Importance of „in vitro” synergism examinations on infections caused by multidrug-resistant pathogens

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Introduction

In view of rapidly rising antibiotic resistance and spread of multiresistant pathogens the clinical use of appropriate antibiotic combinations is crucial in critically ill patients. Cystic fibrosis patients and endocarditis patients mean additional indications. With the emergence of nosocomial multidrug-resistant bacteria use of old generation antibiotics (e.g. fosfomycin, colistin) have come back into prominence. Because of the narrowing options of antimicrobial therapy, life expectancy of patients can be improved with detection of effective synergistic antibiotic combinations.

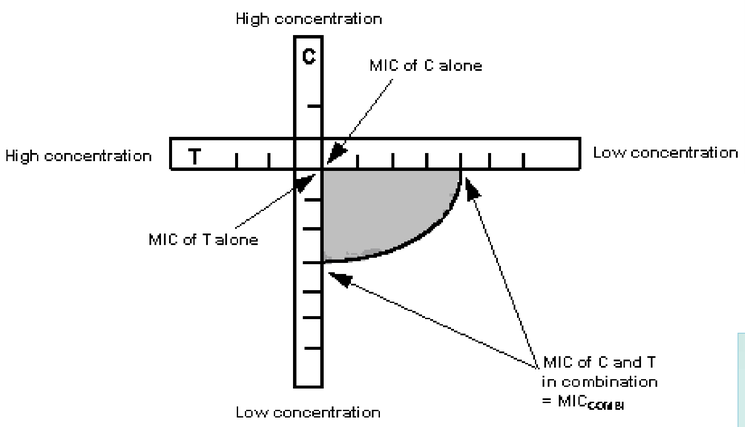
Studies on synergy interactions of antibiotic combinations date back to the 1980s. Detection of synergism was performed at first with the checkerboard titration and killing curve methods. Since execution of these methods are very time-consuming, they can not be introduced in the routine diagnostics. The use of gradient strip diffusion method is easier. There is a daily demand for clinical microbiological laboratories serving intensive care units to give therapeutic counsel, therefore it is important to use a method that is relatively fast and easy to apply. Results can be quantified with the fractional inhibitory concentration (FIC) index, 4 categories are defined: synergistic, additive, indifferent and antagonistic.

Methods

The filter paper-based MTS (MIC Test Strips) synergy method (Liofilchem) does not require special equipment and produces rapid result. The MIC values have to be determined separately for the two (A and B) antibiotics. Position the MIC test strips in the form they intersect each other at the calculated MIC values using MTS Synergy Applicator Platform on the agar plate.

The FIC Index can be calculated with the following formula:

FIC Index=$MIC_{AB}/MIC_{A}+MIC_{BA}/MIC_{B}$



FIC index interpretation

synergistic	**<0.5**
additive	**>0,5 ≤ 1**
indifferent	**<1 ≤ 4,0**
antagonistic	**>4,0**

We examined 45 multi- and pandrug-resistant isolates (mainly nosocomial pathogens) that were cultured in three bacteriological laboratories in Hungary. We have tried to find synergistic antibiotic pairs, which applied in combination make the MIC value in the clinically applicable range. The majority of the enrolled patients were from intensive care unit, surgery, hematology and cystic fibrosis patients from pulmonology. Infections caused by multidrug-resistant *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia and Comamonas testosteronii* have been seen among these patients. The potential combinations are need to be individually selected by each strains. The MIC value interpretation based on the expert rules in antimicrobial susceptibility testing (EUCAST). The FIC index were calculated according to manufacturer's instruction. The incubation period was 24-48 hours in 36°C. We read complete inhibition for the bactericidal antibiotics, but the bacteriostatic ones 80%. The selection of the examined antibiotic combinations were based on the inhibition zone diameters of the disc diffusion antibiogram and the potentially applicable combinations according to literature data. The multidrug-resistant strains (*Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Comamonas testosteronii*) were examined for calculating FIC index with MIC test strip.

Results

In vitro synergism was observed about 15-20 percent of examinations, we can not find any cases of antagonism. The following synergistic combinations were found in cases of *Acinetobacter baumannii* (23 strains): imipenem-amikacin, meropenem-colistin and meropenem-tobramycin. Synergistic effects of ciprofloxacin with amikacin and meropenem in combinations, meropenem-fosfomycin and piperacillin/tazobactam-amikacin were found in *Pseudomonas aeruginosa* (16 strains). Levofloxacin showed synergism with colistin and amikacin against *Stenotrophomonas maltophilia* strains (5 strains).

Table 1. Synergistic antibiotic-pairs in the studied multiresistant strains

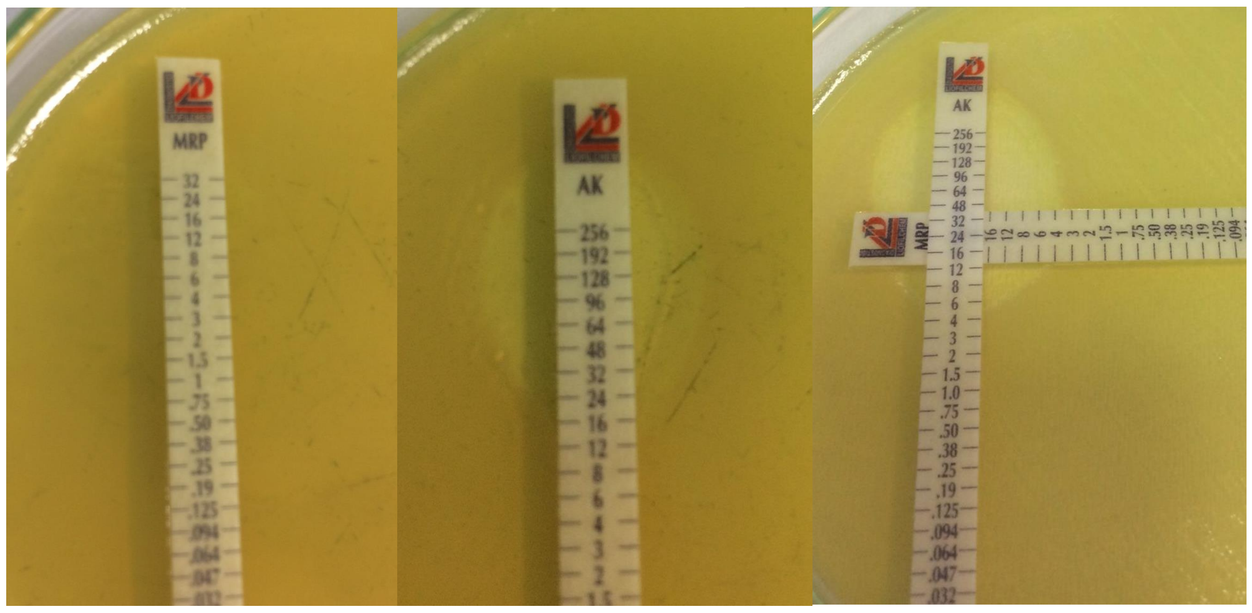
No. strains	Multidrug-resistant bacteria	No. synergistic combination / all tests (%)	Synergistic antibiotic pairs	Type of samples
16	***Pseudomonas aeruginosa***	7/ 38 (18%)	1 meropenem-fosfomycin 1 meropenem-ciprofloxacin 1 meropenem-amikacin 1ciprofloxacin-amikacin 3 piper/tazobactam-amikacin	hemoculture, trachea, sputum, abscess, abdominal fluid, wound, urine
23	***Acinetobacter baumannii***	4/23 (17%)	1 meropenem-tobramicin 1 meropenem-colistin 2 imipenem-amikacin	hemoculture, sputum, wound, trachea, abdominal fluid, abscess
5	***Stenotrophomonas maltophilia***	4 /5	2 levofloxacin-amikacin 1ciprofloxacin-amikacin 1 levofloxacin-colistin	sputum, trachea
1	***Comamonas testosteronii***	2 /4	1 ciprofloxacin- piper/tazobactam 1 gentamicin-piper/tazobactam	sputum

Multiresistant *Pseudomonas aeruginosa* case report

We examined a multiresistant *P. aeruginosa* epidemic strain in Petz Aladar County Teaching Hospital (12. 2016). It was isolated from the urine samples of several urological patients. The nosocomial epidemic was associated with a medical device (cystoscope). The bacteria were able to adhere to the working channel surface of the device because of biofilm formation. The infected patients were treated with intravenous colomycin. Some of them required an additional antibiotic treatment. Therefore we looked for another therapeutic option. In Table 2 can be seen the detected synergistic pairs.

Table 2. Nosocomial *P. aeruginosa* synergy interaction

sample	antibiotics	MIC antibiotics alone	Antibiotic combinations	FIC
urine	**meropenem**	**>32 (R)**	**meropenem/amikacin**	**0,43 synergistic**
	amikacin	**24(R)**	**colistin/amikacin**	**1,00 additive**
	colistin	**2(S)**		



Conclusions

The MTS synergy method is rapid, simple and easy to perform in the clinical microbiological laboratory. The patient’s isolates would require testing on a case-by-case basis. The method can help in clinical situations, in which last resort antibiotics, reviving old antibiotics or synergistic antibiotic combinations provide the only therapeutic options. In the future more clinical studies need to be perform to prove how useful is in vitro synergy testing in clinical situation and follow the outcome of the infection caused by multiresistant pathogen.

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