In vivo virulence of *Staphylococcus aureus* in native versus prosthetic left-sided valve endocarditis

Haytham Elgharably, MD,[a] Jan Claesen, PhD,[b,c] Naseer Sangwan, PhD,[b,c,d] Muhammad Etiwy, MD,[a] Penny Houghtaling, MS,[a] Gary W. Procop, MD, MS,[e] Nabin K. Shrestha, MD,[f] Brian Griffin, MD,[g] Jose L. Navia, MD,[a] Lars G. Svensson, MD, PhD,[a] Daniel J. Wozniak, PhD,[h] and Gosta B. Pettersson, MD, PhD[a]

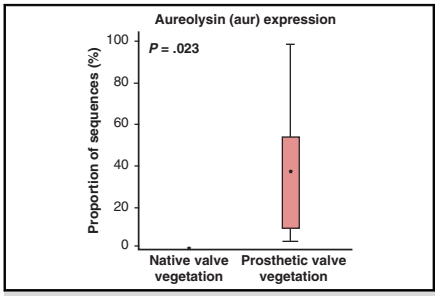
ABSTRACT

Objectives: *Staphylococcus aureus* infective endocarditis is commonly associated with invasive pathology and is worse in prosthetic valve endocarditis. In this study, we aim to examine *S. aureus* virulence and pathological features of native and prosthetic valve infective endocarditis.

Methods: Between 2002 and 2020, 438 patients underwent surgery for left-sided endocarditis caused by *S. aureus* at our center (59% native and 41% prosthetic valve endocarditis). Endocarditis registry was queried, and pathological features were based on the echocardiography and operative findings. In addition, vegetation samples were collected from 6 patients undergoing surgery for infective endocarditis (3 native and 3 prosthetic valve endocarditis). Total RNA was extracted from all specimens, and messenger RNA sequencing was executed for transcriptomic analysis. Data were pooled into STAR aligner, and gene expression related to virulence factors was compared between 2 groups.

Results: Rates of invasive pathology were higher in prosthetic versus native valve infective endocarditis (76% vs 40%, $P < .0001$), which impacted the complexity of surgical procedures and perioperative course, but not in-hospital mortality. Transcriptomic analysis has shown differences in gene expression between vegetation specimens of native and prosthetic valve endocarditis, including genes for stress response, biofilm formation, and virulence factors. The gene *aur* (encodes for aureolysin) was highly upregulated in prosthetic valve vegetations compared with native valve vegetations ($P = .023$).

Conclusions: Prosthetic valve endocarditis caused by *S. aureus* is associated with further invasive pathology compared with native valve endocarditis, which could be related to upregulation of genes responsible for biofilm formation and metalloproteinase production. (JTCVS Open 2025;24:156-69)



Higher expression of *aur* (encodes for aureolysin) in PVE.

CENTRAL MESSAGE

PVE caused by *Staphylococcus aureus* is associated with further invasive pathology compared with NVE, which is secondary to the difference in virulence factors.

PERSPECTIVE

Transcriptomic profiling of *Staphylococcus aureus* colonizing cardiac vegetations has shown upregulation of aureolysin in prosthetic valve vegetation, which could explain the invasive pathology in the surrounding tissues. This finding warrants further investigations to develop novel diagnostics that can guide the clinical decision for the timing of surgery for endocarditis.

From the [a]Department of Thoracic and Cardiovascular Surgery, Cleveland Clinic, Cleveland, Ohio; [b]Department of Cardiovascular and Metabolic Sciences, Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio; [c]Center for Microbiome and Human Health, Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio; [d]Microbial Sequencing & Analytics Resource (MSAAR) Facility, Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio; [e]Department Laboratory Medicine, Pathology and Laboratory Medicine Institute, Cleveland Clinic, Cleveland, Ohio; [f]Department of Infectious Disease, Cleveland Clinic, Cleveland, Ohio; [g]Department of Cardiovascular Medicine, Cleveland Clinic, Cleveland, Ohio; and [h]Departments of Microbial Infection and Immunity and Microbiology, Ohio State University, Columbus, Ohio.

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Address for reprints: Haytham Elgharably, MD, Department of Thoracic and Cardiovascular Surgery, Cleveland Clinic, 9500 Euclid Ave/Desk J4-1, Cleveland, OH 44195 (E-mail: Elgharh@ccf.org).

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Abbreviations and Acronyms
IE = infective endocarditis
NVE = native valve endocarditis
PVE = prosthetic valve endocarditis

Despite the recent advances in medical therapies, infective endocarditis (IE) remains lethal with high mortality rates reaching 25%.[1,2] Advances in medicine have changed the epidemiology and microbiology of IE, but not the outcomes. *Staphylococcus aureus* has become the leading causative pathogen of IE.[3,4] *S. aureus* endocarditis is frequently associated with invasive pathology, especially with prosthetic valve endocarditis (PVE).[5-7] This could explain the increased perioperative mortality risk associated with *S. aureus* infection and PVE.[8-10] Previously, our group presented the hypothesis that IE is a biofilm-associated infection based on a constellation of clinical observations that are in accordance with the criteria of biofilm infection.[1,3] Growing evidence, including microscopic imaging, animal experiments, and in vitro studies, of IE bacterial isolates supports this hypothesis.[1,3,11] However, current studies do not provide a direct in vivo examination of microbial behavior within the cardiac vegetations in patients with IE. This is critical to better understand the mechanisms used by bacteria to evade the host immune response, resist antibiotics, and invade and destroy surrounding tissues. For instance, why is PVE more aggressive than native valve endocarditis (NVE) with respect to tissue invasion (Figure 1)?[5,6] Do bacterial cells modify their gene expression profile upon attachment to the cardiac valve tissue as aggregates of micro-colonies (Figure 2) followed by the production of virulence factors responsible for resistance and tissue invasion? Addressing these questions will have important clinical implications in guiding the current management strategies into more specific diagnostics and effective therapies for IE.

In this study, we hypothesize that PVE caused by *S. aureus* is associated with further invasive pathology than NVE, secondary to difference in virulence. To test this hypothesis, we examined the pathological features in a large cohort of patients who underwent surgery for *S. aureus* IE at our center. Additionally, we examined the difference in gene expression of *S. aureus* isolated from native and prosthetic valve vegetations harvested from 6 patients during surgery for endocarditis.

MATERIAL AND METHODS

Infective Endocarditis Data Registry

In our institution Endocarditis Registry database (a prospective registry established from institutional infectious disease and surgical registries for quality reporting); 2965 patients underwent surgery for IE from January 2002 to December 2020. Of these, 575 (19%) had established diagnosis of *S. aureus* endocarditis, of whom 523 had active endocarditis and 52 had remote endocarditis. Continuous variables are presented as mean ± SD and as 15th, 50th (median), and 85th percentiles; comparisons were made using Wilcoxon rank-sum test. Categorical data are described using frequencies and percentages; comparisons were made using the chi-square test or Fisher exact test when frequency was less than 5. All analyses were performed using SAS statistical software (SAS v9.4; SAS, Inc). Invasive pathology is based on operative findings and defined as extension of infection from aortic valve leaflets into the annulus or beyond (eg, abscess or fistula).

Patients and Clinical Samples

Specimens for transcriptomic profiling (RNA sequencing) were collected from a total of 6 patients (3 with NVE and 3 with PVE; Figure 3). Inclusion criteria involved established diagnosis of IE by an infectious disease specialist in addition to being classified as "definite" endocarditis by Duke Criteria,[12] positive *S. aureus* blood cultures, and referral for cardiac surgery. In this pilot study, samples were collected from the first 6 patients who fulfilled the inclusion criteria. The study protocol was approved by the Cleveland Clinic Institutional Review Board (#16-1521, January 16, 2017), and all participants were provided an information sheet before enrollment. During surgery for endocarditis, explanted valve vegetation specimens were collected and immediately preserved in 1 mL of TRIzol and stored at −80 °C (Invitrogen, Thermo Fisher Scientific, Inc). Samples were given serial codes when sent for RNA-sequencing and bioinformatics analysis to keep results blinded.

Blood Culture Bacterial Isolates

For the 6 patients recruited in the study, corresponding bacterial isolates from positive preoperative blood cultures were obtained from the Cleveland Clinic microbiology laboratory. *S. aureus* blood culture isolate stocks were stored in tryptic soy broth with 15% glycerol (Hardy Diagnostics) at −70 °C until subcultured for testing. Suspensions were prepared at a concentration of 6×10^8 CFU/mL.

Full Prokaryotic Messenger RNA Sequencing: TruSeq Stranded With RiboZero Plus (Human, Mouse, Rat, Bacteria)

RNA extraction was done using a Qiagen RNeasy Plus Mini Kit for all samples (blood culture isolates after growth in media and directly from frozen vegetation specimens). Isolated RNA sample quality was assessed by the BioAnalyzer Pico 6000 RNA Assay (Agilent Technologies Inc) and quantified by Qubit 2.0 RNA HS assay (ThermoFisher). Ribosomal RNA depletion was performed with a Ribo-Zero Plus rRNA Removal Kit (Illumina Inc). Subsequently, libraries were constructed by using a Truseq Stranded Total RNA kit (Illumina). Final library quantity was assessed by Qubit 2.0 (ThermoFisher), and quality was assessed by TapeStation D1000 ScreenTape (Agilent Technologies Inc). The average final library size was approximately 330 bp with an insert size of approximately 200 bp. Illumina 8-nt dual-indices were used. Equimolar pooling of libraries was performed based on QC values and sequenced (Novogene) on an Illumina NovaSeq S4 with a read length configuration of 150 paired-end for 40 M paired-end reads per sample (20 M in each direction).

Bioinformatics Analysis

Quality control of the metagenomic reads was conducted as described previously.[13] Briefly, raw reads were processed for low-quality–based filtering using Trimmomatic pipeline.[14] Host-derived reads were excluded by mapping the reads to the reference human genome (GCF_000001405.40) using BBMap software (sourceforge.net/projects/bbmap/). Reference microbial genomes were annotated using prokka.[15] Quality trimmed reads were processed for taxonomic and functional profiling STAR aligner against the bacterial reference genomes.[16] DAtest

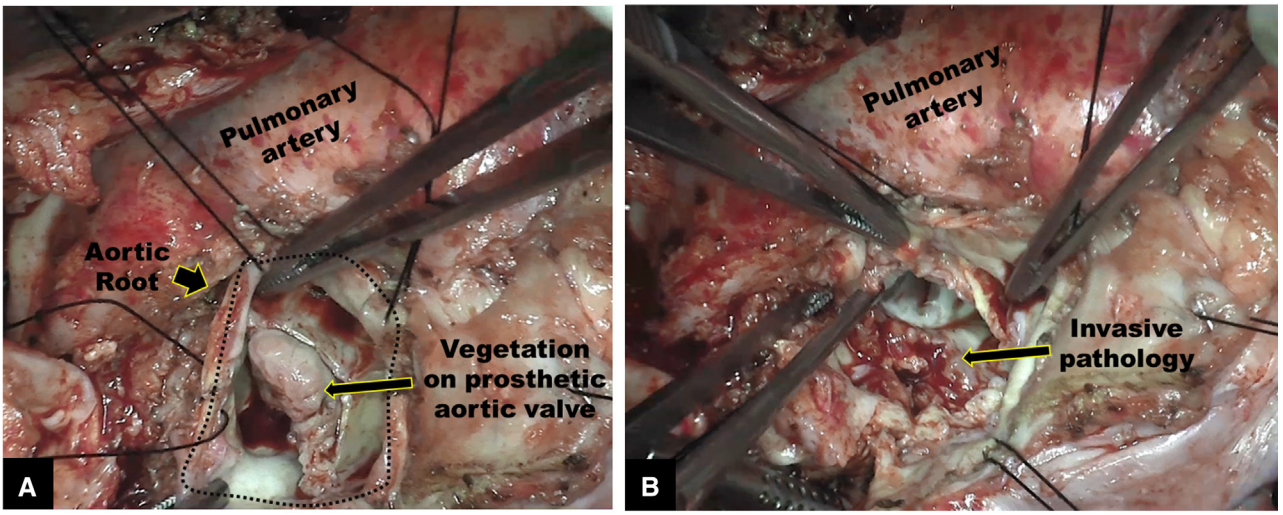

FIGURE 1. Operative photograph showing large vegetation attached to prosthetic aortic valve in a patient with IE (A) and invasion and disintegration of the aortic root tissues after explant of the infected prosthetic valve (B).

package was used to benchmark the statistical tests.[17] Differential feature selection was performed using the Fisher exact *t* test.[18] We assessed the statistical significance ($P < .05$) throughout, and whenever necessary, we adjusted *P* values for multiple comparisons according to the Benjamini and Hochberg method to control the false discovery rate while performing multiple tests on taxa and pathway abundances according to sample types.

RESULTS

Clinical Endocarditis Registry

In our institution's Endocarditis Registry, we identified 438 patients who underwent surgery for left-sided endocarditis caused by *S. aureus*, of whom 260 (59%) had NVE and 178 (41%) had PVE (Tables E1 and E2). For PVE, most cases had involvement of the aortic valve (81%), whereas for patients with NVE the mitral valve was more commonly affected (65%) ($P < .0001$). Invasive disease (76%) with cavity formation (44%) was significantly greater in PVE compared with NVE (40% and 10%, respectively) ($P < .0001$). Surgery for PVE more commonly required reconstruction of the aortic root with the aortic allograft ($P < .0001$), longer cardiopulmonary

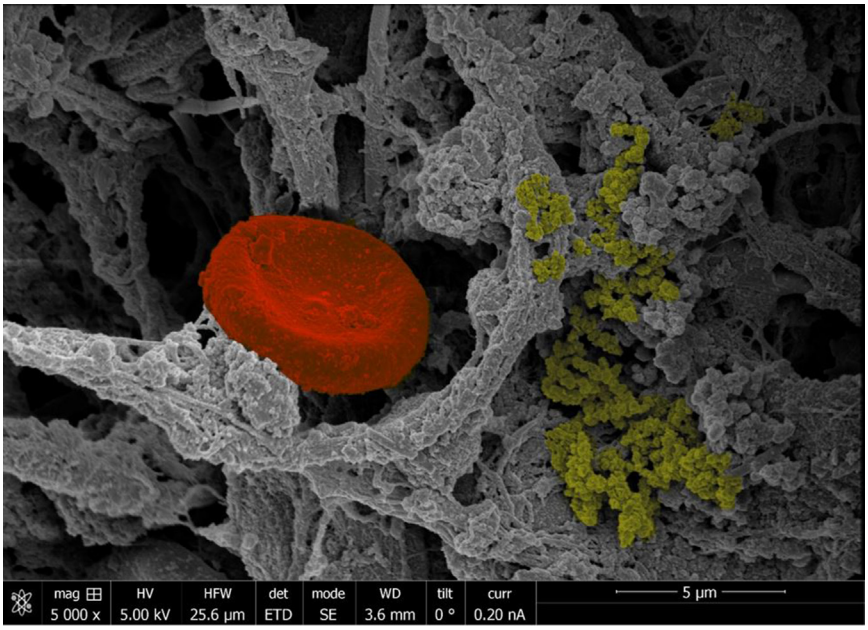

FIGURE 2. Scanning electron microscope image of a porcine bioprosthetic aortic valve explanted from a patient during surgery for endocarditis showing clusters of cocci (*yellow*) clumped together and attached to the extracellular matrix covering the valve leaflet (magnification 5000×). Red blood cell showing in *red* color (Appendix E1).

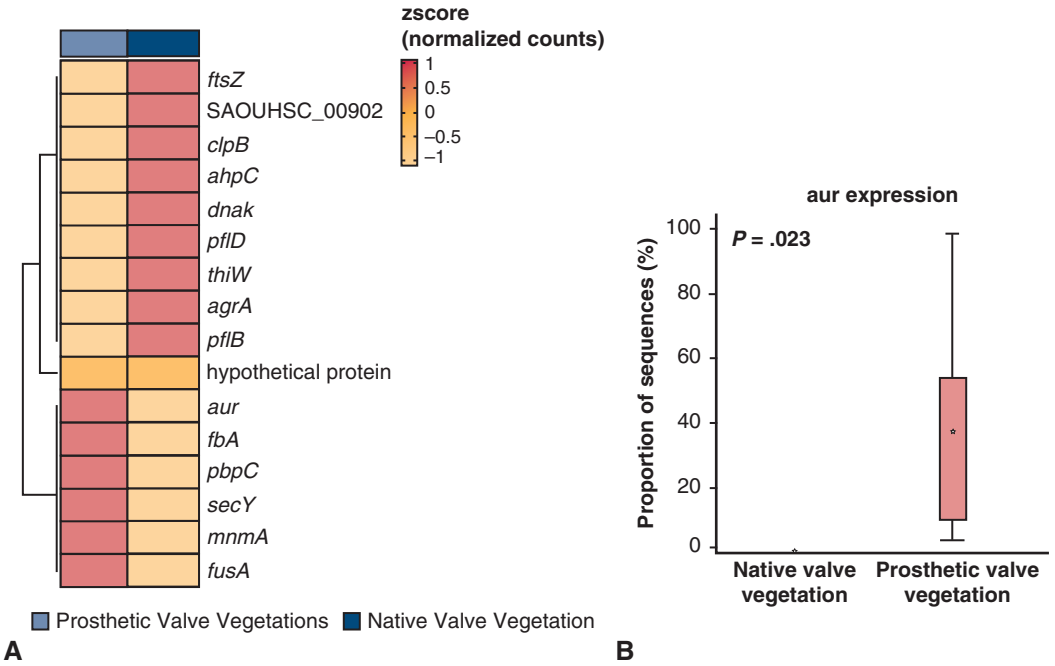

FIGURE 3. A, Heatmap plots showing the difference in the expression of differentially abundant *Staphylococcus* genes between vegetation samples extracted from 3 patients with NVE and 3 patients with PVE during cardiac surgery. B, Difference in aureolysin (*aur*) expression in vegetation samples extracted from 3 patients with NVE and 3 patients with PVE during cardiac surgery.

bypass time ($P < .0001$), and more blood transfusion support ($P < .0001$) compared with surgery for NVE. Allograft is our preferred prosthesis for invasive PVE pathology to reconstruct the destroyed root and possibly resistance to reinfection, whereas focal invasion in NVE can be managed with a pericardial patch and aortic valve replacement. Patients with PVE required a longer length of stay in the intensive care unit ($P = .034$) and prolonged mechanical ventilation ($P = .0008$), but there was no difference in hospital death when compared with patients with NVE ($P = .67$). After multivariable adjustment of baseline variables, there was no difference in outcomes between the groups (Appendix E2, Tables E3 and E4).

Transcriptomic Profiling of *S. aureus* in Cardiac Vegetations

We performed transcriptomic analyses of *S. aureus* isolated from cardiac valve vegetations harvested during surgery for left-sided endocarditis (a summary of the 6 patients' clinical data is presented in Table 1). We interrogated our data to compare the *S. aureus* transcriptional profile in native (n = 3) versus prosthetic (n = 3) valve vegetations: Prosthetic valve vegetation-colonizing bacteria demonstrated upregulation of genes responsible for biofilm formation (*mnmA, secY*), virulence factors (*aur, pbpC*), and stress response/metabolism (*fbA, mnmA, fusA*). Native valve vegetation-colonizing bacteria demonstrated upregulation of genes responsible for biofilm formation (*dnaK, agrA*), virulence factors (*clpB, spsA/SAOUHSC_00902*), cell division (*ftsz*), and stress response/metabolism (*ahpC, pflD, thiW, pflB*) (Figure 3, *A*, and Table E5).

S. aureus Virulence Factor Aureolysin

Expression of *aur,* encoding for the metalloprotease aureolysin, was greater in prosthetic valve vegetation (3 patients) compared with native valve vegetation (3 patients) ($P = .023$) (Figure 3, *B*).

Transcriptomic Profiling of *S. aureus* in Blood Cultures

We performed transcriptomic analyses of *S. aureus* isolated from blood cultures collected before surgery from the 6 subjects recruited in the study (3 patients with NVE and 3 patients with PVE). In NVE blood cultures, *S. aureus* upregulated genes responsible for biofilm formation (*dnak, ebpB, ebpA*), virulence factors (*fusA, clpB*), clumping (*clfB*), and stress response/metabolism (*cydB, raiA*). In PVE blood culture, *S. aureus* upregulated genes responsible for virulence factors and stress response (*aur, ahpC*) (Figure 4 and Table E6).

DISCUSSION

Biofilm-Associated Endocarditis

The initial event of IE pathogenesis is endothelial damage followed by aggregation of platelets and fibrin.[2] During periods of bacteremia, the site of thrombotic endocarditis

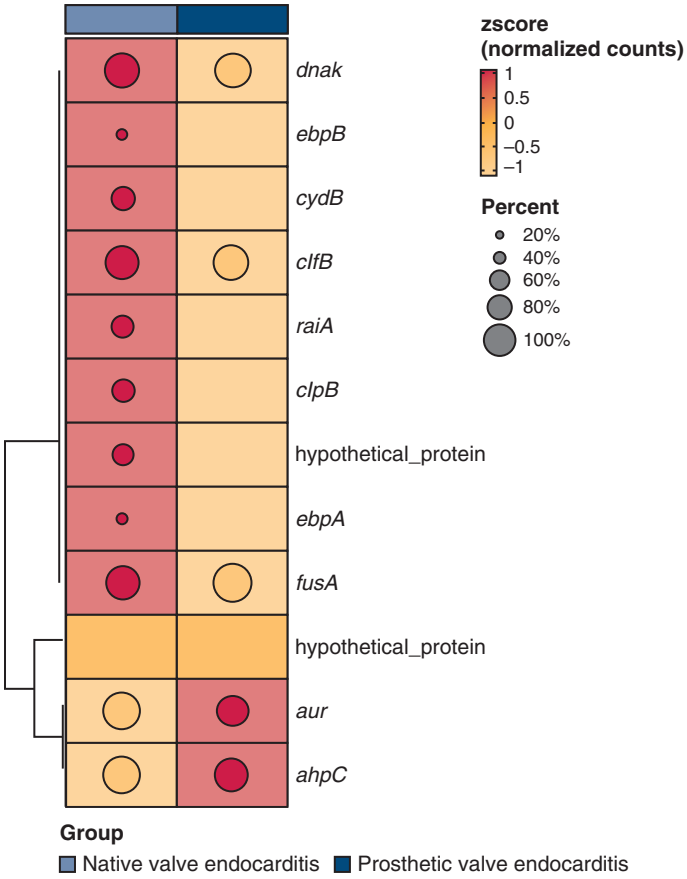

FIGURE 4. Heatmap plots showing the difference in the expression of differentially abundant *Staphylococcus* genes in blood culture samples collected from 3 patients with NVE and 3 patients with PVE before cardiac surgery.

becomes colonized by bacterial cells, which then grow as microcolonies clumping with platelets and fibrin meshwork and forming endocarditis vegetation (Figures 1 and 2). Currently, it is generally accepted that these microcolonies within the endocarditis vegetations grow as biofilm structures. This insight arose from multiple factors, such as the recognition of the role of biofilm in persistent infection,[19] a constellation of clinical observations that can be explained by a biofilm concept,[3] and growing evidence suggestive of biofilm involvement in IE,[1] including experimental work in animal models of IE or in vitro studies using clinical isolates from patients with endocarditis[11,20,21] and imaging studies.[22,23] Nevertheless, our study is the first to provide direct in vivo evidence by analyzing *S. aureus* transcriptome within cardiac vegetations.

Understanding In Vivo Behavior of *S. aureus* in Infective Endocarditis

It is instrumental to study the expression of *S. aureus* virulence factors in patients because their expression patterns in animal models did not correspond to in vitro reports.[24] In addition, studying *S. aureus* colonizing cardiac vegetations provides a higher yield of clinically relevant data without changes in gene expression that can be induced by growth culture conditions in blood cultures isolates or by systemic antibiotics that would have poor penetration into the vegetation biofilms. Transcriptomic analysis of *S. aureus* colonizing cardiac vegetation has shown antibiotic resistance genes such as *pbpC* (encoding a penicillin-binding protein) and *fusA* (conferring fusidic acid resistance) were upregulated in prosthetic valve vegetations compared with native valve vegetations. *secY* encodes for protein translocase subunit SecY, which is part of the general bacterial secretion system and impacts biofilm growth. *fba* encodes for fructose-bisphosphate aldolase, which supports bacterial metabolism during infection. Both *secY* and *fba* were upregulated in prosthetic valve vegetations. *mnmA* encodes for catalytic enzyme involved in protein synthesis and bacterial phenotypic traits including biofilm formation.[25]

agrA is a transcriptional activator of the *agr* quorum sensing system, the unique bacterial mechanisms for cell-to-cell communication that controls gene expression involved in population behaviors including biofilm formation and dispersion.[26] *dnaK,* which in involved in biofilm formation and adherence to eukaryotic cells, is a chaperone

TABLE 1. Clinical data of the specimens used for the transcriptomic analyses

Subjects	Preoperative antibiotics	Surgery	Valve standard culture	Valve universal bacterial PCR	Valve pathology
Case 1 Native left-sided endocarditis	Vancomycin	Findings: vegetations and perforation of aortic valve leaflet Procedure: AVR with bioprosthesis	*Staphylococcus aureus*	*S. aureus*	Gram-positive cocci clusters in fibrinous vegetations
Case 2 Native left-sided endocarditis	Vancomycin	Findings: vegetation on anterior mitral leaflet Procedure: mitral valve repair	Mitral: Negative	Mitral: *S. aureus*	N/A
Case 3 Prosthetic left-sided endocarditis	Vancomycin	Findings: vegetation on the mitral valve prosthesis sewing cuff Procedure: reoperation mitral valve replacement with bioprosthesis	Negative	*S. aureus*	Fibrinous vegetations, clusters of bacterial cocci
Case 4 Prosthetic left-sided endocarditis	Oxacillin	Findings: vegetation on the aortic valve prosthesis, aortic root abscess Procedure: reoperation aortic root replacement with aortic allograft	*S. aureus*	*S. aureus*	Gram-positive cocci in clusters
Case 5 Prosthetic left-sided endocarditis	Oxacillin Rifampin	Findings: aortic root abscess Procedure: Reoperation aortic root replacement with aortic allograft	Negative	*S. aureus*	Gram stain negative for micro-organisms
Case 6 Native left-sided endocarditis	Vancomycin	Findings: vegetations on the mitral valve leaflets Procedure: MVR with bioprosthesis	*S. aureus* *Staphylococcus lugdunensis*	*S. lugdunensis*	Clusters of Gram-positive cocci in the fibrinous vegetations

Universal Bacterial PCR, Bacterial DNA detected with 16s rRNA gene primer set; *PCR*, polymerase chain reaction; *AVR*, aortic valve replacement; *N/A*, not available; *MVR*, mitral valve replacement.

in the *S. aureus* heat shock system. In native valve vegetations, *agrA* and *dnaK* genes were upregulated, indicating biofilm formation in NVE, which is less widely accepted compared with biofilms in PVE.[11] Clp are ATPases involved in intracellular replication and stress response that contribute to pathogenicity of *S. aureus* colonizing native valve vegetations.[27] PflD and PflB are both part of pyruvate-formate-lyase, which is involved in fermentation and biofilm formation. Both were upregulated in native valve vegetations. The results of the transcriptomic analysis of the vegetation samples were supported by the transcriptomic analysis of the blood culture samples from the same 6 subjects, which showed upregulation of genes responsible for biofilm formation, and more important, upregulation of *aur* in PVE blood cultures. These findings suggest that *S. aureus* isolated from blood cultures was seeded from the valve vegetations.

Aureolysin (*aur*)

The *aur* gene, which encodes for zinc metalloprotease aureolysin, was highly expressed in vegetations of PVE compared with NVE. Aureolysin is an important virulence factor responsible for evasion of host immune response, cleavage self-surface proteins (spread), and cleavage of host proteins (invasion).[28,29] Among all *S. aureus* proteases, aureolysin is the most effective virulence factors against complement cascade activation.[30] In addition to evasion of host immune response, aureolysin contributes to a phenomenon called "staphylocoagulation" via cleavage of prothrombin into thrombin, which enables *S. aureus* to be sealed within blood clots and escape phagocytosis.[31] In contrast, aureolysin activates urokinase and inactivates α_2-antiplasmin and plasminogen activator inhibitor-1, which results in dissemination of bacterial cell and promotes pathogenic invasion of the host tissues.[32] Within

the context of IE, these properties contribute to *S. aureus* clumping within the vegetation meshwork, but more important they might be important factors in fragmentation of the vegetation that results in distal embolization phenomena. Additionally, aureolysin cleaves *S. aureus* surface-associated proteins that further promote the switch from adherent to mobile and invasive phenotype.[31,33] With these properties, aureolysin regulates the biofilm growth cycle through mediating both coagulation and dissemination while protecting against host immune defenses. Thus, aureolysin provides a crucial virulence factor for *S. aureus* pathogenicity and persistence. It also can explain the aggressive invasive pathology associated with PVE.[5] *S. aureus* uses proteolytic enzymes to degrade tissues to secure a nutrient source that can result in leaflet perforation or damage in NVE. In PVE, *S. aureus* proteolytic enzymes would degrade the tissues surrounding the prosthesis, which may result in abscess formation or valve dehiscence. This could provide an explanation for the difference in *S. aureus* virulence between NVE and PVE.

Clinical Implications

The transcriptomic analysis supported by clinical pathology findings provides an invaluable in vivo demonstration of the tactics used by *S. aureus* to establish chronic infection and biofilm formation within cardiac vegetation in both NVE and PVE, and thus cannot be sterilized by antibiotics. Moreover, aureolysin production explains some of the associated phenomena of endocarditis, such as embolization and invasion. These findings should be taken into consideration when deciding on the timing for surgical intervention. Antibiotics can sterilize blood cultures, but not prosthetic valves colonized by *S. aureus* biofilm. Thus, delaying surgery for antibiotic response could result in progression of invasive pathology such as prosthesis dehiscence. The findings in this study suggest earlier intervention for PVE caused by *S. aureus*. Novel anti-biofilm therapeutics are under investigations that could be applied clinically in the future to control biofilm-associated infections.[11] Therapeutic targeting of *S. aureus* gene expression is an appealing approach to alter its virulence,[34] which highlights the importance of studying *S. aureus* transcriptome in IE.

In addition, the RNA-sequencing data of *S. aureus* virulence can set up the development of specific diagnostics to monitor the development of biofilm or invasive pathology (eg, aureolysin). In a novel study, Selan and colleagues[35] developed an enzyme-linked immunosorbent assay to detect antibodies against *S. aureus* biofilm antigens (slime polysaccharide antigens) in patients with surgical vascular graft infection. They have demonstrated elevated titers of immunoglobulin-M antibodies against *S. aureus* polysaccharide antigens in patients with ongoing infection as a sensitive and specific noninvasive diagnostic tool to detect *S. aureus* biofilm infection. Similar novel diagnostics can be developed to detect development of biofilm in clinical IE or invasion based on further in vivo analysis of *S. aureus* virulence factors.

Limitations

An important limitation to our study is the sample size. However, in this pilot study, we have demonstrated the feasibility of examining the in vivo gene expression of *S. aureus* in patients with IE using clinical samples, which are an invaluable resource to study molecular aspects of IE. Considering the small sample size and variability of gene expression among different samples, RNA-sequencing data analysis was presented as abundance of gene expression in each group with percentage among the group samples. To avoid any bias of using one reference *S. aureus* genome of a specific taxa, RNA-sequencing analysis was performed using combined database of merged bacterial reference genomes. Gene expression in blood culture *S. aureus* isolates can be altered by culture methods (however, the same for NVE and PVE samples) and antibiotic therapy. The analysis of the Endocarditis Registry data is retrospective and limited by its observational nature, which is subject to investigator bias. Despite these limitations, this study provides the first in vivo analysis of *S. aureus* virulence within cardiac valve vegetations in patients with active IE. We examined *S. aureus* transcriptome as the most common organism causing IE with significant morbidity and mortality.[10] Other organisms that need to be studied are *Streptococci* and *Enterococci*, which together with *S. aureus* are responsible for 80% of IE.[1]

CONCLUSIONS

Using a transcriptomics approach, we have demonstrated the feasibility to study in vivo virulence of *S. aureus* in clinical endocarditis. In this pilot study of 6 patients, *S. aureus* colonizing cardiac valve vegetations in both native and PVE expressed genes responsible for antibiotic resistance and biofilm formation. One critical virulence factor produced by *S. aureus* in IE, aureolysin, was highly expressed in PVE compared with NVE, which could explain the invasive pathology commonly associated with PVE. The results of the molecular analysis were complemented by clinical pathology data from a large surgical cohort of *S. aureus* endocarditis. The findings in this pilot study suggest a difference in *S. aureus* virulence between NVE and PVE, which could explain the difference in pathological features and outcomes. Better understanding of in vivo tactics used by *S. aureus* in IE can guide future studies dedicated to advance the current diagnostics and therapeutics to overcome this lethal disease.

Conflict of Interest Statement

H.E. has a financial relationship with Edwards Lifesciences, Artivion, and LifeNet Health. All other authors reported no conflicts of interest.

The *Journal* policy requires editors and reviewers to disclose conflicts of interest and to decline handling or reviewing manuscripts for which they may have a conflict of interest. The editors and reviewers of this article have no conflicts of interest.

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Key Words: biofilm, infective endocarditis, invasion, *Staphylococcus*, virulence

APPENDIX E1

Scanning Electron Microscope Imaging

For scanning electron microscope imaging, an explanted valve prosthesis sample was obtained from an additional patient undergoing surgery for PVE. The explanted surgical sample was fixed in a 2.5% glutaraldehyde solution in 0.2 M phosphate buffer for 3 days. On the fourth day, samples were washed with 0.1 M phosphate buffer and dehydrated using graded concentrations of ethanol. Samples were washed with hexamethyldisilazane (Ted Pella Inc) and left to dry overnight. Before scanning, samples were mounted and coated with gold. A Helios NanoLab 650 system with a field-emission scanning electron microscope gun electro source and focused gallium-ion beam was used for imaging.

APPENDIX E2

Although unadjusted duration of ICU stay is longer in PVE (median 141 hours) compared with NVE (median 97 hours), after multivariable regression analysis incorporating baseline differences, there is no difference between the groups ($P = .22$) (Table E5 shows parsimonious model after stepwise selection). Likewise, a multivariable logistic regression analysis for prolonged ventilation shows similar outcomes between the groups after adjusting for factors that are imbalanced at baseline ($P = .20$) (Table E6). No significant risk factors were found, but differences in the baseline characteristics were driving unadjusted results.

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TABLE E1. Baseline characteristics of patients undergoing surgery for left-sided endocarditis caused by *Staphylococcus aureus* (n = 438)

Variable	Native IE (n = 260)		Prosthetic IE (n = 178)		*P* value
	n*	No. (%) or mean ± SD	n*	No. (%) or mean ± SD	
Demographics					
Age (y)	260	52 ± 15	178	56 ± 14	.005
Female	260	102 (39)	178	51 (29)	.02
Body mass index (kg/m^2)	260	29 ± 8.4	177	29 ± 6.4	.41
Race	254		170		.53
White		222 (87)		152 (89)	
Black		24 (9.4)		15 (8.8)	
Other		8 (3.1)		3 (1.8)	
NYHA functional class	214		137		.0003
I		69 (32)		51 (37)	
II		65 (30)		41 (30)	
III		36 (17)		38 (28)	
IV		44 (21)		7 (5.1)	
Cardiac comorbidities					
Prior cardiac operations	260		178		<.0001
0		213 (82)		0 (0)	
1		43 (17)		120 (68)	
2		3 (1.2)		43 (24)	
≥3		1 (0.38)		15 (8.4)	
Heart failure	258	127 (49)	178	105 (59)	.045
Peripheral arterial disease	260	46 (18)	178	22 (12)	.13
Hypertension	260	171 (66)	178	130 (73)	.11
LV ejection fraction (%)	256	56 ± 9.5	172	53 ± 9.8	.0003
Noncardiac comorbidities					
Preoperative creatinine†	260	1.4 [0.8, 4.3]	178	1.3 [0.9, 3.5]	.66
Preoperative BUN†	260	22 [12, 54]	178	19 [12, 41]	.008
Preoperative bilirubin†	248	0.5 [0.3, 1.1]	170	0.7 [0.3, 1.5]	.0008
Preoperative hematocrit†	260	29 ± 4.5	178	30 ± 4.7	.31
History of smoking	248	143 (58)	173	108 (62)	.33
Pharmacologically treated diabetes	257	76 (30)	176	42 (24)	.16
COPD	260	44 (17)	178	44 (25)	.05
Dialysis	249	60 (24)	169	27 (16)	.045
Stroke	259	125 (48)	178	86 (48)	.99

IE, Infective endocarditis; *NYHA*, New York Heart Association; *LV*, left ventricle; *BUN*, blood urea nitrogen; *COPD*, chronic obstructive pulmonary disease. *Patients with data available. †Median [15th, 85th] percentiles reported due to skewed distribution.

TABLE E2. Operative details and perioperative complications of patients who underwent surgery for left-sided endocarditis caused by *Staphylococcus aureus* (n = 438)

Variable	Native IE (n = 260)		Prosthetic IE (n = 178)		*P* value
	n*	No. (%) or mean ± SD	n*	No. (%) or mean ± SD	
Valve involved	260		178		<.0001
Aortic valve		90 (35)		102 (57)	
Mitral valve		135 (52)		34 (19)	
Aortic and mitral valves		35 (13)		42 (24)	
Pathological features					
Invasive pathology	260	104 (40)	178	135 (76)	<.0001
Vegetations	260	242 (93)	178	143 (80)	<.0001
Cavity formation	260	27 (10)	178	79 (44)	<.0001
Surgery					
AVR with allograft	137	51 (37)	149	121 (81)	<.0001
AVR with mechanical valve	137	4 (2.9)	149	3 (2.0)	.56
AVR with bioprosthetic valve	137	72 (53)	149	24 (16)	<.0001
Cardiopulmonary bypass (min)	259	133 ± 62.8	178	202 ± 75.3	<.0001
Perioperative transfusion (RBC units)†	260	4 [1, 12]	178	7 [3, 18]	<.0001
Outcomes					
ICU length of stay (h)†	260	97 [30, 406]	177	141 [45, 546]	.034
Prolonged ventilation >24 h	258	100 (39)	176	97 (55)	.0008
Renal dysfunction requiring dialysis	189	17 (9)	142	17 (12)	.38
Septicemia	260	25 (10)	178	17 (10)	>.9
Stroke	260	17 (7)	178	9 (5)	.52
Hospital death	260	19 (7)	178	15 (8)	.67

IE, Infective endocarditis; *AVR*, aortic valve replacement; *RBC*, red blood cell; *ICU*, intensive care unit. *Patients with data available. †Median [15th, 85th] percentiles.

TABLE E3. Linear regression model for risk factors of intensive care unit stay

Factor	Parameter estimate	SE	*P* value
PVE (vs NVE)	−0.24336	0.19832	.22
Patient age at date of surgery	0.01401	0.00453	.002
Total No. of cardiac operations	0.21660	0.11512	.06
Intercept*	3.70160	0.28896	<.0001

PVE, Prosthetic valve endocarditis; *NVE*, native valve endocarditis. *Outcome modeled as log(ICU stay) in hours.

TABLE E4. Logistic regression model for risk of prolonged ventilation

Factor	Parameter estimate	SE	Wald chi-square	*P* value
PVE (vs NVE)	0.3727	0.2895	1.65	.20
Patient's age at date of surgery	0.0129	0.00661	3.79	.05
Total No. of cardiac operations	0.1815	0.1661	1.19	.27
Intercept	−1.3542	0.4257	10.12	.0015

C-statistic = 0.61. *PVE*, Prosthetic valve endocarditis; *NVE*, native valve endocarditis.

TABLE E5. List of the top differentially expressed *Staphylococcus aureus* genes in native (n = 3) and prosthetic (n = 3) valve vegetations, and related biological functions

Gene name	Gene function/protein	Biological function	References
aur	Zinc metalloproteinase aureolysin	Evasion of host immune response, cleavage self-surface proteins (spread), cleavage of host proteins (invasion)	E1,E2
dnaK	Molecular chaperone DnaK	Stress response (heat shock protein), biofilm formation	E3,E4
fusA	Elongation factor G′	Protein synthesis, antibiotic resistance, biofilm survival	E5,E6
clpB	Chaperone protein ClpB	Stress response, intracellular multiplication, regulation of virulence factors expression	E7-E9
ftsZ	Cell division protein FtsZ	Bacterial cell division, persisters growth	E10,E11
spsA	Signal peptidase I (SAOUHSC_00902)	Contribute to phagosomal escape, intracellular survival	E12
mnmA	tRNA-specific 2-thiouridylase MnmA	Protein synthesis, bacterial phenotypic traits including biofilm formation	E13
secY	Protein translocase subunit SecY	Quorum sensing, bacterial secretion systems, and protein export	E14,E15
agrA	Accessory gene regulator protein A	Quorum sensing, biofilm formation	E16-E18
pflD	Formate C-acetyltransferase	Metabolism	E19
pflB	Formate acetyltransferase	Metabolism, biofilm tolerance to anaerobic conditions	E20
fbA	Fructose-bisphosphate aldolase	Metabolism during infection	E21,E22
pbpC	Penicillin-binding protein 2	Cell wall biosynthesis and resistance to antimicrobial	E23,E24

TABLE E6. List of the top differentially expressed *Staphylococcus aureus* genes in blood cultures from patients with native (n = 3) and prosthetic (n = 3) valve endocarditis, and related biological functions

Gene name	Gene function/protein	Biological function	References
dnak	Molecular chaperone DnaK	Stress response (heat shock protein), biofilm formation	E3,E4
ebpB	Endocarditis and biofilm-associated pilus minor subunit EbpB	Pilus biogenesis, biofilm formation	E25
cydB	Cytochrome d ubiquinol oxidase, subunit II	Aerobic respiration, virulence factor for colonization, augmentation of multi-species biofilm	E26,E27
clfB	Clumping factor B	Attachment to human fibrinogen, promote bacterial clumping	E28
raiA	Ribosome hibernation promotion factor	Promote survival, recalcitrance, and infection relapse	E29
clpB	Chaperone protein ClpB	Stress response, intracellular multiplication, regulation of virulence factors expression	E7-E9
ebpA	Endocarditis and biofilm-associated pilus tip protein subunit A	Pilus biogenesis, biofilm formation	E30
fusA	Elongation factor G′	Protein synthesis, antibiotic resistance, biofilm survival	E5,E6
aur	Zinc metalloproteinase aureolysin	Evasion of host immune response, cleavage self-surface proteins (spread), cleavage of host proteins (invasion)	E1,E2
ahpC	Alkyl hydroperoxide reductase C	Oxidative stress response, biofilm virulence	E31,E32