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Colistin-daptomycin, colistin-linezolid, colistin-vancomycin combination effects on colistin in multi-resistant *Acinetobacter baumannii* strains

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Abstract

Objective: The most important problem in the treatment of nosocomial *Acinetobacter baumannii* (*A. baumannii*) infections which is increasingly seen in recent years is that almost all strains are resistant to many antibiotics, including carbapenems, and that the extinction of antibiotic options to be used in treatment. This leads the clinicians to new treatment options and suggests the use of combined antibiotics to achieve success in both the treatment of multi-drug-resistant *A. baumannii* (MDRAB) infections as well as to prevent resistance development. We investigated the in vitro activity of colistin in combination with vancomycin, linezolid or daptomycin against MDRAB to determine whether these combinations would be considered for clinical use. .

Methods: The fractional inhibitory concentration (FIC) index was used to determine the antibiotic combination effects and to evaluate the effect of antibiotic combinations on the bacteria.

Results: MIC values of colistin/vancomycin, colistin/linezolid, and colistin/daptomycin in 10 strains (33.3%) gave similar results. Twenty strains gave different MIC results according to antibiotics. The colistin/daptomycin antagonistic ratio was high when the colistin/vancomycin synergy ratio was high compared to the others.

Conclusion: Antibiotic combinations can be used as an alternative treatment approach in multi-drug resistant *A. baumannii* infections.

Keywords: *A. baumannii* , Colistin, Daptomycin, Linezolid, Vancomycin

Introduction

Recently, *A. baumannii* has emerged as one of the important nosocomial pathogens. Difficulties are encountered in the treatment of the infections caused by *A. baumannii* because the microorganism has an intrinsic resistance to many antibiotics and it has the potency of resistance to various classes of antibiotics. The most important problem in the treatment of nosocomial *A. baumannii* infections, which is increasingly seen in recent years, is that almost all strains are resistant to many antibiotics, including carbapenems, and that the extinction of antibiotic options to be used in treatment (1). This leads the clinicians to new treatment options and suggests the use of combined antibiotics to achieve success in both the treatment of multi-drug-resistant *A. baumannii* (MDRAB) infections as well as to prevent resistance development.

The outer membrane is an effective yet selective permeability barrier which distinguishes gram-negative bacteria from gram-positive bacteria (2).

The sensitivity profiles of bacteria to certain fluoroquinolones, β-lactam antibiotics, Erythromycin and even some of the more recent macrolides have been shown to alter by alterations in the composition and size of porins and/or the bacterial outer membrane (3).

At high concentrations colistin produces rapid bactericidal effects. It affects the bacterial outer membrane at lower concentrations and increases the permeability of gram-negative bacteria which facilitates the penetrative ability of other compounds that are usually excluded such as hydrophobic drugs; rifampicin, macrolides, and glycopeptides (including teicoplanin, telavancin, and daptomycin).

In this study, we investigated the in vitro activity of colistin in combination with vancomycin, linezolid or daptomycin against MDRAB to determine whether these combinations would be considered for clinical use.

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Material and method

Bacterial isolates: From 2016 to 2017 samples, total of 30 *A. baumannii* clinical isolates were selected from our hospitals. Identification of the clinical isolates was performed with Vitek MS system (bioMérieux, Marcy-l'Étoile, France). Susceptibility results were obtained using the Vitek®2 (bioMérieux) bacterial identification device per the manufacturer's instructions. Acinetobacter isolates resistant to three or more antibiotic groups were identified as MDRAB. Escherichia coli strain, ATCC 25922, was used as a control in each batch of tests.

E test and Combination method: Colistin minimum inhibitory concentration (MIC) values against *A. baumannii* clinical isolates were determined by the manufacturer's recommendation gradient diffusion method (E-test, bioMérieux, France) and evaluated according to CLSI recommendations. For colistin, ⩽2 mg/l was considered as susceptible, and ⩾4 mg/l was considered as resistant. Since vancomycin, daptomycin and linezolid are used in gram positives; there are no limit values (Table 1). FIC index was used to determine the antibiotic combination effects and to evaluate the effect of antibiotic combinations on the bacteria. To determine the FIC index by gradient diffusion method, the MIC values of A and B antibiotics in combination were recorded. To detect the combination MIC value, the strip B was first placed in the medium, and after waiting for one hour in the room temperature, the strip B was removed, and the strip A was placed in place so that the concentration lines completely overlapped. Following the 16-20 hour incubation period, the MIC numerical value of A was recorded in the presence of B at the cut-off point of the stripe edge of the inhibition zone diameter. The same procedure was repeated placing A before B. To determine the activity of the combination, the FIC index was calculated according to the following formula: MIC value of A in the presence of FIC A=B / A's MIC value alone MIC value of B in the presence of FIC B=A / B's MIC value alone Σ FIC index = FIC A + FIC B Effectiveness of the combinations If Σ FIC ⩽ 0.5; synergy, 0.5 < Σ FIC> 1 indicates partial synergy, Σ If FIC = 1; additive, 1 < Σ FIC> 4 is ineffective, If Σ FIC ⩾ 4 antagonism was assessed. 90 FIC values were calculated for the three antibiotic combinations tested in 30 MDRAB clinical isolates taken to this study. Ineffective, additive, antagonistic, synergistic and partial synergistic interactions were recorded.

Results

Ten of the 30 MDRAB strains taken into the study were isolated from wound samples, 5 were isolated from blood culture, 13 were isolated from lower respiratory tract sample, and 2 were isolated from urine.

Sequence of resistance rates in *A. baumannii* strains has been determined as; gentamicin;14/30, amikacin;21/30, netilmicin;15/30, tobramycin;11/30, imipenem;30/30, meropenem;30/30, ceftazidime;30/30, piperacillin/tazobactam;30/30, ciprofloxacin;30/30, trimethoprim sulfamethoxazole (SXT);26/30, tigecycline; 4/30. E test method did not detect a zone diameter in vancomycin, linezolid, and daptomycin. colistin E test zone diameter, colistin-vancomycin, colistin-linezolid, colistin-daptomycin combination zone diameters and FIC values are shown in the table 1.

Table 1: Minimum inhibitory concentration (µg/ml) range, MIC50 and MIC90 values.

Agent	MIC range	MIC_{50}	MIC_{90}
CT	0.019–256	0.25	0.75
VA	0.019-256	> 256	> 256
DAP	0.019-256	> 256	> 256
LZD	0.019-256	> 256	> 256

CT: Colistin, **VA:** Vancomycin, **LZD:** Linezolid, **DAP:** Daptomycin, **MIC50:** minimum inhibitory concentrations for 50% of the organisms, **MIC90**: minimum inhibitory concentrations for 90% of the organisms;

Discussion

Among the Acinetobacter species, *A. baumannii* is the most common genomic species which cause diseases in humans. This microorganism, which may colonize in the skin of healthy adults and hospital personnel may be a source of long-term hospital infections (4). The synergistic activity of the antibiotics administered in combination therapy is clinically important and in vitro synergy tests are guiding in this context. Therefore, the use of combined antibiotics is recommended to increase the success of treatment and to prevent or reduce the development of resistance. Many studies highlighted that in vitro synergy testing may be guiding in this context (5,6,7). Colistin-ampicillin sulbactam combination is one of the suggested combinations. The synergistic effect of this combination has been shown in many studies. Combinations of rifampicin-colistin, carbapenem-colistin, and tigecycline-colistin have been shown to be synergistic in; in vivo and in vitro studies (8,9). In addition to these commonly used antibiotics combinations with amikacin, phosphomycin, azithromycin, SXT and teicoplanin or vancomycin have been reported. Vidaillac et al (10) found that colistin-SXT combination showed a synergistic effect in colistin resistant *A.baumannii*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* strains in an in vivo study.

Table 2. Colistin-Daptomycin, Colistin-Linezolid, Colistin-Vancomycin combinations' effect and FIC volues.

Sample number	Colistin (MIC)	CT-VA (MIC)	CT-VA FIC	CT-LZD (MIC)	CT-LZD FIC	CT-DAP (MIC)	CT-DAP FIC
1	0.38	0.38	Additive	0.25	Partial synergy	0.38	Additive
2	0.38	0.125	Synergy	0.25	Partial synergy	0.38	Additive
3	1	0.125	Synergy	0.125	Synergy	0.19	Synergy
4	0.125	0.125	Additive	0.25	Ineffective	0.25	Ineffective
5	0.38	0.125	Synergy	0.19	Synergy	0.25	Partial synergy
6	0.25	0.38	Ineffective	0.5	Ineffective	0.5	Ineffective
7	0.38	0.19	Synergy	0.25	Partial synergy	0.125	Synergy
8	0.125	0.064	Synergy	0.25	Ineffective	0.5	Ineffective
9	0.19	0.125	Partial synergy	0.125	Partial synergy	0.125	Partial synergy
10	2	0.75	Synergy	0.25	Synergy	0.75	Synergy
11	0.5	0.125	Synergy	0.38	Partial synergy	0.25	Synergy
12	0.38	0.19	Synergy	0.75	Ineffective	0.25	Partial synergy
13	0.38	0.38	Additive	0.38	Additive	0.25	Partial synergy
14	0.125	0.5	Antagonistic	0.38	Ineffective	0.5	Antagonistic
15	0.125	0.125	Additive	0.125	Additive	0.094	Partial synergy
16	0.25	0.125	Synergy	0.125	Synergy	0.25	Additive
17	0.25	0.5	Ineffective	0.75	Ineffective	0.75	Ineffective
18	0.125	0.38	Ineffective	0.125	Additive	0.125	Additive
19	0.38	0.25	Partial synergy	0.5	Ineffective	0.75	Ineffective
20	0.25	0.75	Ineffective	0.5	Ineffective	0.75	Ineffective
21	0.094	0.75	Antagonistic	0.75	Antagonistic	1	Antagonistic
22	0.5	1	Ineffective	0.38	Partial synergy	0.75	Ineffective
23	0.125	0.25	Ineffective	0.38	Ineffective	0.25	Ineffective
24	0.125	0.38	Ineffective	0.016	Synergy	0.19	Ineffective
25	0.75	0.75	Additive	0.75	Additive	0.5	Partial synergy
26	0.25	0.38	Ineffective	0.75	Ineffective	0.5	Ineffective
27	12	12	Additive	12	Additive	12	Additive
28	0.38	1	Ineffective	0.25	Partial synergy	0.38	Additive
29	0.125	0.19	Ineffective	0.19	Ineffective	0.75	Antagonistic
30	0.25	0.25	Additive	0.19	Partial synergy	2	Antagonistic
Mean MIC Value	0.76	0.73		0.67		0.85	

Table 3. Colistin-Daptomycin, Colistin-Linezolid, Colistin-Vancomycin combinations' effect

	Synergy	Partial synergy	Additive	Ineffective	Antagonistic
CT -VA	9 (30%)	2 (6.6%)	7 (23.3%)	10 (33.3%)	2 (6.6%)
CT-LZD	5 (16.6%)	8 (26.6%)	5 (16.6%)	11 (36.6)	1 (3.3%)
CT-DAP	4 (13.3%)	6 (20%)	6 (20%)	10 (33.3%)	4 (13.3%)

Although colistin is used as a last resort in infections caused by MDRAB strains, there are concerns about toxicity potential and resistance formation. However, the action mechanism of colistin increases the likelihood of synergy with normally inactive compounds against gram negative organisms due to the impermeability of the bacterial outer membrane. Synergy studies with antibacterial agents against gram-positive microorganisms were tested in several studies. The efficacy of colistin/vancomycin was evaluated in the synergic studies in 5 epidemic strains and 34 MDRAB clinical isolates by microdilution and E test methods. For all strains, after exposure to 0.5 µg / ml colistin, significant synergies were demonstrated in at least one method with a reduction of vancomycin MIC> 256 µg / ml to ⩽48 µg / ml for all strains. This increases the likelihood that this combination will be clinically applicable to infections due to MDRAB; it can be administered at lower doses than the currently used doses (11). Although there is a strong interaction between vancomycin and colistin there is concern about the inherent toxicity of combining these agents in clinical practice. In a different study combination of colistin/teicoplanin has been assessed in vitro to determine whether this combination has similar antimicrobial activities because teicoplanin has less nephrotoxic potential than vancomycin. In the study, the combination of teicoplanin and colistin was bactericidal against all tested strains with the in vitro checkerboard method, FIC indices were found to be <0.5 and compatible with synergy. Using the E test method, the MIC value of teicoplanin was found to be lowered to ⩽ 2 mg / L from > 256 mg / L at MIC for colistin (12).

In a different study, four severe infections due to MDRAB were observed. All patients treated with the combination of colistin/vancomycin received a positive result in treatment. Most importantly, no significant adverse events related to the simultaneous administration of the colistin/vancomycin have been observed. In our in vitro experiments the synergistic effect of the colistin/vancomycin combination demonstrated bactericidal activity even at a vancomycin concentration of 16 mg / L reflecting the serum concentrations obtained in patients. In the Pediatric Intensive Care Unit an antimicrobial strategy based on the activity of colistin plus the absence of adverse effects has been found to be effective in life-threatening infections caused by MDRAB in vitro and in vivo. It has been shown that colistin/Vvancomycin combination has synergistic and bactericidal properties against carbapenem-resistant, colistin-sensitive *A. baumannii*, whereas meropenem addition did not increase the in vitro activity of colistin/vancomycin combination (13).

In a different study, vancomycin/colistin mean FIC was found as 0.08 and colistin/azithromycin mean FIC was found as 0.71 in 30 isolates.

Conclusion

These findings indicate that vancomycin-containing regimens may provide therapeutic benefit for MDRAB-associated infections; However, other methods should be used to confirm such a synergy. Also optimal combination therapy in severe infections should be considered in a prospective clinical trial (14). In a study, conducted in 2013 they found that combinations of colistin resistant *A. baumannii* strains isolated from patients previously treated with colistin were synergistic with vancomycin-colistin containing combinations in an in vivo study and in vivo larval experiments have reported that the combination regimen containing colistin-vancomycin-doripenem increases survival compared to monotherapy (15). In combination with linezolid/colistin, linezolid acts against broad spectrum gram positive bacteria by inhibiting the formation of the 70S initiation complex, possibly influencing the treatment of respiratory tract infections, because it reaches high concentrations in the epithelial lining fluid and blood. It is emphasized that especially in patients with renal dysfunction the combination of colistin and linezolid may be effective in the treatment of *A. baumannii* pneumonia and gram positive coinfection (3,16). In studies with daptomycin, synergy testing with 9 colistin-sensitive and 4 colistin-resistant isolates was conducted, and susceptible strains were considered as ineffective, and synergies were found in resistant strains (17). It has been concluded by studies on Galleria menolella larvae that the use of the combination of daptomycin/colistin is not effective in gram negative infections such as Klebsiella pneumoniae, E. coli, and Pseudomonas aeruginosa but may be beneficial in the treatment of *A. baumannii* (18,19).

When daptomycin was given with colistin in the treatment of Galleria mellonella larvae infected with lethal doses of *A. baumannii* this treatment resulted in significantly enhanced survival rates compared with colistin treatment alone (P<0.05). This work suggests that daptomycin/colistin combination is highly active against *A. baumannii* both in vitro and in a simple invertebrate model of infection (19). When investigating the synergistic interaction between the antibiotics forming the combination, interpretation of the combined interaction by evaluating the MIC values of the antibiotics one-by-one may lead to incorrect results. Sometimes synergistic interactions may occur even when one of the antibiotics used in combination is resistant. Even higher synergistic activity can be observed in combination with two resistant antibiotics (20). It would be better to interpret the antibiotics that make up the combination together since the opposite can also be observed. Although the strains collected in our study were taken from different patients at different times the high proportion of carbapenem resistant strains may be due to the clonal interrelated strains of isolated Acinetobacter species in our Intensive care unit. However, since we

can not do any molecular typing method, it is not possible to pinpoint this relationship. MIC values of vancomycin, linezolid, and daptomycin in 10 strains (33.3%) gave similar results. Twenty strains gave different MIC results according to antibiotics (Table 3). In other studies, for example, when the vancomycin MIC value is greater than 256 it is misleading to interpret MIC value by the combination test as a lower value because the effect of the colistin alone is ignored. Individual MIC values and synergy values were also evaluated in our study. The effect of antibiotics on colistin for gram positive factors was interpreted. The colistin/daptomycin antagonistic ratio was high when the colistin/vancomycin synergy ratio was high compared to the others (Table 3). There are few published articles on this subject. It is difficult to make generalizations because the combinational studies are formed with data that are worked with fewer strains. Antibiotic combinations can be used as an alternative treatment approach in multi-drug resistant *A. baumannii* infections. Althought, They are studies that should be planned according to the characteristics of the patient in the clinic and should be supported by prospective clinical or in vivo studies.

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Ethical issues: All Authors declare that Originality of research/article etc... and ethical approval of research, and responsibilities of research against local ethics commission are under the Authors responsibilities. The study was conducted due to defined rules by the Local Ethics Commission guidelines and audits.

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