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In vitro activity of colistin against nonfermentative gram-negative bacilli

Kolistinin non-fermentatif gram-negatif bakterilere in-vitro etkinliğinin değerlendirilmesi

Özlem Kurt Azap, Hande Arslan, Funda Ergin, Emine Kuru İnci, Göknur Yapar

Baskent University Medical Faculty, Department of Clinical Microbiology and Infectious Disease

Aim: Multidrug-resistant gram-negative isolates have become a problem in everyday clinical practice in intensive care units. Colistin was once used for treatment of gram-negative bacteria, but its systemic use has been completely abandoned due to nephrotoxicity. It was aimed to assess the in-vitro susceptibility of several gram-negative isolates to determine a standardized breakpoint for colistin.
Methods: One hundred thirty-two multidrug-resistant nonfermentative gram-negative isolates were tested. Minimum inhibitory concentrations were determined using an agar dilution technique.
Results: Fifty-two of 55 *P. aeruginosa* isolates (94.1%), 46 of 48 *A. baumannii* isolates (95.8%), 15 of 21 *S. maltophilia* isolates (71.4%), and 1 of 8 *B. cepacia* isolates (8.4%) were susceptible to colistin at a breakpoint of ≤ 4 mg/L. The number of susceptible strains dropped at a susceptibility breakpoint of ≤ 2 mg/L: 33 of 55 *P. aeruginosa* isolates (60%), and 41 of 48 *A. baumannii* isolates (85.4%) were susceptible; none of the *S. maltophilia* or *B. cepacia* isolates was susceptible at this breakpoint.
Conclusion: Colistin maintains effective in vitro activity against *P. aeruginosa* and *A. baumannii* when used at a breakpoint of ≤ 4 mg/L. Further clinical trials are needed to determine which susceptibility criterion is more suitable for clinical practice (≤ 4 mg/L or ≤ 2 mg/L), and to determine a standard breakpoint for all gram-negative bacteria.

Key words: ***Colistin, Susceptibility Breakpoint, Nonfermentative Bacteria***

Giriş: Çoklu ilaç direnci olan gram-negatif bakterilerdeki direnç problemi, yoğun bakım ünitelerinde hemen her gün karşılaşılan önemli bir sorundur. Kolistin, gram-negatif bakterilerin tedavisinde kullanılmış ancak nefrotoksisite nedeniyle kullanımdan kaldırılmıştır. Gram-negatif basillerde kolistin duyarlılığı saptanarak, kolistin duyarlılığı için bir sınır değer belirlenmesi amaçlandı.
Gereç ve yöntem: Yüz otuz iki çoklu ilaç direnci olan gram negatif bakteri test edildi. Minimum inhibitör konsantrasyon değerleri agar dilüsyon yöntemi ile belirlendi.
Bulgular: Duyarlılık sınırı ≤ 4 mg/L olarak kabul edildiğinde 55 *P. aeruginosa* suşunun 52'si (%94.1), 48 *A. baumanii* suşunun 46'sı (%95.8), 21 *S. maltophilia* suşunun 15'i (%71.4) ve 8 *B. cepacia* suşunun 1'i (%8.4) kolisitine duyarlı bulundu. Duyarlılık sınırı ≤ 2 mg/L olarak alındığında ise 55 *P. aeruginosa* suşunun 33'ü (%60), 48 *A. baumanii* suşunun 41'i (%85.4) kolistine duyarlı bulunurken, *S. maltophilia* ve *B. cepacia* suşlarında duyarlılık saptanmadı.
Sonuç: Duyarlılık sınırı ≤ 4 mg/L olarak kabul edildiğinde kolistin in vitro koşullarda *P. aeruginosa* ve *A. baumanii* suşlarına etkilidir. Kolistinin gram-negatif bakteriler için duyarlılık sınır değerinin belirlenebilmesi, daha çok sayıda klinik çalışma ile sağlanabilecektir.

Anahtar kelimeler: ***Kolistin, Duyarlılık sınırı, Nonfermentatif bakteri***

Introduction

Multidrug resistance of gram-negative nosocomial isolates has become a problem in everyday clinical practice in the intensive care units (1). Because no fundamentally new anti-infective drugs are currently available, it appears that older drugs need to be evaluated (2). Colistin, a polymyxin (polymyxin E), was used from the 1960s to the early 1980s; however, owing to toxicity considerations (mainly nephrotoxicity, neuromuscular blockage, and neurotoxicity), its systemic use has been completely abandoned (3). As with other polymyxins, co-

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Corresponding Author
Özlem Kurt Azap
Baskent University Medical Faculty, Department of Clinical Microbiology and Infectious Disease, Ankara
E-mail : okurtazap@baskent-ank.edu.tr
Tel : (312) 212 29 12/149
Faks : (312) 2153723

Table 1. Minimum inhibitory concentrations (MICs) (mg/L) of colistin against the nonfermentative gram-negative bacteria tested

	MIC Range	*MIC_{50}*	*MIC_{90}*	*Susceptibility % (n)**	*Susceptibility % (n)***
P. aeruginosa (55)	2 - ≥ 32	2	4	94.5 (52)	60 (33)
A. baumannii (48)	≤ 0.5 - ≥ 32	2	4	95.8 (46)	85.4 (41)
S. maltophilia (21)	4 - ≥ 32	4	≥ 32	71.4 (15)	0 (0)
B. cepacia (8)	4 - ≥ 32	≥ 32	≥ 32	12.5 (1)	0 (0)

***** According to the susceptibility breakpoint of ≤ 4 mg/L based on BSAC criteria (4).
****** According to the susceptibility breakpoint of ≤ 2 mg/L (7).
n: number of susceptible strains

listin is rapidly bactericidal and exerts its effects by acting as a cationic detergent, causing disruption of the integrity of the bacterial cell membrane, with leakage of intracellular contents and cell death. This process is not dependent on bacterial metabolic activity and may be a significant contributing factor to the lack of development of bacterial resistance to colistin (4). Resistance to beta-lactams, quinolones, and aminoglycosides in nonfermentative bacteria continues to increase. As no novel agents have been introduced to combat these multiple-antibiotic-resistant organisms, clinicians may become forced to rediscover colistin (5).

This study sought to evaluate the in vitro activity of colistin against non-fermentative gram-negative bacilli that are difficult-to-treat nosocomial pathogens.

Materials and Methods

One hundred thirty-two multidrug-resistant nonfermentative gram-negative isolates (55 *Pseudomonas aeruginosa*, 48 *Acinetobacter baumannii,* 21 *Stenotrophomonas maltophilia*, and 8 *Burkholderia cepacia*) were tested. Forty-seven of the strains were isolated from wound infections, 39 of them were isolated from urine, 22 of them were isolated from blood cultures, 13 were isolated from body fluids, and 11 were isolated from catheters.

Colistin was obtained from Sigma Chemicals (Sigma-Aldrich Corp, St. Louis, Mo, USA). Isolates were considered to be multidrug-resistant if they are resistant to two or more classes of the following anti-pseudomonal broad-spectrum agents: cefepime, amikacin, ciprofloxacin, piperacillin-tazobactam, and/or meropenem, by using the Kirby-Bauer method of disk diffusion. The susceptibilities of the isolates were studied before during the routine microbiological procedures. Multidrug resistance was confirmed by E-test® (AB Biodisk, Solna, Sweden) for the agents.

Susceptibility testing

Minimum inhibitory concentrations (MICs) were determined using an agar dilution technique according to NCCLS criteria (6). After incubation for 18-24 hours at 35-37°C, the plates were examined for growth. The MIC of the antibiotic was defined as the concentration at which growth was inhibited. MIC values were evaluated for both of the susceptibility breakpoints: ≤2mg/L and ≤4mg/L (4,7).

The quality-control strain used was *Pseudomonas aeruginosa* ATCC 27853, and it was assessed at the same time as the test strains.

Results

The antimicrobial activity of colistin against the multidrug-resistant gram-negative nosocomial isolates is summarized in Table 1. Fifty-two of 55 *P. aeruginosa* isolates (94.1%), and 46 of 48 *A. baumannii* isolates (95.8%) were susceptible to colistin. Fifteen of 21 *S. maltophilia* isolates (71.4%) were susceptible, although the MIC90 is ≥32 mg/L at the breakpoint of ≤4 mg/L. The number of susceptible strains dropped when ≤2 mg/L was accepted as the susceptibility breakpoint: 33 of 55 *P. aeruginosa* isolates (60%) and 41 of 48 *A. baumannii* isolates (85.4%) were susceptible; none of the *S. maltophilia* or *B. cepacia* isolates was susceptible at this breakpoint. Only one (8.4%) of the 8 *B.cepacia* strains were found to be susceptible at the breakpoint of 4mg/L.

Discussion

The emergence of multidrug-resistant nonfermentative gram-negative bacteria causing nosocomial infections possesses a very serious therapeutic problem. Colistin is a cationic polypeptide antibiotic of the polymyxin type that is rapidly bactericidal against gram-negative bacteria (8). Physicians hesitate to use colistin owing to its increased risk of toxicity (mainly nephrotoxicity) and because of its

restricted spectrum. However this reputation has recently been reconsidered in light of the fact that there are no commercially available alternatives for treatment of resistant strains (9).

According to published reports, colistin is bactericidal against *P. aeruginosa* and *A. baumannii* but has no activity against *B. cepacia*, *Proteus* spp., *Serratia* spp., *Providencia* spp., or *Edwardsiella* spp. (10). The results of this study are in broad agreement with other published data and confirm that colistin maintains useful in vitro activity against *P. aeruginosa* and *A. baumannii*, and for some strains of S. *maltophilia*, and has no activity against *B. cepacia* when ≤ 4 mg/L is accepted as the susceptibility breakpoint (4,7, 10). The Working Party on Antibiotic Sensitivity Testing of the British Society of Antimicrobial Chemotherapy (BSAC) has recommended this breakpoint concentration (≤4mg/L) for colistin (4). The susceptibility breakpoint for colistin as measured by broth dilution methods has not been established by current National Committee for Clinical Laboratory Standards (NCCLS) guidelines. It was last available in the 1981 NCCLS Approved Standard M2-A2-S2; however, with the very restricted use of polymyxins, the published information was later withdrawn.

Disc diffusion methods are not recommended because of technical difficulties with the agar diffusion of colistin. Broth dilution titers of ≤ 2mg/L have been accepted as a provisional susceptibility breakpoint by Gales and coworkers (7). In this study, results were interpreted according to the BSAC criteria (4), and 95.8% of *A. baumannii* strains, 94.1% of *P. aeruginosa* strains, and 71.4% of *S. maltophilia* strains were susceptible, whereas the majority of *B. cepacia* strains were resistant. According to these results, colistin may be a good therapeutic option in the treatment of severe infections caused by multidrug-resistant P. aeruginosa and *A. baumannii*. However, when the breakpoint concentration of ≤2mg/L is applied, the susceptibility rate of *P. aeruginosa* drops from 94.1% to 60%, and for *A. baumannii* from 95.8% to 85.4%. None of the *S. maltophilia* and B. cepacia strains is susceptible. This makes the treatment decision difficult, especially for the very common nosocomial pathogen, multidrug-resistant *P. aeruginosa*. Additionally, colistin is no longer an option in the treatment of another difficult-to-treat pathogen, *S. maltophilia*. Determining which breakpoint correlates with better clinical success is important because there are few published studies evaluating the value of colistin in nosocomial infections caused by gram-negative bacteria (3,8,10,11). Colistin also has been used successfully against *Klebsiella* pneumoniae-associated sepsis (9). A reference value is needed to guide antimicrobial therapy.

Besides colistin alone, antibiotic combinations including colistin will be a new therapeutic option for the treatment of multidrug-resistant strains, if in-vitro data correlate well with clinical studies (1,5,12). Again, determining a standardized breakpoint is important for interpretation of these synergy studies.

Further investigations evaluating which breakpoint correlates with the clinical success of colistin are warranted for improved outcomes in laboratory-assisted treatment of nosocomial infections caused by multidrug-resistant nonfermentative gram-negative bacilli.

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