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Articoli originali

Original articles

Evaluation of the *in vitro* colistin susceptibility of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* strains at a tertiary care centre in Western Turkey

Valutazione della sensibilità in vitro a colistina in Pseudomonas aeruginosa *e* Acinetobacter baumannii *isolati presso un ospedale della Turchia occidentale*

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INTRODUCTION

Multidrug-resistant microorganisms are defined as microorganisms, predominantly bacteria, that are resistant to one or more classes of antimicrobial agents [1]. Multidrug resistance, particularly among gram-negative bacteria, has become an important problem in intensive care units worldwide. Overuse of antibiotics, insufficient compliance with infection control programs, and the ease by which drug resistance can spread through bacterial populations are the important factors that contribute to the increase in drug resistance [2].

Polymyxins were first isolated from several species of *Bacillus polymyxa* in 1947 [3]. Colistin (Polymyxin E), was one of the earliest polymyxin antibiotics, widely used for the treatment of gram-negative bacterial infections; however, concerns regarding nephrotoxicity, and the development of less toxic antibiotics, led to its withdrawal from general use [4]. The appearance of multidrug-resistant strains of *A. baumannii* and *P. aeruginosa* has once again led to use of polymyxins [5]. Colistin, like other polymyxins, exhibits rapid bactericidal activity. Colistin directly targets the bacterial membrane by means of electrostatic interactions between the drug and anionic lipopolysaccharide molecules in the outer membrane of gram-negative bacteria. The cationic detergent-like properties of the drug lead to rupture of the cell membrane, resulting in cell death. Resistance to this class of drugs has been shown to occur through both direct mutations as well as adaptation mechanisms [6].

Clinical responses in nosocomial pneumonia caused by multidrug-resistant *P. aeruginosa* and *A. baumannii* isolates vary between 25 and 73.3%, and between 66 and 88% for bloodstream infections. Colistin is more effective in bloodstream infections [7]. Antibiotic resistance has been shown to vary by location, with differences seen even between departments in the same hospital; the resistance profile of multidrug-resistant strains therefore requires enhanced monitoring, especially for empiric treatment. Obtaining regional resistance data is important for establishing guidelines for appropriate antibiotic use, and may help control the rate of antibiotic resistance. In this respect, we aimed to evaluate the susceptibility of multidrug-resistant *P. aeruginosa* and *A. baumannii* isolates both to colistin and other antibiotics at

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MATERIALS AND METHODS

A total of 149 multidrug-resistant *A. baumannii* and *P. aeruginosa* isolates were collected between January 2011 and October 2012 at Tepecik Education and Research Hospital, Izmir, Turkey. Strains were isolated from wound, blood, urine, CSF, and respiratory specimens. Specimens from the Anesthesiology and Reanimation Intensive Care Unit (ICU), Internal Medicine ICU, Pediatric ICU, Neurology ICU, and Organ Transplantation Units were included in this study. Multidrug-resistant bacteria were classified as bacteria exhibiting resistance to one or more classes of antimicrobial agents. Each of the 149 isolates included in this study represents a single strain isolated from a single anatomical site, collected from 149 different patients. Species identification was performed using an automatized Vitek 2.0 (BioMérieux, France) system. Colistin susceptibility was measured by E-test (AB, Sonla, Sweden); the susceptibility profiles of other antibiotics were evaluated using the Kirby-Bauer disk-diffusion method. Minimum inhibitory concentrations (MICs) were determined according to the CLSI M100-S22 criteria. Cutoff values for colistin MICs against *P. aeruginosa* were as follows: ≤2.0 susceptible (S), 4.0 intermediate (I), and ≥8.0 resistant [7]. For *A. baumannii* isolates MIC cutoffs were ≤2.0 for susceptible (S) and ≥4.0 for resistant [8]. Susceptibility to other antimicrobial agents was evaluated using the Kirby-Bauer disk-diffusion method. *Escherichia coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) strains were used as controls.

RESULTS

A total of 149 strains (98 *A. baumannii* and 51 *P. aeruginosa*) were isolated from wounds, urine, blood, CSF, and respiratory specimens. All patients had been admitted to the hospital at the time of sample collection. Ninety-six (64.4%) patients were male and 53 (35.6%) were female. The average age of the patients was 51 years, ranging from 0-85 years.

The susceptibilities to colistin and other antimicrobials of the isolates are shown in Table 1. The

Table 1 - Susceptibilities of A. baumannii and P. aeruginosa strains to colistin and other antibiotics.

Isolate	*Antibiotic susceptibility*										
	CAZ	*FEP*	*TZP*	*SCF*	*AK*	*GN*	*IMP*	*MEM*	*CIP*	*TG*	*COL*
A. baumannii n = 98	1.1%	0.0%	0.0%	11.2%	8.2%	6.1%	0.0%	0.0%	0.0%	55.1%	100%
	CAZ	*FEP*	*TZP*	*SCF*	*AK*	*GN*	*IMP*	*MEM*	*CIP*	*COL*	
P. aeruginosa n = 51	23.5%	5.8%	6.0%	29.4%	11.7%	5.8%	17.6%	11.7%	0.0%	%100	

Abbreviations: CAZ, Ceftazidime; FEP, Cefepime; TZP, Tazobactam/Piperacillin; SCF, Sulbactam/Cefoperazone; AK, Amikacin; GN, Gentamicin; IMP, Imipenem; MEM, Meropenem; CIP, Ciprofloxacin; TG, Tigecycline; COL, Colistin.

Table 2 - Anatomical distribution of clinical isolates.

	Isolates	
Sample	*Number*	*%*
Blood	16	10.7
Urine	18	12.1
Wound	31	20.8
Respiratory specimen	83	55.7
Cerebrospinal fluid	1	0.6
Total	149	100

Table 3 - Anatomical distribution of strains according to species.

	A. baumannii	*P. aeruginosa*
Wound	10	21
Respiratory Specimen	67	16
Urine	7	11
Blood	13	3
CSF	1	0
Total	98	51

Table 4 - Clinical departments where strains were isolated.

Department	*N.*	*%*
Anesthesiology Intensive Care Unit	69	46.3%
Pediatric Intensive Care Unit	17	11.4%
Internal Medicine Intensive Care Unit	33	22.1%
Neurological Intensive Care Unit	29	19.4%
Organ Transplant	1	0.6%
Total	149	100

Table 5 - Colistin MIC50 and MIC90 values (µg/mL).

	MIC_{50}	MIC_{90}
A. baumannii	0.5	1.0
P. aeruginosa	0.75	2.0

anatomical distribution of clinical isolates is shown in Table 2; the distribution of these isolates is broken down by species in Table 3. The departments from which these strains were isolated are presented in Table 4. The MICs of colistin against *A. baumannii* were 0.125-2.0 µg/mL, and 0.25-2.0 µg/mL against *P. aeruginosa*; all multidrug-resistant strains examined were susceptible to colistin. The colistin MIC_{50} and MIC_{90} values of *A. baumannii* and *P. aeruginosa* strains are shown in Table 5.

DISCUSSION

P. aeruginosa and *A. baumannii* are important clinical pathogens, especially among patients in ICUs. Outside of ICUs, the widespread increase in the number of immunocompromised individuals, whether due to old age, chemotherapy, or organ transplantation, has led to a dramatic increase in the number of opportunistic infections. Multidrug resistance, which is common in species such as *P. aeruginosa* and *A. baumannii*, can often lead to treatment failure, resulting in potentially life-threatening complications and increased medical costs [9]. Identification of alternative treatments effective against multidrug-resistant bacteria is therefore necessary.
Colistin (polymyxin E) was one of the first commercially available antibiotics. While toxicity concerns have limited its usage, its potent activity against multidrug-resistant strains of *P. aeruginosa*, *A. baumannii*, and *K. pneumoniae in vitro* suggests that it may be effective for drug-resistant infections. [10]
The standard disc-diffusion method is unreliable for detecting colistin resistance due to the limited diffusion of colistin through culture media. Colistin MIC testing was therefore performed using the E-test method, which has been shown to be reliable for determining colistin susceptibility [11].
The E-test method, along with other methods such as agar dilution or the VITEK 2 system, has been shown to exhibit a high degree of concordance with the broth microdilution reference method [12]. Direct examination of colistin and other polymyxin antibiotics has confirmed these results, with the concordance between E-test and the broth microdilution methods being 100% for colistin and 90% for polymyxin B [13]. In our study, all strains were susceptible to colistin, suggesting that this drug may be a viable option for treating infections caused by multidrug-resistant pathogens.
A number of studies have investigated the colistin susceptibility of multidrug-resistant bacteria. Walkty et al., who investigated the colistin susceptibility of 3480 Gram-negative strains using the CLSI microdilution method, performed the largest study [14]. All isolates examined (*E. coli*, *Klebsiella* spp., *Enterobacter* spp., *A. baumannii* and *P. aeruginosa*) were susceptible to colistin, including 76 multidrug-resistant *P. aeruginosa* strains. Similar results were reported by Sinirtas et al., who evaluated colistin susceptibility in 100 *A. baumannii* strains; all were susceptible to colistin [15]. However, cross-resistance to colistin has been reported. While Somily et al. were able to report 100% colistin susceptibility among *Acinetobacter* species, resistance was identified in 2% of *A. baumannii*, 16% of *P. aeruginosa*, and 21% of *S. maltophila* isolates [16]. Similarly, Lee et al., reported colistin resistance in 7.4% of *P. aeruginosa* strains tested [17].
In our study we evaluated the susceptibility of multidrug-resistant *A. baumannii* and *P. aeruginosa* strains to colistin using the E-test method. *A. baumannii* colistin MIC values were 0.125-2.0 µg/mL, and *P. aeruginosa* MICs were 0.25-2.0 µg/mL, indicating 100% susceptibility, consistent with the reports of Walkty et al. and Sinirtas et al. [14, 15]. Antibiotic resistance has been shown to vary by location, with differences

seen even between departments in the same hospital. Obtaining regional resistance data is important for establishing guidelines for appropriate antibiotic use, which may help to control the rate of antibiotic resistance.
Several other studies from Turkey have investigated colistin susceptibility in multidrug-resistant Gram-negative bacteria; all isolates tested to date were colistin-sensitive. These observations are consistent with previous data from our laboratory regarding drug susceptibilities of multidrug-resistant isolates [18-20]. As our hospital is a regional center with multiple ICUs, treating a large number of organ transplant patients and other immunocompromised populations, multidrug-resistant *A. baumannii* and *P. aeruginosa* strains are isolated frequently. Colistin remains the last choice for treatment of multidrug-resistant strains; however, susceptibility testing should continue to be routinely carried out to guard against treatment failure. The evidence presented here indicates that colistin resistance is not currently a problem in our unit, suggesting that a rational antibiotic use policy should be followed strictly.

Keywords: *P. aeruginosa*, *A. baumannii*, colistin, antibiotic resistance, polymyxin, E-test.

SUMMARY

Multidrug-resistant gram-negative bacteria are an important issue in intensive care units worldwide. Colistin, one of the earliest polymyxin antibiotics, was once widely used for the treatment of gram-negative bacterial infections. However, its use is now limited due to concerns over nephrotoxicity. The appearance of multidrug-resistant species, including *A. baumannii* and *P. aeruginosa*, has led to the re-emergence of this class of drugs. The aim of this study was to evaluate the susceptibility of *A. baumannii* and *P. aeruginosa* isolates to colistin and other antibiotics. The antimicrobial susceptibility of *A. baumannii* and *P. aeruginosa* isolates to colistin and other antibiotics was evaluated between January 2011 and October 2012 at Tepecik Education and Research Hospital in Izmir, Turkey. Clinical isolates were identified using an automatized Vitek 2.0 system. Colistin susceptibility was measured by E-test; the susceptibility profiles of other antibiotics were evaluated using the Kirby–Bauer disk-diffusion method. A total of 149 isolates were included in the study, consisting of 98 *A. baumannii* and 51 *P. aeruginosa* isolates. The MICs of colistin against *A. baumannii* were 0.125-2.0 µg/mL, and 0.25-2.0 µg/mL against *P. aeruginosa*; all multidrug-resistant strains examined in this study were susceptible to colistin. Recently, colistin has re-emerged as an effective treatment for infections due to multidrug-resistant *A. baumannii*, *P. aeruginosa*, and *Klebsiella pneumoniae.* All isolates examined in this study were susceptible to colistin, suggesting it could be a viable alternative for the treatment of infections with multidrug-resistant strains.

RIASSUNTO

*I batteri gram negativi resistenti a molteplici classi di antibiotici (*MDR, multidrug resistant*) rappresentano un importante problema nelle Unità di Terapia Intensiva. Colistina, uno dei primi esponenti della classe delle polimixine, è stato largamente utilizzato nel trattamento delle infezioni batteriche da gram negativi ma problemi di nefrotossicità ne limitano l'uso. L'insorgenza di microrganismi MDR, quali ad esempio* Acinetobacter baumannii *e* Pseudomonas aeruginosa, *ha rilanciato l'utilizzo di questa classe di farmaci. Questo studio è stato condotto nell'intento di valutare la sensibilità di tali microrganismi,* A. baumannii *e* P. aeruginosa, *verso colistina e altri antibiotici, nel periodo compreso tra gennaio 2011 e ottobre 2012 presso il Tepecik Education and Research Hospital di Izmir, Turchia. L'identificazione degli isolati clinici è stata effettuata con il sistema automatico Vitek 2.0. La sensibilità a colistina è stata determinata con l'E-test mentre i profili di sensibilità verso gli altri antibiotici con il metodo di diffusione di Kirby-Bauer. Nello studio sono stati considerati 149 isolati, di cui 98* A. baumannii *e 51* P. aeruginosa. *I valori di MIC di colistina sono risultati compresi tra 0,125 e 2,0 µg/mL per* A. baumannii, *e tra 0,25 e 2,0 µg/mL per* P. aeruginosa. *Tutti gli isolati MDR esaminati in questo studio sono risultati sensibili a colistina. Recentemente, colistina si è riproposta quale opzione terapeutica efficace per il trattamento di infezioni sostenute da isolati MDR di* A. baumannii, P. aeruginosa e Klebsiella pneumoniae. *I risultati osservati nel nostro studio suggeriscono che colistina rappresenta una valida alternativa per il trattamento delle infezioni sostenute da microrganismi MDR.*

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