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Article *in* Indian Journal of Medical Microbiology · October 2017

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An Update on Technical, Interpretative and Clinical Relevance of Antimicrobial Synergy Testing Methodologies

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Abstract

Testing for antimicrobial interactions has gained popularity in the last decade due to the increasing prevalence of drug-resistant organisms and limited options for the treatment of these infections. *In vitro* combination testing provides information, on which two or more antimicrobials can be combined for a good clinical outcome. Amongst the various *in vitro* methods of drug interactions, time-kill assay (TKA), checkerboard (CB) assay and *E*-test-based methods are most commonly used. Comparative performance of these methods reveals the TKA as the most promising method to detect synergistic combinations followed by CB assay and *E*-test. Various combinations of antimicrobials have been tested to demonstrate synergistic activity. Promising results were obtained for the combinations of meropenem plus colistin and rifampicin plus colistin against *Acinetobacter baumannii*, colistin plus carbapenem and carbapenem plus fluoroquinolones against *Pseudomonas aeruginosa* and colistin/polymyxin B plus rifampicin/meropenem against *Klebsiella pneumoniae*. Antagonism was detected in only few instances. The presence of synergy or antagonism with a combination seems to correlate with minimum inhibitory concentration of the agent and molecular mechanism involved in the resistance. Further studies need to be conducted to assess the utility of *in vitro* testing to predict clinical outcome and direct therapy for drug-resistant organisms.

Keywords: *Acinetobacter baumannii*, antimicrobial resistance, checkerboard assay, combination testing, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, time-kill assay

Introduction

In recent times, need for synergy testing has been driven by the following reasons: (i) necessity to extend the antimicrobial spectrum, (ii) possibility of reducing the dosage and toxicity and (iii) possibility of reducing the development of resistance.[1] In addition, the emergence of multidrug resistance (MDR), extensive drug resistance (XDR) and pan-drug resistance (PDR) strains, combined with the lacunae in the development of newer antimicrobial agents, has contributed to the necessity for the synergy testing between various combinations of antimicrobial agents.

The development of drug-resistant organisms is the prime cause for the increase in healthcare-associated infections, especially ventilator-associated pneumonia (VAP) and bacteraemia. Among the hospital-acquired infections (HAIs) due to Gram-negative organisms, MDR-Gram negative bacilli (GNB) infections accounted for 36.8% in a tertiary care centre in Taiwan during a 7-year period (2002–2009).[2] Similar trend was seen in South America, where a tertiary care centre in Brazil recorded 3.7-fold increase in the infection rates due to MDR-GNB during 1999–2008.[3] The development of MDR and carbapenem resistance was increasingly seen, especially for *Acinetobacter baumannii*.[4] However, good infection control practices were able to decrease the overall HAI rates, and the trend remains unchanged for GNB-HAI contributed by carbapenem resistance organisms.[2]

Alternative therapies or treatment strategies for such XDR and carbapenem-resistant (CR) GNBs are limited. Nevertheless, old drugs such as colistin, fosfomycin and tigecycline can be used in combination with other agents. In the past decades, the use of colistin has been restricted by the

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How to cite this article: Laishram S, Pragasam AK, Bakthavatchalam YD, Veeraraghavan B. An update on technical, interpretative and clinical relevance of antimicrobial synergy testing methodologies. Indian J Med Microbiol 2017;35:445-68.

concerns of toxicity and problems in optimisation of dosage. Tigecycline use was hampered by its large distribution volume, leading to sub-inhibitory levels and selection of resistant strains with increase in the geometric mean of minimum inhibitory concentration (MIC).[5] Further, the Food and Drug Administration approved the use of tigecycline only for complicated skin and soft tissue infections, intra-abdominal infections and community-acquired pneumonia. However, it was not approved for the use in VAP because of higher mortality rate. Fosfomycin was reported to have superior *in vitro* activity against CR-*Enterobacteriaceae* isolates but was restricted for the treatment of urinary tract infection (UTI). Very few reports exist on the use of fosfomycin for other systemic infections. Moreover, fosfomycin must be used in combination with other antimicrobial agents because of high rate of resistance mutation. To overcome the aforementioned concerns, it is essential to test different antimicrobial combinations including the agent to which the organism has developed resistance.

The rationale behind the choice of combination therapy is that the antimicrobials will have a synergistic effect when given together. This review summarises the various methods available to determine synergy between different antimicrobial agents and to provide scientific evidence for utility of such combinations in the clinical setting. In particular, special focus is given on *in vitro* efficacy of the combined antimicrobials against drug-resistant *A. baumannii, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

Technical Performance of Methods for the Determination of Interactions between Antimicrobial Agents

Although many test methods are available to determine the interaction between antimicrobial agents, they were not well standardised. Interpretation criteria followed for test results are not defined and remain uncertain. The various testing methods for determining the synergistic activity of antimicrobials are discussed below.

In vitro assay

Time-kill assay

The time-kill assay (TKA) is considered as the standard reference method for the determination of synergy between antimicrobial agents. TKA determines the actual reduction in the viable count of the organism after exposure to the drug combination compared to the most active single agent at different time intervals. This is done by adding a standard inoculum in broths containing the individual antimicrobial agents and its combination. Sub-culturing is done from the broth containing antimicrobials at different time intervals and the bacterial count is done. Colony count is done at shorter time intervals e.g., every 2 h over a 24-h period for drugs having concentration-dependent activity. For drugs having time-dependent killing activity, colony count is done every 3–4 h till 24–48 h. The determination of the synergistic action by TKA is defined as ≥ 2 $\log_{10}$ CFU/ml reduction in the bacterial growth in the combination when compared to the most active single agent. However, antagonism is defined by an increase of ≥ 2 $\log_{10}$ CFU/ml in the combination compared to the most active single agent. Less than 2 $\log_{10}$ CFU/ml difference is interpreted as indifference. Bactericidal effects of the combinations are determined by a decrease of ≥ 3 $\log_{10}$ CFU/ml from the initial inoculum.

Another method of interpretation of the TKA is area under the killing curve (AUKC),[6] where instead of measuring the $\log_{10}$ difference; the result was plotted on a graph with the $\log_{10}$ CFU/ml value in the Y-axis and the time at the X-axis. The AUKC is calculated for single agent and combinations as well. Any statistically significant difference with $P < 0.05$ is taken as a synergistic interaction.[6] This method of interpretation was found to be robust with high precision and less intra-experimental variation but not widely used.

This method allows the testing of one concentration and one ratio of the antimicrobials at one time. The test has to be repeated to observe interactions at other concentrations and ratios. There is also a lack of consensus as to a standard inoculum of the organism to be used though the inoculum size varied from 1 to 5×10^5. The reported concentration of antimicrobials tested in other studies varies from 0.125 × MIC to 4 × MIC.[7] When drug combinations are tested at the MIC or more than MIC concentrations, the test may be hard to interpret because inhibition of the organism by the single agent may preclude demonstration of synergy.

Some authors prefer testing of drug concentrations that are achievable in human serum when standard dosing regimens are administered.[8] Though this strategy incorporates the pharmacokinetic (PK) property of the tested drugs, it does not implicate the concentration of drug at tissues or other sites of infection. Thus, results may not be extrapolated to particular organ system infection such as VAP where the serum concentration of the drug may not reflect the tissue concentration. The drug concentration in the *in vitro* test does not vary, while *in vivo*, there is a variation in the concentration and ratio of the drugs used. This depends on the PK and pharmacodynamic (PD) property of the drugs, dosing interval, strength and route of administration. The drawbacks of TKA include testing of limited antimicrobial concentrations, non-standardised inoculum size and antimicrobial concentration, static concentration of the drug, labour intensive and time-consuming.

Checkerboard assay

The checkerboard (CB) assay utilises a panel of antimicrobial combinations at different concentrations either in the macrobroth (2 ml volume) or microbroth (100 µl volume) method. The range of tested concentrations varies from four to eight times the MIC to at least 1/8–1/16 of the MIC. It is important to include broad range of concentrations because MIC can vary depending on the method used and also within the method (a variation of one/two-fold dilution is allowed within a test system). For the interpretation of result, the fractional

inhibitory concentration (FIC) is calculated for each antibiotic at a given concentration combination by the following formula:

FIC of agent A = MIC of agent A in combination/MIC of agent A alone

The cumulative FIC is then calculated by summing up the FIC of both the agents. 'Synergy' is interpreted when the FIC index is ≤0.5, 'indifference' or 'no interaction' corresponds to the FIC index >0.5–4.0 and 'antagonism' when the FIC index is >4.0.[9]

However, in some studies, authors have defined 'partial synergy' for FIC index between >0.5 and <1 and an 'additive interaction' for FIC index of 1. Reporting of such results has to carefully considered because of the acceptance of inherent one tube dilution variation with this method and possibility of reproducibility error.[9] This was addressed by Rand *et al*., who reported 25% discordance with the CB method and suggested testing in at least five replicates and considering the reading only with ≥80% agreement between the replicates.[10] Another contentious issue with CB assays is the use of different criteria to interpret the test.

E-test

E-test strips containing gradient of antimicrobial agents have been used to determine the synergistic combinations. The different methods are (i) *E*-test cross method, (ii) *E*-test fixed ratio method, (iii) *E*-test agar method and (iv) *E*-test MIC: MIC method.

E-test cross method

Mueller-Hinton agar (MHA) plate is inoculated with 0.5 McFarland matched inoculum, to which *E*-test strips are placed one over the other at 90° angle crossing at the MICs of the individual agent of the organism determined earlier [Figure 1]. After incubation for 18 h, the zone of inhibition is read and the FIC index is calculated and interpreted as described for CB assay.[11]

E-test fixed ratio method

In this method, MHA plates are inoculated with 0.5 McFarland matched inoculum. *E*-test strip of the first agent is placed and incubated at room temperature for 1 h to allow the antimicrobial to diffuse into the medium. After 1 h, it is removed and saved as MIC template. The *E*-test strip for the second agent is then placed directly over the imprint of the first strip [Figure 2]. The FIC index is again calculated and interpreted as described for CB assay.[12,13]

E-test agar method

In this method, MHA plates are incorporated with 0.5 × or 0.125 × MIC of one agent and the *E*-test strip of the second agent is placed over the inoculated surface [Figure 3]. The MIC obtained is compared with the MIC in drug-free medium. The synergy is interpreted when there is more than three-fold reduction in MIC on the drug-incorporated medium.[13]

E-test minimum inhibitory concentration: minimum inhibitory concentration method

In this method, one test strip is placed on the inoculated MHA plate and incubated at room temperature for 1 h to allow diffusion of the agent. After 1 h, the agar is marked adjacent to the previously determined MIC of the agent and removed. The second *E*-test strip is then placed over the imprint of the previous strip such that the mark on the agar corresponds to the MIC of the second agent [Figure 4]. The resulting ellipse of inhibition is read after 20 h of incubation at 37°C. The FIC index is calculated and interpreted as like that of CB assay.

Compared to the other commonly used methods such as TKA and CB assay, *E*-test methods are technically simpler to

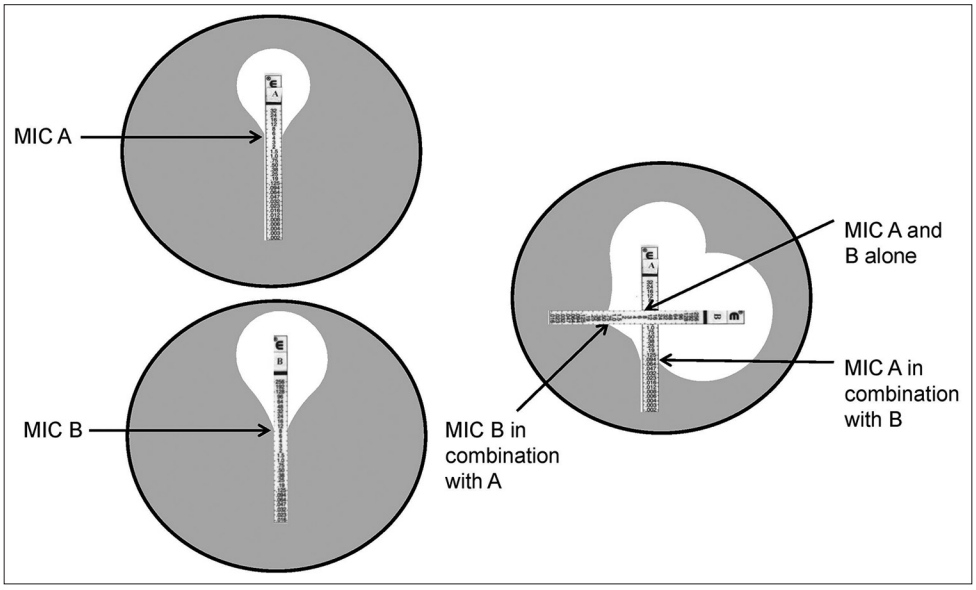

Figure 1: *E*-test cross method. In this example, minimum inhibitory concentration of A is 6 μg/ml and minimum inhibitory concentration of B is 8 μg/ml. After combination of A and B, minimum inhibitory concentration of A is 0.094 μg/ml and minimum inhibitory concentration of B is 0.75 μg/ml. ∑FIC = 0.1 (synergy). FIC: Fractional inhibitory concentration

perform and reproducible. The limitations of *E*-test methods are the inability to determine interaction of more than two antimicrobial combinations and the limited gradient of antimicrobial on the paper strip. For organisms where the MIC is more than the highest concentration on the strip, difficulties may be encountered with calculation of the FIC index and may result in the false interpretations. In addition, detection of antagonistic combinations will be limited for such isolates. With the *E*-test cross method, mild degree of antagonism may not be detected because of overlapping of strips.[11]

In vitro pharmacokinetic model

The various *in vitro* tests for the determination of antimicrobial interactions involve testing of drugs at a static concentration without any change in concentration with time. However, the *in vivo* drug concentrations and ratio keep changing with time. To better simulate these changing conditions, PK models were designed. In a single-compartment model, a glass apparatus with inlet and outlet is maintained at 37°C. Fresh media with antimicrobial agents are loaded and the media from the apparatus are withdrawn using a peristaltic pump at a constant rate mimicking the elimination kinetics of the drug and the half-life at the standard dosing regimen of the drug tested. The compartment is charged with a standard inoculum and the change in organism load is compared between single agent and the combination.[14]

In a two-compartment model, a similar central compartment as above was used with a constant volume with changing antimicrobial concentration. The compartment is connected to three or four dialyser unit which acts as the peripheral compartment. Each peripheral compartment containing 150 ml of the organism culture is exposed to a changing antimicrobial concentration similar to the central compartment. The whole system was then kept at 37°C. This method enables simultaneous testing of up to four isolates.[15,16]

The two models mimic the *in vivo* PK property of the individual agents at the standard dose and regimen. The change in CFU/ml was compared using a standard inoculum for the single-drug administration and the combination regimen at regular time intervals. Synergism is interpreted by decrease of ≥2 $\log_{10}$ CFU/ml compared to the best monotherapy regimen or AUKC analysis can be used to detect synergistic interactions.[15,16]

Hollow fibre infection model

The hollow fibre bioreactor is an important advancement in the *in vitro* combination testing. Currently available *in vitro* testing methods have a drawback of not examining time and concentration of the drug at various exposure concentrations. Hollow fibre model has an advantage of considering PK and PD parameters; thereby it mimics the *in vivo* conditions with dynamic concentration of drug over time. The bioreactor module contains thousands of filters with 200 μ in diameter. The peripheral chamber containing the bacteria is separated from the central compartment via semi-permeable membranes, which allows the flow of nutrients and other molecules in and out while retaining the bacteria. These fibres are designed in such a way that the fibre acts as barriers for the flow of contents. Drug concentration is adjusted through infusions at different intervals and by supplying fresh medium to promote dilution of the drug. By adjusting the volume of central reservoir, a state of dynamic concentration of the drug is created, without

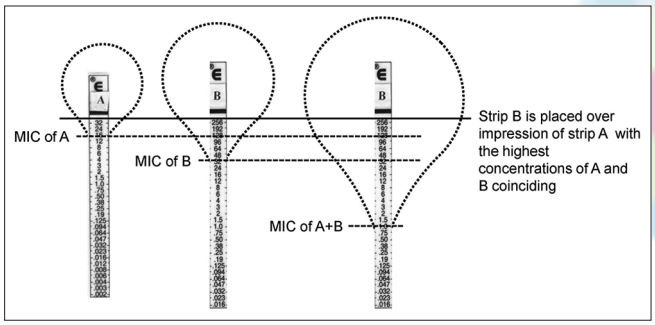

Figure 2: *E*-test fixed ratio method. In this example, minimum inhibitory concentration of A is 16 μg/ml and minimum inhibitory concentration of B is 32 μg/ml. Minimum inhibitory concentration of combination A and B is 1 μg/ml. ∑FIC = 0.09 (synergy). FIC: Fractional inhibitory concentration

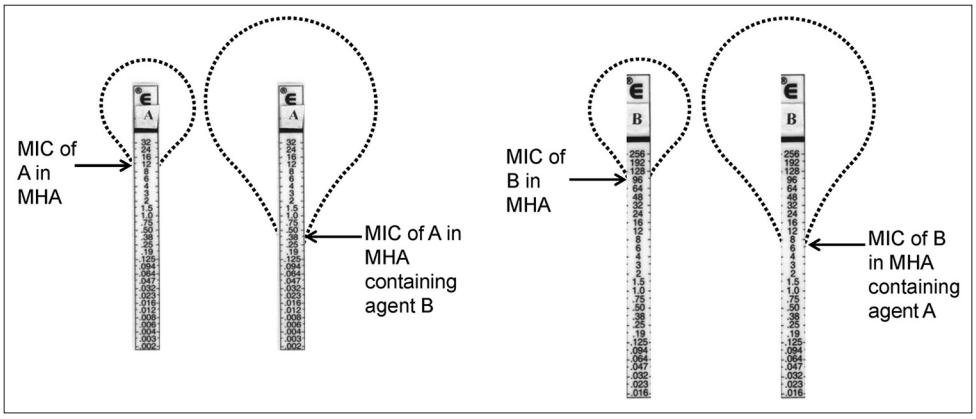

Figure 3: *E*-test agar method. In this example, minimum inhibitory concentration of A is 12 μg/ml and in combination 0.38 μg/ml. Fractional inhibitory concentration of A = 0.03. Minimum inhibitory concentration of B is 96 μg/ml and in combination is 6 μg/ml. Fractional inhibitory concentration of B = 0.06. ∑FIC = 0.09 (synergy). FIC: Fractional inhibitory concentration

diluting the bacterial load in the peripheral compartment. Sampling is done from the peripheral compartment at different intervals to quantify the drug concentration and the bacterial count [Figure 5]. This phenomenon provides the reliable PK and PD profiles, which could be considered for clinical decision-making. Two-compartment hollow fibre infection models provide advantages over one-compartment model with respect to the variable concentration of drug exposure over time. Such *in vitro* PK/PD models are cost-effective and resource intensive. Moreover, it permits the investigation over considerable duration, which is not feasible to perform in animal models. However, this method is technically demanding and requires complex instruments and difficult to standardise.[17]

Critical inhibitory concentration

Determination of critical inhibitory concentration (CIC) was shown to help predict *in vivo* synergistic effect.[18] For the determination of CIC, a pour plate of media inoculated with the organism is prepared. Ten-millimetre holes are made and filled with combinations of the antimicrobials at different concentration ratios and at graded concentrations [Figure 6]. After incubation for 20 h at 37°C, the distance from the edge of the well till the edge of the zone of inhibition is measured (*d*). The square of d (d^2) was then plotted against the concentration of antibiotic at time zero ($\log_e m_0$). A straight line was obtained intercepting the $\log_e m_0$ axis, and antilog of this point of interception gives the CIC value of the combination. A lower CIC indicates a higher killing effect. Using CIC, Chan *et al*. demonstrated the synergistic activity for the combination of amikacin and piperacillin at the ratio of 70:30 for *P. aeruginosa* and was confirmed by TKA and *in vivo* mouse model.[18]

Double disc synergy

Double disc synergy test was conventionally used for the detection of extended-spectrum beta-lactamase (ESBL) production and can also be used for the detection of synergy between antimicrobial combinations. In this method, discs containing the antimicrobials are placed 20 mm (or sum of radii of the zone of inhibition of each drug separately) apart over a lawn culture of the organism and incubated at 37°C. Synergy was indicated by an increase in the zone diameter of ≥2 mm compared to the single agent or bridging of the zone of inhibition [Figure 7].[19,20] An increase of <2 mm in the zone of inhibition is classified as weak synergy, and antagonism is indicated by truncation of the zone of inhibition at the junction of the two antimicrobials. For *P. aeruginosa*, this method was shown to give more synergism for a combination of antimicrobials than CB assay.[19] In addition, double disc synergy test was observed to show more synergy than other methods such as agar-based and broth-based dilution method.[21] Despite the simplicity and easy interpretation of results, this method has not been widely used because of its qualitative nature and subjective interpretation.

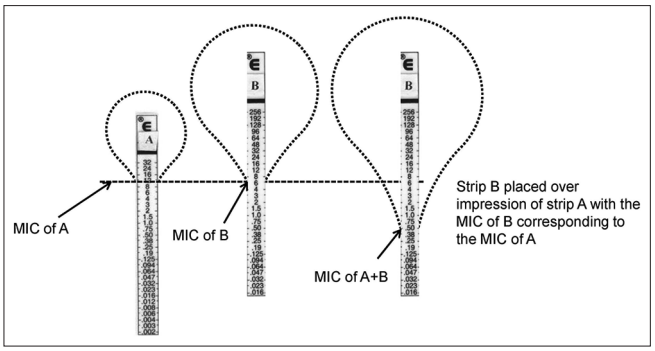

Figure 4: *E*-test minimum inhibitory concentration: minimum inhibitory concentration method. In this example, minimum inhibitory concentration of A is 12 μg/ml and minimum inhibitory concentration of B is 6 μg/ml. Minimum inhibitory concentration of combination A and B is 0.5 μg/ml. ∑FIC = 0.12 (synergy). FIC: Fractional inhibitory concentration

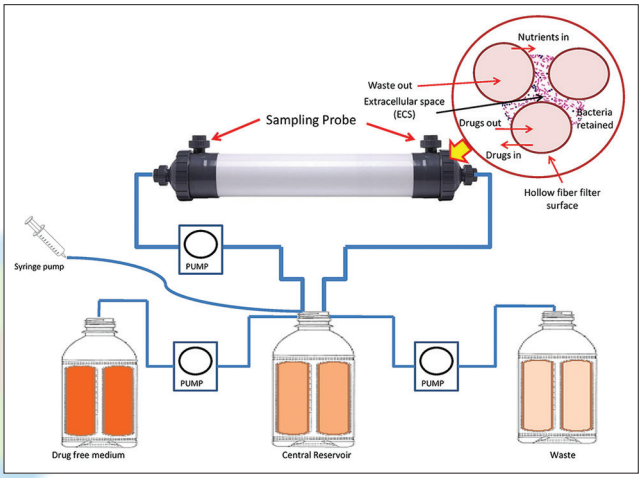

Figure 5: Hollow fibre infection model for *in vitro* antimicrobial combination testing

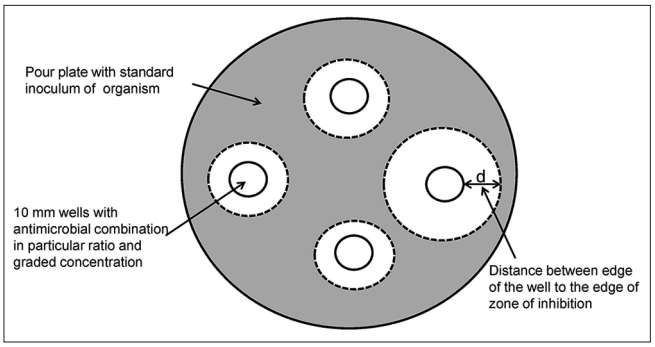

Figure 6: Scheme for critical inhibitory concentration determination. 'd' is the distance between the edge of the well and the edge of zone of inhibition in mm. 'd^2' plotted against concentration of drug gives value of critical inhibitory concentration

Paper strip diffusion

In this method, filter paper strips soaked in different antimicrobial solutions at or above MIC were placed at right angles on the MHA plate inoculated with the test organism. Antibiotics in the filter paper strips are allowed to diffuse

in the medium and are removed after several hours and the plates are incubated for 18–24 h at 37°C. Alternately, the antibiotic soaked strips can be overlaid onto un-inoculated plate media for 24 h for diffusion and the organism inoculated using a membrane transfer technique [Figure 8].[22] The pattern of growth of the organism was interpreted as follows: indifferent (additive) effect is considered as two oval area of inhibition joining at right angles, synergism is indicated by broadening of the inhibition around the angle and antagonism is indicated by indentation or narrowing around the angle.[23] This method provides qualitative result and has not been widely evaluated.

Multiple-combination bactericidal test

Multiple-combination bactericidal test is done in 96-well microtiter plates. Different combination of antimicrobials with a standard inoculum is added into each well and incubated for 48 h. All the non-turbid wells following incubation is sub-cultured onto antimicrobial-free medium and checked for 99.9% killing. Antagonism is defined as growth of the organism on addition of a second antibiotic to a single agent which was bactericidal when tested alone. Though this method detects the extent of bacterial killing, the outcome is not clearly defined. Enhancement of bactericidal activity of a previous non-bactericidal drug in combination can only be made out in terms of a synergistic combination. Its use is limited to the detection of antagonistic combinations rather than a synergistic combinations for agents used for the treatment of respiratory infection in cystic fibrosis patients.[24,25]

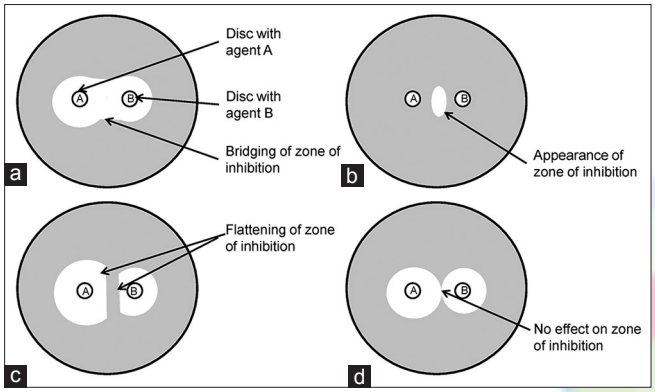

Figure 7: Double disk synergy test. (a) Synergy (bridging of zone of inhibition); (b) synergy (appearance of zone of inhibition in between agent A and B); (c) antagonism (flattening of zone of inhibition); (d) indifference/additive (no effect on zone of inhibition)

Overlay inoculum susceptibility disc method

In this method, solid media incorporated with half the MIC of one agent were used as an agar base over which molten antibiotic-free agar with a standard inoculum of the organism is poured to obtain an overlay inoculum layer. Similar control plates are prepared without antibiotic containing base. Antimicrobial discs are placed over the plate and incubated [Figure 9]. An increase in the inhibition zone diameter (IZD) by 19% corresponds to synergy, <19% synergy corresponds to additive effect and no variation in IZD is an indicative of indifference.[26] Nworu and Esimone demonstrated agreement of this technique with CB with both techniques, showing synergistic interaction between ampicillin and ciprofloxacin for *Staphylococcus aureus* and *Escherichia coli*.[27] However, this method has not been widely evaluated.

Serum bactericidal titre

For the better prediction of the PK property of the antimicrobials tested, synergy can be tested using the serum bactericidal titre (SBT) method. This method takes into account not just the drug elimination kinetics but also the protein binding and the effect of metabolic congeners of the antimicrobial agents.[22] Here, serum from patient or volunteer is collected to get the

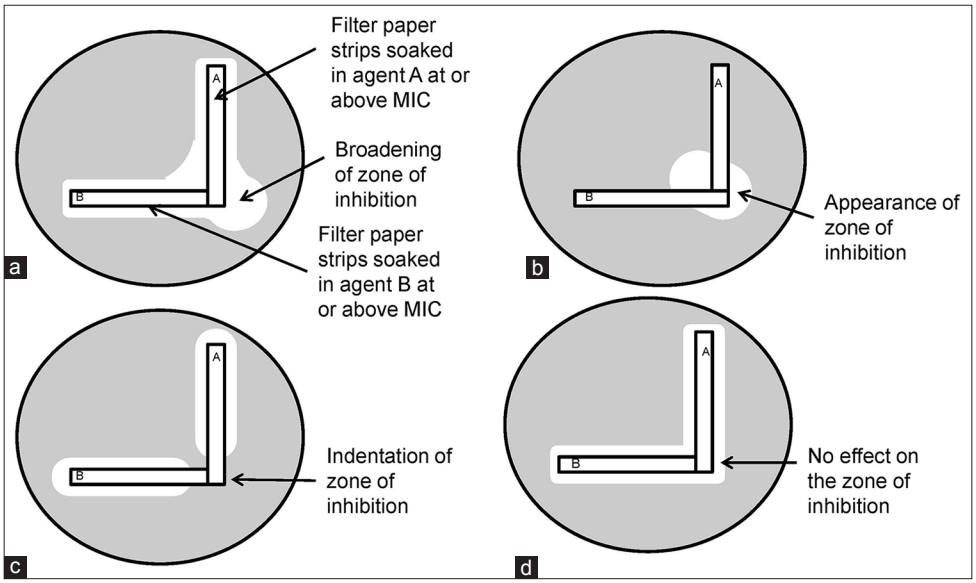

Figure 8: Paper strip diffusion test, (a) synergy (broadening of zone of inhibition at the angle); (b) synergy (appearance of zone of inhibition at the angle); (c) antagonism (indentation and narrowing of zone of inhibition at the angle); (d) indifference/additive (no effect in the zone of inhibition)

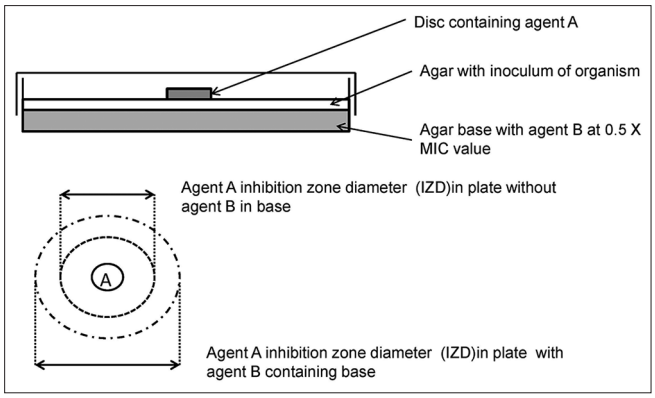

Figure 9: Overlay inoculum susceptibility disk method for determination of synergy. Increase in inhibition zone diameter >19% indicates synergy

peak and the trough level of the antimicrobial in single doses and in combination. The serum is serially diluted and standard inoculum of the organism inoculated. The highest dilution of the patient serum which results in 99.9% killing is designated as the SBT. The minimum bactericidal concentrations of the antimicrobials are determined in Mueller-Hinton broth and the free drug concentrations (drug-f) in serum are determined. The drug interaction is determined using the formula given below:

Drug-A-f/(MBC-A)(SBT) + Drug-B-f/(MBC-B)(SBT)

A value of ≤0.25 indicates synergy, 0.25–4 indicate additive effect and ≥4 indicate antagonism.

Robinson *et al*. compared the SBT with *in vitro* TKA and CB in patients receiving multiple antimicrobial combinations for endocarditis, osteomyelitis or severe septicaemia.[28] Compared to CB assay, SBT detected synergy in 3/10 tests while CB detected synergy in 2/10 tests. One antagonistic combination detected by SBT was determined as synergistic by CB, while two of the synergistic combinations by SBT were determined as antagonistic by CB. TKA detected more number of synergy than either SBT or CB (6/10 at 0.5 × MBC and 5/10 at 1 × MBC). There was no concordance among the three methods when strict definitions are used. However, for four additive combinations tested by SBT, results of synergy or indifference were achieved in the TKA and CB.

Technical difficulties encountered with SBT include difficulty in measuring the drug-f concentration and the need to compare SBT following removal of the antimicrobials from the sample to exclude bactericidal effect due to complement or other inhibitors in the sample.

In vivo models

In vivo studies are essential for the translation of *in vitro* combination testing data to clinical trials for implementation in the clinical setting. The *in vitro* methods does not consider the following factors: pharmacokinetics of the antimicrobials in combination, difference in the route of delivery, humoral and cellular immunity of the host, site of involvement, inoculum of the organism at the infected site, virulence factors of the organism and continuous changing concentration of the antimicrobials as single agent and relative to one another (changing ratio of drug concentration).[29] Animal model studies may confirm or contradict *in vitro* findings based on the PD properties of the antimicrobial agents as well as the host immune response. In addition, *in vivo* models are necessary to determine the optimum dosing strategy.[30]

To better simulate the *in vivo* conditions, various experimental models of infection have been used. Synergy between different drug combinations is determined by statistically significant survival rate or organism load reduction in the combination therapy compared to the most active single-drug regimen. However, using these criteria, additive effect cannot be differentiated from a synergistic activity. Fantin and Carbon suggested to define *in vivo* synergy as 'a significant bactericidal effect of the drug combination in comparison with the sum of the bactericidal effect of each agent alone in comparison with the effect in untreated animal'.[30]

For the mouse pneumonia model, the organisms are inoculated intranasal and kept in hyperoxic condition.[31] For a systemic infection model, organisms are inoculated intraperitoneally in neutropenic mice.[18] Due to ethical and technical considerations, invertebrate models of infections have become an attractive option to study pathogenesis. *In vivo* models involving larva of *Galleria mellonella* (wax moth) has been used for the study of antimicrobial efficacy as infection in this model is amenable to treatment.[32] Hornsey and Wareham demonstrated combination of colistin and vancomycin to be highly effective (>90%) in protecting the larva against infection with both a susceptible and a bla_{OXA-23} producing-resistant strain of *A. baumannii* which showed synergism *in vitro* by CB assay.[33] On the other hand, combination of colistin and teicoplanin was more effective in controlling infection by the susceptible strain than the resistant strain. Monotherapy with vancomycin also showed *in vivo* activity. This has been postulated to be due to ability of vancomycin to enhance the immune response in the larva. O'Hara *et al*. reported a significant improvement in the survival of larva using combinations of doripenem and vancomycin and triple combination of colistin, doripenem and vancomycin in colistin-resistant *A. baumannii* infection. In the same experiment, TKA did not demonstrate synergy with doripenem and vancomycin combination.[34] Hornsey *et al*. also demonstrated the synergistic activity of telavancin and colistin against *A. baumannii*.[35] In spite of its good turnaround time (96 h), simplicity of procedures and clearly defined endpoints, results obtained in the invertebrate model need to be confirmed in vertebrate model as this model may not reflect the exact mammalian *in vivo* milieu.

Comparison of different methods of detection of synergy

Table 1 summarises the relative merits and demerits of the different methods for determination of synergy.

Table 2 gives the comparison of commonly used methods of determination of synergy for *A. baumannii, P. aeruginosa* and *K. pneumoniae*. Synergy is detected most often by TKA followed by CB. *E*-test detected least of synergistic interactions.

Table 1: Relative merits and demerits of methods of determination of antimicrobial interaction

Method	Advantage	Disadvantage
In vitro methods		
E-test	Easy to perform	Different methods used
		Detects less synergy
		Restricted to two drugs combination only
Checkerboard	Relatively easy	Different methods for interpretation
	Multiple concentrations tested	Intra-assay variation
		Static concentration of drugs
Time-kill assay	Reference assay	Limited concentrations tested
	Measures bactericidal effect of combination (both rate and extent of killing)	Time-consuming and labour intensive
		Static concentration of drugs
MCBT	Detects bactericidal effect	Only useful for determination of antagonistic combinations
Double disc synergy	Simple and easy	Qualitative measure
Paper strip diffusion	Simple and easy	Qualitative measure
		Not widely evaluated
OISDM	Relatively easy	Not widely evaluated
CIC	Detects synergism at a particular ratio of the combination	Technical expertise required for performance and interpretation of assay
In vivo PK	Takes PK of drugs in consideration	Technically complex
		May not necessarily reflect *in vivo* conditions
SBT	Takes PK of drug in consideration	Technically complex
		Need for volunteers or patients
Hollow fibre infection model	Takes PK of drug in consideration	Technically complex
In vivo methods		
Mouse models	Takes PK and PD properties of the drugs into account	PK and PD in humans may be different from that of mouse
Larva of *Galleria mellonella*	Simpler model than mouse	Invertebrate model may not mimic conditions in mammals
		Needs confirmation in vertebrate model

MCBT: Multiple combination bactericidal test, OISDM: Overlay inoculum susceptibility disc method, CIC: Critical inhibitory concentration, SBT: Serum bactericidal titre, PK: Pharmacokinetic, PD: Pharmacodynamic

Concordance rates between TKA, CB and *E*-test observed were as follows: 33.3%–100% between CB and TKA; 60%–80.6% between *E*-test and TKA; 83%–84.4% between CB and *E*-test; 52%–75% with all three methods. In spite of more conservative interpretation of synergy, Wareham and Wareham have argued that *E*-test methods may be clinically relevant by giving rapid results of combinations with marked synergy only.[36] Clinical relevance of combinations with only weak synergistic interactions missed by the *E*-test method needs to be studied further by *in vivo* milieu to give evidence for the recommendation of *E*-test for rapid reporting of synergistic combinations.

In spite of the availability of different methods to determine interactions between different antimicrobial agents, lack of standardisation has hampered reliable comparison and compilation of results of different studies. It is also difficult to assess the difference in results due to strain difference, and thus, the reproducibility or clinical efficacy of the combination might vary.

In vitro Synergy of Antimicrobial Combinations in Multidrug-resistant -Gram-negative Bacilli

Emergence of MDR, carbapenem-resistant organism and PDR GNB has triggered the search for synergistic combinations of antimicrobials in the last decade. *A. baumannii, P. aeruginosa* and *K. pneumoniae* are the most commonly studied organisms because of their major role as nosocomial pathogen with frequent drug resistance.

Acinetobacter baumannii

Among commonly studied drug combinations, colistin with either meropenem or rifampicin shows high synergy rates of 96.3% and 94.2% by TKA. Imipenem plus sulbactam/colistin shows moderate rate of synergy (66.6% and 59%, respectively, by TKA). There is a paucity of data to allow adequate comparison of differences among the different carbapenems. In general, all the carbapenems gave a wide range of synergy levels at different combinations which may be accounted by strain difference. Antagonism was noted with combinations sulbactam plus colistin/meropenem; colistin plus meropenem and polymyxin B plus meropenem in few studies. The significance of these observations needs to be further validated by *in vivo* model testing. Table 3 summarises *in vitro* studies done on sulbactam-based combinations and Table 4 summarises polymyxin-based *in vitro* studies done for *A. baumannii.*

Pseudomonas aeruginosa

Available data are very limited to give meaningful interpretation of the combinations tested. However, combinations of colistin plus carbapenem and carbapenem plus higher fluoroquinolone

Table 2: Comparison of different methods for determination of synergy with different antibiotic combinations for *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter* spp.

Reference	Number of isolates	Drug combination	Percentage synergy of isolate/antibiotic combinations tested			Concordance
			CB (%)	TKA (%)	*E*-test* (method) (%)	
ATCC strains						
White *et al*., 1996[11]	4	Czd/Cefe + Cipro/Tobra	4/16 (25)	3/16-4/16 (18.7-25)	2/16 (12.5) (cross)	*E*-test and TKA - 63%-75% CB and TKA - 44%-88% All three - 75%
***Acinetobacter* spp.**						
Bajaksouzian *et al*., 1997[51]	15	Ak + Levo/Oflo/Cipro	0/45 (0)	35/45 (77.7)	-	-
Bonapace *et al*., 2000[52]	10	Pipe, Cefe, Tobra, Trova	12/40 (30)	15/30 (50)	0% (cross)	TKA and *E*-test - 72% TKA and CB - 51%
Pankey and Ashcraft, 2009[53]	8	PB + Mero	-	8/8 (100)	5/8 (62.5%) (MIC: MIC)	62.5%
Gordon *et al*., 2010[54]	6	Col + Van	4/6 (66.6)	4/5 (80)	6/6 (100%) (E-test-agar)	-
Sheng *et al*., 2011[40]	17	Imi + Ak/Cipro/Col/Tige/Amp-sul	32/85 (37.6)	62/85 (72.9)	-	-
Sopirala *et al*., 2010[13]	8	Tige, Col, Imi, Ak	4/40 (10)	14/20 (70)	16/32 (50) (E-test-agar)	CB and *E*-test - 84.4%
Tan *et al*., 2011[55]	16	PB, Rif, Tige	8/48 (17)	19/48 (40)	1/48 (2%) (cross)	All three - 52% TKA and *E*-test - 60% E-test and CB - 83%
Santimaleeworagun *et al*., 2011[56]	8	Sul + Fos	6/8 (75)	6/8 (75)	-	100%
Vidaillac *et al*., 2012[57]	4	Col + Van/SXT	12/12 (100)	12/12 (100)	-	100%
Principe *et al*., 2013[38]	22	Dori + Tige/Col/Ak/Amp-sul/Rif	13/22 (54.2)	8/22 (36.4)	-	-
Galani *et al*., 2014[58]	10 COL-S 4 COL-R	Col + Dapto	-	16/30 isolate-concentration combination (53.3)	-	-
García-Salguero *et al*., 2015[59]	10	Ak + Imi/Mero/Fos/Col/Tige/Plaz + Imi/Mero/Col/Fos/Tige	33/100 (33)	9/64 (14)	-	-
Park *et al*., 2016[60]	69	Col, Dori, Tige	-	75/207 (36.2)	-	-
Hong *et al*., 2016[61]	41	Col + Mero/Rif/Imi/Doxy/Tige Tige + Mero/Imi	-	-	99/287 (34.4) (MIC: MIC)	-
Bae *et al*., 2016[62]	9	Col + Tige/Azi/Ak/SXT/Amp-sul/Cefe/Azt/Mero/Teico/Van/Rif	38/99 (38.3)	-	-	-
Nepka *et al*., 2016[63]	6	Col + SXT	-	6/6 (100)	-	-
Büyük *et al*., 2017[64]	15	Col + Mero/Rif/Cipro/Moxi/Ami Tige + Mero/Rif/Cipro/Moxi/Ak/Col	90/165 (54.5)	-	-	-
P. aeruginosa						
Visalli *et.al*., 1998[65]	12	Levo + Cefe/Czd/Genta/Mero	2/48 (4.1)	34/48 (70.8)	-	33.3%
Di Bonaventura *et al*., 2004[66]	20	Grepa/Levo + Ctr/Ctx	35/80 (43.8)	-	23/80 (31.3)	71.2%
Pankey and Ashcraft, 2005[67]	31	Cipro + Gati	-	13/31 (42)	6/31 (19) (MIC: MIC)	65%
Vidaillac *et al*., 2012[57]	4	Col + Van/SXT	No synergism	2/12 (16.6)	-	83.3%
K. pneumoniae						
Pankey and Ashcraft., 2011[68]	14	PB + Mero	-	9/14 (64)	6/14 (43) (MIC: MIC)	79%
		PB + Rif	-	14/14 (100)	3/14 (21)	21%

Contd...

Table 2: Contd...

Reference	Number of isolates	Drug combination	Percentage synergy of isolate/antibiotic combinations tested			Concordance
			CB (%)	TKA (%)	*E*-test* (method) (%)	
Vidaillac *et al*., 2012[57]	4	Col + Vanco/SXT	3/12 (25)	3/12 (25)	-	100%
Pankey *et al*., 2013[69]	31	PB + Mero	-	23/31 (74)	17/31 (54.8) (MIC: MIC)	80.6%
Gaibani *et al*., 2014[70]	8	Col + Rif Col + Tig Csol + Mero Rif + Tig Rif + Mero Col + Tec	3/8-37.5% (Col + Mero) 6/8-75% (Col + Tig) 8/8-100% (Col + Rif) 0/8-0% (Col + Tec)	8/8-100% (Col + Rif)	8/8-100 (Col+Rif) 2/8-25% (Col+Tig) 1/8-12.5% (Col+Mero) 0/8-0% (Rif+Tig) 0/8-0% (Rif+Mero) 0/8-0% (Col+Tec)	-

**E*-test method showing highest rate of synergy when more than one *E*-test method evaluated. CB: Checker board, TKA: Time-kill assay, Col: Colistin, Cefe: Cefepime, Cipro: Ciprofloxacin, Tobra: Tobramycin, Ak: Amikacin, Levo: Levofloxacin, Oflo: Ofloxacin, Pipe: Piperacillin, Trova: Trovafloxacin, PB: Polymyxin, Mero: Meropenem, Van: Vancomycin, Imi: Imipenem, Tige: Tigecycline, Amp-sul: Ampicillin/sulbactam, Rif: Rifampicin, Sul: Sulbactam, Fos: Fosfomycin, SXT: Trimethoprim/sulphamethoxazole, Dori: Doripenem, Dapto: Daptomycin. Plaz: Plazomicin, Doxy: doxycycline, Azi: Azithromycin, Czd: Ceftazidime, Azt: Aztreonam, Teico: Teicoplanin, Van: Vancomycin, Moxi: Moxifloxacin, Czd: Ceftazidime, Genta: Gentamicin, Ctr: Ceftriaxone, Ctx: Cefotaxime, Gati: Gatifloxacin, Tec: Teicoplanin, MIC: Minimum inhibitory concentration, COL-R: Colistin resistant, *P. aeruginosa*: *Pseudomonas aeruginosa*, *K. pneumoniae*: *Klebsiella pneumoniae*, COL-S: Colistin-susceptible

such as gatifloxacin and levofloxacin seem promising. Except for the combination of colistin with vancomycin and sulphonamides, antagonism was not seen in any of the combinations. Table 5 summarises *in vitro* studies done on antimicrobial combinations in *P. aeruginosa.*

Klebsiella pneumoniae

Combinations of polymyxin B/colistin plus rifampicin/meropenem give promising results. It may be noted that antagonism was detected in combinations of colistin plus ertapenem/imipenem and was found to be correlating with the high MIC of colistin. This needs to be studied further with characterisation of the isolates to understand the underlying mechanism. Table 6 summarises *in vitro* studies done on antimicrobial combinations in *K. pneumoniae.*

Table 7 summarises the most commonly studied combinations of antimicrobials for *A. baumannii, P. aeruginosa* and *K. pneumoniae*. Combined rates were calculated from total number of synergy observed in different studies against the total number of isolates studied.

Correlation of Synergism with Other Factors

Synergy in relation to minimum inhibitory concentration value

There seems to be some degree of relationship between MIC of the combination of antimicrobials tested against a particular organism. Some studies show more synergy in isolates with higher MIC, while other studies have reported contrary findings [Table 8].

For *A. baumannii*, combinations of sulbactam plus meropenem/doripenem and doripenem plus colistin/tigecycline/amikacin/rifampicin exhibited more synergy with isolates with higher MIC for either sulbactam or doripenem.[37,38] However, the actual MIC for the agents seems to have an effect on the level of synergy. Lee *et al*. demonstrated synergism for isolates with moderately high MIC of about 16 μg/ml for sulbactam and 64 μg/ml for meropenem. In contrast, no synergism was noted for isolates with very high MIC of about 128 μg/ml and 256 μg/ml for sulbactam and meropenem, respectively.[39] Combination of colistin and rifampicin also showed synergy for isolates with higher MIC for rifampicin,[8] whereas a combination of ampicillin-sulbactam plus colistin/imipenem and imipenem plus amikacin/colistin/tigecycline showed more synergy with isolates which are colistin susceptible or with lower MIC for imipenem.[40,41]

For *K. pneumoniae*, the combination of doripenem plus colistin showed higher synergy with isolates having high colistin MIC, whereas Clancy *et al*. reported higher synergy with isolates having low doripenem MIC.[42,43] Combination of colistin and imipenem however showed more synergy in isolates with low colistin MIC, with antagonism detected at high MIC.[44]

The variations observed between the tests may be due to the difference in the strain, methodology and geographical area. In particular, the mechanisms of resistance in these isolates were not fully characterised. The question of presence of synergism or antagonism as a function of MIC value for each agent needs to be investigated further to use MIC as a predictor for success of combination therapy. Henceforth, studies must be carried out to decipher the MIC value of individual agents, which is likely to yield synergism or antagonism for a particular combination.

Synergism as a correlate of molecular mechanism of resistance

Another aspect of synergy testing in resistant isolates is its correlation with a particular resistance mechanism involved. Table 9 gives the correlation of synergy with antimicrobial combinations. Very few studies have further investigated

Table 3: Sulbactam-based *in vitro* combination study for *Acinetobacter baumannii*

Method	Reference	Place of study	Isolate	Combination	Percentage synergy	Percentage antagonism
CB	Ozseven *et al*. 2012[71]	Turkey	34 CRAB	Amp/Sul + Imi	88.2	Nil
				Cefe/Sul + Imi	70.6	
				Amp/Sul + Mero	94.1	
				Cefe/Sul + Mero	8.8	
	Kiffer *et al*., 2005[37]	Brazil	48	Sul + Mero	29.2	6.2
	Ji *et al*., 2013[72]	China	40 IMI-S	Sul + Imi/Mero/Cefe	20-27.5 in 40 S	Nil
			40 IMI-R		7.5-25 in 40 R	
	Pongpech *et al*., 2010[73]	Thailand	30 MDR	Sul + Mero	70	6.7% in Sul + Col
				Sul + Col	53.3	
				Sul + Mero + Col	96.7	
	Sheng *et al*. 2011[40]	Tiwan	12 CRAB	Amp/Sul + Imi	16	Nil
	Lee *et al*. 2007[49]	Taiwan	4 MDR CRAB	Sul + Imi	0	Nil
				Sul + Mero	0	
	Ni *et al*., 2013[74]	China	70 MDR	Sul + Tige	64.4	Nil
	Principe *et al*., 2013[38]	Italy	22 MDR	Amp/Sul + Dori	9	Nil
	Pei *et al*., 2012[75]	China	53 CRAB	Cefe/Sul + Mino	73.5	Nil
	Tong *et al*., 2006[76]	China	23 CRAB	Sul + Cefe	33.3	Nil
	Santimaleeworagun *et al*., 2011[56]	Thailand	8 CRAB	Sul + Fos	75	Nil
	Turk Dagi *et al*., 2014[77]	Turkey	40 CRAB	Sul + Imi/Mero/Cefe	45.8	Nil
	Laishram *et al*., 2016[78]	India	50 CRAB	Sul + Mero/Col	34	Nil
	Marie *et al*., 2015[79]	Riyadh	54 MDR	Sul + Mero/Col	49	Nil
E-test	Kempf *et al*., 2012[80]	France	1 COL-R	Sul + Col	100	Nil
	Cikman *et al*., 2013[41]	Turkey	33 IMI-R	Sul + Col	45.5	27.3
	Kiratisin *et al*., 2010[81]	Thailand	40	Cefe/Sul + Dori/Imi/Mero	17.5-32.5	Nil
	Marie *et al*., 2015[79]	Riyadh	54 MDR	Sul + Mero/Col	42.5	Nil
TKA	Ko *et al*., 2004[82]	Tiwan	1 MDR	Sul + Mero	100	Nil
	Sheng *et al*., 2011[40]	Tiwan	12 CRAB	Amp/Sul + Imi	42	Nil
	Song *et al*., 2007[83]	Korea	8 IMI-R	Sul + Imi	87.5	Nil
	Choi *et al*., 2004[84]	Korea	2 IMI-R	Sul + Imi	100	Nil
			2 IMI-S			
	Tripodi *et al*., 2007[8]	Italy	9 MDR CRAB	Amp/Sul + Rif	100	Nil
	Principe *et al*., 2013[38]	Italy	22 MDR	Amp/Sul + Dori	0	Nil
	Tatman-Otkun *et al*., 2004[85]	Turkey	8 MDR	Amp/Sul + Tobra	50	Nil
	Santimaleeworagun *et al*., 2011[56]	Thailand	8 CRAB	Sul + Fos	75	Nil
	Laishram *et al*., 2016[78]	India	50 CRAB	Sul + Mero/Col	40	Nil

CB: Checker board, TKA: Time-kill assay, CRAB: Carbapenem-resistant *Acinetobacter baumannii*, IMI-S: Imipenem susceptible, IMI-R: Imipenem resistant, Amp-sul: Ampicillin/sulbactam, Imi: Imipenem, Cefe: Cefepime, Mero: Meropenem, Sul: Sulbactam, Dor: Doripenem, Fos: Fosfomycin, Col: Colistin, Rif: Rifampicin, COL-R: Colistin resistant, Mino: Minocyline

the resistance mechanism for the study isolates, for which combination testing has been done. In case of *K. pneumoniae*, studies have reported the role of porin channels in determining synergism of the combinations being tested. Similarly, *K. pneumoniae*-producing bla_{NDM} carbapenemase alone showed significantly more synergy than isolates producing bla_{OXA-48}-like carbapenemases.[78] Such correlations with the specific resistance mechanism involved might help predict synergism for a particular combination of antimicrobials for treatment. Thus, determining molecular mechanisms would help direct combination therapy to improve therapeutic success.[45]

In vitro Synergy as a Predictor of Clinical Response

The likelihood of the findings of the *in vitro* synergy studies to be translated into clinical efficacy still remains debatable. The classical example of *in vitro* synergy between aminoglycoside and beta-lactam agents has not stood the test of time. Studies have reported no clinical benefit of combination of beta-lactam plus aminoglycoside combination for Gram-negative infection either in the neutropenic or in the non-neutropenic host.[46,47] Combination therapy may result in adverse effects of nephrotoxicity.

Despite issues of toxicity, combination therapy is the only strategy available for treating infections due to PDR organisms. Very few studies have documented the clinical outcome with combination therapy supported by *in vitro* synergy. Biancofiore *et al*. reported successful treatment of multifocal infection of MDR *A. baumannii* in a 16-year-old female with a combination of colistin, rifampicin and meropenem after synergism between the combinations was proved by CB assay.[48] Lee *et al*.

Table 4: Polymyxin based *in vitro* combination study other than sulbactam for *Acinetobacter baumannii*

Method	Reference	Place of study	Isolate	Combination	Percentage synergy	Percentage antagonism
CB	Timurkaynak *et al*., 2006[86]	Turkey	5 MDR	Col + Rif	80	Nil
				Col + Mero	60	
				Col + Azi	60	
	Biancofiore *et al*., 2007[48]	Italy	1 CRAB	Col + Rif	100	Nil
				Col + Mero	0 (par)	
	Guelfi *et al*., 2008[87]	Brazil	10	PB + Mero	0 (par)	Nil
	Arroyo *et al*., 2009[88]	Spain	35	Col + Tige	0	Nil
	Pongpech *et al*., 2010[73]	Thailand	30 MDR	Col + Imi	100	6.7 in Col + Mero
				Col + Mero	73.3	
	Sopirala *et al*., 2010[13]	USA	8 PDR	Col + Tige	0	Nil
				Col + Imi	25	
	Gordon *et al*., 2010[54]	UK	6	Col + Van	66.6	Nil
	Sheng *et al*., 2011[40]	Tiwan	12 CRAB	Col + Imi	42	Nil
	Santimaleeworagun *et al*., 2011[56]	Thailand	8 CRAB	Col + Imi	0	Nil
				Col + Fos	12.5	
	Tan *et al*., 2011[55]	Singapore	16	PB + Rif	18.75	Nil
				PB + Tige	12.5	
	Ozseven *et al*., 2012[71]	Turkey	34 CRAB	PB + Imi	38.2	Nil
				PB + Mero	2.9	
	Ni *et al*., 2013[74]	China	70 MDR	Col + Tige	24.3	Nil
	Principe *et al*., 2013[38]	Italy	22	Col + Dori	36	Nil
	O'Hara *et al*., 2013[34]	USA	3 COL-R	Col + Dori	66.6	Nil
				Col + Van	100	
	Clock *et al*., 2013[89]	USA	XDR	PB + Dori	2	Nil
				PB + Dori + Rif	10	
				PB + Dori + Tige	2	
	Vidaillac *et al*., 2012[57]	France	4	Col + Van	100	Nil
				Col + Tmp	100	
				Col + Cotri	100	
E-test	Wareham and Bean, 2006[36]	UK	5 CRAB	Col + Imi	20	Nil
	Tan *et al*., 2007[90]	Singapore	13 CRAB	Col + Mino	0	Nil
	Pankey and Ashcraft, 2009[53]	USA	8 CRAB	PB + Mero	62.5	Nil
	Shields *et al*., 2011[91]	USA				
	Sopirala *et al*., 2010[13]	USA	8 PDR	Col + Tige	0	Nil
				Col + Imi	100	
	Gordon *et al*., 2010[54]	UK	6	Col + Van	100	Nil
	Tan *et al*., 2011[55]	Singapore	16	PB + Rif	6.2	6.2 in PB + Tige
				PB + Tige	0	
	Nastro *et al*., 2014[50]	Argentina	4 COL-R	Col + Rif	100	Nil
	Miyasaki *et al*., 2012[92]	USA	20 MDR	Col + Doxy	10	Nil
				Col + Imi	5	
				Col + Rif	5	
TKA	Yoon *et al*., 2004[93]	USA	8 IMI R	PB + Imi	87.5	Nil
				PB + Rif	87.5	
				PB + Imi + Rif	100	
	Tripodi *et al*., 2007[8]	Italy	9 CRAB	Col + Rif	77	Nil
				Col + Imi	0	
	Song *et al*., 2007[83]	Korea	8 CRAB	Col + Rif	100	Nil
	Tan *et al*., 2007[90]	Singapore	13 CRAB	Col + Mino	92	Nil
	Pankuch *et al*., 2008[94]	Germany	51	Col + Mero	96	Nil
	Pankey and Ashcraft, 2009[53]	USA	8 CRAB	PB + Mero	100	Nil
	Rodriguez *et al*., 2010[95]	Argentina	14 MDR	Col + Imi	100	Nil
				Col + Rif	100	

Contd...

Table 4: Contd...

Method	Reference	Place of study	Isolate	Combination	Percentage synergy	Percentage antagonism
	Sopirala *et al*., 2010[13]	USA	8 PDR	Col + Imi	37	Nil
	Pankuch *et al*., 2010[96]	USA	25	Col + Dori	100	Nil
	Liang *et al*., 2011[97]	China	4 CRAB	Col + Mero	100	Nil
				Col + Mino	100	
				Col + Rif	100	
	Sheng *et al*., 2011[40]	Tiwan	12 CRAB	Col + Imi	75	Nil
	Tan *et al*., 2011[55]	Singapore	16	PB + Rif	56.2	Nil
				PB + Tige	43.75	
	Peck *et al*., 2012[98]	Korea	6 CRAB	Col + Imi	50 at 0.5 × MIC	Nil
				Col + Rif	100 at 1 × MIC	
				Col + Tige	33 at 0.5 × MIC	
					100 at 1 × MIC	
					67 at 0.5 × MIC	
					100 at 1 × MIC	
	Vidaillac *et al*., 2012[57]	France	4	Col + Van	100	Nil
				Col + Tmp	100	
				Col + Cotri	100	
	Gordon *et al*., 2010[54]	UK	6	Col + Van	83.3	Nil

CB: Checker board, TKA: Time-kill assay, MDR: Multidrug resistant, CRAB: Carbapenem-resistant *Acinetobacter baumannii*, PDR: Pan-drug resistant, COL-R: Colistin resistant, IMI R: Imipenem resistant, Col: Colistin, Rif: Rifampicin, Mero: Meropenem, Azi: Azithromycin, PB: Polymyxin, Tige: Tigecycline, Imi: Imipenem, Van: Vancomycin, Fos: Fosfomycin, Dori: Doripenem, MIC: Minimum inhibitory concentration, Mino: Minocyline, TMP: Trimethoprim, Cotri: Trimethoprim/sulfamethoxazole

Table 5: *In vitro* studies on antimicrobial combinations against *Pseudomonas aeruginosa*

Method	Reference	Place of study	Isolate	Combination	Percentage synergy	Percentage antagonism
CB	Santos *et al*., 2013[99]	Brazil	4 (2 MDR)	Gen + Imi	25	Nil
				Gen + Pip/Tazo	50	
				Gen + Cefe	25	
				Gen + Czd	25	
				Gen + Cipro	0	
				Tobra + Imi	75	
				Tobra + Pip/Tazo	100	
				Tobra + Czd	75	
				Tobra + Cefe	50	
				Tobra + Cipro	25	
				Fos + Imi	100	
				Fos + Pip/Tazo	100	
				Fos + Cefe	25	
				Fos + Czd	75	
				Fos + Cipro	50	
				Fos + Tobra	50	
				Rif + Imi	75	
				Rif + Pip/Tazo	25	
				Rif + Cefe	25	
				Rif + Czd	50	
				Rif + Cipro	0	
				Rif + Tobra	75	
	Mitsugui *et al*., 2011[100]	Brazil	34	PB/Col + Czd/Cefe/Pip/Tazo	0	Nil
	Dundar and Otkun, 2010[101]	Turkey	12 MDR	Czd + Tobra	67	Nil
				Pip/Tazo + Tobra	50	
				Cipro + Tobra	0	
				Imi + Tobra	0	
				Imi + Cipro	0	

Contd...

Table 5: Contd...

Method	Reference	Place of study	Isolate	Combination	Percentage synergy	Percentage antagonism
			13 S	Czd + Tobra	31	Nil
				Pip/Tazo + Tobra	46	
				Cipro + Tobra	0	
				Imi + Tobra	15	
				Imi + Cipro	8	
	Aoki *et al*., 2009[31]	Japan	7 MDR	Col + Imi	28.5	Nil
				Col + Rif	100	
				Col + Cipro	14.2	
				Col + Ak/Tobra/Pipe/Czd		
	Piccoli *et al*., 2005[102]	Italy	102	Czd + Levo	69.6	6.8
				Czd + Ak	79.4	8.8
	Dawis *et al*., 2003[103]	USA	10	Gati + Cefe	60	Nil
				Gati + Mero	70	
				Gati + Pipe	50	
				Gati + Genta	60	
	Visalli *et al*., 1998[65]	USA	124	Levo + Cefe	7.2	Nil
				Levo + Czd	6.4	
				Levo + Genta	0.8	
				Levo + Mero	5.6	
	Visalli *et al*., 1997[104]	USA	60	Trova + Czd	28.3	Nil
				Trova + Ak	8.3	
				Trova + Imi	23.3	
	Tessier and Quentin, 1997[105]	France	40	Fos + Cipro	15	Nil
				Fos + Ak	7.5	
				Fos + Imi	0	
				Fos + Czd	0	
E-test	He *et al*., 2012[106]	USA	100 CR	Dori + Ak	20	Nil
				Dori + Col	3	
				Dori + Levo	9	
	Samonis *et al*., 2012[107]	Greece	15 MDR	Fos + Imi	46.7	Nil
				Fos + Mero	53.3	
				Fos + Dori	73.3	
				Fos + Col	13.3	
				Fos + Netil	13.3	
				Fos + Tige	13.3	
	Sueke *et al*., 2010[108]	UK	10	Mero + Cipro	10	Nil
	Balke *et al*., 2006[109]	Germany	163 MDR	Czd + Tobra	28.8	Nil
				Mero + Tobra	19	
	Pankey and Ashcraft, 2005[67]	USA	31 Cipro resistant	Gati + Cipro	19	Nil
TKA	Vidaillac *et al*., 2012[57]	France	4	Col + Van	0	100
				Col + Tmp	0	75
				Col + Cotri	25 (seen in COL-R)	75
	Pankuch *et al*., 2008[94]	USA	51	Mero + Cipro	66.6	Nil
				Mero + Col	25.4	
	Pankey and Ashcraft, 2005[67]	USA	31 Cipro resistant	Gati + Cipro	42	Nil
	Giamarellos-Bourboulis *et al*., 2003[110]	Greece	28 MDR	Col + Rif	11.8	Nil
	Ermertcan *et al*., 2001[111]	Turkey	18	Mero + Cipro	22 at 0.5 × MIC 61 at 1 × MIC	Nil
	Gradelski *et al*., 2001[112]	USA	8	Gati + Cefe	37.5	Nil
				Gati + Czd	62.5	
				Gati + Cfper	62.5	

Contd...

Table 5: Contd...

Method	Reference	Place of study	Isolate	Combination	Percentage synergy	Percentage antagonism
				Gati + Imi	75	
				Gati + Ak	37.5	
	Visalli *et al*., 1998[65]	USA	12	Levo + Cefe	83.3	Nil
				Levo + Czd	75	
				Levo + Genta	33.3	
				Levo + Mero	91.6	
	Visalli *et al*., 1997[104]	USA	3	Trova + Czd	100	Nil
				Trova + Ak	100	
				Trova + Imi	100	

MDR: Multidrug resistant, CR: Carbapenem resistant, Cipro: Ciprofloxacin, Imi: Imipenem, pip/tazo: Piperacillin/tazobactam, Cefe: Cefepime, Czd: Ceftazidime, Tobra: Tobramycin, Fos: Fosfomycin, Rif: Rifampicin, PB: Polymyxin, Col: Colistin, Ak: Amikacin, Levo: Levofloxacin, Gati: Gatifloxacin, Genta: Gentamicin, Trova: Trovafloxacin, Mero: Meropenem, Dori: Doripenem, COL-R: Colistin resistant, MIC: Minimum inhibitory concentration, S: Susceptible, Netil: Netilmicin, TMP: Trimethoprim, Cotri: Trimethoprim/sulfamethoxazole

Table 6: *In vitro* studies on antimicrobial combinations against *Klebsiella pneumoniae*

Method	Reference	Place of study	Isolate	Combination	Percentage synergy	Percentage antagonism
CB	Tascini *et al*., 2013[113]	Italy	13 Col R KPC	Col + Rif	100	Nil
				Col + Gen	38.5	
				Col + Mero	38.5	
				Col + Imi	38.5	
				Col + Tige	38.5	
				Tige + Gen	0	
				Tige + Mero	0	
				Tige + Imi	0	
	Clock *et al*., 2013[89]	USA	48 XDR	Dori + Ak	10	4% with Dori + Levo
				Dori + Levo	0	
				Dori + PB	4	
				Dori + Rif	23	
				Dori + Tige	0	
				Dori + PB + Rif	19	
				Dori + PB + Tige	8	
	Vidaillac *et al*., 2012[57]	France	4	Col + Van	25	Nil
				Col + Tmp	25	
				Col + Cotri	25 (in Col R)	
	Elemam *et al*., 2010[114]	USA	12 PB-R KPC	PB + Rif	100	Nil
				PB + Rif	100	
				PB + Imi	100	
				PB + Tige	100	
				PB + Gen	0	
	Dawis *et al*., 2003[103]	USA	10 ESBL	Gati + Cefe	50	Nil
				Gati + Mero	20	
				Gati + Pipe	10	
				Gati + Gen	60	
	Gaibani *et al*., 2014[70]	Italy	8 Col-R KPC-Kp	Col + Mero	37.5	Nil
				Col + Tige	75	
				Col + Rif	100	
				Col + Tec	0	
	Stein *et al*., 2015[115]	Germany	20	Col + Mero	25	Nil
				Mero + Tige	10	
				Mero + Col + Tige	25	
E-test	Nastro *et al*., 2014[50]	Argentina	27 Col R	Col + Rif	100	Nil
	Pankey *et al*., 2013[69]	USA	26 KPC	PB + Mero	57.7	Nil

Contd...

Table 6: Contd...

Method	Reference	Place of study	Isolate	Combination	Percentage synergy	Percentage antagonism
	Pankey and Ashcraft, 2011[68]	USA	14 KPC	PB + Mero	43	Nil
				PB + Rif	21	
	Gaibani *et al*., 2014[70]	Italy	8 Col-R KPC-Kp	Col + Rif	100	Nil
				Col + Tige	25	
				Col + Mero	12.5	
				Rif + Tige	0	
				Rif + Mero	0	
				Col + Tec	0	
TKA	Lee and Burgess, 2013[116]	USA	4 KPC	Col + Dori	100	Nil
				PB + Dori	100	
	Pankey *et al*., 2013[69]	USA	26 KPC	PB + Mero	73	Nil
	Hong *et al*., 2013[45]	USA	12 KPC	Col + Erta	42	25% with Col+Erta
				Col + Dori	50	
				Col + Dori + Erta	67	
	Vidaillac *et al*., 2012[57]	France	4	Col + Van	25	75
				Col + Tmp	50	50
				Col + Cotri	50 (in Col-R)	50
	Jernigan *et al*., 2012[42]	USA	12 KPC	Col + Dori	50	0
				Col + Gen	25	8
				Col + Doxy	8	25
				Dori + Gen	8	17
				Dori + Doxy	25	17
				Gen + Doxy	42	17
	Le *et al*., 2011[117]	USA	4 KPC	Ak + Imi	100	Nil
				Ak + Mero	100	
				Ak + Erta	25	
	Pournaras *et al*., 2011[118]	Greece	4 KPC	Tige + Col	100	Nil
				Tige + Mero	0	
	Pankey and Ashcraft, 2011[68]	USA	14 KPC	PB + Mero	64	Nil
				PB + Rif	100	
	Yim *et al*., 2011[119]	Korea	35 ESBL+AmpC	Tige + Imi	69.2 (18/26)	Nil
				Tige + Ak	57.1 (8/14)	
				Tige + Cipro	35 (7/20)	
	Souli *et al*., 2011[44]	Greece	17 KPC	Fos + Mero	64.7	Nil
				Fos + Col	11.8	
				Fos + Gen	0	
	Vidaillac *et al*., 2009[120]	USA	2 ESBL	Cpt + Ak	100	Nil
				Cpt + Taz	100	
				Cpt + Mero	0	
				Cpt + Azt	0	
				Cpt + Levo	0	
				Cpt + Cefe	0	
				Cpt + Tige	0	
	Souli *et al*., 2009[121]	Greece	42 VIM-1	Col + Imi	33.3	23.8
	Dawis *et al*., 2003[103]	USA	1 ESBL	Gati + Mero	100	Nil
				Gati + Gen	100	
	Clancy *et al*., 2013[43]	USA	23 KPC	Col + Dori	26	Nil
	Gaibani *et al*., 2014[70]	Italy	8 Col-R KPC-Kp	Col + Rif	100	Nil
	Diep *et al*., 2017[122]	USA	2 isolates	PB + Rif	100	Nil
				PB + Mero	100	
				PB + Mero + Rif	100	
	Kulengowski *et al*., 2017[123]	USA	4 KPC-Kp	Mero + PB	100	Nil

KPC: *Klebsiella pneumoniae* carbapenemase, Col: Colistin, Rif: Rifampicin, R: Resistant, Gen: Gentamicin, Mero: Meropenem, Imi: Imipenem, Tige: Tigecycline, XDR: Extensive drug resistance, Dori: Doripenem, Ak: Amikacin, Levo: Levofloxacin, PB: Polymyxin B, Van: Vancomycin, Cefe: Cefepime, Pipe: Piperacillin, ESBL: Extended spectrum beta-lactamase, Gati: Gatifloxacin, Tec: Teicoplanin, Doxy: Doxycycline, Cipro: Ciprofloxacin, Fos: Fosfomycin, Taz: Tazobactam, Azt: Aztreonam, Erta: Ertapenem, CB: Checker board, TKA: Time-kill assay, Cpt: Ceftaroline, TMP: Trimethoprim, Cotri: Trimethoprim/sulfamethoxazole

Table 7: Combined synergy by different methods for *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae

Drug combination	Percentage combined synergy (percentage synergy range)			Remarks
	TKA	CB	*E*-test	
		A. baumannii		
Sul+Imi	66.6 (42-100)	64 (0-88.2)	17.5-32.5	Combination of Col with either Mero or Rif shows high pooled synergy rate while combination of Imi with either Sul or Col shows moderate level of synergy
Sul+Mero	100	57.7 (0-94)		
Sul+Dori	-	9		
Sul+Col	-	53.3	45.5	
Sul+Rif	100	-	-	
Col+Mero	96.3 (96-100)	69.4 (0-73.3)	-	
Col+Imi	59 (0-100)	63.7 (0-100)	69.2 (20-100)	
Col+Dori	100	40 (36-66.6)	-	
Col+Rif	94.2 (77-100)	83 (80-100)	100	
Col+Tige	100	15 (0-24.3)	-	
PB+Mero	100	2.2 (0-2.9)	62.5	
PB+Imi	87.5	38.2	-	
PB+Tige	43.7	12.5	0	
PB+Rif	66.6 (56.2-87.5)	18.7	6.2	
		P. aeruginosa		
Col + Rif	11.8	100	-	There are limited data on a particular combination. Promising combinations include Col plus carbapenem and carbapenem plus fluoroquinolone combinations
Col + Imi	100	44.4 (28.5-100)	-	
Col + Mero	25.4	12	-	
Col + Dori	76	-	3	
Imi + Fos	-	12.7 (0-100)	46.7	
Mero + Fos	-	-	53.3	
Dori + Fos	-	-	73.3	
Imi + Cipro	-	4 (0-8)	-	
Imi + Trova	100	23.3	-	
Imi + Gati	75	-	-	
Mero + Cipro	65.2 (61-66)	-	-	
Mero + Levo	91.6	5.6	-	
Mero + Gati	-	70	-	
Dori + Levo	-	-	9	
Tobra + Imi	-	6.8 (0-15)	-	
Tobra + Mero	-	-	19	
Tobra + Pipe/Taz	-	55 (46-100)	-	
Tobra + Czd	-	45.3 (31-75)	28.8	
Tobra + Cefe	-	50	-	
Tobra + Cipro	-	3.4 (0-25)	-	
		K. pneumoniae		
Col + Imi	33.3	38.5	-	Combination of Col/PB plus Rif/Mero gives promising result and needs to be investigated in *in vivo* models and clinical trials. Antagonism is seen in Col plus Imi/Erta combinations
Col + Mero	-	38.5	-	
Col + Dori	43.1 (26-100)	-	-	
Col + Erta	42	-	-	
Col + Tige	100	38.5	-	
Col + Rif	-	100	100	
PB + Dori	100	4	-	
PB + Mero	70 (64-73)	-	52.5 (43-57.7)	
PB + Rif	100	100	21	
Tige + Mero	0	0	-	
Tige + Imi	69.2	0	-	
Tige + Dori	-	0	-	
Tige + Ak	57.1	-	-	

Contd...

Table 7: Contd...

Drug combination	Percentage combined synergy (percentage synergy range)			Remarks
	TKA	CB	*E*-test	
Tige + Cipro	35	-	-	
Col + Gen	-	38.4	-	
Mero + Gen	-	38.4	-	
Imi + Gen	-	23	-	
Tig + Gen	-	0	-	
Mero + PB	100	-	-	
Mero + Rif + PB	100	-	-	

*Combined synergy is not provided for combinations with only one study. Sul: Sulbactam, Imi: Imipenem, Mero: Meropenem, Dori: Doripenem, Col: Colistin, Rif: Rifampicin, Tige: Tigecycline, PB: Polymyxin B, *P. aeruginosa*: *Pseudomonas aeruginosa*, *A. baumannii*: *Acinetobacter baumannii*, Fos: Fosfomycin, Cipro: Ciprofloxacin, Trova: Trovafloxacin, Gati: Gatifloxacin, Levo: Levofloxacin, Tobra: Tobramycin, Pipe: Piperacillin, Taz: Tazobactam, Czd: Ceftazidime, Cefe: Cefepime, Erta: Ertapenem, Ak: Amikacin, Gen: Gentamicin, *K. pneumonia*: *Klebsiella pneumonia*, TKA: Time-kill assay, CB: Checker board

Table 8: Correlation of interaction of antimicrobial agents with minimum inhibitory concentration value*

Reference	Combination (method)	Isolate (MIC μg/ml)	*n*	Synergy (%)	Antagonism (%)
		A. baumannii			
Kiffer *et al*.[37]	Sul + Mero (CB)	Sul R (≤4)	19	5.2	15.7
		Sul S (≥16)	13	76.9	0
Cikman *et al*.[41]	Amp/Sul + Col (CB)	Col R	12	8.3	75
		Col S	21	66.6	0
Principe *et al*.[38]	Dori + Tige/Col/Ak/Amp-Sul/Rif (CB)	Dori R	17	27	0
		Dori S	5	0	0
Sheng *et al*.[40]	Imi + Ak/Col/Tige/Amp-Sul (CB)	Imi ≥32	5	0	0
		Imi <32	7	20	0
	Imi + Col (TKA)	Imi ≥32	5	40	0
		Imi <32	7	100	0
Ji *et al*.[72]	Sul + Imi/Mero/Pan/Cef (CB)	Imi R	40	7.5-25	0
		Imi S	40	20-27.5	0
		K. pneumonia			
Jernigan *et al*.[42]	Dori + Col (TKA)	Col ≥8	9	66.6	0
		Col ≤4	3	0	0
Souli *et al*.[121]	Col + Imi (TKA)	Col ≥16	18	11.1	55.5
		Col ≤8	24	50	0
Clancy *et al*.[43]	Dori + Col (TKA)	Dori ≥16	19	32	0
		Dori ≤8	4	100	0
Kulengowski *et al*.[123]	Mero + PB (TKA)	Mero ≥4	4	100	0
		PB 0.06	2	100	0
		PB 0.125	2	100	0

*R: Resistant, S: Susceptible. MIC: Minimum inhibitory concentration, *A. baumannii*: *Acinetobacter baumannii*, Sul: Sulbactam, Mero: Meropenem, Col: Colistin, Ak: Amikacin, Amp: Ampicillin, Rif: Rifampicin, CB: Checker board, Imi: Imipenem, Tige: Tigecycline, Cef: Cefpirome, Dori: Doripenem, PB: Polymyxin B, *K. pneumonia*: *Klebsiella pneumonia*, TKA: Time-kill assay, Pan: panipenem

reported favourable outcome with carbapenem and sulbactam combination in four patients (two patients with VAP and two catheter-related bloodstream infection) caused by *A. baumannii*. *In vitro* synergy testing of all four isolates by CB assay showed partial synergy with FIC index ranging from 0.56 to 0.75 for combination of sulbactam and meropenem/imipenem.[49] In one patient with post-neurosurgery bacteraemic meningitis due to CR *A. baumannii* (CRAB), combination of intravenous meropenem and sulbactam leads to reduction in the colony count in cerebrospinal fluid (CSF) from >50,000 CFU/ml to 10,000 CFU/ml in 4 days.[39] Addition of intravenous and intrathecal colistin resulted in clearance of the organism within 2 days both from CSF and blood. The SBT and CSF bactericidal titre increased from fourfold to 32-fold with the three-drug combination compared to two-drug regimen. *In vitro* TKA showed synergism with combination of colistin with meropenem, sulbactam or both. TKA with colistin alone and meropenem plus colistin showed re-growth at 24 h. Though the infection was cleared, the patient expired due to hypoxia secondary to respiratory distress. Nastro *et al*. reported successful treatment for cases with sepsis (n = 1), meningitis (n = 1) and UTI (n = 1) with a combination of

Table 9: Correlation of molecular mechanisms of resistance with the result of *in vitro* antimicrobial interaction study

Reference	Molecular mechanism	*n*	Combination (method)	Percentage synergy
		A. baumannii		
Rodriguez *et al*., 2010[95]	bla_{OXA51} + bla_{OXA58}	3	Imi + Rif (TKA)	0
	bla_{OXA51} + bla_{OXA23}	9	Imi + Rif (TKA)	0
Tripodi *et al*., 2007[8]	bla_{OXA58}	9	Imi/Amp-Sul + Rif	100
			Col + Rif (TKA)	77
Wareham and Bean, 2006[36]	bla_{OXA23}	5	Imi + PB	20
			Rif + PB	20
			Azi + PB (E-test)	0
Santimaleeworagun *et al*., 2011[56]	bla_{OXA23}	8	Col + Sul	0
			Col + Imi	0
			Col + Fos	12.5
			Sul + Imi	0
			Sul + Fos (CB)	75
Song *et al*., 2007[83]	bla_{OXA51}	8	Sul + Imi	87.5
			Col + Rif (TKA)	100
Song *et al*., 2009[124]	bla_{IMP}	1	Col + Rif	0
			Imi + Rif (*in vivo* mouse pneumonia model)	100
	bla_{OXA51}	1	Col + Rif	100
			Imi + Rif	100
	bla_{VIM}	1	Col + Rif	0
			Imi + Rif	100
Miyasaki *et al*., 2012[92]	AME, Ser83leu substitution in GyrA, Ser80Phe substitution in ParC, *adeR*	5	Ak + Imi/Col/Tige (*E*-test)	0
Laishram *et al*., 2016[78]	bla_{NDM}, bla_{OXA51} + bla_{OXA23}	10	Sul + Col	40
	bla_{OXA51} + bla_{OXA23}	39	Sul + Col	18
		K. pneumoniae		
Hong *et al*., 2013[45]	High *omp35/omp36* expression	8	Col + Dori + Erta	100
			Col + Dori (TKA)	63
	Low *omp35/omp36* expression	4	Col + Dori + Erta	0
			Col + Dori (TKA)	25
Clancy *et al*., 2013[43]	Wild type *ompK36* or other mutations	8	Col + Dori (TKA)	75
	IS5 mutants	7	Col + Dori (TKA)	29
	Ins aa 134-135 GD mutants	8	Col + Dori (TKA)	25
Poirel *et al*., 2016[125]	bla_{KPC} (*n*=8)	20	Imp + Mem	40
	bla_{NDM} (*n*=4)		Imp + Dori	20
	bla_{OXA-48} (*n*=6)		Imp + Etp	40
	bla_{NDM} + bla_{OXA181} (2)		Mem + Dori	15
			Etp + Dori	15
			Etp + Mem	0
Laishram *et al*., 2016[78]	bla_{OXA-48} (*n*=23)	50	Col + Mem	30
	bla_{NDM} (*n*=16)		Col + Mem	69
	bla_{NDM} + bla_{OXA-48} (*n*=11)		Col + Mem	46
		P. aeruginosa		
He *et al*., 2012[106]	Efflux	67 (against Dori)	Dori + Ak	23.8
		99 (against Dori/Levo)	Dori + Col	19.5
			Dori + Levo	24.3
	Loss of porin	92	Dori + Ak	4.4
			Dori + Col	3.2
			Dori + Levo	7.3
	AmpC	41	Dori + Ak	9
			Dori + Col	8.6
			Dori + Levo	7.3

Contd...

Table 9: Contd...

Reference	Molecular mechanism	*n*	Combination (method)	Percentage synergy
	Metallo-β-lactamase	3	Dori + Ak	0
			Dori + Col	0
			Dori + Levo	0

Even though synergy levels vary with different mechanisms, antagonism was not noted in any of the combinations tested. TKA: Time-kill assay, Imi: Imipenem, Rif: Rifampicin, Amp: Ampicillin, Sul: Sulbactam, Col: Colistin, PB: Polymyxin B, *A. baumannii*: *Acinetobacter baumannii*, *K. pneumonia*: *Klebsiella pneumonia*, *P. aeruginosa*: *Pseudomonas aeruginosa*, Azi: Azithromycin, Fos: Fosfomycin, Tige: Tigecycline, Dori: Doripenem, Erta: Ertapenem, Levo: Levofloxacin, Ak: Amikacin, CB: Checker board, Imp: Imipenem, Mem: Meropenem, Etp: Ertapenem

Table 10: Correlation of *in vitro* synergy with clinical response

Reference	Clinical condition	Organism	Combination administered	*In vitro* assay result	Clinical outcome
Biancofiore *et al*., 2007[48]	Multifocal (lung, skin, soft tissue) infection	*A. baumannii*	Col + Rif + Mero	Col + Rif CB FIC index 0.3 Mero + Rif CB FIC index 0.25 Col + Mero CB FIC index 1	Bacteria eradicated, patient survived
Lee *et al*., 2007[49]	Pneumonia	*A. baumannii*	Mero + Sul	CB FIC index 0.56	Bacteria eradicated, patient survived
	VAP	*A. baumannii*	Imi + Sul	CB FIC index 0.56	Bacteria eradicated, patient survived
	CRBSI	*A. baumannii*	Imi + Sul	CB FIC index 0.75	Bacteria eradicated, patient survived
	CRBSI	*A. baumannii*	Imi + Sul	CB FIC index 0.56	Bacteria eradicated, patient survived
Lee *et al*., 2008[39]	Post-neurosurgery bacteraemic meningitis	*A. baumannii*	Col + Mero + Sul	Col + Mero synergy by TKA Col + Sul synergy by TKA Col + Mero + Sul synergy by TKA	Bacteria eradicated, patient expired due to respiratory distress

A. baumannii: *Acinetobacter baumannii*, Col: Colistin, Rif: Rifampicin, Mero: Meropenem, CB: Checker board, FIC: Fractional inhibitory concentration, Sul: Sulbactam, TKA: Time-kill assay, VAP: Ventilator-associated pneumonia, CRBSI: Catheter-related bloodstream infection, Imi: Imipenem

colistin and rifampicin against carbapenemase producing GNB.[50] The combination was found to be synergistic for all the isolates by the *E*-test-agar method. Table 10 summarises the studies that have correlated *in vitro* synergy with clinical response.

Conclusion

Combination therapy has gained attention due to increased efficacy and scope for decreasing the toxicity and development of resistance especially against drug-resistant strains. Therefore, it is imperative to investigate the antimicrobials that have to be used in combination for the clinical utility. At present, very few agents are available for treating infections due to PDR pathogens, and combination therapy is found to be the effective strategy to tackle this. Several methods exist for the assessment of synergistic activity of two or more antimicrobial agents. However, wide variation was observed in terms of their technical issues, complexity and interpretation of test results. This signifies the need for global-level standardisation of the various methods for the determination of synergy of antimicrobial combinations. At present, TKA is the reference method which yields considerable level of concordance rate among the various studies. To conclude, majority of the *in vitro* test methods could not predict the clinical success rates. Therefore, prospective clinical trials with *in vitro* synergy testing data are needed to improve the clinical outcome.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Eliopoulos GM, Eliopoulos CT. Antibiotic combinations: Should they be tested? Clin Microbiol Rev 1988;1:139-56.
2. Chen IL, Lee CH, Su LH, Tang YF, Chang SJ, Liu JW, *et al.* Antibiotic consumption and healthcare-associated infections caused by multidrug-resistant gram-negative bacilli at a large medical center in Taiwan from 2002 to 2009: Implicating the importance of antibiotic stewardship. PLoS One 2013;8:e65621.
3. Rubio FG, Oliveira VD, Rangel RM, Nogueira MC, Almeida MT. Trends in bacterial resistance in a tertiary university hospital over one decade. Braz J Infect Dis 2013;17:480-2.
4. Sievert DM, Ricks P, Edwards JR, Schneider A, Patel J, Srinivasan A, *et al.* Antimicrobial-resistant pathogens associated with healthcare-associated infections: Summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2009-2010. Infect Control Hosp Epidemiol 2013;34:1-4.
5. Vila J, Pachón J. Therapeutic options for *Acinetobacter baumannii* infections: An update. Expert Opin Pharmacother 2012;13:2319-36.
6. MacGowan AP, Wootton M, Hedges AJ, Bowker KE, Holt HA, Reeves DS, *et al.* A new time-kill method of assessing the

relative efficacy of antimicrobial agents alone and in combination developed using a representative beta-lactam, aminoglycoside and fluoroquinolone. J Antimicrob Chemother 1996;38:193-203.
7. Zusman O, Avni T, Leibovici L, Adler A, Friberg L, Stergiopoulou T, *et al.* Systematic review and meta-analysis of *in vitro* synergy of polymyxins and carbapenems. Antimicrob Agents Chemother 2013;57:5104-11.
8. Tripodi MF, Durante-Mangoni E, Fortunato R, Utili R, Zarrilli R. Comparative activities of colistin, rifampicin, imipenem and sulbactam/ampicillin alone or in combination against epidemic multidrug-resistant *Acinetobacter baumannii* isolates producing OXA-58 carbapenemases. Int J Antimicrob Agents 2007;30:537-40.
9. Odds FC. Synergy, antagonism, and what the chequerboard puts between them. J Antimicrob Chemother 2003;52:1.
10. Rand KH, Houck HJ, Brown P, Bennett D. Reproducibility of the microdilution checkerboard method for antibiotic synergy. Antimicrob Agents Chemother 1993;37:613-5.
11. White RL, Burgess DS, Manduru M, Bosso JA. Comparison of three different *in vitro* methods of detecting synergy: Time-kill, checkerboard, and E test. Antimicrob Agents Chemother 1996;40:1914-8.
12. Manno G, Ugolotti E, Belli ML, Fenu ML, Romano L, Cruciani M, *et al.* Use of the E test to assess synergy of antibiotic combinations against isolates of *Burkholderia cepacia*-complex from patients with cystic fibrosis. Eur J Clin Microbiol Infect Dis 2003;22:28-34.
13. Sopirala MM, Mangino JE, Gebreyes WA, Biller B, Bannerman T, Balada-Llasat JM, *et al.* Synergy testing by etest, microdilution checkerboard, and time-kill methods for pan-drug-resistant *Acinetobacter baumannii*. Antimicrob Agents Chemother 2010;54:4678-83.
14. Leonard SN, Kaatz GW, Rucker LR, Rybak MJ. Synergy between gemifloxacin and trimethoprim/sulfamethoxazole against community-associated methicillin-resistant *Staphylococcus aureus*. J Antimicrob Chemother 2008;62:1305-10.
15. Mouton JW, den Hollander JG. Killing of *Pseudomonas aeruginosa* during continuous and intermittent infusion of ceftazidime in an *in vitro* pharmacokinetic model. Antimicrob Agents Chemother 1994;38:931-6.
16. den Hollander JG, Horrevorts AM, van Goor ML, Verbrugh HA, Mouton JW. Synergism between tobramycin and ceftazidime against a resistant *Pseudomonas aeruginosa* strain, tested in an *in vitro* pharmacokinetic model. Antimicrob Agents Chemother 1997;41:95-100.
17. Cadwell JJ. The hollow fiber infection model for antimicrobial pharmacodynamics and pharmacokinetics. Adv Pharmacoepidemiol Drug Saf 2012;1:S1:007. doi:10.4172/2167-1052.S1-007.
18. Chan E, Zhou S, Srikumar S, Duan W. Use of *in vitro* critical inhibitory concentration, a novel approach to predict *in vivo* synergistic bactericidal effect of combined amikacin and piperacillin against *Pseudomonas aeruginosa* in a systemic rat infection model. Pharm Res 2006;23:729-41.
19. Mayer I, Nagy E. Investigation of the synergic effects of aminoglycoside-fluoroquinolone and third-generation cephalosporin combinations against clinical isolates of *Pseudomonas* spp. J Antimicrob Chemother 1999;43:651-7.
20. Altoparlak U, Aktas F, Celebi D, Ozkurt Z, Akcay MN. Prevalence of metallo-beta-lactamase among *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolated from burn wounds and *in vitro* activities of antibiotic combinations against these isolates. Burns 2005;31:707-10.
21. Kurien S, Lorian V. Discrepancies between results obtained by agar and broth techniques in testing of drug combinations. J Clin Microbiol 1980;11:527-9.
22. Pillai SK, Moellering RC, Eliopoulos GM. Antimicrobial combinations. In: Lorian V, editor. Antibiotic in Laboratory Medicine. 5th ed., Ch. 9 Philadelphia, USA: Lippincott Williams & Wilkins; 2005. p. 365.
23. Lorian V, Fodor G. Technique for determining the bactericidal effect of drug combinations. Antimicrob Agents Chemother 1974;5:630-3.
24. Aaron SD, Ferris W, Henry DA, Speert DP, Macdonald NE. Multiple combination bactericidal antibiotic testing for patients with cystic fibrosis infected with *Burkholderia cepacia*. Am J Respir Crit Care Med 2000;161:1206-12.
25. Lang BJ, Aaron SD, Ferris W, Hebert PC, MacDonald NE. Multiple combination bactericidal antibiotic testing for patients with cystic fibrosis infected with multiresistant strains of *Pseudomonas aeruginosa*. Am J Respir Crit Care Med 2000;162:2241-5.
26. Chinwuba ZG, Chiori CO, Ghobashy AA, Okore VC. Determination of the synergy of antibiotic combinations by an overlay inoculum susceptibility disc method. Arzneimittelforschung 1991;41:148-50.
27. Nworu CS, Esimone CO. Comparative evaluation of three *in vitro* techniques in the interaction of ampicillin and ciprofloxacin against *Staphylococcus aureus* and *Escherichia coli*. Trop J Pharm Res 2006;5:604-11.
28. Robinson A, Bartlett RC, Mazens MF. Antimicrobial synergy testing based on antibiotic levels, minimal bactericidal concentration, and serum bactericidal activity. Am J Clin Pathol 1985;84:328-33.
29. Amsterdam D. Susceptibility testing of antimicrobials in liquid media. In: Lorian V, editor. Antibiotic in Laboratory Medicine. 5th ed., Ch. 3. Philadelphia, USA: Lippincott Williams & Wilkins; 2005. p. 61.
30. Fantin B, Carbon C. *In vivo* antibiotic synergism: Contribution of animal models. Antimicrob Agents Chemother 1992;36:907-12.
31. Aoki N, Tateda K, Kikuchi Y, Kimura S, Miyazaki C, Ishii Y, *et al.* Efficacy of colistin combination therapy in a mouse model of pneumonia caused by multidrug-resistant *Pseudomonas aeruginosa*. J Antimicrob Chemother 2009;63:534-42.
32. Peleg AY, Jara S, Monga D, Eliopoulos GM, Moellering RC Jr., Mylonakis E, *et al.* Galleria mellonella as a model system to study *Acinetobacter baumannii* pathogenesis and therapeutics. Antimicrob Agents Chemother 2009;53:2605-9.
33. Hornsey M, Wareham DW. *In vivo* efficacy of glycopeptide-colistin combination therapies in a galleria mellonella model of *Acinetobacter baumannii* infection. Antimicrob Agents Chemother 2011;55:3534-7.
34. O'Hara JA, Ambe LA, Casella LG, Townsend BM, Pelletier MR, Ernst RK, *et al.* Activities of vancomycin-containing regimens against colistin-resistant *Acinetobacter baumannii* clinical strains. Antimicrob Agents Chemother 2013;57:2103-8.
35. Hornsey M, Phee L, Longshaw C, Wareham DW. *In vivo* efficacy of telavancin/colistin combination therapy in a galleria mellonella model of *Acinetobacter baumannii* infection. Int J Antimicrob Agents 2013;41:285-7.
36. Wareham DW, Bean DC. *In-vitro* activity of polymyxin B in combination with imipenem, rifampicin and azithromycin versus multidrug resistant strains of *Acinetobacter baumannii* producing OXA-23 carbapenemases. Ann Clin Microbiol Antimicrob 2006;5:10.
37. Kiffer CR, Sampaio JL, Sinto S, Oplustil CP, Koga PC, Arruda AC, *et al. In vitro* synergy test of meropenem and sulbactam against clinical isolates of *Acinetobacter baumannii*. Diagn Microbiol Infect Dis 2005;52:317-22.
38. Principe L, Capone A, Mazzarelli A, D'Arezzo S, Bordi E, Di Caro A, *et al. In vitro* activity of doripenem in combination with various antimicrobials against multidrug-resistant *Acinetobacter baumannii*: Possible options for the treatment of complicated infection. Microb Drug Resist 2013;19:407-14.
39. Lee CH, Tang YF, Su LH, Chien CC, Liu JW. Antimicrobial effects of varied combinations of meropenem, sulbactam, and colistin on a multidrug-resistant *Acinetobacter baumannii* isolate that caused meningitis and bacteremia. Microb Drug Resist 2008;14:233-7.
40. Sheng WH, Wang JT, Li SY, Lin YC, Cheng A, Chen YC, *et al.* Comparative *in vitro* antimicrobial susceptibilities and synergistic activities of antimicrobial combinations against carbapenem-resistant *Acinetobacter* species: *Acinetobacter baumannii* versus *Acinetobacter genospecies* 3 and 13TU. Diagn Microbiol Infect Dis 2011;70:380-6.
41. Cıkman A, Ceylan MR, Parlak M, Karahocagil MK, Berktaş M. Evaluation of colistin-ampicillin/sulbactam combination efficacy in imipenem-resistant *Acinetobacter baumannii* strains. Mikrobiyol Bul 2013;47:147-51.
42. Jernigan MG, Press EG, Nguyen MH, Clancy CJ, Shields RK. The combination of doripenem and colistin is bactericidal and synergistic against colistin-resistant, carbapenemase-producing *Klebsiella pneumoniae*. Antimicrob Agents Chemother 2012;56:3395-8.
43. Clancy CJ, Chen L, Hong JH, Cheng S, Hao B, Shields RK, *et al.*

Mutations of the ompK36 porin gene and promoter impact responses of sequence type 258, KPC-2-producing *Klebsiella pneumoniae* strains to doripenem and doripenem-colistin. Antimicrob Agents Chemother 2013;57:5258-65.
44. Souli M, Galani I, Boukovalas S, Gourgoulis MG, Chryssouli Z, Kanellakopoulou K, *et al. In vitro* interactions of antimicrobial combinations with fosfomycin against KPC-2-producing *Klebsiella pneumoniae* and protection of resistance development. Antimicrob Agents Chemother 2011;55:2395-7.
45. Hong JH, Clancy CJ, Cheng S, Shields RK, Chen L, Doi Y, *et al.* Characterization of porin expression in *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* identifies isolates most susceptible to the combination of colistin and carbapenems. Antimicrob Agents Chemother 2013;57:2147-53.
46. Marcus R, Paul M, Elphick H, Leibovici L. Clinical implications of β-lactam-aminoglycoside synergism: Systematic review of randomised trials. Int J Antimicrob Agents 2011;37:491-503.
47. Paul M, Dickstein Y, Schlesinger A, Grozinsky-Glasberg S, Soares-Weiser K, Leibovici L, *et al.* Beta-lactam versus beta-lactam-aminoglycoside combination therapy in cancer patients with neutropenia. Cochrane Database Syst Rev 2013;6:CD003038.
48. Biancofiore G, Tascini C, Bisà M, Gemignani G, Bindi ML, Leonildi A, *et al.* Colistin, meropenem and rifampin in a combination therapy for multi-drug-resistant *Acinetobacter baumannii* multifocal infection. A case report. Minerva Anestesiol 2007;73:181-5.
49. Lee NY, Wang CL, Chuang YC, Yu WL, Lee HC, Chang CM, *et al.* Combination carbapenem-sulbactam therapy for critically ill patients with multidrug-resistant *Acinetobacter baumannii* bacteremia: Four case reports and an *in vitro* combination synergy study. Pharmacotherapy 2007;27:1506-11.
50. Nastro M, Rodríguez CH, Monge R, Zintgraff J, Neira L, Rebollo M, *et al.* Activity of the colistin-rifampicin combination against colistin-resistant, carbapenemase-producing gram-negative bacteria. J Chemother 2014;26:211-6.
51. Bajaksouzian S, Visalli MA, Jacobs MR, Appelbaum PC. Activities of levofloxacin, ofloxacin, and ciprofloxacin, alone and in combination with amikacin, against *Acinetobacters* as determined by checkerboard and time-kill studies. Antimicrob Agents Chemother 1997;41:1073-6.
52. Bonapace CR, White RL, Friedrich LV, Bosso JA. Evaluation of antibiotic synergy against *Acinetobacter baumannii*: A comparison with etest, time-kill, and checkerboard methods. Diagn Microbiol Infect Dis 2000;38:43-50.
53. Pankey GA, Ashcraft DS. The detection of synergy between meropenem and polymyxin B against meropenem-resistant *Acinetobacter baumannii* using etest and time-kill assay. Diagn Microbiol Infect Dis 2009;63:228-32.
54. Gordon NC, Png K, Wareham DW. Potent synergy and sustained bactericidal activity of a vancomycin-colistin combination versus multidrug-resistant strains of *Acinetobacter baumannii*. Antimicrob Agents Chemother 2010;54:5316-22.
55. Tan TY, Lim TP, Lee WH, Sasikala S, Hsu LY, Kwa AL, *et al. In vitro* antibiotic synergy in extensively drug-resistant *Acinetobacter baumannii*: The effect of testing by time-kill, checkerboard, and etest methods. Antimicrob Agents Chemother 2011;55:436-8.
56. Santimaleeworagun W, Wongpoowarak P, Chayakul P, Pattharachayakul S, Tansakul P, Garey KW, *et al. In vitro* activity of colistin or sulbactam in combination with fosfomycin or imipenem against clinical isolates of carbapenem-resistant *Acinetobacter baumannii* producing OXA-23 carbapenemases. Southeast Asian J Trop Med Public Health 2011;42:890-900.
57. Vidaillac C, Benichou L, Duval RE. *In vitro* synergy of colistin combinations against colistin-resistant *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* isolates. Antimicrob Agents Chemother 2012;56:4856-61.
58. Galani I, Orlandou K, Moraitou H, Petrikkos G, Souli M. Colistin/daptomycin: An unconventional antimicrobial combination synergistic *in vitro* against multidrug-resistant *Acinetobacter baumannii*. Int J Antimicrob Agents 2014;43:370-4.
59. García-Salguero C, Rodríguez-Avial I, Picazo JJ, Culebras E. Can plazomicin alone or in combination be a therapeutic option against carbapenem-resistant *Acinetobacter baumannii*? Antimicrob Agents Chemother 2015;59:5959-66.
60. Park GC, Choi JA, Jang SJ, Jeong SH, Kim CM, Choi IS, *et al. In vitro* interactions of antibiotic combinations of colistin, tigecycline, and doripenem against extensively drug-resistant and multidrug-resistant *Acinetobacter baumannii*. Ann Lab Med 2016;36:124-30.
61. Hong DJ, Kim JO, Lee H, Yoon EJ, Jeong SH, Yong D, *et al. In vitro* antimicrobial synergy of colistin with rifampicin and carbapenems against colistin-resistant *Acinetobacter baumannii* clinical isolates. Diagn Microbiol Infect Dis 2016;86:184-9.
62. Bae S, Kim MC, Park SJ, Kim HS, Sung H, Kim MN, *et al. In vitro* synergistic activity of antimicrobial agents in combination against clinical isolates of colistin-resistant *Acinetobacter baumannii*. Antimicrob Agents Chemother 2016;60:6774-9.
63. Nepka M, Perivolioti E, Kraniotaki E, Politi L, Tsakris A, Pournaras S, *et al. In vitro* bactericidal activity of trimethoprim-sulfamethoxazole alone and in combination with colistin against carbapenem-resistant *Acinetobacter baumannii* clinical isolates. Antimicrob Agents Chemother 2016;60:6903-6.
64. Büyük A, Yilmaz FF, Yurtsever SG, Limoncu MH. Antibiotic resistance profiles and genotypes of *Acinetobacter baumannii* isolates and *in vitro* interactions of various antibiotics in combination with tigecycline and colistin. Turk J Pharm Sci 2017;14:13-8.
65. Visalli MA, Jacobs MR, Appelbaum PC. Determination of activities of levofloxacin, alone and combined with gentamicin, ceftazidime, cefpirome, and meropenem, against 124 strains of *Pseudomonas aeruginosa* by checkerboard and time-kill methodology. Antimicrob Agents Chemother 1998;42:953-5.
66. Di Bonaventura G, Picciani C, Spedicato I, Piccolomini R. E-test method for detecting antibiotic synergy against *Pseudomonas aeruginosa* from neutropenic patients: A cost-effective approach. New Microbiol 2004;27:263-72.
67. Pankey GA, Ashcraft DS. *In vitro* synergy of ciprofloxacin and gatifloxacin against ciprofloxacin-resistant *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2005;49:2959-64.
68. Pankey GA, Ashcraft DS. Detection of synergy using the combination of polymyxin B with either meropenem or rifampin against carbapenemase-producing *Klebsiella pneumoniae*. Diagn Microbiol Infect Dis 2011;70:561-4.
69. Pankey GA, Ashcraft DS, Dornelles A. Comparison of 3 etest® methods and time-kill assay for determination of antimicrobial synergy against carbapenemase-producing *Klebsiella* species. Diagn Microbiol Infect Dis 2013;77:220-6.
70. Gaibani P, Lombardo D, Lewis RE, Mercuri M, Bonora S, Landini MP, *et al. In vitro* activity and post-antibiotic effects of colistin in combination with other antimicrobials against colistin-resistant KPC-producing *Klebsiella pneumoniae* bloodstream isolates. J Antimicrob Chemother 2014;69:1856-65.
71. Ozseven AY, Cetin ES, Aridogan BC, Ozseven L. *In vitro* synergistic activity of carbapenems in combination with other antimicrobial agents against multidrug-resistant *Acinetobacter baumannii*. Afr J Microbiol Res 2012;6:2985-92.
72. Ji J, Du X, Chen Y, Fu Y, Wang H, Yu Y, *et al. In vitro* activity of sulbactam in combination with imipenem, meropenem, panipenem or cefoperazone against clinical isolates of *Acinetobacter baumannii*. Int J Antimicrob Agents 2013;41:400-1.
73. Pongpech P, Amornnopparattanakul S, Panapakdee S, Fungwithaya S, Nannha P, Dhiraputra C, *et al.* Antibacterial activity of carbapenem-based combinations againts multidrug-resistant *Acinetobacter baumannii*. J Med Assoc Thai 2010;93:161-71.
74. Ni W, Cui J, Liang B, Cai Y, Bai N, Cai X, *et al. In vitro* effects of tigecycline in combination with colistin (polymyxin E) and sulbactam against multidrug-resistant *Acinetobacter baumannii*. J Antibiot (Tokyo) 2013;66:705-8.
75. Pei G, Mao Y, Sun Y. *In vitro* activity of minocycline alone and in combination with cefoperazone-sulbactam against carbapenem-resistant *Acinetobacter baumannii*. Microb Drug Resist 2012;18:574-7.
76. Tong W, Wang R, Chai D, Li Z, Pei F. *In vitro* activity of cefepime combined with sulbactam against clinical isolates of

carbapenem-resistant *Acinetobacter* spp. Int J Antimicrob Agents 2006;28:454-6.
77. Turk Dagi H, Kus H, Arslan U, Tuncer I. *In vitro* synergistic activity of sulbactam in combination with imipenem, meropenem and cefoperazone against carbapenem-resistant *Acinetobacter baumannii* isolates. Mikrobiyol Bul 2014;48:311-5.
78. Laishram S, Anandan S, Devi BY, Elakkiya M, Priyanka B, Bhuvaneshwari T, *et al.* Determination of synergy between sulbactam, meropenem and colistin in carbapenem-resistant *Klebsiella pneumoniae* and *Acinetobacter baumannii* isolates and correlation with the molecular mechanism of resistance. J Chemother 2016;28:297-303.
79. Marie MA, Krishnappa LG, Alzahrani AJ, Mubaraki MA, Alyousef AA. A prospective evaluation of synergistic effect of sulbactam and tazobactam combination with meropenem or colistin against multidrug resistant *Acinetobacter baumannii*. Bosn J Basic Med Sci 2015;15:24-9.
80. Kempf M, Djouhri-Bouktab L, Brunel JM, Raoult D, Rolain JM. Synergistic activity of sulbactam combined with colistin against colistin-resistant *Acinetobacter baumannii*. Int J Antimicrob Agents 2012;39:180-1.
81. Kiratisin P, Apisarnthanarak A, Kaewdaeng S. Synergistic activities between carbapenems and other antimicrobial agents against *Acinetobacter baumannii* including multidrug-resistant and extensively drug-resistant isolates. Int J Antimicrob Agents 2010;36:243-6.
82. Ko WC, Lee HC, Chiang SR, Yan JJ, Wu JJ, Lu CL, *et al. In vitro* and *in vivo* activity of meropenem and sulbactam against a multidrug-resistant *Acinetobacter baumannii* strain. J Antimicrob Chemother 2004;53:393-5.
83. Song JY, Kee SY, Hwang IS, Seo YB, Jeong HW, Kim WJ, *et al. In vitro* activities of carbapenem/sulbactam combination, colistin, colistin/rifampicin combination and tigecycline against carbapenem-resistant *Acinetobacter baumannii*. J Antimicrob Chemother 2007;60:317-22.
84. Choi JY, Park YS, Cho CH, Park YS, Shin SY, Song YG, *et al.* Synergic *in-vitro* activity of imipenem and sulbactam against *Acinetobacter baumannii*. Clin Microbiol Infect 2004;10:1098-101.
85. Tatman-Otkun M, Gürcan S, Ozer B, Shokrylanbaran N. Annual trends in antibiotic resistance of nosocomial *Acinetobacter baumannii* strains and the effect of synergistic antibiotic combinations. New Microbiol 2004;27:21-8.
86. Timurkaynak F, Can F, Azap OK, Demirbilek M, Arslan H, Karaman SO, *et al. In vitro* activities of non-traditional antimicrobials alone or in combination against multidrug-resistant strains of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolated from Intensive Care Units. Int J Antimicrob Agents 2006;27:224-8.
87. Guelfi KC, Tognim MC, Cardoso CL, Gales AC, Carrara-Marrone FE, Garcia LB, *et al. In vitro* evaluation of the antimicrobial activity of meropenem in combination with polymyxin B and gatifloxacin against *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. J Chemother 2008;20:180-5.
88. Arroyo LA, Mateos I, González V, Aznar J. *In vitro* activities of tigecycline, minocycline, and colistin-tigecycline combination against multi- and pandrug-resistant clinical isolates of *Acinetobacter baumannii* group. Antimicrob Agents Chemother 2009;53:1295-6.
89. Clock SA, Tabibi S, Alba L, Kubin CJ, Whittier S, Saiman L, *et al. In vitro* activity of doripenem alone and in multi-agent combinations against extensively drug-resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae*. Diagn Microbiol Infect Dis 2013;76:343-6.
90. Tan TY, Ng LS, Tan E, Huang G. *In vitro* effect of minocycline and colistin combinations on imipenem-resistant *Acinetobacter baumannii* clinical isolates. J Antimicrob Chemother 2007;60:421-3.
91. Shields RK, Kwak EJ, Potoski BA, Doi Y, Adams-Haduch JM, Silviera FP, *et al.* High mortality rates among solid organ transplant recipients infected with extensively drug-resistant *Acinetobacter baumannii*: Using *in vitro* antibiotic combination testing to identify the combination of a carbapenem and colistin as an effective treatment regimen. Diagn Microbiol Infect Dis 2011;70:246-52.
92. Miyasaki Y, Morgan MA, Chan RC, Nichols WS, Hujer KM, Bonomo RA, *et al. In vitro* activity of antibiotic combinations against multidrug-resistant strains of *Acinetobacter baumannii* and the effects of their antibiotic resistance determinants. FEMS Microbiol Lett 2012;328:26-31.
93. Yoon J, Urban C, Terzian C, Mariano N, Rahal JJ. *In vitro* double and triple synergistic activities of polymyxin B, imipenem, and rifampin against multidrug-resistant *Acinetobacter baumannii*. Antimicrob Agents Chemother 2004;48:753-7.
94. Pankuch GA, Lin G, Seifert H, Appelbaum PC. Activity of meropenem with and without ciprofloxacin and colistin against *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Antimicrob Agents Chemother 2008;52:333-6.
95. Rodriguez CH, De Ambrosio A, Bajuk M, Spinozzi M, Nastro M, Bombicino K, *et al. In vitro* antimicrobials activity against endemic *Acinetobacter baumannii* multiresistant clones. J Infect Dev Ctries 2010;4:164-7.
96. Pankuch GA, Seifert H, Appelbaum PC. Activity of doripenem with and without levofloxacin, amikacin, and colistin against *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Diagn Microbiol Infect Dis 2010;67:191-7.
97. Liang W, Liu XF, Huang J, Zhu DM, Li J, Zhang J, *et al.* Activities of colistin- and minocycline-based combinations against extensive drug resistant *Acinetobacter baumannii* isolates from Intensive Care Unit patients. BMC Infect Dis 2011;11:109.
98. Peck KR, Kim MJ, Choi JY, Kim HS, Kang CI, Cho YK, *et al. In vitro* time-kill studies of antimicrobial agents against blood isolates of imipenem-resistant *Acinetobacter baumannii*, including colistin- or tigecycline-resistant isolates. J Med Microbiol 2012;61:353-60.
99. Santos DA, Nascimento MM, Vitali LH, Martinez R. *In vitro* activity of antimicrobial combinations against multidrug-resistant *Pseudomonas aeruginosa*. Rev Soc Bras Med Trop 2013;46:299-303.
100. Mitsugui CS, Tognim MC, Cardoso CL, Carrara-Marroni FE, Botelho Garcia L. *In vitro* activity of polymyxins in combination with β-lactams against clinical strains of *Pseudomonas aeruginosa*. Int J Antimicrob Agents 2011;38:447-50.
101. Dundar D, Otkun M. *In-vitro* efficacy of synergistic antibiotic combinations in multidrug resistant *Pseudomonas aeruginosa* strains. Yonsei Med J 2010;51:111-6.
102. Piccoli L, Guerrini M, Felici A, Marchetti F. *In vitro* and *in vivo* synergy of levofloxacin or amikacin both in combination with ceftazidime against clinical isolates of *Pseudomonas aeruginosa*. J Chemother 2005;17:355-60.
103. Dawis MA, Isenberg HD, France KA, Jenkins SG. *In vitro* activity of gatifloxacin alone and in combination with cefepime, meropenem, piperacillin and gentamicin against multidrug-resistant organisms. J Antimicrob Chemother 2003;51:1203-11.
104. Visalli MA, Bajaksouzian S, Jacobs MR, Appelbaum PC. Comparative activity of trovafloxacin, alone and in combination with other agents, against gram-negative nonfermentative rods. Antimicrob Agents Chemother 1997;41:1475-81.
105. Tessier F, Quentin C. *In vitro* activity of fosfomycin combined with ceftazidime, imipenem, amikacin, and ciprofloxacin against *Pseudomonas aeruginosa*. Eur J Clin Microbiol Infect Dis 1997;16:159-62.
106. He W, Kaniga K, Lynch AS, Flamm RK, Davies TA. *In vitro* etest synergy of doripenem with amikacin, colistin, and levofloxacin against pseudomonas aeruginosa with defined carbapenem resistance mechanisms as determined by the etest method. Diagn Microbiol Infect Dis 2012;74:417-9.
107. Samonis G, Maraki S, Karageorgopoulos DE, Vouloumanou EK, Falagas ME. Synergy of fosfomycin with carbapenems, colistin, netilmicin, and tigecycline against multidrug-resistant *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa* clinical isolates. Eur J Clin Microbiol Infect Dis 2012;31:695-701.
108. Sueke H, Kaye SB, Neal T, Hall A, Tuft S, Parry CM, *et al.* An *in vitro* investigation of synergy or antagonism between antimicrobial combinations against isolates from bacterial keratitis. Invest Ophthalmol Vis Sci 2010;51:4151-5.
109. Balke B, Hogardt M, Schmoldt S, Hoy L, Weissbrodt H, Häussler S, *et al.* Evaluation of the E test for the assessment of synergy of antibiotic combinations against multiresistant *Pseudomonas aeruginosa*

isolates from cystic fibrosis patients. Eur J Clin Microbiol Infect Dis 2006;25:25-30.
110. Giamarellos-Bourboulis EJ, Sambatakou H, Galani I, Giamarellou H. *In vitro* interaction of colistin and rifampin on multidrug-resistant *Pseudomonas aeruginosa*. J Chemother 2003;15:235-8.
111. Ermertcan S, Hoşgör M, Tünger O, Coşar G. Investigation of synergism of meropenem and ciprofloxacin against *Pseudomonas aeruginosa* and *Acinetobacter* strains isolated from Intensive Care Unit infections. Scand J Infect Dis 2001;33:818-21.
112. Gradelski E, Valera L, Bonner D, Fung-Tomc J. Synergistic activities of gatifloxacin in combination with other antimicrobial agents against *Pseudomonas aeruginosa* and related species. Antimicrob Agents Chemother 2001;45:3220-2.
113. Tascini C, Tagliaferri E, Giani T, Leonildi A, Flammini S, Casini B, *et al.* Synergistic activity of colistin plus rifampin against colistin-resistant KPC-producing *Klebsiella pneumoniae*. Antimicrob Agents Chemother 2013;57:3990-3.
114. Elemam A, Rahimian J, Doymaz M. *In vitro* evaluation of antibiotic synergy for polymyxin B-resistant carbapenemase-producing *Klebsiella pneumoniae*. J Clin Microbiol 2010;48:3558-62.
115. Stein C, Makarewicz O, Bohnert JA, Pfeifer Y, Kesselmeier M, Hagel S, *et al.* Three dimensional checkerboard synergy analysis of colistin, meropenem, tigecycline against multidrug-resistant clinical *Klebsiella pneumonia* isolates. PLoS One 2015;10:e0126479.
116. Lee GC, Burgess DS. Polymyxins and doripenem combination against KPC-producing *Klebsiella pneumoniae*. J Clin Med Res 2013;5:97-100.
117. Le J, McKee B, Srisupha-Olarn W, Burgess DS. *In vitro* activity of carbapenems alone and in combination with amikacin against KPC-producing *Klebsiella pneumoniae*. J Clin Med Res 2011;3:106-10.
118. Pournaras S, Vrioni G, Neou E, Dendrinos J, Dimitroulia E, Poulou A, *et al.* Activity of tigecycline alone and in combination with colistin and meropenem against *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Enterobacteriaceae* strains by time-kill assay. Int J Antimicrob Agents 2011;37:244-7.
119. Yim H, Woo H, Song W, Park MJ, Kim HS, Lee KM, *et al.* Time-kill synergy tests of tigecycline combined with imipenem, amikacin, and ciprofloxacin against clinical isolates of multidrug-resistant *Klebsiella pneumoniae* and *Escherichia coli*. Ann Clin Lab Sci 2011;41:39-43.
120. Vidaillac C, Leonard SN, Sader HS, Jones RN, Rybak MJ. *In vitro* activity of ceftaroline alone and in combination against clinical isolates of resistant gram-negative pathogens, including beta-lactamase-producing *Enterobacteriaceae* and *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2009;53:2360-6.
121. Souli M, Rekatsina PD, Chryssouli Z, Galani I, Giamarellou H, Kanellakopoulou K, *et al.* Does the activity of the combination of imipenem and colistin *in vitro* exceed the problem of resistance in metallo-beta-lactamase-producing *Klebsiella pneumoniae* isolates? Antimicrob Agents Chemother 2009;53:2133-5.
122. Diep JK, Jacobs DM, Sharma R, Covelli J, Bowers DR, Russo TA, *et al.* Polymyxin B in combination with rifampin and meropenem against polymyxin B-resistant KPC-producing *Klebsiella pneumoniae*. Antimicrob Agents Chemother 2017;61. pii: e02121-16.
123. Kulengowski B, Campion JJ, Feola DJ, Burgess DS. Effect of the meropenem MIC on the killing activity of meropenem and polymyxin B in combination against KPC-producing *Klebsiella pneumoniae*. J Antibiot (Tokyo) 2017;70:974-8.
124. Song JY, Cheong HJ, Lee J, Sung AK, Kim WJ. Efficacy of monotherapy and combined antibiotic therapy for carbapenem-resistant *Acinetobacter baumanni*i pneumonia in an immunosuppressed mouse model. Int J Antimicrob Agents 2009;33:33-9.
125. Poirel L, Kieffer N, Nordmann P. *In vitro* evaluation of dual carbapenem combinations against carbapenemase-producing *Enterobacteriaceae*. J Antimicrob Chemother 2016;71:156-61.