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REVIEW

Infectious disease: how to manage Gram-positive and Gram-negative pathogen conundrums with dual beta-lactam therapy

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Abstract

Antimicrobial resistance is a global public health threat due to its associated increase in mortality, and the most appropriate treatment algorithms for resistant and persistent Gram-positive and Gram-negative infections have yet to be elucidated. Whilst combination therapy has been touted as a viable method to overcome prominent resistant mechanisms represented amongst these microbes, the optimal agents to utilize remains controversial. Beta-lactams have a safe profile and are bactericidal against most Gram-positive and Gram-negative microorganisms. Thus, the use of dual beta-lactam therapy to overcome multidrug-resistant pathogens is of supreme interest. This article reviews the mechanisms of beta-lactam resistance in Gram-positive and Gram-negative bacteria, discusses the rationale for dual beta-lactam use against multidrug-resistant infections (and other scenarios in which this strategy may be most utilized in clinical practice), explores the available in vitro, in vivo and clinical data, and provides considerations for the use of dual beta-lactam therapy against *Enterococcus faecalis, Listeria monocytogenes, Staphylococcus aureus, Enterobacterales, Pseudomonas aeruginosa* and *Acinetobacter baumannii* pathogens.

Keywords: antimicrobial resistance, beta-lactam, combination therapy, double carbapenem therapy, dual therapy, Gram-negative, Gram-positive.

Citation

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Introduction

The World Health Organization has estimated that more than 700,000 hospital deaths globally are attributed to antimicrobial resistance, and this number is projected to increase to 10 million by the year 2050.[1–3] Whilst often discussed separately in terms of therapeutic approach, both Gram-negative and Gram-positive bacteria have the ability to evade the typical mode of action of most available antimicrobial agents. Gram-positive resistance potentiated by *mecA*, *mecB* and *mecC* genes in *Staphylococcus aureus* and *vanA* and *vanB* genes in *Enterococcus* spp. has continued to evolve overtime, resulting in persistent and hard-to-treat infections.[4,5] Gram-negative resistance mechanisms, defined by the Ambler classes of beta-lactamases, overproduction of efflux pumps and loss of porins, have severely limited our antibiotic armamentarium particularly against *Enterobacterales* and non-fermenting Gram-negative organisms such as *Acinetobacter baumannii* and *Pseudomonas aeruginosa*.[6]

Due to the increased prevalence of antimicrobial resistance, there is a growing need for therapeutic strategies that are equipped to overcome advanced microbe resistance. Additionally, the volatile financial predicaments that pharmaceutical companies face when marketing new antimicrobial agents have negatively impacted the availability of novel drug mechanisms to overcome multidrug resistance. Of note, few antibiotics targeted to evade resistance mechanisms present in either Gram-positive or Gram-negative pathogens have been developed in the last 15 years.[7] Due to the current standstill in the antimicrobial pipeline, the optimization of currently available agents has been of high interest. Combination therapies have been recommended as a reasonable approach against both Gram-positive and

Gram-negative susceptible and resistant microorganisms, often with agents that have differing spectra of activity.[8] Nevertheless, the historical antibiotics included in first-line regimens (aminoglycoside plus a beta-lactam, fluoroquinolones plus a beta-lactam) are associated with both negative sequelae and conflicting evidence regarding improved patient clinical outcomes.[9,10]

Irrespective of acquired and intrinsic resistance mechanisms, beta-lactams have remained backbone agents for many infections due to their safety and efficacy profiles.[11] Beta-lactams have many noteworthy qualities, including bactericidal activity and relatively safe administration profiles when compared to other available antibiotics.[12] Additionally, several studies have shown strong activity with beta-lactams against presumed resistant bacterial isolates through maximizing pharmacokinetic and pharmacodynamic parameters via extended infusion dosing due to their time-dependent pharmacodynamic index.[13] Nonetheless, there is a paucity of evidence attesting to the utility of these dosing strategies in most clinical settings, thus emphasizing the need for creative and innovative clinical treatment approaches.

Previously abandoned, dual beta-lactam therapy has been shown to be an effective mitigation strategy against a multitude of severe infections caused by both relatively susceptible and multidrug-resistant Gram-positive and Gram-negative pathogens, ultimately leading to positive patient outcomes.[10,11] Herein, we discuss the mechanisms of resistance limiting beta-lactam therapy in Gram-positive and Gram-negative infections as well as the rationale for dual beta-lactam use to overcome resistance. Further, we provide in vitro, in vivo and clinical evidence describing dual beta-lactam use against the aforementioned organisms. Finally, we offer considerations for the implementation of dual beta-lactam therapy in treatment algorithms and inform potential future directions to produce more robust information on this topic.

Methods

A PubMed search (from 1950 to June 2021) was performed with clinical queries using the key terms "antimicrobial resistance", "multidrug-resistance", "Gram-positive infections", "Gram-negative infections", "dual beta-lactam therapy" and "double carbapenem therapy". The search strategy included review articles, meta-analyses, systemic reviews, in vitro studies and observational studies (prospective and retrospective). The search was restricted to the English language. Articles discussing combination therapies that did not utilize dual-beta lactam regimens were excluded.

Review

Mechanisms of beta-lactam resistance

The target site of the beta-lactams is transpeptidase, also known as the penicillin-binding proteins (PBPs). Beta-lactams are constructed to mimic the D-Ala-D-Ala structure of the bacterial cell wall; however, the catalytic acyl-enzyme in bacteria is absent from the beta-lactam pharmacophore, thus disrupting bacterial transpeptidase and cell-wall synthesis.[14,15] This mode of action is the primary contributor to observed beta-lactam bactericidal activity against most organisms.[15] In respect to the beta-lactam agents, primary resistance mechanisms in Gram-positive and Gram-negative bacteria have vast differences. Nonetheless, the major mechanism of resistance in Gram-positive bacteria is the alteration of PBPs as seen with *S. aureus*, *Listeria monocytogenes* and *Enterococci* spp. resistance.[14,15]

There are obvious differences between the PBP expression of methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA). MSSA encodes eight total PBPs, including PBP1 and PBP2, whilst MRSA encodes nine.[15] PBP2a expression in MRSA bacteria is induced under the irreversible acylation of the protein receptor MecR and expressed by the *mecA* gene.[16] Whilst *L. monocytogenes* has been shown to be inhibited by the beta-lactam class of antibiotics, bactericidal concentrations have proven difficult to achieve.[17–19] The lack in beta-lactam bactericidal activity that results in difficult-to-treat *Listeria* spp. infections has been attributed to the signal transmission of several *L. monocytogenes*-specific genes that adjust the growth rate of the microorganism under beta-lactam pressure.[19] Finally, resistance in *Enterococcus faecium* and *Enterococcus faecalis* presents with distinct differences. *E. faecium* resistance is dependent upon PBP5, which gives the low-affinity enzyme, PBP5fm, whilst weak binding to PBP4 dominates *E. faecalis* beta-lactam resistance.[15,20]

In Gram-negative bacteria, enzymes that can hydrolyse beta-lactam chemical bonds are the primary perpetrators of agent resistance.[21] Currently, there are four Ambler classes of beta-lactamases (class A–D), each with very separate functions in Gram-negative microorganisms.[21]

The class A beta-lactamases include extended spectrum beta-lactamases (e.g. SHV, TEM, CTX-M) and serine carbapenamases (e.g. *Klebsiella pneumoniae* carbapenamases (KPC)).[22] Initially, SHV1 and TEM1 were classified as penicillinases as they rendered the penicillin agents, including those with extended Gram-negative spectra (piperacillin, amoxicillin), ineffective.[6] Consequently, these resistant enzymes evolved and became prominent amongst *Enterobacterales* and conferred resistance to cephalosporin agents, including cefotaxime and ceftriaxone, which have an extended beta-lactam spectrum of activity,[6] thus giving rise to CTX-M beta-lactamases and the moniker, extended spectrum beta-lactamase.[6] Of note, other serine carbapenamases (KPCs), first identified in *Klebsiella* isolates, have begun to spread globally, and have been identified in other *Enterobacterales* (*Escherichia coli*, *Serratia* spp., *Enterobacter* spp.) and non-fermenting Gram-negative microbes (*P. aeruginosa* and *A. baumannii*).[6,23]

The distinctive property of class B or metallo beta-lactamases (MBLs) is their ability to hydrolyse most beta-lactams, including

carbapenems, but not aztreonam.[24] These enzymes carry a zinc requirement and, to date, there are several variants of the IMP, VIM and New Delhi (NDM) MBLs.

Class C beta-lactamases or cephalosporinases, also referred to as AmpC beta-lactamases, are mostly chromosomal enzymes.[6] AmpC beta-lactamases are often present at low production levels; however, high levels of resistance can be induced in the presence of specific induction agents.[25] The utility of cephalosporins in the presence of AmpC remains controversial; nevertheless, specific *Enterobacterales* isolates, including *Enterobacter spp.*, have been shown to be less responsive to cephalosporin therapy in the presence of the class C enzymes.[26]

The oxacillinase (OXA) family of (class D) beta-lactamases have been identified as a subgroup that hydrolyses carbapenems in *A. baumannii* and *P. aeruginosa* bacteria.[27] The OXA enzymes are typically chromosomal; however, they can also be intrinsically present in these microbes.[27] Hydrolysis associated with the OXA carbapenamases is slow; therefore, full carbapenem resistance requires the presence of additional resistance mechanisms such as an under-expression of porin channels and/or over-expression of efflux pumps.[27,28] Table 1 provides a thorough description of resistance mechanisms in various Gram-positive and Gram-negative organisms.

Beta-lactam/beta-lactamase inhibitors

Whilst these mentioned mechanisms of resistance have independent functions, they are often present in concert within Gram-negative species. The infections caused by microorganisms in which these beta-lactamases are present are typically characterized by the carbapenem-resistant *P. aeruginosa* (CRPA), carbapenem-resistant *A. baumannii* (CRAB) or multidrug-resistant (MDR) phenotypes.[28] To counter this, beta-lactamase inhibitors (BLIs) with enzyme degrading properties, such as clavulanic acid, tazobactam, avibactam, vaborbactam, sulbactam and relebactam, have been formulated with beta-lactam (BL) agents to aid in the evasion of the described hydrolysing enzymes.[29] These BLIs have differing levels of activity against the various Ambler classes of beta-lactamases as shown with the increase of activity that tazobactam has against class A beta-lactamases and the high activity that avibactam has against classes C and D beta-lactamases when compared to other BLIs.[30,31] Whilst the BL/BLI co-formulated agents, including ceftolozane/tazobactam, ceftazidime/avibactam, meropenem/vaborbactam, and imipenem/cilastatin/relebactam have been used against these carbapenem-resistant and MDR Gram-negative infections, declines in clinical response continue to exist.[29,32]

Table 1. Resistance mechanisms present in Gram-positive and Gram-negative bacteria.[a]

Organism	Resistance mechanism	Antibiotics affected
Enterococcus spp.[4,15,63,66] (Gram-positive)	PBP-site modifications; PBP5 in *E. faecium*; PBP4 in *E. faecalis*	Penicillins Cephalosporins
Methicillin-susceptible *Staphylococcus* spp. (Gram-positive)[4,15]	PBP-site modifications; PBPs 1 and 2 in methicillin-susceptible *S. aureus*	Penicillins
Methicillin-resistant *Staphylococcus* spp. (Gram-positive)[4,15]	MecA mediated PBP2a expression	Penicillins Cephalosporins Carbapenems
Listeria monocytogenes (Gram-positive)[17,19]	Single transmission-adjusted increases in microorganism growth rates	Penicillins Cephalosporins
Enterobacterales (*Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter* spp.)[47,115] (Gram-negative)	Extended spectrum beta-lactamases (SHV, TEM, CTX-M) (Ambler class A); serine carbapenamases (KPC) (Ambler class A); metallo-beta lactamases (IMP, VIM, NDM) (Ambler class B); AmpC beta-lactamases (Ambler class C); oxacillinases (Ambler class D)	Penicillins Cephalosporins Carbapenems
Pseudomonas aeruginosa (Gram-negative)[47,115]	Metallo-beta lactamases (IMP, VIM, NDM) (Ambler class B); AmpC beta-lactamases (Ambler class C) oxacillinases (Ambler class D)	Penicillins Cephalosporins Carbapenems
Acinetobacter baumannii (Gram-negative)[47,115]	Metallo-beta lactamases (IMP, VIM, NDM) (Ambler class B); oxacillinases (Ambler class D)	Penicillins Cephalosporins Carbapenems

[a]Included in this table are prominent resistance mechanisms characterized in select Gram-positive and Gram-negative organisms as well as commonly affected antibiotics.

Rationale for dual-beta lactam therapy

Synergistic activity and expanded spectra of activity

Synergy is defined as the enhanced activity of multiple agents when used in combination, compared to either agent used as a monotherapy.[33] The utilization of dual-beta lactam therapy has been shown to produce synergistic activity against both persistent or resistant Gram-positive and Gram-negative pathogens.[10] This described synergy is largely based on the complementary binding of the active PBPs, resulting in the complete saturation of the antibiotic binding sites and a potential increase in bactericidal activity.[10,34] Furthermore, the use of two agents in combination provides an expanded spectrum of activity as the agents utilized can provide complementary coverage of Gram-positive and/or Gram-negative microorganisms.

In persistent MSSA infections, ertapenem and cefazolin have been used as a synergistic combination. Hypotheses regarding the observed synergy are attributed to the binding of ertapenem to PBP1, circumventing the downregulation of PBP2 (cefazolin's primary PBP), in *S. aureus*. Thus, highlighting the advantage in utilizing beta-lactams that have complementary PBP binding affinities to maximize antibacterial potency.[35] With that, a similar rationale has been applied to the use of ampicillin and ceftriaxone in *E. faecalis* endocarditis as well as in *L. monocytogenes*-mediated infections.[18] *Enterococcus* spp. and *L. monocytogenes* share several similarities, including their innate resistance to ceftriaxone and the bacteriostatic activity of ampicillin.[18] Nevertheless, cefotaxime (structurally similar to ceftriaxone) is a strong inhibitor of PBP2 and PBP3 in *Enterococcus* spp. and of PBP1, PBP2 and PBP4 in *L. monocytogenes*.[18,36–38] Studies have suggested that the complete saturation of the PBPs by ampicillin and ceftriaxone in these microorganisms presents with increased bactericidal activity when compared to ampicillin utilized as a monotherapy.[18] This synergistic activity has been observed irrespective of the innate resistance of *Enterococcus* spp. and *L. monocytogenes* against cephalosporins.

In Gram-negative infections, the idea of synergy and complementary PBP binding has been recently shown with the use of cefiderocol and meropenem in combination against CRAB.[39] An in vitro time-kill study showed increased bactericidal activity with cefiderocol and meropenem used in combination,[39] this synergy being potentially attributed to cefiderocol, a novel siderophore cephalosporin occupying PBP3 whilst meropenem binds to PBP2, thus potentiating synergistic bactericidal activity.[40] Additionally, antipseudomonal activity with dual beta-lactam regimens, where only one agent is active against *P. aeruginosa*, have been shown to be similar to beta-lactam and aminoglycoside therapy (in this combination, both agents possessed activity against *P. aeruginosa*),[41–43] therefore suggesting synergistic activity as the reasoning for the observed increase in activity.

Nonetheless, the increased binding of beta-lactam agents to PBPs has been reported to cause an overexpression of certain beta-lactamases subsequently, in select circumstances, negatively impacting the opportunity for synergistic activity.[11,41–44] An example of this would be AmpC overexpression observed in *P. aeruginosa,* causative of beta-lactam-mediated PBP4 inactivation (PBP4 saturation).[45,46] Carbapenems are commonly employed against *P. aeruginosa* microorganisms due to increased resistance, and they have been shown to inactivate PBP4.[45] Therefore, in select circumstances, the use of an AmpC-degradable antibiotic (cephalosporin) and a carbapenem against a *P. aeruginosa* isolate may not result in synergistic activity.

Activity via co-formulated beta-lactamase inhibitor

As previously mentioned, BL/BLIs have activity against persistent and MDR Gram-negative bacteria, with that activity being largely attributed to the BLI.[47] Therefore, BL/BLI agents have been utilized in combination with other beta-lactams in primarily MDR Gram-negative infections; for example, the use of ceftazidime/avibactam plus aztreonam against CRPA and other MDR *Enterobacterales*.[48–50] The hypothesis surrounding this increase in activity is based upon an inhibition of class A and C beta-lactamases in the bacteria by avibactam (BLI) and 'bypassing' class B beta-lactamases with aztreonam, restoring antibacterial activity.[48] Further, ampicillin/sulbactam has been utilized in combination with an *A. baumannii*-active carbapenem (meropenem, imipenem/cilastin, doripenem) against CRAB due to a recognized increase in PBP occupancy (PBP1 and PBP3 by sulbactam and PBP2 by the carbapenem), resulting in increases in bactericidal activity.[51,52] Whilst the BLI is credited with the success in these mentioned BL/BLI plus additional BL treatment regimens, they do not exist in many countries, including the US, as lone agents.[47,53,54] Thus, treatment regimens that employ the BL/BLI combination plus an additional BL are often considered dual beta-lactam regimens.

Less toxic alternative

Beta-lactams have been typically used in combination with aminoglycosides, fluoroquinolones and tetracyclines against difficult-to-treat organisms. Nevertheless, these non-beta-lactam antibiotics have poor side-effect profiles, including nephrotoxicity and ototoxicity, which can be irreversible in some cases.[10,11] Therefore, the use of dual beta-lactams in place of these combinations can alleviate safety concerns whilst presenting with similar clinical successes.[10,11,55]

Dual beta-lactam use in Gram-positive microorganisms

For Gram-positive bacteria, dual beta-lactam combination therapy has been evaluated clinically for several organisms, including *E. faecalis*, *L. monocytogenes* and *S. aureus* isolates.

Enterococcus spp. account for approximately 10% of bloodstream infections globally.[56,57] They have also been reported to be the fourth most commonly isolated genus

in endocarditis, responsible for approximately 10% of all cases.[58] *E. faecalis* and *E. faecium* are the two most prevalent *Enterococcus* species in infective endocarditis (IE), with *E. faecalis* accounting for ~97% of cases and *E. faecium* accounting for ~1–2% of cases.[59] Enterococci exhibit an intrinsic resistance to inhibition by some beta-lactam antibiotics secondary to the synthesis of a specific PBP with low affinity for these agents.[60] Consequently, combination therapy utilizing a cell wall-active agent, such as penicillin G or ampicillin, with an aminoglycoside, has been the standard of care in patients with IE caused by *Enterococcus* spp. However, given the increasing incidence of enterococcal strains with high-level aminoglycoside resistance (HLAR), dual beta-lactam therapy has emerged as an alternative treatment option, particularly in *E. faecalis*. The benefits include avoidance of organ toxicities associated with aminoglycoside use, such as nephrotoxicity and ototoxicity, as well as a diminished need to perform therapeutic drug monitoring. An in vitro study against *E. faecalis* strains with HLAR demonstrated a reduction in the minimum inhibitory concentrations of ampicillin when combined with a fixed sub-inhibitory ceftriaxone concentration and found significantly lower residual bacterial titres in aortic valve vegetations of the combination in an experimental endocarditis animal model compared to ampicillin monotherapy.[61] Another animal study concluded the combination of ceftriaxone and ampicillin was as effective as gentamicin and ampicillin for endocarditis in *E. faecalis* strains with no HLAR.[62]

Following these results, an observational, open-label, non-randomized, multicentre study evaluated the safety and efficacy of a 6-week course of ampicillin plus ceftriaxone (AC) in patients with endocarditis due to HLAR *E. faecalis* and in those with non-HLAR *E. faecalis* endocarditis who could not tolerate aminoglycosides. A total of 43 patients were evaluated, 21 with HLAR and 22 with non-HLAR *E. faecalis* endocarditis. Amongst all episodes, the clinical cure rate was 67.4% at 3 months. The mortality rate during treatment was 28.6% and 18.2% in the HLAR and non-HLAR groups, respectively, which is similar to what had been reported in other enterococcal endocarditis series.[63–65] Furthermore, 95.3% of patients overall experienced no adverse effects. The findings of this study support the combination of AC as a potential alternative to beta-lactam-aminoglycoside combination therapy for the management of endocarditis caused by *E. faecalis* given similar efficacy outcomes and improved safety profiles.

To further expound on these findings, Fernández-Hidalgo et al. conducted an observational, non-randomized, comparative, multicentre cohort evaluating AC and ampicillin plus gentamicin (AG) for *E. faecalis* IE. A total of 246 patients were treated with AC (n=159) or AG (n=87) and 32% of episodes treated with AC had isolates expressing HLAR. No differences were observed in mortality during treatment, mortality at 3-month follow-up, treatment failure necessitating a change in antibiotics, or relapse.[66] However, adverse events requiring treatment discontinuation were much more common in the AG group (25% *versus* 1%; p<0.001), mostly due to nephrotoxicity. Findings from this evaluation support the notion that combination therapy with AC was an effective alternative to AG for management of *E. faecalis* IE, including those isolates expressing HLAR.

As the clinical utility of AC increases for *E. faecalis* infections, there are concerns about collateral damage with continued use of ceftriaxone as it has been associated with increased risk of vancomycin-resistant enterococcus gastrointestinal colonization likely owing to its high biliary excretion.[67,68] Ampicillin in combination with other cephalosporins associated with less biliary excretion, including cefepime and ceftaroline, have been evaluated as alternative agents. Whilst promising, the current literature is limited to in vitro data.[69–71]

The combination of ampicillin and ceftriaxone has also been touted as a viable treatment regimen against infections caused by *L. monocytogenes*.[19,72] Similar to *E. faecalis*, *L. monocytogenes* is inherently resistant to cephalosporins such as ceftriaxone and the activity of ampicillin is bacteriostatic.[18,19] In addition, the traditional standard-of-care includes ampicillin and an aminoglycoside. Given these similarities, it has been postulated that the aforementioned antibiotic combination could be beneficial in cases of invasive *L. monocytogenes* infections.[18] The efficacy of combining ampicillin and ceftriaxone was tested against clinical isolates of *L. monocytogenes*, including an endocarditis isolate, in two reports.[18,73] Though rare, endocarditis secondary to listeriosis is associated with a mortality rate of 37–48%.[74,75] In vitro analyses revealed a synergistic effect of ampicillin plus ceftriaxone.[73] A more commonly encountered scenario for this combination includes its empiric use in meningitis for those over 50 years of age (or ampicillin plus cefotaxime in those patients <1 month).[76] Real-world clinical application remains limited, and further research is needed.

S. aureus remains a leading cause of morbidity and mortality and has been reported to account for 20% of nosocomial bloodstream infections.[77,78] Often associated with poor outcomes, antimicrobial selection continues to play an integral role in the management of *S. aureus* bacteraemia for both MSSA and MRSA phenotypes.[79,80] Particularly in cases of persistent infections, a growing body of literature supports combination therapy with various agents for the management of MRSA,[81] many of them involving at least one beta-lactam. Of note, anti-staphylococcal beta-lactams remain the standard of care agents for the treatment of MSSA due to improved clinical outcomes compared to vancomycin.[82] Sakoulas et al.[35] described the synergistic activity and enhanced activity of the first-generation beta-lactam, cefazolin, in combination with ertapenem in vitro and in vivo. This combination was tested against an index MSSA bloodstream isolate in a patient with persistent bacteraemia despite appropriate empirical treatment with ceftaroline and de-escalation to cefazolin. Bacterial clearance was achieved within 24 hours of adding ertapenem to cefazolin. Subsequent

in vitro studies revealed reduced cefazolin heteroresistance and biofilm formation with the addition of ertapenem as well as enhancement of innate immune killing via LL-37. Synergy was also noted in some MRSA strains with this combination despite neither agent having any demonstrable activity against MRSA.[35] This finding corroborates a prior report of beta-lactam (plus BLI) combination of meropenem, piperacillin and tazobactam having synergistic and bactericidal activity against MRSA isolates in vitro.[83] Despite promising in vitro data, clinical application remains scarce and is limited to case reports and case series. A case series published in 2020 evaluated 11 salvage cases (6 with endocarditis) treated with ertapenem and cefazolin after microbiological failure with conventional regimens (e.g. nafcillin, cefazolin) for MSSA bacteraemia. Patients had bacteraemia for a median of 6 days before successful clearance with ertapenem plus cefazolin combination therapy, with the majority of cases achieving clearance within 24 hours.[84] A 2020 case report described similar results in a patient with refractory MSSA bacteraemia and concomitant pneumonia.[85] Of interest, there is currently an ongoing randomized-controlled clinical trial (ClinicalTrials.gov identifier: NCT04886284), expected to conclude in 2022, evaluating the use of cefazolin plus ertapenem in MSSA bacteraemia.[86] Nonetheless, additional literature and, ideally, more prospective or randomized controlled trials are needed to further elucidate the place in therapy for this promising beta-lactam combination. Table 2 summarizes clinical findings with dual beta-lactam therapy against Gram-positive infections.

Dual beta-lactam use in Gram-negative microorganisms

For Gram-negative bacteria, dual beta-lactam combination regimens have been evaluated against carbapenem-resistant *Enterobacterales* (CRE), CRPA and CRAB.

In vitro studies have shown synergistic activity against non-MBL CRE for the combination of meropenem and ceftazidime/avibactam as well as all possible permutations of double-carbapenem combinations. Contrastingly, synergistic activity

Table 2. Compilation of clinical studies evaluating dual-beta lactam therapy against Gram-positive organisms.[a]

Organism	Author	Study design	Antibiotic combination therapy	Clinical scenario	Outcome
Enterococcus	Gavaldá et al., 2007[63]	Observational, open-label, non-randomized, multicentre study	Ampicillin + ceftriaxone	*E. faecalis* endocarditis with HLAR (48.8%) and non-HLAR (51.2%) isolates	43 cases; cure rate at end of therapy of 71.4% (HLAR) *versus* 72.7% (non-HLAR); higher tolerability and similar mortality to previously reported cases
	Fernández-Hidalgo et al., 2013[66]	Observational, non-randomized, comparative, multicentre cohort	Ampicillin + ceftriaxone *versus* ampicillin + gentamicin	*E. faecalis* endocarditis with HLAR-AC group (32%) and non-HLAR isolates	246 cases; No difference in mortality during treatment, mortality at 3-months, treatment failure requiring a change in therapy, or relapse; more adverse events in aminoglycoside group
MSSA	Sakoulas et al., 2016[35]	Case report	Ertapenem + cefazolin	Persistent bacteraemia of 5 days	Single case; bacterial clearance within 24 hours of initiating combination therapy
	Sargi et al., 2020[85]	Case report	Ertapenem + cefazolin	Persistent bacteraemia with concomitant pneumonia	Single case; bacterial clearance after 3 days of combination therapy
	Ulloa et al., 2020[84]	Case series	Ertapenem + cefazolin	Persistent bacteraemia for a median of 6 days	11 cases; 8/11 cases achieved bacterial clearance achieved within 24 hours; bacterial clearance in all cases within 3 days

[a]Included in Table 2 is a compilation of the patient outcomes from clinical studies that investigate the use of various dual-beta lactam combinations against several species of Gram-positive and Gram-negative organisms.

against MBL CRE has been shown for the combination of aztreonam and ceftazidime/avibactam.[10,48,54,87–89] Cefoxitin is a strong beta-lactamase inducer and has been shown to cause antagonism when combined with aztreonam or piperacillin in vitro.[10,90] Against non-MBL *P. aeruginosa*, in vitro studies have shown synergistic activity for combinations of piperacillin and a third-generation cephalosporin, piperacillin and cefepime, ceftazidime and meropenem, and ceftazidime/avibactam and meropenem, whereas synergistic activity against MBL *P. aeruginosa* has been shown for the combination of aztreonam and ceftazidime/avibactam.[10,87,91–94] Further, the combination of aztreonam and piperacillin/tazobactam has been shown to result in antagonism against *P. aeruginosa*.[10,95] Additionally, combinations of meropenem and ampicillin/sulbactam and of imipenem plus ampicillin/sulbactam have been shown to have synergistic activity against CRAB.[96,97]

Double-carbapenem therapy (DCT) for the treatment of infections caused by CRE organisms has emerged as a viable treatment regimen.[98] Various case reports, case series and observational studies have been published describing the activity of DCT. A systematic review and meta-analysis of three cohort or case–control studies evaluated 235 patients and found a lower mortality rate with DCT when compared to the control treatment (colistin, tigecycline and aminoglycoside monotherapies, or combined regimens).[99] The infections evaluated included pneumonia, bacteraemia and urinary tract infections, all caused by *Klebsiella pneumoniae*. There was no statistically significant difference between the treatment arms regarding clinical or microbiological response. The most common DCT regimen was ertapenem plus meropenem, with few patients receiving ertapenem plus doripenem. Ertapenem plus imipenem has not been clinically evaluated due to the potential for an increased risk of neurotoxicity. A more recent meta-analysis of observational studies evaluated 1,849 patients with carbapenem-resistant *K. pneumoniae* and found a significantly lower 28-day to 30-day mortality rate and higher microbiological cure rate with DCT compared to standard antibiotic therapy.[100] However, there was no statistically significant difference in 60-day to 90-day mortality, likely as a result of the observational nature of the studies included.

The treatment of infections due to MBL-producing *Enterobacterales* has been very challenging due to limited treatment options that maintain activity against MBLs. The combination of aztreonam plus ceftazidime/avibactam has been readily evaluated, clinically, against MBL *Enterobacterales*.[101] Whilst case reports and case series have documented clinical success, clinical failure, as well as recurrence after those observed clinical successes, have also been documented.[101–103] A recent prospective, multicentre, observational study enrolled 102 patients with bacteraemia due to MBL-producing *Enterobacterales*; patients either received a combination of aztreonam plus ceftazidime/avibactam or other active antibiotics (OAA), including but not limited to colistin, tigecycline, fosfomycin, gentamicin or meropenem.[49] The rate of 30-day mortality was 19.2% with ceftazidime/avibactam plus aztreonam compared to 44.0% in the OAA group (p=0.007). A propensity score-adjusted analysis also showed significantly lower 30-day mortality, lower clinical failure on day 14, and shorter length of stay with the use of ceftazidime/avibactam and aztreonam compared to OAA. Likely due to predominant colistin and aminoglycoside use, the rate of nephrotoxicity was significantly higher in the OAA group (20.0% *versus* 1.9%; p=0.003). These results are promising for the use of ceftazidime/avibactam and aztreonam against MBL-producing *Enterobacterales*. Furthermore, a single case report has documented the successful use of this combination for the treatment of MBL-producing *P. aeruginosa* in a patient with pneumonia.[101]

Treatment options for CRAB remain scarce with combinations that include ampicillin/sulbactam heavily represented amongst dual beta-lactam therapies.[96,104,105] The combination of ampicillin/sulbactam plus imipenem was evaluated in a retrospective single-centre observational study of 386 patients with healthcare-associated infections, mainly bacteraemia, respiratory and urinary tract infections, caused by MDR *A. baumannii*.[106] Patients received either a tigecycline regimen, either alone or in combination with ceftazidime, ceftriaxone, or piperacillin/tazobactam or only a combination of imipenem and ampicillin/sulbactam. There were no statistically significant differences between the two groups in 30-day mortality. Clinical cure or improvement was significantly higher in the tigecycline group, whereas microbiological eradication was significantly higher in patients receiving imipenem and sulbactam. Moreover, a small randomized clinical trial enrolled 47 patients with ventilator-associated pneumonia due to CRAB to receive either meropenem plus colistin or meropenem plus ampicillin/sulbactam.[96] There were no statistically significant differences between the groups in clinical response or microbial eradication likely due to the study being underpowered.

Clinical experience with dual beta-lactam treatment of non-MBL CRPA is also very limited. A systematic review and meta-analysis of randomized clinical trials comparing dual beta-lactam therapy to beta-lactam plus aminoglycoside therapy found no statistically significant difference in clinical response, although there was a non-significant trend toward higher response with dual beta-lactam therapy.[11] Many of the patients included had febrile neutropenia and were enrolled in the studies in the 1970s and 1980s. There was also no statistically significant difference in microbiological response, including in the subgroup of patients with *P. aeruginosa* infections; however, there was a non-significant trend toward higher response with dual beta-lactam therapy.[11] Compared to beta-lactam plus aminoglycoside therapy, dual beta-lactam therapy was associated with significantly lower nephrotoxicity and ototoxicity but with a higher risk of hypokalaemia and coagulation abnormality, which could have been due to high usage of moxalactam during that time period.[11] Table 3 summarizes in vitro and clinical findings with dual beta-lactam therapy against Gram-negative infections.

Table 3. Compilation of clinical studies evaluating dual-beta lactam therapy against Gram-negative organisms.[a]

Organism	Author	Study design	Antibiotic combination therapy	Clinical scenario	Outcome
Non-MBL CRE	Ceccarelli et al., 2013[116]	Case report	Ertapenem + doripenem	Bacteraemia + pneumonia	Clinical and microbiological response
	Giamarellou 2013[111]	Case series	Ertapenem + meropenem Ertapenem + doripenem	Bacteraemia, urinary tract infection	Clinical and microbiological response in 3/3
	Oliva et al., 2014[117]	Case series	Ertapenem + meropenem	Bacteraemia, aortic periprosthetic infection	Clinical and microbiological response in 3/3; 1 death
	Camargo et al., 2015[118]	Case report	Ertapenem + meropenem	Bacteraemia + pneumonia + intra-abdominal infection	Microbiological failure, switched to ceftaz/avi + ertapenem
	Chua et al., 2015[119]	Case series	Ertapenem + doripenem	Pneumonia, surgical site infection	Clinical and microbiological response in 2/2; both died
	Oliva et al., 2015[120]	Case report	Ertapenem + meropenem	Central venous catheter infection	Clinical and microbiological response
	Tumbarello et al., 2015[121]	Case series	Ertapenem + meropenem	Bacteraemia	3/8 died
	Oliva et al., 2016[122]	Case series	Ertapenem + meropenem	Urinary tract infection, skin infection, hardware infection, pneumonia, multiple site infection	Clinical and microbiological response in 12/15; 1 death
	Cprek et al., 2016[123]	Case series	Ertapenem + meropenem or ertapenem + doripenem	Bacteraemia, pneumonia, intra-abdominal infection, urinary tract infection, skin infection	Clinical response in 7/18; microbiological response in 11/14; 5 deaths
	Montelione 2016 et al., [124]	Case report	Ertapenem + meropenem	Aortic periprosthetic infection	Clinical and microbiological response
	Oliva et al., 2016[122]	Case report	Ertapenem + meropenem	Bacteraemia + surgical site infection + pneumonia	Clinical and microbiological response
	Basaranoglu et al., 2017[125]	Case series	Ertapenem + meropenem	Bacteraemia	Clinical response in 2/3; microbiological response in 3/3
	Nekidy et al., 2017[126]	Case report	Ertapenem + meropenem	Bacteraemia + surgical site infection + urinary tract infection + pneumonia	Clinical and microbiological response
	Souli et al., 2017[127]	Case series	Ertapenem + meropenem	Bacteraemia, urinary tract infection, pneumonia, ventricular drainage infection	Clinical response in 21/27; microbiological response in 20/27; 8/27 died

(Continued)

Table 3. (*Continued*)

Organism	Author	Study design	Antibiotic combination therapy	Clinical scenario	Outcome
	Piedra-Carrasco et al., 2018[128]	Case report	Ertapenem + meropenem	Bacteraemia	Clinical and microbiological response
	Galvao et al., 2018[129]	Case report	Ertapenem + meropenem	Bacteraemia and surgical site infection	Died
	Jiao et al., 2019[11]	Systematic review and meta-analysis of 13 randomized controlled trials	Double beta-lactam *versus* beta-lactam + aminoglycoside	Febrile neutropenia (majority), pneumonia, severe infection	Clinical cure: 67.4% *versus* 64.2% (p=0.09; I^2=0%) Microbiological cure: 66.5% *versus* 58.6% (p=0.08; I^2=0%)
	Li et al., 2020[109]	Systematic review and meta-analysis of three observational studies	Ertapenem + meropenem or ertapenem + doripenem	Bacteraemia, pneumonia, intra-abdominal infection, skin infection, urinary tract infection, multiple site infection	Clinical cure: 67.8% *versus* 54.7% (p=0.05; I^2=25%) Microbiological cure: 61.7% *versus* 43.9% (p=0.07; I^2=19%) Mortality: 24.7% *versus* 41.2% (p=0.009; I^2=0%)
MBL CRE	Rosa et al., 2018[130]	Case series	Ertapenem + meropenem	Urinary tract infection	Clinical and microbiological response in 2/2
	Davido et al., 2017[101]	Case report	Aztreonam + ceftazidime/avibactam	Bacteraemia	Clinical cure, but ultimately died
	Shaw et al., 2017[103]	Case series	Aztreonam + ceftazidime/avibactam	Bacteraemia, urinary tract infection, intra-abdominal infection, pneumonia	Clinical cure in 6/10 but 2 of the 6 had recurrence; 3/10 died
	Emeraud et al., 2019[102]	Case report	Aztreonam + ceftazidime/avibactam	Urinary tract infection	Clinical and microbiological cure
	Falcone et al., 2020[49]	Observational study	Aztreonam + ceftazidime/avibactam	Bacteraemia	Clinical cure: 75% *versus* 48% (p=0.005) Mortality: 19.2% *versus* 44% (p=0.007)
Non-MBL CRPA	Jiao et al., 2019[11]	Systematic review and meta-analysis of 13 randomized controlled trials	Double beta-lactam *versus* beta-lactam + aminoglycoside	Febrile neutropenia (majority), pneumonia, severe infection	Clinical cure: 67.4% *versus* 64.2% (p=0.09; I^2=0%) Microbiological cure: 66.5% *versus* 58.6% (p=0.08; I^2 =0%) Microbiological cure in PA subgroup: 58.5% *versus* 60.6% (p>0.05)
CRAB	Lee et al., 2013[106]	Observational study	Sulbactam + imipenem/cilastatin or tigecycline-based treatment	Bacteraemia, urinary tract infection, pneumonia, other	Clinical cure: 50% *versus* 69.2% (p<0.001) Microbiological cure: 11.7% *versus* 1.1% (p<0.001) Mortality: 53.3% *versus* 53.3% (p=0.93)

(Continued)

Table 3. (*Continued*)

Organism	Author	Study design	Antibiotic combination therapy	Clinical scenario	Outcome
MBL CRPA	Davido et al., 2017[101]	Case report	Aztreonam + ceftazidime/avibactam	Pneumonia	Clinical cure and survival
CRAB	Lee et al., 2013[106]	Observational study	Sulbactam + imipenem/cilastatin or tigecycline-based treatment	Bacteraemia, urinary tract infection, pneumonia, other	Clinical cure: 50% *versus* 69.2% ($p<0.001$) Microbiological cure: 11.7% *versus* 1.1% ($p<0.001$) Mortality: 53.3% *versus* 53.3% ($p=0.93$)
	Khalili et al., 2018[96]	Randomized controlled trial	Ampicillin/sulbactam + meropenem or colistin + meropenem	Pneumonia	Clinical cure: 75% *versus* 69.6% ($p=0.75$) Microbiological cure: 87.5% *versus* 91.3% ($p=0.59$) Mortality: 41.67% *versus* 39.13% ($p>0.99$)

[a]Included in Table 3 is a compilation of the patient outcomes from clinical studies that investigate the use of various dual-beta lactam combinations against several species of Gram-positive and Gram-negative organisms.
Non-MBL CRE, non-metallo beta-lactamase carbapenem-resistant *Enterobacterales*; MBL CRE, metallo beta-lactamase carbapenem-resistant *Enterobacterales*; Non-MBL CRPA, non-metallo beta-lactamase carbapenem-resistant *Pseudomonas aeruginosa*; MBL CRPA, metallo beta-lactamase carbapenem-resistant *Pseudomonas aeruginosa*; CRAB, carbapenem-resistant *Acinetobacter baumanii*

Discussion (clinical applications and future considerations)

With the available antimicrobials currently on the market paired with the characteristic differences of various infections, there are nearly infinite clinical scenarios that could be tailored to each unique patient. Overall, the results available from in vitro and clinical studies evaluating dual beta-lactam therapy have been predominantly favourable.

For Gram-positive organisms, dual beta-lactam therapy is most utilized in the clinical realm for *E. faecalis* IE, empiric coverage of community-acquired meningitis in extremes of age, and in cases of refractory MSSA infections.[59,76,84] Endocarditis caused by *E. faecalis* was historically treated with ampicillin plus an aminoglycoside; however, this regimen is falling out of favour due to similar outcomes and an enhanced safety profile noted with ceftriaxone plus ampicillin as well as rising aminoglycoside resistance and the lack of need to measure aminoglycoside serum concentrations.[55,59,62]

Common causes of community-acquired bacterial meningitis include *Streptococcus pneumoniae*, *Neisseria meningitidis* and, in patients less than 1 month of age or older than 50 years of age, *L. monocytogenes*.[107,108] Commonly, ceftriaxone (or cefotaxime in neonates) and vancomycin are utilized for coverage of *S. pneumoniae* and *N. meningitidis*, whilst ampicillin is added in this population due to its in vitro potency against *L. monocytogenes* and most extensive experience against this organism.[17,72,76] Lastly, dual beta-lactam therapy may be utilized in serious cases of *S. aureus*. Despite there being limited-to-no data describing this approach within infections caused by MRSA, positive clinical reports are accumulating, promoting the use of the cefazolin and ertapenem combination therapy as a salvage regimen against infections caused by MSSA.[84]

For Gram-negative organisms, the combination of beta-lactams may be used in patients clinically to treat drug-resistant organisms, including CRE, CRPA and CRAB.[11,49,99] Interestingly, positive outcomes of DCT against CRE have been reported, particularly when utilizing high-dose regimens and extended infusions[109] (Table 4). Despite being classified as resistant by the Clinical Laboratory and Science Institute, the increased dose and extended infusion enhance exposures, which optimize the time-dependent pharmacodynamic index of the carbapenems. Specifically, when ertapenem is used, the mechanism of synergy is theorized to be due to the preferential affinity of KPC to ertapenem in comparison to the other carbapenems.[110] Ertapenem is utilized as a 'suicide substrate', consuming the carbapenamases, allowing higher concentrations of the other carbapenem (which is likely hydrolysed to a lesser extent) to be available to inhibit cell wall synthesis.[98,109,111] Particularly against MBL-producing CRE, the combination of ceftazidime/avibactam plus aztreonam has shown promise clinically due to the inability of these enzymes to inactivate aztreonam. However, these isolates typically harbour other resistance mechanisms that inhibit aztreonam (co-production of other beta-lactamases such as ESBLs and KPCs), thus requiring the addition of the combination agent ceftazidime/avibactam as avibactam is not currently commercially available as a single agent.[49,50,112] Whilst the

Table 4. Common dosing strategies for dual beta-lactam therapy.[a]

Pathogen	Infection	Dosing	Duration of therapy[b]	Reference
Gram-positive organisms				
MSSA	Bacteraemia	Ertapenem 1 g q24h Cefazolin 2 g q8h	2 weeks, followed by 4 weeks of cefazolin monotherapy	Sakoulas et al., 2016[35]
E. faecalis	Endocarditis	Ampicillin 2 g q4h Ceftriaxone 2 g q12h	6 weeks	Gavalda et al., 2007[55,62]
		Penicillin 18–24 mu continuous infusion Ceftriaxone 2 g q12h	6 weeks	Trittle et al., 2020[131]
Gram-negative organisms				
Non-MBL CRE	Pneumonia, bacteraemia, and urinary tract, skin and soft tissue	Ertapenem 1–2 g q24h Meropenem 2g q8h (3–4 hour infusion)	10–28 days	Li et al., 2020[99]
MBL CRE	Unknown, urinary tract, intravascular device, skin and soft tissue, respiratory tract, and intra-abdominal	Ceftazidime/avibactam 2.5 g q8h Aztreonam 2 g q8h	7–14 days	Falcone et al., 2016[50]
CRPA	Hollow-fibre infection model	Ceftolozane/tazobactam 3 g q8h Meropenem 2 g q8h	14 days	Montero et al., 2018[132]
CRAB	Respiratory, bacteraemia and urinary tract	Imipenem/cilastatin 500 mg q6h Ampicillin/sulbactam 1 g q6h	9–19 days	Lee et al., 2013[83]

MSSA, methicillin-susceptible *Staphylococcus aureus*; Non-MBL CRE, non-metallo beta-lactamase carbapenem-resistant *Enterobacterales*; MBL CRE, metallo beta-lactamase carbapenem-resistant *Enterobacterales*; CRPA, carbapenem-resistant *Pseudomonas aeruginosa*; CRAB, carbapenem-resistant *Acinetobacter baumannii*.
[a]Included in Table 4 are dosing strategies, with dual beta-lactam therapy, utilized in the referenced clinical studies to overcome resistant Gram-negative and Gram-positive organisms.
[b]Dosing strategies may vary considerably based on patient/infection-specific factors (e.g. renal insufficiency, lack of source control, etc.)

availability of newer BL/BLI agents has shown activity against non-MBL producing CRE and CRPA, the cost and widespread availability of these agents will likely serve as a barrier in overall usage.[113] Furthermore, dual combination of beta-lactams may be forced once again in CRAB due to the in vitro and synergistic activity of sulbactam but it is only commercially available in the United States as the combination product ampicillin/sulbactam.[105,114] Although studies have been underwhelming, there may be instances in clinical practice in certain patient scenarios in which carbapenem plus ampicillin/sulbactam is used in CRAB or dual beta-lactams are utilized against CRPA.

Although the aforementioned sections have summarized common 'textbook' usages of dual beta-lactam therapy, there are other factors in which this approach may be used within the clinical setting. There are numerous host factors, such as allergies and potential toxicities of alternative agents, and other considerations (i.e. specific infectious diseases, multisite infections, multiple species of pathogens) that may lead to the use of combination beta-lactams. An example showcasing this would be in patients with true allergies to alternative antimicrobial agents (e.g. non-beta-lactam antimicrobials), who have an infection caused by a pathogen with suspected resistance patterns that could further preclude the use of non-beta-lactam agents. In this scenario, clinicians may need to utilize a combination beta-lactam approach to attempt to adequately eradicate their infection. As previously mentioned, beta-lactams are amongst the safest antimicrobials on the market. Dual beta-lactam therapy may be utilized against resistant infections in patients at substantial risk for antimicrobial-associated adverse effects, in whom the risks of using a non-dual beta-lactam regimen may outweigh the benefits. Furthermore, other patients may have multisite infections that could require this approach. For example, a patient may be receiving combination therapy with vancomycin plus cefazolin to treat an MRSA bacteraemia. However, the isolation of a concomitant KPC-producing

microorganism as the causative of pneumonia would warrant the use of an additional beta-lactam agent such as ceftazidime/avibactam or meropenem/vaborbactam. Therefore, the scenarios that require the application of dual beta-lactam therapy are vast and vary from case to case.

Conclusion

Ultimately, the use of dual beta-lactam therapy against Gram-positive and Gram-negative organisms has shown mostly promising data in in vitro, in vivo and clinical studies. At the very least, the data have shown that some combinations can produce similar activity to that of more harmful dual therapies, and the increase in activity is likely potentiated due to synergistic mechanisms. Given the major importance of appropriately treating bacterial infections during a time of growing antimicrobial resistance, we believe that the provided information supports future investigations with more robust studies defining the role for dual beta-lactam therapy, if any. Nevertheless, it is important that these studies prioritize investigating exposure target requirements, optimal patient populations, and promising dosing strategies to utilize when employing dual beta-lactam therapy in the management of Gram-positive and Gram-negative infections.

Key practice points

- Due to the development and propagation of multidrug resistance amongst Gram-positive and Gram-negative bacteria, the investigation of innovative treatment regimens is imperative.
- The use of dual beta-lactam regimens against resistant and persistent infections caused by Gram-positive and Gram-negative organisms has been associated with positive outcomes.
- Synergistic drug therapy combinations that include penicillin plus cephalosporins, as well as carbapenems plus cephalosporins, have been shown to decrease the microbial counts and improve clinical outcomes in infections caused by Gram-positive bacteria.
- Against multidrug-resistant Gram-negative microorganisms, the use of therapeutic regimens combining beta-lactam/beta-lactam inhibitors with carbapenems has resulted in the resolution of bacterial infections.
- Additional research is necessary to define the best placement for dual-beta lactam regimens in current practice.

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