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In vitro synergistic effect of colistin in combination with meropenem and rifampin against carbapenem–resistant e. *coli* and *k. pneumoniae*

Abstract

Combination therapy is being investigated in order to improve the clinical success of colistin. We assessed the activity of colistin in combination with meropenem/rifampin against carbapenem–resistant isolates. Synergy occurred *in vitro* in all the tested isolates in which colistin minimal inhibitory concentration (MIC) values were at sub–MIC levels. There was no significant difference observed in our results between checkerboard and plate synergy method. Our findings indicated the utility of plate synergy method in order to predict the activity of specific antibiotic combinations. More importantly, these combinational drugs could be a good candidate for carbapenem–resistant bacterial infections.

Keywords: combination therapy, checkerboard, plate synergy method, synergism, colistin

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Abbreviations: MIC: Minimal Inhibitory Concentration; FIC: Fractional Inhibitory Concentration; LPS: Lipopolysaccharide; OM: Outer Membrane

Introduction

Antibiotic resistance poses a real threat to treat infections caused by pathogenic bacteria.[1] As there are increasing numbers of reports on bacterial resistance to antibiotics especially during monotherapy, there is a renewed interest towards combination therapy.[2] Synergistic effects of various drug combinations are under study and some combinations are prescribed clinically.[3,4] The problems with existing synergy tests are; there are no exact gold–standard methods to perform the test in clinical laboratories, the available reference tests such as checkerboard and time–kill analysis are time–consuming, requires predetermined minimal inhibitory concentration (MIC) values and interpretation of results are difficult.[5–7] Combination therapy is essential at least for the treatment of patients to whom monotherapy fails. Carbapenem and colistin are considered as an antibiotic of last resort and rifampin is frequently used in combination with other antibiotics to increase its efficiency. In order to make combination therapy more precise, an *in vitro* synergy test methods should be more rapid and clinically dependable.[5] In this study, two different synergy tests (checkerboard and plate synergy method) were compared using the combination of colistin–meropenem and colistin–rifampin.

In this study, ten clinical isolates were included; five *Escherichia coli* and five *Klebsiella pneumoniae* collected from diagnostic centers in Chennai, Tamil Nadu. All the isolates were found to be resistant to rifampin and carbapenem by agar dilution method, and screened for carbapenem resistant genes such as NDM, IMP, VIM, KPC, GIM, AIM, BIC where as two each of *E. coli* (EC4, EC5) and *K. pneumoniae* (KP4, KP5) were identified as NDM (New Delhi metallo beta–lactamase) producer by polymerase chain reaction. For evaluation of synergistic activity of colistin in combination with meropenem and rifampin, both checkerboard and plate synergy methods were followed.[7] Initially, MIC was performed (following CLSI guidelines) for colistin and meropenem, the concentrations of antibiotics used throughout this study was 0.06, 0.12, 0.25, 0.50, 1, 2, 4, 8, 16, 32, 64, 128 µg/ml. For determining the checkerboard results for colistin in combination with meropenem and rifampin, micro–broth dilution method (Muller–Hinton broth in 96–well microtiter plate) was followed using the lowest and the highest concentrations of antibiotics as follows; colistin (0.06–8 µg/ml), meropenem (0.06–32 µg/ml) and rifampin (0.5–16 µg/ml). Fractional Inhibitory Concentration (FIC) index was calculated using the FIC of drug A+FIC of drug B whereas FIC is defined as the minimal inhibitory concentration (MIC) of drugs in combination divided by MIC of drugs alone. FIC of ≤1.0 was considered as synergistic, >1.0 as an additive and ≥2.0 as antagonistic, (only) for a combination of colistin with meropenem.[8] For plate synergy method, colistin MIC was determined using E–strips (Hi–media, India). The required concentrations of antibiotics such as meropenem (0.06–32 µg/ml) and rifampin (0.5–16 µg/ml) were prepared in Muller–Hinton agar plates and colistin E–strip was placed, a plate without antibiotic combination served as a control. The entire synergy test results were interpreted based on colistin in combination with meropenem or rifampin and all the experiments were replicated trice to confirm the results.

In this study, the synergistic comparison was made for colistin in combination with meropenem and rifampin. All the results were interpreted using EUCAST guidelines considering clinical breakpoint of colistin as >2 µg/ml [9] Initially, MIC results showed that all the ten isolates were meropenem–resistant; in addition, all the isolates were resistant to colistin when tested alone. When colistin micro–broth dilution MIC values were compared with E–strip MIC values, nearly the same results were obtained with the negligible dispute. In checkerboard method, colistin in combination with meropenem showed that, for the tested *E. coli* and *K. pneumoniae*, colistin MIC values (0.12, 0.25, 1, 2 µg/ml) were below the clinically acceptable range (Table 1). FIC index values for colistin–meropenem were EC1=0.16, EC2=0.13, EC3=2.0, EC4=2.0, EC5=2.15, KP1=0.28, KP2=2.0, KP3=2.0, KP4=0.24, and KP5=2.0. For colistin–meropenem plate synergy method, there was no much variation in colistin MIC results were observed while comparing with checkerboard method (Table 1). In all the tested isolates, meropenem concentration of 4 µg/ml and colistin concentration of ≤2 µg/ml was considered as sufficient

for bacterial inhibition. In the case of colistin combined with rifampin by checkerboard method, the colistin MIC values (µg/ml) were 0.12 (EC4), 0.25 (KP2), 1.0 (EC2, EC5, KP1, KP3) and 2.0 (EC1, EC3, KP4, KP5). Similarly, in plate synergy method using rifampin (Figure1) the colistin MIC values were 0.1 µg/ml (one *E. coli*, one *K. pneumoniae*) and 1.0 µg/ml (4 *E. coli*, 4 *K. pneumoniae*). The MIC values of colistin were found to be below the susceptibility breakpoint in all the tested isolates either with the combination of colistin and meropenem or rifampin.

Table 1 Minimal Inhibitory Concentrations (MIC) of colistin, in combination with rifampin and meropenem against MDR *E. coli and K. pneumoniae*

Drug Combinations and Bacterial Isolates	Colistin MIC (µg/mL) Micro-broth Dilution Method	Meropenem MIC (µg/mL) Agar Dilution Method	Colistin MIC (µg/mL)	†Colistin MIC in Presence of Meropenem (µg/mL) [Checker Board Method]	*Colistin MIC in Presence of Meropenem (µg/mL) Plate Synergy Method	†Colistin MIC in Presence of Rifampin (µg/mL) [Checker Board Method]	*Colistin MIC in Presence of Rifampin (µg/mL) Plate Synergy Method
EC1	8	32	7.5	1	0.1	2	1
EC2	8	32	3	0.12	0.1	1	1
EC3	8	32	3	1	1	2	1
EC4	8	32	7.5	1	1	0.12	1
EC5	8	32	3	0.25	0.1	1	0.1
KP1	8	32	15	1	0.1	1	1
KP2	8	32	7.5	1	1	0.25	1
KP3	8	32	15	2	1	1	0.1
KP4	8	32	7.5	0.12	0.1	2	1
KP5	8	32	7.5	1	0.1	2	1

Resistance break point for colistin (EUCAST) is >2 and meropenem (CLSI) is ≥4.

Bolded numerical represents the values below the clinical breakpoint for colistin (EUCAST).

*For plate synergy method, concentration of meropenem and rifampin used were 4µg/ml and 8µg/ml respectively.

†Colistin MIC values represents the data obtained using 4 µg/ml of meropenem and 8µg/ml of rifampin respectively

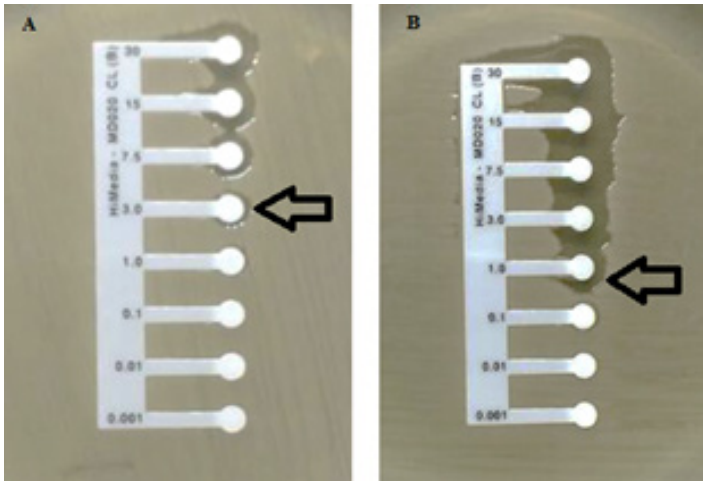

Figure 1 Plate synergy method for detection of synergism between colistin and rifampin.

A) Minimum inhibitory concentrations of colistin on Mueller Hinton agar alone and

B) Supplemented with rifampin 8µg/ml against New Delhi metallo beta-lactamase (NDM) producing *Escherichia coli.*

Conclusion

The mechanism of action of colistin is known to interact with the outer membrane (OM) of Gram–negative bacteria and disrupt the lipopolysaccharide (LPS) layer.[10,7] When colistin is combined with meropenem or rifampin the mechanism of synergistic activity is thought to be the perturbation of the OM by colistin that favours the easy penetration of meropenem/rifampin at intracellular concentrations that enable inhibition of protein synthesis eventually leading to cell death.[7] Colistin is known to be binding with the plastic materials during *in vitro* studies that cause limitations in testing methods.[11] Though there are controversies regarding the MIC values obtained with micro–broth dilution method and E–strip method for colistin.[11] our results showed negligible variations with none of the isolates were misjudged between sensitive and resistance. Earlier studies also showed that colistin–rifampicin and colistin–meropenem/imipenem could exert synergistic effects.[12] The *in vitro* synergistic activity obtained for both colistin–meropenem and colistin–rifampin were below the clinically acceptable range that can have a significant clinical advantage. However, when tested clinically, the exact concentrations needed for the combination of drug A and drug B should not be neglected, and the inhibition of drug need not be

reduced. In this study, checkerboard and plate synergy tests showed similar results suggesting that plate synergy method is easy to perform and with the lesser percentage of error while comparing with standard checkerboard methods that are complicated. In conclusion, the use of plate synergy method to test clinical drug combinations is an effective strategy and the use of combination therapy (colistin–meropenem and colistin–rifampin) to treat carbapenem–resistant bacterial infections is beneficial.

Acknowledgments

None.

Conflicts of interest

None.

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