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Epidemiology of Colistin-Resistant Acinetobacter Baumannii in Shiraz, Iran

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ABSTRACT

Acinetobacter baumannii is a Gram-negative pathogen and often a cause of nosocomial infections such as bacteremia, pneumonia, meningitis, and urinary tract infections. A. baumannii is known as an emerging cause of nosocomial infections worldwide; it is categorized as one of the six highly hazardous microorganisms by the (Infectious) Diseases Society of America. Multidrug-resistant strains of A. baumannii have been reported in recent decades, which may be a result of the wide use of antimicrobial agents. Colistin is the last line treatment for multidrug-resistant A. baumannii, and unfortunately, its resistance to colistin is being reported from all around the world. This cross-sectional study was carried out during 12 months on 100 A. baumannii samples isolated from ulcers, urine, respiratory secretions, and blood of patients admitted to the intensive care unit of Shahid Rajai Hospital in Shiraz. The bacteria were recognized through microscopy and biochemical tests using a Microgen kit. The disk diffusion method with colistin disk (MAST, England) and E-test were utilized to identify the resistance to colistin. A total of 100 A. baumannii bacteria were identified, of which 94 were sensitive and 6 were resistant to colistin, respectively, according to the disk diffusion test. In resistant samples, the MIC number for E-test was 64 µg/mL. The results showed that the resistance of A. baumannii to colistin, as the last line treatment, is rising in Iran.

KEYWORDS: Acinetobacter baumannii; multidrug resistance; Colistin, Microgen kit; E-test

INTRODUCTION

Microorganisms responsible for nosocomial infections impose many problems in terms of treatment failure and mortality in patients, particularly due to antibiotic resistance. Among these bacteria, Acinetobacter baumannii is an important human pathogen which has been highly regarded in recent years [1].

A. baumannii is a non-motile, Gram-negative, non-fermentative, oxidase negative, catalase-positive, and obligate aerobic pathogen. It often causes nosocomial infections such as bacteremia, pneumonia, meningitis, and urinary tract infections. A. baumannii is known as an emerging cause of nosocomial infections worldwide; it is categorized as one of the six highly hazardous microorganisms by the (Infectious) Diseases Society of America [2]. The bacteria can survive for long times on skin, equipment, and various inanimate materials in different wards of hospitals, especially in burn wards and intensive care units; this paves the way for further dissemination of these organisms and hence infections in patients hospitalized in these wards. Multidrug resistance is of special importance in A. baumannii, as it is becoming resistant to all antibiotics including beta-lactams, aminoglycosides, tetracyclines, fluoroquinolones, and colistin [3].

Colistin is the mere active antibiotic and the last treatment for A. baumannii. Polymyxins are a class of cationic polypeptide antibiotics and are composed of a positively charged deca-peptide molecule which is bound to a fatty acid chain; either 6-methyl-octaenoic acid or 6-methyl-pentaenoic acid. The main difference between polymyxin B and E molecules is their amino acid content [4]. Cationic molecules of polymyxin B and colistin compete and exchange Ca^{2+} and Mg^{2+} ions, which typically stabilize the lipopolysaccharide molecules of the outer membrane of Gram-negative bacteria. The molecules are composed of a multi-cationic peptide ring containing ten amino acids and a fatty acid side chain. Both compounds have bactericidal properties; they target bacterial cell wall and disrupt the membrane permeability, and ultimately lead to cell death. Polymyxins exert their bactericidal activity through binding to the bacterial cell membrane and disrupting its permeability, resulting in leakage of intracellular components. Polymyxins show anti-endotoxin activity as well [5]. Colistin disassembles the outer membrane through interaction with lipid A unit of lipopolysaccharide (LPS) and quickly destroys Gram-negative bacteria [6]. Given the toxic effects of colistin on kidneys and nerve cells, and the advent of less toxic antibiotics, the clinical use of colistin was almost banned. Researchers re-evaluated the toxicity of colistin, and in comparison with previous reports, found evidence of low toxicity when using less intense colistin. Improved formulation of colistimethate sodium, avoiding of nephrotoxic and neurotoxic drugs

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at the same time, and determining the exact dose of colistin has ameliorated its use. Colistin has been used for the treatment of multidrug-resistant bacteria in recent years, and unfortunately resistance to colistin is being reported [4].

Asia and Europe show the most serious status in terms of resistance to colistin and have a higher number of reports and more resistant A. baumannii in comparison with North and South America.

MATERIALS AND METHODS

This study was carried out during 12 months in the intensive care unit of Shahid Rajai Hospital in Shiraz. Sampling was performed from ulcers, urinary tract, blood, and respiratory secretions of patients admitted to ICU. The bacteria were cultured in Eosin Methylene Blue, blood agar, and MacConkey's agar media. After inoculation, the samples were placed on the relevant culture medium. The cultured plates were then incubated at 37 °C for 18-24 hours. A. baumannii was recognized using the tests catalase, oxidase, TSI, and motility; other biochemical tests were performed subsequently by a Microgen kit.

ID-GN A Microgen Kit

In this method, a single colony from the culture medium is used to prepare a bacterial suspension in 3MI saline according to McFarland 0.5 turbidity standard. The plastic strip over the kit was removed and 2 or 3 drops of the suspension (100 µL) was added to the wells in each strip. Oil was dripped into the wells which were then covered by the plastic strip. The strips were then incubated at 37 °C for 24 hours, and the necessary reagents were added to the wells. The results were obtained based on comparison of color formed in the wells with the Color Guide, and to find the diagnosis code, they were inserted in a report form, and ultimately the 4-digit code was entered in Microgen specific software.

Antibiogram

All suspensions and plates were numbered, and antibiogram was performed on 100 purified samples through the following general procedure. To prepare a suspension of A. Baumannii with turbidity equal to that of half McFarland, a number of A. Baumannii colonies were dissolved in some sterile normal saline to produce a suspension with half McFarland turbidity; a swab was entered in the suspension and its extra solution was squeezed, the swab was then streaked slowly in different directions on Mueller Hinton agar medium so that its entire surface was covered uniformly with the bacteria; after the surface was dried (not to be more than a quarter), antibiotics disks (MAST Co., England) were placed on the medium at least 2 cm apart from each other using sterile forceps; the plates were then incubated at 35 °C for 18-24 hours. Finally, the zone of inhibition was measured with a ruler and matched with CLSI tables for different antibiotics.

E-test

To perform E-test, a suspension of A. baumannii was prepared equivalent to the half McFarland standard using sterile saline. A sterile swab was entered in the suspension and its extra solution was squeezed, the swab was then streaked slowly in different directions on Mueller Hinton agar medium so that its entire surface was covered uniformly with the bacteria. E-test strips were placed on the culture medium and the plates were incubated at 35 °C for 18-24 hours. The confluence of inhibition zone with the strips shows the MIC number of colistin for inhibition of A. baumannii.

RESULTS

All 100 isolates identified as A. baumannii in this study had the same biochemical pattern. A. baumannii produced pale pink mucoid colonies on blood agar (without hemolysis and pigment) and MacConkey's agar media. At the end of this study, 100 strains of A. baumannii were recovered in Shahid Rajai Hospital using microscopy and biochemical tests and by eliminating unrelated and repeated isolates. Most isolates were isolated from ulcers (Table 1).

Table 1: Distribution of A. baumannii isolates based on clinical samples

	Samples				
	Ulcer	**Respiratory**	**Blood**	**Urine**	**Others**
Number	60	20	5	10	5
Percentage	60	20	5	10	5

Regarding the resistance of A. baumannii to colistin in Shahid Rajai Hospital in Shiraz, 6 and 94 isolates were resistant and sensitive to colistin, respectively. The MIC number of colistin-resistant A. baumannii in E-test was more than 64 µg/mL (Table 2).

Table 2: The pattern of drug resistance in A. baumannii isolates in Shahid Rajai Hospital in Shiraz by E-test

Antibiotic	Breakpoint (µg/mL)	Resistant (%)	Medium (%)	Sensitive (%)	MIC (µg/mL)
Colistin	Sensitive ≤ 2	0	0	94	≤ 1
	Resistant ≥ 4	6	0	0	64 ≤

DISCUSSION

High resistance of Acinetobacter baumannii to different antibiotics and prevalence of multidrug-resistant strains (MDR), especially in immunosuppressed patients, makes difficult the control and treatment of the disease. Infections caused by this microorganism have a negative impact on clinical outcomes and treatment costs. As a nosocomial-inducing bacterium, A. baumannii creates many health problems in hospitals in Iran like the rest of the world; therefore, knowledge of its resistance to colistin is of great importance in the treatment of A. Baumannii-induced infections. In comparison with similar studies in other regions of Iran, the frequency of colistin-resistant isolates of A. Baumannii was high in this study. The difference in these findings may represent the increasing resistance of these strains to colistin, so that comparison of the studies dates confirms this increment over time. In this research, 6% of the isolates were resistant to colistin which was the greatest resistance rate compared with other studies.

Reports are increasing after the first report of colistin-resistant Acinetobacter spp. in 1999 in the Czech Republic [7]. Li et al. described colistin-resistant A. baumannii for the first time in 2006 [8]. The resistant of A. baumannii to colistin in clinical isolates is a serious danger, suggesting that if colistin is inappropriately used, a rapid increase in resistance and treatment failure are likely to occur. The antimicrobial surveillance department including the United States of America, Europe, Latin America, and Asia-Pacific region indicated that the resistance of A. baumannii to colistin remained at a low level from 2001 to 2011 (0.9%-3.3%) [9-11]. Other reports of colistin-resistant A. baumannii were obtained from Asia, Europe, North America, and South America. Ten reports from Europe, including 2 case reports, provided information about colistin-resistant A. Baumannii [12-13]. The rate was less than 7% in most of these reports; [14], however the rates of two reports from Bulgaria and Spain were 16.7% and 19.1%, respectively [15-16]. Interestingly, this rate was 40.7% in another report from Spain in which the cases were recruited from a three-care hospital between 2000 and November 2006. According to [15], the rate in 7 out of 8 reports from Asia was less than 12%. Ko et al. reported the highest rate of colistin resistance as 30.6% in Korea [17-20]. Out of 3 reports from the United State [21], 2 had a relatively low resistance not more than 1.2% [22-23]. The rate of resistance was not more than 1.7% in South America [24]. Asia and Europe have the most serious situation in terms of resistance to colistin, and have more reports and higher resistance rates, while North and South America had a lower rate of colistin-resistant A. baumannii and fewer reports in this regard.

Comparison of the results of this research with similar studies indicates increasing prevalence of nosocomial infections caused by colistin-resistant A. baumannii. According to the result obtained in this study and other regions of the world, the isolates of A. baumannii resistant to colistin are increasing in Iran, and infections caused by these microorganisms will adversely affect clinical outcomes and treatment costs. Spread of colistin-resistant A. baumannii in Iran is similar to Korea in Asia and shows that this colistin-resistant organism is increasing in Asia. Since no new drug is on the way to replace the existing drugs against Gram-negative pathogens, and no wide coverage vaccine exists against these infections, the only way to mitigate the spread of infection is to control the bacteria prevalence. This is only achievable by completely understanding the causes, dynamics, and complexity of the occurrence of these organisms.

Conclusion

This research isolated colistin-resistant A. baumannii strains from hospitals in Shiraz; this necessitates adopting appropriate treatment regimen and application of detailed strategies for prevention of nosocomial infections caused by these bacteria.

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