Combination antibiotic therapy for multidrug-resistant Gram-negative bacteria

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Abstract
Combination antibiotic therapy for Gram-negative sepsis is controversial. The present review provides a brief summary of the existing knowledge on combination therapy for severe infections with multidrug-resistant Pseudomonas spp., Acinetobacter spp., and Enterobacteriaceae. Empirical combination antibiotic therapy is recommended for severe sepsis and septic shock to reduce mortality related to inappropriate antibiotic treatment. Because definitive combination therapy has not been proven superior to monotherapy in meta-analyses, it is generally advised to de-escalate antibiotic therapy when the antibiotic susceptibility profile is known, although it cannot be excluded that some subgroups of patients might still benefit from continued combination therapy. Definitive combination therapy is recommended for carbapenemase-producing Enterobacteriaceae and should also be considered for severe infections with Pseudomonas and Acinetobacter spp. when beta-lactams cannot be used. Because resistance to broad-spectrum beta-lactams is increasing in Gram-negative bacteria and because no new antibiotics are expected to become available in the near future, the antibacterial potential of combination therapy should be further explored. In vitro data suggest that combinations can be effective even if the bacteria are resistant to the individual antibiotics, although existing evidence is insufficient to support the choice of combinations and explain the synergistic effects observed. In vitro models can be used to screen for effective combinations that can later be validated in animal or clinical studies. Further, in the absence of clinical evidence, in vitro data might be useful in supporting therapeutic decisions for severe infections with multidrug-resistant Gram-negative bacteria.

Key words: Acinetobacter, combination therapy, enterobacteriaceae, in vitro, multidrug-resistant, pseudomonas, sepsis, synergy

Introduction
Combination antibiotic therapy is frequently used to treat severe Gram-negative infections but is controversial and debatable. Potential achievements with combinations as compared with monotherapy include a broader antibacterial spectrum, synergistic effects, and reduced risk for emerging resistance during therapy. In the absence of evidence-based treatment options, combinations are increasingly employed to enhance the antibacterial effects of available drugs against multidrug-resistant strains. However, excessive use of combinations should be avoided because it might be associated with increased risk for toxicity, superinfections, selection of resistant strains, and higher costs.

The aim of the present review is to present and discuss existing knowledge on combination therapy for severe infections with Gram-negative bacteria, as well as to examine the potential use of antibiotic combinations and in vitro studies to manage the growing threat of multidrug-resistant Pseudomonas spp., Acinetobacter spp., and Enterobacteriaceae.

Empirical combination therapy for Gram-negative sepsis
The results of published clinical studies and meta-analyses on combination therapy for Gram-negative sepsis are diverse and contradictory (1–5). In a review article, combination therapy was associated with reduced mortality only in the subgroup of
**Pseudomonas aeruginosa** bacteraemia (1). In another review combination therapy was superior to monotherapy for severely ill patients, particularly those in septic shock (2). According to a recent Cochrane review, the addition of an aminoglycoside to a broad-spectrum beta-lactam does not reduce the overall mortality in patients with Gram-negative sepsis, but is associated with an increased risk for adverse events and is therefore discouraged (3).

The conflicting results might be explained by variations between studies with regard to patient characteristics, severity of infections, infection sites, causative bacteria, and antibiotic treatment. Delayed appropriate antibiotic therapy is known to be strongly associated with increased mortality in patients with septic shock (6), and broad-spectrum combination therapy will increase the probability for appropriate therapy as compared with single antibiotics (4,6–9). Therefore, empirical combination therapy is recommended for severe sepsis and septic shock with Gram-negative bacteria, particularly for neutropenic patients and patients at high risk of being infected with multidrug-resistant strains (10). The optimal choice of antibiotics depends on the local resistance epidemiology as well as individual risk factors for resistance, including recent antibiotic use, hospitalization, and previous colonization or infection with resistant strains (5).

**Definitive combination therapy for Gram-negative sepsis**

Definitive combination therapy including two antibiotics to which the bacteria are susceptible has been suggested to improve clinical outcome as compared with monotherapy for critically ill or neutropenic patients and severe infections with *Pseudomonas* spp. (11,12). Further, it has been argued that combinations should be used to prevent emergence of resistance during therapy (13). However, recent meta-analyses conclude that the existing clinical evidence is insufficient to support the use of definitive combination therapy for these reasons and that combination therapy is associated with an increased risk for ototoxicity, nephrotoxicity, bacterial superinfections, and selection of resistant strains (3,5). It has therefore been recommended to de-escalate antibiotic treatment to the most appropriate single agent as soon as the antibiotic susceptibility profile of the causative pathogen is known (5,10).

However, the non-inferiority with monotherapy reported in these meta-analyses refers to treatment with a broad-spectrum beta-lactam (3,5) and might not be valid for severe Gram-negative infections when these antibiotics cannot be used due to resistance or intolerance. For example, clinical studies strongly suggest that combination therapy is superior to monotherapy for carbapenemase-producing *Enterobacteriaceae*, even when the isolated bacteria are susceptible *in vitro* to the individual drugs (14–16). Monotherapy with an aminoglycoside is equally effective as beta-lactam antibiotics for urinary tract infections, but not for other infections, severe sepsis, or septic shock (17). Tigecycline is associated with higher mortality rates than carbapenems for severe Gram-negative infections, especially for hospital-acquired pneumonia, and has been questioned because of a bacteriostatic effect and reports of emerging resistance and breakthrough bacteraemia during therapy (18–22). Based on existing clinical data and a high risk for resistance development when used alone, monotherapy is not recommended for colistin or parenteral fosfomycin (14,15,21,23–25).

Thus, when beta-lactams are not suitable, prolonged or definitive combination therapy might be warranted for severe Gram-negative infections to improve the insufficient clinical efficacy of available treatment options.

**Suggested antibiotic combinations**

Clinical data to support the choice of antibiotic combinations are sparse and conflicting. Outcome might be difficult to assess for the severely ill patients included in these studies because of frequent changes in antibiotic therapy, co-morbidity and high all-cause mortality. Moreover, the results for specific combinations might differ between studies because of differences in patient material, infections, antibiotics used, dosage regimens, treatment durations, and strain-dependent factors.

Combination therapy for suspected Gram-negative sepsis and severe infections with *Pseudomonas* spp. typically includes a broad-spectrum beta-lactam and an aminoglycoside or a fluoroquinolone. However, colistin combinations are increasingly used as a last-resort treatment for multidrug-resistant strains (1,2,5,7–10,21). Combinations that include an aminoglycoside, ampicillin/sulbactam, a carbapenem, colistin, or rifampin have been successful against multidrug-resistant *Acinetobacter* spp. (26–29). Colistin–tigecycline and other combinations including an aminoglycoside, a carbapenem, colistin, fosfomycin, rifampin, or tigecycline have been advocated for carbapenemase-producing *Enterobacteriaceae* (14,16,21,30,31). Based on retrospective analysis, it has been recommended to use combinations including a carbapenem for these bacteria if the carbapenem minimum inhibitory concentration (MIC) is ≤4 mg/L (30).
Combination therapy for MDR Gram-negative bacteria

Combinations effective in vitro

In vitro, antibiotic combinations are usually evaluated with the checkerboard method or by time-kill experiments using static antibiotic concentrations. According to standard definitions, synergy depicts an enhanced antibacterial effect with the combination after 24 hours as compared with the effects of the individual antibiotics. The results from published in vitro studies are conflicting, which may be due to differences in methods, antibiotic concentrations, bacterial inocula, and strain-dependent factors. However, in many of these studies antibiotic combinations have demonstrated synergistic or bactericidal effects against bacteria that have been resistant to the individual drugs.

For example, synergistic effects have been demonstrated for double and triple antibiotic combinations including an aminoglycoside, an anti-pseudomonal beta-lactam, colistin, a fluoroquinolone, a macrolide, or rifampin against multidrug-resistant Pseudomonas spp. (32–36). Double and triple antibiotic combinations including an aminoglycoside, ampicillin/subbactam, a carbapenem, colistin, rifampin, tigecycline, or vancomycin have been effective against multidrug-resistant Acinetobacter spp. (35,37–40). For carbapenemase-producing Enterobacteriaceae, double and triple antibiotic combinations that include an aminoglycoside, aztreonam, a carbapenem, colistin, rifampin, tigecycline, or fosfomycin have demonstrated synergistic or bactericidal effects in vitro (25,35,41–44).

Mechanisms of synergy

The mechanisms of synergy are often not fully understood, but plausible explanations exist for some antibiotics. Colistin, which is frequently a component of effective combinations, increases the permeability of other antibiotics through the bacterial outer membrane by a detergent mechanism (45). This mechanism can counteract acquired resistance mediated by decreased antibiotic permeability (e.g. porin loss), and will also enable antibiotics that are not traditionally considered treatment options for Gram-negative bacteria to exert their actions. For instance, the addition of rifampin to colistin and meropenem/doripenem has resulted in synergistic effects in vitro against multidrug-resistant Pseudomonas spp., Acinetobacter spp., and carbapenemase-producing Enterobacteriaceae and has been reported as successful treatment in case reports (35,44,46,47). Synergy has sometimes been demonstrated for combination therapy that comprises several beta-lactams. For example, ertapenem–doripenem has been used against carbapenemase-producing Klebsiella pneumoniae (48,49). In these combinations synergy is probably achieved because beta-lactams, when hydrolysed, act as competitive beta-lactamase inhibitors (50).

Discussion and conclusions

Empirical combination antibiotic therapy is recommended for severe sepsis and septic shock caused by Gram-negative bacteria to reduce mortality related to inappropriate antibiotic treatment. Definitive combination therapy has not been proven superior to monotherapy with a broad-spectrum beta-lactam for patients with Gram-negative sepsis but is associated with an increased risk for toxicity and bacterial superinfections. However, the performed meta-analyses might have been insufficiently powered to detect benefits of definitive combination therapy in certain subgroups (e.g. critically ill or neutropenic patents and Pseudomonas aeruginosa bacteraemia). Definitive combination therapy is advocated for carbapenemase-producing Enterobacteriaceae and should also be considered for Pseudomonas and Acinetobacter spp. in situations in which beta-lactam monotherapy cannot be used because alternative antibiotics alone are often insufficient for severe infections.

Because resistance to carbapenems and other broad-spectrum beta-lactams is increasing, and because there is a lack of new antibiotics, it is urgent to explore the potential of combination therapy to enhance the antibacterial effects of available drugs. Clinical data to support the choice of combinations are insufficient. In vitro data suggest that combination therapy can be effective even if the bacteria are resistant to the individual drugs, but the results vary greatly between studies. A better appreciation of the mechanisms of synergy would facilitate the understanding of results obtained and help to predict the effects of other antibiotic combinations. For example, colistin is more likely to overcome impermeability than changes in the target molecule, and ertapenem can act as a competitive carbapenemase inhibitor in the periplasmic space only if the antibiotic molecules can penetrate the bacterial outer membrane.

For several reasons, the clinical relevance of in vitro findings is uncertain. However, in vitro models can be used to perform a large-scale screening for synergistic combinations to be further explored in animal studies and prospective clinical studies. In addition, in situations where there are no evidence-based treatment options, in vitro data can be useful to support therapeutic decisions for severe infections with multidrug-resistant Gram-negative bacteria.
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References

1. Saif N, Handelsman J, Maki DG. Does combination antimicrobial therapy reduce mortality in Gram-negative bacteremia? A meta-analysis. Lancet Infect Dis. 2004;4:519-27.
2. Kumar A, Safdar N, Kethireddy S, Chateau D. A survival benefit of combination antibiotic therapy for serious infections associated with sepsis and septic shock is contingent only on the risk of death: a meta-analytic/meta-regression study. Crit Care Med. 2010;38:1651–64.
3. Paul M, Lador A, Grozinys-Glasberg S, Leibovici L. Beta lactam antibiotic monotherapy versus beta lactam-aminoglycoside antibiotic combination therapy for sepsis. Cochrane Database Syst Rev. 2014;1:CD003344.
4. Kumar A, Zarychanski R, Light B, Parrillo J, Maki D, Simon D, et al. Early combination antibiotic therapy yields improved survival compared with monotherapy in septic shock: a propensity-matched analysis. Crit Care Med. 2010;38:1773–85.
5. Tamma PD, Cosgrove SE, Maragakis LL. Combination therapy for treatment of infections with Gram-negative bacteria. Clin Microbiol Rev. 2012;25:450–70.
6. Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Crit Care Med. 2006;34:1589–96.
7. Traugott KA, Echevarria K, Maxwell P, Green K, Lewis JS. Monotherapy or combination therapy? The Pseudomonas aeruginosa conundrum. Pharmacotherapy. 2011;31:598–608.
8. Micek ST, Welch EC, Khan J, Perverze M, Doherty JA, Reichley RM, et al. Empiric combination antibiotic therapy is associated with improved outcome against sepsis due to Gram-negative bacteria: a retrospective analysis. Antimicrob Agents Chemother. 2010;54:1742–8.
9. Martinez JA, Cobos-Trigueros N, Soriano A, Almela M, Ortega M, Marco F, et al. Influence of empiric therapy with a beta-lactam alone or combined with an aminoglycoside on prognosis of bacteremia due to gram-negative microorganisms. Antimicrob Agents Chemother. 2010;54:3590–6.
10. Dellige-RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, et al. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. Crit Care Med. 2013;41:580–637.
11. Ceftazidime combined with a short or long course of amikacin for empirical therapy of Gram-negative sepsis in cancer patients with granulocytopenia. The EORTC International Antimicrobial Therapy Cooperative Group. N Engl J Med. 1987;317:1692–8.
12. Hilf M, Yu VL, Sharp J, Zuravlev JJ, Korvick JA, Muder RR. Antibiotic therapy for Pseudomonas aeruginosa bacteremia: outcome correlations in a prospective study of 200 patients. Am J Med. 1989;87:540–6.
13. Mouton JW. Combination therapy as a tool to prevent emergence of bacterial resistance. Infection. 1999;27:524–8.
14. Hirsch EB, Tam VH. Detection and treatment options for Klebsiella pneumoniae carbapenemases (KPCs): an emerging cause of multidrug-resistant infection. J Antimicrob Chemother. 2010;65:1119–25.
15. Zarkotou O, Pournaras S, Tselioti P, Dragoumanos V, Pirigia V, Ranellou K, et al. Predictors of mortality in patients with bloodstream infections caused by KPC-producing Klebsiella pneumoniae and impact of appropriate antimicrobial treatment. Clin Microbiol Infect. 2011;17:1798–803.
16. Qureshi ZA, Paterson DL, Potoski BA, Kilia MC, Sandovks G, Sordillo E, et al. Treatment outcome of bacteremia due to KPC-producing Klebsiella pneumoniae: superiority of combination antimicrobial regimens. Antimicrob Agents Chemother. 2012;56:2108–13.
17. Vidal L, Gaiter-Gvili A, Borok S, Fraser A, Leibovici L, Paul M. Efficacy and safety of aminoglycoside monotherapy: systematic review and meta-analysis of randomized controlled trials. J Antimicrob Chemother. 2007;60:247–57.
18. FDA Drug Safety Communication: Increased risk of death with Tygacil (tigecycline) compared to other antibiotics used to treat similar infections. 2010. Available at http://www.fda.gov/Drugs/DrugSafety/ucm369580.htm [Accessed 17 March 2014].
19. Freire AT, Melnyk V, Kim MJ, Datsenko O, Dyublik O, Glumcher F, et al. Comparison of tigecycline with imipenem/cilastatin for the treatment of hospital-acquired pneumonia. Diagn Microbiol Infect Dis. 2010;68:140–51.
20. Souli M, Kontopodou FV, Papadomichelakis E, Galani I, Armaganidis A, Giarrarello H. Clinical experience of serious infections caused by Enterobacteriaceae producing VIM-1 metallo-beta-lactamase in a Greek University Hospital. Clin Infect Dis. 2008;46:847–54.
21. Miyakis S, Pefanis A, Tsakis A. The challenges of antimicrobial drug resistance in Greece. Clin Infect Dis. 2011;53:177–84.
22. Gordon NC, Wareham DW. A review of clinical and microbiological outcomes following treatment of infections involving multidrug-resistant Acinetobacter baumannii with tigecycline. J Antimicrob Chemother. 2009;63:775–80.
23. Paul M, Bishara J, Levcovich A, Chowers M, Goldberg E, Singer P, et al. Effectiveness and safety of colistin: prospective comparative cohort study. J Antimicrob Chemother. 2010;65:1019–27.
24. Kontopidou F, Plachouras D, Papadomichelakis E, Koukos G, Galani I, Poulikou G, et al. Colonization and infection by colistin-resistant Gram-negative bacteria in a cohort of critically ill patients. Clin Microbiol Infect. 2011;17:E9–11.
25. Souli M, Galani I, Boukalas S, Gorgoulis MG, Chryssouli Z, Kanellopoulou 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.
26. Kuo SC, Lai CC, Liao CH, Hsu CK, Chang YL, Chang CY, et al. Multidrug-resistant Acinetobacter baumannii bacteremia: clinical features, antimicrobial therapy and outcome. Clin Microbiol Infect. 2007;13:196–8.
27. Motauakkil S, Charrar B, Hachimi A, Nejmi H, Benslama A, Elmdaghri N, et al. Colistin and rifampicin in the treatment of nosocomial infections from multiresistant Acinetobacter baumannii. J Infect. 2006;53:274–8.
28. Shields RK, Kwak EJ, Potoski BA, Doi Y, Adams-Haduch JM, Silveria 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.
29. Bassetti M, Repetto E, Righi E, Boni S, Diverio M, Molinari MP, et al. Colistin and rifampicin in the treatment of multidrug-resistant Acinetobacter baumannii infections. J Antimicrob Chemother. 2008;61:417–20.

30. Daikos GL, Markogiannakis A. Carbapenemase-producing Klebsiella pneumoniae: (when) might we still consider treating with carbapenems? Clin Microbiol Infect. 2011;17:1135–41.

31. Michalopoulos A, Virtzili S, Rafaillidis P, Chalevelakis G, Damala M, Falagas ME. Intravenous fosfomycin for the treatment of nosocomial infections caused by carbapenem-resistant Klebsiella pneumoniae in critically ill patients: a prospective evaluation. Clin Microbiol Infect. 2010;16:184–6.

32. Fish DN, Choi MK, Jung R. Synergic activity of cephalosporins plus fluoroquinolones against Pseudomonas aeruginosa with resistance to one or both drugs. J Antimicrob Chemother. 2002;50:1045–9.

33. Saiman L, Chen Y, Gabriel PS, Knirsch C. Synergistic activities of macrolide antibiotics against Pseudomonas aeruginosa, Burkholderia cepacia, Stenotrophomonas maltophilia, and Alcaligenes xylosoxidans isolated from patients with cystic fibrosis. Antimicrob Agents Chemother. 2002;46:1105–7.

34. 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.

35. Urban C, Mariano N, Rahal JJ. In vitro double and triple bactericidal activities of doripenem, polymyxin B, and rifampin against multidrug-resistant Acinetobacter baumannii, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Escherichia coli. Antimicrob Agents Chemother. 2010;54:2732–4.

36. Bergen PJ, Tsuji BT, Bulitta JB, Forrest A, Jacob J, Sidjabat HE, et al. Synergistic killing of multidrug-resistant Pseudomonas aeruginosa at multiple inocula by colistin combined with doripenem in an in vitro pharmacokinetic/pharmacodynamic model. Antimicrob Agents Chemother. 2011;55:5685–95.

37. Kiffer CR, Sampaio JL, Sinto S, Oplustil CP, Koga PC, Arruda AC, et al. In vitro synergy test of meropenem and sulfactam against clinical isolates of Acinetobacter baumannii. Diagn Microbiol Infect Dis. 2005;52:317–22.

38. Li J, Nation RL, Owen RJ, Wong S, Spelman D, Franklin C. Antibiograms of multidrug-resistant clinical Acinetobacter baumannii: promising therapeutic options for treatment of infection with colistin-resistant strains. Clin Infect Dis. 2007;45:594–8.

39. Principe L, D’Arezzo S, Capone A, Petrosillo N, Visca P. In vitro activity of tigecycline in combination with various antimicrobials against multidrug resistant Acinetobacter baumannii. Ann Clin Microbiol Antimicrob. 2009;8:18.

40. 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.

41. Elefman 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.

42. Bercot B, Poirel L, Dortet L, Nordmann P. In vitro evaluation of antibiotic synergy for NDM-1-producing Enterobacteriaceae. J Antimicrob Chemother. 2011;66:2295–7.

43. Pouraras S, Vronti 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.

44. Tangden T, Hickman RA, Forsberg P, Lagerback P, Giske CG, Cars O. Evaluation of double and triple antibiotic combinations for VIM- and NDM-producing Klebsiella pneumoniae by in vitro time-kill experiments. Antimicrob Agents Chemother. 2014;58:1757–62.

45. Rahal JJ. Antimicrobial resistance among and therapeutic options against gram-negative pathogens. Clin Infect Dis. 2009;49:S4–10.

46. Biancofiore G, Tascini C, Bisa M, Gemignani G, Bindi ML, Leonardi A, et al. Colistin, meropenem and rifampin in a combination therapy for multi-drug-resistant Acinetobacter baumannii multilocal infection. A case report. Minerva Anestesiol. 2007;73:181–5.

47. Morelli P, Ferrario A, Tordato F, Piazza A, Casari E. Successful treatment of post-neurosurgical multidrug-resistant Pseudomonas aeruginosa meningo-encephalitis with combination therapy of colistin, rifampicin and doripenem. J Antimicrob Chemother. 2014;69:857–9.

48. Bulik CG, Nicolau DP. Double-carbapenem therapy for carbapenemase-producing Klebsiella pneumoniae. Antimicrob Agents Chemother. 2011;55:3002–4.

49. Ceccarelli G, Falcone M, Giordano A, Mezzatassa ML, Caio C, Stefani S, et al. Successful ertapenem-doripenem combination treatment of bacteremic ventilator-associated pneumonia due to colistin-resistant KPC-producing Klebsiella pneumoniae. Antimicrob Agents Chemother. 2013;57:2900–1.

50. Drawz SM, Bonomo RA. Three decades of beta-lactamase inhibitors. Clin Microbiol Rev. 2010;23:160–201.