

Original Article

Colistin, an option for treatment of multiple drug resistant *Pseudomonas aeruginosa*

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Abstract

Introduction: Multi-drug resistant (MDR) *P. aeruginosa* is constantly increasing and causing severe issues in combatting widely spread health problems. The aim of this study was to assess colistin susceptibility in MDR *P. aeruginosa* isolates obtained from different infection sites.

Methods: Ninety clinical isolates of *P. aeruginosa* were collected from different hospitals of the Tabriz University of Medical Sciences. All isolates were identified using standard microbiology tests. The disk diffusion susceptibility testing was performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Antibiotic disks used in this study included ciprofloxacin, levofloxacin, ceftazidime, amikacin, gentamicin, cefepime, imipenem, meropenem, ticarcillin/clavulanic acid, piperacillin, aztreonam, and colistin. The MIC (Minimum Inhibitory Concentration) of colistin was determined by the agar dilution method according to CLSI guidelines.

Results: MDR isolates were found in 75.6%, in which there was a high frequency in wound specimens (23.3%), followed by blood (17.8%), urine (15.6%), trachea (13.3%), and peritoneum (5.6%). High resistance rate (above 50%) was observed with piperacillin, aztreonam, ceftazidime, cefepime, meropenem, gentamicin, amikacin, ciprofloxacin, and levofloxacin. All isolates were found to be susceptible to colistin through the disk diffusion method; however, two isolates were non-susceptible in the agar dilution method.

Conclusion: The present study shows a high frequency of MDR *P. aeruginosa* in our subjects, the limitations of empirical therapy, and the need for susceptibility testing. The most effective antibiotic against MDR *P. aeruginosa* was colistin. Therefore, colistin may be an alternative antimicrobial agent for infections due to MDR *P. aeruginosa*.

Keywords:

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Abbreviations:

CLSI: Clinical and Laboratory Standards Institute;
MIC: Minimum Inhibitory Concentration;
MDR: Multi-drug resistant;
P. aeruginosa:
Pseudomonas aeruginosa;
ATCC: American Type Culture Collection

Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is one of

the most common causes of nosocomial and healthcare-associated infections (Rossolini and Mantengoli, 2005). *P. aeruginosa* is one of the most important microorganisms responsible for infections;

such as lung infections, urinary tract infections, surgical site infections, and sepsis (Lee and Ko, 2014).

Various anti-pseudomonas antibiotic agents are utilized currently for the management of infections caused by this pathogen, including ticarcillin, carbenicillin, piperacillin, tazobactam, tobramycin, gentamicin, amikacin, ciprofloxacin, ceftazidime, imipenem, and aztreonam. However, resistance to these antibiotics is becoming more common and this emphasizes the increasing anxiety about the efficient treatment of infections caused by this microorganism (Song et al., 2003; Li et al., 2005; Rossolini and Mantengoli, 2005). *P. aeruginosa* has the capacity to expand resistance to several classes of antimicrobial agents, provoking the appearance of multi-drug resistant (MDR) isolates (Rossolini and Mantengoli, 2005). The increasing use of antibiotics, rising numbers of invasive actions and immunocompromised individuals in health care settings, with the development of intrinsic and acquired resistance to antimicrobial agents in the microorganism lead to frequent MDR *P. aeruginosa* (Shahcheraghi et al., 2010). Infections caused by multidrug-resistant isolates are associated with increased prices, duration of hospitalization, and, particularly, morbidity and mortality rates (Gales et al., 2012).

As no new agents have been established to manage these MDR organisms, and as it seems unlikely that any novel agents will be introduced soon, clinicians may become obliged to employ drugs used in the past (such as colistin) regardless of their toxicity. Indeed, there is a reappearance of attention in the use of colistin (Falagas et al., 2005; Lee and Ko, 2014).

Colistin, also known as polymyxin E, is an old and cationic polypeptide antibiotic with considerable *in vitro* effects against *P. aeruginosa*, for which it is currently the only accessible active antibiotic (Michalopoulos and Karatza, 2010). It is bactericidal to Gram-negative bacteria, and these organisms can expand resistance to this agent via adaptation mechanisms or mutation (Falagas et al., 2005). Cross-resistance with other antimicrobial agents has not been described, with greater than ever utilization of colistin, colistin-resistant *P. aeruginosa* strains have been emerging around the world (Bialvaei and Samadi Kafil, 2015).

In this regard, the aim of this study was to evaluate the antimicrobial activity of colistin against MDR *P. aeruginosa* that is resistant to traditional antibiotics.

Materials and methods

Bacterial strains

Ninety *P. aeruginosa* isolates were collected from different hospitals of Tabriz, Iran. The sources of specimen used were blood, urine, trachea, peritoneum, and wounds. Gram staining and standard biochemical tests were performed for identification of isolates in the Microbiology Department of Tabriz University of Medical Sciences during 2014-2015.

Antimicrobial susceptibility testing

The disk diffusion susceptibility testing was performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Antibiotic disks (MAST, England) used in this study, included ciprofloxacin (5µg), levofloxacin (5µg), ceftazidime (30µg), amikacin (30µg), gentamicin (10µg), cefepime (30µg), imipenem (10µg), meropenem (10µg), piperacillin (100µg), ticarcillin/clavulanic acid (75/10µg), aztreonam (30µg) and colistin (10µg). The results of susceptibility testing were validated using the American Type Culture Collection quality control strain *P. aeruginosa* ATCC 27853 (Wayne, 2011). In this study, MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories (Magiorakos et al., 2012).

Determination of colistin MIC

The agar dilution method was used to determine the colistin Minimum Inhibitory Concentration (MIC) according to the CLSI guidelines. The colistin concentrations used ranged from 0.25 to 256 µg/ml. Mueller-Hinton agar (Merck, German) plates containing *P. aeruginosa* without antimicrobial agent were used as positive control of bacterial growth. The MIC was defined as the lowest concentration that inhibited the evident growth of the bacteria (Wayne, 2011).

Statistical method

The results were analyzed using descriptive statistics in SPSS software for Windows (version 19 SPSS Inc., Chicago, IL, USA).

Table1: Antibiotic susceptibility patterns of *P. aeruginosa* isolates obtained from different specimens and frequency of MDR isolates by disk diffusion test

Sources n(%)	Trachea	Blood	Urine	Peritoneum	Wound	Total
	17 (18.9)	22 (24.4)	16 (17.8)	7 (7.8)	28 (31.1)	90 (100)
Antibiotics						
PIP	9 (10)	14 (15.6)	11 (12.2)	4 (4.4)	15 (16.7)	53 (58.9)
TIC	7 (7.8)	11 (12.2)	10 (11.1)	3 (3.3)	13 (14.4)	44 (48.9)
AZT	13 (14.4)	13 (14.4)	11 (12.2)	4 (4.4)	17 (18.9)	58 (64.4)
CAZ	13 (14.4)	11 (12.2)	11 (12.2)	4 (4.4)	21 (23.3)	60 (66.7)
FEP	13 (14.4)	14 (15.6)	11 (12.2)	3 (3.3)	16 (17.8)	57 (63.3)
IMI	9 (10)	9 (10)	5 (5.6)	1 (1.1)	14 (15.6)	38 (42.2)
MEM	12 (13.3)	13 (14.4)	9 (10)	4 (4.4)	16 (17.8)	54 (60)
GM	13 (14.4)	12 (13.3)	10 (11.1)	3 (3.3)	16 (17.8)	54 (60)
AMK	7 (7.8)	11 (12.2)	12 (13.3)	5 (5.6)	13 (14.4)	48 (53.3)
CIP	9 (10)	16 (17.8)	10 (11.1)	5 (5.6)	19 (21.1)	59 (65.6)
LVX	14 (15.6)	14 (15.6)	10 (11.1)	4 (4.4)	17 (18.9)	59 (65.6)
CL	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
MDR	12 (13.3)	16 (17.8)	14 (15.6)	5 (5.6)	21 (23.3)	68 (75.6)

PIP=piperacillin, TIC= ticarcillin/clavulanic acid AZT=aztreonam, CAZ=ceftazidime, FEP=cefepime, IMI=Imipenem, MEM=meropenem, GM=gentamicin, AMK=Amikacin, CIP=ciprofloxacin, LVX=levofloxacin, CL=colistin, MDR=multi-drug resistant

Results

We evaluated 90 *P. aeruginosa* isolates which were obtained from different clinical specimens. Table 1 shows antibiotic susceptibility patterns of *P. aeruginosa* isolates obtained from different specimens and frequency of MDR isolates by disk diffusion test. The highest rate of resistance was in ceftazidime with 66.7%. In contrast, the highest sensitivity rates were discovered in colistin and the carbapenem group (imipenem).

All isolates were colistin sensitive by the disk diffusion test but 2 (2.2%) isolates were non-susceptible in the agar dilution method. The range of colistin MIC was from 0.5 to 64 µg/mL. Figure 1 shows the distribution of colistin MIC in bacterial isolates.

In the present study, MDR isolates were observed in 75.6% of all isolates with high frequency in wound specimens (23.3%) followed by blood (17.8%), urine (15.6%), trachea (13.3%), and peritoneum specimens (5.6%). Among MDR isolates, the highest prevalence of resistance to antibiotic was detected in aztreonam (60%) and followed by cefepime (58.9%), levofloxacin (57.8%) and the lowest resistance was observed in

colistin (2.2%) and followed by imipenem (35.6%). In this study, 12%, 22.2 % and 41.1% of MDR isolates were resistant to 3, 4 and 5 antibiotics, respectively.

Discussion

Despite the advances in hospital care and the introduction of a wide variety of antimicrobial agents, *P.aeruginosa* continues to be a common cause of nosocomial infections. This bacterium is one of the most important microorganisms, which cause various clinical problems as a result of high resistance to antimicrobial agents (Vitkauskiene et al., 2010).

In this study, similar to the other studies carried out by Akingbade et al, (2012) and Maita and Boonbumrung, (2014), high-level resistance was observed with monobactam, cephalosporins, and fluoroquinolones. The introduction of carbapenems into clinical practice represents a great advance for the treatment of serious bacterial infections caused by β-lactam-resistant isolates (Yousefi et al., 2010). The findings of this research indicate that 42.2% of *P. aeruginosa* isolates were resistant to imipenem. Burjanadze et al, (2007) and Saderi and Owlia, (2015) reported 68.5% and 34.1% imipenem resistant

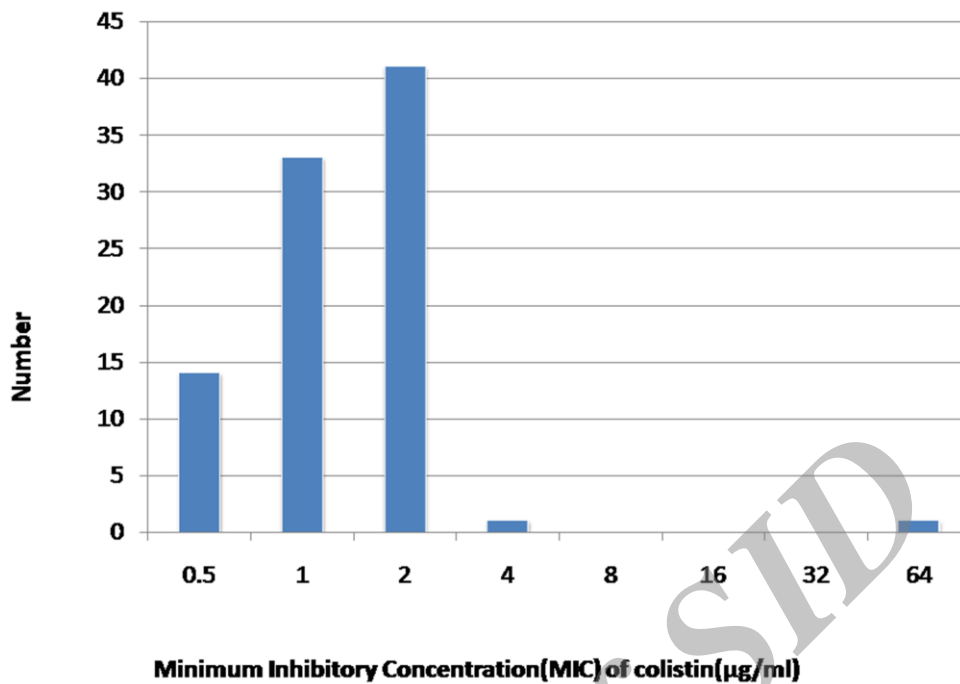


Fig.1. Distribution of colistin MIC (minimum inhibitory concentration) in bacterial isolates

P. aeruginosa, respectively. Carbapenems resistance in *P. aeruginosa* has been developed mainly due to the production of metallo-beta-lactamases. The genes of metallo-beta-lactamases are located in integrons, and some of these genes are horizontally transferable by conjugative plasmids, thus may be lead to MDR *P. aeruginosa* (Yousefi et al., 2010).

MDR Gram-negative bacteria have emerged as the most important threat to hospitalized patients and have been related with mortality rates ranging from 30 to 70%. In this study, 75.6% of tested isolates were identified as MDR and non-susceptible to at least one agent in three or more antimicrobial categories. Yoon et al, (2010) from Korea, reported that 56% of MDR isolates based this definition. High prevalence of MDR was reported in the studies defined MDR as resistant to ≥ 3 classes of antibiotics; 100% by Moazami-Goudarzi and Eftekhari (2013) and 60% by Bayani et al., (2013) from Iran. In other countries, low prevalence of MDR was usually reported; Morales et al, (2012) 33.3% from Spain and De Francesco et al., (2013) 20% from Italy. Geographic diversity in antimicrobial susceptibility was described in other studies (Tacconelli et al., 2002). Patient's demographics, access to therapeutic care, and illicit drug usage are some of the factors

explaining differences in antibiotic susceptibility patterns (Saderi and Owlia, 2015). Many factors may play a role in the acquisition of MDR *P.aeruginosa* such as urinary catheterization, nasogastric feeding, an imbalance in gut flora, previous hospitalization, a severity of illness, surgery, immunosuppression and antibiotic exposure with carbapenems and quinolones (Cao et al., 2004; Nouér et al., 2005; Montero et al., 2010). An emergence of bacterial strains resistant to aztreonam, ceftizoxime, levofloxacin and cefepime, unfortunately, decreases the effectiveness of these drugs for empirical therapy. The range of obtainable therapeutic choices has become severely narrowed in recent years, predominantly for hospital MDR *P. aeruginosa* strains.

The findings of this study are similar to a study, carried out by Akhi et al (2015) that indicated all *P. aeruginosa* isolates were susceptible to colistin in the disk diffusion method. When the administration of a β -lactam, aminoglycoside, or quinolone is unsuccessful, the polymyxins, particularly colistin, remain final drug option for MDR *P. aeruginosa* infections. There are some investigational and clinical studies in the literature on the subject of synergistic effects of colistin with other antimicrobial agents against MDR Gram-negative organisms (Zavascki et al., 2007). These studies showed that the

combination of colistin with an anti-pseudomonas agent such as imipenem, piperacillin, aztreonam, ceftazidime, azlocillin, rifampin or ciprofloxacin was more efficient than only colistin against MDR *P. aeruginosa* (Gunderson et al., 2003; Zavascki et al., 2007; D'Souza et al., 2014).

Also, resistance to colistin is infrequently observed despite a daily selective pressure in patients receiving colistin by inhalation (Zavascki et al., 2007). However, increasing administration of colistin for antibiotic therapy of infections by MDR organisms may lead to the emergence of colistin-resistant strains in some countries (Bialvaei and Samadi Kafil, 2015). The worldwide prevalence of *P. aeruginosa* resistance to colistin is low and may be different between regions and over time. In the previous studies, colistin-resistant *P. aeruginosa* from Iran (Saderi and Owlia, 2015), Singapore (Tan and Ng, 2006a), Thailand (Tunyapanit et al., 2013), the USA, and South America (Keen et al., 2010) were 9.1%, 30%, 2%, 5.5%, and 9%, respectively and suggested colistin as appropriate option for treatment of MDR *P. aeruginosa* (Keen et al., 2010). However, the resistance rate to colistin is usually less than 10% and is higher in South East Asia (Keen et al., 2010; Bialvaei and Samadi Kafil, 2015) and African countries (Igumbor et al., 2000). These variations may be due to differences in methodology. Some laboratories apply only the disk diffusion method, and polymyxins diffuse weak due to high molecular weight. Thus, one could underestimate resistance when using the disk diffusion assay (Bialvaei and Samadi Kafil, 2015). In addition, CLSI does not have a breaking point for determination of intermediate resistance to colistin in the disk diffusion method (Wayne, 2011). In our study, two isolates were susceptible to colistin in the disk diffusion method while these isolates were determined as non-susceptible by the agar dilution method. In agreement with our results, Tunyapanit et al (2013) detected one resistant isolate by disk diffusion while 2 isolates were non-susceptible by E-Test. Disk susceptibility method described by Tan and Ng (2006b) is unreliable at identifying colistin-resistant isolates. These researchers have suggested dilution methods as the method of choice for susceptibility testing of colistin. Other researchers have also described the agar dilution method as an appropriate method to detect heteroresistant isolates (Lo-Ten-Foe et al.,

2007).

In the present study, susceptibility to fosfomycin and tigecycline was not evaluated for determination of pan-drug resistance and extensive drug resistance. We also suggest detection of colistin resistance mechanisms and molecular typing of resistant isolates may help to identify the origin and design program to control the spread of resistant strains.

Conclusion

Our findings show a high frequency of MDR *P. aeruginosa* in health centers, which requires appropriate programs to prevent the spread of these strains. The most effective antibiotic was colistin; therefore, this drug should be the last resort for the treatment of infections caused by MDR *P. aeruginosa*. We suggest the dilution method for the detection of colistin-resistant isolates, due to reliability, over the disk diffusion method. Furthermore, performing MIC susceptibility testing before administration of colistin in the treatment of MDR *P.aeruginosa* infections is also recommended.

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Conflict of interest

The authors have reported no conflict of interest.

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