolated gram-negative bacilli by gram staining. To identify gram-negative bacilli, differential tests such as oxidase, TSI, Simon citrate medium, urea medium, MR-VP were used.

According to the CLSI standard, the resistance pattern in the isolated strains against the third-generation cephalosporin antibiotics, i.e., cefixime, ceftriaxone, ceftazidime, cefotaxime was evaluated by disk diffusion method on Müller-Hinton agar medium and Kirby-Bauer standard method. The resistant strains were isolated and these strains were examined for resistance to carbapenem antibiotics. In the isolated strains, insensitivity to one or more of the carbapenem antibiotics, including imipenem, ertapenem, and meropenem, was examined for carbapenems production by Modified Hodge tests (MHT) [16].

After collecting the data and entering the information into SPSS26 software, the necessary controls were performed. Results were examined by Chi-square test and analyzed using descriptive statistics such as frequency tables, percentage, mean, etc. The significance level of 0.05% test was used to interpret the data.

The research is in line Declaration of Helsinki. The Ethics Committee of (XXX).

Unique identifying number is: researchregistry7657.

The methods are stated in line with STROCSS guidelines [17],

3. Results

In this study, 5043 samples (2591 blood samples, 2171 urine samples and 281 stool samples) of patients admitted to different wards of the hospital were collected. After initial screening, 291 cases of gram-negative bacilli were identified. The highest frequency was related to urine samples with 193 cases (66.3%). Highest number of cases were obtained from emergency ward, 126 cases (43.3%) and the lowest were that from chemotherapy ward with 1 case (0.3%). Also, the highest frequency was related to *E. coli* with 150 cases (51.5%).

Based on the results of agar disk diffusion test, 14 of 291 (4.8%) gram-negative bacilli showed resistance to meropenem disks on Müller-Hinton agar. These 14 samples were equally distributed between both, men and women and there was no statistically significant relationship between the resistance of meropenem and the sex of patient ($p = 0.843$). 6 out of 14 cases that from patients aged 30–64 years. According to the Chi-square test, there was no significant relationship between antibiotic resistance to meropenem and patients' age ($p = 0.636$). The highest number of antibiotic-resistant isolates of meropenem was isolated from urine samples (7 out of 14) but was not statistically significant ($p = 0.395$). The NICU ward had the highest frequency with 4 positive cases. *Klebsiella pneumoniae* was also the predominant species among the isolates (10 out of 14).

MHT method showed that out of 14 strains resistant to meropenem, 12 (85.8%) strains were producing KPC enzyme, of which 6 cases were related to the samples of patients aged 30–64 years (the highest frequency) ($p = 0.053$) (Table 1). According to MHT test results, 12 positive isolates were evenly distributed between men and women (Fig. 1). Out of 12 isolates producing KPC enzyme, the highest frequency was related to urine samples with (50%) 7 cases and the lowest frequency was related to stool samples (no positive case was found), which was statistically significant ($p = 0.027$) (Fig. 2). Among the sampling wards, the highest frequency was related to the NICU and the male internal ward, with 4 (28.6%) and 3 (21.4%) positive cases, respectively (Table 1). There was a significant relationship between KPC-producing gram-negative bacilli and hospital wards of the prepared samples but it was not statistically significant ($p = 0.051$) (Table 2). Among bacteria, *Klebsiella pneumoniae*, with 9 positive cases, was the most gram-negative bacilli producing KPC enzyme ($p = 0.463$) (Table 3).

4. Discussion

This study was performed on samples obtained from hospitalized patients to evaluate the frequency of gram-negative KPC-producing bacteria. Carbapenem resistance in gram-negative bacteria has become a global concern. Carbapenem-resistant *K. pneumoniae* (CRKP) has emerged in many countries as a result of intercontinental and intracontinental expansion [18,19]. The warning level of carbapenem resistance presents specific challenges for the management of a variety of infections caused by non-fermentative agents due to the low permeability of the outer bacterial membrane to several antibiotics, including

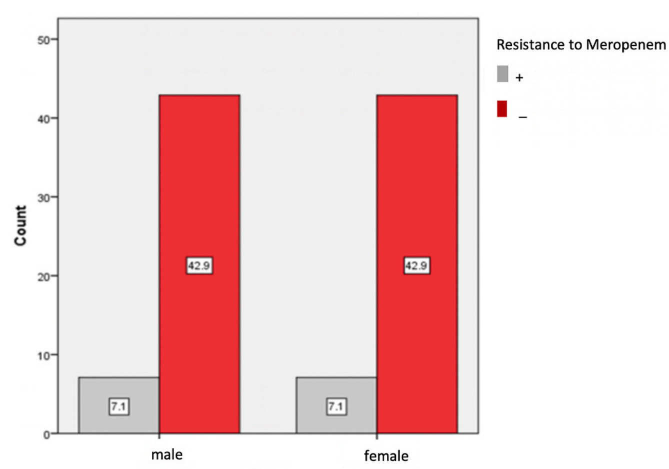

Fig. 1. Frequency of gram-negative bacilli based on KPC enzyme and sex.

Table 1
Consensus table of gram-negative bacilli based on KPC enzyme and age groups.

KPC	+		-(		Total		P value
Age	(Percentage)	number	(Percentage)	number	(Percentage)	number	
≤18	28.6	4	0	0	28.6	4	0.053
19–29	0	0	0	0	0	0	
30–64	42.8	6	0	0	42.8	6	
≥65	14.3	2	14.3	2	28.6	4	
Total	85.7	112	14.3	2	100	14	

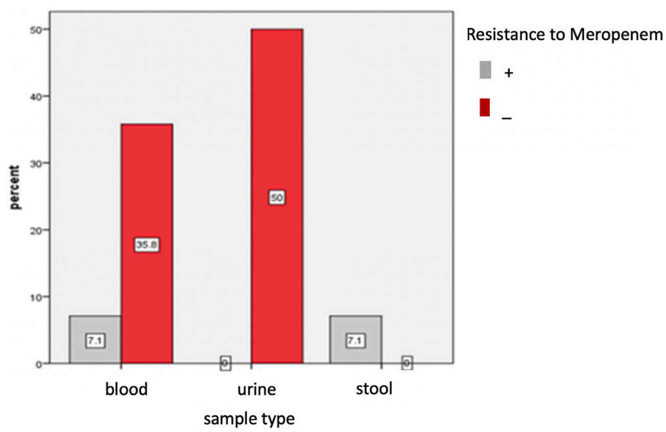

Fig. 2. Distribution of gram-negative bacilli based on KPC enzyme and sample type.

Table 2
Consensus table of gram-negative bacilli based on KPC enzyme and hospital wards.

KPC	+	–	Total	P value
Ward	(Percentage) number	(Percentage) number	(Percentage) number	
NICU	4 (28.6)	0 (0)	4 (28.6)	0.051
male internal	3 (21.4)	0 (0)	3 (21.4)	
General Internal	2 (14.3)	0 (0)	2 (14.3)	
heart	1 (7.1)	0 (0)	1 (7.1)	
ICU Surgery	1 (7.1)	0 (0)	1 (7.1)	
Chemotherapy	1 (7.1)	0 (0)	1 (7.1)	
Male surgery	0 (0)	1 (7.1)	1 (7.1)	
Internal ICU	0 (0)	1 (7.1)	1 (7.1)	
total	12 (85.7)	2 (14.3)	14 (100)	

Table 3
Consensus table of gram-negative bacilli based on KPC enzyme and type of bacteria.

KPC	+	–	Total	Pvalue
Type of bacteria	(Percentage) number	(Percentage) number	(Percentage) number	
Klebsiella pneumoniae	9 (64.3)	1 (7.1)	10 (71.5)	0.463
Acinetobacter baumannii	1 (7.1)	1 (7.1)	2 (14.3)	
Alcaligenes	1 (7.1)	0 (0)	1 (7.1)	
Escherichia coli	1 (7.1)	0 (0)	1 (7.1)	
Total	12 (85.7)	2 (14.3)	14 (100)	

carbapenems [20,21]. One of the main mechanisms of carbapenem resistance is the hydrolysis of carbapenems by carbapenemase enzyme among carbapenem-resistant Enterobacteriaceae clinical isolates that spreads rapidly among them because these enzymes can be encoded not only by chromosomal genes but also by mobile elements such as plasmids and transposons [22–24].

Frequent use and abuse of these drugs, combined with the transmissibility of resistance determinants mediated by mobile elements (plasmids, transposons, and other integrative conjugative elements), has contributed to the spread of resistance to β-lactams by *Klebsiella pneumoniae* [25].

In this study, out of 5043 samples collected, 291 g-negative bacilli were identified that the most common of which were related to *E. coli*, Maltophilia and *Klebsiella pneumoniae*, respectively. As in a cohort study conducted in Isfahan from 2012 to 2013, after isolating 500 strains of Enterobacteriaceae from clinical samples of patients by biochemical methods and PCR, the most isolated organism was a type of E. coli and urinary samples [26]. In our study, 14 of the 219 g-negative bacilli showed resistance to meropenem disc, which was less frequent than some studies [27–29]. In these prospective studies, *Pseudomonas aeruginosa* strains were studied. *Pseudomonas aeruginosa* did not show any resistance in our study. However, it seems that the small volume of *Pseudomonas aeruginosa* samples in the present study has an effect on the frequency of resistant cases of this isolate and may change if the sample size increases. The highest percentage of meropenem-resistant species was *Klebsiella pneumoniae*. Among the 219 g-negative bacilli identified, the highest and lowest cases were related to inpatient emergency and chemotherapy wards, respectively. In a survey-based study conducted by Ramezanzadeh et al. in 2013–2014 in Sanandaj teaching hospitals, 2289 g-negative bacilli were isolated from clinical specimens. Of these, the most isolated gram-negative isolates were obtained from women's wards with 625 cases. Also, the highest and lowest abundance among gram-negative bacilli was related to *Escherichia coli* and Morganella [30].

In our study, using MHT test, we showed that 12 of the 14 strains resistant to meropenem produce KPC enzyme, of which *Klebsiella pneumoniae* (64.3%) was the most common gram-negative bacilli producing carbapenemase in the collected samples, although not significant. This result was similar to the results obtained from previous studies at the genome level, which showed Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is a major cause of hospital infections worldwide [31,32]. In a cross-sectional prospective study performed by Krishna Dhungana et al. at Human Organ Transplant Center, the most KPC-producing isolates, isolated from 1500 clinical samples were obtained from the urine sample with 78.9% and the most frequent KPC producers obtained from urine sample was *E. coli* (57.8%) followed by 10.5% *K. pneumoniae* [33]. The difference in the results of different studies in terms of the type of common species, can be due to the type of patient samples, inpatient wards, and also the geographical area of the study. In our study, as a prospective study of Arezoo Saadatian Farivar et al. conducted on 81 samples, the prevalence of *Klebsiella pneumoniae* was higher in urine samples taken from patients [34]. These strains can become an important challenge for community and hospital as they spread among patients in hospitals and complicate the treatment process.

Studies have shown that patients in the intensive care unit (ICU) are at high risk for carbapenem-resistant Klebsiella infections, which is in parallel to the findings from our study. Because these infections can infect the other patients, and hospital staff, antibiotic resistance should be monitored [35,36].

Methods like pulse-field gel electrophoresis and multi-locus sequence typing can improve the quality of the results presented in this study. Furthermore, our study does not provide the data the source of infection, co-morbidities and patient outcomes. One of the significant challenges encountered during the research was obtaining samples from critically ill patients. For this reason, we did not include such patients in our study.

5. Conclusion

The results of studies with a wider statistical community to identify maximum carbapenem-resistant organisms can help us better understand this immediate threat and enable physicians to choose the most appropriate antibiotics. The results of this study show that beta-lactam-resistant strains, especially carbapenems, are considered as a growing problem in hospitals, especially in the intensive care unit. Also, there is a significant relationship between KPC-producing gram-negative bacilli and the type of sample, and urine samples were the most common.

The research followed the tents of the Declaration of Helsinki. The Ethics Committee of Lorestan University of Medical Sciences approved this study (IR.LUMS.REC.1399.098).

Human and animal rights

No animals were used in this research. All human research procedures followed were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013.

Consent for publication

Informed consent was obtained from each participant.

Availability of data and materials

All relevant data and materials are provided with in manuscript.

Funding source

None.

Registration of research studies

Name of the registry: N/a
Unique Identifying number or registration ID: IR.LUMS.REC.1399.098

Hyperlink to the registration (must be publicly accessible): https://ethics.research.ac.ir/ProposalCertificateEn.php?id=146648&Print=true&NoPrintHeader=true&NoPrintFooter=true&NoPrintPageBorder=true&LetterPrint=true.

Authors' contribution

Dr. Ali Kharazmkia and Samareh Mir: conceptualized and designed the study, drafted the initial manuscript, and reviewed and revised the manuscript.

Mehran Amirizadeh and, Zahra Goudarzi: Designed the data collection instruments, collected data, carried out the initial analyses, and reviewed and revised the manuscript.

Dr.Mehdi Birjandi and Alireza Barfipoursalar: Coordinated and supervised data collection, and critically reviewed the manuscript for important intellectual content.

Guarantor

Dr. Ali Kharazmkia.

Declaration of competing interest

The authors deny any conflict of interest in any terms or by any means during the study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.amsu.2022.103690.

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