Top Med Chem (2018) 25: 69–88
DOI: 10.1007/7355_2017_23

Published online: 26 July 2017

Synergistic Antibiotic Combinations

Karen Bush

Abstract Synergy between antibiotics is a strictly defined microbiological phenomenon, requiring two bioactive agents to exhibit enhanced bacterial killing when the two are combined. Because of increasing antibiotic resistance, and few new drugs to treat multidrug-resistant bacteria, combination therapy is often used in the clinical setting. Frequently, these combinations have demonstrated synergistic activity both in vitro and in animal models before being used therapeutically. Antibiotic combinations are more likely to be used in patients with drug-resistant staphylococcal or enterococcal infections, as well as in patients whose diseases are caused by carbapenem-resistant *Enterobacteriaceae*, *Pseudomonas aeruginosa*, or *Acinetobacter* spp. Although well-defined combinations have been approved by regulatory authorities as single agents, such as trimethoprim–sulfamethoxazole or β-lactamase inhibitor combinations, many combinations are used empirically with no clinical data to support their use. Because combination therapy will continue to be used in the absence of supportive clinical data, it will be important in the future to investigate mechanistic principles that may lead to predictive models for successful patient outcomes.

Keywords Antibiotic, Combination, Multidrug-resistant, Resistance

Contents

K. Bush (✉)
Indiana University Bloomington, Bloomington, IN 47405, USA
e-mail: karbush@indiana.edu

1 Introduction

Bacterial infections occur every day, in every country, in every age group, in every ethnic population. For thousands of years the human race has struggled to combat these infections, with limited success. The introduction of the sulfonamides in the 1930s [1], followed shortly thereafter by penicillin [2] and aminoglycosides [3], began to make the world complacent about the ability to overcome bacterial disease. However, following the use of these antibiotics, resistance arose more rapidly than expected [4], beginning with yearly increases in penicillin resistance in staphylococci in the 1940s [5, 6]. As novel resistance mechanisms to all antibiotics continue to emerge, resistant bacteria are becoming one of the most critical threats to human health worldwide. According to the Centers for Disease Control and Prevention (CDC) "Antibiotic resistance has been called one of the world's most pressing public health problems" [7]. In 2016 the United Nations issued a declaration addressing antibiotic resistance, with the UN Secretary General stating that "Antimicrobial resistance (AMR) poses a fundamental, long-term threat to human health, sustainable food production and development" [8].

In response to these concerns, the CDC provided a listing of those antibiotic-resistant pathogens deemed to be serious or urgent threats to human health in 2013 [9]. Among the most prominent are the urgent threat of carbapenem-resistant *Enterobacteriaceae* (CRE) and the serious threats of multidrug-resistant (MDR) *Pseudomonas aeruginosa*, *Acinetobacter* spp., and methicillin-resistant *Staphylococcus aureus* (MRSA) [9]. This document was followed in 2017 by the World Health Organization (WHO) report identifying the three most critical priorities as carbapenem-resistant *Acinetobacter baumannii*, *P. aeruginosa*, and *Enterobacteriaceae*, including extended-spectrum β-lactamase (ESBL)-producing as well as carbapenemase-producing isolates [10]. The WHO noted specifically that *Mycobacterium tuberculosis* was not included because it had already been identified as a global priority for which new treatments are urgently needed.

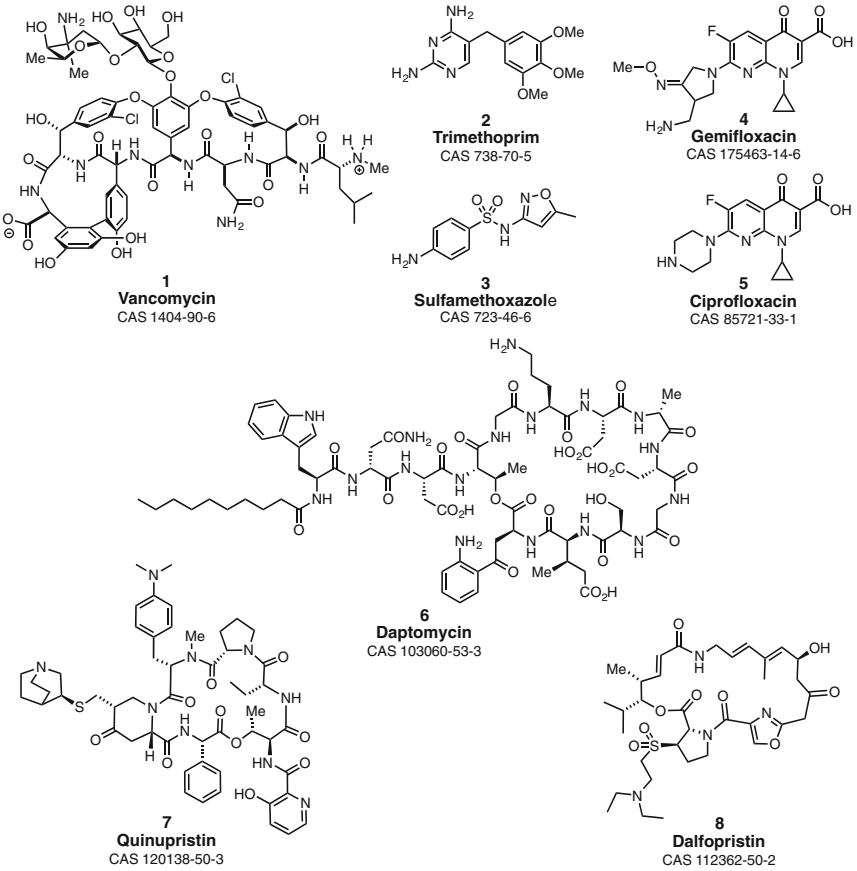
As the race between man and bug continues, fewer therapeutic options remain for the treatment of antibiotic-resistant infections. Although novel antibacterial drugs are both in development and have been introduced recently to the market, many of these agents encounter resistance within a short time following their introduction. Even with the new agents, monotherapy may not be sufficiently effective to treat serious infections caused by pathogens that are multidrug- or pan-resistant [11]. As a result, physicians have been relying on combination therapies to address these issues. Curiously, in a large meta-analysis of 12 recent

clinical studies that enrolled 3,571 patients treated empirically for ventilator-associated pneumonia (VAP), no statistical difference was observed in outcomes in patients treated with monotherapy compared to those who received combination therapy when following the American Thoracic Society guidelines [12]. However, these studies did not look at a subpopulation of patients with infections caused by MDR pathogens. Many experts believe that combination therapy should always be used as empiric therapy against MDR infections, especially when caused by CRE, *P. aeruginosa,* or *Acinetobacter* spp. [13]. This approach is based on the well-established principles used in the treatment of tuberculosis where monotherapy is never indicated due to the rapid selection of resistance, and new combinations of drugs are continually being tested in the clinic [14]. In addition to the enhanced antibacterial effects that can be gained by using more than one agent, combinations of known antibiotics may have the potential to reduce selection of resistance [15–17]. However, a recent study by Vestergaard et al. showed that the combination of ciprofloxacin and ceftazidime, when tested in vitro against *P. aeruginosa*, tended to select for broad-spectrum resistance due to mutational inactivation of *mex*R, the repressor gene that regulates expression of the multidrug efflux pump MexAB-OprM [18].

In this chapter, combinations of antibacterial agents are discussed using as the primary focus the literature from 2015 to 2017 describing combinations of agents shown to demonstrate microbiological synergy against pathogens of serious medical concern. The emphasis is on combinations of approved antimicrobial drugs, rather than proposed combinations with investigational agents such as the addition of the novel oxadiazoles to potentiate the activity of β-lactams against MRSA [19], or with agents that do not possess antibacterial activity. The use of adjuvants to improve pharmacological properties of an active agent is not covered.

2 Microbiological Synergy

Synergy is a well-defined concept in microbiological terminology. It is defined as the inhibition of microbial growth by two bioactive agents that exhibit a positive interaction [20]. According to a consensus in the clinical microbiology field [20–22], drug combinations may act in "synergy," may show "antagonism," or may have "no interaction" or "indifference." Investigators may test for synergism in vitro using checkerboard assays in which the concentrations of the two drugs are varied in a two-dimensional array and minimum inhibitory concentrations (MICs) are recorded. Although disk-diffusion synergy testing has also been described as a method to test for synergy, Sy et al. showed that broth dilution assays were more predictive of synergy than assays using disk diffusion, based on validation in time-kill studies of the combination of vancomycin (**1**) (Scheme 1) and β-lactam antibiotics against MRSA [23]. Most microbiologists validate checkerboard synergy results by monitoring the microbial growth of the target organism in the presence of each agent alone and in combination over a 24 h period



Scheme 1 Structures of the glycopeptide vancomycin; the folate antagonists trimethoprim and sulfamethoxazole; the fluoroquinolones gemifloxacin and ciprofloxacin; the cyclic lipopeptide daptomycin, and the synergistic inhibitors of the streptogramin-class, quinupristin–dalfopristin

in time-kill studies. Interpretations of the results from checkerboard assays are calculated using the "fractional inhibitory concentration" (FIC) index (FICI), as shown in the following consensus agreed upon in the early 2000s.

FIC = MIC for drug in combination/MIC for drug alone
FICI = FIC for drug A + FIC for drug B

FICI $\leq$ 0.5, Synergy
FICI $>$ 0.5–4.0, No interaction/nonsynergistic/nonantagonistic
FICI $>$ 4.0, Antagonism

These interpretations were accepted in order to avoid terms such as "additivity," "indifferent," or "partial synergy" that were previously used to describe data ranging from 0.5 to 4.0, within experimental error of an FICI value [20]. MIC

values that are determined in assays using drug concentrations in a series of twofold dilutions exhibit an inherent reading error of +/− one doubling dilution. Thus, valid data may fall into a fourfold range of being accurate, e.g., an MIC of 1 μg/mL may actually be 0.5 or 2 μg/mL and experimental variations of one twofold dilution for the MICs for each drug would give FICI values that remained in the "no interaction" range.

Data from killing curves, or time-kill assays, also have strict definitions for the interpretation of synergy, and are often used to validate synergistic combinations identified from checkerboard assays [24]. In these studies bacteria growing in log phase are incubated in media containing each drug alone or the drugs in combination and compared to a growth control that has no drug added to the medium. The concentration of at least one of the drugs should be low enough so as to not affect the growth of the organism when tested alone. At selected time points aliquots are removed and colony forming units (CFUs) are counted. Synergism is observed if these two criteria are met: [1] a decrease of at least 2 $\log_{10}$ CFU/mL is observed compared to the CFU/mL of the more active drug after 24 h; and [2] the final bacterial count at 24 h must be at least 2 $\log_{10}$ CFU/mL lower than the starting inoculum.

3 Approved Antibiotic Combinations

3.1 Folate Pathway Inhibitors

Relatively early in the history of antibiotic development, trimethoprim (**2**), a dihydrofolate reductase (DHFR) inhibitor, was shown to potentiate the activity of sulfonamide drugs that block the conversion of *p*-aminobenzoic acid into dihydrofolic acid [25]. Combination of trimethoprim with sulfamethoxazole (**3**) results in broad-spectrum, synergistic, bactericidal activity against a wide range of pathogens, which include MRSA, streptococci, *E. faecalis*, *Neisseria* spp., and many enteric bacteria. This combination is a well-prescribed and orally active therapy for the treatment of common infections such as urinary tract infections (UTI) and otitis media, particularly in patients with allergies to other antibiotics [26]. Although strong synergy is observed in vitro for the combination, clinical practice suggested that this synergy did not always carry over to the treatment of patients [27], except for the treatment of toxoplasmosis, brucellosis, nocardiosis, chancroid, and pneumonia due to *Pneumocystis carinii* [28]. Resistance to trimethoprim can occur as a result of several different events, including the acquisition of a plasmid encoding a DHFR that confers high-level resistance [29]. Unfortunately, the use of the combination did not tend to reduce the emergence of trimethoprim resistance, but trimethoprim appears to reduce the incidence of sulfonamide resistance [28]. Triple combinations including trimethoprim–sulfamethoxazole have also been considered. Gemifloxacin (**4**) in combination with trimethoprim–

sulfamethoxazole has demonstrated synergistic bactericidal activity against community-acquired-MRSA (CA-MRSA) in both time-kill studies and animal models [55]. Combinations of vancomycin or ciprofloxacin (**5**) tested in vitro in time-kill assays with trimethoprim–sulfamethoxazole were also synergistic against vancomycin-intermediate *S. aureus* (VISA) or heterogeneous vancomycin-intermediate *S. aureus* (hVISA) [56]. In a clinical study (Table 1), the combination of daptomycin (**6**) and trimethoprim–sulfamethoxazole resulted in microbiological cures of 24 of the 28 patients infected with either daptomycin-susceptible or daptomycin-resistant MRSA; 17 of the 17 isolates that could be recovered demonstrated synergistic behavior in time-kill assays [54].

3.2 Streptogramins

Quinupristin–dalfopristin represents the only approved streptogramin combination. Quinupristin (**7**) is a cyclic depsipeptide analog of the naturally occurring pristinamycin IA, and dalfopristin (**8**) is a polyunsaturated cyclic macrolactone derivative of the natural product pristinamycin IIA, all members of the streptogramin family [57]. Combined in a molar ratio of 30:70, the combination has potent synergistic activity against Gram-positive bacteria, including MRSA and MDR-streptococci. In contrast to the behavior of many agents with Gram-positive activity, *E. faecalis* demonstrates intrinsic resistance due to production of an ABC (ATP-binding cassette) homologue, Lsa(A), whereas the generally more resistant *E. faecium* is naturally susceptible. Resistance to the streptogramins in *E. faecium* has been reported both in vitro and in vivo due to production of a variant of Lsa (A) with a point mutation [58]. Over time, the drug combination has not been used extensively in the clinic, due in part to a relatively high incidence of localized phlebitis during infusion, and observed elevations in serum aminotransferase levels in a small percentage of patients [57].

3.3 β-Lactamase Inhibitor Combinations

Probably the most commonly used antibiotic-combination therapy involves the addition of a β-lactamase inhibitor (BLI) to a β-lactam that is labile to hydrolysis by β-lactamases. Prescribing information reported for the years 2004–2014 shows that 65% of all United States hospital prescriptions are for β-lactam antibiotics, and of these over half are for BLI combinations [59]. Because this set of combinations has been reviewed extensively in the past few years [59–61], particularly with respect to newer combinations in clinical development, the following discussion is centered on FDA-approved BLI combinations (Scheme 2).

Beginning in 1986 and proceeding until 2014, three BLIs were approved for therapeutic use, with all the inhibitors matched with a penicillin counterpart:

Table 1 Empiric antibiotic combinations using FDA-approved antibacterial agents

Antibiotic	Antibiotic in combination	Organism affected	Studies to support synergy	Reference
Colistin	Azithromycin	*Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*	Time-kill curves	[30]
	Chloramphenicol	*Klebsiella pneumoniae*	Time-kill curves	[31]
	Doripenem	*Pseudomonas aeruginosa*	In vitro, hollow fiber studies	[17]
	Rifampin	*Acinetobacter baumannii*	Checkerboard; time-kill curves	[32]
	Tazobactam	*Acinetobacter baumannii*	Time-kill curves	[33]
	Tigecycline	*Acinetobacter baumannii*, CRE[a], *Klebsiella pneumoniae*	Checkerboard; time-kill curves clinical data	[34–37]
	Vancomycin	*Acinetobacter baumannii*	Checkerboard; time-kill curves	[32]
Daptomycin	Ceftaroline	MRSA	Bacteremic patients	[38]
	β-Lactams	MRSA, enterococci	Checkerboard; time-kill curves	[39–41]
	Dalbavancin	MRSA	Checkerboard	[42]
	Gentamicin	MRSA, enterococci	Checkerboard; time-kill curves (variable results)	[40, 43–45]
	Linezolid	MRSA	Checkerboard	[42]
	Sulbactam, tazobactam	MRSA, hVISA, VISA[b]	Time-kill curves	[33]
	Tigecycline	MRSA	Checkerboard; time-kill curves, surgical site infection model	[46]
Levofloxacin	Linezolid	*Bacillus anthracis*	Synergy in checkerboard against Sterne strain; indifference or antagonism in models	[47]
Vancomycin	β-Lactams	MRSA	16 studies based on in vitro and in vivo animal models	Summarized in [43, 48, 49]
	Ceftaroline	MRSA	In vitro PK/PD model; six clinical case studies	[50, 51]
	Flucloxacillin	MRSA	Bacteremic patients	[48]

(continued)

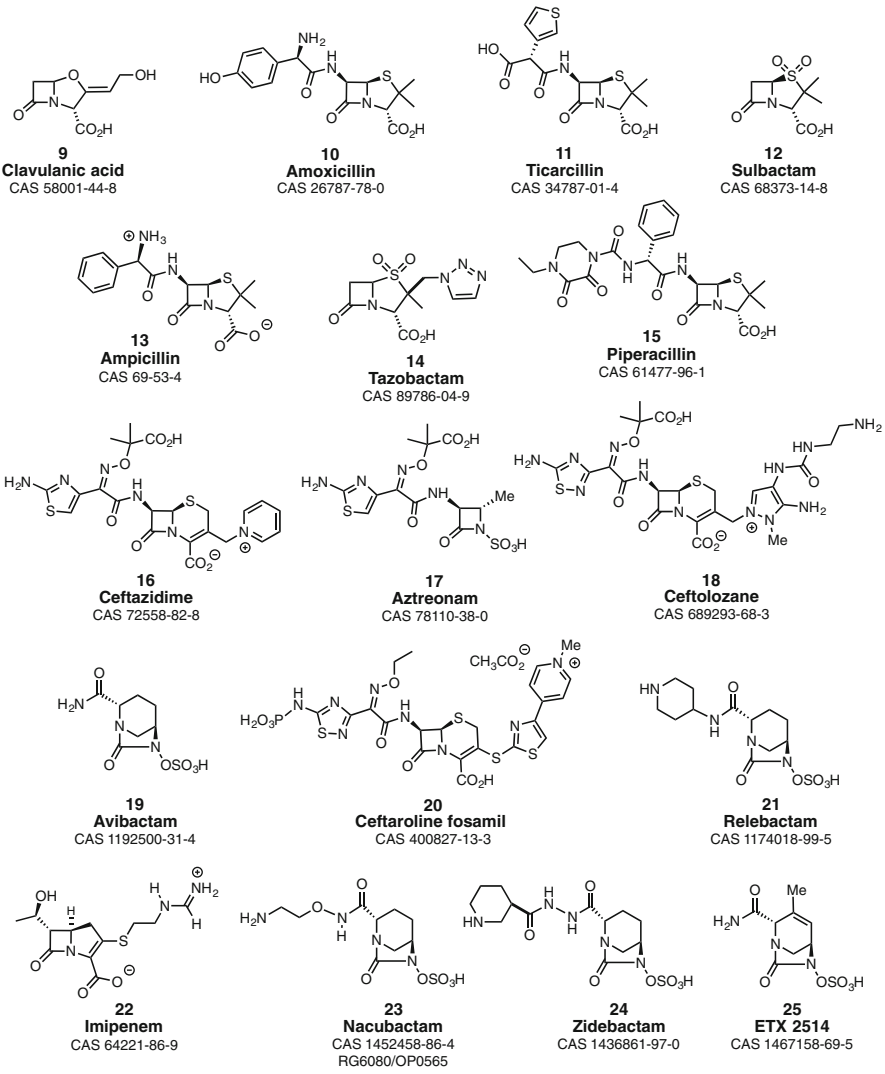
Table 1 (continued)

Antibiotic	Antibiotic in combination	Organism affected	Studies to support synergy	Reference
	Gentamicin	MRSA	Checkerboard; time-kill curves; not supported by clinical studies	[52, 53]
	Trimethoprim–sulfamethoxazole	MRSA	Clinical studies in daptomycin-resistant patients	[54]

[a]*CRE* carbapenem-resistant Enterobacteriaceae
[b]*MRSA* methicillin-resistant *Staphylococcus aureus*, *hVISA* heteroresistant vancomycin-intermediate *S. aureus*, *VISA* vancomycin-intermediate *S. aureus*

clavulanic acid (**9**) with amoxicillin (**10**) or ticarcillin (**11**); sulbactam (**12**) with ampicillin (**13**); and tazobactam (**14**) with piperacillin (**15**). These inhibitors act as suicide inactivators with inhibitory activity against class A penicillinases and broad-spectrum β-lactamases that do not hydrolyze carbapenems or β-lactams with aminothiazole oxime side chains such as ceftazidime (**16**) or aztreonam (**17**) [62]. These BLI-penicillin combinations had broad-spectrum bactericidal activity against MSSA, streptococci, and enteric bacteria. Piperacillin–tazobactam also was efficacious against pseudomonal infections, primarily due to the antipseudomonal activity of piperacillin. The three BLIs were developed during the time that ESBLs were not known (clavulanic acid and sulbactam combinations), or during the time that ESBLs were considered to be rarities in clinical practice (piperacillin–tazobactam). However, that situation changed during the late 1990s when ESBLs became global problems. Although the inhibitors usually demonstrated inhibitory activities against most ESBLs when tested in vitro in isolated enzyme assays, they fell down in efficacy when the combinations were tested against ESBL-producing organisms that harbored additional β-lactamases. As early as 2000 in Canada, 71% of organisms that produced an ESBL were reported to produce at least one other β-lactamase, resulting in <31% susceptibility to either amoxicillin-clavulanic acid or piperacillin–tazobactam [63]. In addition to the ESBLs, the emergence of carbapenemases in the early 2000s posed additional problems for the inhibitors; none of the inhibitors affected the activity of metallo-β-lactamases (MBLs), and had poor activity when tested in penicillin combinations in whole cell assays with organisms that produced serine carbapenemases such as the KPC enzymes [64], in spite of comparable tazobactam concentrations that effectively inhibited either isolated KPC or broad-spectrum TEM enzymes [65, 66]. Notably, almost all carbapenemase-producing organisms also produce additional β-lactamases in a similar manner as seen with the ESBLs [67], thus exacerbating the situation.

In an attempt to address the decreased response to BLI combinations in ESBL-producing organisms, in 2014 the FDA approved the combination of ceftolozane (**18**), a potent antipseudomonal cephalosporin, with tazobactam, using a different tazobactam dosing regimen from that used for the piperacillin–tazobactam



Scheme 2 Structures of the penicillin β-lactams amoxicillin, ticarcillin, ampicillin, and piperacillin; the cephalosporins β ceftazidime and ceftolozane; the anti-MRSA cephalosporin pro-drug ceftaroline fosamil; the monobactam aztreonam; the carbapenem imipenem; the classical β-lactamase inhibitors clavulanic acid, sulbactam, and tazobactam; and the non-classical DBO-class β-lactamase inhibitors avibactam, relebactam, nacubactam, zidebactam, and ETX2514

combination to allow for more favorable pharmacodynamics [68, 69]. Although the combination had high susceptibility rates when tested against *E. coli* producing a single CTX-M-14 or CTX-M-15 ESBL [70], the agent is probably most useful as an antipseudomonal drug, exhibiting >90% susceptibility in contemporary meropenem-resistant *P. aeruginosa* isolates [71].

The argument may be made that these combinations do not fit the classical definition of synergism, in that a β-lactamase inhibitor is not necessarily considered to have antibacterial activity in its own right. However, many BLIs are known to bind to essential PBPs and may exhibit at least some weak growth inhibition. Examples include clavulanic acid with MICs as low as 0.1 μg/mL for *Neisseria gonorrhoeae* and 6.3 μg/mL for *Haemophilus influenzae* [72] and sulbactam that inhibits PBPs 1 and 3 in *A. baumannii*. [73]. Some of the more recent BLIs have even greater antibacterial activity on their own, thereby qualifying as legitimate synergistic agents when combined with a companion β-lactam.

In 2015 the FDA and EMA approved the combination of ceftazidime with avibactam (**19**), a non-β-lactam BLI with weak antibacterial activity due to binding to PBP4 in *S. aureus* and PBP2 in Gram-negative bacteria [74]. Although avibactam MICs as low as 4 μg/mL against *E. coli* have been reported, MICs >64 μg/mL against non-enteric bacteria and *S. aureus* have been also detected [74, 75]. Avibactam is a potent, covalent, reversible inhibitor of most class A, C, and D β-lactamases [76, 77], and an irreversible inhibitor of the KPC-2 carbapenemase [77]. Combinations of avibactam at subinhibitory concentrations were capable of potentiating ceftazidime such that MICs could be reduced as much as 1,000-fold in enteric bacteria producing KPC and/or ESBL enzymes [78, 79]. Avibactam may also be combined with the anti-MRSA cephalosporin ceftaroline (**20**), potentially to provide efficacy against mixed infections that include Gram-positive pathogens as well as ESBL- or KPC-producing Gram-negative bacteria [80]. Avibactam is also being studied in combination with aztreonam (a monobactam with stability against MBL hydrolysis) in Phase 2 clinical studies (https://clinicaltrials.gov/ct2/home), thus potentially providing at least some coverage of MBL-producing organisms [81].

After confirmation of the potent β-lactamase-inhibitory activity of avibactam, its diazabicyclooctane (DBO) structure was modified extensively by medicinal chemists at multiple pharmaceutical companies to provide "second generation" DBO derivatives such as relebactam (**21**), being developed in combination with imipenem (**22**) [82]. Some of these newer DBOs have enhanced antibacterial activity, such as nacubactam (RG6080/OP0595) (**23**) [83], zidebactam (**24**) [84], or ETX2514 (**25**) [85], again due to binding to PBP2 to provide a dual mechanism of action in Gram-negative bacteria. The combination of the PBP2-binding inhibitors with cephalosporins or monobactams has been proposed to offer a selective advantage in terms of the emergence of resistance [83]. The high affinity of these β-lactams for PBP3 drives their antibacterial activity, so it is possible that resistance due to target modifications will require mutations in both PBP2 and PBP3 to achieve high-level resistance. However, in the short time in which the newer combinations have been used clinically, resistance has been reported in patients treated with ceftazidime–avibactam. Mutations have been reported in KPC-3 with multiple point mutations in different patients [86], conferring resistance to avibactam combinations, but restoring susceptibility to meropenem. Mutations in *E. coli* PBP3 have also been reported in historical clinical isolates, appearing as

gene duplications resulting in four amino-acid insertions that result in 4- to 32-fold increases in MICs for ceftazidime, as well as for ceftaroline and avibactam [87, 88].

4 Empirical Antibiotic Combinations

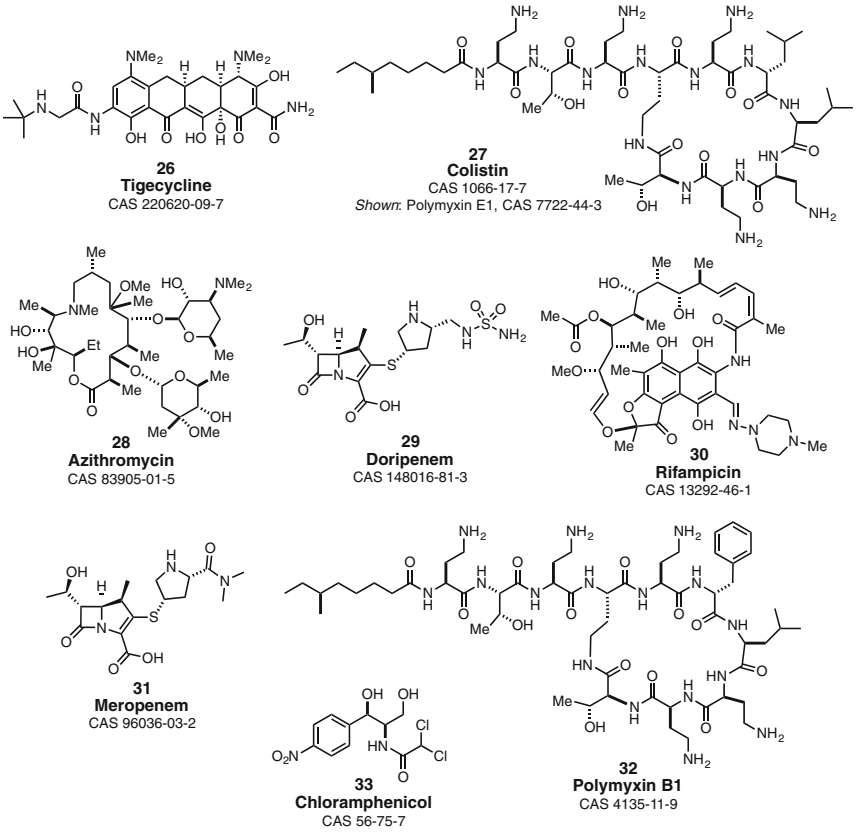
Empirical antibiotic combinations refer to combinations used clinically in the absence of an approved indication by a regulatory agency. In vitro microbiological synergy data may exist to support the use of combination, but there are few, if any, controlled clinical trials that support the use of these agents as effective therapies. These combinations are listed in Table 1.

4.1 Gram-Negative Infections

Gram-negative bacteria are frequently named among the greatest threats to human health [9, 10]. Among the most worrisome are the nonfermentative bacteria *Acinetobacter* spp. and *P. aeruginosa*, as well as the carbapenem-resistant Enterobacteriaceae (CRE). Combination therapy is frequently recommended for initial empiric treatment of patients infected with these organisms [13, 89]. In vitro studies have even suggested that two carbapenems may be synergistic against KPC- or OXA-48-producing Enterobacteriaceae [90]. Limited clinical data based on retrospective data have suggested that a carbapenem (meropenem) in combination with another sensitive drug may be successful in treating CRE infections if the meropenem MIC was <8 μg/mL [91]. In another set of retrospective clinical data from 26 published studies, CRE-infected patients treated with a tigecycline (**26**) combination were statistically more likely to have lower mortality, both in the ICU and at a 30-day follow-up evaluation, compared to patients treated with monotherapy [34] (Scheme 3).

Antimicrobial peptides, especially colistin (**27**), a member of the polymyxin class, have become drugs of last resort for the treatment of infections caused by MDR Gram-negative pathogens. However, liabilities associated with colistin are the perception of increased nephrotoxicity compared to the β-lactams and macrolides, and increasing colistin-resistance [17, 30]. For this reason, combinations with other, safer antibiotics have been examined. One of the more unusual combinations involves a colistin-azithromycin (**28**) duo that demonstrated synergistic activity by time-kill assays against *A. baumannii*, *K. pneumoniae,* and *P. aeruginosa*. The macrolide with poor antibacterial activity against Gram-negative bacteria is presumed to be synergized due to the membrane-permeabilizing properties of colistin [30].

Other colistin combinations that have been studied in vitro include combinations with β-lactams against *A. baumannii* (tazobactam) [33] and *P. aeruginosa* (doripenem) (**29**) [17]. In the latter combination, suppression of resistance was



Scheme 3 Structures of the glycylcycline tigecycline; the polymyxins E1 (primary structural component of colistin) and B1; the macrolide azithromycin; the carbapenems doripenem and meropenem; the rifamycin-class, rifampicin; and chloramphenicol

noted for both colistin and doripenem [17]. Against *A. baumannii*, colistin also synergized the antibacterial activity of rifampicin (**30**) or vancomycin [32] or tigecycline [35, 36]. The latter combination also demonstrated synergy against *K. pneumoniae*, both in vitro and in clinical studies [37]. Triple combinations of colistin with meropenem (**31**) and tigecycline demonstrated synergistic activity against MDR *K. pneumoniae*, but it was no greater than that observed with double colistin combinations with either agent alone [92]. Similar to the observed behavior with colistin, polymyxin B (**32**) was able to synergize the activity of chloramphenicol (**33**) in vitro against MDR *K. pneumoniae* [31]. However, the additive possibilities for toxicity probably do not warrant serious consideration for clinical usage [93]. Clinical data to support combinations therapy to treat infections caused by MDR Gram-negative bacteria are still sparse, especially with regard to controlled trials [34, 89], and further efforts to correlate in vitro synergy with clinical outcomes are needed.

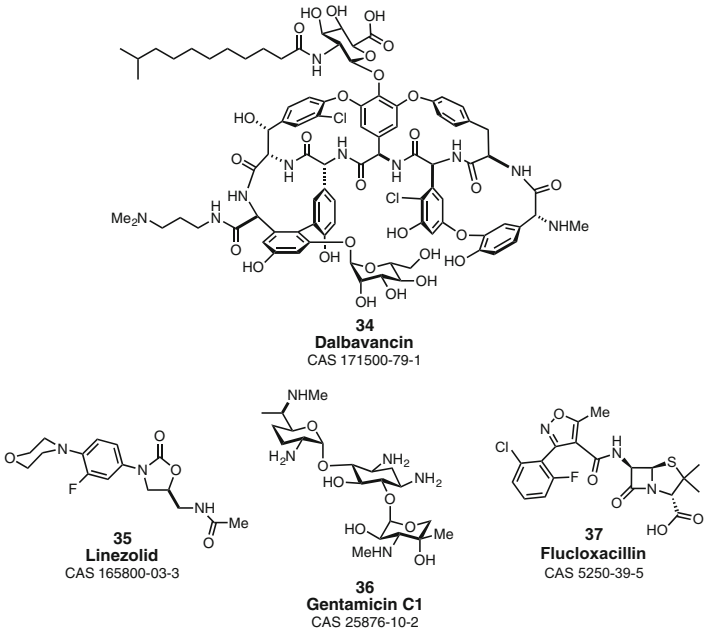
4.2 Gram-Positive Infections

Combination therapy for treatment of Gram-positive infections has been discussed extensively in the literature, with recent reviews tackling the issue with respect to MDR infections caused by MRSA [43] and vancomycin-resistant enterococci (VRE) [94]. Combinations that have been studied either in vitro or in clinical studies are summarized in Table 1. The anti-MRSA cephalosporin ceftaroline has been used successfully as a companion to vancomycin, based on in vitro pharmacodynamic studies and on retrospective case reports [50, 51]. Ceftaroline in combination with daptomycin to treat 20 patients with MRSA bacteremia resulted in a shortened time to eradication compared to standard therapy; in addition, the combination caused a sensitization to bacterial killing by neutrophils [38]. Mechanistically ceftaroline has been shown to bind to a specific allosteric site as well as the active site of PBP2a in MRSA, thus allowing for the possibility of ceftaroline allosteric binding to enhance the binding of other β-lactams to the active site [95].

Daptomycin combinations with drugs in various antibacterial classes have also been studied. Synergy has been observed in time-kill studies with daptomycin combinations containing sulfone-containing β-lactamase inhibitors against MRSA [33, 96] or for combinations with other β-lactams [39–41], especially β-lactams such as meropenem and imipenem that bind preferentially to PBP1 [39]. The combination of daptomycin with dalbavancin (**34**), molecules with similar chemical structures and functions with respect to bacterial killing, was synergistic for MRSA using checkerboard assays; likewise, the protein synthesis inhibitor linezolid (**35**) was also synergistic in the same study [42]. For the synergistic daptomycin-gentamicin (**36**) combination, resistance rates were lower in vitro when tested against MRSA [44]. The daptomycin–tigecycline was synergistic in vitro in checkerboard and time-kill assays, in addition to a surgical site infection model [46] (Scheme 4).

Other antibiotics that kill bacteria by interfering with cell-wall assembly include vancomycin and the β-lactam antibiotics, agents with variable activity against MDR Gram-positive bacteria. Vancomycin, a commonly prescribed agent for treatment of infections caused by MRSA and vancomycin-susceptible enterococci, has demonstrated synergy against these organisms with a number of agents both in vitro and in clinical trials to treat the most serious of these infections. In clinical studies, vancomycin combined with the antistaphylococcal flucloxacillin (**37**) shortened the duration of bacteremia caused by MRSA [48] and was successfully combined with trimethoprim–sulfamethoxazole to treat patients infected with daptomycin-resistant MRSA [54]. Triple β-lactam combinations with in vitro synergistic activity against MRSA include meropenem–piperacillin–tazobactam, a combination shown to suppress the emergence of resistance [49]. This finding is notable in that each of these β-lactams individually has limited anti-MRSA activity.

Linezolid (**35**), a bacteriostatic protein synthesis inhibitor, has been studied in combinations with bactericidal drugs for potential treatment of infections caused by toxin-producing or spore-forming Gram-positive bacteria. These combinations are



Scheme 4 Structures of the lipoglycopeptide dalbavancin; the oxazolidinone linezolid; the aminoglycoside gentamicin C1; and the antistaphylococcal penicillin flucloxacillin

based on the hypothesis that linezolid could inhibit the formation of toxins or spores, at the same time that the organism is being killed by the companion drug. However, the results for these combinations have been mixed. The combination of vancomycin with linezolid has resulted in conflicting reports about synergistic activity against staphylococci [43]. Although in vitro studies demonstrated that linezolid could inhibit toxin production by *S. aureus* [52, 53], possibly serving to decrease virulence, this result has not been validated in animal infection models [43]. Similarly, in studies with *Bacillus anthracis*, linezolid inhibited toxin production when used alone, but the combination with levofloxacin that was synergistic in time-kill studies did not significantly affect spore or toxin formation compared to linezolid alone [47].

Combinations of the aminoglycoside gentamicin with a variety of other agents have also been examined against MRSA. However, clinical data based on patients who received gentamicin together with vancomycin showed no significant improvement in 6-month recurrence rates [45]. When low dose gentamicin was administered with either vancomycin, daptomycin, or an antistaphylococcal penicillin to treat patients suspected to have *S. aureus* native valve endocarditis, a significant decrease in creatinine clearance was reported [97]. Thus, this combination is not clinically advisable.

5 Future Directions

Antibiotic combinations will continue to be used to treat seriously ill patients, because any delay in providing appropriate therapy increases morbidity and mortality [98, 99]. Many of these combinations will be used empirically based on in vitro synergy testing or sporadic case reports, because of the lack of controlled randomized clinical trial data. In vitro synergy testing of newer agents with reduced antimicrobial activity against resistant organisms will undoubtedly lead to the identification of effective combinations with established antibiotics. However, at this time, mechanistic explanations for many of the synergistic combinations are lacking. It is hoped that further studies delineating the biochemical or microbiological explanations for the observed synergies will be undertaken, so as to guide the identification of additional useful combinations of drugs that may be used to treat the most deleterious and life-threatening pathogens. Perhaps, in the process, the selection of resistance to these agents will be diminished as a result of multiple targets that must be mutated in order for resistance to emerge. The study of antibiotic combinations, therefore, will continue to be of high interest in the pursuit of treatment options for MDR and pan-resistant bacteria.

References

1. Blake FG (1940) Chemotherapy with the sulfonamide derivatives: general principles. Bull N Y Acad Med 16:197–207
2. Chain E, Florey HW, Gardner AD et al (1940) Penicillin as a chemotherapeutic agent. Lancet 236:226–228
3. Waksman SA, Woodruff HB (1942) Selective antibiotic action of various substances of microbial origin. J Bacteriol 44:373–384
4. Kirby WMN (1944) Extraction of a highly potent penicillin inactivator from penicillin resistant staphylococci. Science 99:452–453
5. Barber M, Whitehead JE (1949) Bacteriophage types in penicillin-resistant staphylococcal infection. Br Med J 2:565–569
6. Medeiros AA (1997) Evolution and dissemination of β-lactamases accelerated by generations of β-lactam antibiotics. Clin Infect Dis 24:S19–S45
7. CDC (2017) Get smart: know when antibiotics work. https://www.cdc.gov/getsmart/community/about/index.html. Accessed 5 Feb 2017
8. Dall C (2016) UN leaders pledge to fight antimicrobial resistance. http://www.cidrap.umn.edu/news-perspective/2016/09/un-leaders-pledge-fight-antimicrobial-resistance. Accessed 2 Feb 2017
9. CDC (2013) Antibiotic resistance threats in the United States, 2013. CDC, Atlanta
10. WHO (2017) WHO publishes list of bacteria for which new antibiotics are urgently needed. Press Release by WHO, Geneva
11. Mutlu GM, Wunderink RG (2006) Severe pseudomonal infections. Curr Opin Crit Care 12:458–463. doi:10.1097/01.ccx.0000244127.92612.b4
12. Arthur LE, Kizor RS, Selim AG et al (2016) Antibiotics for ventilator-associated pneumonia. Cochrane Database Syst Rev 10:CD004267. doi:10.1002/14651858.CD004267.pub4

13. Perez F, El Chakhtoura NG, Papp-Wallace KM et al (2016) Treatment options for infections caused by carbapenem-resistant Enterobacteriaceae: can we apply "precision medicine" to antimicrobial chemotherapy? Expert Opin Pharmacother 17:761–781. doi:10.1517/14656566.2016.1145658
14. Kurz SG, Furin JJ, Bark CM (2016) Drug-resistant tuberculosis: challenges and progress. Infect Dis Clin N Am 30:509–522. doi:10.1016/j.idc.2016.02.010
15. Drago L, De Vecchi E, Nicola L et al (2005) In vitro selection of resistance in *Pseudomonas aeruginosa* and *Acinetobacter* spp. by levofloxacin and ciprofloxacin alone and in combination with beta-lactams and amikacin. J Antimicrob Chemother 56:353–359. doi:10.1093/jac/dki204
16. Chait R, Craney A, Kishony R (2007) Antibiotic interactions that select against resistance. Nature 446:668–671. doi:10.1038/nature05685
17. Ly NS, Bulitta JB, Rao GG et al (2015) Colistin and doripenem combinations against *Pseudomonas aeruginosa*: profiling the time course of synergistic killing and prevention of resistance. J Antimicrob Chemother 70:1434–1442. doi:10.1093/jac/dku567
18. Vestergaard M, Paulander W, Marvig RL et al (2016) Antibiotic combination therapy can select for broad-spectrum multidrug resistance in *Pseudomonas aeruginosa*. Int J Antimicrob Agents 47:48–55. doi:10.1016/j.ijantimicag.2015.09.014
19. Janardhanan J, Meisel JE, Ding D et al (2016) In vitro and in vivo synergy of the oxadiazole class of antibacterials with β-lactams. Antimicrob Agents Chemother 60:5581–5588. doi:10.1128/AAC.00787-16
20. Odds FC (2003) Synergy, antagonism, and what the chequerboard puts between them. J Antimicrob Chemother 52:1. doi:10.1093/jac/dkg301
21. Greco WR, Bravo G, Parsons JC (1995) The search for synergy: a critical review from a response surface perspective. Pharmacol Rev 47:331–385
22. Johnson MD, MacDougall C, Ostrosky-Zeichner L et al (2004) Combination antifungal therapy. Antimicrob Agents Chemother 48:693–715
23. Sy CL, Huang TS, Chen CS et al (2016) Synergy of β-lactams with vancomycin against methicillin-resistant *Staphylococcus aureus*: correlation of disk diffusion and checkerboard methods. J Clin Microbiol 54:565–568. doi:10.1128/JCM.01779-15
24. American Society for Microbiology (2017) Instructions to authors. Antimicrob Agents Chemother 1–25. aac.asm.org. Accessed 1 June 2017
25. Bushby SR, Hitchings GH (1968) Trimethoprim, a sulphonamide potentiator. Br J Pharmacol Chemother 33:72–90
26. Gleckman R, Alvarez S, Joubert DW (1979) Drug therapy reviews: trimethoprim-sulfamethoxazole. Am J Hosp Pharm 36:893–906
27. Brumfitt W, Hamilton-Miller JM (1994) Limitations of and indications for the use of co-trimoxazole. J Chemother 6:3–11
28. Brumfitt W, Hamilton-Miller JM (1995) Combinations of sulphonamides with diaminopyrimidines: how, when and why? J Chemother 7:136–139
29. Wylie BA, Amyes SG, Young HK et al (1988) Identification of a novel plasmid-encoded dihydrofolate reductase mediating high-level resistance to trimethoprim. J Antimicrob Chemother 22:429–435
30. Lin L, Nonejuie P, Munguia J et al (2015) Azithromycin synergizes with cationic antimicrobial peptides to exert bactericidal and therapeutic activity against highly multidrug-resistant Gram-negative bacterial pathogens. EBioMedicine 2:690–698. doi:10.1016/j.ebiom.2015.05.021
31. Abdul Rahim N, Cheah SE, Johnson MD et al (2015) Synergistic killing of NDM-producing MDR *Klebsiella pneumoniae* by two 'old' antibiotics-polymyxin B and chloramphenicol. J Antimicrob Chemother 70:2589–2597. doi:10.1093/jac/dkv135
32. Leite GC, Oliveira MS, Perdigao-Neto LV et al (2016) Antimicrobial combinations against pan-resistant *Acinetobacter baumannii* isolates with different resistance mechanisms. PLoS One 11:e0151270

33. Sakoulas G, Rose W, Berti A et al (2017) Classical β-lactamase inhibitors potentiate the activity of daptomycin against methicillin-resistant *Staphylococcus aureus* and colistin against *Acinetobacter baumannii*. Antimicrob Agents Chemother 61:e01745–e01716
34. Ni W, Han Y, Liu J et al (2016) Tigecycline treatment for carbapenem-resistant Enterobacteriaceae infections: a systematic review and meta-analysis. Medicine 95(11): e3126. doi:10.1097/MD.0000000000003126
35. Taccone FS, Rodriguez-Villalobos H, De Backer D et al (2006) Successful treatment of septic shock due to pan-resistant *Acinetobacter baumannii* using combined antimicrobial therapy including tigecycline. Eur J Clin Microbiol Infect Dis 25:257–260. doi:10.1007/s10096-006-0123-1
36. Principe L, D'Arezzo S, Capone A et al (2009) In vitro activity of tigecycline in combination with various antimicrobials against multidrug resistant *Acinetobacter baumannii*. Ann Clin Microbiol Antimicrob 8:18. doi:10.1186/1476-0711-8-18
37. Humphries RM, Kelesidis T, Bard JD et al (2010) Successful treatment of pan-resistant *Klebsiella pneumoniae* pneumonia and bacteraemia with a combination of high-dose tigecycline and colistin. J Med Microbiol 59:1383–1386. doi:10.1099/jmm.0.023010-0
38. Sakoulas G, Moise PA, Casapao AM et al (2014) Antimicrobial salvage therapy for persistent staphylococcal bacteremia using daptomycin plus ceftaroline. Clin Ther 36:1317–1333
39. Berti AD, Theisen E, Sauer JD et al (2015) Penicillin binding protein 1 is important in the compensatory response of *Staphylococcus aureus* to daptomycin-induced membrane damage and is a potential target for β-lactam-daptomycin synergy. Antimicrob Agents Chemother 60:451–458
40. Snydman DR, McDermott LA, Jacobus NV (2005) Evaluation of in vitro interaction of daptomycin with gentamicin or beta-lactam antibiotics against *Staphylococcus aureus* and enterococci by FIC index and timed-kill curves. J Chemother 17:614–621. doi:10.1179/joc.2005.17.6.614
41. Smith JR, Barber KE, Raut A et al (2015) β-Lactam combinations with daptomycin provide synergy against vancomycin-resistant *Enterococcus faecalis* and *Enterococcus faecium*. J Antimicrob Chemother 70:1738–1743. doi:10.1093/jac/dkv007
42. Aktas G, Derbentli S (2017) In vitro activity of daptomycin combined with dalbavancin and linezolid, and dalbavancin with linezolid against MRSA strains. J Antimicrob Chemother 72:441–443. doi:10.1093/jac/dkw416
43. Davis JS, Van Hal S, Tong SY (2015) Combination antibiotic treatment of serious methicillin-resistant *Staphylococcus aureus* infections. Semin Respir Crit Care Med 36:3–16. doi:10.1055/s-0034-1396906
44. Jiang JH, Peleg AY (2015) Daptomycin-nonsusceptible *Staphylococcus aureus*: the role of combination therapy with daptomycin and gentamicin. Genes 6:1256–1267. doi:10.3390/genes6041256
45. Lemonovich TL, Haynes K, Lautenbach E et al (2011) Combination therapy with an aminoglycoside for *Staphylococcus aureus* endocarditis and/or persistent bacteremia is associated with a decreased rate of recurrent bacteremia: a cohort study. Infection 39:549–554. doi:10.1007/s15010-011-0189-2
46. Silvestri C, Cirioni O, Arzeni D et al (2012) In vitro activity and in vivo efficacy of tigecycline alone and in combination with daptomycin and rifampin against Gram-positive cocci isolated from surgical wound infection. Eur J Clin Microbiol Infect Dis 31:1759–1764. doi:10.1007/s10096-011-1498-1
47. Head BM, Alfa M, Sitar DS et al (2017) In vitro evaluation of the effect of linezolid and levofloxacin on *Bacillus anthracis* toxin production, spore formation and cell growth. J Antimicrob Chemother 72:417–420. doi:10.1093/jac/dkw427
48. Davis JS, Sud A, O'Sullivan MV et al (2016) Combination of vancomycin and β-lactam therapy for methicillin-resistant *Staphylococcus aureus* bacteremia: a pilot multicenter randomized controlled trial. Clin Infect Dis 62:173–180. doi:10.1093/cid/civ808

49. Gonzales PR, Pesesky MW, Bouley R et al (2015) Synergistic, collaterally sensitive β-lactam combinations suppress resistance in MRSA. Nat Chem Biol 11:855–861. doi:10.1038/nchembio.1911
50. Barber KE, Rybak MJ, Sakoulas G (2015) Vancomycin plus ceftaroline shows potent in vitro synergy and was successfully utilized to clear persistent daptomycin-non-susceptible MRSA bacteraemia. J Antimicrob Chemother 70:311–313
51. Gritsenko D, Fedorenko M, Ruhe JJ et al (2017) Combination therapy with vancomycin and ceftaroline for refractory methicillin-resistant *Staphylococcus aureus* bacteremia: a case series. Clin Ther 39:212–218
52. Bernardo K, Pakulat N, Fleer S et al (2004) Subinhibitory concentrations of linezolid reduce *Staphylococcus aureus* virulence factor expression. Antimicrob Agents Chemother 48:546–555
53. Stevens DL, Ma Y, Salmi DB et al (2007) Impact of antibiotics on expression of virulence-associated exotoxin genes in methicillin-sensitive and methicillin-resistant *Staphylococcus aureus*. J Infect Dis 195:202–211. doi:10.1086/510396
54. Claeys KC, Smith JR, Casapao AM et al (2015) Impact of the combination of daptomycin and trimethoprim-sulfamethoxazole on clinical outcomes in methicillin-resistant *Staphylococcus aureus* infections. Antimicrob Agents Chemother 59:1969–1976. doi:10.1128/AAC.04141-14
55. Leonard SN, Kaatz GW, Rucker LR et al (2008) Synergy between gemifloxacin and trimethoprim/sulfamethoxazole against community-associated methicillin-resistant *Staphylococcus aureus*. J Antimicrob Chemother 62:1305–1310. doi:10.1093/jac/dkn379
56. Kang YR, Chung DR, Kim J et al (2016) In vitro synergistic effects of various combinations of vancomycin and non-β-lactams against *Staphylococcus aureus* with reduced susceptibility to vancomycin. Diagn Microbiol Infect Dis 86:293–299. doi:10.1016/j.diagmicrobio.2016.08.009
57. Linden P (1999) Quinupristin-dalfopristin. Curr Infect Dis Rep 1:480–487
58. Isnard C, Malbruny B, Leclercq R et al (2013) Genetic basis for in vitro and in vivo resistance to lincosamides, streptogramins A, and pleuromutilins (LSAP phenotype) in *Enterococcus faecium*. Antimicrob Agents Chemother 57:4463–4469. doi:10.1128/AAC.01030-13
59. Bush K, Bradford PA (2016) β-Lactams and β-lactamase inhibitors: an overview. Cold Spring Harb Perspect Med 6:a025247. doi:10.1101/cshperspect.a025247
60. Drawz SM, Papp-Wallace KM, Bonomo RA (2014) New β-lactamase inhibitors: a therapeutic renaissance in an MDR world. Antimicrob Agents Chemother 58:1835–1846. doi:10.1128/AAC.00826-13
61. Bush K (2015) A resurgence of β-lactamase inhibitor combinations effective against multidrug-resistant Gram-negative pathogens. Int J Antimicrob Agents 46:483–493. doi:10.1016/j.ijantimicag.2015.08.011
62. Drawz SM, Bonomo RA (2010) Three decades of β-lactamase inhibitors. Clin Microbiol Rev 23:160–201. doi:10.1128/CMR.00037-09
63. Mulvey MR, Bryce E, Boyd D et al (2004) Ambler class A extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella* spp. in Canadian hospitals. Antimicrob Agents Chemother 48:1204–1214
64. Mushtaq S, Ge Y, Livermore DM (2004) Comparative activities of doripenem versus isolates, mutants, and transconjugants of Enterobacteriaceae and *Acinetobacter* spp. with characterized beta-lactamases. Antimicrob Agents Chemother 48:1313–1319
65. Yigit H, Queenan AM, Rasheed JK et al (2003) Carbapenem-resistant strain of *Klebsiella oxytoca* harboring carbapenem-hydrolyzing beta-lactamase KPC-2. Antimicrob Agents Chemother 47:3881–3889
66. Bush K, Jacoby GA, Medeiros AA (1995) A functional classification scheme for β-lactamases and its correlation with molecular structure. Antimicrob Agents Chemother 39:1211–1233
67. Bush K (2013) Carbapenemases: partners in crime. J Glob Antimicrob Resist 1:7–16. doi:10.1016/j.jgar.2013.01.005

68. Chandorkar G, Huntington JA, Gotfried MH et al (2012) Intrapulmonary penetration of ceftolozane/tazobactam and piperacillin/tazobactam in healthy adult subjects. J Antimicrob Chemother 67:2463–2469. doi:10.1093/jac/dks246
69. Miller B, Hershberger E, Benziger D et al (2012) Pharmacokinetics and safety of intravenous ceftolozane-tazobactam in healthy adult subjects following single and multiple ascending doses. Antimicrob Agents Chemother 56:3086–3091. doi:10.1128/AAC.06349-11
70. Estabrook M, Bussell B, Clugston SL et al (2014) In vitro activity of ceftolozane-tazobactam as determined by broth dilution and agar diffusion assays against recent U.S. *Escherichia coli* isolates from 2010 to 2011 carrying CTX-M-type extended-spectrum β-lactamases. J Clin Microbiol 52:4049–4052. doi:10.1128/JCM.02357-14
71. Buehrle DJ, Shields RK, Chen L et al (2016) Evaluation of the in vitro activity of ceftazidime-avibactam and ceftolozane-tazobactam against meropenem-resistant *Pseudomonas aeruginosa* isolates. Antimicrob Agents Chemother 60:3227–3231. doi:10.1128/AAC.02969-15
72. Neu HC, Fu KP (1978) Clavulanic acid, a novel inhibitor of β-lactamases. Antimicrob Agents Chemother 14:650–655
73. Penwell WF, Shapiro AB, Giacobbe RA et al (2015) Molecular mechanisms of sulbactam antibacterial activity and resistance determinants in *Acinetobacter baumannii*. Antimicrob Agents Chemother 59:1680–1689. doi:10.1128/AAC.04808-14
74. Asli A, Brouillette E, Krause KM et al (2016) Distinctive binding of avibactam to penicillin-binding proteins of Gram-negative and Gram-positive bacteria. Antimicrob Agents Chemother 60:752–756. doi:10.1128/AAC.02102-15
75. Lagace-Wiens PR, Tailor F, Simner P et al (2011) Activity of NXL104 in combination with beta-lactams against genetically characterized *Escherichia coli* and *Klebsiella pneumoniae* isolates producing class A extended-spectrum β-lactamases and class C β-lactamases. Antimicrob Agents Chemother 55:2434–2437. doi:10.1128/AAC.01722-10
76. Ehmann DE, Jahic H, Ross PL et al (2012) Avibactam is a covalent, reversible, non-β-lactam beta-lactamase inhibitor. Proc Natl Acad Sci U S A 109:11663–11668. doi:10.1074/jbc.M113.485979
77. Ehmann DE, Jahić H, Ross PL et al (2013) Kinetics of avibactam inhibition against class A, C, and D β-lactamases. J Biol Chem 288:27960–27971. doi:10.1074/jbc.M113.485979
78. Stachyra T, Levasseur P, Pechereau MC et al (2009) In vitro activity of the β-lactamase inhibitor NXL104 against KPC-2 carbapenemase and *Enterobacteriaceae* expressing KPC carbapenemases. J Antimicrob Chemother 64:326–329. doi:10.1093/jac/dkp197
79. Livermore DM, Mushtaq S, Warner M et al (2008) NXL104 combinations versus Enterobacteriaceae with CTX-M extended-spectrum β-lactamases and carbapenemases. J Antimicrob Chemother 62:1053–1056. doi:10.1093/jac/dkn320
80. Li H, Estabrook M, Jacoby GA et al (2015) *In vitro* susceptibility of characterized β-lactamase-producing strains tested with avibactam combinations. Antimicrob Agents Chemother 59:1789–1793. doi:10.1128/AAC.04191-14
81. Marshall S, Hujer AM, Rojas LJ et al (2017) Can ceftazidime-avibactam and aztreonam overcome β-lactam resistance conferred by metallo-β-lactamases in Enterobacteriaceae? Antimicrob Agents Chemother 61:e02243-16. doi:10.1128/AAC.02243-16
82. Hirsch EB, Ledesma KR, Chang K-T et al (2012) In vitro activity of MK-7655, a novel β-lactamase inhibitor, in combination with imipenem against carbapenem-resistant Gram-negative bacteria. Antimicrob Agents Chemother 56:3753–3757. doi:10.1128/AAC.05927-11
83. Morinaka A, Tsutsumi Y, Yamada M et al (2015) OP0595, a new diazabicyclooctane: mode of action as a serine β-lactamase inhibitor, antibiotic and β-lactam 'enhancer'. J Antimicrob Chemother 70:2779–2786. doi:10.1093/jac/dkv166
84. Livermore DM, Mushtaq S, Warner M et al (2017) In vitro activity of cefepime/zidebactam (WCK 5222) against Gram-negative bacteria. J Antimicrob Chemother 72:1373–1385. doi:10.1093/jac/dkw593

85. Durand-Reville T (2017) Discovery of ETX2514, a novel, rationally designed inhibitor of class A, C and D beta-lactamases, for the treatment of Gram-negative infections. In: Abstr 253rd ACS general meeting, San Francisco, CA, Apr 2017
86. Shields RK, Chen L, Cheng S et al (2017) Emergence of ceftazidime-avibactam resistance due to plasmid-borne bla_{KPC-3} mutations during treatment of carbapenem-resistant *Klebsiella pneumoniae* infections. Antimicrob Agents Chemother 61:e02097–16. doi:10.1128/AAC.02097-16
87. Alm RA, Johnstone MR, Lahiri SD (2015) Characterization of *Escherichia coli* NDM isolates with decreased susceptibility to aztreonam/avibactam: role of a novel insertion in PBP3. J Antimicrob Chemother 70:1420–1428. doi:10.1093/jac/dku568
88. Zhang Y, Kashikar A, Brown CA et al (2017) An unusual *E. coli* PBP3 insertion sequence identified from a collection of carbapenem-resistant Enterobacteriaceae (CRE) tested in vitro with ceftazidime-, ceftaroline- or aztreonam-avibactam combinations. Antimicrob Agents Chemother 61 (accepted manuscript posted online 30 May 2017). doi:10.1128/AAC.00389-17
89. Nabarro LE, Veeraraghavan B (2015) Combination therapy for carbapenem-resistant Enterobacteriaceae: increasing evidence, unanswered questions, potential solutions. Eur J Clin Microbiol Infect Dis 34:2307–2311. doi:10.1007/s10096-015-2486-7
90. Poirel L, Kieffer N, Nordmann P (2016) In vitro evaluation of dual carbapenem combinations against carbapenemase-producing Enterobacteriaceae. J Antimicrob Chemother 71:156–161. doi:10.1093/jac/dkv294
91. Tumbarello M, Trecarichi EM, De Rosa FG et al (2015) Infections caused by KPC-producing *Klebsiella pneumoniae*: differences in therapy and mortality in a multicentre study. J Antimicrob Chemother 70:2133–2143. doi:10.1093/jac/dkv086
92. Stein C, Makarewicz O, Bohnert JA et al (2015) Three dimensional checkerboard synergy analysis of colistin, meropenem, tigecycline against multidrug-resistant clinical *Klebsiella pneumonia* isolates. PLoS One 10:e0126479. doi:10.1371/journal.pone.0126479
93. Čivljak R, Giannella M, Di Bella S et al (2014) Could chloramphenicol be used against ESKAPE pathogens? A review of in vitro data in the literature from the 21st century. Expert Rev Anti Infect Ther 12:249–264. doi:10.1586/14787210.2014.878647
94. O'Driscoll T, Crank CW (2015) Vancomycin-resistant enterococcal infections: epidemiology, clinical manifestations, and optimal management. Infect Drug Resist 8:217–230. doi:10.2147/IDR.S54125
95. Otero LH, Rojas-Altuve A, Llarrull LI et al (2013) How allosteric control of *Staphylococcus aureus* penicillin binding protein 2a enables methicillin resistance and physiological function. Proc Natl Acad Sci U S A 110:16808–16813
96. Henson KER, Yim J, Smith JR et al (2017) β-Lactamase inhibitors enhance the synergy between β-lactam antibiotics and daptomycin against methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 61:e01564–e01516
97. Cosgrove SE, Vigliani GA, Fowler VG Jr et al (2009) Initial low-dose gentamicin for *Staphylococcus aureus* bacteremia and endocarditis is nephrotoxic. Clin Infect Dis 48:713–721. doi:10.1086/597031
98. Alp E (2016) Right first time! Ann Transl Med 4:331. doi:10.21037/atm.2016.08.52
99. Micozzi A, Gentile G, Minotti C et al (2017) Carbapenem-resistant *Klebsiella pneumoniae* in high-risk haematological patients: factors favouring spread, risk factors and outcome of carbapenem-resistant *Klebsiella pneumoniae* bacteremias. BMC Infect Dis 17:203. doi:10.1186/s12879-017-2297-9