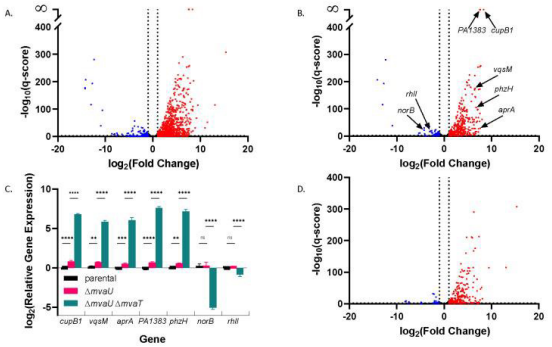
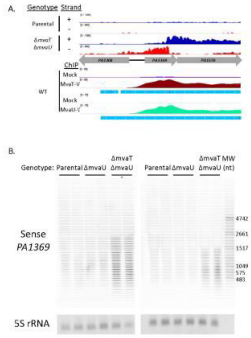
Loss of MvaT and MvaU causes global changes in gene expression



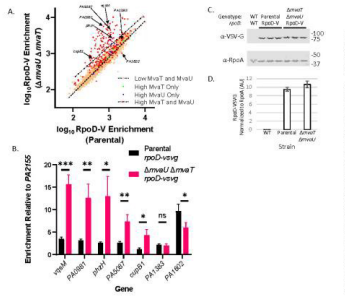
A. RNA-seq was performed in cells lacking *pilY1* (parental) and in cells also lacking *mvaT* and *mvaU*. A volcano plot demonstrates transcripts with statistically significant differential expression in *ΔmvaT ΔmvaU* vs parental (q<0.01, horizonal dotted line) and absolute value of fold change ≥2 (lateral of vertical dotted lines). Red and blue points indicate transcripts with increased and decreased abundance in *ΔmvaT ΔmvaU* relative to the parental, respectively. Black points indicate transcripts whose differential expression does not meet statistical significance or whose fold change was less <2.
B. A volcano plot of only the coding (sense) transcripts for annotated genes is shown.
C. Candidate genes, indicated in the volcano plot (B), were validated by qRT-PCR.
*p<0.05, **p<0.01, ***p<0.001, ***p<0.0001
D. A volcano plot of only transcripts antisense to annotated genes is shown.

Loss of MvaT and MvaU results in increased sense and antisense transcription



Loss of MvaT and MvaU results in increased sense- and antisense transcription.
A. RNA-seq was performed in cells lacking *pilY1* (parental, *mvaU*$^{+}$ *mvaT*$^{+}$) and in cells also lacking *mvaT* and *mvaU*. Reads are showed aligned to plus (blue) and minus (red) strands, scaled equally among plus and minus stranded reads, respectively. A representative region showing both sense (blue) and antisense (red) transcripts only in cells lacking *mvaT* and *mvaU* is shown for the *PA1369* gene, an annotated conserved hypothetical protein. ChIP-seq performed in strains that are wild-type with exception of an in-frame epitope tag on either *mvaT* (MvaT-V) or *mvaU* (MvaU-V) demonstrate MvaT and MvaU occupancy in the region of increased sense and antisense transcripts in the *ΔmvaT ΔmvaU* strain. RNA-seq and both MvaT-V and MvaU-V ChIP-seq were performed in biologic triplicate. Representative tracks for a single replicate are shown.
B. A Northern blot was performed with RNA from cells with the parental genotype, cells lacking *mvaU* only, and cells lacking both *mvaU* and *mvaT*. The membrane was hybridized with probes that bind to the coding strand (sense) or non-coding strand (antisense) of *PA1369*. Increases in both sense and antisense transcripts of apparently different size are seen only when cells lack both *mvaT* and *mvaU*. 5S rRNA is shown as a loading control. Two biological replicates for each strain are shown.

Loss of both MvaT and MvaU results in genome-wide redistribution of RNA polymerase



Loss of MvaT and MvaU results in redistribution of RNA polymerase subunit σ^{70} genome-wide.
A. RpoD-V enrichment scores were ascertained summed across each annotated gene. Each point represents the log RpoD-V enrichment for an annotated gene in the parental strain (x coordinate) and *ΔmvaU ΔmvaT* strain (y coordinate). Genes whose association is unchanged by deletion of *mvaT* and *mvaU* will follow a y=x distribution. Plotted genes were subdivided by enrichment for MvaT-V and MvaU-V: not enriched for MvaT-V and MvaU-V (peach), enriched for MvaT-V but not MvaU-V (green), enriched for MvaU-V but not MvaT-V (blue), and enriched for both MvaT-V and MvaU-V (red). Comparison of linear regression for the dual-enriched (high MvaT and MvaU, dot-dash) and dual-unenriched (low MvaT and MvaU, dashed) demonstrates a statistically significant difference in y-intercept (p<0.0001) for the two trendlines.
B. Candidate genes were evaluated for enrichment of RpoD-V by ChIP-qPCR in the parental (black) and *ΔmvaU ΔmvaT* strain (magenta). Error bars represent the standard deviation of a biological triplicate performed in technical triplicate. Significance was determined using the two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli, with Q = 1% and without the assumption of consistent standard deviation.
C. Protein was isolated from cells of the parental strain with epitope-tagged RpoD-V and a similar strain also lacking *mvaT* and *mvaU*, each grown in biological triplicate. Quantitative western blot was performed using antibodies against the VSVG epitope tag on RpoD and a loading control (α subunit of RNA polymerase). Protein isolated from wild-type cells was loaded as a single negative control lane.
D. VSVG signal is shown normalized to the loading control.
*p≤0.05, **p<0.01, ***p<0.001, ***p<0.0001

Conclusion. Our findings suggest that the ability of H-NS-like proteins to repress intragenic transcription is a universal function of these proteins and describe a second mechanism by which MvaT and MvaU may contribute to the growth of *P. aeruginosa*.

Disclosures. **All Authors**: No reported disclosures

1193. Human Transcriptomic Analysis of Periprosthetic Joint Infection

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Session: P-53. Microbial Pathogenesis

Background. Periprosthetic joint infection (PJI), a devastating complication of total joint replacement, is of incompletely understood pathogenesis and may sometimes be challenging to clinically distinguish from other causes of arthroplasty failure.

Methods. We characterized human gene expression in 93 specimens derived from surfaces of resected arthroplasties, comparing transcriptomes of subjects with infection- *versus* non-infection-associated arthroplasty failure.

Results. Differential gene expression analysis confirmed the association of 28 previously investigated biomarkers with PJI- bactericidal/permeability increasing protein (BPI), cathelicidin antimicrobial peptide (CAMP), chemokines CCL3, CCL4, and CXCL2, colony stimulating factor 2 receptor (CSF2RB), colony stimulating factor 3 (CSF3), alpha-defensin (DEFA4), receptor CD64B, intercellular adhesion molecule 1 (ICAM1), IFNG, IL13RA2, IL17D, IL1A, IL1B, IL1RN, IL2RA, IL2RG, IL5RA, IL6, IL8, lipopolysaccharide binding protein (LBP), lipocalin (LCN2), lactate dehydrogenase C (LDHC), lactotransferrin (LTF), matrix metallopeptidase 3 (MMP3), peptidase inhibitor 3 (PI3), and vascular endothelial growth factor A (VEGFA), as well as identified three novel molecules with diagnostic potential for detection of PJI- chemokine CCL20, coagulation factor VII (F7), B cell receptor FCRL4. Comparative analysis of infections caused by *staphylococcal versus* non-*staphylococcal* and *Staphylococcus aureus versus Staphylococcus epidermidis* showed significant elevated expression of IL13, IL17D, and metalloprotease protein MMP3 in *staphylococcocal* infections, and increased expression of IL1B, IL8, and platelet factor PF4V1 in *S. aureus* infections. Pathway analysis of over-presented genes suggested activation of host immune response and cellular maintenance and repair functions in response to invasion of infectious agents.

Conclusion. Our study provides new potential targets for diagnosis of PJI and targets for differentiation of PJI-associated infectious agents.

Disclosures. Matthew P. Abdel, MD, Dr. Abdel receives royalties from Stryker on certain hip and knee products, and is a paid consultant for Stryker. (Consultant) Robin Patel, MD, Accelerate Diagnostics (Grant/Research Support)CD Diagnostics (Grant/Research Support)Contrafect (Grant/Research Support)Curetis (Consultant) GenMark Diagnostics (Consultant)Heraeus Medical (Consultant)Hutchison Biofilm Medical Solutions (Grant/Research Support)Merck (Grant/Research Support)Next Gen Diagnostics (Consultant)PathoQuest (Consultant)Qvella (Consultant)Samsung (Other Financial or Material Support, Dr. Patel has a patent on Bordetella pertussis/parapertussis PCR issued, a patent on a device/method for sonication with royalties paid by Samsung to Mayo Clinic, and a patent on an anti-biofilm substance issued.) Selux Dx (Consultant)Shionogi (Grant/Research Support)Specific Technologies (Consultant)

1194. Identification and Characterization of Extracellular Inducers of Persistence in Staphylococcus epidermidis and Staphylococcus aureus

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Elyse C. Curry, Ryan G. Hart, Danni Y. Habtu and Neal R. Chamberlain

Session: P-53. Microbial Pathogenesis

Background. This study describes the identification and partial characterization of persistence inducing factors (PIF) from *Staphylococcus aureus* (*S. aureus*) and *Staphylococcus epidermidis* (*S. epidermidis*). Persistence is an epigenetic process that results in tolerance of bacterial cells to antibiotic treatment, which can result in chronic human infections.

Methods. Others have demonstrated a significant increase in persister numbers during mid-log phase. Inducers of this mid-log increase have yet to be identified in staphylococci. Optical density at 600 nm (OD_{600}) was used instead of time to determine when persister numbers increased during logarithmic growth. Concentrated culture filtrates (CCF) from *S. epidermidis* RP62A and *S. aureus* SH1000 were obtained at various OD_{600}'s and following incubation at 16 h. The CCF's were used to develop a persistence inducing factor (PIF) assay. The PIF assay was used to partially characterize PIF from *S. epidermidis* RP62A and *S. aureus* SH1000 for relative molecular weight, temperature and protease sensitivity and inter-species communications.

Results. Optimal OD_{600}'s for the *S. epidermidis* RP62A and *S. aureus* SH1000 PIF assays were 2.0 and 0.5, respectively. The highest PIF activity for both species was from CCF following incubation overnight (16 h). *S. epidermidis* RP62A's PIF activity was decreased by storage at 4° C (2 weeks or longer) but not following incubation at 20° C (16 h), 37° C (1 h) or 100° C (15 min). *S. aureus* SH1000's PIF activity was decreased following storage at 4° C (2 week or longer) and after boiling at 100°C for 5 min but not after incubation at 37° C (1 h). PIF activity from both species was less than 3,000 Mrr. Proteinase-K treatment of *S. aureus* SH1000 PIF decreased activity but did not decrease PIF activity of *S. epidermidis* RP62A. PIF from *S. epidermidis* RP62A did not increase persister numbers when used to treat *S. aureus* SH1000 cells nor did PIF from *S. aureus* SH1000 increase persister numbers in *S. epidermidis* RP62A cells.

Conclusion. Previous attempts to discover PIF's for staphylococcal species were unsuccessful due to the time-based means used to identify mid-log. Both staphylococcal species appear to produce unique, extracellular, low-molecular-weight inducers of persistence (PIF) when assayed using an OD_{600}-based PIF assay.

Disclosures. **All Authors**: No reported disclosures

1195. Influence of Histoplasma capsulatum Infection on Endothelin-1 mRNA Gene Expression in Bone Marrow Derived Macrophages

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Session: P-53. Microbial Pathogenesis

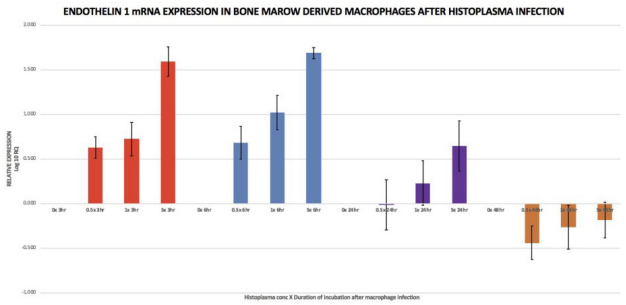
Background. Endothelin-1 (ET-1) is increasingly recognized as an immune modulator; it exerts a pro-inflammatory effect by increasing the release of cytokines like interferon gamma. ET-1 is secreted by a variety of cells such as macrophages, neurons and endothelial cells. Activation of the endothelin system has been implicated

in the pathogenesis of sepsis caused by bacteria, viruses and even parasites. However, there are no published studies that have explored the role of ET-1 in *Histoplasma capsulatum* infection. Studying the role of ET-1 in histoplasmosis is important because understanding its role in the host defense mechanism may serve as the foundation for future discovery of novel therapeutic options.

Methods. Bone marrow cells were isolated from mice and set up for tissue culture. Bone marrow derived macrophages (BMDM) were harvested after 5-7 days of incubation, and infected with varying ratios (0.5,1 and 5) of yeasts to macrophages. RNA was extracted from the BMDM after 3, 6, 24 and 48 hours of infection. For comparison, RNA was also extracted from uninfected BMDM at the same time points. Real-time PCR (polymerase chain reaction) was performed on complementary DNA. ET-1 (*Edn1*) messenger RNA (mRNA) gene expression was quantified relative to the expression of the house keeping /endogenous control gene that encodes for beta-2 microglobulin (*B2m*).

Results. In BMDM infected with *H. capsulatum* there was upregulation of *Edn1* after 3, 6 and 24 hours of infection. During this same time points, the expression of ET-1 mRNA in the uninfected BMDM remained constant. Expression of *Edn1* was highest in the BMDM infected with 5x *H. capsulatum* after 3 and 6 hours of infection. After 24 hours, the expression of ET-1 mRNA decreased markedly in all concentrations of *H. capsulatum*. At 48 hours post-infection the *Edn1* was downregulated in the 0.5,1 and 5-fold quantities of *H. capsulatum* across all time the time intervals.

Figure 1



Conclusion. Results from this study indicate that *H. capsulatum* infection induced an upregulation of the *Edn-1* in BMDM. This may correlate with an increase in levels of ET-1 production by the BMDM in the face of *H. capsulatum* infection. These results provide a platform in which to examine the influence of ET-1 on the host response to this fungus.

Disclosures. **All Authors**: No reported disclosures

1196. Influence of antibiotic use on the effectiveness and safety of immune checkpoint inhibitors in Japan.

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Session: P-53. Microbial Pathogenesis

Background. It has been reported that antibiotic use changes the gut microbiome and alters the outcome of treatment with immune checkpoint inhibitors (ICIs). However, in Asia, this has not been well studied, and there is insufficient evidence to support these reports.

Methods. In this study, we investigated the concurrent use of antibiotics and the administration of PD-1 inhibitors in Japanese patients, and examined the relationship between antibiotics and the clinical benefit or safety of PD-1 inhibitors.

Results. In total, 152 patients were analyzed: 62 patients received systemic antibiotics within 2 months before or 1 month after the first dose of PD-1 inhibitors (the antibiotic group); the remaining patients comprised the non-antibiotic group. There was a significantly higher proportion of patients under 65 years of age in the antibiotic group. Overall survival (OS) was not reached in the antibiotic and non-antibiotic groups, and there was no statistically significant difference between the two groups (HR = 1.48) (Figure 1). Progression-free survival (PFS) was 3.29 months in the antibiotic group and was significantly shorter than that in the non-antibiotic group (5.99 months, HR = 1.75) (Figure 2). Multivariate analysis by Cox regression analysis also showed that PFS was shorter in the antibiotic group (HR=1.63). As age may be a confounding factor, we performed a stratified analysis, a common method used to adjust for bias. The results of the stratified log-rank test after adjustment for age showed that the PFS was significantly shorter in the antibiotic group. There were no statistically significant differences between the two groups in the response rate, incidence of adverse events of Grade 3 or above, and laboratory data (Table 1).

Figure 1

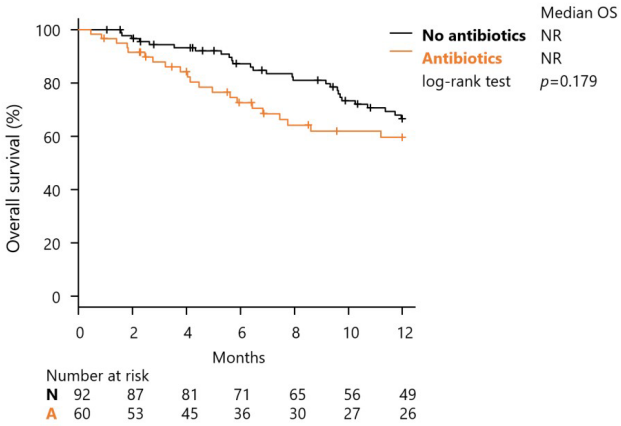

Figure 2

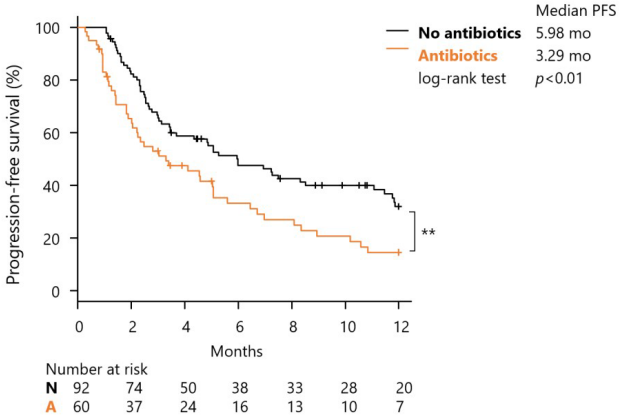

Table 1

	No antibiotics n=92		Antibiotics n=60		*p* value	
	All Grade	Grade 3	All Grade	Grade 3	All Grade	Grade 3
Any adverse event	75	11	50	9	0.831	0.629
Infusion reaction	0	0	2	0	0.154	
Fatigue	26	0	17	0	1.000	
Itching	25	1	13	1	0.566	
Rash	27	3	11	0	0.179	
Diarrhea	9	0	5	0	1.000	
Nausea	7	0	3	0	0.741	
Decreased appetite	14	0	10	0	0.823	
Joint pain	6	0	4	0	1.000	
Muscle pain	6	0	2	0	0.480	
Fever	10	0	13	0	0.104	
Anemia	10	3	8	1	0.798	
Pneumonitis	9	1	10	3	0.221	
Hyperthyroidism	10	1	3	0	0.248	
Hypothyroidism	17	0	9	0	0.663	
Hypophysitis	4	3	2	0	1.000	
Type 1 DM	1	1	0	0	1.000	
Myocarditis	0	0	1	1	0.395	
Joint inflammation	0	0	2	0	0.154	
Increase in AST level	12	0	14	4	0.124	
Increase in ALT level	12	0	13	2	0.183	
Increase in γ-GTP level	17	1	16	1	0.236	
Increase in T-Bil level	2	0	2	0	0.648	
Increase in SCr level	7	0	5	0	1.000	
Otherwise	5	0	7	0	0.220	

CTCAE v4.0, Fisher's exact test

Conclusion. Our results suggest that the use of antibiotics may affect the anticancer treatment outcomes of Japanese patients who are administered PD-1.

Disclosures. **All Authors**: No reported disclosures