Prevalence of Gram Negative Infections Caused by Acinetobacter and Pseudomonas Severely Resistant to Treatment and Evaluation of Their Antibiotic Susceptibility Based on Minimum Growth Inhibitor Concentration

Introduction: *Currently, we are witnessing the formation of various species of gram negative microorganisms, including Enterobacteriaceae, Pseudomonas aeruginosa and acinetobacter, resistant to antibiotics such as MDR, XDR and PDR. This study is important to confirm microbial resistance to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. Regarding XDR gram-negative microorganisms isolated from samples, it was considered necessary to determine MIC.*

Method: *Patients suspected of various infections with septicemia diagnosed in different wards of the Firoozgar Hospital were enrolled. Quantitative value of minimum growth inhibitor concentration (MIC) was determined for infections caused by highly resistant gram-negative bacteria (acinetobacter and Pseudomonas species) (XDR) reported by antibiogram disk.*

Results: *sample size was 117, of which 41.9% were female and 58.1% were male. Regarding Colistin, 80% of the cultures were resistant and 12% were intermittent; this value was 52% in the MIC test. Regarding tigecycline, 100% of the acinetobacter samples were susceptible to this antibiotic. Most of cultures which had antibiotic resistance were acinetobacter (61.4%) and pseudomonas (39.6%).*

Discussion: *Acinetobacter baumannii is susceptible to tigecycline. Emergence of multi-drug resistance in Pseudomonas aeruginosa and A. baumannii is a major concern in the world, because several drugs, except polymyxins, are available to treat these infections. A significant resistance was found in MIC to Colistin (31.1%). Thus, there is resistance to Colistin, which is one of the last lines of antibiotic treatment.*

Conclusion: *This study shows an increase in percentage resistance of these bacteria to antibiotics. This trend is a worrying process for antibiotic treatment of diseases.*

Keywords: Acinetobacter, Pseudomonas, Colistin, tigecycline, MIC

Introduction

Due to widespread use of antibiotics, antibiotic resistance is one of the major causes of failure in treatment of many microbial diseases. Several definitions of multi-drug resistant (MDR), extensively drug resistant (XDR) and pandrug resistant (PDR) bacteria are used to classify different patterns of bacterial resistance present at different levels of the health system. Acinetobacter is able to collect various mechanisms to resist against antibiotic treatment; this results in emergence of strains resistant to all antibiotics (1). Since 1980s, drug resistant strains have become increasingly common causes of hospital infection (2-5). The term multi-drug resistance does not have a standard definition in Acinetobacter; it sometimes means resistance to three or more drugs known as a treatment for Acinetobacter infections (e.g., quinolones,

cephalosporins, and carbapenems). The term pan resistant is used to describe Acinetobacter species which are resistant to all antimicrobial agents, except Colistin (6). A group of international experts gathered together by ECDC[1] and CDC[2] to introduce a common international language for explaining profiles required for bacterial resistance based on antibiotic treatment failure points explained by CLSI[3], EUCAST[4] and FDA[5]. By definition, MDR is an acquired lack of therapeutic response to at least one agent in three or more antimicrobial classifications; XDR is lack of therapeutic response to at least one agent in all but two or less antimicrobial classifications; PDR is acquired lack of therapeutic response to all antibacterial agents in all classifications (7). Currently, we are witnessing the formation of various species of gram negative microorganisms, including Enterobacteriaceae, Pseudomonas aeruginosa and Acinetobacter resistant to antibiotic treatment, including MDR, XDR and PDR (Table 1, 2, 3). As it seems, the reported cases of XDR gram-negative bacterial agents are increasing (8-10), which increases the concern of medical community to treat these infections. Different sensitivity methods are used in vitro, including disc diffusion method and minimum inhibitory concentration (MIC). Disc diffusion method is used conventionally for determining antibiotic susceptibility because of its ease of use and its low cost. In microbiology, minimum inhibitory concentration (MIC) is minimal antimicrobial concentration which inhibits visible growth of microorganisms after one night of incubation; it is important to confirm microbial resistance to an antimicrobial agent and also to monitor activity of new antimicrobial agents (11). MIC is generally considered as the most fundamental laboratory measure for activity of an antimicrobial agent against an organism (12). Regarding isolated XDR gram-negative microorganisms, it is necessary to determine the MIC from patient samples. Regarding XDR gram-negative microorganisms isolated from samples, it seems essential to determine MIC.

Materials and Methods

Patients suspected of various infections with septicemia diagnosis including respiratory tract, urinary/genital tract, and meningitis infections who were hospitalized in different wards of the Firouzgar Hospital in a six month period from March to September 2009 underwent the required

[1] European Centre for Disease Prevention and Control
[2] Centers for Disease Control and Prevention
[3] Clinical Laboratory Standards Institute
[4] European Committee on Antimicrobial Susceptibility Testing
[5] United States Food and Drug Administration

work ups including blood, throat secretion, tracheal tube, CSF and urine sampling. Antibiotic disc method was used to assess antibiotic susceptibility or resistance in early studies. Then, infections caused by XDR gram-negative bacilli (Acinetobacter and Pseudomonas) reported by antibiogram disc method were sent to the Microbial Resistance Research Center of the Iran University of Medical Sciences for quantitative determination of MIC. There, samples were again subjected to MIC by E-test. Data was analyzed using SPSS software. In order to determine descriptive objectives, mean, median, range of variations and standard deviation were used based on the type of variables. Chi-square and independent t-test were used to determine analytical objectives of the study.

Results

The sample size was 117, of which 41.9% were women and 58.1% were male. Their mean age was 57.78 (22.39). The mean number of hospitalization days was 39 days (±28 days); 95% of patients with resistant infections were hospitalized for 11-67 days.

Mortality rate was 55%. Different antibiotics were used in different wards of the hospital. Meropenem colistin and meropenem ciprofloxacin were commonly used diet for treating these infections (11.7% and 12%, respectively).

63% of samples were taken from patient throats. Regarding colistin which was studied here, the results of Pseudomonas resistance were significantly different in MIC and culture.

Regarding colistin, a significant percentage of resistance (31%) was observed because disc diffusion was not applied on probable resistant and intermittent samples and only MIC was done. Moreover, more than half of cases of Acinetobacter baumannii (68.9%) were susceptible to colistin in MIC.

Another important result of the study is better performance of MIC to disc diffusion in resistant strains ($p = 0.001$).

Table 1: diseases

Disease	N	%
Urosepsis	13	11.1
pneumosepsis	21	17.9
VAP	57	48.7
Sepsis with uncertain origin	2	1.7
Meningitis	7	6.0
Abdominal infections	2	1.7
Septic arthritis	1	0.9

SBP	3	2.6
Infectious wound	8	6.8
Endocarditis	1	0.9
UTI	2	1.7
Total number of patients	117	100%

Table 2: Frequency and percentage of antibiotic susceptibility of Pseudomonas aeruginosa in culture

Antibiotic	Susceptibility			N (%)
	Resistant	Susceptible	Intermittent	
Amikacin	55 (41.0%)	3 (2.2%)	3 (2.2%)	61 (45.5%)
Imipenem	57 (42.5%)	10 (7.5%)	2 (1.5%)	69 (51.5%)
Ceftazidime	54 (40.3%)	7 (5.2%)	2 (1.5%)	63 (47.0%)
Ciprofloxacin	39 (29.1%)	3 (2.2%)	1 (0.7%)	43 (32.1%)
Piperacillin/tazobactam	59 (44.0%)	4 (3.0%)	3 (2.2%)	66 (49.3%)
Aztreonam	0	0	0	0
Fosfomycin	0	0	0	0
Colistin	48 (37.3%)	12 (10.4%)	0	60 (47.8%)

Table 3: Frequency and percentage of antibiotic susceptibility of Acinetobacter baumannii in culture

Antibiotic	Susceptibility			N (%)
	Resistant	Susceptible	Intermittent	
Amikacin	58 (43.3%)	1 (0.7%)	0	59 (44.0%)
Imipenem	92 (68.7%)	0	0	92 (68.7%)
Ceftazidime	89 (66.4%)	0	0	89 (66.4%)
Ciprofloxacin	70 (94.1%)	0	0	70 (94.1%)
Piperacillin/tazobactam	95 (70.9%)	0	0	95 (70.9%)
co-trimoxazole	86 (64.2%)	3 (2.2%)	1 (0.7%)	90 (67.2%)
Tetracycline	1 (0.7%)	0	0	1 (0.7%)
Colistin	0	0	0	0
Tigecycline	0	92	0	92 (68.7%)

Table 4: comparison of susceptibility of Pseudomonas to colistin in culture and MIC

	Sensitivity type			**N**	**P-value**
	Resistant	Intermittent	Susceptible		
Culture	48 (80.0%)	12 (20.0%)	0 (0.0%)	60	0.001
MIC	28 (52.8%)	11 (20.8%)	14 (26.4%)	53	
	78 (66.7%)	25 (21.4%)	14 (12.0%)	117 (100.0%)	

Table 5: comparison of susceptibility of Acinetobacter baumannii to colistin in culture and MIC

	Sensitivity type			**N**	**P-value**
	Resistant	Intermittent	Susceptible		
Culture	74 (100.0%)	0	0	74	0.001
MIC	23 (31.1%)	0	51 (68.9%)	74	

Table 6: comparison of susceptibility of Pseudomonas to Tigecycline in culture and MIC

	Sensitivity type	**N**	**P-value**

	Resistant	Intermittent	Susceptible		
Culture	-	-	-	60	
MIC			53 (100.0%)	53	

100

101 **Table 7: comparison of susceptibility of Acinetobacter baumannii to Tigecycline in culture and MIC**

Bacterial culture	**Sensitivity type**			**N**	**P-value**
	Resistant	Intermittent	Susceptible		
Culture	-	-	-	74	
MIC			74 (100.0%)	74	

102 **Discussion**

103 The sample size was 117, of which 41.9% were women and 58.1% were male. Their mean age
104 was 57.78 (22.39). Therefore, the age of patients has no significant effect on the rate of
105 antibiotic-resistant infections and antibiotic-resistant infections may occur at any age.
106 Several species of bacteria have emerged as major contributors to bacteremia which are very
107 important because of the lack of susceptibility of their strains to the last line of antibiotics. Thus,
108 Staphylococcus aureus, Streptococcus pneumoniae, Enterococcus faecalis, Enterococcus
109 faecium, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter
110 baumanii have been identified as a major threat, and have been subject to active monitoring and
111 annual reporting in most European countries since 1998 (13).
112 The prevalence of antibiotic-resistant bacteria has increased worldwide. In 2013, the US Centers
113 for Disease Control and Prevention reported that at least two million people in the United States
114 suffer from serious infections annually due to bacterial resistance and more than 23,000 people
115 with this antibiotic-resistant infection lose their lives. Resistance rates in countries vary because
116 of differences in the use of antimicrobial agents and prevention of resistant bacteria. In addition
117 to resistance rate, resistance states are also different in countries and even in cities of one
118 country. Therefore, careful monitoring of antibiotic-resistant bacteria throughout the country is
119 becoming a treatment guideline (14).
120 Hospitals worldwide have witnessed an increasing trend in gram negative bacteremia, which has
121 become a major concern with regard to the nature of its survival in hospital settings and
122 reduction in sensitivity to available antibiotics. One of the most disturbing findings in recent
123 years is the presence of antibiotic-resistant bacteria. Gram-negative bacteria such as
124 Pseudomonas and Acinetobacter members of this group of bacteria; Pseudomonas is resistant to

the last line of antibiotics (Carbapenems) as well as three key antibiotic groups (fluoroquinolones, cephalosporins third generation, and aminoglycosides) (15, 16).

Mortality rate was 55%. Severity of the underlying disease was effective on mortality rate. Diabetes and neurosurgery were the most frequent diseases among the underlying diseases. Different antibiotics were used in different wards. Meropenem colistin and meropenem ciprofloxacin were commonly used diet for treating these infections (11.7% and 12%, respectively).

The mean number of hospitalization days was 39 days (±28 days). Thus, 95% of patients with resistant infections were hospitalized for 11 to 67 days. It can be concluded that the higher the hospitalization rate is, the higher the percentage of resistant infections will be.

In a study which evaluated the resistance to acinetobacteria, more than 70% of Acinetobacter was resistant to any antibiotic and more than 90% was resistant to fluoroquinolone and carbapenems. In various reports published, acinetobacter levels were reported zero in Finland and Norway and over 90% in Croatia, Romania, and Greece (13). Thus, there is a difference in level of antibiotic resistance between countries of the European Union and Iran.

Regarding colistin which was studied here, the results of Pseudomonas resistance were significantly different in MIC and culture.

Regarding colistin, a significant percentage of resistance (31.1%) was observed because disc diffusion was not applied on probable resistant and intermittent samples and only MIC was done. Thus, there is resistance to Colistin, which is one of the last lines of antibiotic treatment.

MIC test is significantly more able to show resistance. More than half of the cases of Acinetobacter baumanii (68.9%) have been shown to be sensitive to Colistin in MIC.

Regarding Acinetobacter baumanii, all bacteria were susceptible to tigecycline, indicating a high effectiveness of this drug. Pseudomonas aeruginosa and Acinetobacter baumanii is a major pathogen in hospital infections. The emergence of multi-drug resistance in Pseudomonas aeruginosa and A. buomani is a major concern in the world, because several drugs, except poly-myxins, are available to treat these infections. Pseudomonas resistance to Carbapenem was about 35% in 2015, higher than the rate reported by Lee et al in 2009 (23%) (17). Additionally, resistance rate of acinetobacter against carbapenem gradually increased to 80% (81). In contrast, ampicillin-sulbactam resistance decreased to 46% in 2015. Accordingly, ampicillin sulbactam can be a therapeutic option for MRAB in combination with Colistin (18).

This study shows an increase in percentage resistance of these bacteria to antibiotics. This trend is a worrying process for antibiotic treatment. Moreover, this study suggest MIC for future studies to evaluate resistance and susceptibility of samples.

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