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EFFECTIVENESS OF COLISTIN WITH RIFAMPICIN AND MEROPENEM AGAINST COLISTIN-RESISTANT *ACINETOBACTER BAUMANNII* STRAINS: AN *IN VITRO* STUDY

Adriana Mosca[1], Lidia Dalfino[2], Federica Romanelli[1], Stefania Stolfa[1] Raffaele Del Prete[1], Luigi Santacroce[3*]

1. ***Department of Interdisciplinary Medicine, Sect. of Microbiology, University of Bari, Italy.***
2. ***Clinical Department of Anesthesiology, University Hospital of Bari, Italy.***
3. ***Ionian Department, Microbiology and Virology Lab, University Hospital of Bari, Italy.***

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ABSTRACT

Introduction: Antibiotic resistance is an important issue that may worsen the clinical evolution of high risks patients. The increasing use of colistin for the treatment of carbapenem-resistant *Acinetobacter baumannii* infections has led to the emergence of colistin resistance. Since *A. baumannii* is an important nosocomial pathogen and is responsible for severe infections, the use of antimicrobial combinations has been strongly recommended. Materials & Methods: This study aims to evaluate the *in vitro* effect of colistin/rifampicin and colistin/meropenem combinations against colistin-resistant *A. baumannii* isolates using two rapid E-test methods. Results: Synergy was observed with colistin in combination with rifampicin against all the isolates. Otherwise, colistin/meropenem combination showed indifference by E-test cross formation and synergy dependent by meropenem concentration by the E-test gradient. Discussion and conclusion: Nowadays *A. baumannii* is one of the most important emerging, opportunistic pathogens that can affect critical patients. Colistin, the main antibiotic effective against such bacterium, when associated with rifampicin shows an enhanced activity.

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Introduction

Antibiotic resistance is a global emergency [33], so in recent years WHO and the EU have released the "Global action plan on antimicrobial resistance" and the "Council conclusions on the next steps under a One Health approach to combat antimicrobial resistance", respectively, to attempt to limit its effects. [1-4] Medical device exhibits the advancement of the medical field and conjointly providing a larger contribution on lives. [34] Antibiotic resistance development has been a major issue for managing infections both in community and hospital settings. [35]

According to these guidelines, research is currently aimed worldwide to diminish the spreading of bacterial resistant strains and to isolate and produce new, effective antibiotics. [5, 6]

However, many studies are currently aimed to define the usefulness and the antibacterial activity of essential oils and probiotics or synbiotics used as antibiotic substitutes or supportive antimicrobial therapy. [7-13]

Acinetobacter baumannii is ubiquitous and can be found as colonizer of the skin, respiratory and digestive tract in both patients and healthcare professionals. It is an obliged aerobic Gram-negative coccobacillus, can survive in the environment for long periods, even up to 30 days, and may act as an opportunistic pathogen able to develop multidrug resistance against the major antibiotic classes including carbapenem. Microbes are also becoming resistant to available antimicrobial drugs,

Corresponding Author: Luigi Santacroce; Ionian Department (DJSGEM), Microbiology and Virology Lab, University Hospital of Bari, 70124 Bari, Italy. Email: luigi.santacroce @ uniba.it

thus requiring alternative medications. [36] In recent years it emerged as one of the leading nosocomial pathogens, particularly in severely ill patients and, in the last decade, carbapenem-resistance associated with resistance to the other routinely tested antibiotics has become common for the majority of *A. baumannii* strains. [14, 15] The impressive number of acquired mechanisms of resistance makes it difficult to select an appropriate antimicrobial agent and leads to the renewed clinical use of colistin which is increasingly used as the last line of defense despite its nephrotoxicity and neurotoxicity. [16, 17] More recently, resistance to colistin has been reported in *A. baumannii* clinical isolates probably because of its use in monotherapy, restricting further treatment options. [18]

The rapid emergence of resistance to colistin has been attributed to the high frequency of heteroresistant isolates which are rapidly selected as dominant resistant populations during colistin treatment. [19] Also, monotherapy is less effective than combination therapy, despite in vitro tests revealed drug susceptibility; thus, antimicrobial combinations are explored as potential therapeutic options. [20] Antimicrobial Stewardship Program has been an essential program in hospitals. One of its objectives is to prevent the emergence of antimicrobial resistance. [32] Bacterial infections that had been willingly cured by AM are lasting longer due to resistance emergence and this will lead to intense morbidity and mortality. [37]

Previous *in vitro* studies have investigated the benefits of different combination therapies against colistin-susceptible *A. baumannii*, especially the activity of imipenem, tigecycline, rifampicin and colistin alone or in combination. [21, 22]

A combination of antimicrobial agents, with a synergistic effect *in vitro*, has been associated with a better clinical outcome. Therefore, the clinicians need it to be performed synergist tests but both checkerboard and time-kill kinetic tests are time-consuming and unsuitable for daily laboratory routine.

To be clinically useful, a synergistic test should be rapid and easy to perform. [23] To study antibiotic synergy, E-test strips have been used in two different ways: in a cross formation and Mueller Hinton agar supplemented with an antibiotic. [24, 25]

This study aimed to evaluate in vitro the activity of colistin in combination with rifampicin or meropenem against colistin-resistant *A. baumannii* strains using two different E-test methods for the detection of synergy.

Materials and Methods

Twenty colistin-resistant *Acinetobacter calcoaceticus baumannii complex* strains collected at Policlinico University Hospital, Bari, Italy, were selected for the study. The strains were identified by biochemical profiling using the Vitek2 system (bioMerieux, France) and confirmed as *A. baumannii* by MALDI-TOF assay (bioMerieux, France). Antibiotic susceptibility was assessed using the GN202card of the Vitek2 system and the results were interpreted according to the current EUCAST clinical breakpoints. Colistin resistance was confirmed by broth microdilution (Merlin). [26]

To test synergy, MICs of colistin, meropenem, and rifampicin were determined by E-test. Briefly, each strain was matched to a 0.5 McFarland turbidity standard and inoculated in Mueller-Hinton agar plates. E-test strips (Liofilchem) were placed on the inoculated agar plates and incubated overnight at 35°C degrees in ambient air.

E-test cross formation

Each isolate was matched to a 0.5 McFarland standard and streaked on Mueller-Hinton agar plates. The E-test strips of COL/MER and COL/RIF were placed on Mueller-Hinton agar in a cross formation, with a 90° angle at the intersection between the scales at their respective value of MICs. The plates were incubated at 35-37 °C overnight and the zones of inhibition were read as the value where the eclipse intersected the scale on E-test strips. The nature of drug interaction (synergy, indifference or antagonism) was determined based on the calculated fractional inhibitory concentration (FIC) index as described for the checkerboard method.

E-test agar dilution

Mueller Hinton agar, supplemented with rifampicin or meropenem were prepared at a final concentration of 32, 16, 8, 4, 2 µg/ml, respectively. Plates were inoculated with the same bacterial suspension used for E-test cross formation, E-test strips of colistin were applied and MICs of colistin were read after 24 hours of incubation at 35-37 °C.

Results

All *A. baumannii* strains were resistant to colistin (MIC_{50} and MIC_{90} > 16µg/ml), ciprofloxacin, gentamicin, imipenem, meropenem, and trimethoprim/sulfamethoxazole.

Table 1 shows MICs obtained for COL and RIF alone and MICs obtained for the respective antibiotic combination. The concordance among both methods of synergy testing was observed for all isolates tested. In fact, with the E-test cross formation, the FIC index ranging from 0.18 to 0.5 (FICI $\leq$0.5 was considered synergistic) while with E-test gradient dilution MICs of COL decreased below the susceptibility breakpoint (MIC $\leq$2 µg/ml).

Table 1: Comparison of results by E-test cross formation and E-test gradient dilution for synergistic activity of colistin (COL) and rifampicin (RIF) combination. (-) indicates no growth

	E-test Cross formation					E-test Gradient dilution with RIF (ug/ml)				
	MICs (ug/ml)					COL MIC (ug/ml)				
Strains.	**COL alone**	**COL col+rif**	**RIF alone**	**RIF col+rif**	**FICI**	**32**	**16**	**8**	**4**	**2**
1	256	48	32	8	0.43	0.25	0.38	0.5	0.75	1
2	256	32	32	8	0.37	0.5	0.5	0.75	0.75	0.75
3	256	48	32	6	0.36	0.38	0.5	0.75	1	1
4	256	48	32	3	0.27	0.5	1	1	2	2
5	256	48	32	8	0.43	0.5	0.75	1	1	1.5
6	256	32	32	8	0.37	0.38	0.5	0.5	0.75	1
7	256	24	32	8	0.34	0.25	0.12	0.125	0.12	0.09
8	256	24	32	8	0.34	0.38	0.38	0.5	0.75	1
9	256	48	32	6	0.36	0.38	0.5	0.25	0.75	0.75
10	256	6	32	8	0.27	0.38	0.38	0.38	0.5	1
11	8	1.5	32	12	0.55	0.25	0.38	0.5	0.5	0.25
12	256	16	32	3	0.15	0.38	0.75	0.75	0.75	0.75
13	256	16	2	0.5	0.31	-	-	-	-	-
14	256	48	2	0.5	0.45	-	-	-	-	0.75
15	12	2	6	0.75	0.28	-	-	-	-	0.25
16	6	1.5	32	6	0.43	0.38	0.38	0.75	0.75	0.25
17	6	2	32	16	0.8	0.38	0.5	0.75	0.75	1.5
18	6	2	32	6	0.5	0.25	0.25	0.5	0.5	0.5
19	12	3	32	4	0.37	0.25	0.25	0.19	0.38	0.5
20	12	3	32	12	0.62	0.38	0.38	0.38	0.5	0.75

The results of the COL and MER combination are shown in table 2. With E-test cross formation, the FIC index ranged from 0.21 to 1.75. In detail, for 4 strains the antimicrobial combination was a synergistic while for all the other was indifferent. With E-test gradient dilution, the results showed synergistic activity-dependent to meropenem concentration in the medium.

Table 2. Comparison of results by E-test cross formation and E-test agar dilution for synergistic activity of colistin (COL) and rifampicin (RIF) combination. (-) indicates no growth

	E-test Cross formation					E-test Gradient dilution with MER (ug/ml)				
	MIC (ug/ml)					COL MIC (ug/ml)				
Strains	**COL alone**	**COL col+mer**	**MER alone**	**MER Mer+col**	**FICI**	**32**	**16**	**8**	**4**	**2**
1	256	128	12	6	1	-	-	1	6	16
2	256	128	32	8	0.7	-	1	1.5	3	3
3	256	32	32	6	0.3	-	1.5	3	6	256
4	256	64	32	8	0.5	-	16	256	256	256
5	256	128	32	16	1	-	1.5	4	256	256
6	256	128	32	8	0.7	-	1.5	1.5	2	2
7	256	128	32	16	1	-	0.38	1.5	2	4
8	256	128	32	12	0.8	-	0.75	1.5	4	4
9	256	256	16	6	1.3	-	-	3	12	12
10	256	128	32	12	0.8	-	1.5	8	256	256
11	8	4	32	16	1	-	1	1.5	2	2
12	256	24	32	4	0.2	-	1	1.5	6	4
13	256	128	32	12	0.8	-	-	1	4	4
14	256	96	32	8	0.6	-	0.75	1.5	1.5	2
15	12	6	32	8	0.7	0.5	0.75	1.5	1.5	1.5
16	6	6	32	32	2					
17	6	3	32	16	1	-	0.75	1.5	3	3

18	6	1.5	32	6	0.4	-	1.5	1.5	2	1.5
19	12	6	32	6	0.6	-	2	3	16	8
20	12	6	32	16	1	-	0.38	0.75	1	0.75

At last, MICs of colistin decreased below to susceptibility breakpoint only in presence of meropenem concentration of 32-16 µg/ml.

Discussion

A. baumannii, commonly known for causing ventilator-associated pneumonia (VAP), bloodstream infections and sepsis, especially in critically ill hosts, is becoming difficult to treat because of the limited options available. [27]
The therapy of carbapenem-resistant strains often requires the use of colistin, but alarming resistance rates are rising and are slowly becoming a routine phenotype for this organism. [28, 29]
Colistin-resistant *A.baumannii* occurred among patients who received colistin to treat infection due to *A. baumannii* carbapenem-resistant but colistin susceptible (Nuovo). The colistin resistance is strongly associated with Lipid A modification by the addition of phosphoethanolamine. [19, 30]
Performing synergy tests may be helpful for the choice of combination therapy, especially in care units where patients are critically ill and where resistant strains are more common and difficult to treat. Currently, in patients with MDR gram-negative pneumonia, the colistin combination therapy turned out to be more successful than colistin monotherapy. [31, 32]
Then, a reliable in vitro test that could accurately predict the synergy of antibiotic combinations, simple to perform and reproducible would be an important contribution for its use in clinical settings.
We assessed in vitro activity of colistin in combination with meropenem and rifampicin against 20 colistin-resistant *A. baumannii* strains, using two rapid E-test methods. Our data showed that colistin/rifampicin combination presents high synergistic activity in vitro with both methods for all the tested strains; on the other hand, for colistin/meropenem combination there was no concordance of results. With E-test agar dilution the synergistic activity correlated to the concentration of meropenem and it was synergistic at 32-16 µg/ml. However, this study demonstrates that the colistin/rifampicin combination is effective against colistin-resistant strains while the colistin/meropenem combination may be synergistic only in the presence of an adequate concentration of meropenem.

Conclusions

Antimicrobial resistance is an increasingly serious global health problem for both humans and animals, which limits or renders treatment options less effective while decreasing the quality of life and increasing the cost of healthcare. In the EU, every year, are an estimated 25,000 deaths for infections caused by antimicrobial-resistant bacteria.
At this rate, it is assumed that by 2050 the number of people who died prematurely worldwide due to antimicrobial resistance will be 300 million.
Nowadays *A. baumannii* is one of the most important emerging, opportunistic pathogens that can affect critical patients. It requires complex antibiotic therapy, including colistin, the effect of which can be enhanced by associating rifampicin as demonstrated by our results.

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