Clinical Study

Clinical Response and Outcome in Patients with Multidrug Resistant Gram-negative Infections

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ABSTRACT

Objective: In this study, frequency and antimicrobial sensitivity pattern of multidrug resistant (MDR) microorganisms were evaluated in a referral teaching hospital in Iran.

Methods: Patients with MDR Gram-negative pathogens were followed during the course of hospitalization. Demographic data, baseline diseases, type of biological sample, isolated microorganism, type of infection, antibiotic regimen before the availability of the culture result and change in the antibiotic regimen following receiving the antibiogram results, response to the treatment regimen, and duration of hospitalization and patient's outcome were considered variables for each recruited patient.

Findings: In 71% of the patients, antibiotic regimens were changed according to the antibiogram results. A carbapenem alone or plus amikacin or ciprofloxacin were selected regimens for patients with extended-spectrum beta-lactamase (ESBL) infections. For patients with probable carbapenem-resistant *Enterobacteriaceae* infections, a carbapenem plus colistin was the most common antibiotic regimen. Clinical response was detected in 54.5% of the patients who were treated based on the antibiogram results. Clinical response was higher in the ESBL producers (ESBL-P) than the non-ESBL-P infections (75% vs. 52%). However, this difference was not significant (P = 0.09). Most nonresponders (80%) had sepsis due to *Klebsiella* species. Finally, 41.9% of the patients were discharged from the hospital and 58.2% died.

Conclusion: Same as other countries, infections due MDR microorganisms is increasing in the recent years. This type of resistance caused poor clinical response and high rate mortality in the patients.

KeyWords: *Ethiopia, expectation, pharmaceutical services, satisfaction, service quality*

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Introduction

The rapid spread of resistance among common pathogenic microorganisms is a serious challenge around the word. This phenomenon affects antibiotics' effectiveness and limits available options for the treatment of common infections in human.[1,2]

Serious infections due to beta-lactamase producing microorganisms, especially in hospitalized patients are increasing now. Several mechanisms for antibiotic resistance have been introduced in Gram-negative bacteria. Both enzymatic and nonenzymatic pathways cause resistance to third-generation cephalosporins, aminoglycosides, fluoroquinolones, and carbapenems. Antibiotic resistance occurs following mutation in chromosomal genes or by horizontal transfer of genes between different microorganisms. The main mechanism of antimicrobial resistance in *Enterobacteriaceae* family is transferring of plasmid encoding extended-spectrum beta-lactamase (ESBL).[1-6] ESBL producers (ESBL-P) are Gram-negative microorganisms which almost always belong to the *Enterobacteriaceae* species. These Gram-negative bacteria secret ESBL enzyme in periplasmic space and hydrolyze the beta-lactam ring in penicillins, cephalosporins, and aztreonam. In general, carbapenems and cephamycines are resistant to this enzyme. ESBL-P pathogens can cause severe and life-threatening infections such as bacteremia, sepsis, pneumonia, and meningitis.[3,4] In the United States, 26,000 infections and 17,000 deaths per 2012 were due to ESBL-P species.[5]

According to the Centers for Disease Control and Prevention (CDC) report, more than 19% of healthcare-associated infections are resistant to extended-spectrum cephalosporins. In the United States, 37% of nosocomial infections were due to ESBL-P *Enterobacteriaceae* species. The mortality rate was 57%

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more common in patients with bloodstream infection caused by ESBL-P than nonproducers.[5] Prolong hospitalization, presence of invasive medical devices, receiving total parenteral nutrition, age <12 weeks, prior treatment with cephalosporins and aminoglycosides, recent surgery, and hemodialysis are defined as risk factors for colonization with ESBL-P species.[7]

Antibiotic resistance is a critical issue in developing countries. The incidence of infections due to resistant microorganisms is increasing in the recent years in Iran.[8,9] In this study, frequency and antimicrobial sensitivity pattern of multidrug resistant (MDR) Gram-negative microorganisms were evaluated in a referral teaching hospital in Tehran, Iran.

Methods

This cross-sectional study was performed between December 2014 and January 2016 in Imam Khomeini Hospital, a referral teaching hospital affiliated to the Tehran University of Medical Sciences, Tehran, Iran.

Patients with nosocomial infections (acquired 48–72 following the hospital admission) were included. Biologic clinical samples including urine, cerebrospinal fluid (CSF), blood, and tracheal secretions that were referred to the central laboratory department from different wards of the hospital were analyzed according to the clinical and laboratory standard institute instructions.[10] Antimicrobial sensitivity patterns of all isolates were recognized by using standard antibiotic disks on Mueller-Hinton agar. Following antibiotic disks from HiMedia, Bioscience Company, India, was used for the primary antibiogram and ESBL screening; ciprofloxacin (5 μg), ceftriaxone (30 μg), cefotaxime (30 μg), ceftazidime (30 μg), amikacin (30 μg), ampicillin-sulbactam (10/10 μg), imipenem (10 μg), and meropenem (10 μg). After 24 h of incubation, if an inhibitory concentration zone was <25 mm for ceftriaxone, 27 mm for cefotaxime or 22 mm for ceftazidime, phenotypic confirmatory test was performed with double disk synergy test. For this test, cefotaxime/clavulanic acid (30/10 μg) and ceftazidime/clavulanic acid (30/10 μg) discs were used.[10] Increasing of ≥5 mm in the inhibition zone diameter in double synergy test versus the antibiotic tested alone was considered in favor of ESBL-P isolates. Non-ESBL isolates that were resistant to imipenem or meropenem was categorized as probable carbapenem-resistant Gram-negative microorganisms.[10]

Patients, in whom MDR Gram-negative pathogens were confirmed phenotypically, were detected and followed by the clinical pharmacists during the course of hospitalization. MDR was defined as resistance to at least three classes of antibiotics (aminoglycosides, anti-MRSA cephalosporins, antipseudomonal penicillins + beta-lactamase inhibitors, carbapenems, and nonextended spectrum cephalosporins; first and second generation cephalosporins, extended-spectrum cephalosporins; and third and fourth generation cephalosporins, cephamycins, fluoroquinolones, folate pathway inhibitors, glycylcyclines, monobactams, penicillins, penicillins + beta-lactamase inhibitors, polymyxins, phosphonic acids, phenicols, and tetracyclines).[11]

Demographic data, baseline diseases, type of biological sample, isolated microorganism, type of infection, antibiotic regimen before availability of the culture result, and change in the antibiotic regimen following receiving the antibiogram results, response to the treatment regimen, duration of hospitalization, and patient's outcome were considered variables for each recruited patient. Cultures compatible with patient clinical status were measured as true infection according to the CDC definitions for health-care associated infections.[11] Patients with positive culture without these criteria were considered as colonized.

Statistical analyses were performed by the IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. (IBM Corp., Armonk, NY). Continuous data were expressed as a mean ± standard deviation (SD). Categorical variables were reported as percentages. Chi-square or Fisher exact test was used for comparing the categorical variables between the groups. Continuous variables were compared by the independent *t*-test. $P < 0.05$ was defined as statistically significant.

Results

During the study period, fifty patients with MDR Gram-negative infections including confirmed ESBL or probably carbapenem-resistant *Enterobacteriaceae* (CRE) were detected. The mean ± SD of patients' age was 59.02 ± 17.96 years old and thirty (60%) of them were males. Tracheal secretions (17 [34%]), urine (15 [30%]), blood (8 [16%]), soft tissue (3 [6%]), peritoneal fluid (2 [4%]), CSF (1 [2%]), and pleural fluid (1 [2%]) were positive in the patients. Most patients were hospitalized in Intensive Care Unit (35 [70%]) and general ward (8 [16%]), followed by emergency, neurosurgery, and Coronary Care Unit wards. *Klebsiella* species (78%), *Escherichia coli* (20%), and *Enterobacter cloacae* (2%) were isolated microorganisms from the patients' biological samples.

Antimicrobial sensitivity pattern of the microorganisms is shown in Table 1. Most active antibiotics were carbapenems and aminoglycosides, respectively. All of isolated *E. coli* and *E. cloacae* but only 56% of isolated *Klebsiella*s species were sensitive to carbapenems. The result of antimicrobial susceptibility tests revealed that 30% of the isolated microorganisms were resistant to carbapenems that may be CRE. However, most of these species (86.7%) were ESBL negative. According to the double disk synergy test, 17 (34%) of all isolates were ESBL-P and others were ESBL-negative. ESBL was positive in 58.8% and 41.2% of isolated *Klebsiella* species and *E. coli*, respectively. Based on the CDC definition, the clinical condition was compatible with the isolates in 62% of all patients and 25.8% of patients with ESBL-P infections. In 71% of the cases, antibiotic regimens were changed according to the antibiogram results. A carbapenem alone or plus amikacin or ciprofloxacin were selected regimens for patients with ESBL infections. For patients with probable CRE infections, a carbapenem plus colistin was the most common antibiotic regimen. Clinical response was detected in 54.5% of the patients who were treated based on the

antibiogram results. Clinical response was higher in the ESBL-P than the non-ESBL-P infections (75% vs. 52%). However, this difference was not significant (P = 0.09). Most nonresponders (80%) had sepsis due to *Klebsiella* species. Finally, 41.9% of the patients were discharged from the hospital and 58.2% died. Characteristics of patients with ESBL-P and probably CRE infections were summarized in Tables 2 and 3, respectively.

Discussion

Inappropriate antibiotic administration and consequent increasing number of MDR pathogens including ESBL-P and CRE are a serious worldwide concern in recent years.[12-14] Rapid growing of ESBL-P and CRE among community and hospitalized patients is a global threat, especially in critically ill patients.[15] Considering that only limited new antimicrobial agents have been introduced in recent years; in some situations, we do not have an effective weapon against these pathogens.[13,15]

Following extensive use of cephalosporins in last years, resistance rate of *Enterobacteriaceae* family to these agents is increasing around the world. Cephalsporins-resistant rate of these microorganisms was 30% among 11 countries of Asia in 2010. This rate received to 87% in 2014 at Latin America.[2]

Table 1: Resistance pattern of isolated Gram-negative pathogens

Antimicrobial agents	Sensitive (%)		Intermediate (%)		Resistance (%)		Not reported (%)	
	CRE	ESBL	CRE	ESBL	CRE	ESBL	CRE	ESBL
Third generation cephalosporins	-	-	-	-	95.5	97.9	4.5	2.1
Ciprofloxacin	11.2	20.8	4.8	2.1	80	68.8	4	8.3
Ampicillin-sulbactam	28.9	16.7	1.1	4.2	65.4	62.5	4.6	16.7
Piperacillin-tazobactam	6.4	12.5	4.6	4.2	80.7	52.1	8.3	31.3
Aminoglycosides	43.2	60.4	3.8	4.2	51.2	33.3	1.8	2.1
Carbapenems	0	64.6	3.6	2.1	93.4	29.2	3	4.2

ESBL=Extended spectrum beta-lactamase-producing, CRE=Carbapenem-resistant enterobacteriaceae

Table 2: Characteristics of patients with extended-spectrum β-lactamase-producing infections

Age (year)	Sex	Source of microorganism	Type of microorganism	ESBL positive or negative	Baseline diseases	Primary antibiotic regimen (before the culture result)	Secondary antibiotic regimen (after the culture result)	Duration of hospitalization (day)	Clinical response	Outcome
69	Female	Urine	*E. coli*	Positive	HTN	Meropenem Vancomycin	Meropenem Amikacin	86	Yes	Death
68	Male	Urine	*E. coli*	Negative	Bladder cancer	Ciprofloxacin Piperacillin/tazobactam Metronidazole Ceftriaxone	Piperacillin/tazobactam	8	No	Death
55	Male	Blood	*Klebsiella* spp.	Negative	HTN and DM	Meropenem Ciprofloxacin Vancomycin	Meropenem Ciprofloxacin	12	Yes	Discharge
76	Male	Blood	*Klebsiella* spp.	Negative	None	Meropenem Vancomycin Piperacillin/tazobactam Linezolid	Meropenem Ciprofloxacin	None	Yes	Discharge
43	Male	Tracheal	*Klebsiella* spp.	Negative	Asthma and COPD and Esophagus candidiasis	Imipenem Ciprofloxacin Vancomycin	Imipenem Ciprofloxacin	11	Yes	Death
27	Male	Tracheal	*Klebsiella* spp.	Negative	HTN and CVA and seizure	Meropenem Vancomycin Piperacillin/tazobactam Ceftriaxone	None	11	No	Death

Contd...

Table 2: Contd...

Age (year)	Sex	Source of microorganism	Type of microorganism	ESBL positive or negative	Baseline diseases	Primary antibiotic regimen (before the culture result)	Secondary antibiotic regimen (after the culture result)	Duration of hospitalization (day)	Clinical response	Outcome
82	Male	Peritoneal fluid	*E. coli*	Positive	HTN	Imipenem Ciprofloxacin Metronidazole	Imipenem	33	Yes	Discharge
55	Male	Blood	*Klebsiella* spp.	Negative	DM and DLP and IHD and COPD and HCV	Imipenem Ciprofloxacin Vancomycin	Imipenem Ciprofloxacin	26	No treated	Death
53	Male	Tracheal	*Klebsiella* spp.	Positive	Brain tumor	Amikacin Meropenem Ciprofloxacin Vancomycin	Amikacin Meropenem	9	Yes	Discharge
66	Male	CSF	*Klebsiella* spp.	Negative	Pituitary adenoma	Vancomycin Meropenem Colistin Cefazolin	Meropenem Colistin	68	Yes	Death
56	Male	Tracheal	*Klebsiella* spp.	Negative	Seizure	Vancomycin Meropenem Cefazolin Ciprofloxacin	Meropenem Ceftriaxone	29	Yes	Death
65	Male	Tracheal	*Klebsiella* spp.	Negative	None	Ceftriaxone Vancomycin Ampicillin Ciprofloxacin Piperacillin/tazobactam Meropenem	Meropenem	15	In-appreciable	Death
68	Female	Urine	*E. coli*	Positive	HTN and DM and IHD	Vancomycin Ampicillin Metronidazole Cefixime	Imipenem	42	Yes	Discharge
63	Male	Tracheal	*Klebsiella* spp.	Positive	None	Meropenem Vancomycin Ciprofloxacin Colistin Ampicillin/sulbactam Ceftriaxone	Colistin Ampicillin/sulbactam	40	No	Death
33	Male	Blood	*Klebsiella* spp.	Negative	None	Meropenem Vancomycin Imipenem Piperacillin/tazobactam Clindamycin	Meropenem Colistin	73	Yes	Discharge

Contd...

Table 2: Contd...

Age (year)	Sex	Source of microorganism	Type of microorganism	ESBL positive or negative	Baseline diseases	Primary antibiotic regimen (before the culture result)	Secondary antibiotic regimen (after the culture result)	Duration of hospitalization (day)	Clinical response	Outcome
68	Female	Tracheal	*Klebsiella* spp.	Negative	HTN and DM	Meropenem Ciprofloxacin Vancomycin Colistin Cefazolin	Meropenem Ciprofloxacin	94	Yes	
55	Male	Blood	*Klebsiella* spp.	Negative	ESRD	Meropenem Vancomycin Ciprofloxacin Colistin	Meropenem	64	Yes	Discharge
67	Female	Tracheal	*Klebsiella* spp.	Negative	DM and HF	Imipenem Ciprofloxacin	Meropenem	46	No	Death
27	Male	Tracheal	*Klebsiella* spp.	Negative	ESRD	Ceftriaxone Meropenem Vancomycin Ciprofloxacin Colistin	Colistin	59	No	Death
71	Female	Blood	*Klebsiella* spp.	Negative	HTN and DM	Vancomycin Ciprofloxacin Piperacillin/tazobactam Clindamycin metronidazole	None	20	No treated	
38	Male	Blood	*E. coli*	Negative	HIV and IDU	Meropenem Vancomycin Ceftriaxone	Meropenem	29	Yes	Discharge
87	Male	Urine	*E. coli*	Positive	Alzheimer's disease	Imipenem Vancomycin Ampicillin/sulbactam	Meropenem Ciprofloxacin	53	No	Discharge
28	Male	Soft tissue	*E. coli*	Negative	IDU	Imipenem Vancomycin	Imipenem	48	Yes	Discharge
57	Female	Soft tissue	*E. coli*	Negative	DM and eye mucormycosis	Piperacillin/tazobactam Vancomycin	Piperacillin/tazobactam	40	Yes	Discharge
17	Female	Soft tissue	*E. coli*	Positive	Ovarian cancer	Piperacillin/tazobactam Metronidazole Clindamycin Ampicillin Gentamicin Metronidazole Ceftizoxime	Piperacillin/tazobactam	15	Yes	Discharge

Contd...

Table 2: Contd...

Age (year)	Sex	Source of microorganism	Type of microorganism	ESBL positive or negative	Baseline diseases	Primary antibiotic regimen (before the culture result)	Secondary antibiotic regimen (after the culture result)	Duration of hospitalization (day)	Clinical response	Outcome
76	Male	Tracheal	*Klebsiella* spp.	Negative	DM and CABG and CVA and HTN	Clindamycin Imipenem Vancomycin Ampicillin/sulbactam	Imipenem	64	No	Death
71	Male	Tracheal	*Klebsiella* spp.	Negative	None	Colistin Meropenem Ciprofloxacin Vancomycin Ampicillin/sulbactam	Colistin Meropenem	31	No	Death
70	Male	Peritoneal fluid	*E. coli*	Positive	CLL	Imipenem Ciprofloxacin Ceftriaxone Trimethoprim/sulfamethoxazole Azithromycin	Imipenem Ciprofloxacin	29	No	Death
68	Male	Blood	*Klebsiella* spp.	Negative	None	Imipenem Vancomycin	Imipenem	72	No	Death
34	Male	Tracheal	*Klebsiella* spp.	Negative	HCV	Ciprofloxacin Vancomycin Azithromycin Clindamycin	Meropenem	58	Yes	Discharge
64	Female	Tracheal	*Klebsiella* spp.	Negative	Breast cancer	Meropenem Vancomycin Colistin Ciprofloxacin Imipenem Metronidazole	Meropenem Colistin	79	No	Death

ESBL=Extended spectrum beta-lactamase-producing, CABG=Coronary artery bypass graft, DM=Diabetes mellitus, HTN=Hypertension, HCV=Hepatitis C virus, CLL=Chronic lymphocytic leukemia, IDU=Injection drug user, ESRD=End-stage renal disease, HF=Heart failure, IHD=Ischemic heart disease, *E. coli=Escherichia coli, DLP=Dyslipidemia*

Only in limited studies, the prevalence of ESBL-P pathogens was evaluated in Iran and ranged from 43.6% in Ilam to 74% in Milad Hospital.[15] However, the average rate of ESBL-P microorganisms was 42.2% in Iran.[16] In a recent study, more than 50% of isolated microorganisms from bile specimens were ESBL-P.[17] The most isolated ESBL-P were *Klebsiella* species followed by *E. coli*.[4] In European hospitals, more than 80% of isolated *E. coli* and *Klebsiella pneumonia* were belonged to the ESBL-P category.[2]

In the present study, the frequency of ESBL-P pathogens was lower than the previous reports from our country. In a report from three hospitals of Iran, all isolated ESBL-P microorganisms were sensitive to carbapenems.[15] However, in our study, some of ESBL-P species and most of ESBL negative strains were CRE. All CRE were *Klebsiella* species.

To interpret the result of clinical responses, limitations of the study should be considered. The sample size of the study was small for assessment of the treatment outcome. ESBL-P pathogens were identified phenotypically but were not confirmed by the genotypic assay method. Genotypic assay is not easily available method in our hospitals and only is applied for research purpose. There are several clinical diagnostic laboratory tests for detection of ESBL-P microorganisms.[18-21] Although double disk synergy test is a common and practical method for ESBL confirmation but some isolates may be missed by this test. The sensitivity of this method could be reduced by microorganisms

Table 3: Characteristics of patients with probable carbapenem resistant enterobacteriaceae infections

Age (year)	Sex	Source of microorganism	Type of microorganism	ESBL positive or negative	Baseline diseases	Primary antibiotic regimen (before the culture result)	Secondary antibiotic regimen (after the culture result)	Duration of hospitalization (day)	Clinical response	Outcome
33	Male	Blood	*Klebsiella* spp.	Negative	None	Meropenem Vancomycin Imipenem Piperacillin/tazobactam Clindamycin	Meropenem Colistin	73	Yes	Discharge
55	Male	Blood	*Klebsiella* spp.	Negative	ESRD	Meropenem Vancomycin Ciprofloxacin Colistin	Meropenem	64	Yes	Discharge
55	Male	Blood	*Klebsiella* spp.	Negative	DM/DLP/IHD/COPD/HCV	Imipenem Ciprofloxacin Vancomycin	Imipenem Ciprofloxacin	26	No treated	Death
71	Female	Blood	*Klebsiella* spp.	Negative	HTN and DM	Vancomycin Ciprofloxacin Piperacillin/tazobactam	None	20	No treated	
71	Male	Tracheal	*Klebsiella* spp.	Negative	None	Colistin Meropenem Ciprofloxacin Vancomycin Ampicillin/sulbactam	Colistin Meropenem	31	No	Death
64	Female	Tracheal	*Klebsiella* spp.	Negative	Breast cancer	Meropenem Vancomycin Colistin Ciprofloxacin Imipenem Metronidazole	Meropenem Colistin	79	No	Death

ESBL=Extended spectrum beta-lactamase-producing, DM=Diabetes mellitus, IHD=Ischemic heart disease, HCV=Hepatitis C virus, DM=Diabetes mellitus, HTN=Hypertension, COPD=Chronic obstructive pulmonary disease, DLP=Dyslipidemia

that show low-ESBL activity.[18] It has been shown that 13.63% of ESBL positive strains were not recognized by double disk method.[18] Therefore, some of non-ESBL strains in our study may be false negatives of the test. In this study, CRE isolates were detected based on the results of the disk diffusion method and were not confirmed based on the phenotypic and genotypic assays.

Most MDR Gram-negative strains frequently carry both carbapenemase and ESBL genes. Specific methods such as bromic acid in combination with clavulanate are recommended to unmask the underlying ESBLs among *Enterobacteriaceae* family with carbapenemase enzyme. However, carbapenems-hydrolyzing ability of non-ESBL species is not impossible.[22]

Pharmacokinetic parameters such as inadequate tissue penetration of antimicrobial agents can influence the clinical responses in *in vivo* settings.[23] Most of the recruited patients had at least one of the following severe comorbidities including malignancies, respiratory disorders, ischemic heart disease, heart failure, diabetes mellitus, renal failure, cerebrovascular accident, hepatitis, immunodeficiency, and sepsis. A high rate mortality rate among our patients may be related to these conditions.

Unfortunately like other countries, CRE prevalence in our country is increasing in the recent years. Empiric administration of carbapenems should be restricted to patients with risk factors of infections with ESBL-P bacteria and in specific clinical situations. To limit the use of last-line antibiotics such as carbapenems, availability of accurate phenotypic, and genotypic methods for detection

of ESBL-P and carbapenemase strains is essential in clinical practice.

Authors' Contribution

Masoume Malekolkottab contributed in data gathering. Lida Shojaei contributed in drafting the manuscript and data gathering. Hossein Khalili contributed in data interpretation and manuscript editing. Mahsa Doumanlu performed laboratory analysis.

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Conflicts of interest

There are no conflicts of interest.

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