# POSTANTIBIOTIC EFFECT OF COLISTIN ALONE AND COMBINED WITH VANCOMYCIN OR MEROPENEM AGAINST *ACINETOBACTER BAUMANNII* WITH WELL DEFINED RESISTANCE MECHANISMS

Branka Bedenić[1,2], Nataša Beader[1,2], Esmina Prahin[1], Karmen Godič-Torkar[3], Ljiljana Mihaljević[2], Jasmina Vraneš[1]

[1]Department of Microbiology, School of Medicine, University of Zagreb, Zagreb, Croatia; [2]Department of Clinical and Molecular Microbiology, Clinical Hospital Center Zagreb, Zagreb, Croatia
[3]Department for Sanitary Engeneering, Faculty of Health Sciences, University of Ljubljana, Ljubljana, Slovenia

## BACKGROUND

Carbapenem-resistance in *A. baumannii* is reported increasingly worldwide. Colistin is now being administered as salvage therapy in patients in whom none of other antibiotics are active against their isolates. Persistant suppression of bacterial growth after short antimicrobial exposure is called the postantibiotic effect (PAE). Previous studies found short postantibiotic effect of colistin on *A. baumannii* However, only a few studies have evaluated the potential for synergy between colisitin and other antibiotics against *A. baumannii*. The aim of this study was to determine in vitro synergy and PAE of colistin combined with other antibiotics (vancomycin or meropenem) against carbapenem-resistant *A. baumannii* strains with defined resistance mechanisms. It was hypothesised that vancomycin or meropenem would prolog the PAE of colistin since it was previously found that they exert synergism with colistin in time-kill kinetics and also in clinical trials.

### Bacteria

The experiments were performed on four *A. baumannii* strains with previously characterized carbapenem-hydrolyzing oxacillinases (CHDL) which included:*A. baumannii* strain 5 positive for OXA-23-like, *A. baumannii* strain 7 positive for OXA-24/40-like, *A. baumannii* strain 9 positive for OXA-58-like, *A. baumannii* positive for OXA-143.

### Determination of minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBC)

MICs of colistin, vancomycin and meropenem were determined by the broth microdilution method according to the CLSI guidelines. 10 µL of the sample from the wells with no visible growth was subcultured on Mueller-Hinton agar in order to determine the MBC. The The interactions of colistin combinations with meropenem or vancomycin were tested by chequerboard MIC technique, yielding the fractional inhibitory concentration index (FICI) as described previously. FICI was calculated for each antibiotic combination using the following formula: $FIC_A$ + $FIC_B$ = FICI where $FIC_A$= MIC of colistin in combination with either vancomycin or meropenem/MIC of colistin alone, and $FIC_B$ = MIC of either vancomycin or meropenem in combination with colistin/MIC of either vancomycin or meropenem alone. The FICI was interpreted as follows: synergy = FICI ⩽ 0.5; no interaction = FICI >0.5-⩽ 4; antagonism = FIC >4. The confirmatory method was two-well method using concentrations of 0.25 x MIC and 2 x MIC of colistin alone

### Time- kill experiments (TK)

Time-kill experiments were carried out by exposing test cultures to colistin alone and combined with vancomycin and meropenem and establishing bacterial numbers by viable counting.

### Determination of PAE

Inocula of $10^7$ CFU/mL were exposed to 4 x MIC of colistin alone or combined with vancomycin (10 mg/L) or meropenem (10 mg/L) for 1h, after which antibiotic was eliminated by centrifugation and washing of the pellet in saline twice The PAE was defined according to the following formula: PAE = T-C, where T is the time required for the viable counts of the antibiotic exposed cultures to increase by 1 $log_{10}$ above the counts observed immediately after dilution, and C is the corresponding time for the unexposed cultures.

*Corresponding address: Branka Bedenić,
Department of Microbiology, School of Medicine,
University of Zagreb, Zagreb, Croatia, tel: +385 1 2367304, fax: +385 1 4

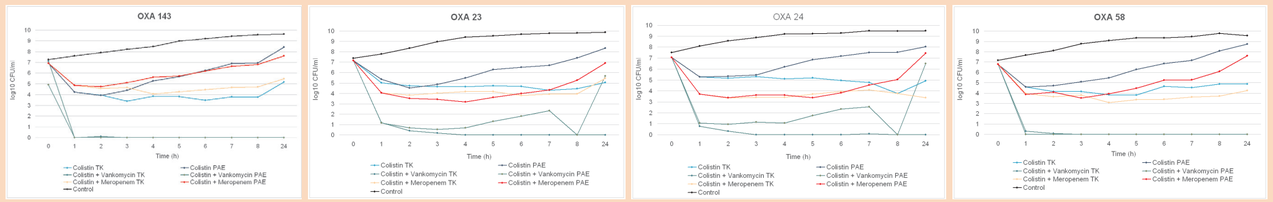


**Figure 1.** Time-kill curves and postantibiotic effect (PAE) of colistin alone and combined with either vancomycin or meropenem against *Acinetobacter baumannii* strains positive for OXA-23, OXA-24, OXA-58 nad OXA-143 β-lactamase.

## RESULTS

### Minimum inhibitory concentrations (MICs)

MICs and MBCs are shown in Table 1. All strains were susceptible to colistin but resistant to meropenem and vancomycin.

### Synergy studies

The results of the chequerboard analysis indicated synergism between colistin and vancomycin with OXA-23 and OXA-24 positive strains (FICI of 0.5 and 0.25, respectively) but not with OXA-58 and OXA-143 (FICI of 1). When colistin was combined with meropenem the FICI was 0.0154 for OXA-23, 0.03046 for OXA-24, 0.00077 for OXA-58 and 0.125 for OXA-143 positive strain indicating synergism as shown in Table 2. Two well method revealed the growth of all strains at 0.25 x MIC of colistin alone but absence of growth when the strains were exposed to 2 x MIC of colistin alone. No growth was observed when the strains were exposed to 0.25 x MIC and 2 x MIC of colistin combined with either vancomycin or meropenem confirming synergy with both drugs against all tested strains (Table 2).

### Time- kill experiments

Colistin alone produced rapid bacterial killing with 1.5 log10 (OXA-23, OXA-24) to 2 log10 (OXA-58, OXA-143) reduction in bacterial counts after 1 h exposure. However, after initial killing, no further reduction in CFU was noticed after first hour as shown in Fig 1 and Table 2. Colistin combined with vancomycin demonstrated the rapid bacterial killing with less than 1log10 (OXA-23, OXA-24) or no detectable bacterial counts (OXA-58, OXA-143) after 1 h exposure and with no regrowth within 24 h. Colistin combined with meropenem exerted the 3 to 4 log10 reduction of viable cells in all strains with no significant regrowth except in OXA-23 positive strain. The combination of colistin with vancomycin demonstrated more then 2 log10 reduction in CFU compared to colistin alone. When colistin was combined with meropenem synergism was observed only with OXA-24-positive strain. Other strains did not demonstrate synergism in TK.

### Determination of PAE

Colistin alone exhibited the negative (-0.07 h) (OXA-143), short (0.2-1,1 h) (OXA-24, OXA-58) or moderate PAE (3.2 h) for OXA-23 positive strain. When combined with vancomycin, the PAE was moderate (1.7-3.5) with OXA-23 and OXA-24 positive strains while with OXA-58 and OXA-143 positive strains it was not possible to calculate PAE because in two experiments there was no regrowth after exposure to antibiotics. The combination with meropenem resulted in short (0.2 h) (OXA-143), moderate (2.4-2.5 h) (OXA-24, OXA-58) or long PAE of 4.7 h (OXA-23) as shown in Table 3.

OXA-23 positive strain displayed longer duration of PAE induced by colistin alone and combined with meropenem or vancomycin compared to other strains.

**Table 1.** Minimum inhibitory (MIC) and minimum bactericidal concentrationS (MBC) of colistin, vancomycin and meropenem.

| | MIC (mg/L) | | | MBC (mg/L) | | |
|---|---|---|---|---|---|---|
| Strain | Colistin | vancomycin | meropenem | colistin | vancomycin | meropenem |
| Acb-OXA-23 | 2 | >128 | 64 | 2 | >128 | >128 |
| Acb-OXA-24 | 2 | >128 | >128 | 2 | >128 | >128 |
| Acb-OXA-58 | 2 | >128 | >128 | 4 | >128 | >128 |
| Acb-OXA-143 | 0.5 | >128 | 64 | 2 | >128 | >128 |

**Table 2.** Synergistic effect of colistin combined with either vancomycin or meropenem with three different methods.

| Strain | Chequerboard analysis colistin+ vankomycin (FICI) | Chequerboard analysis colistin + Meropenem (FICI) | Two well method Colistin+ vancomycin | Two well method colistin+ meropenem | Time-kill colistin +vankomycin | Time-kill colistin + meropenem ?2log10 reduction in CFU/ml |
|---|---|---|---|---|---|---|
| OXA-23 | + (0.031) | + (0.015) | + | + | + (no regrowth) | - |
| OXA-24 | + (0.25) | + (0.03046) | + | + | + (no regrowth) | + (2log10) |
| OXA-58 | - (0.56) | + (0.00077) | + | + | + (no regrowth) | - |
| OXA-143 | + (0.003) | + (0.125) | + | + | + (no regrowth) | - |

**Table 3.** Postantibiotic effect of colistin alone and combined with vancomycin and meropenem. The duration of postantibiotic effect is expressed in hours.

| **Colistin alone** | | | | | | | |
|---|---|---|---|---|---|---|---|
| | OXA-23 | | OXA-24 | | OXA-58 | | OXA-143 |
| Mean: | 3,240 | Mean: | 0,298 | Mean: | 1,138 | Mean: | -0,078 |
| SD: | 2,676 | SD: | 1,310 | SD: | 0,961 | SD: | 1,942 |
| **Colistin + vancomycin** | | | | | | | |
| | OXA-23 | | OXA-24 | | OXA-58 | | OXA-143 |
| Mean | 3,598 | Mean: | 1.75 | | NA* | | NA* |
| SD: | 0,750 | SD: | 0.07 | | NA* | | NA* |
| **Colistin+meropenem** | | | | | | | |
| Mean: | 4,717 | Mean: | 2,563 | Mean: | 2,411 | Mean: | 0,293 |
| SD: | 2,033 | SD: | 3,288 | SD: | 2,485 | SD: | 2,498 |

*NA-not applicable: in two experiments there was no regrowth after exposure to colistin combined with vancomycin.

## CONCLUSION

In our study we demonstrated significant synergism between colistin and meropenem or vancomycin in both the time kill kinetics and PAE. In vitro synergism in time-kill effect and checquerboard testing was previously reported for colistin combined with vancomycin, ciprofloxacin, trimethoprime or sulphametoxazole against multiresistant Gram-negative bacteria by other authors. Vancomycin cannot penetrate the cell wall of Gram-negative bacteria but colistin disrupts the outer membrane and enables penetration of vancomycin when they are administered in combination. Meropenem also acts on bacterial cell wall synthesis and can be used in combination with antibiotics which act on the cytoplasmic membrane function.

Synergism was more pronounced in chequerboard analysis when colistin was combined with meropenem than with vancomycin as demonstrated by lower FICI values in chequerboard analysis. However, in time-kill analysis there was no synergisms between colistin and meropenem except in OXA-24-positive strain. The possible explanation is the production of CHDL (OXA-23-like, OXA-24-like, OXA-58-like and OXA-143-like) which are released from periplasmic space into the medium after colistin disrupts outer membrane of *A. baumannii* and destroy meropenem. Vancomycin is not hydrolyzed by CHDL and thus can exert synergism. Zusman et al reported synergy rate between polymixins and carbapenems aganst *A. baumannii* of 71% in TK but only 32% in chequerboard analysis . This is in contrast with our results which demonstrated synergy in chequerboard analsis and 2 well method but not in TK.

There was a discrepancy between chequerboard and two well method with OXA-58 and OXA-143 positive strains which displayed synergism between colistin and vancomycin in two well method but not in chequerboard test. In time kill experiments colistin alone demonstrated rapid killing, however, regrowth occurred after 24 h, probably due to development of heteroresistance as described by Li J et al. Heteroresisant mutants causing regrowth after five to six hours developed even at colistin concentration corresponding to 64 x MIC (23-24). Poudyall et al reported that colistin monotherapy led to resistance development in almost 100% of the strains within 24 h while with combination therapy carbapenem successfully supressed colistin resistant population. When vancomycin or meropenem were added no regrowth was demonstrated because development of heteroresistance was prevented.

From the clinical point of view, the prolongation of colistin PAE when combined with other antibiotics could provide a rationale for the modification of the dosing interval and could be important for the optimization of the treatment regimen and the minimization of drug-induced side effects. Furthermore, combined therapy could prevent development of heteroresistance in *A. baumannii* which often happens during colistin monotherapy . The results of the study are in concordance with previous studies which demonstrated prolonged PAE of colistin against multidrug-resistant *A. baumannii* in contrast from the results reported by Owen et al.who found negative PAE values induced by colistin. In our study negative PAE values were noticed only with OXA-143 producing strain. Negative PAE values induced by colistin were also observed with Klebsiella pneumoniae. The duration of PAE in this study was strain dependent and the longest with OXA-23 producing organism. Previous studies also found that antibiotic combination produce longer PAE than each of the antibiotic alone. Ozbek et al reported that addition of tigecycline to colistin prolonged the PAE.

Limitation of the study is the limited number of tested strains (one strain with each type of CHDL, similar resistance profiles of the tested strains (all displayed resistance to meropenem and had identical MICs of colistin) and experimental design which included only one method for determination of PAE (viable count method). Bioluminiscent method has advantage over viable count because the β-lactam antibiotics induce the formation of filaments which contain biomass equivalent to over 20 colonies but it was not performed in this study .