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Staphylococcus aureus gene expression in a rat model of infective endocarditis

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Abstract

Background

Diabetes mellitus is a frequent underlying comorbidity in patients with *Staphylococcus aureus* endocarditis, and it represents a risk factor for complications and a negative outcome. The pathogenesis of staphylococcal endocardial infections in diabetic hosts has been poorly characterized, and little is known about *S. aureus* gene expression in endocardial vegetations.

Methods

We utilized a rat model of experimental *S. aureus* endocarditis to compare the pathogenesis of staphylococcal infection in diabetic and nondiabetic hosts and to study the global *S. aureus* transcriptome in endocardial vegetations in vivo.

Results

Diabetic rats had higher levels of bacteremia and larger endocardial vegetations than nondiabetic control animals. Microarray analyses revealed that 61 *S. aureus* genes were upregulated in diabetic rats, and the majority of these bacterial genes were involved in amino acid and carbohydrate metabolism. When bacterial gene expression in vivo (diabetic or nondiabetic endocardial vegetations) was compared to in vitro growth conditions, higher in vivo expression of genes encoding toxins and proteases was observed. Additionally, genes involved in the production of adhesins, capsular polysaccharide, and siderophores, as well as amino acid and carbohydrate transport and metabolism were upregulated in endocardial vegetations. To test the contribution of selected upregulated genes to the pathogenesis of staphylococcal endocarditis, isogenic deletion mutants were utilized. A mutant defective in production of the siderophore staphyloferrin B was attenuated in the endocarditis model, whereas the virulence of a surface adhesin (Δ*sdrCDE)* mutant was similar to that of the parental *S. aureus* strain.

Conclusions

Our results emphasize the relevance of diabetes mellitus as a risk factor for infectious endocarditis and provide a basis for understanding gene expression during staphylococcal infections in vivo.

Background

Infective endocarditis is an invasive human disease with an estimated incidence of 5 to 16 cases per 100,000 person-years [1,2] and an in-hospital mortality rate of 18-20% [3]. *Staphylococcus aureus* is the most frequent etiologic agent of endocarditis in developed countries [1,3] and is associated with an aggressive disease course, a poor outcome, and complications such as local destruction of valve tissue, septic emboli, and persistent bacteremia [3]. Antibiotic resistance is widespread among clinical *S. aureus* isolates, and bacteremia due to methicillin-resistant *S. aureus* (MRSA) often requires the use of expensive or less effective antibiotics.

Risk factors for endocarditis include injection drug use, prosthetic heart valves, structural heart defects, and comorbidities, such as diabetes [2,4,5]. Among patients with endocarditis, *S. aureus* is more frequently isolated from diabetic than nondiabetic individuals [6]. Moreover, diabetic patients suffer from a higher mortality due to *S. aureus* endocarditis than nondiabetic patients [7]. Although defects in the immune response to bacterial infections in diabetic patients have been postulated [8], the mechanism(s) behind the increased susceptibility to invasive *S. aureus* infections remain elusive.

Several *S. aureus* virulence factors have been implicated in the pathogenesis of endocarditis. Fibronectin binding protein A (FnBPA) and clumping factor A (ClfA) mediate staphylococcal adherence to endothelial cells [9,10]. Damage to cardiac valves results in exposure of the subendothelial matrix and deposition of fibrin and platelets at the site of endovascular injury. ClfA plays a role in *S. aureus* binding to platelets—an interaction that is critical to the induction of staphylococcal endocarditis [11]. Staphylococci recovered from rabbit endocardial vegetations are highly encapsulated [12]. Other factors shown to play a

role in the pathogenesis of endocarditis include gene regulators such as *sar* and *agr* [13], alpha toxin [14], the sortase enzyme SrtA [15] and the proline permease PutP [16].

Although numerous studies have described in vivo expression of selected staphylococcal genes [17,18]), few have investigated overall patterns of bacterial gene expression in an infected host. Analyses of global gene expression patterns have focused primarily on in vitro conditions, and few descriptions of the *S. aureus* transcriptome during the course of an infection have been reported [19,20]. The *S. aureus* transcriptional profile during growth in broth culture correlates poorly with gene expression in mammalian infections, and further work to characterize *S. aureus* gene regulation in vivo is necessary [21].

Our goal was to (i) assess the impact of concurrent diabetes mellitus on the course of endocarditis in a well defined experimental infection model, (ii) compare the transcriptional profile of *S. aureus* in established endocardial vegetations of diabetic and nondiabetic hosts versus growth in vitro, and (iii) study the role of genes highly expressed in vivo as virulence factors in *S. aureus* endocarditis.

Methods

Bacteria and culture conditions

To evaluate differences in endocarditis disease severity and for microarray analyses, diabetic or nondiabetic rats were challenged with the methicillin resistant *S. aureus* strain COL. To establish the role of selected genes in the pathogenesis of *S. aureus* endocarditis, we utilized *S. aureus* strain Newman and its isogenic mutants. Newman Δ*sdrCDE* [22] was kindly provided by Dr. Timothy Foster, and Newman Δ*sbnE* [23] was generously provided by Dr. David Heinrichs. Staphylococci were cultivated in tryptic soy broth (TSB) to the mid-logarithmic phase, washed in PBS, and diluted to yield an inoculum of ~3×10^4 CFU/rat.

Rat endocarditis model

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All animal experiments were approved by the Harvard Medical School Standing Committee on Animals or by the local authorities in Regensburg. Diabetes was induced one day prior to surgery by injecting male Sprague-Dawley rats (~200 g; Charles River Laboratories) in the tail vein with streptozotocin (60 mg/kg); control rats received citrate buffer only [24]. Animals were considered diabetic if blood glucose levels exceeded 250 mg/dl after 24 h.

The rat model of catheter-induced *S. aureus* endocarditis was described previously [25]. Catheterized diabetic or nondiabetic rats were challenged intravenously with ~3×10^4 CFU *S. aureus* 48 h after surgery. Heparinized blood was collected daily from each animal by tail vein puncture and plated quantitatively. Surviving rats were euthanized on day 3 after challenge. Catheter tips (~2 cm) were removed, sonicated in PBS, and plated quantitatively. The kidneys, spleens and aortic valve vegetations were weighed, homogenized, and cultured quantitatively. Homogenized vegetations were pelleted, suspended in RNAprotect Bacteria Reagent (Qiagen) overnight at 4 °C, and stored at -80 °C.

RNA isolation

Total RNA was prepared from homogenized vegetation pellets from single animals after digestion with 4 mg/ml proteinase K (Qiagen) for 30 min. *S. aureus* COL cells were lysed with 0.5 ml zirconia silica beads (Fisher Scientific) in a dental amalgamator, and RNA was purified with the RNeasy Mini kit (Qiagen), treated with DNase I (Invitrogen), and stored at -80 °C. RNA integrity and absence of eukaryotic RNA were confirmed by denaturing gel electrophoresis. RNA from planktonic cultures was isolated from bacteria harvested from mid-logarithmic (5 h) or stationary phase (18 h) TSB cultures incubated at 37 °C.

Microarray analysis

Ten nanograms RNA samples were amplified using ExpressArt Nanokits (Amsbio), according to the manufacturer's recommendations for bacterial mRNA amplification, except that the final transcription and labeling step was performed using Enzo BioArray Highyield RNA Transcript Labeling kits to incorporate biotinylated ribonucleotides into final RNA products. Each biotinylated RNA sample (1 μg) was hybridized to Affymetrix *S. aureus* GeneChips following the manufacturer's recommendations for antisense prokaryotic arrays. GeneChips were washed, stained, and scanned, as previously described [26]. Each condition (diabetic or nondiabetic rat and logarithmic- or stationary-phase culture) was analyzed in duplicate. GeneChip signal intensity values for each qualifier were then normalized, averaged and analyzed using GeneSpring 6.2 software (Silicon Genetics), as described [26]. Genes were considered to be induced in a vegetation if they were determined to be present by Affymetrix algorithms in the endocarditis condition, and if they exhibited a >2-fold increase in RNA titer (Student's *t*-test; $P \leq 0.05$) compared to the corresponding planktonic growth condition. Genes were considered down regulated in the vegetations if they were present in either planktonic condition and had an expression level <50% of that observed in the corresponding planktonic growth condition (Student's *t*-test; $P \leq 0.05$). All Genechip data have been deposited in the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) microarray repository under accession number GSE62390.

Quantitative PCR

cDNA was synthesized using the AffinityScript Multiple Temperature cDNA Synthesis Kit (Agilent Technologies), 5 μl of hot-denatured DNA-free RNA and 100 pmol of random hexamer primers. Products were precipitated in ethanol, resuspended in DEPC-water and stored at −20 °C. Quantification was performed using a LightCycler 2.0 apparatus (Roche) and QuantiTect SYBR Green PCR Kits (Qiagen), using 2 μl of cDNA and 20 pmol of each primer. Primers (Additional file 1: Table S1) were synthesized by Eurofins MWG.

Statistical analysis

Student's *t*-tests were used to compare gene expression data. Mann–Whitney *U* tests were performed to compare quantitative culture data, and the log rank test was used to compare survival distributions. A *P* value of ≤ 0.05 was considered significant.

Results

Infective endocarditis was more severe in diabetic than nondiabetic rats

To assess the impact of diabetes on the pathogenesis of *S. aureus* endocarditis induced by strain COL, we compared the infection in rats with streptozotocin-induced diabetes to that of nondiabetic rats. All rats showed increasing bacteremia levels from days 1 to 3 (Figure 1A). Diabetic rats had significantly higher levels of bacteremia than nondiabetic rats on days 2 and 3 after inoculation (Figure 1A). Diabetic rats had larger endocardial vegetations (Figure 1B) with significantly more bacteria per vegetation (Figure 1C). We also observed trends toward elevated bacterial burdens in kidneys, spleens and explanted catheters from diabetic rats compared to nondiabetic rats (Figure 1D), although these differences did not reach statistical significance.

Figure 1 Endocarditis was more severe in diabetic rats than in nondiabetic rats. (A) Diabetic animals had significantly higher levels of bacteremia on day 2 and day 3 after bacterial challenge. **(B)** Diabetic animals had larger vegetations, and **(C)** the vegetations were associated with a significantly higher bacterial burden. **(D)** The bacterial burden in the kidneys, spleens and catheters were not significantly different between diabetic and nondiabetic rats.

Differential *S. aureus* gene expression in diabetic and nondiabetic rats

We hypothesized that staphylococcal gene expression might be influenced by hyperglycemia in the diabetic host. Microarray analyses, however, indicated that overall gene expression was similar between the two groups of animals challenged with *S. aureus* COL. A total of 61 transcripts were expressed higher under diabetic in vivo conditions (selected genes are presented in Table 1). Most of these genes were associated with metabolic processes such as amino acid and carbohydrate metabolism or cell homeostasis. LightCycler analyses confirmed higher expression of ATP synthase *atpA* (12.9 ± 4.3 fold) and glyceraldehyde dehydrogenase *gapA* (10.6 ± 2.1 fold) in vegetations from diabetic rats compared to nondiabetic rats. Only two genes (SACOL1895 and a 102 bp fragment, both with unknown functions) were expressed significantly lower in the vegetations of diabetic compared to nondiabetic animals.

Table 1 List of selected genes differentially expressed in diabetic and nondiabetic vegetations

Gene	Diabetic vs. nondiabetic	Gene product	Functional group
acpS	2.6	holo-(acyl-carrier-protein) synthase	Lipid transport and metabolism
alr	2.9	alanine racemase	Cell wall/membrane biogenesis
argG	10.0	argininosuccinate synthase	Amino acid transport and metabolism
argH	15.3	argininosuccinate lyase	Amino acid transport and metabolism
atpA	3.8	ATP synthase F1, alpha subunit	Energy production and conversion
atpE	2.4	ATP synthase F0, C subunit	Energy production and conversion
atpH	3.3	ATP synthase F1, delta subunit	Energy production and conversion
cap5F	2.5	capsular polysaccharide biosynthesis	Cell wall/membrane biogenesis
cap5K	3.5	capsular polysaccharide biosynthesis	Cell wall/membrane biogenesis
ccrB	2.0	cassette chromosome recombinase B	
dnaE	2.6	DNA polymerase III, alpha subunit	Nucleotide metabolism, replication, recombination
fabD	3.3	malonyl CoA-acyl carrier protein transacylase	Lipid transport and metabolism
gapA	4.4	glyceraldehyde 3-P dehydrogenase	Carbohydrate transport and metabolism
geh	2.3	glycerol ester hydrolase	Miscellaneous
glpP	2.2	glycerol uptake operon regulatory protein	Transcription, translation and post-translational mod.
glyS	4.2	glycyl-tRNA synthetase	Transcription, translation and post-translational mod.
hemX	2.2	hemX protein	Transcription, translation and post-translational mod.
infB	2.6	translation initiation factor IF-2	Transcription, translation and post-translational mod.
kdpE	2.4	DNA-binding response regulator	Regulatory
ligA	2.3	DNA ligase, NAD-dependent	Nucleotide metabolism, replication, recombination
menD	2.1	carboxylic acid synthase	Coenzyme transport and metabolism
moeA	3.4	molybdopterin biosynthesis, putative	Coenzyme transport and metabolism
nrdF	2.7	ribonucleoside-diphosphate reductase 2	Nucleotide metabolism, replication, recombination
pls	3.7	Plasmin sensitive protein	Virulence
recQ	2.8	ATP-dependent DNA helicase	Nucleotide metabolism, replication, recombination
rexA	3.2	exonuclease	Nucleotide metabolism, replication, recombination
ribD	2.7	riboflavin biosynthesis protein	Coenzyme transport and metabolism
ribE	2.4	riboflavin synthase, alpha subunit	Coenzyme transport and metabolism
ruvB	2.2	Holliday junction DNA helicase	Nucleotide metabolism, replication, recombination
sasA	4.8	LPXTG cell wall surface protein	Virulence
sirC	3.1	iron ABC transporter, permease protein	Inorganic ion transport and metabolism
ureC	7.5	urease, alpha subunit	Amino acid transport and metabolism

Differential *S. aureus* gene expression in endocardial vegetations

To compare *S. aureus* gene expression in vivo vs. in vitro, we isolated bacterial RNA from endocardial vegetations on day 3 post-challenge and from staphylococci in the logarithmic or stationary growth phase. Overall gene expression in diabetic and nondiabetic animals was similar, but differences between in vivo and in vitro growth conditions tended to be more pronounced in vegetations from diabetic hosts (Table 2). Microarray analyses revealed that 116 and 109 genes were expressed higher in vegetations from nondiabetic and diabetic rats, respectively, compared to both planktonic conditions, 98 and 103 genes were expressed higher in vivo compared to logarithmic phase cultures only, and 81 and 65 genes were higher

in vivo compared to stationary phase cultures only. Additionally, we observed that 223 and 152 genes were expressed lower under in vivo conditions in nondiabetic and diabetic animals, respectively, compared to both planktonic conditions, 208 and 199 genes were expressed lower in vivo compared to logarithmic phase cultures only, and 183 and 106 genes were lower in vivo compared to stationary phase cultures only. The largest group of genes differentially expressed in vegetations was genes of unknown function (hypothetical proteins). The next largest groups were genes involved in transcription, translation and posttranslational modification, amino acid transport and metabolism, cell wall and membrane biogenesis, nucleotide metabolism and replication, carbohydrate transport and metabolism, and virulence/immune evasion mechanisms (Figure 2). Numerous virulence-associated genes, such as toxins and proteases, were expressed higher in endocardial vegetations than in planktonic cultures.

Table 2 Selected genes that were differentially expressed in endocardial vegetations

Gene		Nondiabetic vs. log	vegetation vs. stat.	Diabetic vs. log	vegetation vs. stat.	Gene product
Virulence associated genes						
hla	SACOL1173	22.9	n.s.	17.5	n.s	alpha-hemolysin precursor
hld	SACOL2022	38.5	n.s.	37.8	n.s.	delta-hemolysin
hlgA	SACOL2419	18.5	5.4	6.3	2.1	gamma-hemolysin, component A
hlgB	SACOL2421	11.8	2.7	n.s	n.s	gamma hemolysin, component B
hlgC	SACOL2422	16.5	2.4	n.s	n.s	gamma hemolysin, component C
lukF	SACOL2004	11.1	18.7	4.9	8.1	leukocidin F subunit
lukS	SACOL2006	11.9	14.7	6.4	8.0	leukocidin S subunit
nuc	SACOL0860	n.s.	n.s.	2.9	7.8	thermonuclease
seb	SACOL0907	8.0	n.s.	4.1	n.s.	staphylococcal enterotoxin B
splB	SACOL1868	10.7	5.7	n.s.	n.s.	serine protease
splC	SACOL1867	n.s.	n.s.	3.4	2.5	serine protease
splD	SACOL1866	n.s.	n.s.	3.8	2.9	serine protease
splE	SACOL1865	28.4	11.6	16.9	7.5	serine protease
aur	SACOL2659	3.3	2.4	2.2	n.s.	zinc metalloproteinase aureolysin
clpB	SACOL0979	n.s.	n.s.	4.4	8.1	ATP-dependent protease
ssaA	SACOL2581	5.7	13.1	2.6	5.1	staphylococcal secretory antigen; staphyloxanthin biosynthesis protein
sodA	SACOL1610	-5.7	-4.5	-5.7	-4.5	superoxide dismutase
Cell wall and capsule synthesis						
cap5A	SACOL0136	8.5	2.8	8.8	3.0	capsular polysaccharide biosynthesis protein
cap5B	SACOL0137	7.3	2.6	7.4	2.6	capsular polysaccharide biosynthesis protein
cap5D	SACOL0139	6.3	2.2	7.4	3.8	capsular polysaccharide biosynthesis protein
cap5E	SACOL0140	13.4	10.2	9.1	6.8	capsular polysaccharide biosynthesis protein
cap5F	SACOL0141	5.3	3.6	n.s.	n.s.	capsular polysaccharide biosynthesis protein
cap5G	SACOL0142	4.3	2.5	6.6	4.0	capsular polysaccharide biosynthesis protein
cap5I	SACOL0144	3.3	n.s.	4.1	2.3	capsular polysaccharide biosynthesis protein
cap5J	SACOL0145	n.s.	n.s.	2.2	2.4	capsular polysaccharide biosynthesis protein
dltA	SACOL0935	-8.5	-2.9	-6.2	-2.1	D-alanine-ligase subunit 1
dltB	SACOL0936	-10.8	-3.4	-9.5	-2.8	D-alanyl-lipoteichoic acid biosynthesis protein
dltC	SACOL0937	-4.9	n.s.	-4.9	n.s.	D-alanine-ligase subunit 2
dltD	SACOL0938	-13.5	-3.5	-7.4	-2.0	D-alanine transfer protein

Surface proteins						
clfA	SACOL0856	5.8	n.s.	5.6	n.s.	clumping factor A
clfB	SACOL2652	n.s.	n.s.	n.s.	3.1	clumping factor B
fnbA	SACOL2511	3.4	2.7	n.s.	n.s.	fibronectin binding protein A
sasF	SACOL2668	12.2	10.5	14.4	12.9	LPXTG cell wall surface anchor family protein
sdrC	SACOL0519	12.3	8.7	8.6	6.5	Ser-Asp repeat protein
pls	SACOL0050	-2.3	-3.3	n.s.	-4.3	surface protein
sasA	SACOL2676	n.s.	-9.8	2.2	-4.2	LPXTG cell wall surface anchor family protein
spa	SACOL0095	-6.3	-4.9	-5.7	-4.5	immunoglobulin G binding protein A prec.
iron uptake and iron regulated genes						
sbnA	SACOL0112	n.s.	n.s.	48.9	22.2	siderophore biosynthesis protein
sbnB	SACOL0101	n.s.	n.s.	25.9	19.7	siderophore biosynthesis protein
sbnD	SACOL0103	n.s.	n.s.	18.4	17.1	siderophore biosynthesis protein
sbnE	SACOL0104	n.s.	n.s.	23.0	18.4	siderophore biosynthesis protein
sbnG	SACOL0106	n.s.	n.s.	21.6	25.2	siderophore biosynthesis protein
sbnH	SACOL0107	n.s.	n.s.	15.4	16.8	siderophore biosynthesis protein
sbnI	SACOL0108	n.s.	n.s.	23.5	30.0	siderophore biosynthesis protein
sirR	SACOL0691	2.3	n.s.	n.s.	n.s.	iron dependent repressor
sirB	SACOL0098	2.9	n.s.	n.s.	n.s.	iron compound ABC transporter, permease
	SACOL2167	n.s	n.s.	2.9	n.s.	iron complex transport substrate-binding protein
	SACOL2169	4.4	n.s.	4.2	n.s.	aerobactin biosynthesis protein
	SACOL2170	n.s.	n.s.	2.6	n.s.	major facilitator transporter
transport						
isdD	SACOL1142	3.1	n.s.	2.3	n.s.	Heme ABC transporter
dapF	SACOL2479	18.2	18.9	15.2	15.6	diaminopimelate epimerase family protein
	SACOL2478	9.2	9.4	7.8	8.3	conserved hypothetical protein
	SACOL2477	17.6	12.8	20.9	15.1	conserved hypothetical protein
opp1A	SACOL2476	18.1	11.1	10.8	13.9	peptide ABC transporter
opp1B	SACOL2475	4.3	3.4	11.2	9.0	peptide ABC transporter, permease
opp1C	SACOL2474	15.9	12.9	30.4	24.0	peptide ABC transporter, permease
opp1D	SACOL2473	n.s.	n.s.	8.0	7.3	peptide ABC transporter ATP-binding protein
opp1F	SACOL2472	n.s.	n.s.	21.5	20.9	peptide ABC transporter ATP-binding protein
norD	SACOL2471	17.8	12.3	27.0	18.5	transporter, putative
epiG	SACOL1871	2.4	n.s.	2.6	n.s.	lantibiotic ABC transporter protein
epiE	SACOL1872	4.2	n.s.	4.4	n.s.	lantibiotic ABC transporter protein
epiF	SACOL1873	5.5	n.s.	5.7	n.s.	lantibiotic ABC transporter protein
malE	SACOL0193	n.s.	n.s.	3.1	2.5	maltose ABC transporter, maltose-binding
malF	SACOL0194	7.4	3.9	6.0	3.5	maltose ABC transporter, permease protein
malK	SACOL0192	14.7	14.7	6.2	6.8	maltose ABC transporter, ATP-binding protein
opuD	SACOL2176	2.0	2.4	n.s.	n.s.	osmoprotectant transporter, BCCT family
tet38	SACOL0122	21.2	12.2	13.1	8.9	tetracycline resistance protein, putative
ulaA	SACOL0400	12.9	5.6	7.3	3.2	PTS system ascorbate-specific transporter
uhpT	SACOL0200	125	29	78.1	18.1	sugar phosphate antiporter
kdpA	SACOL2068	n.s.	n.s.	8.5	15.0	potassium-transporting ATPase, C subunit

kdpB	SACOL2067	n.s.	n.s.	9.0	13.3	potassium-transporting P-type ATPase, B unit
norB	SACOL1475	25.1	27.5	16.1	17.3	drug transporter, quinolone resistance protein
	SACOL1476	24.8	22.4	18.7	18.6	transmembrane amino acid transporter
ilvA	SACOL1477	34.0	25.0	28.7	22.7	threonine dehydratase
ald	SACOL1478	42.3	24.0	31.2	17.6	alanine dehydrogenase
mntH	SACOL1114	-9.5	-6.3	-9.4	-6.3	Mn2+/Fe2+ transporter
	SACOL1115	-3.9	-4.0	-4.3	-4.4	hypothetical protein
mntC	SACOL0688	3.7	-5.8	3.5	-6.3	ABC transporter substrate-binding protein
mntB	SACOL0689	4.1	-5.7	4.5	-4.7	ABC transporter permease
mntA	SACOL0690	8.5	-2.7	5.6	-4.4	ABC transporter ATP-binding protein
	SACOL0157	-11.4	-12.0	-12.9	-12.8	conserved hypothetical protein
	SACOL0158	-6.8	-15.2	-6.1	-13.4	ABC transporter, ATP-binding protein
	SACOL0159	-6.9	-8.2	-5.0	-6.1	ABC transporter, permease protein
	SACOL0160	-4.9	-7.1	-5.5	-7.9	conserved hypothetical protein
metabolism						
adhE	SACOL0135	22.9	22.3	30.9	31.1	alcohol dehydrogenase, iron-containing
ald	SACOL1478	42.3	24.0	31.2	17.6	alanine dehydrogenase
arcB1	SACOL1181	n.s.	n.s.	2.2	2.5	ornithine carbamoyltransferase
arcC	SACOL1182	21.8	15.3	12.9	9.9	carbamate kinase
arcD	SACOL1183	8.7	5.9	6.8	4.8	arginine/ornithine antiporter
arcA	SACOL2657	12.4	12.2	8.4	8.5	arginine deiminase
arcB2	SACOL2656	6.4	7.6	4.6	5.3	ornithine carbamoyltransferase
arcD	SACOL2655	30.1	43.5	27.6	43.1	arginine/ornithine antiporter
argG	SACOL0963	n.s.	n.s.	3.4	6.4	argininosuccinate synthase
argH	SACOL0964	n.s.	n.s.	5.4	13.3	argininosuccinate lyase
deoB	SACOL0124	4.0	2.2	4.4	2.3	phosphopentomutase
deoD	SACOL0121	7.1	7.8	8.9	10.0	purine nucleoside phosphorylase
gltA	SACOL1742	6.9	-6.7	4.8	-9.4	citrate synthase
gntK	SACOL2515	6.9	4.9	n.s.	n.s.	gluconokinase
ilvA	SACOL1477	34.0	25.0	28.7	22.7	threonine dehydratase
nirB	SACOL2398	21.8	19.6	12.8	11.9	nitrite reductase [NAD(P)H], large subunit
nirD	SACOL2397	8.1	6.1	4.7	3.9	nitrite reductase [NAD(P)H], small subunit
pflA	SACOL0205	94.7	125.7	116.5	157.6	pyruvate formate-lyase-activating enzyme
pflB	SACOL0204	44.7	46.9	59.9	65.6	formate acetyltransferase
rbsK	SACOL0253	6.9	2.5	4.9	n.s.	ribokinase
	SACOL2396	19.0	14.3	7.4	6.1	uroporphyrin-III C-methyl transferase
	SACOL1476	24.8	22.4	18.7	18.6	amino acid permease
regulators						
agrA	SACOL2026	n.s.	n.s.	4.8	n.s.	accessory gene regulator protein A
agrC	SACOL2025	-2.6	-3.3	4.5	-2.2	accessory gene regulator protein C
agrD	SACOL2024	2.3	-4.0	5.2	n.s.	accessory gene regulator protein D
gntR	SACOL2516	5.0	4.1	n.s.	n.s.	gluconate operon transcriptional repressor
purR	SACOL0539	n.s.	2.3	n.s.	n.s.	pur operon repressor
rsbV	SACOL2056	n.s.	3.6	n.s.	3.7	anti-anti-sigma factor RsbV
rsbW	SACOL2055	n.s.	2.7	n.s.	3.0	anti-sigma B factor
sarA	SACOL0672	n.s.	2.5	n.s.	2.1	transcriptional regulator
sarS	SACOL0096	-14.7	-7.2	-8.7	-4.2	transcriptional regulator
sigB	SACOL2054	n.s.	n.s.	n.s.	2.1	alternative sigma factor

fur	SACOL1541	n.s.	n.s.	-2.1	-3.1	transcriptional regulator, Fur family
araC	SACOL2378	-3.6	-2.7	n.s.	n.s.	transcriptional regulator, AraC family
tetR	SACOL2374	n.s.	n.s.	-2.0	-2.4	transcriptional regulator, TetR family
vraR	SACOL1942	-4.8	-3.2	-3.7	-2.5	DNA-binding response regulator
vraS	SACOL1943	-4.3	-2.8	-3.3	-2.2	sensor histidine kinase

n.s. difference not significant.

Figure 2 Number of genes differentially expressed in endocardial vegetations from nondiabetic (A) and diabetic (B) rats. Absolute number of genes down (left) or upregulated (right) in endocardial vegetations compared to in vitro exponential (red bars), stationary (blue bars), or both growth phases (black bars). The four groups with the highest number of genes expressed higher in vivo were amino acid transport and metabolism, carbohydrate transport and metabolism, virulence / immune evasion, and transcription, translation and posttranslational modification.

A selection of *S. aureus* genes differentially expressed in endocardial vegetations compared to planktonic growth conditions in vitro is presented in Table 2. Among the virulence-associated up-regulated genes were the alpha-, delta-, and gamma-hemolysins and the bi-component leukocidins LukS and LukF, which were expressed higher under in vivo conditions compared to in vitro conditions. Likewise, genes encoding the metalloprotease aureolysin, the serine proteases SplBCDE, and the staphylococcal secretory antigen SsaA were all upregulated in vivo. Staphylococcal enterotoxin B (*seb*) showed higher expression in vegetations and in stationary growth phase compared to *S. aureus* in logarithmic growth phase. Neither *srtA* (encoding sortase A) nor *putP* (proline permease) showed differential expression in vivo. The superoxide dismutase *sodA* was significantly down regulated in endocardial vegetations compared to both in vitro conditions.

The capsule synthesis operon (*cap5A* to *cap5J*) was expressed higher in vegetations than in either planktonic growth phases, whereas the *dltABCD* operon (encoding enzymes mediating D-alanylation of teichoic acids in the cell envelope) was down regulated in vivo compared to in vitro. Regarding the *S. aureus ica* locus that encodes poly-N-acetyl glucosamine, apparently COL does not synthesize this surface-associated polymer since it has a mutated *icaC* gene (SACOL2692). Most of the other *ica* genes were not expressed differentially in vivo or in vitro.

Among staphylococcal surface proteins, clumping factor A (ClfA) and B (ClfB) genes were expressed higher in vivo compared to logarithmic or stationary growth phase planktonic cultures, respectively. The fibronectin binding protein A was significantly upregulated in the vegetations of nondiabetic rats. Genes encoding the LPXTG anchored proteins SdrC (a beta-neurexin binding protein) and SasF (associated with resistance to linoleic acid) were up regulated in vivo compared to both in vitro conditions. Of note, expression of both *spa* (endoding protein A) and *pls* (encoding a surface-associated plasmin-sensitive protein) were significantly lower in vivo than in vitro.

Regarding iron acquisition, we observed high in vivo expression of the *sbn* operon encoding proteins required for the synthesis of the nonhydroxamate siderophore staphyloferrin B. *SbnABDEGHI* were significantly upregulated in vegetations from diabetic animals compared to logarithmic and stationary phase planktonic cultures. The same trend and magnitude of changes in expression were also observed in nondiabetic vegetations, but were not considered

statistically significant. In addition, *sirB*, part of the *sirABC* operon encoding transport of staphyloferrin B into the bacterial cell [23,27], showed significant up regulation in nondiabetic rats with endocarditis. Some of the genes encoding the siderophore staphyloferrin A (SACOL2167, SACOL2169, and SACOL2170 [28]) were upregulated in endocardial vegetations (Table 2).

Several of the iron-regulated *S. aureus* genes were also involved in transport. Among the Isd genes that mediate heme uptake, only *isdD* (a heme ABC transporter) was consistently up regulated in vivo and only vs. logarithmic phase *S. aureus*. Other iron-regulated genes expressed significantly higher in vivo included the oligopeptide permease and *norD* genes of the *opp1ABCDFnorD* operon [29]. Numerous genes encoding transporters were highly expressed by *S. aureus* in endocardial vegetations. The maltose transport (*malEFK*) genes were almost uniformly upregulated in vivo by staphylococci recovered from infected vegetations of nondiabetic and diabetic rats. Similarly, *kdpAB* genes encoding a potassium-transporting ATPase were upregulated in diabetic vegetations compared to both in vitro growth conditions. Other upregulated transporters include *epiGEF* involved in lantibiotic transport and *tet38 and norB*, mediating drug resistance. Of note, *mntH* (a Mn2+/Fe2+ transporter) was down regulated in vivo compared to both planktonic conditions, where the *mntABC* operon (involved in Mn2+ uptake, [30]) was upregulated compared to logarithmic phase *S. aureus* and down regulated compared to stationary phase cells. A large uncharacterized transporter operon (SACOL2471-2479) was upregulated in vivo, whereas the SACOL0157 to SACOL0160 operon had significantly lower expression levels in vegetations than in planktonic cultures.

Genes associated with metabolic pathways and expressed higher in vegetations were numerous and included an alcohol dehydrogenase (*adhE*), nitrite reductase (*nirBD*), and genes implicated in arginine (*argGH* and two *arc* operons) and pyruvate metabolism (*pflAB*).

Transcription of a few staphylococcal regulatory genes was enhanced in vivo. The transcriptional regulator GntR showed higher expression in vivo in nondiabetic rats. In contrast, genes encoding the staphylococcal accessory regulator SarS, the iron regulated repressor Fur, the regulators VraR and VraS, and the transcriptional regulator AraC were expressed lower in vivo than in vitro (Table 2). The *agr* locus did not show consistent changes in vivo compared to in vitro (Table 2), although in diabetic rats the locus was upregulated compared to in vitro logarithmic-phase cultures.

Confirmation of upregulated genes in endocardial vegetations by real-time PCR

We used quantitative PCR to confirm differential expression of selected genes in vivo. Genes were selected based on (i) upregulation in microarray analysis, (ii) being a potential virulence factor and (iii) not having been implicated in the pathogenesis of endocarditis previously. In vitro or in vivo expression of *sbnC, sdrC* and *splB* was thus quantified using the LightCycler real-time PCR system. Although the magnitude of the differences in gene expression varied, we observed an overall good correlation between gene expression measured by microarray or LightCycler. All three genes tested showed a trend towards higher gene expression levels in vivo, but only *splB* showed significantly higher gene expression in endocardial vegetations compared to both planktonic growth phases (60.4 fold upregulation versus logarithmic growth phase, $P < 0.01$, and 8.6 fold versus stationary growth phase, $P = 0.03$). Expression levels of *sbnC* and *sdrC* in endocardial vegetations were significantly higher compared to

logarithmic growth phase *S. aureus* (502.3 fold for *sbnC*, $P < 0.01$, and 25.1 fold for *sdrC*, $P < 0.01$).

Role of selected genes in the pathogenesis of *S. aureus* endocarditis

To assess the contribution of selected genes preferentially expressed in vivo to the pathogenesis of *S. aureus* endocarditis, we performed virulence studies in the nondiabetic rat endocarditis model. We chose genes that had not previously been implicated as important in endocarditis – the *sbn* locus (encoding staphyloferrin B) and the *sdrCDE* locus (involved in fibrinogen-mediated *S. aureus* adherence to platelets under in vitro shear flow conditions [31]). Rats infected with Newman Δ*sbnE* survived longer than rats infected with the parental isolate (Figure 3A; $P = 0.047$, Log-rank analysis), and they had lower bacteremia levels at all time points, although the differences reached significance only on day 2 ($P = 0.039$) (Figure 3B). Likewise, rats infected with strain Newman experienced greater weight loss ($P = 0.002$) than rats infected with the *sbnE* mutant (Figure 3C). No significant differences were observed in the bacterial burden in kidneys (Figure 3D) or vegetations (Figure 3E) from rats infected with Newman or the Δ*sbnE* mutant. In contrast, rats infected with strain Newman or the Δ*sdrCDE* mutant had similar survival times (not shown), bacteremia levels (Additional file 1: Figure S1A), and weight loss (Additional file 1: Figure S1B). Likewise, the bacterial burdens in the kidneys (Additional file 1: Figure S1C) and endocardial vegetations (Additional file 1: Figure S1D) were comparable for wild type and Δ*sdrCDE* strains.

Figure 3 Comparative virulence of strain Newman and its isogenic *sbnE* deletion mutant. (A) Compared to rats infected with strain Newman, rats infected with the *sbnE* mutant (defective in the synthesis of the siderophore staphyloferrin **B)** lived significantly longer than rats infected with the parental strain, **(B)** had significantly lower levels of bacteremia on day 2, and **(C)** lost significantly less weight 48 h after bacterial inoculation. **(D)** Bacterial burdens in the kidneys and **(E)** vegetations were not significantly different between wild type and mutant strain.

Discussion

Diabetic patients have a higher risk of staphylococcal infections than nondiabetic hosts, and diabetic patients who develop *S. aureus* infective endocarditis are likely to experience a poor outcome [2,5]. We have demonstrated in an animal model of *S. aureus* endocarditis that diabetes is associated with a more severe disease course, as reflected by higher levels of bacteremia and larger endocardial vegetations. We used the vegetations recovered from infected rats on infection day 3 to investigate the in vivo transcriptome of *S. aureus* in the setting of an acute infection. This time point allowed maximal recovery of organisms multiplying within the host and allowed us to recover sufficient bacterial RNA for analysis.

Characterization of gene regulation and gene expression under in vivo conditions is a prerequisite for alternative treatment approaches, including the development of new drug targets and vaccination strategies. The *S. aureus* transcriptome under in vivo conditions has not been well characterized. Global *S. aureus* gene expression in vitro has been investigated after in vitro exposure to microbicides [32], in response to mild acid [33], during growth in biofilm [34], in blood [35] and after phagocytosis [36]. Changes in *S. aureus* gene expression during early adaptation to the mouse lung were described [19], and Date et al. recently reported global *S. aureus* gene expression in human abscesses and infected murine kidneys

[20]. Our study is the first description of the *S. aureus* transcriptome during an established endocardial infection. We used rich medium (TSB) as an in vitro comparator condition, although minimal medium such as RPMI may better reflect iron-limited conditions in vivo. The choice of TSB as the in vitro comparator medium may explain some of the differences in gene expression reported (e.g. iron rich medium versus iron depleted in vivo conditions). However, one of the aims of our study was to compare the gene expression pattern observed in endocarditis vegetations to gene expression found during growth under standard laboratory conditions. Our approach thus ensures comparability to previously published in vitro data (including previous microarray data, e.g. [26,36-38]) and highlights the important contribution of in vivo conditions (e.g. iron starvation) to *S. aureus* gene expression during an infection. An additional limitation of our study is the lack of a glucose supplemented in vitro control arm. However, the fact that we did not observe significant differences in *S. aureus* virulence gene expression between nondiabetic and diabetic rats makes this additional control less important.

S. aureus COL is a well defined and sequenced MRSA strain that we used for the experiments with diabetic vs. nondiabetic rats and the microarray analyses. Moreover, all gene expression studies and subsequent analyses were performed with strain COL. A related MSSA strain (Newman [39]) was used for the single gene knockout studies because of the availability of relevant mutant strains. Mutations in strains Newman and COL have been described [40-42], and both strains are members of clonal complex 8 and readily produce endocarditis in rats.

We observed that >100 genes were upregulated in endocardial vegetations compared to in vitro cultures. However, the number of genes significantly down regulated in vivo was approximately two-fold higher than the number of genes upregulated in vivo. This may be due to the fact that in vivo *S. aureus* gene expression was analyzed from vegetations three days after bacterial challenge. Date et al. [20] reported similar gene expression patterns in human abscesses and infected murine kidneys for some *S. aureus* genes such as the serine proteases *spl*, gamma-hemolysin and the *opp1* operon. Other genes with higher expression levels in abscesses such as the *isd* genes, however, were not differentially expressed in endocardial vegetations in our study.

A majority of the genes that were differentially expressed in endocardial vegetations have not yet been assigned a function or are classified as hypothetical proteins. ClfA is a critical virulence factor in *S. aureus* endocarditis [10] and *clfA* transcript levels have been reported to increase after the late log growth phase in vitro [43]. In our study, *clfA* was upregulated in vivo versus logarithmic growth phase cells, whereas fibronectin binding protein A was found to be significantly upregulated in nondiabetic vegetations only. Both are likely to be critical for initiation of infection, as has been described in previous studies [44,45]. Although acapsular strains provoke endocarditis at lower inocula than encapsulated isolates [46], *S. aureus* recovered from vegetations were shown to produce large quantities of capsular polysaccharides [12], consistent with our observation that the *cap5* genes were upregulated in established vegetations. Capsule production enhances bacteremia in vivo and renders the bacterium resistant to uptake and killing by neutrophils [47]. Moreover, active and/or passive immunization strategies targeting capsular polysaccharides were able to protect rodents against *S. aureus* bacteremia [48] and endocarditis [25]. In contrast to the *cap5* genes, the *dlt* operon was down regulated in endocardial vegetations. The *dlt* genes encode proteins mediating D-alanylation of lipoteichoic and wall teichoic acids, and transcription of the *dlt* genes is repressed in the presence of cations such as magnesium salt [49]. Higher *dlt*

expression has been associated with daptomycin resistance [50], and the observation that *dlt* is down regulated in endocardial vegetations could thus support clinical and preclinical data on the use of daptomycin in staphylococcal endocarditis [51,52].

Other virulence factors upregulated in vivo include exotoxins (hemolysins and leukotoxins) and proteases. The serine protease genes *splA – splE* are located on a pathogenicity island [39], and these proteases play a role in the degradation and inactivation of antibacterial peptides [53]. Both Spl proteases and aureolysin (*aur*) are associated with detachment of *S. aureus* from established biofilms [54] and may promote bacteremia by dispersing staphylococci from endocardial vegetations. Exotoxins upregulated in endocardial vegetations include two-component pore-forming cytolysins such as gamma-hemolysin and LukFS that are able to lyse erythrocytes and leukocytes [55]. Other factors previously implicated in the pathogenesis of endocarditis such as the sortase enzyme SrtA [15] and the proline permease PutP [16] were not differentially expressed in endocardial vegetations compared to planktonic conditions.

To acquire iron from the host organism, *S. aureus* synthesizes two major nonhydroxamate siderophores (staphyloferrin A and staphyloferrin B [28,56]), as well as proteins that mediate import and utilization of iron bound to heme or transferrin [57]. Although heme was identified as a preferred iron source for *S. aureus* [57], we found neither the iron surface determinant *(isd*) nor the heme transport system (*hts*) genes significantly upregulated in endocardial vegetations (with the exception of *isdD* compared to exponential phase cultures). In contrast, the staphyloferrin B synthesis operon (*sbnA – sbnI*) was preferentially upregulated in vivo. There was also a trend towards higher expression in vivo of the *fhuCBG* operon encoding an ABC transporter for iron(III)-hydroxamates [58]. However, *fhuD1* and *fhuD2*, encoding iron(III)-hydroxamate-binding lipoproteins, were not upregulated in endocarditis (not shown). The observed differences in iron uptake systems may in part be attributable to different experimental conditions and the animal models employed (e.g. [59]).

Dale et al. [23] constructed a Newman mutant (*ΔsbnE*) deficient in staphyloferrin B synthesis, and introduction of *sbnE* on a plasmid complemented the inability of the mutant to produce the siderophore under iron-limiting conditions. The Newman *ΔsbnE* mutant was compromised in the *S. aureus* renal abscess model [23]. The Newman *ΔsbnE* was attenuated in the endocarditis model since rats inoculated with the mutant showed lower bacteremia levels, reduced weight loss and increased survival compared to animals challenged with the parental strain. Although the reduction in virulence is modest, this can likely be attributed to the multitude of virulence factors involved in the pathogenesis of staphylococcal endocarditis. Thus, staphyloferrin B appears to promote staphylococcal virulence in abscesses and endocarditis.

Genes encoding multiple transporters were highly expressed in endocardial vegetations, including amino acid permeases, the lantibiotic transporter proteins EpiEFG, a maltose transporter, a potassium ATPase, the *norD-opp1* operon and genes associated with drug resistance such as *tet38* and *norB*. NorB promoted bacterial survival in a subcutaneous abscess model [17], and it has broad substrate specificity. Upregulation of *norB* under conditions of low pH or reduced aeration has been reported [60]. Similarly, the efflux pump transporter NorD is iron-regulated and highly expressed in staphylococcal abscesses [29]. *NorD* is part of an operon with five upstream oligopeptide permease genes (*opp1ABCDF*), and all were significantly upregulated in endocardial vegetations. NorB and NorD efflux

pumps may eliminate toxic metabolites or antibacterial factors produced by the host *in vivo*, although their substrates remain to be defined.

Among the surface molecules expressed higher in endocardial vegetations were the LPXTG-containing protein SasF, and the β-neurexin binding protein SdrC. Genes within the *sdr* region (*sdrCDE)* have been reported to be differentially transcribed [61], and *sdr* genes other than *sdrC* were not upregulated in endocardial vegetations. SdrC could mediate initial contact with host cells or cell adhesion molecules, such as β-neurexin [62], although our experimental endocarditis data indicate that it was not critical for the establishment of endocarditis. SdrC may play a role in complications of infectious endocarditis, such as metastatic seeding to neuronal tissues.

The largest group of genes differentially expressed in vivo were involved in amino acid transport and metabolism. The chromosomal arginine deiminase pathway (*arcABCD*) was strongly upregulated in endocardial vegetations. Arginine metabolism may serve to counter acidification or serve as an energy source under anaerobic conditions [63]. The latter is supported by the fact that nitrate reductase (NirBD) and pyruvate formate lyase (PflAB, [64]) were also upregulated in endocardial vegetations. Expression of the superoxide dismutase gene s*odA*, which is induced under microaerophilic conditions [65], was reduced in endocardial vegetations. Although staphylococcal vegetations grow in the oxygen-rich bloodstream at the aortic valve, the microenvironment within the vegetation may be oxygen-starved. Of note, we also observed differential gene expression of several gene clusters with yet unknown function (e.g. upregulation of SACOL2477 to SACOL2479 and downregulation of SACOL0157 to SACOL0160 in endocardial vegetations). Their role in staphylococcal infections and contribution to the pathogenesis of endocarditis in particular remains to be determined.

S. aureus gene expression is controlled by a complex network of regulatory proteins. In a previous study, expression of *S. aureus agr* in rabbit endocardial vegetations was shown to increase over time and to correlate with bacterial densities in vegetations [66]. We observed that the *agr* operon was only consistently upregulated in endocardial vegetations from diabetic rats compared to cultures harvested from the logarithmic growth phase. The *S. aureus fur* regulator, which represses genes involved in iron acquisition, cytolysins, and immunomodulatory proteins [67], was also expressed lower in endocardial vegetations than in vitro, as was the transcriptional regulator *sarS*. Differential expression of other regulatory genes included down regulation of *vraSR* (involved in resistance to cell wall active antibiotics and antimicrobial peptides [68]) and the *araC* family regulator (linking environmental chemical signals and virulence factors [69]) and upregulation of the gluconate repressor operon *gntR*. The *saeS* gene was significantly down regulated in vivo in the vegetations of diabetic rats.

With regard to genes differentially expressed in endocardial vegetations from diabetic and nondiabetic rats, we found 61 genes with higher expression levels in vegetations from diabetic animals. Although bacterial densities in the vegetations from both groups were similar (not shown), vegetations from diabetic animals were significantly larger and contained more bacteria than their nondiabetic counterparts. The composition or size of these vegetations may have an influence on staphylococcal gene expression in endocarditis. Of note, several of the differentially expressed genes with high upregulation (*argG*, *argH*, *glyS*, *ureC*) were previously reported to be upregulated in biofilms versus planktonic cultures [26]. Other genes found to be upregulated in diabetic vegetations (*gapA*, *hemX*, *nrdF*) were

demonstrated to be under the control of glucose and CcpA [70]. In summary, we cannot exclude that differences in gene expression observed between diabetic and nondiabetic rats with endocarditis reflect differences in the size of the vegetations involved (possibly attributable to an altered innate immune response in diabetic hosts). However, there is also evidence that *S. aureus* may sense (and possibly benefit from) increased glucose levels in hosts with diabetes mellitus.

Conclusions

In summary, we have demonstrated that diabetes mellitus led to higher bacterial burdens in an experimental model of *S. aureus* endocarditis. Gene expression patterns in endocardial vegetations differed from those observed during in vitro growth with overall down regulation of transcription. Endocarditis promoted higher expression of genes encoding toxins, surface proteins, and enzymes involved in capsule biosynthesis, iron homeostasis, and glucose and amino acid metabolism. Our findings indicate that gene expression of the staphyloferrin B operon is upregulated in endocarditis, and that production of this siderophore promoted staphylococcal virulence in this infection model. These results may form a basis for future analyses of specific genes, regulators, and pathways that are critical in endocarditis and other infections. Our findings may thus contribute to a better understanding of *S. aureus* pathogenesis in vivo and lead to alternative approaches in prevention or treatment of staphylococcal endocarditis.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

FH and JCL designed the experiments; CR and PMD designed microarray experiments. All animal experiments were performed by FH and JCL; RNA isolation and qPCR experiments were performed by FH, CR and PMD performed and analyzed the microarray experiments. All other data were analyzed by FH, BS and JCL FH and JCL wrote the manuscript, and BS critically reviewed it. All authors read and approved the final manuscript.

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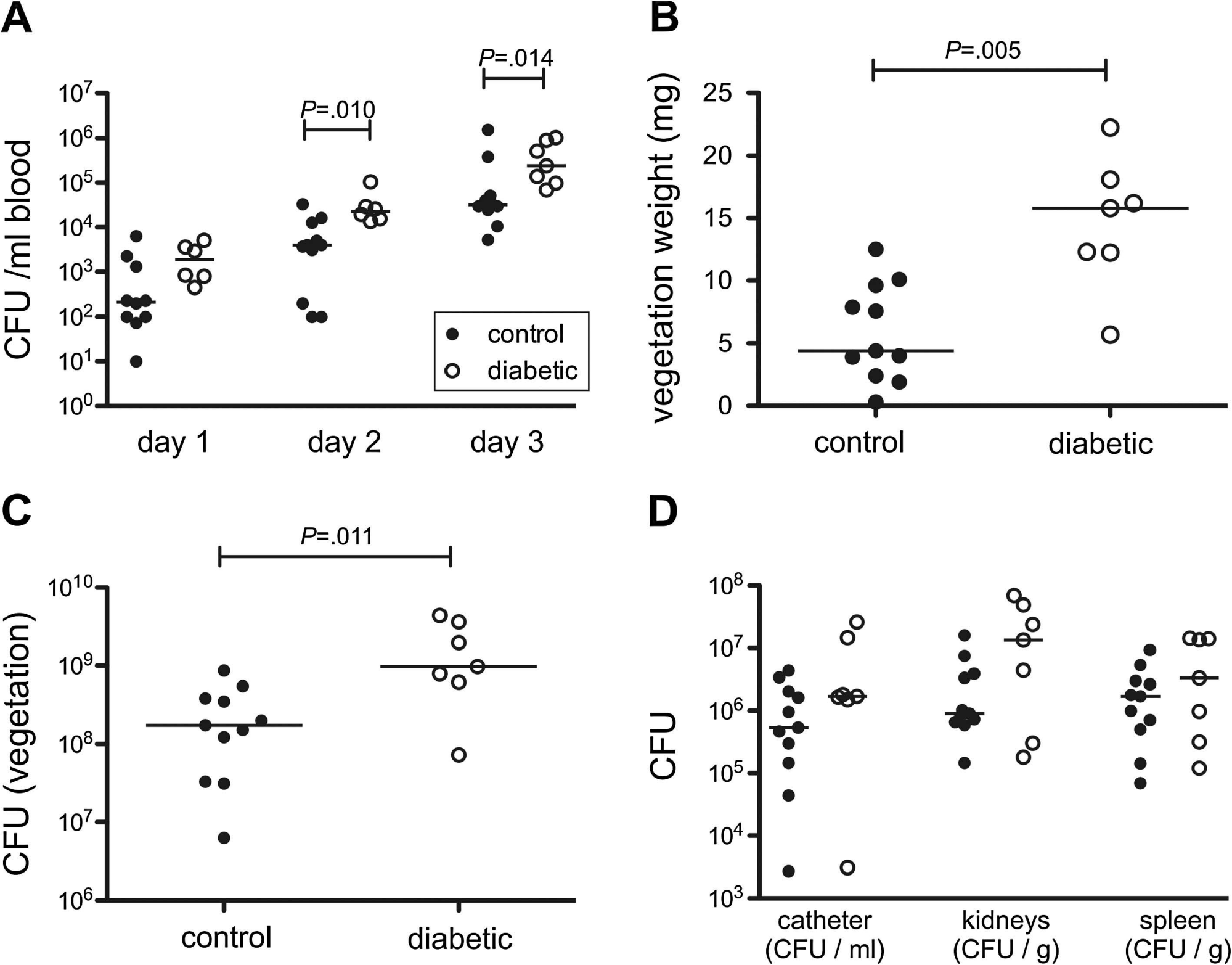
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control
diabetic
day 1
day 2
day 3
B
vegetation weight (mg)
25
20
15
10
5
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P=.005
control
diabetic
C
CFU (vegetation)
10^{10}
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control
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D
CFU
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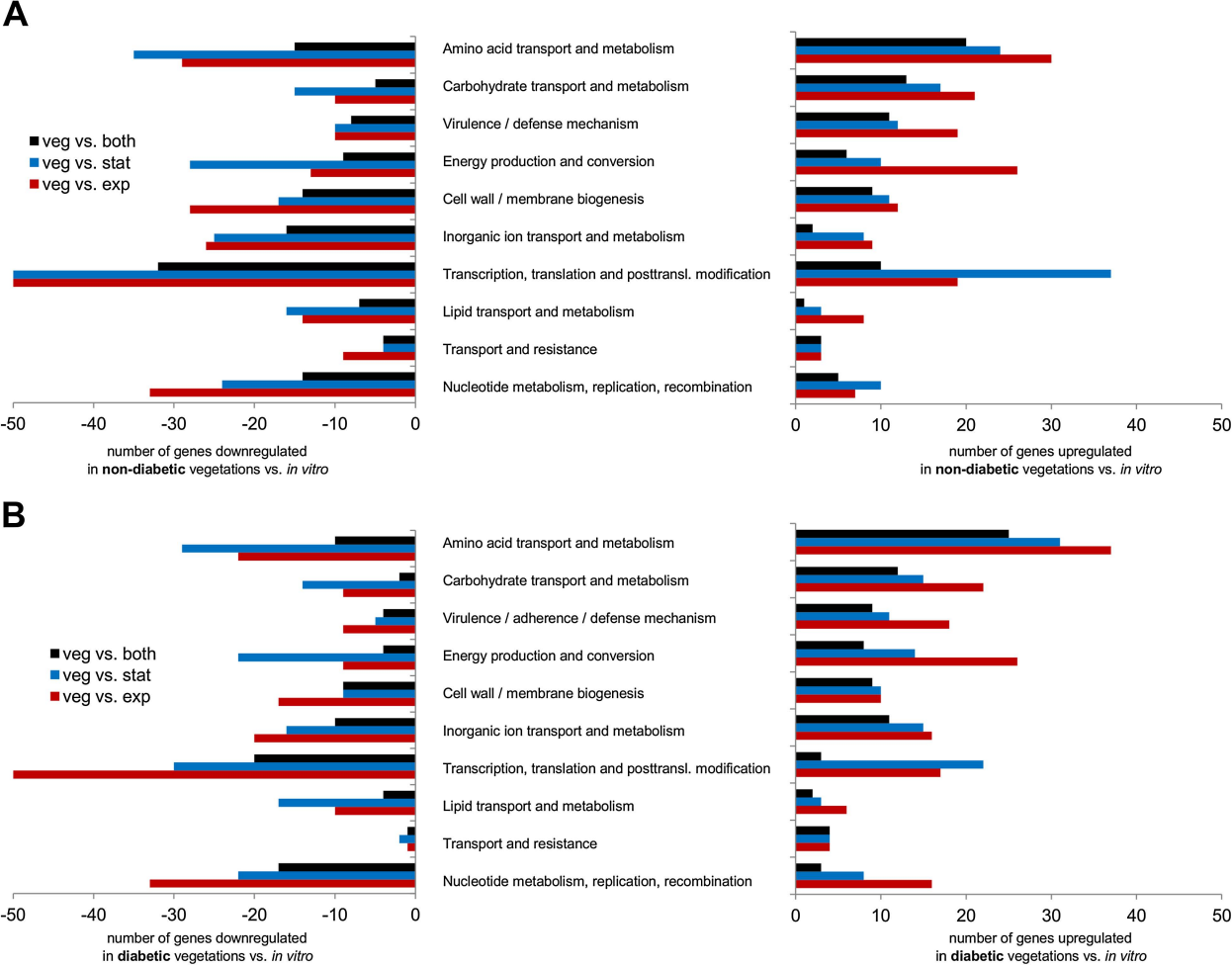
A
veg vs. both
veg vs. stat
veg vs. exp
Amino acid transport and metabolism
Carbohydrate transport and metabolism
Virulence / defense mechanism
Energy production and conversion
Cell wall / membrane biogenesis
Inorganic ion transport and metabolism
Transcription, translation and posttransl. modification
Lipid transport and metabolism
Transport and resistance
Nucleotide metabolism, replication, recombination
-50
-40
-30
-20
-10
0
number of genes downregulated in non-diabetic vegetations vs. in vitro
0
10
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30
40
50
number of genes upregulated in non-diabetic vegetations vs. in vitro
B
veg vs. both
veg vs. stat
veg vs. exp
Amino acid transport and metabolism
Carbohydrate transport and metabolism
Virulence / adherence / defense mechanism
Energy production and conversion
Cell wall / membrane biogenesis
Inorganic ion transport and metabolism
Transcription, translation and posttransl. modification
Lipid transport and metabolism
Transport and resistance
Nucleotide metabolism, replication, recombination
-50
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number of genes downregulated in diabetic vegetations vs. in vitro
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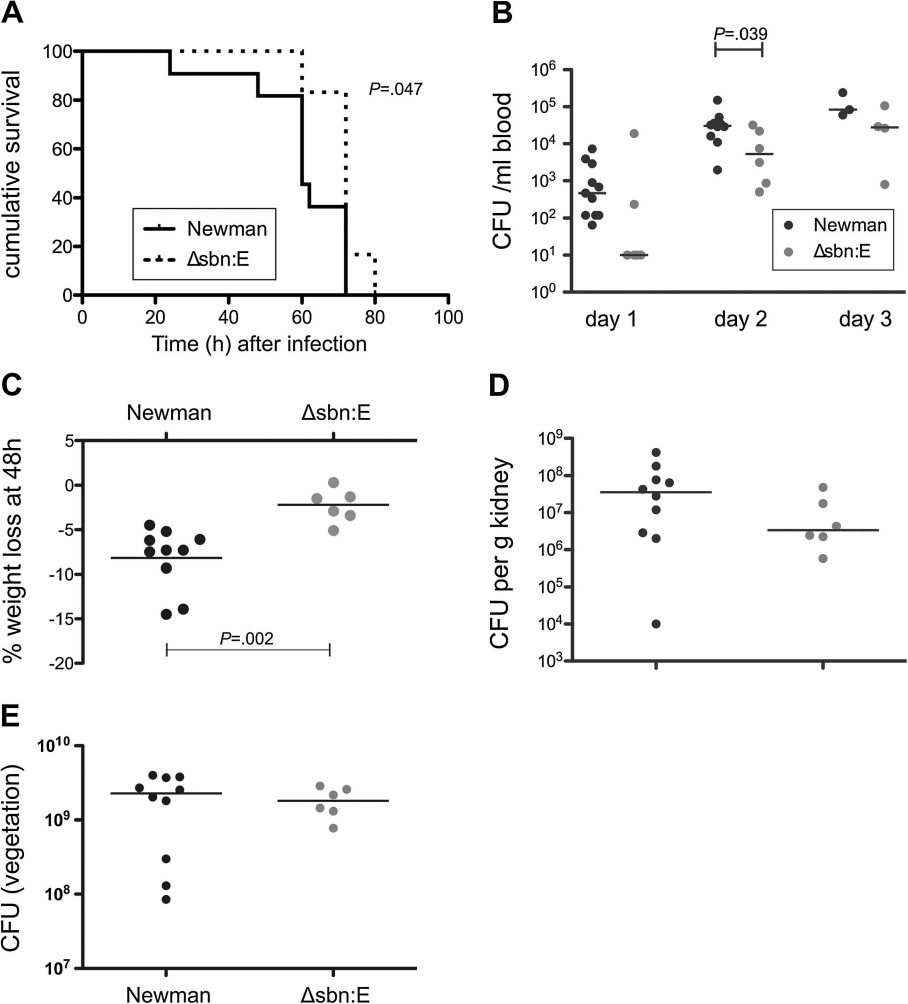
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80
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0
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Time (h) after infection
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Newman
Δsbn:E
B
CFU /ml blood
P=.039
day 1
day 2
day 3
Newman
Δsbn:E
C
Newman
Δsbn:E
% weight loss at 48h
P=.002
D
CFU per g kidney
E
CFU (vegetation)
Newman
Δsbn:E

Additional files provided with this submission:

Additional file 1. Figure S1. Comparative virulence of strain Newman and its isogenic sdrCDE deletion mutant. (A) Infection with the mutant strain led to comparable levels of bacteremia, (B) weight loss at 48 h, (C) bacterial burden in the kidneys an (endocardialk)
http://genomemedicine.com/content/supplementary/s13073-014-0093-3-s1.docx