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A Review of Combination Antimicrobial Therapy for *Enterococcus Faecalis* Bloodstream Infections and Infective Endocarditis

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Title: A review of combination antimicrobial therapy for *Enterococcus faecalis* bloodstream infections and infective endocarditis

Running Title: Treatment for *E. faecalis infections*

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Summary: The purpose of this review to highlight available treatment options, their limitations, and provide direction for future investigative efforts to aid in the treatment of severe *E. faecalis* infections, namely infective endocarditis.

ABSTRACT

Enterococci, one of the most common causes of hospital-associated infections, are responsible for substantial morbidity and mortality. *Enterococcus faecalis*, the more common and virulent species, cause serious high-inoculum infections, namely infective endocarditis, that are associated with cardiac surgery and mortality rates that remained unchanged for the last 30 years. The best cure for these infections are observed with combination antibiotic therapy; however, optimal treatment has not been fully elucidated. It is the purpose of this review to highlight treatment options, their limitations, and provide direction for future investigative efforts to aid in the treatment of these severe infections. While ampicillin plus ceftriaxone has emerged as a preferred treatment option, mortality rates continue to be high, and from a safety standpoint, ceftriaxone, unlike other cephalosporins, promotes colonization with vancomycin resistant-enterococci due to high biliary concentrations. More research is needed to improve patient outcomes from this high mortality disease.

INTRODUCTION

Severe enterococcal infections including infective endocarditis (IE), are associated with mortality rates as high as 20-40% and have remained unchanged for the last three decades despite advances in antimicrobial therapy.[1] Although *Enterococcus faecalis* and *Enterococcus faecium* are the two most clinically relevant species, *E. faecalis* accounts for approximately 97% of all IE cases predominantly impacting the elderly and patients with comorbidities.[2] *E. faecalis*, unlike *E. faecium,* is less frequently multidrug resistant.[2] However, lack of bactericidal activity of beta-lactams[3], and ability to form biofilm at higher rates than *E. faecium* (87-95% vs. 16-29%, respectively)[4, 5], makes treatment of *E. faecalis* infections particularly challenging, and may contribute to the unchanging mortality rates. Consequently, combination antimicrobial therapy is required for deep-seated *E. faecalis* infections, and with over 50% of isolates expressing aminoglycoside resistance, treatment options are becoming limited.[6] It is the purpose of this review to highlight available treatment options, their limitations, and provide direction for investigation of future novel combination therapies including ampicillin plus non-ceftriaxone beta-lactams and daptomycin combination therapy, to further aid in the treatment of *E. faecalis* IE.

METHODS

Studies were identified by conducting PubMed, and EMBASE searches using the following keywords in one or more combinations with '*Enterococcus faecalis*': infective, endocarditis, bacteremia, bloodstream, infection, treatment, guideline, antibiotic, combination, synergy, resistant, biofilm, clinical, diagnosis, epidemiology, in vitro, in vivo, simulated endocardial vegetation, experimental, and beta-lactamase. Manual searches of reference lists of relevant articles found from initial searches were also conducted. No limitation was placed on publication time period. Studies were selected based on authors' (MB and MKL) judgment of relevance to topic.

ORIGIN OF COMBINATION THERAPY

For serious *E. faecalis* infections, such as IE, bactericidal agents often as combination therapy are preferred.[2] Beta-lactam antibiotics lack bactericidal activity against enterococci when used as monotherapy, making treatment of systemic infections particularly challenging.[3] Although *E. faecalis* are often susceptible to ampicillin, treatment failure of 60%, and lack of bactericidal activity of cell-wall active agents (i.e. penicillin G, ampicillin, vancomycin) prompted efforts to identify combination therapies that would yield a bactericidal effect in severe infections.[1-3] Originally, penicillin or ampicillin was combined with gentamicin or streptomycin to facilitate intra-cellular uptake of aminoglycosides.[3] The recognition of in vitro bactericidal synergism between beta-lactams and aminoglycosides was supported by observational clinical data and led to improvements in IE cure rates up to 75%.[3] However, rising high-level aminoglycoside resistance (HLAR), which may range to up to 63%[1, 6, 7] prompted the need for alternative therapy. Subsequently, dual beta-lactam combination therapy emerged as a viable, safe treatment option for severe infections with *E. faecalis*.

DUAL BETA-LACTAM THERAPY

In Vitro and Experimental Animal Data

In 1995, Mainardi and colleagues were the first to report synergy between amoxicillin and cefotaxime in *E. faecalis*.[8] The results showed that the MIC for amoxicillin decreased substantially in the presence of cefotaxime, as did the MIC of cefotaxime in the presence of amoxicillin. The proposed mechanism of synergy is that partial saturation of essential penicillin binding proteins (PBPs) 4 and 5 by amoxicillin, coupled with complete saturation of non-essential PBPs 2 and 3 by cefotaxime leads to a bactericidal effect.[8] Taken together, the combination of cefotaxime and amoxicillin exploits the optimal inactivation of PBPs 2, 3, 4 and 5, thereby producing synergism on *E. faecalis.* Presumably the marked impairment in cell wall synthesis is the basis for this effect.

In 1999, Gavalda and colleagues further explored beta-lactam combinations by evaluating the activity of ampicillin plus ceftriaxone (AC) against *E. faecalis* strains with HLAR.[9] They confirmed Mainardi's synergistic findings, and observed up to a fourfold reduction in ampicillin MIC in the presence of ceftriaxone. Furthermore, rabbits treated with AC in HLAR *E. faecalis* endocarditis had lower bacterial vegetation counts than rabbits treated with ampicillin alone.[9] In 2003, Gavalda et al. evaluated the utility of AC versus ampicillin plus gentamicin (AG) against *E. faecalis* with or without HLAR in rabbits with catheter-induced endocarditis.[10] They determined that the two combinations were comparable in efficacy, and further concluded that AC may be an alternative to AG particularly in special populations, such as patients with renal insufficiency (Table 1).[10]

Human Data

Clinical data have since evaluated the combination of AC against HLAR and non-HLAR *E. faecalis* IE.[6, 11, 12] In 2007, Gavalda et al. assessed the efficacy and safety of AC in 21 patients with HLAR, and 22 patients with non-HLAR *E. faecalis* IE in a multicenter, open-label clinical trial.[6] In this observational study of enterococcal IE, it was concluded that in addition to AC being a safe and effective treatment option for HLAR IE, it is a reasonable alternative for patents at risk for nephrotoxicity infected with non-HLAR organisms.[6] Subsequently, Fernandez-Hidalgo and colleagues conducted a large, non-randomized, multicenter, cohort study comparing the safety and efficacy of AC and AG in 246 episodes (159 subjects in AC group; 87 subjects in AG group) of IE caused by *E. faecalis*.[11] The authors concluded that the two combinations were equally effective as there was no difference in mortality while on antimicrobial treatment and during the 3-month follow-up, relapse, or treatment failures requiring alternate therapy. However, patients treated with AG had significantly higher rates of adverse events (i.e. renal impairment) requiring therapy withdrawal.[11] These findings coincide with a retrospective study of prospectively

collected data that evaluated 69 episodes of IE caused by *E. faecalis* (30 subjects in AG group; 39 subjects in AC group).[12] Similar to Fernandez-Hidalgo and colleagues, the authors did not observe a difference in in-hospital mortality or 1-year mortality between AG and AC groups, and found that patients on AG had higher rates of treatment-induced renal failure than patients receiving AC. Interestingly, the authors captured epidemiologic data that demonstrates a significant increase in IE caused by HLAR-producing *E. faecalis* over the course of 14 years, along with an increase in AC therapy, although the small sample size limits definite conclusions (Table 1).[12]

Clinical trials are limited in IE

As a result of these two clinical studies [6, 11], the 2015 national IE guidelines have been updated to recommend double beta-lactam therapy (i.e. AC) as a treatment option for HLAR infections, and a reasonable alternative to aminoglycosides for non-HLAR *E. faecalis* infections (Class IIa; Level of Evidence B recommendation).[2] Of note, isolates with gentamicin resistance may be susceptible to streptomycin, and vice versa, although monitoring for streptomycin concentrations is often difficult and inefficient for clinicians since it is not available within most hospitals. The guideline recognizes that the AC regimen has several limitations – notably 1) all data were retrospectively collected without randomization, 2) treatment recommendations were center-dependent; therefore, unmeasured confounding factors as well as treatment and indication bias impacting these results cannot be ruled out and 3) gentamicin dosing and therapeutic drug monitoring were not consistent across all centers, and higher levels may have contributed to the observed increase in renal impairment.[11, 12] While data supporting the use of AC has limitations, it is important to note that studies recommending AG treatment are observational and have similar limitations.[2, 3]

As it currently stands, data providing support for optimal drug, dose and duration for the currently available treatment options remain controversial. A recent study investigated optimal gentamicin treatment duration in 84 patients with non-HLAR *E. faecalis* IE by comparing two groups: patients admitted prior to the Danish 2007 guideline modification versus patients admitted after guideline modification that recommended reducing gentamicin treatment duration from 4-6 weeks to 2 weeks.[13] Forty-one patients received gentamicin for a median of 28 days (IQR, 18-42), and 43 patients received a median of 14 days (IQR, 7-15). There was no difference between groups for the primary outcome of 1-year event-free survival (27 [66%] vs. 29 [69%], p =0.75) measured from the end of treatment. No differences in complications, relapse, in-hospital mortality, baseline renal function, and 14-day renal function were observed between groups. However, patients receiving 14-day treatment with gentamicin therapy experienced a significantly lower reduction in renal function at discharge compared to those receiving the full course, as measured by estimated glomerular filtration rate (median -11 versus -1mL/min, p =0.009).[13] They concluded that patients may be adequately treated with two weeks of gentamicin, thereby avoiding renal impairment that is associated with long duration of aminoglycoside therapy.[13] However, this study was limited by a small sample size, and insufficient power, thereby leaving the optimal duration of therapy unclear.

Interestingly, other studies demonstrate that toxicity resulting in gentamicin discontinuation occurred after approximately two weeks of treatment.[11, 12] Although Fernández-Hidalgo did not directly evaluate a shorter gentamicin treatment duration, the authors describe outcomes of gentamicin treatment failure due to adverse events, namely renal dysfunction. For the 25% of patients that failed AG therapy, the median duration of therapy with gentamicin was 14 days (IQR, 12–20 days).[11] Furthermore, ten patients did not receive combination therapy after stopping gentamicin and completed their treatment course with ampicillin monotherapy.[11] Pericas et al. reported that 43% of patients in the AG group had to discontinue treatment due to toxicity; thirteen

patients were switched to AC therapy after a median of 18 days (range, 5-30 days; IQR, 15-24.5 days).[12] Overall these data indicate that gentamicin toxicity is associated with longer treatment durations, and a two-week treatment course may be reasonable.

CONCERN FOR DEVELOPMENT OF RESISTANCE

Enterococcal resistance to beta-lactams is primarily acquired by overproduction of PBP5, and by amino acid substitutions that result in altered binding site and reduced beta-lactam interaction with PBP5.[14] Additionally, rare isolates of *E. faecalis* produce beta-lactamase enzymes, which in theory could compromise beta-lactam therapy against enterococcal endocarditis, and further limit the available treatment options.[3, 15] While the impact of enterococcal beta-lactamase in low-inoculum infections is difficult to detect, the impact in high-inoculum infections, such as endocarditis, has not been fully elucidated. Data suggest that although most beta-lactamase enzymes are inducible, enterococcal beta-lactamase is produced constitutively, and at substantially lower amounts.[3, 15] Furthermore, the enzyme remains membrane-bound, making detection of phenotypic resistance difficult unless high-inocula are used.[3, 15]

CEFTRIAXONE SAFETY AND ADVERSE EVENTS CONCERNS

Currently, AC combination therapy is the only tested option for the treatment of IE and bacteremia due to HLAR *E. faecalis* with supportive clinical data. While seemingly safe as compared to AG, safety risk associated with ceftriaxone use should not be negated. In addition to being an independent risk-factor for *Clostridium difficile* infections[16] numerous clinical and observational studies implicate ceftriaxone as a major risk factor for occurrence of vancomycin resistant *E. faecium* (VRE) infection, including bacteremia.[17, 18] This is in addition to a wealth of animal studies that have linked ceftriaxone use to promotion of gastrointestinal (GI) colonization by VRE.[19, 20] It is suggested that the high biliary excretion of ceftriaxone, with levels that exceed GI concentrations of 5,000 µg/ml, promote overgrowth of ampicillin- and vancomycin-resistant *E.*

faecium, whose MIC for ceftriaxone typically exceeds 10,000 µg/ml.[20] This ability of ceftriaxone to "select" for drug-resistant enterococci poses not only a risk to individual patients, but also threatens public health by contributing to developing of resistance in multiple organisms in the hospital environment. Consequently, studies investigating alternative treatment options, particularly novel beta-lactam combinations, are crucial to expand the therapeutic armamentarium against these organisms.

OTHER COMBINATION THERAPIES AND FUTURE RESEARCH POTENTIAL

Novel Dual Beta-Lactam Combinations

Unlike ceftriaxone, other cephalosporin antibiotics, such as cefepime [19] and ceftaroline [21] do not appear to promote VRE colonization. When cefepime, cefotetan, ceftriaxone and ceftazidime were studied in the GI tract of mice, it was noted that cefepime was the least likely of the four to cause VRE colonization (no difference in colonization compared to 0.9% sodium chloride), while ceftriaxone and cefotetan reached the highest levels of colonization.[19] This is presumably a result of minimal biliary excretion of cefepime and ceftaroline, and lack of antianaerobic effect of cefepime. The combination of ampicillin plus ceftaroline demonstrated efficacy similar to AC in several in vitro pharmacodynamics studies.[22, 23] A recent in vitro study evaluated high-inoculum *E. faecalis* against ampicillin in combination with ceftaroline, cefepime, and ceftriaxone in an in vitro pharmacodynamic model simulating human concentration-time profiles.[22] The data indicated that AC activity was similar to ampicillin plus ceftaroline, and ampicillin plus cefepime. Although ceftaroline and cefepime are not associated with VRE colonization, their utilization necessitates careful evaluation for safety and development of resistance. Dual beta-lactam therapy warrants further investigation, not only for efficacy, but also for the development of resistance and optimal dosing.

Daptomycin plus Beta-Lactam Therapy

Daptomycin, a lipopeptide antibiotic with activity against Gram-positive bacteria, is of interest in treating enterococcal infections due to its activity against *E. faecalis* and *E. faecium,* including VRE. Recent data has indicated that the combination of daptomycin with beta-lactam antibiotics has synergistic effects.[24, 25] Daptomycin activity can be potentiated due to beta-lactam-mediated shifts in surface charge of enterococci, causing increased uptake of the drug. While daptomycin combination therapy is more often observed in patients with resistant strains of *E. faecium,* case reports of successful utilization of daptomycin combination therapy in patients with severe *E. faecalis* infections have been published.[24, 26]

Sierra-Hoffman et al*.* report using daptomycin (6mg/kg Q48h) in combination with ampicillin (1g Q6h) for the treatment of mitral valve IE in an 89-year-old female with stage 4 chronic kidney disease.[26] The patient was not a surgical candidate, and received 6 weeks of treatment. Subsequent surveillance blood cultures 2 weeks after cessation of therapy remained negative, and patient remained alive without signs or symptoms of IE at her 1-year follow up.[26] Although this case report used a 6mg/kg/day dose, several in vitro, in vivo and clinical outcome studies suggest higher doses (10-12mg/kg/day) are associated with better patient outcomes, particularly in severe infections [27-29]. This suggests that synergistic combinations may be daptomycin dose-sparing. Further studies exploring dosing for synergistic combinations of daptomycin and beta-lactams are warranted.

Daptomycin (8 mg/kg/day) plus ceftaroline was successfully used in a case report of a 63-year-old male with recurrent aortic valve endocarditis caused by HLAR *E. faecalis.*[24] Therapy was initiated after patient failed 6 weeks of AC therapy as evidenced by recurrent signs and symptoms of IE, and doubling in vegetation size from 5mm to 10mm. This combination was selected due to unpublished observations of synergy against several bacteremia-causing enterococci.[24] A fourfold reduction in daptomycin MIC, as well as increased daptomycin binding to the

enterococcal cell membrane in the presence of ceftaroline was observed.[24] Smith and colleagues evaluated several beta-lactams in combination with daptomycin.[25] Similar to Sakoulas et al*.,* the authors found that ceftaroline demonstrated the greatest daptomycin MIC reduction (average 19.1**+/-**17.6 –fold [baseline daptomycin MIC/ daptomycin combination MIC]), followed by (in decreasing order) cefepime, ceftriaxone, ampicillin, ertapenem, cefazolin and cefotaxime.[25] Time-kill studies demonstrated synergy with daptomycin in combination with ceftaroline, ampicillin, ertapenem, ceftriaxone, and cefepime. Inconsistent synergy was noted with daptomycin and cefotaxime. No synergy was observed with daptomycin in combination with cefazolin, possibly due to differences between PBP binding profiles of beta-lactam antibiotics.[25]

Fosfomycin Combinations

Fosfomycin demonstrated synergy in combination with daptomycin in in vitro studies.[30] However, a follow-up in vivo aortic valve endocarditis study in rats infected with HLAR, beta-lactamase producing strain of *E. faecalis* demonstrated no difference between the number of valves sterilized by daptomycin-alone versus daptomycin plus fosfomycin when administered as a continuous infusion through the left internal jugular vein.[31] More recent in vitro data demonstrated synergy with fosfomycin in combination with ceftriaxone[32], rifampin, tigecycline, and teicoplanin (unavailable in the US), and antagonism with ampicillin.[33] Teicoplanin is particularly interesting for further investigation as previous in vitro data demonstrate advantage over vancomycin against *E. faecalis*.[34] Despite in vitro synergy, current fosfomycin use is limited to uncomplicated UTIs and should not be used to treat severe infections due to limited systemic absorption when administered orally.[35] Intravenous formulations of fosfomycin are currently unavailable in the US, but may have future utility. A recent study of in vitro and in vivo (guinea pig model) use of intraperitoneal fosfomycin demonstrated promising activity against both planktonic and biofilm-forming *E. faecalis* when fosfomycin was used in combination with gentamicin, and daptomycin [36] demonstrating a need for further investigation.

Miscellaneous Combinations

Several other in vitro and in vivo studies have been conducted evaluating combination therapy.[37-40] Synergistic combinations and their respective study designs are summarized in Table 2. Of particular interest, Arias et al. evaluated a beta-lactamase stable cephalosporin, ceftobiprole (currently unavailable in the US), and observed efficacy against bla+ and VanB-resistant strains of *E. faecalis* in addition to synergy when used in combination with aminoglycosides.[37] Overall, ceftobiprole demonstrates high affinity for enterococcal PBPs, and requires further exploration in human subjects.

CONCLUSION

Although aminoglycoside-containing regimens have been the standard of enterococcal IE treatment, the rise in resistance and availability of less nephrotoxic agents have led to novel treatment options.[2] Double beta-lactam therapies have emerged as a novel strategy in the treatment of serious high-inoculum enterococcal infections due of their favorable side effect profiles, and tolerability during long-term use. Currently, AC is the only combination beta-lactam therapy supported by clinical data for the treatment of IE and bacteremia due to HLAR enterococci. However, AC combination is not without risk (i.e. resistance, VRE colonization). Therefore, there is a critical need to investigate novel drug combinations, and explore dosing strategies that optimize dose and overall exposure needed to improve efficacy and suppress the emergence of resistance.

Disclaimer

The views expressed are those of the authors and do not necessarily reflect the position or policy of the United States Department of Veterans Affairs.

Potential Conflict of Interest

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