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1 **Major Article**

2

3 **Title:** Investigating Transfusion-Related Sepsis using Culture-Independent Metagenomic

4 Sequencing

5

6 **Running Title:** Tracking Septic Transfusions with mNGS

7

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42
43 **Main Point:** Transfusion-transmitted infections cause significant morbidity and are challenging
44 to prevent and diagnose. We found that culture-independent metagenomic sequencing of blood
45 products afforded rapid and precise assessment of pathogen identity, abundance and genetic
46 relatedness, enhancing traditional hospital infection control strategies.
47
48 **Keywords:** Healthcare infections, platelet transfusion, septic transfusion, metagenomic
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54 **Abstract**

55 **Background.** Transfusion-related sepsis remains an important hospital infection control
56 challenge. Investigating septic transfusion events is often restricted by the limitations of bacterial
57 culture in terms of time requirements and low yield in the setting of prior antibiotic
58 administration.

59 **Methods.** In three Gram-negative septic transfusion cases, we performed mNGS of direct
60 clinical blood specimens in addition to standard culture-based approaches utilized for infection
61 control investigations. Pathogen detection leveraged IDSeq, a new open-access microbial
62 bioinformatics portal. Phylogenetic analysis was performed to assess microbial genetic
63 relatedness and understand transmission events.

64 **Results.** mNGS of direct clinical blood specimens afforded precision detection of pathogens
65 responsible for each case of transfusion-related sepsis, and enabled discovery of a novel
66 *Acinetobacter* species in a platelet product that had become contaminated despite
67 photochemical pathogen reduction. In each case, longitudinal assessment of pathogen burden
68 elucidated the temporal sequence of events associated with each transfusion-transmitted
69 infection. We found that informative data could be obtained from culture-independent mNGS of
70 residual platelet products and leftover blood specimens that were either unsuitable or
71 unavailable for culture, or that failed to grow due to prior antibiotic administration. We
72 additionally developed methods to enhance accuracy for detecting transfusion-associated
73 pathogens sharing taxonomic similarity to contaminants commonly found in mNGS library
74 preparations.

75 **Conclusions.** Culture-independent mNGS of blood products afforded rapid and precise
76 assessment of pathogen identity, abundance and genetic relatedness. Together, these
77 challenging cases demonstrated the potential for metagenomics to advance existing methods
78 for investigating transfusion-transmitted infections.

79

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80 **Introduction**

81 While transfusion-associated infections have globally decreased over the past 50 years,
82 bacterial contamination of the platelet supply remains a significant public health challenge, with
83 approximately one in 1,500 – 5,000 units containing detectable organisms[1–5]. Room
84 temperature storage facilitates more bacterial growth in platelets compared to other blood
85 products, with skin flora, asymptomatic donor bacteremia, and direct introduction from
86 environmental sources accounting for the majority of contaminants[3,6]. Even though current
87 culture-based screening approaches may miss a significant fraction of contaminated units,
88 clinically reported sepsis occurs in only one in 15,000-100,000 transfusions, presumably due to
89 frequent concurrent antibiotic administration, low bacterial inoculum, or attribution of transfusion-
90 related outcomes to a patient's pre-existing infection or illness[3,7].

91 Pathogen-reduction of platelet products using amotosalen, a nucleic acid cross-linking
92 agent activated by ultraviolet (UV)-A irradiation, has been routinely performed in Europe for over
93 a decade and recently received United States Food and Drug Administration approval[8].
94 Photoinactivation is broadly effective against diverse bacterial pathogens, and while failure at
95 high bacterial loads has been described, few reports of sepsis have been reported following
96 treatment[9,10].

97 Here we describe three transfusion-related Gram-negative sepsis investigations
98 including one involving a pathogen-reduced platelet product. For each, we performed
99 metagenomic next generation sequencing (mNGS) in addition to standard microbiologic
100 diagnostics and found that mNGS enabled rapid and precise taxonomic identification and
101 phylogenetic analysis of bacterial isolates, as well as culture-independent assessment of direct
102 clinical samples.

103

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104 **Methods**

105 Ethics Statement

106 Investigations were carried out according to a no-subject-contact study protocol
107 approved by the UCSF Institutional Review Board (IRB# 17-24056) which permitted analysis of
108 de-identified leftover clinical microbiology samples from collaborating institutions and
109 subsequent review of study subjects' electronic medical records. No decisions regarding
110 antibiotics or other patient-specific treatment interventions were made using sequencing data.

111

112 Microbial culture

113 Patient blood cultures were performed via inoculation into BD Bactec Plus Aerobic and
114 Lytic Anaerobic media (Becton Dickinson). This same approach was used for residual platelet
115 transfusion cultures in cases one and three, and for residual red blood cell segment cultures in
116 case 2. Species identification was performed using MALDI-TOF mass spectrometry (Bruker).
117 Details on culture time to positivity are described in **Supplemental Table 1**. Sterility testing was
118 carried out as described in the **Supplemental Methods**.

119

120 Sequencing

121 Sample processing and DNA extraction were carried out as described in the
122 **Supplemental Methods**. Ten to 100 ng of DNA from each sample was sheared with
123 fragmentase (New England Biolabs) and used to construct sequencing libraries with the
124 NEBNext Ultra II Library Prep Kit (New England Biolabs). Adaptor ligated samples underwent
125 amplification with dual unique indexing primers. Libraries were quantified, pooled, and
126 underwent paired end 150 base pair sequencing on an Illumina MiSeq or NextSeq 550.
127 **Supplemental Table 2** lists the number of reads obtained for each sample.

128

129 Bioinformatics and phylogenetic analyses

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130 Detection, taxonomic identification, and abundance quantitation of microbes from raw
131 sequencing reads was first performed using the IDseq pipeline according to described
132 protocols[11]. To control for background environmental and reagent contaminants, no-template
133 water control samples were incorporated alongside extracted nucleic acid and carried forward
134 throughout library preparation and sequencing. Genome assembly was performed by first
135 trimming the raw sequencing reads in fastq files using TrimGalore[12] and assembling using
136 Unicycler[13] with default parameters. To identify the closest related species in cases one and
137 two, BLAST+[14] against the National Center for Biotechnology Information (NCBI) nt database
138 and the Mash/Mini Hash search via PATRIC[15,16] were employed to analyze assembled and
139 annotated contiguous sequences (contigs). For case three, sequence type was determined
140 using SRST2[17]; other *Klebsiella pneumoniae* sequences in NCBI databases belonging to the
141 same sequence type were found using a Mash/Mini Hash search via PATRIC[15,16].
142 Phylogenetic analysis for case one and case three was used to determine the
143 relationship between the samples in this study and the most closely related NCBI genomes.
144 First, trimmed reads were aligned against a reference genome (case one: *A. baumannii*
145 CP017642.1, case three: *K. pneumoniae* CP015392.1) using Snippy v4.3.6[18]. Then a SNP
146 alignment was obtained by variant calling using bcftools v1.9[19]. We filtered SNPs with
147 QUAL<30, and filtered genotypes with major allele depth (FMT/AD) < 10 or major allele
148 frequency (FMT/AF) < .9. Finally, the maximum likelihood phylogeny based on the SNP
149 alignments was built in RAxML v8.2.12[20] using '-m ASC_GTRCAT --asc-corr=lewis' options.
150
151

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152 **Results**

153

154 **Investigation #1: *Acinetobacter* septic platelet transfusion**

155 Patient A, a 59 year old man with relapsed acute lymphoblastic leukemia undergoing
156 chemotherapy, received two doses of pathogen-reduced apheresis platelets on the day of
157 planned home discharge. No side effects were experienced after transfusion of the first platelet
158 unit, however after receiving the second unit two hours later, he developed chills and rigors
159 followed by fever and critical hypotension. He received fluids and vancomycin before being
160 transferred to the intensive care unit where meropenem, vasopressors, respiratory support and
161 continuous renal replacement therapy were administered. He recovered fully and was
162 discharged home 16 days later on parenteral antibiotics.

163 Blood cultures obtained 4.5 hours post-transfusion returned positive for *Acinetobacter*
164 *calcoaceticus/baumanii (ACB) complex*. Residual material obtained from a saline rinse of the
165 returned platelet bag also grew *ACB complex* as well as *Staphylococcus saprophyticus*. Culture
166 of a non-transfused platelet co-component from the same donor, concurrently pathogen-
167 inactivated with the transfused product five days earlier, was negative. At the time of the
168 investigation, hypotheses considered included failure of the pathogen-reduction process,
169 contamination of the pathogen-reduced platelet bag during transport, or pre-existing sub-clinical
170 bacteremia in the patient.

171 To investigate this case further, we first sequenced the cultured *ACB* complex isolate
172 from the patient's blood and the *ACB* complex isolate from a wash of the transfused platelet
173 bag. In total, sequencing and analysis for this case was performed in 45 hours. *De novo*
174 genome assembly revealed a novel *Acinetobacter* species most closely related (92% average
175 nucleotide identity) to *A. nosocomialis* and *A. pitii,* members of the *ACB* complex **(Figure 1A)**.
176 Phylogenetic analysis revealed zero single nucleotide polymorphisms (SNPs) over the 3.9 Mb

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177 genome between the novel *Acinetobacter* species cultured from the blood and from the platelet
178 bag, indicating that the two isolates were identical.
179 Culture-independent mNGS detected a high abundance of *ACB complex* in the
180 transfused product (230,000 reads per million, rpM) and in the patient's plasma following
181 transfusion (190 rpM) **(Figure 1B)**, as determined by summing the reads found by IDSeq
182 aligning to species within the ACB complex (NCBI taxid 909768) which includes *A.*
183 *calcoaceticus, A. baumanii, A. nosocomialis* and *A. pitii* among others**.** By comparison, the
184 untransfused co-components and patient's plasma before transfusion had very few alignments
185 to *ACB complex*, in the range of expected background levels (0.25 and 2.2 rpM, respectively).
186 Assessment of these low abundance alignments at the species level revealed that they most
187 likely represented mis-assigned reads from other *Acinetobacter* species, notably *A. johnsonii*.
188 More specifically, the co-components and pre-transfusion samples had only a small percentage
189 of *Acinetobacter* genus reads that best aligned to an *ACB species*, while in the transfused
190 product and post-transfusion samples, the vast majority of genus *Acinetobacter* reads aligned to
191 *ACB species* (**Supplemental Figure 1A**). The orders-of-magnitude difference in percent reads
192 mapping to *ACB complex*, along with the change in composition of *Acinetobacter* assignments
193 between samples, indicated that only the transfused product and patient's post-transfusion
194 plasma were contaminated by a species within the *ACB complex*. Although the sequence of the
195 cultured isolate in this case allowed us to properly identify the strain phylogenetically, it was not
196 required to complete the mNGS analysis that distinguished pathogen and environmental
197 contaminant.
198
199
200 **Investigation #2: Fatal *Pseudomonas aeruginosa* septic transfusion**
201 Patient B, a 77 year old man, was admitted for management of acute on chronic heart
202 failure and implantation of a right ventricular assist device. During surgery, he received three

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203 units of platelets, three units of fresh frozen plasma, and two units of cross-matched packed red
204 blood cells (PRBCs). Intraoperatively, the patient became hemodynamically unstable. Cultures
205 drawn three hours post-surgery ultimately returned positive for *Pseudomonas aeruginosa,*
206 raising concern for a septic transfusion reaction (**Figure 2**).

207 Despite administration of antipseudomonal antibiotics, the patient's clinical stability
208 continued to deteriorate, and he did not survive beyond post-operative day three. mNGS was
209 retrospectively performed on plasma samples collected pre- and post-transfusion from the
210 patient, on aliquots from the blood culture bottles that eventually turned positive, and on residual
211 packed red blood cells (PRBCs) remaining following transfusion. Following receipt of samples
212 from the affected hospital, library preparation, sequencing and preliminary analysis time totaled
213 72 hours. No remaining material from the platelets or transfused plasma was available for
214 mNGS or other diagnostic testing. Cultured bacterial isolates were unavailable for sequencing.

215 mNGS revealed no *P. aeruginosa* in the pre-transfusion plasma sample nor in the
216 residual PRBCs, but identified a high abundance of *P. aeruginosa* in post-transfusion plasma
217 (**Figure 2**) that decreased over time in the setting of antibiotic treatment. Serial measurement of
218 procalcitonin demonstrated a normal pre-transfusion level (0.034 ng/mL, reference interval:
219 <0.15 ng/mL) but significantly elevated concentrations following surgery (range: 42.73 to >100
220 ng/mL [above detection limit]). Phylogenetic analysis indicated strong relatedness to *P.*
221 *aeruginosa* strain BWHPSA041.

222 Several environmentally ubiquitous *Pseudomonas* species are common contaminants of
223 mNGS library preparation reagents and thus we used IDSeq[11] to determine the percent of
224 *Pseudomonas* genus reads that mapped specifically to *P. aeruginosa.* In all six post-transfusion
225 plasma samples, as well as the blood culture bottle samples, over 99% of genus *Pseudomonas*
226 reads mapped best to *P. aeruginosa*, while in water controls an average of 7.2% (range 0-
227 12.1%) mapped best to *P. aeruginosa* (**Supplemental Figure 1B).** This result indicated that the

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228 *P. aeruginosa* observed in the post-transfusion plasma samples was not the result of
229 contamination from mNGS library preparation reagents.
230
231 **Investigation #3: Fatal *Klebsiella* septic platelet transfusion**
232 Two immunocompromised pediatric patients, 2 and 3 years of age, developed septic
233 shock following transfusion of platelets derived from a single donor, as recently reported[6].
234 Patient C, who had undergone successful autologous hematopoietic stem cell transplantation,
235 developed hypotension, tachycardia and vomiting 15 minutes into the transfusion. Despite
236 initiation of vancomycin and cefepime, fluid resuscitation, vasopressor support and intubation,
237 he died within five hours. Blood cultures ultimately returned positive for *Klebsiella pneumoniae*,
238 as did a culture from residual material in the platelet bag.
239 Five hours earlier, Patient D, who was receiving empiric cefepime for neutropenic fever,
240 had undergone transfusion with the second platelet unit derived from the same donor. He
241 decompensated into septic shock nine hours following transfusion but ultimately survived
242 following fluid resuscitation and vasopressor support. Blood cultures remained negative in the
243 setting of concurrent antibiotic treatment, initially precluding determination of whether sepsis
244 was due to a *Klebsiella*-contaminated platelet transfusion or to another etiology.
245 Culture-independent mNGS of pre- and post-transfusion plasma or serum samples
246 revealed a marked increase in *Klebsiella pneumoniae* following transfusion in both patients,
247 although patient D, who had negative blood cultures, had 12.9-fold fewer *Klebsiella* rpM
248 detected compared to patient C (**Figure 3**). Genome assembly and phylogenetic analysis
249 revealed only one SNP across the 4.2 Mb core genome of *K. pneumoniae* among Patient C's
250 plasma sample, Patient C's blood culture isolate, and the residual platelet bag culture isolate.
251 The residual platelet product in the bag given to Patient D was discarded by the hospital nursing
252 staff and unavailable for either culture or mNGS. Across approximately 2700 bases of the *K.*
253 *pneumoniae* genome detected in Patient D's plasma with a read depth greater than 10, zero

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254 SNPs relative to the other three samples were identified. The sequence type of *K. pneumoniae*
255 in all four samples was determined to be ST491, and the closest ST491 sequence on Genbank
256 differed from these sequences by 212 SNPs.

257 Together these data confirmed that the platelet unit represented a single source of
258 infection and that despite Patient D having negative blood cultures, both patients became
259 bacteremic with the same strain of *K. pneumoniae* transfused from the contaminated platelet
260 components. As previously reported, routine culture-based screening of the donor's platelets at
261 24 hours performed by the blood supplier remained negative at five days, although an additional
262 platelet unit from the same donor shipped to a different hospital and quarantined before
263 transfusion grew *K. pneumonaie* that was highly related based on whole genome sequencing
264 analysis[6].

265 **Discussion**

266 Bacterial contamination of platelet products remains an important and underrecognized
267 hospital infection control challenge despite existing screening methods and psoralen-based
268 pathogen-reduction strategies[1–5]. Rapid recognition of potential transfusion-associated sepsis
269 can permit quarantine of untransfused co-components from a potentially contaminated supply
270 chain and assist with root cause analysis. Traditionally, culture-based methods including pulsed-
271 field gel electrophoresis and more recently, whole genome sequencing, have been the central
272 diagnostic tools for septic transfusion investigations. Here, we found that culture-independent
273 mNGS extended the utility of these methods by directly detecting pathogens from clinical
274 samples to assess genetic relatedness, obtain precise strain information, and interrogate levels
275 of pathogen in a patient's bloodstream throughout the course of a septic transfusion event.

276 In each case examined, mNGS provided detailed and precise information that clarified
277 the sequence of events resulting in transfusion-related sepsis. In case one, for example,
278 potential explanations considered included: 1) failure of the pathogen inactivation process; 2)
279 contamination of the platelet bag from an environmental source after pathogen reduction; and 3)

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280 pre-existing occult bacteremia resulting in retrograde introduction of bacteria into the platelet
281 bag during transfusion. Temporal mNGS assessment of patient plasma demonstrated abundant
282 *Acinetobacter* DNA in post-transfusion samples and in washes of the transfused platelet bag,
283 but not in any pre-transfusion samples, consistent with a septic transfusion event and not pre-
284 existing bacteremia.

285 The findings of high abundance *Acinetobacter sp.* in the transfused unit but not
286 untransfused co-components suggested that contamination occurred following pathogen
287 reduction treatment, potentially from an environmental source during product handling, transport
288 or storage. Transfusion-related sepsis from pathogen-reduced platelet products has been
289 reported but is extremely rare[9,10]. This case suggests that pathogen reduction technology
290 should still be accompanied by rigorous infection control precautions during downstream
291 processing and potentially undergo the same culture-based sterility testing as other platelet
292 products. Discovery that the implicated pathogen in case one represented a novel species of
293 *Acinetobacter* also highlighted the unique ability of sequencing-based diagnostics for unbiased
294 microbe discovery.

295 In case two, the possibility of an occult but developing bloodstream infection present
296 prior to transfusion was also considered as a potential explanation for the patient's post-surgical
297 sepsis. As in case one, assessment of plasma samples collected before and after transfusion
298 clarified the sequence of events and demonstrated that *P. aeruginosa* was only detectable post-
299 transfusion. Evidence for a septic transfusion event was further corroborated by temporal
300 measurement of procalcitonin, a host inflammatory biomarker with specificity for bacterial
301 infection[22,23]. Post-transfusion procalcitonin levels above the upper limit of detection in the
302 context of normal pre-surgical levels provided further evidence that a septic transfusion event
303 had occurred. The absence of detectable *P. aeruginosa* in the transfused PRBC segments
304 suggested that platelets or plasma may have been the source of contamination, although
305 neither sample type was available for confirmation.

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306 All three cases notably demonstrated that mNGS afforded high resolution taxonomic
307 identification without a need to isolate a pathogen in culture. This allowed for post-hoc analyses
308 of banked clinical specimens obtained both pre- and post-transfusion that were either unsuitable
309 or unavailable for culture. For instance, even though no cultured isolates were available in case
310 two, direct mNGS of leftover blood products allowed for precise identification of the most closely
311 related *Pseudomonas aeruginosa* strain, which incidentally was recovered in 2013 from a
312 patient's wound in Massachusetts, USA.

313 Confirming transmission of a pathogen during septic transfusion events is essential for
314 hospital infection control, but in some cases is not possible because culture fails to identify a
315 microbe. This problem was highlighted by case three, in which blood cultures from patient D
316 remained negative despite the development of post-transfusion septic shock. Patient D was
317 receiving a prophylactic antibiotic with activity against *Klebsiella*, which likely inhibited bacterial
318 growth in culture, precluding definite confirmation of a septic transfusion event related to that
319 experienced by Patient C. Culture-independent mNGS not only confirmed the presence of *K.*
320 *pneumoniae* in the blood of both patients but also established that it was identical to the isolate
321 derived from transfused platelet product. This unfortunate fatal transfusion case demonstrated
322 the capability of mNGS to provide definitive confirmation and characterization of septic
323 transfusion events in cases where culture fails to yield an isolate.

324 *Acinetobacter* and *Pseudomonas* are environmentally ubiquitous and common
325 contaminants of mNGS and 16S rRNA gene sequencing library preparation reagents[21,24],
326 and as such can add considerable complexity to investigations in which accurately assessing
327 the abundance of transfused pathogens belonging to these genera is critical. To address this,
328 we employed two complementary approaches that may be broadly useful for future
329 investigations: 1) assessing compositional changes of species within the relevant genus as a
330 proxy for environmental contamination, and 2) focusing analysis exclusively on the exact
331 species implicated in the transfusion event. In case one, for example, these approaches

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332 clarified that the novel *Acinetobacter* species was present only in the residual transfused
333 product and in the patient's plasma following transfusion, but not in the other co-components
334 nor in the patient's bloodstream prior to the event. In case two, the lack of a *Pseudomonas*
335 cultured isolate limited our ability to perform phylogenetic analyses, but mNGS clearly identified
336 the most closely related species to the causative agent and exhibited a stark compositional
337 difference between the pre- and post-transfusion samples, demonstrating that metagenomic
338 bioinformatics tools like IDSeq can make species-level assignments of individual reads or
339 contigs even in the absence of culture-based whole genome sequencing.

340 Rapid assessment of septic transfusion events is critical to ensure related contaminated
341 products can be swiftly quarantined, and probable sources of contamination identified. We
342 found that mNGS and pathogen analysis could be reliably performed in under 48 hours, faster
343 than the turnaround time for blood culture at many institutions. Cost, time and infrastructure
344 requirements currently make sequencing impractical at many healthcare institutions, however
345 new platforms such as the Illumina iSeq and Oxford Nanopore Minion will undoubtedly increase
346 the broad applicability of this technology for rapid hospital epidemiologic investigations.

347 As genomic approaches become more widely used for investigating transfusion-related
348 infections, rapid exchange of pathogen genomic information via open access databases could
349 accelerate identification of related cases, enhancing infection control efforts of emerging
350 outbreaks. Indeed, the findings described here have contributed to a multicenter United States
351 Centers for Disease Control and Prevention investigation which has identified the novel
352 *Acinetobacter sp*. from Patient A in related cases from Utah and Connecticut[25].

353 In summary, transfusion-related sepsis continues to cause excess mortality and
354 morbidity despite the introduction of pathogen-reduction technologies. We found that culture-
355 independent mNGS complemented current best available methods for investigation of
356 transfusion-related sepsis by extending traditional whole genome sequencing-based
357 phylogenetics of cultured isolates and by permitting longitudinal assessment of pathogen

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358 abundance pre- and post-transfusion from direct clinical specimens. While additional studies are
359 needed to validate these methods, implementation of mNGS for both investigation and
360 prevention of transfusion-related infections may enhance existing practices.

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361 **Data Availability**

362 Raw sequencing data is available via NCBI BioProject Accession ID: PRJNA544865.

363

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367

368 **Conflicts of Interest Statement:**

369 The authors declare no conflicts of interest regarding the publication of this manuscript.

370

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375 evaluating transfusion-transmitted infections.

376

377 **Authorship Contributions**

378 C.L., E.C., J.K. and S.M. wrote the paper

379 J.K. and L.L. performed phylogenetic analyses

380 C.L., E.C., J.K., L.L. and PC performed additional data analyses

381 A.N., A.N., D.Y., M.M., M.Z., N.T. and S.B. directed clinical aspects of the investigations

382 C.T. and J.D. directed molecular aspects of the investigations

383 A.L., B.P., C.L., E.C., P.H., and J.Q. performed sample extractions and metagenomic library
384 preparation

385 M.P. served as clinical research coordinator

386 M.T. and R.S. performed sequencing and quality control

387

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457 **Figure Legends**

458 **Figure 1. *Acinetobacter* septic transfusion investigation**

459 **A)** Maximum likelihood phylogenetic tree based on SNP alignments demonstrates relatedness
460 of the novel *Acinetobacter species* isolated from both patient A and the residual transfused
461 platelet product relative to closely related species within the *Acinetobacter*
462 *calcoaceticus/baumannii* (ACB) complex. **B)** Abundance of *ACB* complex in the transfused
463 platelet product and co-components (left panel) and in patient A's plasma (right panel),
464 determined by culture-independent metagenomic sequencing and measured in reads per
465 million, rpM.

466

467 **Figure 2. *Pseudomonas* septic transfusion investigation**

468 Abundance of *Pseudomonas aeruginosa* in patient B's plasma throughout the course of the fatal
469 septic transfusion event, determined by culture-independent metagenomic sequencing and
470 measured in reads per million (rpM, lower panel). Procalcitonin level (ng/mL) over the course of
471 the septic transfusion event is plotted in the upper panel.

472

473 **Figure 3. *Klebsiella* septic transfusion investigation**

474 Abundance of *Klebsiella pneumoniae* in plasma from patients C (upper panel) and D (lower
475 panel) during the course of related septic transfusion events, as determined by culture-
476 independent metagenomic sequencing and measured in reads per million (rpM). Patient C, who
477 did not survive the event, had post-transfusion blood cultures return positive for *K. pneumoniae*
478 that was highly related (1 SNP across the 4.2 Mb core genome) to the *K. pneumoniae* isolated
479 from the residual transfused platelet product. Patient D, who was receiving antibiotics with
480 activity against *Klebsiella* prior to transfusion, survived, but had negative post-transfusion blood
481 cultures, precluding definitive confirmation of a related second septic transfusion event in the
482 absence of culture-independent metagenomic sequencing.

Figure 1

A

Acinetobacter sp. from patient A

Patient A isolate 1

Patient A isolate 2

Platelet bag isolate

Acinetobacter nosocomialis

CP010368.1

FDAARGOS_131

CP014019.1

CP029351.1

CP033520.1

Acinetobacter pitii

CP017642.1

CP027183.1

CP012587.1

Acinetobacter baumanii

B

$5x10^5$

$5x10^4$

$5x10^3$

$5x10^2$

500

400

300

200

100

0

A. calcoaceticus/baumannii complex rpM

Co-component 1

Co-component 2

Transfused platelets

04:52

Time

Transfusion (12:23-12:50)

14:41

14:41

15:23

Figure 2

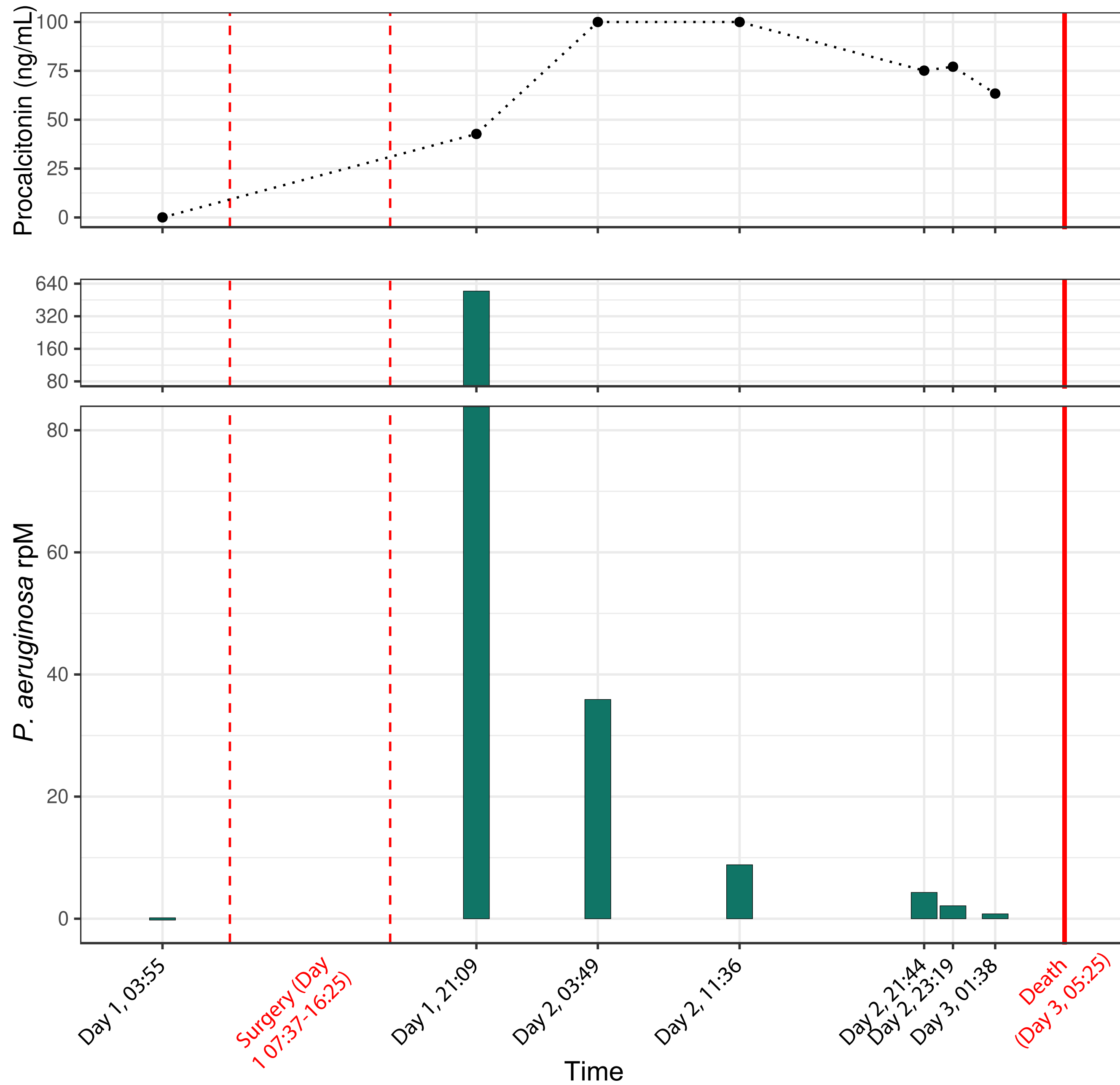
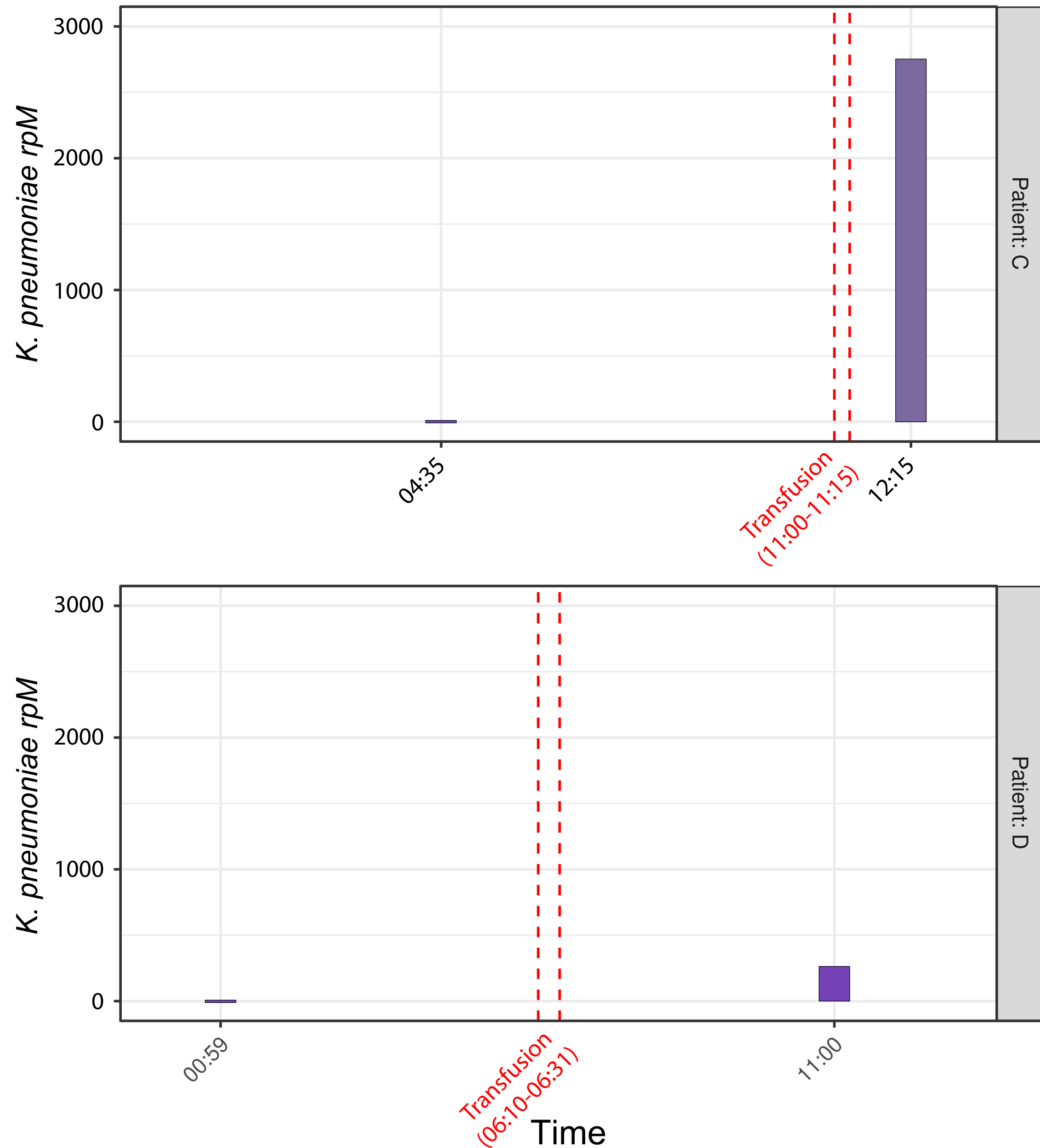


Figure 3



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Investigating Transfusion-Related Sepsis using Culture-Independent Metagenomic Sequencing, Supplemental Materials

Supplemental Methods

Sample Processing and Nucleic Acid Extraction

Samples were inactivated by adding DNA/RNA Shield (Zymo) and bashed with ceramic beads on a TissueLyser (Qiagen) for either two minutes at $15s^{-1}$ or one minute at $30s^{-1}$. For case one, metagenomic samples were extracted with the Quick-DNA/RNA Miniprep Kit (Zymo) and cultured isolate samples were extracted with the Quick DNA Fungal/Bacterial kit (Zymo). Case two samples were extracted with the Quick-DNA/RNA Microprep Plus kit (Zymo). For case three, metagenomic samples were extracted with the Duet DNA/RNA kit (Zymo) and cultured isolate samples were extracted with the AllPrep DNA/RNA kit (Qiagen) on a Qiacube robot.

Sterility Testing

The United States Food and Drug Administration (FDA) does not require primary culture testing for pathogen-reduced platelet products, as such no sterility testing was done for the platelet products from case 1. For the remaining cases, apheresis platelet products were subjected to sterility testing by the blood supplier using a standard FDA-approved automated culture methods and protocol. This involved culture performed by the blood supplier 24 hours after collection of the donor's platelets. Eight mL of platelet product was inoculated into an aerobic BacT bottle and incubated at 37 degrees Celsius for 5 days. In case 3, The BacT bottle remained negative at the end of 5 days of incubation. False negativity is a known limitation of culture-based platelet sterility testing and may reflect low levels of inoculum at the time of platelet collection that are not picked up by sampling a small volume of product. Transfusion services are not currently required to perform additional cultures or point of release tests for bacterial contamination prior to issuing platelets to patients. Plasma products are not subjected to sterility testing by the blood supplier or by the transfusion service.

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Supplemental Table 1: Microbial Culture Data

Case	Pathogen	Culture type	Time to positivity	Time to AST reporting
1	*Acinetobacter*	Product initial wash	14h 41m	66h 39m
1	*Acinetobacter*	Product repeat wash	6h 58m	59h 46m
1	*Acinetobacter*	Central blood	15h 54m	66h 4m
1	*Acinetobacter*	Peripheral blood	24h 34m	135h 41m
2	*Pseudomonas*	Central blood	18h 19m	59h 22m
2	*Pseudomonas*	Peripheral blood	27h 14m	
3	*Klebsiella*	Central blood red port	7h 6m	
3	*Klebsiella*	Central blood white port	5h 6m	42h 16m
3	*Klebsiella*	Peripheral blood	95h 7m	
3	*Klebsiella*	Platelet product	1h 6m	62h 21m

Legend: AST = antimicrobial susceptibility testing.

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Supplemental Table 2: Sequencing reads collected per sample.

Case	Sample Name	Day	Time	Total Reads	Pathogen Reads	Pathogen
1	H2O-1a	n/a	n/a	12292	0	*ACB complex*
1	H2O-1b	n/a	n/a	4196	0	*ACB complex*
1	Co-component-1	n/a	n/a	33437780	87	*ACB complex*
1	Co-component-2	n/a	n/a	105027102	190	*ACB complex*
1	Patient A	Day (-1)	5:01	111863490	58	*ACB complex*
1	Patient A	Day 0	3:17	91789296	23	*ACB complex*
1	Patient A	Day 1	4:52	86230676	91	*ACB complex*
1	Patient A	Day 1	14:41a	89397082	19092	*ACB complex*
1	Patient A	Day 1	14:41b	150000000	30666	*ACB complex*
1	Patient A	Day 1	15:23	148209522	21096	*ACB complex*
1	H2O-2a	n/a	n/a	5532686	1290	*ACB complex*
1	Transfused platelets	n/a	n/a	150000000	34367349	*ACB complex*
1	Isolate-a (Platelet bag)	n/a	n/a	10273280	-	*ACB complex*
1	isolate-b (Platelet bag)	n/a	n/a	7746330	-	*ACB complex*
1	isolate-c (Patient)	n/a	n/a	6458218	-	*ACB complex*
2	H2O-1a	n/a	n/a	56452	3	*P. aeruginosa*
2	H2O-1b	n/a	n/a	1427720	155	*P. aeruginosa*
2	H2O-1c	n/a	n/a	283232	32	*P. aeruginosa*
2	Blood culture bottle-1	Day 2	16:06	150000000	1223	*P. aeruginosa*
2	Blood culture bottle-2	Day 2	16:06	150000000	1533	*P. aeruginosa*
2	H2O-2a	n/a	n/a	9523090	399	*P. aeruginosa*
2	Patient B	Day 1	3:55	150000000	5	*P. aeruginosa*
2	Patient B	Day 1	21:09	150000000	81995	*P. aeruginosa*
2	Patient B	Day 2	3:49	118341948	4248	*P. aeruginosa*
2	Patient B	Day 2	11:36	99059392	875	*P. aeruginosa*
2	Patient B	Day 2	21:44	62467588	269	*P. aeruginosa*
2	Patient B	Day 2	23:19	83908304	178	*P. aeruginosa*
2	Patient B	Day 3	1:38	110713036	87	*P. aeruginosa*
2	PRBC-1	n/a	n/a	150000000	24	*P. aeruginosa*
2	PRBC-2	n/a	n/a	150000000	234	*P. aeruginosa*
3	Patient C	Day 1	4:35	125893204	31	*K. pneumoniae*
3	Patient C	Day 1	12:15	45615102	1255715	*K. pneumoniae*
3	Patient D	Day 1	0:59	10769438	42	*K. pneumoniae*
3	Patient D	Day 1	11:00	42995136	113123	*K. pneumoniae*
3	H2O-1a	n/a	n/a	2734220	217	*K. pneumoniae*
3	Isolate-a (Patient C)	n/a	n/a	6601096	-	*K. pneumoniae*
3	Isolate-b (Platelet bag)	n/a	n/a	5800012	-	*K. pneumoniae*

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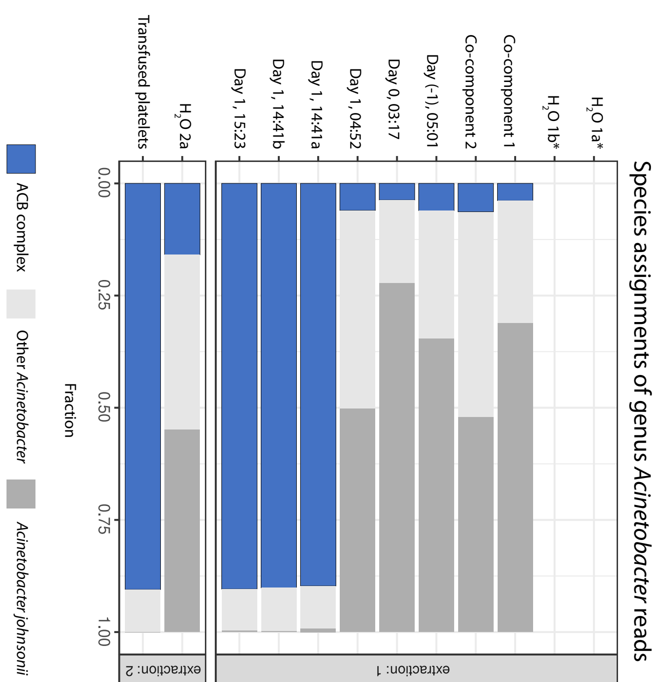

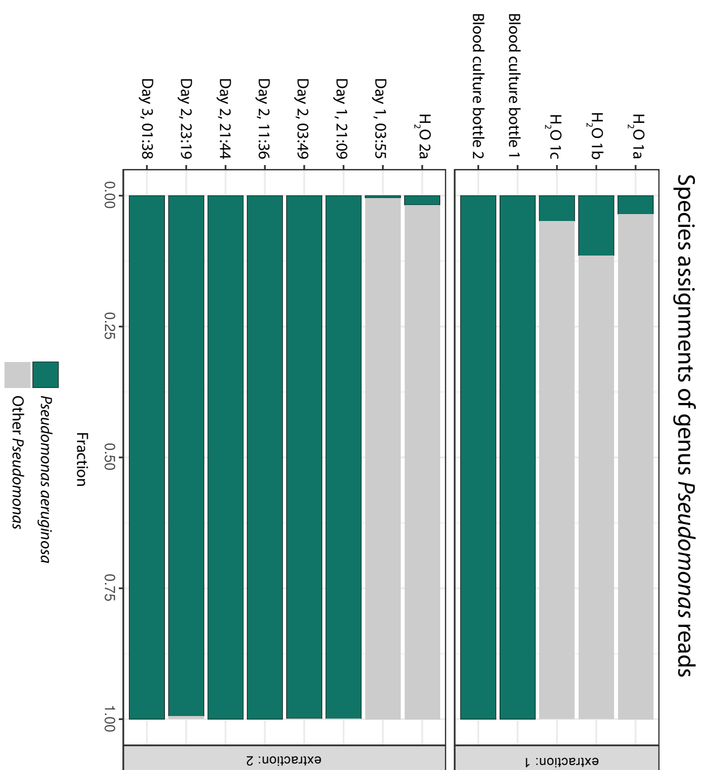

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Supplemental Figure 1. Fraction of species-specific sequencing reads aligning to transfusion-transmitted pathogens.

A) Fraction of species-specific *Acinetobacter* read assignments for samples evaluated by culture-independent metagenomic sequencing in case 1, including water controls. The co-components and pre-transfusion samples (upper panel) had only a small fraction of genus *Acinetobacter* reads that best aligned to *Acinetobacter calcoaceticus/baumannii* (ACB) complex while the transfused product (lower panel) and patient's post-transfusion plasma (upper panel) was comprised mostly of reads aligning to the ACB complex. Asterisks indicate samples with no reads to genus *Acinetobacter*. **B)** Fraction of species-specific *Pseudomonas* sequencing read assignments for plasma samples evaluated by culture-independent metagenomic sequencing for case 2, including water controls. Samples from the blood culture bottles which eventually turned positive (upper panel) and from the patient's plasma post-transfusion (lower panel) were comprised almost entirely of reads best aligning to *Pseudomonas aeruginosa,* while the patient's plasma before transfusion and water control samples had mostly reads aligning to other *Pseudomonas* species.