P 1553

Synergistic effects of rifampicin, nitrofurantoin, fosfomycin and colistin against NDM-1 positive *Klebsiella pneumoniae* and *Escherichia coli* using the new MTS synergy method

Janis Weeks & Timothy R. Walsh

Department of Medical Microbiology and Infectious Diseases, Institute of Infection and Immunity, Cardiff University, Heath Park, Cardiff UK

Introduction.

NDM-1 positive *Klebsiella pneumoniae* and *Escherichia coli* are highly resistant to most antimicrobials and represent particular challenges for antibiotic treatment therefore exploring the synergistic effects of both active and old antibiotics can potentially increase our therapeutic options. However, most synergistic methods are arduous and correspondingly expensive. In order to potentially overcome these difficulties, herein we evaluated the performance of a new gradient-diffusion (MTS) synergy method (MTS) against NDM-1 positive *K. pneumoniae* and *E. coli* non-clonal isolates.

Methods.

17 NDM-1 positive *K. pneumoniae* (12) and *E. coli* (5) non-clonal isolates collected from 4 different countries were tested in duplicate for reproducibility. Drug combinations were: fosfomycin/rifampicin, fosfomycin/nitrofurantoin, fosfomycin/colistin, rifampicin/colistin and colistin/nitrofurantoin. Where synergy was observed, standard FICs (micro broth dilution (MBD)) were used to validate results. First MTS MICs were determined on MH agar. The MTS synergy method was performed on a MTS platform and the MTS strips aligned along the MIC. An MTS applicator was then used to carefully transfer the two MTS at 90 degrees to a MH agar plate. Plates were incubated overnight and synergy (SYN), Additive (ADD), indifference (IND) and antagonism (ANT) interpreted according to the manufacturers' instructions (Liofilchem, Italy).

Results.

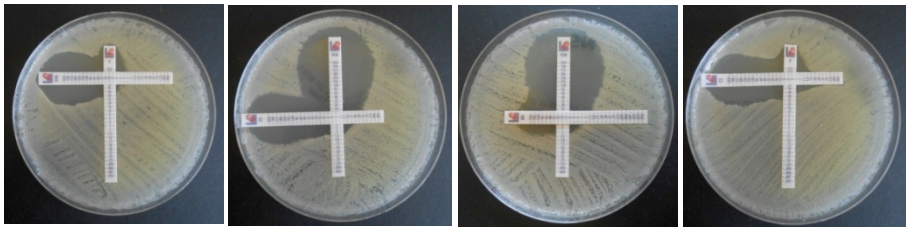

Fig. Examples of MTS combinations with NDM-1 producers. Left – right; ***E . coli* 33-5** nitrofurantoin (F) and fosfomycin (FOS), ***K. pneumoniae* 2** with fosfomycin (FOS) and colistin (CS), ***K. pneumoniae* 34** with rifampicin (R) and fosfomycin (FOS), and ***E . coli* 60** with colistin (CS) and nitrofurantoin (F).

Results.

Out of 85 strain drug combinations, SYN was seen in 11/85 and ADD in 22/85 with the majority of the combinations displaying IND but no evidence of ANT. Only 4/17 strains were prone to SYN and 10/17 to ADD. Nitrofurantoin/colistin and nitrofurantoin/fosfomycin were the most active combinations. (Table). Comparison of MTS with MBD showed very good correlation with approx. 80% agreement and where difference did occur these were very minor and were usually between additive and no effect.

Table. Synergistic effects of rifampicin, nitrofurantoin, fosfomycin and colistin against NDM-1 positive *K. pneumoniae* and *E. coli* comparing MTS with MBD. Cells highlighted in blue show interpretation differences and cells highlighted in red are interpretation agreement.

Strain	Fos/Nit		Fos/Rif		Fos/Col		Col/Nit		Col/Rif	
	MTS	MBD	MTS	MBD	MTS	MBD	MTS	MBD	MTS	MBD
K1	0.13 (SYN)	0.23 (SYN)	0.21 (SYN)	0.32 (SYN)	0.16 (SYN)	0.32 (SYN)	0.63 (ADD)	0.0.9 (ADD)	1.3	1.1
60	1.2	1.3	1.2	1.0 (ADD)	1.2	1.3	0.38 (SYN)	0.21 (SYN)	1.1	1.3
23	2.0	1.5	2.0	1.9	1.8	1.5	1.7	1.5	1.7	1.5
29	1.2	1.2	0.8 (ADD)	1.0 (ADD)	1.5	1.3	1.8	1.8	1.5	1.7
45	1.0 (ADD)	1.0 (ADD)	1.7	1.5	1.1	0.9 (ADD)	1.0 (ADD)	0.9 (ADD)	1.8	1.5
26	0.8 (ADD)	1.0 (ADD)	0.8 (ADD)	1.0	0.9 (ADD)	0.9 (ADD)	1.0 (ADD)	1.1	0.6 (ADD)	0.6 (ADD)
2	0.9 (ADD)	0.8 (ADD)	0.7 (ADD)	0.8 (ADD)	0.7 (ADD)	0.9 (ADD)	1.8	1.7	0.9 (ADD)	1.0 (ADD)
19	1.3	1.5	1.3	1.3	1.1	1.0 (ADD)	2	2	1.5	1.7
33-5	1.0 (ADD)	1.2	1.1	1.1	1.3	1.1	0.48 (SYN)	0.33 (SYN)	2.0	2.0
43	1.5	1.5	1.5	1.5	1.3	1.3	1.6	1.5	1.8	1.8
14	1.4	1.5	1.3	1.5	1.3	1.3	0.8 (ADD)	1.0 (ADD)	1.0 (ADD)	0.9 (ADD)
34	0.48 (SYN)	0.9 (ADD)	1.0 (ADD)	1.0 (ADD)	0.44 (SYN)	0.44 (SYN)	0.9 (ADD)	0.9 (ADD)	1.0 (ADD)	0.8 (ADD)
8	0.95 (ADD)	1.2	0.95 (ADD)	0.95 (ADD)	1.3	1.5	1.4	1.0 (ADD)	1.4	1.3
57	1.5	1.5	1.3	1.3	0.95 (ADD)	1.0 (ADD)	1.3	1.5	0.6 (ADD)	0.48 (SYN)
5	0.34 (SYN)	0.48 (SYN)	0.44 (SYN)	0.44 (SYN)	0.42 (SYN)	0.65 (ADD)	0.21 (SYN)	0.32 (SYN)	1.4	1.4
15	1.6	1.4	1.3	1.5	1.6	1.5	1.5	1.7	1.7	1.5
21	1.2	1.3	1.6	1.6	1.6	1.6	2.0	2.0	1.6	1.5

Conclusions.

The MTS synergy method provides a simple, rapid and extremely cost effect method exploring the combination of antibiotics against highly resistant bacteria. As Enterobacteriaceae become increasingly resistant such methods will becoming increasingly useful to maximize therapy and patient outcome.

Email: walshtr@cardiff.ac.uk