Pathogens and Disease, 76, 2018, fty084

doi: 10.1093/femspd/fty084
Advance Access Publication Date: 16 November 2018
Research Article

RESEARCH ARTICLE

Anti-Ace monoclonal antibody reduces *Enterococcus faecalis* aortic valve infection in a rat infective endocarditis model

Kavindra V. Singh[1,2,*,†], Kenneth L. Pinkston[3,‡], Peng Gao[3,§], Barrett R. Harvey[3,4] and Barbara E. Murray[1,2,4]

[1]Division of Infectious Diseases, Department of Internal Medicine, The University of Texas Health Science Center at Houston, 6431 Fannin St. Houston, TX 77030, USA, [2]UTHealth's Center for Antimicrobial Resistance and Microbial Genomics (CARMiG), 6431 Fannin St., Houston, TX 77030, [3]Center for Molecular Imaging, Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases, 1825 Pressler St, The University of Texas Health Science Center at Houston, Houston, TX 77030, USA and [4]Department of Microbiology and Molecular Genetics, The University of Texas Health Science Center at Houston, 6431 Fannin St. Houston, TX 77030, USA

*Corresponding author: Division of Infectious Diseases, Department of Internal Medicine, University of Texas Health Science Center at Houston, 6431 Fannin Street, MSB 2.112, Houston, Texas 77030. Tel: +713-500-6758; Fax: +713-500-6745; E-mail: Kavindra.singh@uth.tmc.edu
†Kavindra V. Singh and Kenneth L. Pinkston contributed equally to this work.
‡Current affiliation: Chief Operations Officer, IDA Therapeutics, LLC Houston, TX 77021.
§Current affiliation: Chief Operations Officer, IDA Therapeutics, LLC Houston, TX 77021.
K.V.S. has received grants from Paratek Pharmaceuticals and Merck.
B.E.M. has received grants from Paratek Pharmaceuticals and Merck, and served on the advisory board for Cempra and Paratek.
One sentence summary: Anti-Ace mAb_{70} prevents *E. faecalis* endocarditis in rats.
Editor: Nicholas Carbonetti

ABSTRACT

Ace (Adhesin to collagen from *Enterococcus faecalis*) is a cell-wall anchored protein that is expressed conditionally and is important for virulence in a rat infective endocarditis (IE) model. Previously, we showed that rats immunized with the collagen binding domain of Ace (domain A), or administered anti-Ace domain A polyclonal antibody, were less susceptible to *E. faecalis* endocarditis than sham-immunized controls. In this work, we demonstrated that a sub nanomolar monoclonal antibody (mAb), anti-Ace mAb_{70}, significantly diminished *E. faecalis* binding to ECM collagen IV in *in vitro* adherence assays and that, in the endocarditis model, anti-Ace mAb_{70} pre-treatment significantly reduced *E. faecalis* infection of aortic valves. The effectiveness of anti-Ace mAb against IE in the rat model suggests it might serve as a beneficial agent for passive protection against *E. faecalis* infections.

Keywords: *Enterococcus faecalis*; pathogenesis; ace collagen adhesion; infective endocarditis; protective vaccine; immunoprophylaxis

Received: 1 October 2018; **Accepted:** 15 November 2018

INTRODUCTION

Enterococci are gram-positive cocci of intestinal origin and have been recognized as the 3rd most common cause of community onset infective endocarditis (IE) with *Enterococcus faecalis* accounting for majority (Tleyjeh *et al.* 2005; Murdoch *et al.* 2009; Slipczuk *et al.* 2013). The therapeutic problems posed for patients with *E. faecalis* IE were well recognized as early as the 1950s. From the 1980's onwards, *E. faecalis* strains have also been recognized as important causes of healthcare-associated infections (UTIs, bacteremia, intra-abdominal and wound infections and endocarditis) (Tleyjeh *et al.* 2005; Murdoch *et al.* 2009; Slipczuk *et al.* 2013) and increasingly antibiotic resistant (Arias and Murray 2009; Nigo *et al.* 2014). Thus, the study of potential virulence properties and immuno-protective agents may play an important role in discovering non-antibiotic means to treat, prevent or modulate *E. faecalis* infections and set the stage for preventative strategies for *Enterococcus faecium*, an even more difficult to treat organism.

Our previously published studies identified a family of genes encoding MSCRAMM-like proteins and one of these, called Ace (for Adhesin to collagen of *E. faecalis*), has been studied in detail (Nallapareddy *et al.* 2000; Nallapareddy *et al.* 2000; Nallapareddy and Murray 2006; Singh *et al.* 2010). Similar to Cna from *Staphylococcus aureus*, Ace contain features characteristic of the LPXTG cell wall-anchored family of proteins (Symersky *et al.* 1997; Rich *et al.* 1999; Nallapareddy *et al.* 2000). The A region of Ace contains the collagen binding domain and has significant sequence similarity to the corresponding domain of Cna.

Ace is ubiquitous (Duh *et al.* 2001) in *E. faecalis* and conserved among diverse isolates, albeit with at least four variants due to variation in the number of repeats of the B domain (Nallapareddy *et al.* 2000); we previously showed that Ace elicits an antibody response in patients with *E. faecalis* IE (Nallapareddy *et al.* 2000) and that Ace is important for IE pathogenesis in a rat IE model (Singh *et al.* 2010). Ace domain A has been shown to mediate the adherence of *E. faecalis* cells to collagen type I (CI), collagen type IV (CIV), laminin (Rich *et al.* 1999; Nallapareddy *et al.* 2000; Tomita and Ike 2004) and dentin (Kowalski *et al.* 2006). Conditional *in vitro* surface expression of Ace (i.e. growth in brain heart infusion (BHI) at 46°C, with 40% serum (BHIS), or with bile) correlates with conditional adherence of *E. faecalis* strains to collagens and laminin (Nallapareddy *et al.* 2000; Nallapareddy and Murray 2006; Pinkston *et al.* 2011; Roh *et al.* 2015). Previous studies have shown that affinity purified anti-Ace polyclonal antibodies from serum of patients with *E. faecalis* IE or from animals immunized with rAce domain A inhibited *in vitro* adherence of *E. faecalis* strains to collagen and laminin (Nallapareddy *et al.* 2000; Nallapareddy *et al.* 2000). Hall *et al.*, showed that anti-Ace_{40} monoclonal antibody (targeting the ligand-binding domain A of Ace) inhibited binding of recombinant Ace to human CI and CIV and inhibited binding of Ace-coated fluorescent beads to epithelial cell (Hall *et al.* 2007).

Utilizing an anti-Ace domain A mAb selected from a panel of mAbs developed against rAce domain A, designated anti-Ace mAb_{70} (Gao, Pinkston and Nallapareddy 2010), we previously demonstrated its utility in monitoring levels of Ace domain A protein expression to improve our understanding of factors regulating surface display (Gao, Pinkston and Nallapareddy 2010; Pinkston *et al.* 2011; Gao *et al.* 2013). In this study, we further characterize anti-Ace mAb_{70} for its affinity to rAce domain A, its ability to inhibit *E. faecalis* adhesion to collagen, and its effectiveness in preventing infection in a rat model of IE. Our findings demonstrate that anti-Ace mAb_{70} significantly reduces aortic valve infection by *E. faecalis* when given prophylactically in a rat model of IE.

MATERIALS AND METHODS

Bacterial strain and growth conditions

Enterococcus faecalis strain OG1RF (a strain we have extensively used for IE and whose genome has been sequenced and closed) (Murray *et al.* 1993; Bourgogne *et al.* 2006) was used in the rat endocarditis model. *Enterococcus faecalis* OG1RF was grown either on BHI agar (Difco Laboratories) for *in vitro* experiments or in BHI broth with 40% horse serum for *in vivo* experiments. Enterococcosel™ Agar (EA) (Becton Dickinson) supplemented with rifampicin (RIF) 100 μg mL^{-1} was used to plate tissue homogenates for bacterial recovery.

Anti-Ace mAb70 kinetic evaluation by Biacore T 100 system

To generate quantitative surface plasmon resonance (SPR) measurements of the equilibrium dissociation constant (K_D) of anti-Ace mAb_{70}, binding on and off rates to rAce domain A were measured using SPR and a Biacore T100 instrument (GE Healthcare). Two flow cells of a CM5 chip were coated with goat anti-mouse (Fc-specific) (Jackson ImmunoResearch) to approximately 8000 resonance units with an NHS/EDC Amine Coupling Kit (BR-1000-50; GE Healthcare). All measurements were made at 25°C using HBS EP (10 mM HEPES, 150 mM NaCl, 3 mM ethylenediaminetetraacetic acid, 0.05% v/v polysorbate 20, pH 7.4) as running buffer. For kinetic analysis, approximately 100 RU units of anti-Ace mAb 70 was captured on flow cell 2, followed by rAce domain A analyte run over both lanes 2 and 1 (in HBS EP). Analyte was tested over a 2-fold dilution series of 5 different molar concentrations from 100–6.25 nM. Flow cells were regenerated after each run with 100 mM phosphoric acid.

In vitro inhibition assay against CIV using anti-Ace mAb_{70}

Microlon 600 ELISA plates (Greiner) were coated with Human Collagen IV (Sigma) at 10 μg mL^{-1} in PBS, 100 μL/well overnight at 4°C. Collagen-coated ELISA plates were blocked with 200 μL/well PBS-1% Bovine Serum Albumin (BSA) for 1 h at room temperature. Overnight bacterial cultures grown in 20 mL BHI at 37°C, 240 rpm, were diluted to OD_{600} = 0.05 into 125 mL centrifuge tubes containing 20 mL BHI broth and incubated an additional 60 min with shaking. Bacteria were harvested and resuspended in PBS–BSA containing either anti-Ace mAb_{70} or a murine gamma globulin (MGG) control (Jackson ImmunoResearch, West Grove, PA) at indicated concentrations (10 000–0.3 ng mL^{-1}) to an optical density of 1.0. Bacterial suspensions were pipetted onto prewashed (PBS) collagen-coated plates at 100 μL/well, covered and incubated an additional 1 h at room temperature under static conditions. Micro-well contents were aspirated and washed three-times with PBS. The remaining attached bacterial cells were fixed with 100 μL/well Bouin's solution for 30 min. Following an additional PBS wash step, a 1% solution of crystal violet was applied for another 30 min. The crystal violet was removed with sequential Milli-Q water flushes and solubilized with a 200 μL solution of 80:20 ethanol: acetone. Absorbance was determined with a Multiskan EX plate reader with 595 nm filter. Net adherence values were determined by absorbance

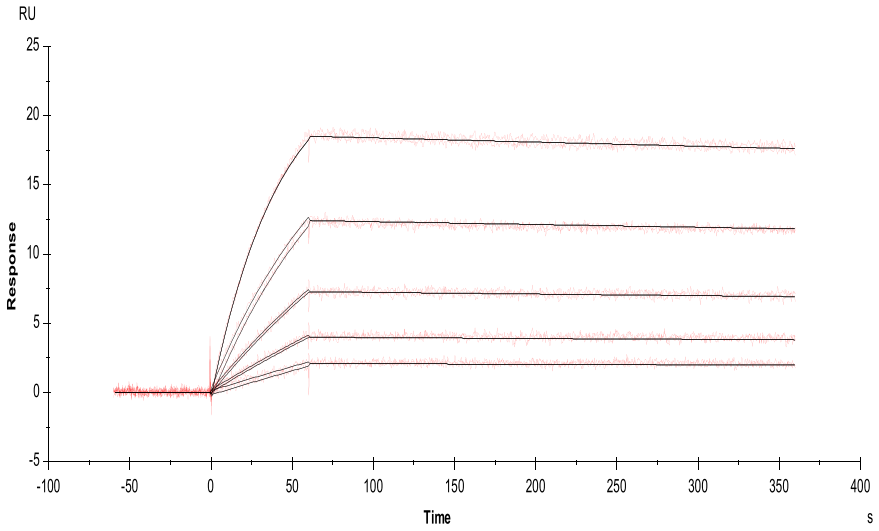

Figure 1. Kinetic evaluation of anti-Ace mAb_{70} affinity for recombinant Ace domain A. To determine Kon and Koff kinetic measurements of the anti-Ace mAb_{70} binding domain with recombinant Ace domain A interaction, a dilution series (100–6.25nM) run in duplicate of Ace domain A (analyte) was evaluated for a 1:1 binding interaction with anti-Ace mAb_{70} immobilized to SPR surface (ligand). Results are representative of three independent experiments.

subtraction of the PBS-only from the collagen IV coated wells and results were plotted.

Rat endocarditis

Endocarditis was produced in male Sprague–Dawley rats weighing ~200 g using previous methodologies (Singh *et al.* 2005; Singh *et al.* 2010; Pinkston *et al.* 2014). In brief, animals were anesthetized with isoflurane for placement of intravascular catheters. The right carotid artery exposed and a sterile polyethylene catheter (e.g. Intramedic PE 10; Clay Adams, Parsippany, NJ) was inserted through a small incision and advanced to 4 cm into the left ventricle. The catheter was ligated immediately and left in place for the duration of the experiment. The incision was closed with sutures. The animal protocol was pre-approved by UTHSC, Animal Welfare Committee, Houston (AWC), TX, USA and all the approved guidelines were followed during the course of this investigation.

Passive immunization and protection against *E. faecalis* infection

Rats were injected intravenously (i.v.) via the tail vein with 2 mg/kg of anti-Ace mAb_{70} or murine control IgG at 24 h post-catheterization and 1 h prior to bacterial inoculation (Singh *et al.* 2010; Pinkston *et al.* 2014). A total of 11 rats were given anti-Ace mAb_{70} and 10 rats were given control IgG (Pinkston *et al.* 2014). *Enterococcus faecalis* OG1RF ($8–9 \times 10^7$) grown in BHIS (Pinkston *et al.* 2014) and premixed in saline was then injected i.v. *via* the tail vein, 25 h after catheter placement (Pinkston *et al.* 2014) and 1 h after the injection of anti-Ace mAb_{70} or control IgG.

Animals were euthanized 24 h post infection. Hearts were aseptically removed from all euthanized animals. Aortic valves containing vegetations were removed from the hearts, weighed and homogenized in 1 mL of 0.9% saline. Sequential dilutions of homogenized tissues were carried out and the entire volume of each dilution including the undiluted sample was plated onto EA + RIF100 μg/mL plates to enumerate bacteria.

Statistics

Bacterial CFU/g from each rat vegetation were log-transformed and unpaired *t*-test was performed comparing anti-Ace mAb_{70} versus control IgG groups to obtain *P* values (Hagberg *et al.* 1984). The geometric means of the bacterial CFU/g were also calculated in each group. Cultures yielding no growth were scored as sterile and were assigned a value of 1 CFU for statistical analysis of geometric means. Fisher's exact test was used for comparing the total number of infected/non-infected rats between anti-Ace mAb_{70} versus control IgG groups to obtain *P* values. Data/graphs were generated using Prism for Windows (version 4.00; GraphPad Software). Overall, differences were considered significant at a P level of <0.05.

RESULTS AND DISCUSSION

Affinity of anti-Ace mAb_{70} for rAce domain A and *in vitro* inhibition assay

Utilizing the GE biacore evaluation software, we evaluated the kinetics of anti-Ace mAb_{70} interaction with rAce domain A, using the 1:1 binding model. Results demonstrated that anti-Ace mAb_{70} has a sub nanomolar affinity to rAce domain A, with an on rate (K_{on}) of 2.37×10^5 (1/Ms) and an off rate (K_{off}) of 1.66×10^{-4}, yielding an overall affinity measurement (KD) of 0.7 nM (Fig. 1). We have previously demonstrated by flow cytometry analysis that anti-Ace mAb_{70} readily recognizes natively displayed Ace on the surface of *E. faecalis* cells at early time points, which is when the metalloprotease gelatinase (GelE) is not present (Pinkston *et al.* 2011). Our results here demonstrated that anti-Ace mAb_{70} efficiently inhibited *E. faecalis* cells from adhering to collagen IV *in vitro*, compared to the MGG control (Fig. 2).

Passive immunization and protection against *E. faecalis* infection

In the rat endocarditis model, as seen in Fig. 3, we noted that 9 of 10 control IgG treated rats (90%) developed *E. faecalis*

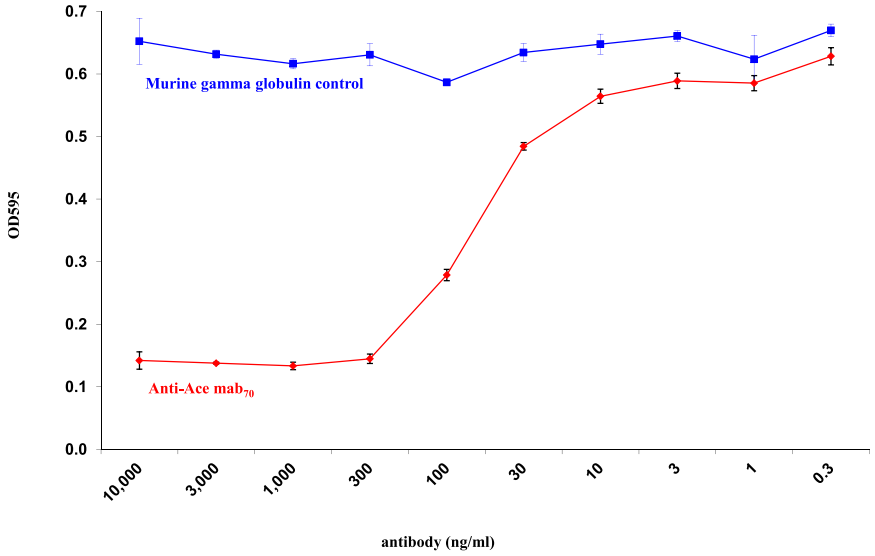

Figure 2. Prevention of *E. faecalis* OG1RF adherence to immobilized collagen IV. *Enterococcus faecalis* adherence to immobilized collagen was evaluated at increasing levels of anti-Ace mAb_{70} concentration. Levels of cell adherence were measured by crystal violet absorbance at OD_{595}. Murine gamma globulin was used as control.

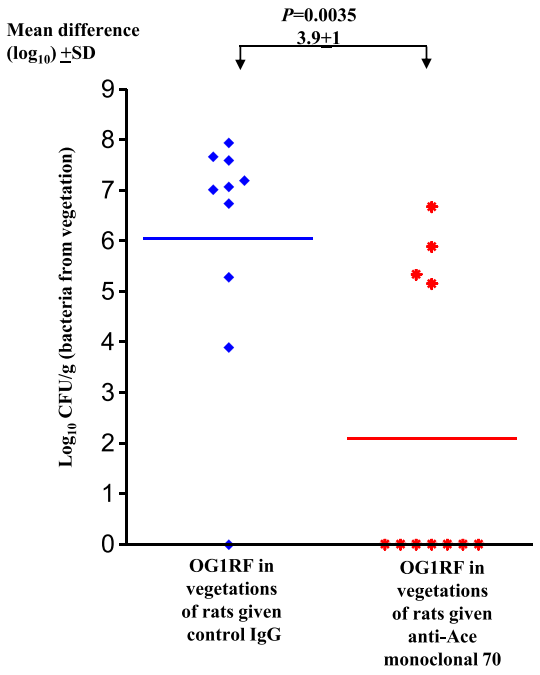

Figure 3. Passive immunization (anti-Ace mAb_{70} versus control IgG) in the rat endocarditis model. In the panel on the left, solid diamonds represent OG1RF log_{10} CFU/g recovered 24 h post-infection from vegetations from control IgG pre-treated rats. In the panel on the right, stars represent OG1RF log_{10} CFU/g recovered 24 h post infection from vegetations from anti-Ace mAb_{70} pre-treated rats. Horizontal bars represent the geometric means. Significantly fewer rats were infected by OG1RF in anti-Ace mAb_{70} administered rats (2 mg/kg) ($P = 0.0237$ by Fisher's exact test). Rats (anti-Ace mAb_{70} pre-treatment) showed a geometric mean ± SD decrease of 3.9 ± 1 log_{10} OG1RF CFU/g from vegetations versus control IgG pre-treatment ($P = 0.0035$ by unpaired t-test of log transformants).

endocarditis compared with only 4 of 11 rats (36%) in the anti-Ace mAb_{70} treated group (Fisher's exact test P 0.0237). Control IgG treated rats showed 3.9 ± 1 log_{10} more CFU/g than the anti-Ace mAb 70 treated group (P 0.0035) in vegetations recovered from heart valves (Fig. 3).

Development of endocarditis can be initiated by injury to the valvular endothelium, disrupting the normal valve structure which exposes the underlying tissues, including ECM material resulting in deposition of host proteins, e.g. fibrin, as well as platelets at the site of injury leading to the formation of a sterile thrombotic vegetation. This endovascular lesion can be colonized by circulating bacteria in blood, leading to an infected vegetation. Both valvular and aortic tissues are collagen rich (Angrist and Oka 1963) and collagen is also found in sterile vegetations (Angrist and Oka 1963).

We previously demonstrated that polyclonal Ab to rAce A domain significantly prevents IE via both active immunization and using affinity purified Ig in passive immunization (Singh *et al.* 2010). Because passive immunization studies would be much more practical for at-risk hospitalized patients and because mAb can be scaled up and are relatively safe, we evaluated monoclonal antibodies as a passive protective agent against *E. faecalis* experimental endocarditis and found it significantly prevented infection and reduced log_{10} CFU/g. However, in 4/11 rats protection was not seen. It has been reported that, in humans, response to vaccination is variable because different hosts respond variably in immunization studies (Kimman, Vandebriel and Hoebee 2007). We suspect similar response to immunogens/vaccines occurs in animals with some rats showing less or no protection.

We earlier described Ebp pili as another important factor in the pathogenesis of *E. faecalis* endocarditis as well as urinary tract infections and biofilm formation (Nallapareddy *et al.* 2006; Singh, Nallapareddy and Murray 2007). More recently, we showed that monoclonal antibody to EbpC, the major (core) subunit of *E. faecalis* pili, labeled polymerized pilus structures, inhibited biofilm formation in plates, and significantly prevented the establishment of *E. faecalis* IE in a rat model. In addition, radiolabeled EbpC was detected at the site of *E. faecalis* infection, demonstrating molecular specificity during imaging of an established IE in rat model. The effectiveness of this anti-EbpC monoclonal provides further support of use of monoclonal antibodies as a preventative tool in IE.

In summary, we have demonstrated here that a monoclonal Ab, with subnanomolar affinity for the collagen adhesion

domain A of Ace, (i) inhibited *in vitro* adherence of *E. faecalis* to CIV and (ii) conferred significant protection against endocarditis in passive immunization studies. Taken together, these results indicate that Ace is a promising target for prophylactic and therapeutic strategies against *E. faecalis* endocarditis and anti-Ace mAb_{70} provides novel data in support of the development of vaccines or immunotherapeutics that could be useful for the prevention of enterococcal infections.

ACKNOWLEDGEMENTS

We thank Karen Jacques-Palaz for technical assistance. This work was supported in part by NIH grant R01 AI047923-14 from NIAID to B.E.M.

Conflict of interest. None declared.

REFERENCES

Angrist AA, Oka M. Pathogenesis of bacterial endocarditis. *JAMA* 1963;**183**:249–52.

Arias CA, Murray BE. Antibiotic-resistant bugs in the 21st century–a clinical super-challenge. *N Engl J Med* 2009;**360**:439–43.

Bourgogne A, Hilsenbeck SG, Dunny GM *et al.* Comparison of OG1RF and an isogenic *fsrB* deletion mutant by transcriptional analysis: the Fsr system of *Enterococcus faecalis* is more than the activator of gelatinase and serine protease. *J Bacteriol* 2006;**188**:2875–84.

Duh RW, Singh KV, Malathum K *et al.* In vitro activity of 19 antimicrobial agents against enterococci from healthy subjects and hospitalized patients and use of an *ace* gene probe from *Enterococcus faecalis* for species identification. *Microb Drug Resist* 2001;**7**:39–46.

Gao P, Pinkston KL, Nallapareddy SR. *Enterococcus faecalis rnjB* is required for pilin gene expression and biofilm formation. *J Bacteriol* 2010;**192**:5489–98.

Gao P, Pinkston KL, Bourgogne A *et al.* Library screen identifies Enterococcus faecalis CcpA, the catabolite control protein A, as an effector of Ace, a collagen adhesion protein linked to virulence. *J Bacteriol* 2013;**195**:4761–8.

Hagberg L, Hull R, Hull S *et al.* Difference in susceptibility to gram-negative urinary tract infection between C3H/HeJ and C3H/HeN mice. *Infect Immun* 1984;**46**:839–44.

Hall AE, Gorovits EL, Syribeys PJ *et al.* Monoclonal antibodies recognizing the *Enterococcus faecalis* collagen-binding MSCRAMM Ace: conditional expression and binding analysis. *Microb Pathog* 2007;**43**:55–66.

Kimman TG, Vandebriel RJ, Hoebee B. Genetic variation in the response to vaccination. *Community Genet* 2007;**10**:201–17.

Kowalski WJ, Kasper EL, Hatton JF *et al.* *Enterococcus faecalis* adhesin, Ace, mediates attachment to particulate dentin. *J Endod* 2006;**32**:634–7.

Murdoch DR, Corey GR, Hoen B *et al.* Clinical presentation, etiology, and outcome of infective endocarditis in the 21st century: the International Collaboration on Endocarditis-Prospective Cohort Study. *Arch Intern Med* 2009;**169**:463–73.

Murray BE, Singh KV, Ross RP *et al.* Generation of restriction map of Enterococcus faecalis OG1 and investigation of growth requirements and regions encoding biosynthetic function. *J Bacteriol* 1993;**175**:5216–23.

Nallapareddy SR, Murray BE. Ligand-signaled upregulation of *Enterococcus faecalis ace* transcription, a mechanism for modulating host-*E. faecalis* interaction. *Infect Immun* 2006;**74**:4982–9.

Nallapareddy SR, Qin X, Weinstock GM *et al.* *Enterococcus faecalis* adhesin, *ace*, mediates attachment to extracellular matrix proteins collagen type IV and laminin as well as collagen type I. *Infect Immun* 2000;**68**:5218–24.

Nallapareddy SR, Singh KV, Duh RW *et al.* Diversity of *ace*, a gene encoding a microbial surface component recognizing adhesive matrix molecules, from different strains of *Enterococcus faecalis* and evidence for production of ace during human infections. *Infect Immun* 2000;**68**:5210–7.

Nallapareddy SR, Singh KV, Sillanpaa J *et al.* Endocarditis and biofilm-associated pili of *Enterococcus faecalis*. *J Clin Invest* 2006;**116**:2799–807.

Nigo M, Munita JM, Arias CA *et al.* What's new in the treatment of enterococcal endocarditis? *Curr Infect Dis Rep* 2014;**16**:431.

Pinkston KL, Gao P, Diaz-Garcia D *et al.* The Fsr quorum-sensing system of *Enterococcus faecalis* modulates surface display of the collagen-binding MSCRAMM Ace through regulation of gelE. *J Bacteriol* 2011;**193**:4317–25.

Pinkston KL, Singh KV, Gao P *et al.* Targeting pili in enterococcal pathogenesis. *Infect Immun* 2014;**82**:1540–7.

Rich RL, Kreikemeyer B, Owens RT *et al.* Ace is a collagen-binding MSCRAMM from *Enterococcus faecalis*. *J Biol Chem* 1999;**274**:26939–45.

Roh JH, Singh KV, La Rosa SL *et al.* The two-component system GrvRS (EtaRS) regulates ace expression in *Enterococcus faecalis* OG1RF. *Infect Immun* 2015;**83**:389–95.

Singh KV, Nallapareddy SR, Murray BE. Importance of the *ebp* (endocarditis- and biofilm-associated pilus) locus in the pathogenesis of *Enterococcus faecalis* ascending urinary tract infection. *J Infect Dis* 2007;**195**:1671–7.

Singh KV, Nallapareddy SR, Nannini EC. Fsr-independent production of protease(s) may explain the lack of attenuation of an *Enterococcus faecalis* fsr mutant versus a *gelE-sprE* mutant in induction of endocarditis. *Infect Immun* 2005;**73**:4888–94.

Singh KV, Nallapareddy SR, Sillanpaa J *et al.* Importance of the collagen adhesin ace in pathogenesis and protection against *Enterococcus faecalis* experimental endocarditis. *PLoS Pathog* 2010;**6**:e1000716.

Slipczuk L, Codolosa JN, Davila CD *et al.* Infective endocarditis epidemiology over five decades: a systematic review. *PLoS One* 2013;**8**:e82665.

Symersky J, Patti JM, Carson M *et al.* Structure of the collagen-binding domain from a *Staphylococcus aureus* adhesin. *Nat Struct Mol Biol* 1997;**4**:833–8.

Tleyjeh IM, Steckelberg JM, Murad HS *et al.* Temporal trends in infective endocarditis: a population-based study in Olmsted County, Minnesota. *JAMA* 2005;**293**:3022–8.

Tomita H, Ike Y. Tissue-specific adherent *Enterococcus faecalis* strains that show highly efficient adhesion to human bladder carcinoma T24 cells also adhere to extracellular matrix proteins. *Infect Immun* 2004;**72**:5877–85.