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Listeria monocytogenes endocarditis: case report, review of the literature, and laboratory evaluation of potential novel antibiotic synergies

Monika Kumaraswamy^{a,b,*}, Carter Do^c, George Sakoulas^c, Poochit Nonejuie^d, Guan Woei Tseng^e, Helen King^a, Joshua Fierer^{a,b}, Joe Pogliano^d, Victor Nizet^{c,f}

^a Division of Infectious Diseases, University of California San Diego, La Jolla, CA 92093, USA

^b Infectious Diseases Section, VA San Diego Healthcare System, San Diego, CA 92161, USA

^c Department of Pediatrics, University of California San Diego, La Jolla, CA 92093, USA

^d Division of Biological Sciences, University of California San Diego, La Jolla, CA 92093, USA

^e School of Medicine, China Medical University, Taichung 40402, Taiwan

^f Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, La Jolla, CA 92093, USA

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ABSTRACT

Endocarditis is a rare but serious manifestation of *Listeria monocytogenes* (LM). However, the optimal treatment strategy for LM endocarditis has yet to be established. Current antibiotic strategies for listeriosis include penicillin G or ampicillin (AMP) monotherapy, or AMP + gentamicin combination therapy which is often favored for endocarditis. The primary objective of our investigation was to assess the utility of AMP + ceftriaxone (CRO) and AMP + daptomycin (DAP) against LM, modeling less nephrotoxic antibiotic combinations traditionally used to manage resistant enterococcal endocarditis. Here we report a case of LM endocarditis, review the world literature, and evaluate alternative treatment strategies for listeriosis utilizing *in vitro* and *ex vivo* studies. The combination of AMP + CRO and AMP + DAP were each noted to have synergistic activity against a LM endocarditis isolate. Additionally, co-incubation of the isolate with sub-lethal concentrations of antibiotics (AMP, CRO, DAP, AMP + CRO or AMP + DAP) sensitized the bacterium to whole blood killing while pretreatment with CRO and DAP (at 1/4 MIC) sensitized the bacterium to neutrophil killing. However, these effects did not reflect potentiation of antibiotic activity to human cathelicidin peptide LL-37, which is abundant in neutrophils and highly active against LM. Interestingly, AMP pretreatment of the LM endocarditis isolate resulted in increased DAP binding to the bacterium when assessed by fluorescence microscopy. These *in vitro* and *ex vivo* studies suggest further investigation of combination therapy using AMP + CRO or AMP + DAP as an alternative treatment for LM infection is warranted.

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1. Introduction

1.1. Background

The Gram-positive facultative intracellular coccobacillus *Listeria monocytogenes* (LM) is an important foodborne and opportunistic pathogen. Widely distributed in nature, LM is found in soil, vegetation, and stool of healthy mammals, and has been isolated from many foods including processed/delicatessen meats, soft cheeses, pâtés and cantaloupe. LM can survive many modern food processing technologies by tolerating high salt and low pH conditions, and can multiply even at refrigeration temperatures.

Human LM infection involves ingestion of contaminated foods resulting in an infection spectrum ranging from self-limiting febrile gastroenteritis in healthy individuals to life threatening bacteremia, meningitis, cerebritis, rhombencephalitis, and focal disease in high risk groups including neonates, the elderly, pregnant women, and immunosuppressed patients. LM has the third highest mortality rate (16%) among foodborne infections in the United States, after *Vibrio vulnificus* (35%) and *Clostridium botulinum* (17%), and accounts for 19% of foodborne related deaths overall [1].

Endocarditis, a rare but serious complication afflicting ~8% of listeriosis patients occurs on native or prosthetic valves, with frequent embolic complications and an associated mortality of 37–50% [2,3]. First reported by Hoepflich and Chernoff in 1955, only 68 cases of LM endocarditis have since been described in the medical literature [4,5]. The scarcity of patient cases and absence of randomized controlled treatment studies means the optimal antibiotic strategy for LM endocarditis has yet to be established. Commonly utilized treatment

* Corresponding author. Division of Infectious Diseases, University of California San Diego, 9500 Gilman Drive, Mail Code 0711, La Jolla, CA 92093-0711, USA.

E-mail address: mkumaraswamy@ucsd.edu (M. Kumaraswamy).

strategies include penicillin G (PCN) or ampicillin (AMP) monotherapy, or AMP + gentamicin (GENT) combination therapy. *In vitro*, PCN or AMP cause a delayed bactericidal effect on LM following prolonged exposure. In contrast, AMP + GENT show rapid and potent synergistic bactericidal activity *in vitro* and *in vivo*, making this combination first line therapy for LM in humans [6–8]. Of note, retrospective clinical studies have failed to reveal consistent *in vivo* AMP + GENT synergy nor superior outcomes when compared to simple AMP monotherapy [9].

Like LM, traditional standard of care for *Enterococcus faecalis* endocarditis has been a cell wall-active agent (e.g. AMP) + aminoglycoside (e.g. GENT). However, over time, alternative enterococcal therapeutic strategies have emerged. Dual β -lactam therapy with AMP + ceftriaxone (CRO) for enterococcal endocarditis (with or without high level aminoglycoside resistance) yields clinical cure rates equivalent to AMP + GENT while avoiding renal and oto-toxicity potentially associated with aminoglycosides [10]. AMP + the cyclic lipopeptide antibiotic daptomycin (DAP) may also show benefit in antibiotic-resistant or recalcitrant *Enterococcus* infections based on *in vitro* studies and limited clinical data [11].

Here we review 100 cases of native and prosthetic valve LM endocarditis identified in the literature with an emphasis on therapeutic outcomes, and evaluate alternative antimicrobial strategies against LM. We assessed the efficacy of AMP + CRO and AMP + DAP, antibiotic combinations traditionally associated with the treatment of resistant enterococcal infections, against a cadre of LM strains from various food and clinical sources including a contemporaneous clinical isolate from a patient with LM endocarditis using minimum inhibitory concentration (MIC) testing, checkerboard synergy assays, kinetic killing curves, and bacterial cytological profiling. In addition, *ex vivo* studies evaluating human whole blood and neutrophil killing were performed on LM exposed to different antibiotic concentrations.

1.2. Brief history of source case

A 79-year-old man with end stage renal disease on hemodialysis via a tunneled left internal jugular catheter presented with fever, chills, generalized weakness, poor appetite and malaise. His medical history included hypertension, dyslipidemia, diabetes mellitus, systolic heart failure, aortic valve stenosis, regurgitation of all four major heart valves, pulmonary hypertension, atrial fibrillation, alcohol and tobacco dependence, anemia, gout and an episode of *E. faecalis* bacteremia one year earlier. Multiple peripheral and catheter-derived blood cultures and the removed catheter (tip) grew LM (hereafter isolate LM SDVA) (Supplementary Fig. S1). Trans-thoracic echocardiogram revealed a 1.25 cm x 1.0 cm aortic valve mass (Supplementary Fig. S2) which resolved following 6 weeks of renally dosed AMP 2g IV every 12 h. Unfortunately, the patient died 9 months later from complications of congestive heart failure.

1.3. Review of reported cases

A comprehensive search for all human cases of LM endocarditis was performed using the MEDLINE database (1946 to March 2017). The search terms used included “*Listeria monocytogenes*,” “*Listeria monocytogenes* endocarditis,” “*Listeria* endocarditis,” “endocarditis,” “*Listeria* prosthetic valve endocarditis,” “prosthetic valve endocarditis,” and “*Listeria* bacteremia.” Two criteria were required for inclusion: (1) LM isolation from blood or tissue, and (2) diagnosis of endocarditis. Cases published in English, Spanish, French, German, Danish, Swedish, Portuguese and Japanese were included. Ultimately, this search revealed 100 cases of endocarditis due to LM in addition to the case presently reported (Table 1). Patient age averaged 65 years, with a range from 5 to 87 years. LM endocarditis occurred in 62 men and 37 women with a male-to-

female ratio of 1.7 to 1. For the 98 cases with reported outcomes, overall mortality was 30% (29 deaths, 69 survivors) with mortality in cases reported since 2000 reduced to 16% (5 deaths, 26 survivors) (Table 1 and 2). Underlying cardiac and non-cardiac conditions were reported in 93 of 100 patients and are shown in Table 2. Overall, 71% of patients had an underlying cardiac condition, the most common being a prosthetic valve (43 patients) followed by underlying valvular disease—aortic, mitral and/or tricuspid (24 patients). The most common underlying non-cardiac conditions included diabetes mellitus (12 patients) and malignancy (11 patients).

A comparison of the age, male-to-female ratio, site of cardiac involvement and outcome (based on the antibiotic regimen and medical vs. medical + surgical treatment) in patients with identified native valve and prosthetic valve endocarditis is illustrated in Table 3. The mean age in years of patients with native valve LM endocarditis was 51 (range 10 to 83; M:F 1.4 to 1) and with prosthetic valve LM endocarditis was 68 (range 27 to 87; M:F 2.2 to 1). For cases with a reported outcome, overall mortality for native valve LM endocarditis was 40% (20 deaths, 30 survivors) and for prosthetic valve LM endocarditis was 22% (9 deaths, 32 survivors). The 3 most common sites of valvular involvement for native valve LM endocarditis were aortic (14 patients), aortic + mitral (14 patients) and mitral (12 patients) while the 3 most common sites of valvular involvement for prosthetic valve LM endocarditis were mitral (23 patients), aortic (14 patients) and aortic + mitral (2 patients).

Ultimately, 59 patients received solely medical (antibiotic) treatment and 30 patients received medical + surgical treatment. Outcomes were similar in both groups. Forty-two of 59 patients (71%) who received medical treatment alone survived while 23 of 30 patients (77%) who received medical + surgical treatment survived. The survival rate for those who underwent medical or medical + surgical treatment was higher among patients with prosthetic valve endocarditis in contrast to native valve endocarditis. Survival rates of 77% (5 deaths, 17 survivors) and 82% (3 deaths, 14 survivors) were noted for patients with prosthetic valve endocarditis who underwent medical and medical + surgical treatment, respectively while survival rates of 65% (13 deaths, 24 survivors) and 56% (4 death, 5 survivors) were noted for native valve endocarditis patients who underwent medical and medical + surgical treatment, respectively. Increased survival in patients with prosthetic valves may reflect greater clinical suspicion for underlying endocarditis, rapid initiation of antibiotic therapy and increased use of surgery.

A vast array of antibiotic regimens with varying outcomes has been employed in the management of LM endocarditis (Table 4). The two most commonly utilized antibiotic strategies included PCN, AMP or amoxicillin (AMX) only (14 patients), and one of these β -lactam agents + aminoglycoside (49 patients). Survival rates for those treated with β -lactam alone vs. β -lactam + aminoglycoside were comparable at 79% and 73%, respectively. However, the survival rate for those treated with β -lactam alone was 90% (1 death, 9 survivors) for native valve endocarditis and 50% (2 deaths, 2 survivors) for prosthetic valve endocarditis, while the survival rate for those treated with β -lactam + aminoglycoside was 59% (9 deaths, 13 survivors) for native valve endocarditis vs. 77% (5 deaths, 21 survivors) for prosthetic valve endocarditis (Table 3). Thus, improved outcomes using β -lactam alone were observed in patients with native valve endocarditis, while survival among patients with prosthetic valve endocarditis was better in those treated with the combination of a β -lactam and an aminoglycoside.

2. Material and methods

2.1. Bacterial strains, media & antibiotics

The clinical isolate LM SDVA was utilized in all experiments performed. Susceptibility and synergy testing was also performed on

Table 1
Reported cases of *Listeria monocytogenes* endocarditis.

Case	Age, Sex	Underlying Condition (Including Cardiac Pathology)	Antibiotic Regimen	Valve Type	Medical or Medical + Surgical Treatment	Outcome	Reference ^b
1	42, M	Rheumatic Heart Disease, Periodontoclasia	Penicillin + Dihydrostreptomycin + Erythromycin	Native	Medical	Survived	[1]
2	Unknown, F	Severe Anemia	Ciprofloxacin	Native	Medical	Survived	[2]
3	35, F	Chorea, Septic Abortion	Penicillin + Streptomycin + Erythromycin	Native	Medical	Died	[2]
4	28, M	Rheumatic Heart Disease	Penicillin + Streptomycin	Native	Medical	Survived	[3]
5	55, M	Alcoholism, Metastatic Pancreatic Carcinoma	None	Native	None	Died	[3]
6	40, F	Rheumatic Heart Disease	Oxytetracycline + Streptomycin	Native	Medical	Survived	[4]
7	22, F	Hodgkin's Lymphoma, Adrenal Insufficiency, Steroid Treatment, Zoster	None	Native	None	Died	[5]
8	49, M	Rheumatic Heart Disease, Diabetes Mellitus, Alcoholism	Penicillin + Streptomycin + Erythromycin (+Isoniazid)	Native	Medical	Died	[6]
9	56, M	Alcoholism, Facial Basal Cell Carcinoma	Penicillin + Erythromycin	Native	Medical	Survived	[7]
10	10, M	Kwashiorkor	Penicillin + Chloramphenicol	Native	Medical	Survived	[8]
11	23, M	Aortic Stenosis	Penicillin + Streptomycin	Native	Medical + Surgical	Died	[9]
12	31, M	Aortic Stenosis	Ampicillin + Streptomycin (Followed By Amphotericin B For Fungal Endocarditis)	Native	Medical + Surgical	Died	[10]
13	64, M	Angina Pectoris	Penicillin + Gentamicin	Native	Medical	Died	[11]
14	32, M	Heart Failure	Chloramphenicol + Oleandomycin + Erythromycin	Native	Medical	Survived	[12]
15	54, M	Unknown	Penicillin + Streptomycin	Native	Unknown	Survived	[13]
16	76, M	Hemolytic Anemia, Autoimmune Radiculitis, Steroid Therapy	None	Native	None	Died	[13]
17	27, F	End Stage Renal Disease, Hemodialysis, Renal Transplant	Ampicillin + Gentamicin	Native	Medical	Survived	[14]
18	69, M	End Stage Renal Disease, Hemodialysis, Renal Transplant, Heart Failure, Myocardial Infarction, Bicuspid Aortic Valve, Atherosclerosis, Hypertension, Angina Pectoris	Ampicillin + Oxacillin	Native	Medical	Died	[14]
19	75, M	Atrial Fibrillation, Heart Failure	Penicillin	Native	Medical	Survived	[15]
20	72, F	Aortic Stenosis, Valve Replacement, Heart Failure	Penicillin + Kanamycin	Prosthetic	Medical + Surgical	Died	[16]
21	64, M	Angina Pectoris	Unknown	Unknown	Unknown	Died	[17]
22	23, M	Aortic Stenosis	Unknown	Native	Unknown	Died	[17]
23	67, F	Rheumatic Heart Disease, Streptococcal Endocarditis, Milroy Disease (Hereditary Lymphedema), Heart Failure, Cellulitis	Cephalothin (First Generation Cephalosporin)	Native	Medical	Died	[18]
24	16, F	Rheumatic Heart Disease	Penicillin	Native	Medical	Survived	[19]
25	55, F	Rheumatic Heart Disease	Penicillin	Native	Medical	Survived	[20]
26	25, F	Pregnancy, Diabetes Mellitus	Ampicillin (For 4 Weeks Then Followed By Amoxicillin For 2 Weeks)	Native	Medical	Survived	[21]
27	64, M	Heart Failure, Valve Replacement	Penicillin	Prosthetic	Medical	Died	[22]
28	53, M	Hypertrophic Cardiomyopathy, Anemia	Penicillin	Native	Medical	Survived	[23]
29	58, F	Rheumatic Heart Disease, Valve Replacement	Penicillin + Tobramycin	Prosthetic	Medical	Survived	[24]
30	23, F	Septic Abortion	Penicillin + Cephalothin + Streptomycin	Native	Medical	Died	[25]
31	Unknown, M	Unknown	Unknown	Unknown	Medical + Surgical	Survived	[26]
32	66, M	Chronic Lymphocytic Leukemia	Penicillin + Streptomycin	Native	Medical	Survived	[27]
33	27, M	Marfan Syndrome, Valve Replacement, Started On Peritoneal Dialysis Over Hospital Course	Penicillin	Prosthetic	Medical	Died	[28]
34	56, F	Mitral Valve Prolapse	Penicillin	Native	Medical	Survived	[29]
35	63, M	Chronic Rectal Fistula, Mitral Stenosis, Valve Replacement	Penicillin + Streptomycin	Prosthetic	Medical	Survived	[30]
36	69, F	Mitral Regurgitation, Valve Replacement	Ampicillin + Gentamicin	Prosthetic	Medical	Survived	[31]
37	34, F	None	Penicillin	Native	Medical	Survived	[32]
38	43, M	Redo Valve Replacement	Ampicillin + Amikacin	Prosthetic	Medical	Died	[33]
39	74, M	Atrial Fibrillation	Ampicillin + Netilmicin (For 3–4 Weeks Followed by Amoxicillin For 4 Weeks)	Native	Medical	Died	[34]
40	68, M	Diabetes Mellitus	Unknown	Unknown	Unknown	Survived	[35]
41	72, F	Unknown	Unknown	Prosthetic	Unknown	Survived	[36]

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Table 1 (continued)

Case	Age, Sex	Underlying Condition (Including Cardiac Pathology)	Antibiotic Regimen	Valve Type	Medical or Medical + Surgical Treatment	Outcome	Reference ^b
42	69, F	Myocardial Infarction, Diabetes Mellitus, Hypertension, Heart Failure, Right Iliofemoral Endarterectomy, Arterial Venous Graft, Bilateral Carotid Endarterectomies, Aortic Aneurysm Resection, Aortic Iliac Graft Placement, End Stage Renal Disease, Hemodialysis, Vascular Infection, Vineberg Procedure (Forerunner To CABG)	Vancomycin + Gentamicin	Native	Medical	Survived	[37]
43	77, F	Unknown	Unknown	Unknown	Unknown	Survived	[38]
44	55, M	Myocardial Infarction, Seizure Episode	Penicillin + Netilmicin	Native	Medical + Surgical	Survived	[39]
45	58, M	Colon Adenocarcinoma	Ampicillin + Netilmicin	Native	Medical + Surgical	Died	[40]
46	75, F	Aortic Stenosis, Streptococcal Endocarditis, Lung Adenocarcinoma (Diagnosed During Admission), Colon Adenocarcinoma, Chronic Anemia	Ampicillin + Gentamicin	Native	Medical	Died	[40]
47	83, F	Colon Adenocarcinoma, Atherosclerosis	Ampicillin (Initially Then Pivampicillin)	Native	Medical	Died	[40]
48	52, F	Cirrhosis	Ampicillin + Gentamicin	Native	Medical	Survived	[41]
49	55, M	HIV, Ischemic Heart Disease, Seizures?	Penicillin + Netilmicin	Native	Medical + Surgical	Survived	[42]
50	69, M	Valve Replacement, Rheumatoid Arthritis, Alcoholism	Ampicillin + Streptomycin	Prosthetic	Medical + Surgical	Survived	[43]
51	70, F	Coronary Artery Bypass Graft, Atherosclerosis	Ampicillin + Gentamicin	Native	Medical	Survived	[44]
52	73, F	Valve Replacement, Aortic Insufficiency	Ampicillin + Gentamicin	Prosthetic	Medical + Surgical	Survived	[45]
53	46, M	Valve Replacement	Amoxicillin + Netilmicin	Prosthetic	Medical	Survived	[46]
54	55, Unknown	HIV, Myocardial Infarction	Penicillin + Netilmicin	Native	Medical + Surgical	Survived	[47]
55	35, M	HIV, Intravenous Drug Use, Candida endophthalmitis, Cirrhosis	Co-trimoxazole	Native	Medical	Died	[48]
56	67, M	Valve Replacement, Left Atrial Thrombus, Colon Adenocarcinoma, Mitral Insufficiency	Vancomycin + Gentamicin	Prosthetic	Medical	Survived	[49]
57	78, M	Valve Replacement, Pacemaker, Mitral Regurgitation, Aortic Regurgitation	Ampicillin + Tobramycin	Prosthetic	Medical + Surgical	Survived	[50]
58	56, F	Valve Replacement	Co-trimoxazole	Prosthetic	Medical + Surgical	Survived	[51]
59	71, M	Valve Replacement, Vertebral Fracture, Spondylodiscitis	Ampicillin + Amikacin	Prosthetic	Medical + Surgical	Survived	[52]
60	61, M	Rheumatic Heart Disease, Valve Replacement	Ampicillin + Gentamicin	Prosthetic	Medical	Survived	[53]
61	69, M	Valve Replacement, Colon Adenocarcinoma, Multiple Bouts of Endocarditis (E. faecalis, S. sanguis, K. pneumoniae), Mitral Valvuloplasty	Vancomycin + Gentamicin	Prosthetic	Medical	Survived	[54]
62	78, F	Valve Replacement	Ampicillin	Prosthetic	Medical	Survived	[55]
63	65, M	Hemochromatosis, Duodenal Ulcer, Laparotomy	Ampicillin + Tobramycin	Native	Medical	Died	[56]
64	67, M	Alcoholism, Valve Replacement	Ampicillin + Gentamicin	Prosthetic	Medical + Surgical	Survived	[57]
65	76, F	Urinary Tract Infection, Valve Replacement	Ampicillin + Gentamicin	Prosthetic	Medical	Died	[58]
66	41, F	Liver Transplant, Immunosuppressive Therapy, CMV Hepatitis, Hickman's Catheter	Ampicillin (Followed By Penicillin) + Gentamicin	Native	Medical	Survived	[59]
67	74, M	Varicose Vein Stripping, Valve Replacement	Amoxicillin + Gentamicin	Prosthetic	Medical + Surgical	Survived	[60]
68	79, M	Hepatitis B Virus, Valve Replacement	Ampicillin + Gentamicin	Prosthetic	Medical + Surgical	Survived	[61]

(continued on next page)

Table 1 (continued)

Case	Age, Sex	Underlying Condition (Including Cardiac Pathology)	Antibiotic Regimen	Valve Type	Medical or Medical + Surgical Treatment	Outcome	Reference ^b
69	69, F	Valvuloplasty, Valve Replacement, Pacemaker	Ampicillin (For 3 Weeks Then Co-trimoxazole + Rifampicin + Teicoplanin For 4–5 Weeks)	Prosthetic	Medical	Survived	[62]
70	42, M	HIV, Diabetes Mellitus, Chronic Renal Failure, Heart Failure, Redo Valve Replacement	Ampicillin + Gentamicin	Prosthetic	Medical	Survived	[63]
71	81, M	Valve Replacement	Ampicillin + Gentamicin (Then Lifelong Suppressive Therapy With Amoxicillin)	Prosthetic	Medical + Surgical	Survived	[64]
72	77, M	Rheumatic Heart Disease, Valve Replacement	Vancomycin + Gentamicin	Prosthetic	Medical + Surgical	Survived	[65]
73	68, M	Rheumatic Heart Disease, Valve Replacement	Penicillin + Gentamicin	Prosthetic	Medical + Surgical	Survived	[65]
74	70, M	Aortic Stenosis, Colon Polyp	Ampicillin + Gentamicin (Stopped After 7 Days Due To Renal Insufficiency)	Native	Medical + Surgical	Survived	[66]
75	76, M	Valve Replacement, Defibrillator, Chronic Renal Insufficiency, Polymyalgia Rheumatica, Barrett's Esophagus, GERD, Chronic Gastritis, Diverticulosis, Colon Polyp, Duodenal Ulcer, Iron Deficiency Anemia, Transient Ischemic Attack	Vancomycin (For 2 Weeks Then Linezolid For 4 Weeks)	Prosthetic	Medical + Surgical	Survived	[67]
76	76, M	Hypertension, Hyperlipidemia, Coronary Artery Bypass Graft, Valve Replacement, Tobacco Use, Invasive Rectal Carcinoma	Unknown	Prosthetic	Medical + Surgical	Died	[68]
77	74, M	Valve Replacement, Aortic Stenosis	Meropenem + Gentamicin	Prosthetic	Medical	Survived	[69]
78	71, M	Hypertension, Atherosclerosis, Alcoholism, Alcoholic Hepatitis, Chronic Renal Insufficiency, Tobacco Use, Dental Procedure	Penicillin	Native	Medical	Survived	[70]
79	80, F	Listeria Meningitis (1 Year Prior), Valve Replacements (Mitral + Aortic)	Ampicillin + Gentamicin	Prosthetic	Unknown	Unknown	[71]
80	73, F	Diabetes Mellitus, Valve Replacement	Co-trimoxazole + Aminoglycoside (Type Unknown)	Prosthetic	Unknown	Unknown	[71]
81	87, M	Valve Replacement, Atrial Fibrillation, Heart Failure, Prostate Cancer, Hemorrhoids	Ampicillin (6 Weeks) + Tobramycin (3 Weeks) (Followed By Amoxicillin For 9 Weeks)	Prosthetic	Medical	Survived	[72]
82	67, M	Valve Replacement	Linezolid + Co-trimoxazole	Prosthetic	Medical + Surgical	Survived	[73]
83	5, M	None	Levofloxacin + Rifampin	N/A ^a	Medical + Surgical	Survived	[74]
84	78, F	3rd Degree Heart Block, Pacemaker, Heart Failure, Tricuspid Regurgitation, Coronary Artery Bypass Graft, Hypoglycemia	Ampicillin + Gentamicin	Native	Medical	Died	[75]
85	42, F	Psoriatic Arthritis On Infliximab	Ampicillin	Native	Medical	Survived	[76]
86	74, M	HIV	Unknown	N/A ^a	Medical + Surgical	Survived	[77]
87	74, M	Rheumatic Heart Disease, Atrial Fibrillation, Atrophic Gastritis	Ampicillin + Gentamicin	Native	Medical + Surgical	Died	[78]
88	58, F	Rheumatic Heart Disease, Valvuloplasties, Valve Replacement, Atrial Fibrillation, Chronic Renal Insufficiency, Right Nephrectomy	Ampicillin + Gentamicin	Prosthetic	Medical + Surgical	Died	[78]
89	44, F	Multiple Sclerosis	Amoxicillin + Gentamicin	N/A ^a	Medical + Surgical	Survived	[79]
90	77, M	Coarctation of the Aorta, Valve Replacements (Mitral + Aortic), Hypertension, Heart Failure, Hypothyroidism	Ampicillin + Gentamicin	Prosthetic	Medical	Survived	[80]
91	73, F	Breast Cancer With Metastases, Diabetes Mellitus	Ampicillin + Gentamicin	N/A ^a	Medical	Survived	[81]

(continued on next page)

Table 1 (continued)

Case	Age, Sex	Underlying Condition (Including Cardiac Pathology)	Antibiotic Regimen	Valve Type	Medical or Medical + Surgical Treatment	Outcome	Reference ^b
92	70, M	Valve Replacement, Aortic Stenosis, Atrial Fibrillation, Pacemaker, Diabetes Mellitus, Cerebrovascular Accident, Rheumatoid Arthritis on Methotrexate, Chronic Obstructive Pulmonary Disease	Meropenem + Ciprofloxacin	Native	Medical	Died	[82]
93	54, F	None	Amoxicillin + Gentamicin + Co-trimoxazole	Native	Medical	Survived	[83]
94	54, M	Congenitally Corrected Transposition of the Great Vessels, Valve Replacements (Mitral + Aortic)	Ampicillin + Gentamicin	Prosthetic	Medical	Survived	[84]
95	36, F	Pregnancy, Bicuspid Aortic Valve	Ampicillin + Gentamicin	Native	Medical + Surgical	Survived	[85]
96	84, M	Chronic Renal Failure, Diabetes Mellitus, Collagenopathy, Chronic Corticosteroid Use, Valve Replacement	Meropenem + Daptomycin	Prosthetic	Medical	Survived	[86]
97	84, M	Valve Replacement	Ampicillin + Gentamicin	Prosthetic	Medical	Survived	[86]
98	80, F	Diabetes Mellitus, Valve Replacement	Ampicillin + Gentamicin	Prosthetic	Medical	Survived	[86]
99	79, M	Diabetes Mellitus, Valve Replacement	Ampicillin + Gentamicin	Native	Medical	Survived	[86]
100	63, M	Hypertension, Diabetes Mellitus, Atrial Flutter, Valve Replacement	Penicillin + Rifampicin (For 4–6 Weeks Then Amoxicillin For 18 Weeks)	Prosthetic	Medical + Surgical	Survived	[87]

^a Mass/Vegetation Identified Along Right Atrium, Left Atrium And/Or Left Ventricular Outflow Tract.

^b Please see supplementary data section for table references.

4 additional strains: LM L028 (a virulent hemolytic carriage strain isolated from the feces of a healthy pregnant woman), LM 2203 (isolated during an outbreak affecting pregnant women and associated with intrauterine/cervical infections), LM 10403 (streptomycin-

resistant strain isolated from a human skin lesion), and LM EGD-e (isolated from an epidemic involving laboratory animals and associated with foodborne illness). All isolates were stored in tryptic soy broth (TSB) containing 50% glycerol at -80°C until use. AMP, CRO and DAP were purchased from Sigma Aldrich and cathelicidin LL-37 from the American Peptide Company. The media, Mueller Hinton Broth (Spectrum Chemicals), was supplemented with cations (Ca^{2+} 20–25 mg/L and Mg^{2+} 10–12.5 mg/L [CA-MHB]) and 5% lysed horse blood (LHB).

Table 2

Underlying cardiac and non-cardiac conditions reported in 100 patients diagnosed with *Listeria monocytogenes* endocarditis.

Variable	<i>Listeria monocytogenes</i> Endocarditis (n = 100)
Age, Mean Years (Range)	65 (5 to 87)
Ratio of Male-to-Female Patients	1.7
Underlying Cardiac Conditions (Top 10):	(No. of Patients)
Prosthetic Valve	43
Valvular Disease	24
Rheumatic Fever or Heart Disease	13
Heart Failure	10
Atrial Fibrillation/Flutter	7
Angina Pectoralis or Myocardial Infarction	7
Hypertension	6
Pacemaker/Defibrillator	4
Coronary Artery Bypass Graft or Vinberg	4
Atherosclerosis	4
Underlying Non-cardiac Conditions (Top 10):	(No. of Patients)
Diabetes Mellitus	12
Malignancy	11
Chronic Kidney Disease or End Stage Renal Disease	7
Alcoholism	6
Immunosuppressive Therapy	5
Anemia	5
Human Immunodeficiency Virus	5
Hemodialysis or Peritoneal Dialysis	4
Renal or Liver Transplant	3
Pregnancy or Septic Abortion	3
Medical or Medical + Surgical Therapy:	Proportion of Patients With Cure (% Survival)
Medical	42/59 (71%)
Medical + Surgical	23/30 (77%)
Overall Survival	69/98 (70%)

2.2. Minimum inhibitory concentration, checkerboard and time kill assays

Broth microdilution antimicrobial susceptibility testing, checkerboard and time kill assays were performed in CA-MHB + 5% LHB and in accordance with CLSI guidelines [12,13]. Checkerboard synergy, additivity and antagonism were defined by the fractional inhibitory concentration index (FICI): $\text{FICI} \leq 0.5$ defined synergy, >0.5 to ≤ 1 additivity, >1 to <4 indifference, and ≥ 4 antagonism. Bactericidal vs. bacteriostatic activity was determined by time kill assays using antibiotic combinations and concentrations identified to be synergistic by checkerboard assay. Time kill assay bactericidal activity was defined as a reduction in viable bacteria by $\geq 3 \log_{10}$ colony forming units (cfu)/mL, and bacteriostatic activity was defined as a reduction in viable bacteria by $<3 \log_{10}$ cfu/mL at 24 h compared with the starting inoculum.

2.3. Fluorescence microscopy

Fluorescence microscopy was performed as previously described with the following modifications [11]. LM VASD was grown overnight (14–16 h) in Luria broth (LB) at 37°C in the presence or absence of $\frac{1}{4}$ MIC AMP (0.25 $\mu\text{g}/\text{mL}$). Overnight cultures were then diluted 1:100 into fresh LB in the presence or absence of $\frac{1}{4}$ MIC AMP

Table 3Reported clinical characteristics and outcomes in patients with native and prosthetic valve endocarditis due to *Listeria monocytogenes*.

Variable	Native Valve Endocarditis (n = 51)	Prosthetic Valve Endocarditis (n = 41)
Age, Mean Years (Range)	51 (10 to 83)	68 (27 to 87)
Ratio of Male to Female Patients	1.4	2.2
Site of Cardiac Involvement:	(No. of Patients)	(No. of Patients)
Aortic	14	14
Mitral	12	23
Tricuspid	3	1
Aortic + Mitral	14	2
Aortic + Tricuspid	1	0
Mitral + Tricuspid	0	1
Unknown	7	0
Antibiotic Therapy:	Proportion of Patients With Cure (% Survival)	Proportion of Patients With Cure (% Survival)
Penicillin or Ampicillin or Amoxicillin Only	9/10 (90%)	2/4 (50%)
Penicillin or Ampicillin or Amoxicillin + Aminoglycoside	13/22 (59%)	21/26 (77%)
Penicillin or Ampicillin or Amoxicillin Only and Penicillin or Ampicillin or Amoxicillin + Aminoglycoside	22/32 (69%)	23/30 (77%)
Medical or Medical + Surgical Therapy:		
Medical	24/37 (65%)	17/22 (77%)
Medical + Surgical	5/9 (56%)	14/17 (82%)
Overall Survival	30/50 (60%)	32/41 (78%)

(0.25 µg/mL). When an OD₆₀₀ = 0.5 was reached, 4 µg/mL of Bodipy-fluorescein-labeled DAP (4X MIC but a pharmacologically achievable concentration) was added to exponentially growing bacteria. Bacteria were then incubated with Bodipy-fluorescein-labeled DAP (Cubist Pharmaceuticals) for 40 min at room temperature before being washed with PBS x 3, counterstained with 2 µg/mL DAPI (nucleic acid) (Molecular Probes) and then transferred onto a 1.2% agarose pad containing 20% LB for microscopy and image analysis using ImageJ software v1.48f and CellProfiler 2.0.

2.4. Neutrophil isolation

Human neutrophils were isolated from healthy donors using the PolymorphPrep system (Axis-Shield) as per manufacturer's instructions and under protocols approved by the UCSD Human Subjects Institutional Review Board.

2.5. Neutrophil killing assay

Neutrophil assays were performed as previously described with the following modifications [14]. Human neutrophils were resuspended in RPMI 1640 to 2 x 10⁶ cells/mL and then used to seed the wells of a 96-well plate at 2 x 10⁵ cells/well. Neutrophils were then infected at a multiplicity of infection (MOI) = 10 bacteria/neutrophil with untreated LM (control) and LM pretreated overnight with ¼ MIC antibiotic (AMP 0.25 µg/mL, CRO 4 µg/mL or DAP 1 µg/mL). Plates

were centrifuged at 500 x g for 10 min then incubated for 45 min at 37 °C in 5% CO₂. Serial dilutions in sterile PBS and Triton-X 100 (0.02%) were plated on trypticase soy agar (TSA) plates for bacterial enumeration. The percentage of surviving bacteria was calculated for comparison to the initial inoculum.

2.6. Whole blood assay

Stationary phase bacteria were washed twice, diluted to an inoculum of 10⁴ cfu in 50 µL PBS and mixed with 300 µL heparinized human blood and 50 µL PBS with or without antibiotics in siliconized tubes. Final concentrations of tubes with ¼ MIC antibiotic concentrations were AMP 0.25 µg/mL, CRO 4 µg/mL, DAP 1 µg/mL, AMP/CRO 0.25/4 µg/mL and AMP/DAP 0.25/1 µg/mL. Tubes were incubated at 37 °C and rotated for 3 h. Serial dilutions performed using sterile PBS and Triton-X 100 (0.025%) were plated on TSA plates for bacterial enumeration. The survival index was defined as cfu enumerated at the end of the assay divided by cfu at time point 0 h.

2.7. Statistics

Statistical analyses were performed using GraphPad Prism 6.0f (GraphPad Software). One-way analysis of variance (ANOVA) or two-way ANOVA were utilized where appropriate. *P* values <0.05 were regarded to be statistically significant.

Table 4

Antibiotic treatment regimens and survival.

Antibiotic Regimen	Survived	Died	%Survival
Penicillin or Ampicillin or Amoxicillin Only	11	3	79
Penicillin or Ampicillin or Amoxicillin + Aminoglycoside	36	13	73
Penicillin or Ampicillin or Amoxicillin + Aminoglycoside + Other	2	3	40
Penicillin or Ampicillin or Amoxicillin + Non-aminoglycoside	3	1	75
Vancomycin + Aminoglycoside	4	0	100
Vancomycin or Linezolid Only	1	0	100
Co-trimoxazole ± Other	2	1	67
Meropenem + Other	2	1	67
Fluoroquinolone ± Other	2	0	100
Other Combination Regimens	2	1	67
Unknown Regimen	4	3	57
No Antibiotics	0	3	0

Table 5
Antimicrobial susceptibility of *Listeria monocytogenes* strains to AMP and corresponding combinational therapy with CRO and DAP assessed by MIC and checkerboard assays.^a

Strain	MIC ($\mu\text{g/mL}$)					Checkerboard FICI (Interpretation)	
	AMP	CRO	DAP	AMP/CRO	AMP/DAP	FICI _{AMP-CRO}	FICI _{AMP-DAP}
<i>L. monocytogenes</i>							
LM SDVA	1	16	32	0.25/4	0.25/1	0.5(S)	0.28125(S)
LM L028	1	8	8	0.5/2	0.5/2	0.75(A)	0.75(A)
LM 2203	1	8	16	0.25/4	0.25/8	0.75(A)	0.75(A)
LM 10403S	0.5	8	32	0.5/1	0.25/16	1.25(I)	1(A)
LM EDGE	1	8	16	0.25/2	0.5/4	0.5(S)	0.75(A)

^a AMP, Ampicillin; CRO, Ceftriaxone; DAP, Daptomycin; FICI, Fractional Inhibitory Concentration Index; MIC, minimum inhibitory concentration; S, Synergy; A, Additivity; I, Indifference.

3. Results

3.1. In vitro susceptibilities of LM to the antibiotics AMP, CRO, DAP, AMP/CRO, AMP/DAP and AMP/LL-37

MIC testing, checkerboard assays and time kill assays were performed for AMP, CRO, DAP and combinations of AMP with CRO, DAP or LL-37 to assess antimicrobial activity against the clinical endocarditis isolate, LM SDVA (Table 5, Table 6 and Fig. 1). In addition, MIC testing and checkerboard assays were performed using 4 other strains of LM (L028, 2203, 10403S, and EDG-e) (Table 5 and Table 6). All 5 LM

Table 6
Antimicrobial susceptibility of *Listeria monocytogenes* strains to AMP and corresponding combinational therapy with LL-37 assessed by MIC and checkerboard assays.^a

Strain	MIC ($\mu\text{g/mL}$)			Checkerboard FICI (Interpretation)
	AMP	LL-37	AMP/LL-37	FICI _{AMP-LL-37}
<i>L. monocytogenes</i>				
LM SDVA	1	1	0.03125/1	1.03125(I)
LM L028	1	2	0.03125/2	1.03125(I)
LM 2203	1	2	0.03125/2	1.03125(I)
LM 10403S	0.5	2	0.03125/2	1.0625(I)
LM EDGE	1	2	0.03125/2	1.03125(I)

^a AMP, Ampicillin; LL-37, Cathelicidin; FICI, Fractional Inhibitory Concentration Index; MIC, minimum inhibitory concentration; I, Indifference.

isolates exhibited susceptibility to AMP (MIC₉₀ 0.5–1 $\mu\text{g/mL}$), resistance to CRO (MIC₉₀ 8–16 $\mu\text{g/mL}$), resistance to DAP (MIC₉₀ 8–32 $\mu\text{g/mL}$) and susceptibility to LL-37 (MIC₉₀ 1–2 μM). Despite resistance to specific individual antibiotics, synergy defined as an FICI of ≤ 0.5 for the checkerboard assays was observed for LM SDVA treated with AMP + CRO and with AMP + DAP. However, bactericidal activity, defined as a reduction in viable bacteria by ≥ 3 log₁₀ cfu/mL via time kill assays was observed only for LM SDVA treated with AMP + DAP but not with AMP + CRO (Fig. 1). In sum, synergy (FICI ≤ 0.5) was observed for 2/5 and 1/5 of the LM strains treated with AMP + CRO and AMP + DAP respectively, while additivity (FICI > 0.5 to ≤ 1) was observed for all other strains with the exception of AMP + CRO for LM 10403S (FICI = 1.25; indifference). No synergy or additivity was appreciated for any LM strains using the combination AMP + LL-37.

3.2. LM pretreated with sub-lethal concentrations of AMP exhibits increased binding to Bodipy-labeled DAP

As a complement to the antimicrobial susceptibility assays illustrating synergy of AMP + DAP against LM SDVA, DAP membrane binding studies were performed using Bodipy-fluorescein-labeled DAP and LM SDVA grown in the presence vs. absence of $\frac{1}{4}$ MIC AMP (0.25 $\mu\text{g/mL}$). LM SDVA grown in AMP exhibited greater binding to labeled DAP compared to LM SDVA grown in antibiotic free bacteriological medium when visualized by fluorescence microscopy, and measured by spot and cell edge intensity (Fig. 2).

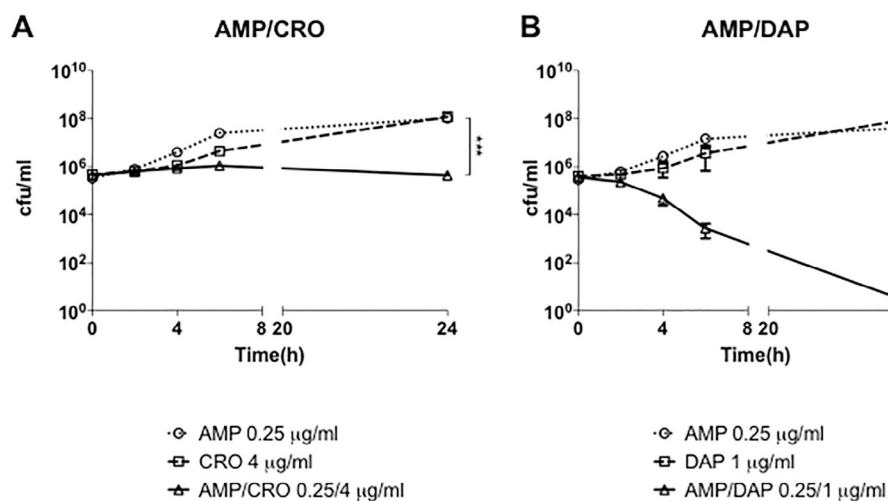


Fig. 1. Time kill curve demonstrating (A) ampicillin + ceftriaxone and (B) ampicillin + daptomycin activity against *Listeria monocytogenes* SDVA in cation-adjusted Mueller Hinton broth supplemented with 5% lysed horse blood. Bactericidal synergy, defined as a ≥ 3 log₁₀ decrease in cfu/mL for time kill assays, was observed only for ampicillin + daptomycin at $\frac{1}{4}$ MIC of each agent in combination. Data are plotted as mean \pm SEM and represent the combination of three experiments performed in triplicate. *** $P < 0.001$ by two-way ANOVA. AMP, ampicillin; CRO, ceftriaxone; DAP, daptomycin.

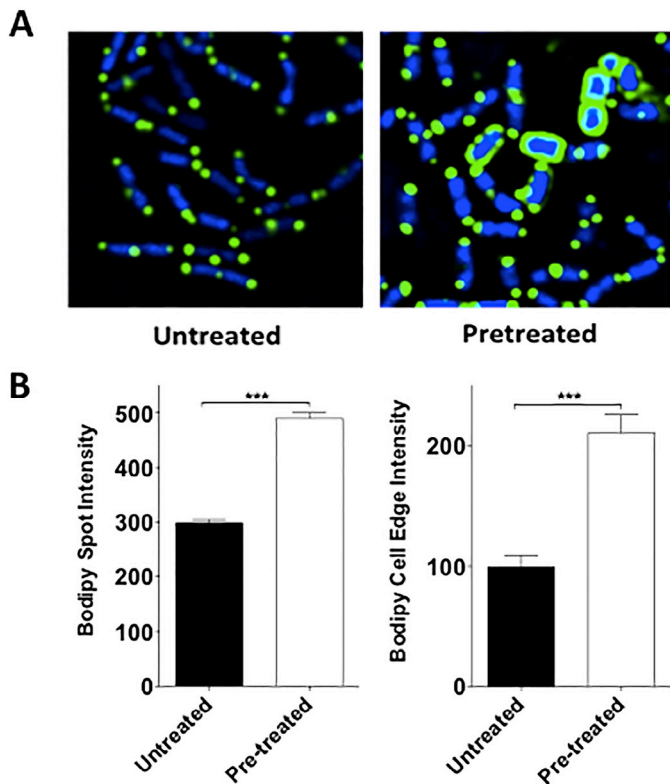


Fig. 2. Binding of fluorescently-labeled daptomycin to *Listeria monocytogenes* (LM) SDVA pretreated without or with ampicillin. (A) Fluorescence microscopy was performed using log-phase LM SDVA grown in the presence or absence of $\frac{1}{4}$ MIC (0.25 $\mu\text{g}/\text{mL}$) ampicillin, and following exposure to Bodipy-fluorescein-labeled daptomycin (green) and nucleic acid counterstaining with DAPI (blue). (B) Bar graphs generated from software analysis of multiple random fluorescent microscopy fields of cells exposed to Bodipy-fluorescein-labeled daptomycin. Bodipy spot and cell edge intensity were noted to be $>1.5\text{X}$ and $>2\text{X}$ higher for cells grown in the presence of antibiotic than cells grown in the absence of antibiotic. Data are plotted as mean \pm SEM and represent the combination of three experiments performed in triplicate. *** $P < 0.001$ by one-way ANOVA.

3.3. AMP, CRO & DAP enhance whole blood mediated killing of LM SDVA

Overnight pretreatment of LM SDVA with sub-bacteriostatic concentrations ($\frac{1}{4}$ MIC) of CRO or DAP sensitized the bacterium to human neutrophil killing, while pretreatment with $\frac{1}{4}$ MIC AMP had no effect (Fig. 3A). However, enhanced whole blood killing of LM SDVA was observed in the presence of all antibiotics at $\frac{1}{4}$ MIC (AMP 0.25 $\mu\text{g}/\text{mL}$, CRO 4 $\mu\text{g}/\text{mL}$, DAP 1 $\mu\text{g}/\text{mL}$, AMP/CRO 0.25/4 $\mu\text{g}/\text{mL}$ or AMP/DAP 0.25/1 $\mu\text{g}/\text{mL}$) when compared to LM SDVA in the absence of antibiotic (Fig. 3B).

4. Discussion

Prospective and randomized clinical studies of LM endocarditis identifying optimal antibiotic therapy are lacking due to the scarcity of cases. *In vitro*, LM appears susceptible to nearly all antibiotics. However, traditional cell free *in vitro* susceptibility testing does not always translate to clinical efficacy for intracellular pathogens and often fails to adequately account for interactions with the innate immune system. *In vivo*, intracellular LM concealed from the extracellular environment may be protected from potent antibiotics with poor intracellular penetration and activity. The concentration of antibiotic at remote sites of infection like endocarditis or intracellularly may remain consistently below the MIC over the course of human antimicrobial therapy. Greater knowledge and insight of sub-inhibitory antibiotic concentrations, simultaneous interactions of two groups of antibiotics (potential synergy) and interactions with components of the innate immune system such as endogenous antimicrobial peptides, serum and phagocytic cells may provide guidance toward identifying optimal therapeutic strategies against LM beyond AMP + GENT.

Potential of killing *E. faecalis*, a Gram-positive opportunistic pathogen like LM, by dual β -lactam or β -lactam + lipopeptide combinations for treatment of endocarditis has emerged as a successful alternative therapeutic strategy to AMP + GENT but without the associated adverse aminoglycoside toxicity [10]. However, a review of the world literature reveals its application has yet to be evaluated against *Listeria*. We hypothesized the alternative anti-enterococcal therapeutic strategies, AMP + CRO and AMP + DAP, may also be effective against LM.

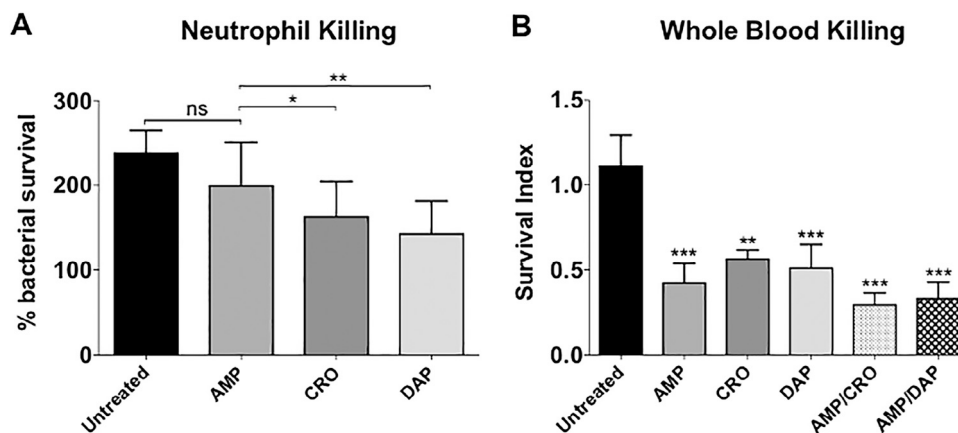


Fig. 3. Neutrophil and whole blood killing assays for *Listeria monocytogenes* SDVA. (A) Percentage survival of untreated bacteria vs. bacteria pretreated overnight with $\frac{1}{4}$ MIC ampicillin, ceftriaxone or daptomycin in a neutrophil killing assay. (B) Survival of bacteria following co-incubation with human whole blood and antibiotics ($\frac{1}{4}$ MIC ampicillin, ceftriaxone, daptomycin, ampicillin + ceftriaxone or ampicillin + daptomycin) or no antibiotics in a whole blood killing assay. Survival index = (cfu at end of assay)/(cfu at time 0). Data are plotted as mean \pm SEM and represent the combination of three experiments performed in triplicate. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ or n.s., no statistical significance by two-way ANOVA comparing pretreated or treated bacteria to untreated bacteria. AMP, ampicillin; CRO, ceftriaxone; DAP, daptomycin.

LM possess five penicillin binding proteins (PBP1–5), enzymes essential for bacterial cell wall synthesis. However, unlike other PBPs only PBP3 is identical in all *Listeria* species [7]. Inhibition of PBP3, an enzyme involved in the final stages of peptidoglycan synthesis, has lethal consequences for LM cells and is a major target of β -lactam antibiotics such as PCN and AMP [7]. PCN and AMP bind to all LM PBP to varying degrees but have the greatest affinity for PBP3 and PBP5. The third generation cephalosporin, CRO, exhibits weak activity against LM due to a lack of affinity for PBP3 and is clinically ineffective [7]. However, CRO is known to be a good inhibitor of PBP1, PBP2 and PBP4 [15]. Therefore we reasoned combination antimicrobial therapy with AMP + CRO targeting complementary PBPs with inhibition of 5 out of 5 key LM PBPs would enhance killing to a greater extent than each agent alone. In our current investigation utilizing 5 CRO-resistant LM isolates, we indeed observed AMP + CRO synergy or additivity against 80% (4/5) of LM isolates via checkerboard assays.

The lipopeptide antibiotic, DAP, alone exhibits potent activity against certain drug resistant Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus spp.* (VRE) but is further enhanced in combination with AMP [11,16]. DAP irreversibly binds to the cytoplasmic cell membrane and oligomerizes to form a pore or ion channel resulting in membrane depolarization and cell death. However, *in vitro* activity against LM using standard MIC testing is variable and clinical experience is lacking. We studied 5 DAP resistant LM isolates and confirmed *in vitro* AMP + DAP bactericidal synergy against one isolate of LM associated with endocarditis (LM SDVA) via checkerboard and time kill assays, and additivity against the other 4 LM isolates studied via checkerboard. Bactericidal killing of LM SDVA was observed at clinically relevant but sub-MIC levels of AMP + DAP even though $\frac{1}{4}$ MIC AMP and $\frac{1}{4}$ MIC DAP alone had no measurable effect on inhibiting bacterial growth. Additionally, LM SDVA grown in $\frac{1}{4}$ MIC AMP bound Bodipy-labeled DAP to a greater extent than LM SDVA grown in antibiotic free bacteriological medium when visualized by fluorescence microscopy. These findings suggest AMP enhances target membrane binding by DAP.

The present findings are consistent with prior studies demonstrating favorable interactions between β -lactam antibiotics (AMP, oxacillin, nafcillin, ceftaroline, etc.) and the lipopeptide antibiotic DAP against Gram-positive bacteria. Staphylococcal and enterococcal pre-exposure to AMP has been shown to reduce bacterial net positive surface charge and increase susceptibility to killing by cationic antimicrobials such as DAP [11,16]. We propose two possible mechanisms for AMP enhancement of DAP activity. First and as suggested previously, AMP may release lipoteichoic acid from the cell surface, which could then translate to either enhanced cell wall autolysin activity or reduced substrates for D-alanylation as a means of bacterial reduction in net negative surface charge [17]. Secondly, sub-lethal AMP concentrations may result in a reduction in cell wall cross linking allowing for greater DAP access to bacterial membrane targets.

Like DAP, the endogenous cationic host defense antimicrobial peptide cathelicidin LL-37 forms pores to disrupt bacterial membranes. LL-37 is abundantly produced by epithelial cells and neutrophils, is known to exert antimicrobial activity within the neutrophil phagolysosome, and likely plays a significant role in neutrophil intracellular bacterial killing [18]. All 5 LM isolates were noted to be highly sensitive to LL-37 (MIC 1–2 μ M). However, the presence of LL-37 did not potentiate the killing of any of the 5 LM isolates by AMP in combination (FICI >1; indifference) nor did pretreatment of LM SDVA with $\frac{1}{4}$ MIC AMP sensitize LM SDVA to neutrophil killing (Table 6 and Fig. 3A). In contrast, pretreatment of LM SDVA with sub-lethal CRO or DAP (at $\frac{1}{4}$ MIC) enhanced neutrophil killing of the bacterial strain (Fig. 3A). However, exposure to $\frac{1}{4}$ MIC of AMP, CRO, DAP, AMP/CRO or AMP/DAP sensitized LM SDVA to whole blood killing where neutrophils, serum and platelets

can all contribute to innate immune bacterial clearance (Fig. 3A) [19]. Additionally, a trend toward reduced bacterial survival with combinations of AMP + CRO or AMP + DAP compared to AMP, CRO or DAP alone were noted with whole blood killing but did not reach statistical significance.

In conclusion, these findings illustrate potentially beneficial interactions between LM, different antibiotic combinations, and components of host innate immunity. Certain antibiotics deemed ineffective by conventional antimicrobial susceptibility testing may nevertheless exhibit favorable properties in combination therapy or in conjunction with host defense. Limitations of our study include the modest number of LM isolates tested (including only one endocarditis isolate), a lack of extension to *in vivo* models (e.g. mice, rats, guinea pigs, rabbits, etc.) and clinical experience comparing or contrasting the efficacy of AMP + CRO or AMP + DAP therapy to AMP + GENT or AMP monotherapy in patients. Nevertheless, these results indicate alternative therapeutic strategies employed against sensitive and resistant *Enterococcus spp.* may also have clinical utility against LM infections. Additionally, AMP, CRO and DAP may be beneficial beyond their direct antimicrobial properties in combination and enhance bacterial clearance by components of the innate immune system. Future prospective clinical trials in humans evaluating and comparing the efficacy of antibiotic combinations such as AMP + CRO, AMP + DAP, AMP + GENT and AMP monotherapy would be required to definitively determine the optimal therapeutic strategy for LM endocarditis but will likely never be achieved given the paucity and sporadic nature of these serious infections.

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Competing interests: GS has received speaking honoraria from Allergan, Sunovion, and Theravance as well as honoraria and consulting fees from The Medicines Company.

Ethical approval: Not required.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.ijantimicag.2017.12.032](https://doi.org/10.1016/j.ijantimicag.2017.12.032).

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