Title: Precision Identification of Diverse Bloodstream Pathogens from the Gut Microbiome

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Abstract

Bloodstream infection is the most common infectious complication in hematopoietic cell transplantation recipients. To evaluate the genomic concordance of bloodstream pathogens and bacterial strains within the intestinal microbiome using whole genome sequencing, we developed StrainSifter, a bioinformatic pipeline to compare nucleotide variation between bacterial isolate strains and stool metagenomes. We applied StrainSifter to bloodstream isolates and stool metagenome samples from hematopoietic stem cell transplant recipients with bloodstream infections. StrainSifter is designed to identify single nucleotide variants between isolate and metagenomic short reads using stringent alignment, coverage, and variant frequency criteria for strain comparison. We identified enteric BSI isolates that were highly concordant with those in the gut microbiota, as well as highly concordant strains of typically non-enteric bacteria. These findings demonstrate the utility of StrainSifter in strain matching and provide a more precise investigation of the intestine as a reservoir of diverse pathogens capable of causing bloodstream infections.

26 **Introduction**

27 Microbial pathogenicity and transmission depend in part on strain-level variability in both gene content
28 and at the single-nucleotide variant (SNV) level, as different strains of the same organism can vary widely
29 in their ability to cause disease (Snitkin et al. 2011; Lieberman et al. 2013). Whole genome sequencing
30 (WGS) has facilitated the exploration of strain-level determinants of virulence and has enabled the precise
31 tracking of pathogen transmission (Snitkin et al. 2011; Snitkin et al. 2012). Methods for strain tracking
32 and comparison most commonly rely on either sequencing a defined number of genes or identifying
33 single nucleotide variants (SNVs) across the whole genome. For example, whole genome sequencing of
34 cultured *Klebsiella pneumoniae* stool isolates in ICU patients demonstrated the role of *K. pneumoniae*
35 colonization in systemic infections (Gorrie et al. 2017; Martin et al. 2016; Nielsen et al. 2017) and
36 genome-wide SNV comparison is routinely employed for epidemiologic strain tracking (Salipante et al.
37 2015; Snitkin et al. 2012; Harris et al. 2013; Chen et al. 2017; Kovanen et al. 2014). While strain
38 comparisons have primarily been performed on bacterial isolates, newer computational tools (MIDAS,
39 StrainPhlAn, and metaSNV) profile strain variation between metagenomic samples over time without the
40 bias inherent in culturing isolates (Costea et al. 2017; Nayfach et al. 2016; Truong et al. 2017).
41

42 Despite the increasing use of WGS for strain comparison, bioinformatic tools have not been developed for
43 the specific purpose of comparing disease-causing bacterial isolates to metagenomic samples at the
44 nucleotide level within the same patient. It is desirable to achieve the highest resolution of comparison
45 across the whole genome of an organism in order to precisely compare clinical isolates to strains within a
46 sampled metagenome. Additionally, most tools depend on the availability of reference genomes and are
47 thus often restricted to studying well characterized bacterial pathogens, limiting the types of organisms
48 that are available for strain comparisons. To date, no reference-free tool exists with the stringent filtering
49 steps necessary to identify genomic variants specific to a single strain between genomes and
50 metagenomes. We sought to compare strain-level identity between bloodstream infection isolates and
51 stool metagenomes using samples from immunocompromised patients at elevated risk of systemic

52 infections, with the goal of better understanding the origins of bloodstream infection in this patient
53 population.
54
55 Hematopoietic stem cell transplant (HCT) recipients are at high risk for bloodstream infections, which
56 occur in 20-60% of patients primarily during periods of neutropenia and mucositis (Blennow et al. 2014;
57 Gudiol et al. 2014; Mikulska et al. 2009). Prior to engraftment of donor stem cells, HCT recipients are
58 particularly susceptible to translocation of enteric organisms from the intestinal microbial reservoir into
59 the bloodstream. During periods of mucositis, primary bloodstream infections with enteric Gram-negative
60 and Gram-positive organisms are thought to arise from mucosal barrier injury (See et al. 2017). In
61 contrast, non-enteric commensal and environmental bacteria access the bloodstream through other routes
62 including intravenous lines and sites where skin epithelial integrity has become compromised. Existing
63 methods for strain comparison have been helpful in identifying the origins of bloodstream infections in
64 HCT patients using culture-based pulsed-field gel electrophoresis (PFGE), or PCR-based multi-locus
65 sequence typing (MLST) (Tancrede and Andremont 1985; Samet et al. 2013). Although these methods
66 are rapid, affordable, and standardized across many organisms, they have several limitations: 1) they
67 require isolation and culture of candidate pathogens from the stool or other suspected pathogen reservoirs,
68 a step that can introduce selection bias, and 2) they often rely on detailed characterization of a small
69 number of genes and therefore lack the discriminatory power required for robust, genome-wide strain
70 comparison (W. Li, Raoult, and Fournier 2009). No studies to date have investigated the origins of
71 bloodstream infections in HCT or other immunocompromised patients at the level of single nucleotide
72 variation across entire genomes.
73
74 In this work, we present StrainSifter, a straightforward bioinformatics pipeline based on whole genome
75 sequencing to comprehensively and precisely compare single nucleotide variants between isolate
76 genomes and metagenomes without bias introduced by culturing. Additionally, unlike most previous
77 strain comparison bioinformatic tools (MIDAS and StrainPhlAn), variant calling within our pipeline does

not depend on the availability of a reference genome database, a particularly relevant consideration in immunocompromised patients who develop infections with lesser known and poorly studied opportunistic pathogens. We have applied this tool to track bacterial strain translocation from the gut to the bloodstream in a unique cohort of immunocompromised patients at high risk for translocation and bloodstream infection, specifically patients with hematologic malignancies who have undergone hematopoietic stem cell transplantation. We find that StrainSifter is able to identify strains of both intestinal mucosa-associated (*Enterococcus faecium*, *Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae, and Streptococcus mitis*) and typically non-mucosal bacteria (*Staphylococcus epidermidis* and *Pseudomonas aeruginosa*) within the gut microbiome as being concordant with the same strain cultured from the blood of patients with bloodstream infections. Intriguingly, this suggests that both enteric and classically non-enteric pathogens can colonize and even thrive within the intestinal reservoir of immunocompromised hosts.

Methods

Cohort selection

A retrospective cohort study, approved by the institutional review board under the IRB protocol #8903 (PI: Dr. David Miklos, co-PI: Dr. Ami Bhatt), was performed at Stanford Hospital. Informed consent was obtained from all subjects prior to specimen collection. At the time of cohort identification (July 2017), a stool biospecimen collection containing 964 stool samples from 402 patients was available for investigation. This collection consisted of convenience samples collected from autologous and allogeneic stem cell transplantation patients at Stanford University Hospital between October 5 2015 and June 9 2017. Patients were included in this study if a stool sample had been collected within 30 days prior to an episode of bloodstream infection (BSI) for which a blood isolate was also available. From this final cohort, we sequenced all stool samples in our collection within 60 days prior to and 30 days after BSI.

Bloodstream isolate identification

104 Bloodstream isolates from HCT patients who received medical care at Stanford University Hospital were
105 obtained from the Stanford Hospital Clinical Microbiology Laboratory. All isolates considered typical
106 bloodstream pathogens by National Healthcare Safety Network (NHSN) guidelines are stored in a
107 glycerol suspension at -80°C for 12 months ("Centers for Disease Control and Prevention" 2018). Blood
108 culture isolates considered to be skin-associated bacteria (including viridans group *Streptococcus spp*. and
109 coagulase-negative *Staphylococcus spp.*) were saved if they were recovered in two or more blood culture
110 sets as per NHSN criteria ("Centers for Disease Control and Prevention" 2018). Isolates were identified
111 by standard biochemical testing and matrix-assisted laser desorption and ionization time-of-flight mass
112 spectrometry (MALDI-TOF) (Bruker Daltonics, Bremen, Germany).
113
114 **Sample processing**
115 Bloodstream bacterial isolates were plated on brain heart infusion agar with 10% horse blood. DNA was
116 extracted from isolates using the Gentra Puregene Yeast/Bact. Kit per manufacturer's instructions. Stool
117 samples were collected and stored at 4°C for up to 24 hours prior to homogenization, aliquoting and
118 storage at 80°C. DNA was extracted from stool using the QIAamp DNA Stool Mini Kit (QIAGEN®) per
119 manufacturer's instructions, with an initial bead-beating step prior to extraction using the Mini-
120 Beadbeater-16 (BioSpec Products) and 1 mm diameter Zirconia/Silica beads (BioSpec Products). Bead-
121 beating consisted of 7 rounds of alternating 30 second bead-beating bursts followed by 30 seconds
122 cooling on ice. DNA concentration for all samples was measured using Qubit® Fluorometric Quantitation
123 (Life Technologies). DNA sequencing libraries from both isolates and stool were prepared using the
124 Nextera XT DNA Library Prep Kit (Illumina®) with isolates and stool microbiota libraries prepared at
125 separate times following DNAse treatment of all lab surfaces to avoid cross-contamination. Library
126 concentration was measured using Qubit® Fluorometric Quantitation (Life Technologies) and library
127 quality and size distributions were analyzed with the Bioanalyzer 2100 (Agilent). Prepared libraries were
128 multiplexed and subjected to 100 base pair, paired-end sequencing on the HiSeq 4000 platform
129 (Illumina®).

130

131 **Shotgun sequencing analysis**

132 *Preprocessing*

133 Sequencing data were demultiplexed by unique barcodes (bcl2fastq v2.20.0.422, Illumina®). Read
134 quality was assessed using FastQC v0.11.4. Reads were deduplicated to remove PCR and optical
135 duplicates using SuperDeduper v1.4 with the start location in the read at 5 bp (-s 5) and minimum length
136 of 50 bp (-l 50). Deduplicated reads were trimmed using TrimGalore v0.4.4, a wrapper for CutAdapt
137 v1.16, with a minimum quality score of 30 for trimming (-q 30), minimum read length of 50 (--length 50)
138 and the "--nextera" flag to remove Illumina Nextera adapter sequences. Draft genomes of bacterial
139 isolates were assembled using SPAdes v3.8.0 (Bankevich et al. 2012) with default parameters. Summary
140 statistics for each BSI assembly were generated using 'basic_assembly_stats.py' from GAEMR v1.0.1
141 ("GAEMR" n.d.).

142

143 *Taxonomic classification*

144 Bloodstream isolates were taxonomically classified by aligning draft genome contigs to the BLAST nt
145 database and identifying the most common top hit for each contig. Gut metagenomic reads were
146 taxonomically classified via the One Codex platform, a web-based tool for assigning read-level
147 classifications based on unique *k*-mer signatures relative to a curated reference database [2017 database]
148 (Minot, Krumm, and Greenfield 2015).

149

150 *Phylogenetic tree building and variant identification with the StrainSifter pipeline*

151 StrainSifter is a pipeline deployed via Snakemake with modules for variant calling and phylogenetic tree
152 building, available at GitHub (https://github.com/bhattlab/strainsifter). StrainSifter accepts as input an
153 assembled bacterial draft genome and two or more short read data sets (isolate or metagenomic), and can
154 report a phylogenetic tree of input samples as well as pairwise SNV counts. To build the phylogenetic
155 trees reported in this manuscript, an NCBI RefSeq reference genome for each infectious species (based on

156 clinical laboratory classifications) was provided to StrainSifter. For the variant counting reported herein,
157 BSI draft genomes were supplied to StrainSifter. For both analyses, all stool and BSI short read datasets
158 were provided as input. For both phylogeny and SNV-counting modules, preprocessed short reads are
159 first aligned to the reference genome using the Burrows-Wheeler aligner (BWA) v0.7.10 (Li and Durbin
160 2009). Alignments are filtered to include only high-confidence alignments with mapping quality of at
161 least 60 using the "view" tool from the SAMtools suite (v1.7)(Li et al. 2009) (samtools view -b -q 60),
162 and further filtered using BamTools "filter" (v2.4.0) to include only reads with the desired number or
163 fewer mismatches (i.e. for five or fewer mismatches: bamtools filter -tag 'NM:<6') (Barnett et al. 2011)
164 For phylogenetic tree construction, reads with 5 or fewer mismatches were included; for determining
165 strain single-nucleotide variants, reads were limited to 1 or fewer mismatches. Per-base coverage is
166 calculated from each resulting BAM file using bedtools genomecov (v2.26.0) and processed with custom
167 python scripts to identify samples meeting a minimum average coverage of 5X across at least 50% of the
168 genome. Only samples meeting the coverage requirement are continued through the pipeline. Pileup files
169 are created from BAM files using SAMtools "mpileup", and are analyzed using custom python scripts to
170 identify bases occurring with at least 0.8 frequency at positions covered 5X or greater. Only bases with a
171 minimum phred score of >=20 are considered. Consensus sequences for each sample are created, wherein
172 bases that cannot be confidently determined given the described parameters are called as "N".
173
174 To create a phylogenetic tree, core positions are identified on a per-species basis, where core positions are
175 defined as positions in the reference genome where a base could be confidently called for all samples
176 analyzed. To generate phylogenetic trees, core positions with variants in at least one sample are identified
177 and concatenated into fasta files. Fasta files are aligned using MUSCLE v3.8.31 (Edgar 2004) and a
178 maximum-likelihood phylogenetic tree is computed using FastTree v2.1.7 (Price, Dehal, and Arkin 2010).
179 Phylogenetic trees are visualized in R using the ape, phangorn, and ggtree packages. Pairwise SNVs are
180 determined from the consensus sequences using a custom python script.
181

182 *Synthetic multilocus sequence typing*

183 Metagenomic short reads were assembled using metaSPAdes v3.8.0 (Nurk et al. 2017). Multilocus
184 sequence typing (MLST) schemes and sequences were downloaded from the PubMLST database
185 ("PubMLST" n.d.). MLST gene sequences were aligned to metagenome assemblies, and the top hit for
186 each alignment was chosen based on E-value, percent identity, and alignment length. Only MLST
187 sequences that were present in the metagenomic assembly with 100% identity across the entire length of
188 the sequence were reported. MLST types generated by our in-house analysis were confirmed with the
189 SRST2 synthetic multilocus sequence typing tool (Inouye et al. 2014).

190

191 *Plots*

192 Plots were generated using the R programming language using the ggplot2, reshape2, and dplyr packages.

193

194 **Results**

195 **Sample collection and cohort**

196 During the study period, we identified 74 patients within our stool biospecimen collection with a
197 bloodstream infection (77 BSI episodes). Of these, we identified 30 patients (32 bloodstream isolates)
198 with at least 1 collected stool sample within 30 days prior to their bacterial BSI. From our final cohort of
199 30, we sequenced all stool samples collected between 60 days prior to and 30 days after the date of BSI,
200 83 samples in total. Patients had a median of 2 stool samples (range 1-8), with the sampling distribution
201 presented in Figure 1. Stools were collected at a median of 9 days prior to BSI (range -58 to +31).
202 Clinical characteristics of the 30 patients are listed in Table 1 (see Table S1 for individual patient data).
203 All patients in the cohort were on at least one antibiotic within 30 days prior to BSI, with
204 fluoroquinolones being the most commonly administered antibiotic.

205

206 **Table 1.**

Baseline characteristics		**N (%)**
Age (years)		
	<30	4 (13%)
	≤30-39	4 (13%)
	≤40-49	6 (20%)
	≤50-59	3 (10%)
	≤60	11 (37%)
Sex (%male)		17 (57%)
Underlying diagnosis		
	AML	7 (23%)
	ALL	6 (20%)
	CML	2 (7%)
	Lymphoma	7 (23%)
	MDS/Myelofibrosis	6 (20%)
	Other	2 (7%)
Conditioning regimen		
	Myeloablative	16 (53%)
	Reduced intensity	9 (30%)
	Non-myeloablative	5 (17%)
Transplant source		
	Peripheral blood	20 (67%)
	Bone marrow	8 (27%)
	Double umbilical cord blood	2 (7%)
Type of donor		
Autologous		3 (10%)
Allogeneic		27 (90%)
	Matched related donor	10 (37%)

	Matched unrelated donor	14 (52%)
	Mismatched unrelated donor	3 (11%)
TPN within 30 days		15 (50%)
Antibiotics within 30 days*		30 (100%)
	Fluoroquinolones	26 (87%)
	Beta-lactam	14 (47%)
	Carbapenem	6 (20%)
	Vancomycin (IV)	12 (40%)
Bacteremia species		
Gram-positive	*Staphylococcus aureus*	8 (25%)
	MSSA	5 (16%)
	MRSA	3 (9%)
	Coagulase-negative Staphylococcus spp.	4 (13%)
	Streptococcus mitis	7 (22%)
	Enterococcus faecium	2 (6%)
	Rothia mucilaginosa	2 (6%)
Gram-negative	*Escherichia coli*	4 (13%)
	Klebsiella spp.	3 (9%)
	Klebsiella pneumoniae	2 (6%)
	Klebsiella oxytoca	1 (3%)
	Enterobacter cloacae	1 (3%)
	Pseudomonas aeruginosa	1 (3%)

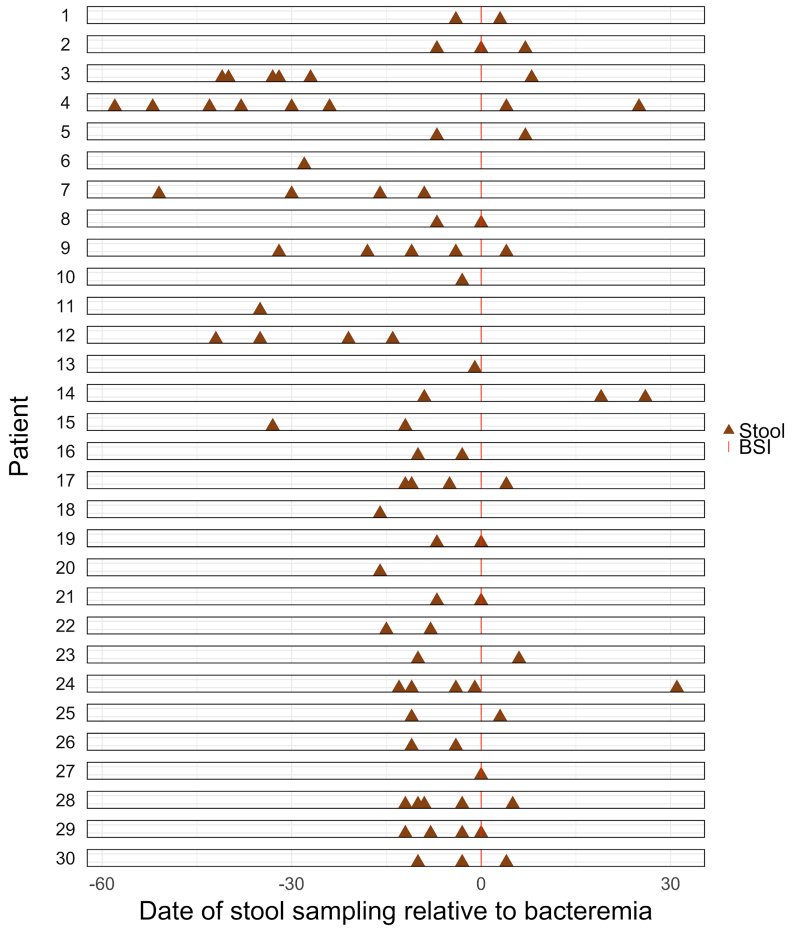
List of abbreviations: AML (acute myeloid leukemia); ALL (acute lymphoblastic leukemia); CML (chronic myeloid leukemia).

***Antibiotics are not exclusive and will not add up to 100%**

213 **Figure 1. Stool sampling**

214 Timeline of stool sampling relative to BSI and hematopoietic cell transplant for patients included in the

215 study.



216

217

218 **Infectious taxa are present in the gut microbiome near the time of infection**

219 We sequenced an average of 68,704,214 reads per stool sample (20,298,792-148,162,892) and 27,999,060

220 reads per isolate (10,652,310-61,058,906) (Table S2). Isolate reads were assembled into draft genomes

221 (Table S3).

222

223 MALDI-TOF is unable to discriminate between some closely related taxa (e.g. *Streptococcus* spp.), so we

224 sought to confirm the clinical laboratory BSI classifications by aligning draft genome contigs to the

225 BLAST nt database. Of 32 BSIs, we identified 3 for which WGS and MALDI-TOF results disagreed.
226 Two *Streptococcus* isolates that had been classified as *Streptococcus mitis* by MALDI-TOF were
227 identified as *Streptococcus oralis,* and one as *Streptococcus pseudopneumoniae* by WGS (Table S4).
228
229 To determine community composition of pre- and post-BSI stool samples, we taxonomically classified
230 gut metagenomic reads via the One Codex platform. Considering species level read classifications, we
231 observe the target species in the gut at a threshold of 0.1% or greater relative abundance for 19 of 83
232 stools (23%) or 20 of 95 stool-BSI pairs (21%) taking into account episodes of polymicrobial BSI; one
233 patient developed a BSI with two bacterial species, both of which were present in the stool above
234 threshold level (Table 2; full taxonomic classifications in table S5). Read-level taxonomic classifiers are
235 often unable to discriminate between closely related species, so we also compared BSIs and stool read
236 classifications at the genus level. We observe the target genus in the gut community for 27% of stools, or
237 23% of stool-BSI pairs (Table S5, S6).
238
239 **Table 2.**

Patient	Day relative to BSI	BSI organism	Relative abundance (%)
1	-4	Staphylococcus aureus	0
1	3	Staphylococcus aureus	0
2	-7	Enterobacter cloacae	0
2	0	Enterobacter cloacae	0.74
2	7	Enterobacter cloacae	0
2	-7	Klebsiella pneumoniae	0
2	0	Klebsiella pneumoniae	3.47
2	7	Klebsiella pneumoniae	0.01
3	-27	Escherichia coli	87.15
3	-32	Escherichia coli	97.25
3	-33	Escherichia coli	96.4
3	-40	Escherichia coli	0.05

3	-41	Escherichia coli	0.43
3	8	Escherichia coli	0.01
3	-27	Staphylococcus aureus	0
3	-32	Staphylococcus aureus	0
3	-33	Staphylococcus aureus	0
3	-40	Staphylococcus aureus	0
3	-41	Staphylococcus aureus	0
3	8	Staphylococcus aureus	0
4	-24	Staphylococcus aureus	0
4	-30	Staphylococcus aureus	0
4	-38	Staphylococcus aureus	0
4	-43	Staphylococcus aureus	0
4	-52	Staphylococcus aureus	0
4	-58	Staphylococcus aureus	0
4	25	Staphylococcus aureus	0
4	4	Staphylococcus aureus	0
5	-7	Staphylococcus aureus	0
5	7	Staphylococcus aureus	0
6	-28	Klebsiella oxytoca	0
7	-16	Escherichia coli	0.79
7	-30	Escherichia coli	0.22
7	-51	Escherichia coli	2.05
7	-9	Escherichia coli	0.38
8	-7	Rothia mucilaginosa	0
8	0	Rothia mucilaginosa	0
9	-11	Escherichia coli	0.31
9	-18	Escherichia coli	0
9	-32	Escherichia coli	0
9	-4	Escherichia coli	0.03
9	4	Escherichia coli	0
10	-3	Staphylococcus aureus	0

11	-35	Escherichia coli	0.04
12	-14	Staphylococcus aureus	0
12	-21	Staphylococcus aureus	0
12	-35	Staphylococcus aureus	0.65
12	-42	Staphylococcus aureus	0.09
13	-1	Staphylococcus epidermidis	2.51
14	-9	Klebsiella pneumoniae	0
14	19	Klebsiella pneumoniae	0
14	26	Klebsiella pneumoniae	0
15	-12	Staphylococcus epidermidis	0
15	-12	Staphylococcus epidermidis	0
15	-33	Staphylococcus epidermidis	0
15	-33	Staphylococcus epidermidis	0
16	-10	Streptococcus mitis	0
16	-3	Streptococcus mitis	0
17	-11	Streptococcus mitis	0
17	-12	Streptococcus mitis	0
17	-5	Streptococcus mitis	0
17	4	Streptococcus mitis	0.03
18	-16	Staphylococcus epidermidis	0
18	-16	Staphylococcus epidermidis	0
19	-7	Pseudomonas aeruginosa	0
19	0	Pseudomonas aeruginosa	0.83
20	-16	Staphylococcus epidermidis	0
21	-7	Staphylococcus aureus	0.01
21	0	Staphylococcus aureus	0.25
22	-15	Streptococcus mitis	0
22	-8	Streptococcus mitis	2.33
23	-10	Enterococcus faecium	11.61
23	6	Enterococcus faecium	0
24	-1	Rothia mucilaginosa	0.04

24	-11	Rothia mucilaginosa	0
24	-13	Rothia mucilaginosa	0
24	-4	Rothia mucilaginosa	0
24	31	Rothia mucilaginosa	0
25	-11	Enterococcus faecium	0.36
25	3	Enterococcus faecium	94.09
26	-11	Streptococcus mitis	0
26	-4	Streptococcus mitis	0
27	0	Staphylococcus aureus	0.51
28	-10	Streptococcus mitis	0
28	-12	Streptococcus mitis	0.04
28	-3	Streptococcus mitis	0
28	-9	Streptococcus mitis	0.02
28	5	Streptococcus mitis	0
29	-12	Streptococcus mitis	0
29	-3	Streptococcus mitis	0
29	-8	Streptococcus mitis	0.01
29	0	Streptococcus mitis	0.03
30	-10	Streptococcus mitis	0
30	-3	Streptococcus mitis	0
30	4	Streptococcus mitis	0

Gut domination with an infectious pathogen does not always precede bloodstream infection

We sought to determine whether bloodstream infection is preceded by gut microbiota domination by a pathogen as has been previously reported (Ubeda et al. 2010; Taur et al. 2012). We defined domination as 30% species relative abundance or greater of an organism in the gut, as previously described (Taur et al. 2012). Of the 19 stools in which the BSI organism was detected, only 4 stool samples (21%) were dominated by the BSI pathogen. Stool samples from patient 3 were dominated by *Escherichia coli* (>85% relative abundance) 27, 32, and 33 days prior to the development of an *E. coli* BSI (Figure 2A; table 2);

249 *Enterococcus faecium* dominated the intestinal microbiome (94% relative abundance) in patient 25 three
250 days after *E. faecium* BSI (Figure 2B; table 2).
251
252 Several patients experienced BSI with organisms present at relatively lower abundance in the gut prior to
253 bloodstream infection: *Staphylococcus epidermidis* at approximately 2.5% relative abundance in the gut
254 of patient 13 one day prior to their *S. epidermidis* infection, *Pseudomonas aeruginosa* at low abundance
255 (0.83%) in the gut of patient 19 on the day of infection, and *Klebsiella pneumoniae* at 3.5% relative
256 abundance in the gut of patient 2, 9 days prior to BSI (Figure 2C,D; table 2).
257
258 We also observe gut domination by non-BSI-causing pathogens. Patient 14 experienced a *K. pneumoniae*
259 BSI, yet stool samples at two timepoints were dominated by other pathogens: *E. coli* at 67% relative
260 abundance 9 days prior to BSI and *E. faecium* at 85% relative abundance 19 days after BSI (Table 2).
261
262

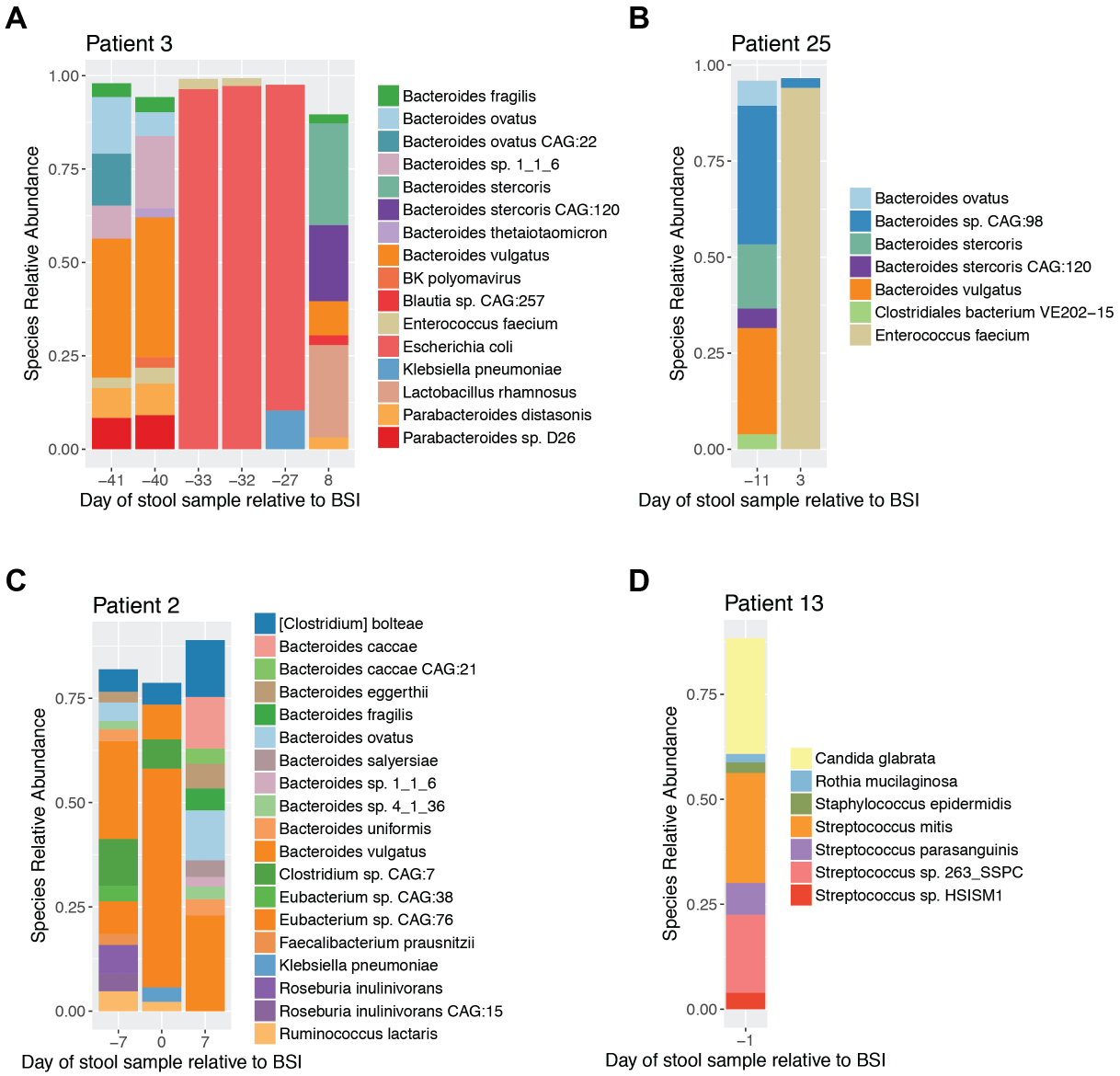
263 **Figure 2. Gut domination by a pathogen does not always precede bloodstream infection.** Domination

264 by *E. coli* (A) and Enterococcus faecium (B) occurs prior to bacteremia, while *K. pneumoniae* (C) and *S.*

265 *epidermidis* (D) are present in the gut microbiota of their respective patients prior to BSI but not at high

266 abundance. Plots show species present at 2% relative abundance or greater (host reads excluded).

267

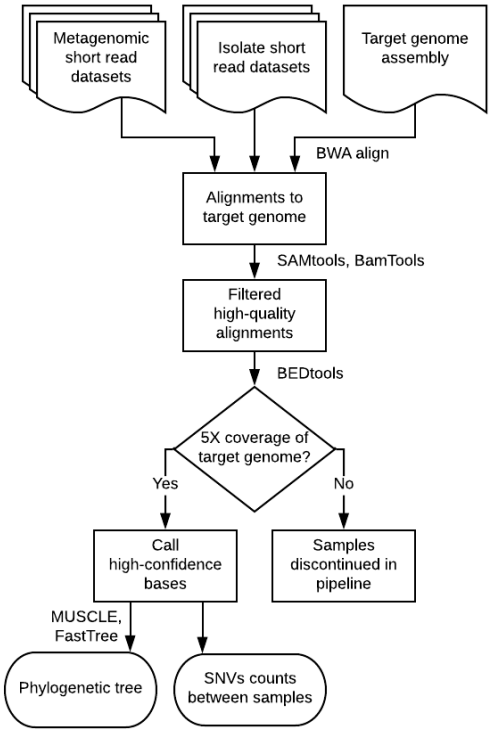


268

269 **Phylogenetic analysis indicates close relatedness of gut and BSI strains**

270 We developed the StrainSifter pipeline to perform nucleotide-level comparisons between genomes and

271 metagenomes. StrainSifter is deployed as a Snakemake workflow, and the pipeline steps are described in
272 Figure 3. Briefly, the inputs to StrainSifter are a draft genome (the "target" genome) and one or more
273 other short read datasets (isolate genomes or metagenomes). StrainSifter detects whether the target
274 organism is present with sufficient abundance in the short read datasets and outputs phylogenetic trees
275 and SNV counts between samples. Despite an abundance of research studies focused on strain-level
276 comparisons of microbes, there is still no clear definition of what constitutes a "strain," and it is unclear
277 whether genomes that differ by a few nucleotides should be considered distinct strains. To investigate the
278 overall relatedness of strains of each BSI species in our metagenomes and isolates, we first took a
279 comparative phylogenomics approach by applying StrainSifter to assess inter-patient strain relatedness.
280 Rather than compare strains from the same patient to publicly-available reference genomes, we instead
281 compared the phylogenetic relatedness of a patient's gut and BSI strains to strains from the other patient
282 samples in our collection. We reasoned that strains from the same geographic location are more likely to
283 be closely phylogenetically related than strains that are both spatially and temporally distant, so
284 comparing inter-patient strain relatedness in the context of the other strains in our sample collection
285 should more precisely indicate strains or lineages unique to a patient.
286
287 **Figure 3. StrainSifter pipeline**
288 Flowchart showing input, analysis steps, and output of the StrainSifter pipeline.



For each BSI species, we used StrainSifter to identify metagenomic samples with at least 5X coverage of the NCBI representative genome for each target organism, found core SNVs present in each sample, and inferred maximum-likelihood phylogenetic trees from multiple sequence alignments of concatenated core SNVs. Reference genomes were selected based on the clinical laboratory MALDI-TOF classification of BSI isolates. Phylogenetic trees demonstrate a higher level of intra-patient versus inter-patient genetic relatedness among colonizing and infecting organisms (Figure 4). BSI and intestinal strains of typically enteric species such as *Enterococcus faecium* (patient 25), *Klebsiella pneumoniae* (patient 2), *E. coli* (patient 3, patient 7), and *S. mitis* (patient 22) are closely phylogenetically related. Unexpectedly, we observe close intra-patient strain relatedness between gut and BSI for typically non-enteric taxa including *S. epidermidis* strains (patient 13) and *Pseudomonas aeruginosa* (patient 19, not pictured). Phylogenetic trees for *P. aeruginosa* and *Enterobacter cloacae* are not shown as these species were not observed in any other samples.

Single-nucleotide variants reveal strain identity

304 We next used StrainSifter to compare single-nucleotide variants (SNVs) between strains in our sample
305 collection. We executed the StrainSifter pipeline for each draft genome, with all stool short read datasets
306 as input. StrainSifter identified samples with sufficient coverage (at least 5X coverage across 50% or
307 more of the genome) of the target organism in the gut metagenome and determined the number of variants
308 shared between each pair of input samples that contained the target strain.
309
310 We observe 1 SNV between the BSI and gut content of Patient 13 who developed a *S. epidermidis* BSI
311 (Table 3). This indicates that the bloodstream strain is concordant to the strain found in the gut 1 day
312 prior. Patient 3 developed an *E. coli* bloodstream infection and we observe zero SNVs between the BSI
313 strain and all samples 33, 32, and 27 days prior to the BSI, indicating that the identical E. coli BSI strain
314 colonized the gut over a month before the onset of infection. Further, we observe zero discriminating
315 SNVs between identical strains of *Pseudomonas aeruginosa* in both the blood and stool specimens.
316 Notably, all stool samples containing the BSI-causing organism at sufficient coverage to profile with
317 StrainSifter were more closely related to their respective BSI isolate based on SNV distance than to any
318 sample from other patients. None of the 30 patients included in our study had sufficient *Staphylococcus*
319 *aureus* in their stool samples to profile with StrainSifter, indicating that this organism infrequently
320 colonizes the gut of HCT patients, even at low abundance.
321
322 **Table 3.**

BSI organism	Sample 1	Sample 2	SNVs	Bases compared	SNVs per base compared	Total bases	Percentage of bases compared
Enterobacter cloacae	Patient 2 BSI	Patient 2 stool day 0	5	4499921	1.11E-06	4552154	98.85%
Enterococcus faecium	Patient 25 BSI	Patient 25 stool day 3	2	2931442	6.82E-07	2935264	99.87%
Escherichia coli	Patient 3 BSI	Patient 3 stool day -33	0	5032224	0.00E+00	5056158	99.53%
Escherichia coli	Patient 3 BSI	Patient 3 stool day -32	0	5032230	0	5056158	99.53%

Escherichia coli	Patient 3 BSI	Patient 3 stool day -27	0	5030454	0	5056158	99.49%
Escherichia coli	Patient 7 BSI	Patient 7 stool day -51	259	4388254	5.90E-05	5267890	83.30%
Klebsiella pneumoniae	Patient 2 BSI	Patient 2 stool day 0	4	5379270	7.44E-07	5390737	99.79%
Klebsiella pneumoniae	Patient 14 BSI	Patient 14 stool day -9	72	3924126	1.83E-05	5407718	72.57%
Pseudomonas aeruginosa	Patient 19 BSI	Patient 19 stool day 0	0	6830248	0	6882743	99.24%
Staphylococcus epidermidis	Patient 13 BSI	Patient 13 stool day -1	1	2592411	3.86E-07	2604609	99.53%
Streptococcus mitis	Patient 22 BSI	Patient 22 stool day -8	28	1345797	2.08E-05	2084062	64.58%

323

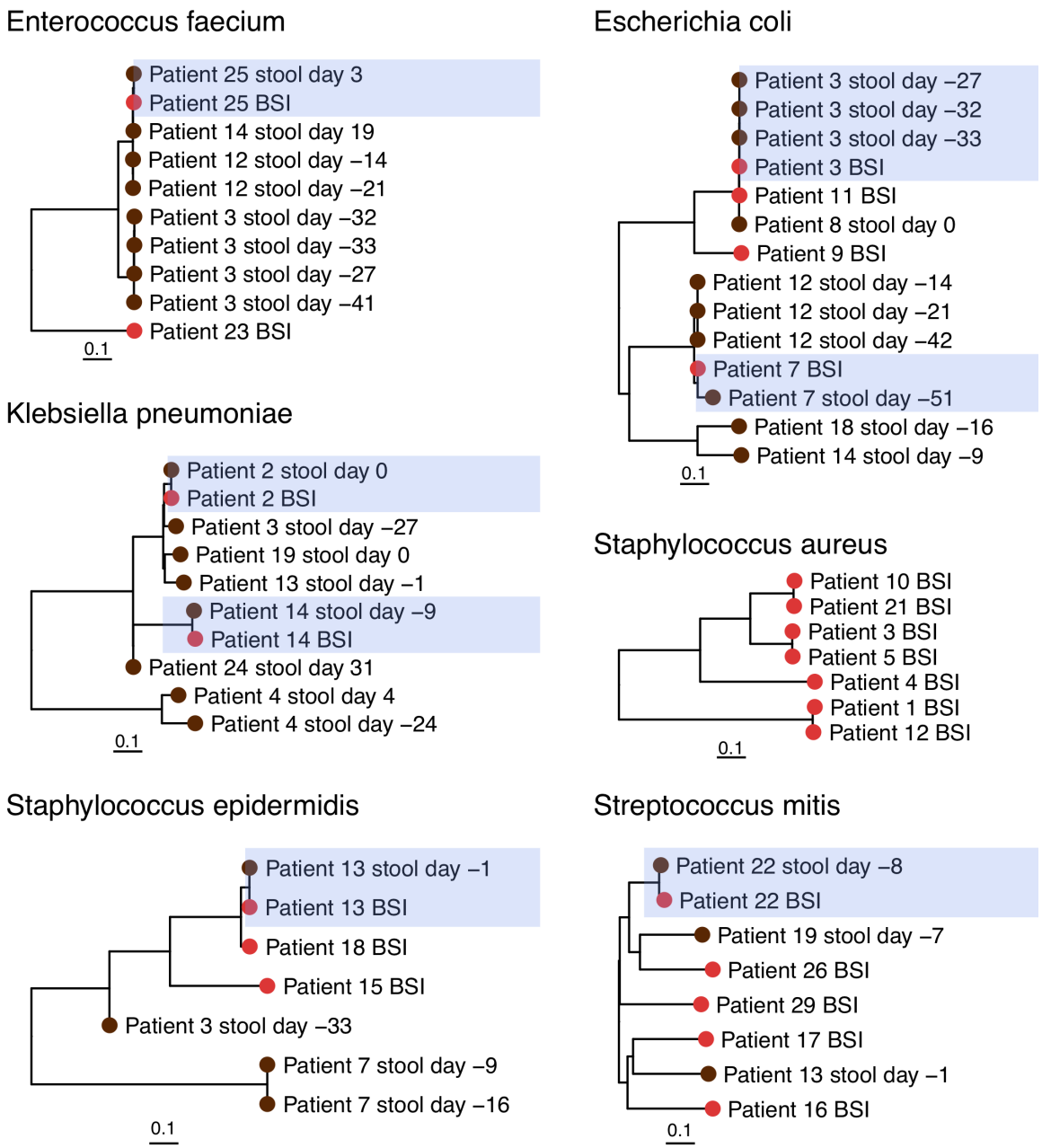
324

325 **Figure 4. Intra-patient samples are more closely related than inter-patient samples.** Branch tip

326 colors indicate stool and BSI samples from the same patient. Samples from the same patient are more

327 closely phylogenetically related than other samples.

328



329

330

331 ***In silico* multilocus sequence typing is concordant with StrainSifter results**

332 To compare whole-genome sequencing-based approaches to traditional strain typing methods, we
333 performed *in silico* multilocus sequence typing (MLST) on BSI genomes and assembled stool
334 metagenomes (Table 3). In many instances, MLST types for gut metagenome strains could not be
335 resolved or could only be partially detected, likely due to low abundance or absence of the target
336 organism in the gut community. MLST data is concordant with StrainSifter SNV profiling in only 4
337 infections for which MLST schemes exist for the bloodstream isolate of interest (Table S7). MLST types
338 could not be determined for either of patient 2's BSI isolates (*K. pneumoniae* and *E. cloacae*) because the
339 sequences at several loci in each genome were not present in the respective MLST databases. We were
340 unable to type *Rothia mucilaginosa* and *Streptococcus mitis*, as standard MLST schemes do not exist for
341 these organisms.
342
343 **Discussion**
344
345 **StrainSifter is a useful pipeline for strain detection within metagenomes**
346 High resolution strain comparisons are required to identify the origins of bloodstream infections using
347 metagenomic samples. While many bioinformatic tools exist for strain comparison between clinical
348 isolates or SNV profiling between metagenomes, no pipeline exists for the specific purpose of detecting
349 the presence of a single strain in a metagenome. In this study, we have developed StrainSifter, a
350 straightforward and facile pipeline for identifying phylogenetic relationships between isolate and
351 metagenomic WGS samples.
352
353 In our study, StrainSifter was able to precisely and comprehensively compare gut metagenomic content to
354 the genomes of diverse bloodstream isolates using high resolution WGS. Although techniques such as *in*
355 *silico* MLST are able to compare genomes to metagenomes, MLST loci are only 450 to 500 base pairs on
356 average, and standard 7-gene MLST schemes therefore analyze less than 4 kilobases of DNA - on the
357 order of 0.1% of the average bacterial genome. By examining genome-wide variants, StrainSifter greatly

358 increases the confidence of strain comparisons.

359

360 Using StrainSifter, we found that the enteric organisms *E. faecium, E.coli*, *K. pneumoniae*, *E. cloacae*,
361 and *S. mitis* were concordant between blood and stool microbiota samples. This confirms what has
362 previously been reported in the literature (Tancrede and Andremont 1985; Samet et al. 2013). Previously
363 published studies have indicated that gut domination with a pathogen is a risk factor for subsequent
364 bloodstream infection (Taur et al. 2012; Ubeda et al. 2010; "Centers for Disease Control and Prevention"
365 2018). While *E. coli* and *E. faecium* domination events were observed preceding BSI, we find *K.*
366 *pneumoniae*, *E. cloacae,* and *S. epidermidis* in relatively low abundance in stool samples on the day of
367 BSI and one day prior.

368

369 Interestingly, we also find that typically non-enteric organisms *S. epidermidis* and *P. aeruginosa* were not
370 only present in the gut, but strains within the gut microbiota and bloodstream were concordant. Gut
371 colonization by *S. epidermidis* and *P. aeruginosa* is unexpected given current National Health and Safety
372 guidelines delimiting enteric versus non-enteric organisms (Taur et al. 2012; Ubeda et al. 2010; "Centers
373 for Disease Control and Prevention" 2018; Advani et al. 2017). Yet, colonization by atypical organisms
374 may not be surprising given the degree of disruption of the intestinal microbiota by antibiotics,
375 chemotherapy, and radiation. While *P. aeruginosa* intestinal colonization has been previously
376 documented (Nesher et al. 2014), intestinal colonization with coagulase-negative *Staphylococcus* is not
377 well described. In our own study, multiple stool samples were determined to have more than 80% relative
378 abundance of *S. epidermidis*. In addition, we observe the same colonizing strain of coagulase-negative
379 *Staphylococcus* over time in the same individual (patient 7), arguing against only transient membership
380 within the intestinal microbiome.

381

382 Previous research using existing strain typing and culture-based strategies has only tentatively
383 demonstrated evidence for gut translocation of coagulase-negative *Staphylococcus spp.* and *P. aeruginosa*

384 in immunocompromised patients (Wade et al. 1982; Rotstein et al. 1988; Nesher et al. 2014; MacFie et al.
385 1999; Costa et al. 2006). Here our straightforward approach allows for greater precision and confidence in
386 strain comparisons between the gut microbiota and infecting organism in the bloodstream. Given that our
387 analysis focused entirely on the stool microbiota, we cannot rule out the possibility of the same strain of
388 coagulase-negative *Staphylococcus* colonizing the entire body surface--including the gut, nares, skin, or
389 central line--from which the infection may have originated instead. Previous research has suggested,
390 however, that the majority of patients with coagulase-negative *Staphylococcus* bloodstream infection are
391 colonized with a multitude of strains that differ between the intestine and the skin (Herwaldt et al. 1992).
392
393 Determining whether two strains are related based on the number of SNVs that differ between their
394 genomes is not straightforward. While approximate bacterial mutation rates due to genetic drift have been
395 established, most estimates are derived from experiments in culture media. As gut and bloodstream
396 environments impose many selective pressures beyond genetic drift, such estimates are likely not relevant
397 in the clinical context. For instance, it is possible that bacterial pathogens that originate from the intestine
398 and translocate into the bloodstream may acquire single nucleotide variants over time due to selective
399 pressures. For this reason, it is difficult to define a threshold number of SNVs beyond which two strains
400 are not considered to be the same. Regardless of the absolute number of SNVs between strains or across
401 all strains analyzed with StrainSifter, strains from the same patient consistently share fewer SNVs
402 compared to those from other patients.
403
404 Although StrainSifter can precisely identify shared variants between genomes and metagenomes, it is
405 limited to profiling only the dominant strain of a given organism in a community. However, it has been
406 shown that gut metagenomes frequently contain only one predominant strain of each species, so
407 StrainSifter is likely to function optimally in most situations (Truong et al. 2017). As is the case with
408 existing tools for metagenomic strain comparison, StrainSifter is unable to analyze low-abundance strains
409 since adequate coverage (5X) is required for confident variant calling.

410

411 **Future directions**

412 StrainSifter can be used to precisely identify strains within relatively biomass-rich metagenomic samples
413 without relying on external reference genome databases in which representation of opportunistic
414 pathogens may be lacking. Compared to stool, samples from the nares, skin, or the environment typically
415 have far less microbial biomass, limiting the number of sequencing reads that can be obtained. In order to
416 provide the most precise epidemiologic information about the origins of bloodstream and other infections,
417 StrainSifter will need to be tested in low microbial biomass samples in a larger prospective study of
418 immunocompromised patients. Given the high resolution of WGS-based strain comparisons, we may
419 indeed discover instances of organisms thought to originate from non-enteric sites instead colonizing and
420 translocating from the intestine in severely immunocompromised patients, as was potentially
421 demonstrated by *S. epidermidis* and *P. aeruginosa* in our study. Given the growing body of research on
422 therapies to improve gut microbiota diversity and bolster colonization resistance against pathogens, more
423 precisely identifying the origins of bloodstream infections in the stem cell transplant population may
424 influence how hospitals and healthcare providers can most effectively work to prevent infections
425 (Galloway-Peña, Jenq, and Shelburne 2017). Finally, once StrainSifter is able to establish the origins of
426 an infection, the wealth of information offered by WGS can be interrogated to identify potential
427 functional differences, including in gene content and metabolic pathways, between strains colonizing the
428 gut, nares, or skin that result in disseminated infections compared to those that do not.

429

430 In conclusion, we have shown that StrainSifter offers a straightforward bioinformatic pipeline to precisely
431 compare bacterial isolates to metagenomic samples, circumventing the need for external reference
432 genome databases or culture-based methods of strain comparison. We successfully applied StrainSifter to
433 blood culture isolates and stool samples from hematopoietic stem cell transplant patients and found
434 classically non-enteric bacteria in the gut microbiome that were concordant with bloodstream isolates. We
435 anticipate that StrainSifter will be an easy-to-use tool in the context of hospital or infectious outbreak

436 epidemiologic studies, and that characterization of the microbiome dynamics that occur prior to infection
437 may help us precisely identify potential reservoirs of pathogens.

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451

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453 **References**

454 Advani, Sonali, Rachael Lee, Mariann Schmitz, Martha Long, and Bernard Camins. 2017. "2015 NHSN
455 CAUTI Definition Change and Its Impact on CLABSI Rates at an Academic Medical Center." *Open*
456 *Forum Infectious Diseases* 4 (suppl_1): S180–S180.

457 Bankevich, Anton, Sergey Nurk, Dmitry Antipov, Alexey A. Gurevich, Mikhail Dvorkin, Alexander S.
458 Kulikov, Valery M. Lesin, et al. 2012. "SPAdes: A New Genome Assembly Algorithm and Its
459 Applications to Single-Cell Sequencing." *Journal of Computational Biology: A Journal of*
460 *Computational Molecular Cell Biology* 19 (5): 455–77.

461 Barnett, Derek W., Erik K. Garrison, Aaron R. Quinlan, Michael P. Strömberg, and Gabor T. Marth.
462 2011. "BamTools: A C++ API and Toolkit for Analyzing and Managing BAM Files."
463 *Bioinformatics* 27 (12): 1691–92.

464 Blennow, O., P. Ljungman, E. Sparrelid, J. Mattsson, and M. Remberger. 2014. "Incidence, Risk Factors,
465 and Outcome of Bloodstream Infections during the Pre-Engraftment Phase in 521 Allogeneic
466 Hematopoietic Stem Cell Transplantations." *Transplant Infectious Disease: An Official Journal of*
467 *the Transplantation Society* 16 (1): 106–14.

468 "Centers for Disease Control and Prevention." 2018. Bloodstream Infection Event (Central Line-
469 Associated Bloodstream Infection and Non-Central Line-Associated Bloodstream Infection). The
470 Centers for Disease Control and Prevention Web Site. February 2018.
471 http://www.cdc.gov/nhsn/PDFs/pscManual/4PSC_CLABS current.pdf.

472 Chen, Yi, Yan Luo, Heather Carleton, Ruth Timme, David Melka, Tim Muruvanda, Charles Wang, et al.
473 2017. "Whole Genome and Core Genome Multilocus Sequence Typing and Single Nucleotide
474 Polymorphism Analyses of Listeria Monocytogenes Associated with an Outbreak Linked to Cheese,
475 United States, 2013." *Applied and Environmental Microbiology* 83 (15): 1–15.

476 Costa, Silvia F., Antonio A. Barone, Marisa H. Miceli, Inneke M. van der Heijden, Robson E. Soares,
477 Anna S. Levin, and Elias J. Anaissie. 2006. "Colonization and Molecular Epidemiology of

478 Coagulase-Negative Staphylococcal Bacteremia in Cancer Patients: A Pilot Study." *American*
479 *Journal of Infection Control* 34 (1): 36–40.
480 Costea, Paul Igor, Robin Munch, Luis Pedro Coelho, Lucas Paoli, Shinichi Sunagawa, and Peer Bork.
481 2017. "metaSNV: A Tool for Metagenomic Strain Level Analysis." *PloS One* 12 (7): 1–9.
482 Edgar, Robert C. 2004. "MUSCLE: Multiple Sequence Alignment with High Accuracy and High
483 Throughput." *Nucleic Acids Research* 32 (5): 1792–97.
484 "GAEMR." n.d. Accessed April 19, 2018. https://www.broadinstitute.org/software/gaemr/.
485 Galloway-Peña, Jessica R., Robert R. Jenq, and Samuel A. Shelburne. 2017. "Can Consideration of the
486 Microbiome Improve Antimicrobial Utilization and Treatment Outcomes in the Oncology Patient?"
487 *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research* 23
488 (13): 3263–68.
489 Gorrie, Claire L., Mirjana Mirčeta, Ryan R. Wick, David J. Edwards, Nicholas R. Thomson, Richard A.
490 Strugnell, Nigel F. Pratt, et al. 2017. "Gastrointestinal Carriage Is a Major Reservoir of Klebsiella
491 Pneumoniae Infection in Intensive Care Patients." *Clinical Infectious Diseases: An Official*
492 *Publication of the Infectious Diseases Society of America* 65 (2): 1–8.
493 Gudiol, C., C. Garcia-Vidal, M. Arnan, I. Sánchez-Ortega, B. Patiño, R. Duarte, and J. Carratalà. 2014.
494 "Etiology, Clinical Features and Outcomes of Pre-Engraftment and Post-Engraftment Bloodstream
495 Infection in Hematopoietic SCT Recipients." *Bone Marrow Transplantation* 49 (6): 824–30.
496 Harris, Simon R., Edward J. P. Cartwright, M. Estée Török, Matthew T. G. Holden, Nicholas M. Brown,
497 Amanda L. Ogilvy-Stuart, Matthew J. Ellington, et al. 2013. "Whole-Genome Sequencing for
498 Analysis of an Outbreak of Meticillin-Resistant Staphylococcus Aureus: A Descriptive Study." *The*
499 *Lancet Infectious Diseases* 13 (2): 130–36.
500 Herwaldt, L. A., R. J. Hollis, L. D. Boyken, and M. A. Pfaller. 1992. "Molecular Epidemiology of
501 Coagulase-Negative Staphylococci Isolated from Immunocompromised Patients." *Infection Control*
502 *and Hospital Epidemiology: The Official Journal of the Society of Hospital Epidemiologists of*
503 *America* 13 (2): 86–92.

504 Inouye, Michael, Harriet Dashnow, Lesley-Ann Raven, Mark B. Schultz, Bernard J. Pope, Takehiro
505 Tomita, Justin Zobel, and Kathryn E. Holt. 2014. "SRST2: Rapid Genomic Surveillance for Public
506 Health and Hospital Microbiology Labs." *Genome Medicine* 6 (11): 90.
507 Kovanen, Sara M., Rauni I. Kivistö, Mirko Rossi, Thomas Schott, Ulla-Maija Kärkkäinen, Tamara
508 Tuuminen, Jaakko Uksila, Hilpi Rautelin, and Marja-Liisa Hänninen. 2014. "Multilocus Sequence
509 Typing (MLST) and Whole-Genome MLST of Campylobacter Jejuni Isolates from Human
510 Infections in Three Districts during a Seasonal Peak in Finland." *Journal of Clinical Microbiology*
511 52 (12): 4147–54.
512 Lieberman, Tami D., Kelly B. Flett, Idan Yelin, Thomas R. Martin, Alexander J. McAdam, Gregory P.
513 Priebe, and Roy Kishony. 2013. "Genetic Variation of a Bacterial Pathogen within Individuals with
514 Cystic Fibrosis Provides a Record of Selective Pressures." *Nature Genetics* 46 (1): 82–87.
515 Li, H., and R. Durbin. 2009. "Fast and Accurate Short Read Alignment with Burrows–Wheeler
516 Transform." *Bioinformatics* 25 (14): 1754–60.
517 Li, Heng, Bob Handsaker, Alec Wysoker, Tim Fennell, Jue Ruan, Nils Homer, Gabor Marth, Goncalo
518 Abecasis, Richard Durbin, and 1000 Genome Project Data Processing Subgroup. 2009. "The
519 Sequence Alignment/Map Format and SAMtools." *Bioinformatics* 25 (16): 2078–79.
520 Li, Wenjun, Didier Raoult, and Pierre-Edouard Fournier. 2009. "Bacterial Strain Typing in the Genomic
521 Era." *FEMS Microbiology Reviews* 33 (5): 892–916.
522 MacFie, J., C. O'Boyle, C. J. Mitchell, P. M. Buckley, D. Johnstone, and P. Sudworth. 1999. "Gut Origin
523 of Sepsis: A Prospective Study Investigating Associations between Bacterial Translocation, Gastric
524 Microflora, and Septic Morbidity." *Gut* 45 (2): 223–28.
525 Martin, Rebekah M., Jie Cao, Sylvain Brisse, Virginie Passet, Weisheng Wu, Lili Zhao, Preeti N. Malani,
526 Krishna Rao, and Michael A. Bachman. 2016. "Molecular Epidemiology of Colonizing and Infecting
527 Isolates of Klebsiella Pneumoniae." *mSphere* 1 (5): 1–12.
528 Mikulska, Malgorzata, Valerio Del Bono, Anna Maria Raiola, Barbara Bruno, Francesca Gualandi,
529 Domenico Occhini, Carmen di Grazia, Francesco Frassoni, Andrea Bacigalupo, and Claudio Viscoli.

530 2009. “Blood Stream Infections in Allogeneic Hematopoietic Stem Cell Transplant Recipients:
531 Reemergence of Gram-Negative Rods and Increasing Antibiotic Resistance.” *Biology of Blood and*
532 *Marrow Transplantation: Journal of the American Society for Blood and Marrow Transplantation*
533 15 (1): 47–53.
534 Minot, Samuel S., Niklas Krumm, and Nicholas B. Greenfield. 2015. “One Codex: A Sensitive and
535 Accurate Data Platform for Genomic Microbial Identification.” *bioRxiv*, 027607.
536 Nayfach, Stephen, Beltran Rodriguez-Mueller, Nandita Garud, and Katherine S. Pollard. 2016. “An
537 Integrated Metagenomics Pipeline for Strain Profiling Reveals Novel Patterns of Bacterial
538 Transmission and Biogeography.” *Genome Research* 26 (11): 1612–25.
539 Nesher, L., K. V. I. Rolston, D. P. Shah, J. T. Tarrand, V. Mulanovich, E. J. Ariza-Heredia, and R. F.
540 Chemaly. 2014. “Fecal Colonization and Infection with Pseudomonas aeruginosa in Recipients of
541 Allogeneic Hematopoietic Stem Cell Transplantation.” *Transplant Infectious Disease: An Official*
542 *Journal of the Transplantation Society* 17 (1): 33–38.
543 Nielsen, Karen Leth, Marc Stegger, Kristoffer Kiil, Paul A. Godfrey, Michael Feldgarden, Berit Lilje,
544 Paal S. Andersen, and Niels Frimodt-Møller. 2017. “Whole-Genome Comparison of Urinary
545 Pathogenic Escherichia Coli and Faecal Isolates of UTI Patients and Healthy Controls.”
546 *International Journal of Medical Microbiology: IJMM* 307 (8): 497–507.
547 Nurk, Sergey, Dmitry Meleshko, Anton Korobeynikov, and Pavel A. Pevzner. 2017. “metaSPAdes: A
548 New Versatile Metagenomic Assembler.” *Genome Research* 27 (5): 824–34.
549 Price, Morgan N., Paramvir S. Dehal, and Adam P. Arkin. 2010. “FastTree 2--Approximately Maximum-
550 Likelihood Trees for Large Alignments.” *PloS One* 5 (3). Public Library of Science: e9490.
551 “PubMLST.” n.d. Accessed April 20, 2018. https://pubmlst.org/.
552 Rotstein, C., D. Higby, K. Killion, and E. Powell. 1988. “Relationship of Surveillance Cultures to
553 Bacteremia and Fungemia in Bone Marrow Transplant Recipients with Hickman or Broviac
554 Catheters.” *Journal of Surgical Oncology* 39 (3): 154–58.
555 Salipante, Stephen J., Dhruba J. SenGupta, Lisa A. Cummings, Tyler A. Land, Daniel R. Hoogestraat,

556 and Brad T. Cookson. 2015. “Application of Whole-Genome Sequencing for Bacterial Strain Typing
557 in Molecular Epidemiology.” *Journal of Clinical Microbiology* 53 (4): 1072–79.
558 Samet, A., A. Śledzińska, B. Krawczyk, A. Hellmann, S. Nowicki, J. Kur, and B. Nowicki. 2013.
559 “Leukemia and Risk of Recurrent Escherichia Coli Bacteremia: Genotyping Implicates E. Coli
560 Translocation from the Colon to the Bloodstream.” *European Journal of Clinical Microbiology &*
561 *Infectious Diseases: Official Publication of the European Society of Clinical Microbiology* 32 (11):
562 1393–1400.
563 See, Isaac, Minn M. Soe, Lauren Epstein, Jonathan R. Edwards, Shelley S. Magill, and Nicola D.
564 Thompson. 2017. “Impact of Removing Mucosal Barrier Injury Laboratory-Confirmed Bloodstream
565 Infections from Central Line-Associated Bloodstream Infection Rates in the National Healthcare
566 Safety Network, 2014.” *American Journal of Infection Control* 45 (3): 321–23.
567 Snitkin, E. S., A. M. Zelazny, P. J. Thomas, F. Stock, D. K. Henderson, T. N. Palmore, J. A. Segre, and
568 Nisc Comparative Sequencing Program. 2012. “Tracking a Hospital Outbreak of Carbapenem-
569 Resistant Klebsiella Pneumoniae with Whole-Genome Sequencing.” *Science Translational Medicine*
570 4 (148): 148ra116–48ra116.
571 Snitkin, Evan S., Adrian M. Zelazny, Clemente I. Montero, Frida Stock, Lilia Mijares, NISC Comparative
572 Sequence Program, Patrick R. Murray, and Julie A. Segre. 2011. “Genome-Wide Recombination
573 Drives Diversification of Epidemic Strains of Acinetobacter Baumannii.” *Proceedings of the*
574 *National Academy of Sciences of the United States of America* 108 (33): 13758–63.
575 Tancrede, C. H., and A. O. Andremont. 1985. “Bacterial Translocation and Gram-Negative Bacteremia in
576 Patients with Hematological Malignancies.” *The Journal of Infectious Diseases* 152 (1): 99–103.
577 Taur, Ying, Joao B. Xavier, Lauren Lipuma, Carles Ubeda, Jenna Goldberg, Asia Gobourne, Yeon Joo
578 Lee, et al. 2012. “Intestinal Domination and the Risk of Bacteremia in Patients Undergoing
579 Allogeneic Hematopoietic Stem Cell Transplantation.” *Clinical Infectious Diseases: An Official*
580 *Publication of the Infectious Diseases Society of America* 55 (7): 905–14.
581 Truong, Duy Tin, Adrian Tett, Edoardo Pasolli, Curtis Huttenhower, and Nicola Segata. 2017. “Microbial

582 Strain-Level Population Structure & Genetic Diversity from Metagenomes." *Genome Research* 27
583 (4): 626–38.
584 Ubeda, Carles, Ying Taur, Robert R. Jenq, Michele J. Equinda, Tammy Son, Miriam Samstein, Agnes
585 Viale, et al. 2010. "Vancomycin-Resistant Enterococcus Domination of Intestinal Microbiota Is
586 Enabled by Antibiotic Treatment in Mice and Precedes Bloodstream Invasion in Humans." *The*
587 *Journal of Clinical Investigation* 120 (12): 4332–41.
588 Wade, J. C., S. C. Schimpff, K. A. Newman, and P. H. Wiernik. 1982. "Staphylococcus Epidermidis: An
589 Increasing Cause of Infection in Patients with Granulocytopenia." *Annals of Internal Medicine* 97
590 (4): 503–8.

591