Overview Methicillin-Resistant Staphylococcus Aureus Combination Treatment Options in Vivo and in Vitro

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Abstract

The spread of multidrug-resistant Staphylococcus aureus continues to threaten global health. Methicillin-resistant Staphylococcus aureus (MRSA) is one of the common causes of bacterial infections in hospitals and communities. Despite vancomycin being an effective treatment for MRSA, from 2006 to 2020, vancomycin-resistant MRSA increased 3.5-fold, from 5% to 7%. Bacterial genome mutations, as well as bacteria's ability to transfer genetic material with other bacteria, allowing them to obtain resistance genes from different strains, are all factors contributing to the development of vancomycin resistance in MRSA. As a result, combination therapy may be a potential treatment for MRSA infection. We searched in PubMed and Google Scholar, and our search yielded 92 articles, out of which 74 full-text articles were reviewed and 56 were selected for this study. This literature review examines combination therapies for MRSA infections, such as β-Lactams with vancomycin, linezolid and imipenem, daptomycin and ceftaroline. The review yielded several studies looking at the synergy between β-lactams and vancomycin. Although linezolid and rifampicin demonstrate synergy against MRSA in vivo and in vitro in various invasion diseases, more clinical research is required to prove their efficacy. Furthermore, daptomycin plus ceftaroline shows synergy for refractory staphylococcal bacteremia in vivo and in vitro. Combining ceftaroline and daptomycin has two benefits: they work synergistically together and make the innate host defense peptide cathelicidin leucine-leucine-37 (LL-37) more sensitizing. Ceftaroline plus daptomycin was recently used in MRSA biofilm infections, demonstrating a potentially promising treatment as the first combination used without side effects in humans.

Keywords: Bacteremia, Vancomycin, Daptomycin, Ceftaroline, Staphylococcus aureus, MRSA.

Introduction

In the last few decades, Staphylococcus aureus has evolved into a form increasingly resistant to antibiotics, called methicillin-resistant Staphylococcus aureus (MRSA). MRSA is resistant to beta-lactams such as methicillin and oxacillin. Resistance to MRSA is caused by the production of penicillin-binding protein 2a (PBP2a); this enzyme attaches to the antibiotic's beta-lactam ring, preventing it from binding to its target and making it ineffective. MRSA infection has become more common over time, especially in people who have undergone surgery, have medical devices installed, or have poor immune systems [1,2]. Staphylococcus aureus is known to colonize the skin in approximately 1 in 30 people and can be transferred by physically touching an infected individual or touching surfaces or objects where the pathogen is present [3].

Colonization with Staphylococcus aureus does not cause symptoms, but it can predispose to deep-seated infections. It can cause redness and swelling, and if it progresses further, it can cause fever, aches, and confusion [3]. Infection with Staphylococcus aureus can cause sepsis and infective endocarditis, as well as septic arthritis, pneumonia, and device-related infections. In England alone, 11,938 cases of MRSA were reported between April 2017 and March 2018 [3], a 3.8% increase over the previous year's figures and a 36.2% increase over the 2011/2012 period [4]. Without adequate treatment, the condition can be life-threatening. A study showed that 23% of patients who are carriers for MRSA will develop MRSA infections within one year, 39% of which were pneumonia, with MRSA contributing to death in 5% of cases [5]. Therefore, MRSA represents a significant and pressing health and community health issue that needs to be addressed through novel and evidence-based management strategies. Therefore, this literature review aims to examine combination therapies discovered against MRSA in vivo and in vitro and analyze their known limitations.

Methods for searching:
We searched in PubMed and Google Scholar for MRSA combination treatment options in vivo and in vitro. We searched using the following search terms: MRSA combination treatment, MRSA and combination therapy, methicillin-resistant Staphylococcus aureus and combination treatment, MRSA triple combination, linezolid and rifampicin, linezolid and fosfomycin, linezolid and imipenem, vancomycin and B-lactams, with all their synonyms. Inclusion criteria were in vivo and in vitro research published in English. Exclusion criteria were articles published in languages other than English. We searched in PubMed and Google Scholar, and our search yielded 92 articles, out of which 74 full-text articles were reviewed, and 56 were selected for this study.

MRSA History

In 1881, Staphylococcus aureus was first described in the papers of Alexander Ogston [6,7]. Following introduction of methicillin in 1961, the first case of MRSA was observed later that year by Jevons and his associated scientists, and the pathogen was widespread throughout Europe in the 1970s [8]. MRSA has emerged increasingly since 1960s and has spread globally to become the leading cause of community acquired bacterial infections [9]. The increasing emergence of MRSA has led to a considerable rise in vancomycin use. Vancomycin-resistant strains of staphylococcus aureus are generated by a change in the peptidoglycan terminal, resulting in diminished vancomycin attachment and the inability to suppress cell wall formation. Resistance is also caused by the creation of aberrant peptides in the cell wall that engage vancomycin and inhibit it from attaching to its receptor or by increased peptidoglycan, leading to thickened cell walls [10,11]. 2 % of MRSA were resistant to vancomycin before 2006, 5 % from 2006 to 2014, and 7 % from 2015 to 2020, indicating a 3.5-fold increase in the prevalence of vancomycin-resistant MRSA from 2006 to 2020 [11].

Current MRSA treatment methods

Current treatment of MRSA includes vancomycin, daptomycin, linezolid, clindamycin, tigecycline, ceftobiprole, bactrim and doxycycline. These agents have advantages and limitations as monotherapy for MRSA [9,12-14]. Daptomycin has been demonstrated effective in treating MRSA infections, including bacteraemia, endocarditis, and complicated skin and soft tissue infections (SSTIs) [14,15]; however, it is not a suitable treatment for pneumonia because it is inactivated by lung surfactant. Daptomycin is also not indicated to treat infections of the central nervous system, due to its poor penetration of tissue into the cerebral spinal fluid [9,15]. Phase III clinical studies have shown that 4 mg/kg of daptomycin in complicated SSTIs and 6 mg/kg in MRSA bacteremia are non-inferior to vancomycin [14]. A case-control study supports switching to daptomycin in MRSA bacteremia patients with high vancomycin minimum inhibitory concentration values (>1 µg/mL) [16].

Linezolid has outstanding tissue penetration and is efficacious for treating pneumonia and SSTIs [17]. Moreover, the treatment time for patients with MRSA complicated SSTIs is shorter than with vancomycin. Itani found that the average length of intravenous treatment with linezolid was much shorter than with vancomycin (5.3 days vs. 9.8 days; P-value = 0.001) [18]. Itani further demonstrated that the median and mean lengths of stay in the linezolid cohort were 5.0 and 7.7 days, respectively, compared to 7.0 and 8.9 days in the vancomycin cohort (P-value = 0.016) [18]. It remains controversial whether it is superior to vancomycin for treating MRSA infections. Side effects of linezolid include several serious complications, such as bone marrow suppression, kidney failure, and lactic acidosis [13,14,19].

Clindamycin is a successful regime for treating SSTIs caused by MRSA and is effective against community associated MRSA. Although clindamycin has been shown to have efficacy in treating joint and bone infections, its resistance rate and bacteriostatic nature have increased [13]. Tigecycline is active in vitro against gram-positive and gram-negative bacteria and is an excellent option for treating MRSA because of its broad coverage. However, it is bacteriostatic, and there are insufficient studies to support its use in clinical practice, especially for MRSA infections. It is also likely not an effective treatment for hospital acquired MRSA (HA-MRSA) pneumonia. Furthermore, it has a huge protein structure, which results in low serum levels, restricting its effectiveness in bacteraemia cases [13,14,19]. According to the Food and Drug Administration's safety warning, patients with severe infections treated with tigecycline have a higher overall mortality rate (4%) than those treated with a comparator drug (3%) [20,21]. Although no significant differences in effectiveness between tigecycline and comparator treatments have been seen in complicated SSTIs, tigecycline is often prescribed as a second- or third-line therapy for MRSA infections when other therapies are ineffective [21,22].

Ceftaroline is bactericidal, broad spectrum, and active against MRSA, as it has a high affinity for MRSA penicillin-binding proteins, mainly PBP2a, as well as gram-negative bacteria. Ceftaroline can treat acute bacterial skin and skin structure infections (SSSI) and community-acquired pneumonia. Although some evidence indicates that it can treat severe MRSA infections, like orthopedic infections and bacteremia, it is noninferior to vancomycin with aztreonam to treat complicated MRSA in SSTIs [23]. Another treatment option is ceftobiprole, which is approved in some European countries. Although ceftobiprole has a spectrum broader than ceftaroline, is bactericidal, and used for treatment in community-acquired pneumonia and hospital-acquired pneumonia, it cannot treat ventilator-associated pneumonia [9,24]. In previous research clinical cure rates for ventilator-associated pneumonia patients were 23.1% with ceftobiprole and 36.8% in patients treated with ceftazidime and linezolid, with ceftobiprole failing to demonstrate non-inferiority [25]. The reasons behind ceftobiprole's inability to demonstrate non-inferiority are not entirely clear. It has been proposed that presence of biofilm-embedded organisms may have an adverse impact [25]. Alternative explanations include need for greater doses and longer infusions to produce appropriate therapeutic concentrations of ceftobiprole in patients with CLCr ≥ 150 ml/min, a condition found in 29% of ceftobiprole-treated patients with ventilator-associated pneumonia [25-27]. Clinical effectiveness data limitations, medication product scarcity, and safety concerns have all hampered use of traditional monotherapies. However, many alternative solutions have been established to overcome the challenges of MRSA infections, like an antimicrobial peptide, bacteriophage therapy, newly discovered antibiotics, antivirulence factors, and antibiotic combination therapy [28,29].

Review of current combination therapy

Combination antibiotics, such as streptomycin and penicillin in the 1950s and trimethoprim with sulfonamides in 1986, are historically proven to be more effective and have a larger range than antibiotic monotherapy [30,31]. A decade ago, Silver reported that most successful antibiotic therapies have multiple targets [32]. Combination therapy is advantageous because it broadens the spectrum of antimicrobial coverage against pathogens. Furthermore, it enables synergistic effects, which improve efficacy. It also decreases development resistance and reduces the toxicity of antibiotics because lower concentrations of drugs are used to attain the synergistic effect. Antibiotic combination therapy can also help improve patient safety and reduce resistance and the cost of discovering new antibiotics [29,33]. Although vancomycin is gold standard for MRSA therapy, monotherapy with a glycopeptide for MRSA has shortcomings, including a slow bactericidal effect, development of drug resistance, and weak tissue penetration. Theoretically, combination therapy might overcome these shortcomings by combining two or three agents to address these deficiencies. Combination treatments can improve tissue and biofilm penetration while reducing resistance and the cost of discovering new antibiotics [12,34,35].

Principally, data on combination therapy have come from in vitro tests. These tests have the following possible results: synergy, antagonism, and indifference. The method used to measure synergy is based on agar diffusions, such as antibiotic discs or E-test strips, checkerboard testing, and time-kill curves. For checkerboard testing, different dilutions of antibiotic combinations are placed in a 96-well plate to achieve a variety of minimum inhibitory concentrations (MICs) for each antibiotic. This method can be used to estimate the degree of synergy, which is expressed as frictional inhibitor concentration index (FICI). A value ≤ 0.5 indicates synergy, whereas a value ≥ 2.0 indicates antagonism; however, the intermediate values indicate indifference.

Time-kill curve assay is a dynamic methodology rather than an assessment of synergy at one time point, which better reflects clinical use. In this method, bacteria are cultured in liquid media with different concentrations: either one antibiotic or a combination of antibiotics. Consequently, the time-kill kinetics and speed of bacterial killing can be calculated over time. In time-kill analysis, synergy (bactericidal and bacteriostatic) is defined as decrease of ≥ 2 log colony forming unit (CFU) of bacteria/mL from the CFU/mL for the strongest component. Eventually, in vitro is used to determine appropriate antibiotic doses in animals and humans. Any synergy is obtained by adding one or two additional drugs, which might increase the bactericidal activity. This method is used to assess existing antibiotics against a target organism [29,34,36].

MRSA Triple combination

One of the most effective combination therapies for MRSA, which was a triple combination of a subclass of β-lactams, studied in vivo and in vitro was reported to display highly synergistic activity. This triple therapy of β-lactams used three old antibiotics, meropenem, piperacillin and tazobactam (ME/PI/TZ) that were clinically approved as monotherapies and found that they act synergistically as a bactericidal agent against MRSA strain N315 and other clinical isolates of MRSA [37]. The FICI of the ME/PI/TZ combination was evaluated in vitro using the checkerboard assay, and a value of 0.11 was obtained, indicating that combination had highly synergistic bacterial killing activity against MRSA N315. However, when only meropenem and tazobactam were used in combination, the FICI was 0.67, indicating that the combination of these two drugs was less synergistic.

In contrast, combination of meropenem and piperacillin was also shown to be synergistic, as was combination of piperacillin and tazobactam [37]. The ME/PI/TZ combination was tested in vivo in a neutropenic mouse model infected with MRSA N315 to determine efficacy of combination. Results of the ME/PI/TZ treatment in the mice model demonstrated that the triple combination had strong killing activity against MRSA N315 and that all mice survived six days post infection. Therefore, combinations of old antibiotics could overcome resistance to MRSA in human infections [37]. Moreover, this triple combination suppressed resistance development in MRSA N315 in vitro and in vivo in 11-day trials [37]. In contrast, treatment with two or one of them resulted in development of resistance in 1-8 days. Mechanism of ME/PI/TZ triple therapy is as follows: Tazobactam inhibits the staphylococcal penicillinase BlaZ, protecting piperacillin from hydrolysis by penicillinase. Then piperacillin can bind to penicillin-binding protein 2 (PBP2) to inhibit transpeptidase activity in MRSA. Meropenem can bind to a penicillin-binding protein (PBP1) and prevent transpeptidation and bind to PBP2a allosteric active site. Therefore, meropenem or other β-lactams open active sites that can affect and inhibit cell wall synthesis machinery in MRSA [37,38]. Despite the fact that MRSA strains are resistant to most β-lactams because of PBP2a, the combination restores efficacy of β-lactams against MRSA [37]. Although in vitro and in vivo studies have shown synergy, resistance suppression over prolonged dose intervals, and collateral antibiotic susceptibility, more human studies are needed to provide additional information on this promising MRSA treatment.

Recently, Yoneda et al. showed that this combination of ME/PI/TZ is highly effective against 207 clinical staphylococcal strain isolates, including MRSA, methicillin-susceptible Staphylococcus aureus, ceftarolinenon-susceptible MRSA, and coagulase-negative staphylococci. In this study, ME/PI/TZ combination was found effective, especially against the USA 300-SCCmec (strain of gram-positive coccus bacteria) type IV isolate, which has spread globally. These samples were taken from clinical isolate patients to investigate antibiotic susceptibility testing using MICs. All species demonstrate high sensitivity to triple combination of ME/PI/TZ in MICs [39]. Also, combination of ME/PI/TZ is effective against vancomycin resistant Enterococcus, Staphylococcus epidermidis, and Staphylococcus pseudintermedius [39]. These findings may prove that the combination of ME/PI/TZ might be a novel way to tackle severe MRSA infections. Additionally, combination of ME/PI/TZ is also safe and less expensive than a new anti-MRSA [37-39].

Linezolid combination treatment

Linezolid is antibiotic authorized to treat serious MRSA infections, including endocarditis, pneumonia, meningitis, bacteremia, and SSTIs. Linezolid is a broad-spectrum antibiotic and inhibits protein synthesis in bacteria by binding ribosomal ribonucleic acid involved in the 30S and 50S ribosomal subunits [40,41]. In addition, linezolid might suppress expression of virulence factors and decrease toxin production in gram-positive bacteria [42]. It has been shown highly effective against MRSA infections when combined with other antibiotics because linezolid is bacteriostatic. In 2014, Cabellos et al. studied efficacy of the linezolid and rifampin combination using time-killing curves and rabbit model meningitis [43]. The results revealed that combination had good efficacy, with outstanding antibacterial activity and a synergistic effect after 24 hours in vitro and in vivo at 1/2 the MIC [43,44]. However, the study indicated that there was no antagonism with the combination (linezolid plus rifampin) in vitro and in vivo, the combination mechanism was not explained, and the study's time period was limited; therefore, further investigations are needed.

Linezolid resistance is mediated by plasmid encoding cfr gene [45,46]. Zhou et al. tested the same combination (linezolid with rifampicin) in murine pneumonia infected with cfr-positive and cfr-negative MRSA strains [45,46]. In this study, seven clinical MRSA (ST764) strains were isolated from sputum specimens from patients with pulmonary infections. The effects of combination of linezolid and rifampicin were assessed in vitro using the checkerboard test, and the FICI obtained was equal to or less than 0.5, which indicated a synergistic effect between them. Furthermore, time-killing curves were used to confirm this result, also indicating a synergistic effect over 24 hours. In vivo, the efficacy and dose response of combination have been assessed in neutropenic mice, and the results indicated that linezolid with rifampicin had profound treatment efficacy in vivo over 24 hours. However, this study used only one MRSA genotype, ST764 (HA-MRSA), which is present in Asia, and the number of MRSA strains (seven) examined was small. Therefore, more in vivo investigations are needed, and other infections causing MRSA, such as bacteremia, endocarditis, and SSTIs, should be examined.

In 2020, Zhou et al. studied efficacy of the linezolid plus rifampicin combination against MRSA with or without cfr gene. They tested 10 strains of MRSA isolated from samples from hospitalized patients with pulmonary infections; these

strains included 161402, 161400, 161494, and 161813, as well as ST 5 HA-MRSA lineage, which causes bacteremia and necrotizing fasciitis, and ST 398 MRSA, which causes zoonotic infections that can result in pneumonia and SSSI. In vitro, time-kill curves and concentration-effect tests were performed, and the in vitro results indicated synergy. When the combination was assessed in vivo against murine bacteremia and SSSI, results indicated that the treatment was effective. An advantage of rifampicin is that it decreases fibronectin binding in Staphylococcus aureus when used as a combination therapy [45,47]. This characteristic could be useful in treatment of persistent and recurrent MRSA bacteremia [45,48]. In one report, a patient diagnosed with cellulitis and erythema was treated with a combination of linezolid and rifampicin, and after three days, all symptoms were significantly reduced [44] (Table 1).

Daptomycin with ceftaroline

Daptomycin's mechanism of action involves depolarization, permeabilization, and ion leakage in cell membrane, leading to cell death [51]. In 2014, Sakoulas et al. studied combination of daptomycin and ceftaroline as a possible option to treat refractory staphylococcal bacteremia. The combination was found effective in 26 cases of refractory bacteremia, 20 of which were MRSA. In vitro, checkerboard tests, timekilling, binding assays, and cathelicidin LL-37 killing assays were used to test the combination, which was verified to be synergistic. However, it takes a median of ten days to eradicate bacteremia, with an approximate range of 3 to 23 days. However, a combination of daptomycin and ceftaroline eradicated bacteremia in 2 days (a range of 1 to 6 days). However, ceftaroline alone has also been tested in vitro and was found to kill bacteria slower than daptomycin. Therefore, combining ceftaroline with daptomycin provides dual advantages through synergy and sensitization to cathelicidin LL37, which is an innate host defense peptide. It was reported that sensitization (daptomycin and ceftaroline) to cathelicidin LL37 might help attenuate the virulence of the pathogen. Consequently, combining ceftaroline and daptomycin might be an excellent option for treating refractory staphylococcal bacteremia [52]. However, further human studies are needed to evaluate the resistance mechanism of this combination, as well as its side effects in humans.

In 2015, Barber et al. evaluated daptomycin plus ceftaroline against three clinical biofilm-producing MRSA strains in vitro using a pharmacokinetic/pharmacodynamic model to simulate catheter-related biofilms. Their results indicated bactericidal activity and reduction in bacteria density at 72 hours. They found that daptomycin with ceftaroline improved the killing of biofilm-producing staphylococci in contrast to monotherapy [53]. However, the duration of this study was restricted to 72 hours, and different results might be obtained with other materials, such as prostheses. More in vivo and in vitro examinations are needed, as well as in humans, to clarify the effects against biofilm-producing MRSA [53]. Recently, in 2020, a combination of daptomycin with ceftaroline in humans was evaluated in a cohort study comprised of a 500-bed community teaching hospital to determine its effectiveness in treating staphylococcal infections associated with medical devices. The study assessed and compared the clinical synergy between rifampin with adjuvant therapy and combination daptomycin and ceftaroline in 116 inpatients aged 18 or older. The primary outcomes for the patients included normal white blood cell count, a temperature less than 38°C, and no remaining symptoms, all of which were achieved with the daptomycin with ceftaroline combination therapy. Furthermore, the secondary outcome was not statistically different in their patient groups, except for a longer hospital stay in the daptomycin with ceftaroline group, 9 vs. 15 days, but there were also no symptoms [54]. Daptomycin with ceftaroline represents a novel combination therapy to treat refractory or relapsing staphylococcal infections associated with medical devices.

Daptomycin with rifampin

In vitro and animal studies have revealed general trends of antagonism or indifference when rifampin is added to daptomycin. For example, a combination daptomycin with rifampin or gentamicin in vitro model was found to antagonize or delay bactericidal activity in daptomycin when used alone [55]. Likewise, combination of daptomycin and rifampin or gentamicin in time-killing experiments and rabbit endocarditis models showed no increase in efficacy of daptomycin against MRSA [56]. However, efficacy of daptomycin plus rifampin in infections associated with prosthetic devices has previously been reported in a rat foreign body infection model and a retrospective review [57,58]. In this combination, rifampin can efficiently penetrate the biofilm and exert its bactericidal activity independently of cell cycle. This combination is suitable for treating slow-growing infections in prosthetic devices. Case studies have also indicated synergy and clinical progress in vitro [59,60]. Rose et al. reported that daptomycin plus rifampin salvage therapy was the most widely used therapy when they examined 12 patients with chronic MRSA infections that usually involved developing biofilms, 10 of whom were treated clinically. Even though a checkerboard test indicated synergy in 75% (9/12) of patients and the anticipated treatment efficacy was 100%, the time-killing curve was not synergistic. These results indicate that the checkerboard test is essential for assessing any combination in future investigations. Furthermore, the finding supports use of daptomycin with rifampin as potential treatment for infections that form biofilms [61], although additional human studies are required.

β-Lactams with vancomycin

Several studies have tested the efficacy of vancomycin with β-lactams against MRSA strains and found indications of synergy in vitro and in vivo. These studies used diverse methodologies, such as disc diffusion assays, E-tests, time-killing tests, or checkboard assays. Most found that the combination is highly effective in killing bacteria, but not all strains, as described in Table 2 [62-68]. Among these studies, there were no clear common characteristics among the strains. However, these studies did show a general trend toward the combination's effectiveness [34]. As a result, additional studies should be performed to examine efficacy of these combinations (Table 2).

Table 1: Combination linezolid therapy for MRSA in vivo and in vitro

Antibiotic combination therapy used	Setting	Procedures	Interaction	Outcomes	Diseases treated	Limitation	References
Linezolid plus rifampicin	In vitro and in vivo (rabbit meningitis model) tests	Time-killing and bacteria counting assays, pharmacokinetic and pharmacodynamic tests	Synergistic	Linezolid with rifampicin showed synergistic anti-bacterial activity at 24 hours	Meningitis	Mechanism of combination has not been determined and further in vivo studies are needed.	[43]
Linezolid plus rifampicin	In vitro and in vivo (murine pneumonia model) tests	Time-killing assay, checkerboard assay, pharmacokinetic profiles	Synergistic	Effective	Pneumonia	Mechanism of combination was not determined in study, and additional in vivo studies on other diseases are needed.	[46]
Linezolid plus rifampicin	In vitro and in vivo murine models of bacteraemia and skin and skin structure infection (SSSI)	Time-killing assay, checkerboard assay, and pharmacokinetic profiles	Synergistic	Linezolid plus rifampicin showed efficacy	Bacteremia infections and skin and soft tissue infection (SSTI)	Further human studies are needed.	[45]
Linezolid and fosfomycin	In vitro and in vivo (biofilm-infection rat model) tests	Time-killing assay, checkerboard assay, and bacteria counting	Synergistic	Linezolid with fosfomycin display anti-biofilm activity against MRSA strains both in vitro and in vivo.	Biofilm associated with MRSA infection, especially catheter infections	Although fosfomycin was shown to destroy the outer layer of bacteria cells and prevent first step in cell wall biosynthesis, more in vivo studies are needed.	[49]
Linezolid and imipenem	In vitro and in vivo (rabbit endocarditis model) tests	Time-killing assay, checkerboard assay, and bacteria counting	Synergistic	Linezolid with imipenem demonstrated synergy and bactericidal activity against MRSA strains after 5 days	Endocarditis	Although this study reports a promising and comprehensive treatment for severe MRSA infections, it did not study the synergistic mechanism between two drugs. Further investigations are needed.	[50]

Table 2: Combination β-lactam with vancomycin

Combination therapy	Setting	Organism	Methods	Outcomes	Disease treated	Limitation	References
Vancomycin + cefpirome, cefoperazone, or ceftazidime	In vitro	MRSA	Checkerboard test	Synergy was observed among cefpirome + vancomycin and cefoperazone + vancomycin but ceftazidime + vancomycin was not synergistic	Not mentioned	The study lacked time killing assays and in vivo studies. Further investigations are needed.	[67]
Vancomycin + piperacillin, tazobactam, ampicillin, sulbactam, imipenem, or nafcillin	In vitro	MRSA	Time-killing curve in infected fibrin clots.	Synergy was observed	Not mentioned	More testing is needed; checkboard tests and in vivo studies are needed.	[66]
Vancomycin+ oxacillin, ceftriaxone, ceftazidime, or Augmentin (clavulanic acid + amoxicillin)	In vitro and in vivo (only tested nafcillin for sterilising vegetations and renal abscesses)	MRSA	Checkerboard, disk diffusion, agar dilution, and time-kill tests	Synergy was observed in vitro for all combinations, but in vivo, they only assessed vancomycin with nafcillin. It demonstrated synergy.	Endocarditis	More studies are needed to determine mechanism of action of drugs, and all combinations need to be investigated in vivo.	[62]
Vancomycin + cefepime	In vitro	MRSA	Time-killing assay and checkerboard test	Synergistic according to time killing assay, and the checkboard test indicated additivity or indifference for some strains of MRSA	Not mentioned	More testing is needed in animals and in vitro to confirm the results.	[65]
Vancomycin + cefotaxime	In vitro	MRSA	Checkerboard assay and time kill curves	Synergy was demonstrated in 43 out of 50 MRSA strains	Severe pneumonia	Further examinations in animal model are needed.	[64]
Vancomycin + imipenem	In vitro	MRSA	E-test, time-kill curve method, checkerboard test	Synergistic when using sub-MIC of vancomycin	Not mentioned	Further investigations using the checkboard assay and in vivo studies are needed.	[68]
Vancomycin + piperacillin/tazobactam or oxacillin	In vitro	MRSA	Disk diffusion and E-test studies and time-kill curves over 24 hours	Synergistic	Not mentioned	More examinations in vivo are needed.	[63]

Conclusions

MRSA remains one of the common resistant infections, and vancomycin monotherapy is most effective treatment; however, as MRSA strains become more resistant to antibiotic therapies, combination therapy, such as vancomycin with β-lactam synergy in vitro and in vivo, may be one treatment option for MRSA. Although linezolid and rifampicin demonstrated synergy against bacteremia and SSSI caused by MRSA, more clinical research is required to prove their efficacy. Combination treatment has bactericidal activity, which may compensate for the drawbacks of monotherapy. Ceftaroline and daptomycin were recently utilized in MRSA biofilm infections, suggesting a potentially viable therapy as the first combination tested in people without adverse effects. This could be a unique therapy for staphylococcal device infection. Although combination therapy might be a potential treatment for MRSA invasion infections, more studies are needed before clinical trials. Furthermore, for future studies, a time-killing assay, checkerboard test, and MIC in vitro and in vivo should be used to provide accurate results to demonstrate bactericidal activity. Also, evaluation of a suppressed mechanism and two to three combinations are important to support study of combinations. Importantly, clinical use and controlled studies should be applied to evaluate synergy in humans and identify side effects and limitations.

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