SKIN MICROBIOME

Contribution of the patient microbiome to surgical site infection and antibiotic prophylaxis failure in spine surgery

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Despite modern antiseptic techniques, surgical site infection (SSI) remains a leading complication of surgery. However, the origins of SSI and the high rates of antimicrobial resistance observed in these infections are poorly understood. Using instrumented spine surgery as a model of clean (class I) skin incision, we prospectively sampled preoperative microbiomes and postoperative SSI isolates in a cohort of 204 patients. Combining multiple forms of genomic analysis, we correlated the identity, anatomic distribution, and antimicrobial resistance profiles of SSI pathogens with those of preoperative strains obtained from the patient skin microbiome. We found that 86% of SSIs, comprising a broad range of bacterial species, originated endogenously from preoperative strains, with no evidence of common source infection among a superset of 1610 patients. Most SSI isolates (59%) were resistant to the prophylactic antibiotic administered during surgery, and their resistance phenotypes correlated with the patient's preoperative resistome ($P = 0.0002$). These findings indicate the need for SSI prevention strategies tailored to the preoperative microbiome and resistome present in individual patients.

INTRODUCTION

Surgical site infection (SSI) is the most common and costly complication of modern surgery, affecting about 1 in 30 procedures (*1*). Compared with other hospital-acquired conditions, SSI rates have seen little improvement over recent reporting periods (*2*–*4*) despite wide adherence to standard infection prevention measures (*4*, *5*). Moreover, focused efforts to identify and correct deficiencies in established best practices for SSI prevention have not translated to reduced rates of infection (*6*), suggesting both the limited potential of current strategies to drive substantial further reductions in SSI and the need for new data to guide development of preventative approaches.

Future quality improvement in this arena remains limited by a poor fundamental understanding of both the origins of SSI and the high rates of resistance to prophylactic antimicrobial agents observed in these infections (*7*). Whereas the potential for surgical wounds to become inoculated with endogenous bacteria may be intuitive for contaminated or "clean-contaminated" procedures involving nonsterile spaces (for example, in colorectal surgery) (*8*), the pathogenesis of SSI in procedures involving clean skin incisions in the era of modern surgical antisepsis remains a matter of debate (*9*–*12*). Infection prevention strategies, particularly in orthopedic surgery, have historically emphasized the importance of environmental cleaning, sterile processing, and operating room attire, which target "exogenous" sources of infection from nosocomial reservoirs. In contrast, prior microbiological studies (*13*–*15*) indicate that many wound infections may arise from "endogenous" reservoirs of colonizing microbiota carried by the patient. Yet, seminal studies on endogenous wound infection predate the era of next-generation sequencing and have largely been limited to *Staphylococcus aureus*, leaving unaddressed the broader range of pathogens, including Gram-negative and anaerobic organisms, that are commonly implicated in SSI (*16*). A more generalized model of SSI as an infectious process of predominantly endogenous origin (including in clean procedures and non-staphylococcal infections) has therefore not been widely adopted. Surgical culture, health care system initiatives, research studies, product development, legal proceedings, and many guidelines consequently continue to place emphasis on hospital-centered rather than patient-centered factors, such as operating room traffic, surgical attire, air flow, equipment decontamination, and interpersonal transmission (*17*–*23*). Only recently has there been a call to broadly reexamine the role of the patient microbiome as a central factor in SSI pathogenesis (*9*, *10*).

Instrumented spine surgery represents a useful model for studying the pathobiology of SSI after clean skin incisions [termed "class I" surgical wounds, with no entry into alimentary, respiratory, or urogenital tracts or breach in sterile procedure (*24*)] for several reasons. Similar numbers of women and men undergo spine surgery for a variety of indications across the patient life span, allowing greater generalizability. Because of their frequency and complexity, more health care resources are expended on spinal fusion than any other elective surgical procedure in the United States (*25*). Infections after these procedures also occur at a predictable rate (3 to 5%) (*26*, *27*), involve a wide range of pathogens, and are routinely cultured before antibiotic administration because of the need for extended treatment durations. Last, we and others have previously described an anatomic gradient in the pathogens causing SSI after instrumented spine surgery, transitioning along the length of the back

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from Gram-positive to Gram-negative infections (*26*, *27*). This unique feature provides a complementary avenue for comparing the microbiome of the surgical site with the microbial epidemiology of SSI.

The objectives of this prospective study were to characterize the contributions of the preoperative patient microbiome and resistome to SSI after instrumented spine surgery. Using multiple forms of genomic analysis, including techniques for species-targeted whole-genome enrichment of metagenomic sequencing libraries [genome capture sequencing (GenCap-Seq)] (*28*), we sought to determine (i) whether anatomic differences in the organisms causing SSI at various surgical anatomic locations (*29*) correlate with differences in the preoperative skin microbiome, (ii) whether the causative SSI strain(s) identified by clinical wound culture are present in the patient's preoperative microbiota, and (iii) whether the preoperative resistome is related to the development of prophylaxis-resistant infection. In parallel, we also aimed to assess whether, under routine operating circumstances (in the absence of overt nosocomial outbreaks), SSIs occurring in a shared operative environment frequently involve cryptic transmission of exogenous, nosocomial strains across patients.

RESULTS

Characteristics of cohort and infections

Among 210 adult patients undergoing posterior spinal fusion, adequate preoperative nasal, rectal, and skin specimens (Fig. 1, "microbiome arm") were obtained from 204 (97.1%). Demographic and clinical characteristics of the cohort are shown in table S1. SSI developed among 14 (6.8%) enrolled patients. Details of these 14 cases including procedural indication, surgical antibiotic prophylaxis, causative organisms, resistance profiles, closest reference sequence, and sequence read archive (SRA) accession information are included in data file S1. During the same period, 1406 additional patients underwent spine surgery in the same environment, including lower-risk noninstrumented and minimally invasive spine procedures, with an infection rate of 4.2% (Fig. 1, "genomic surveillance only arm").

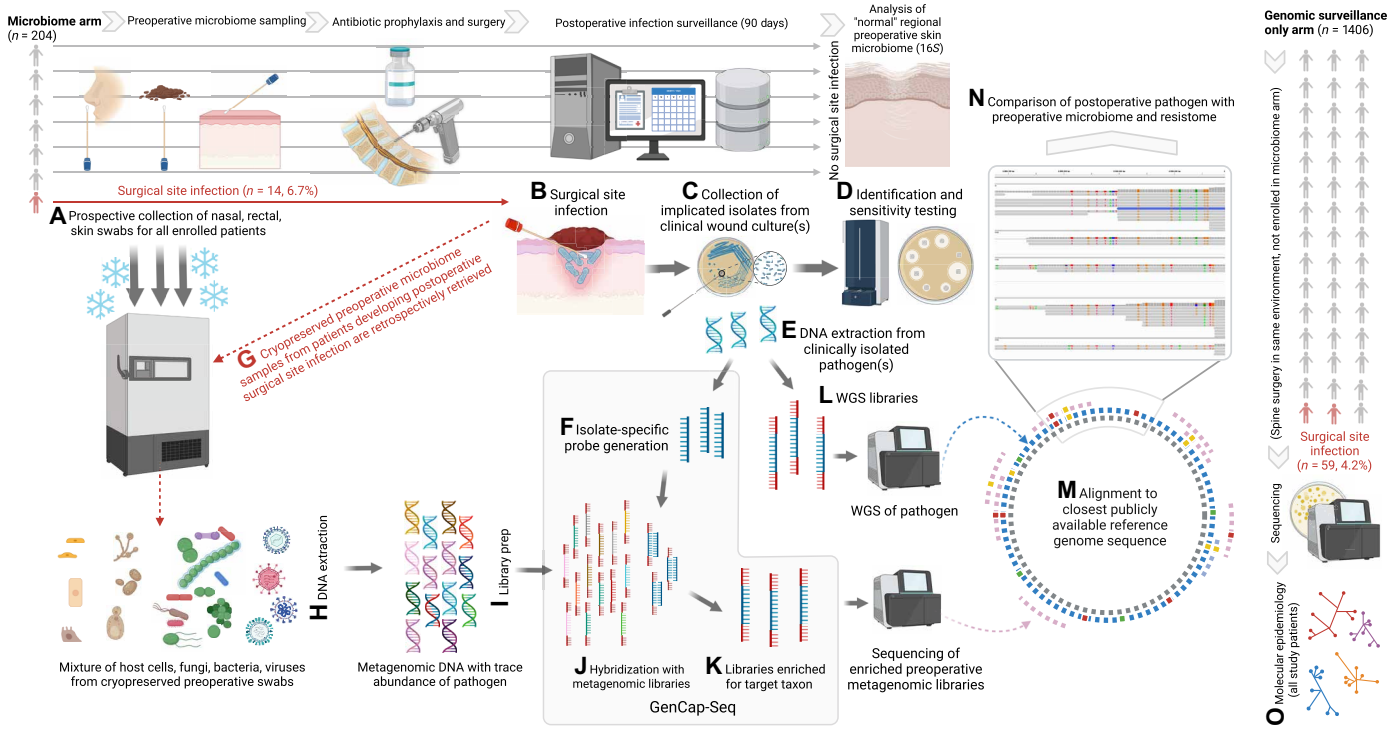

Fig. 1. Schematic overview of study design. (**A**) Preoperative swabs from the nares, rectum, and skin overlying the intended surgical site were prospectively collected on the day of surgery before surgical skin antisepsis and administration of surgical antibiotic prophylaxis and were immediately cryopreserved. Enrolled patients were monitored for the occurrence of culture-positive SSI (**B**). For cases subsequently complicated by SSI, deep swabs and tissue specimens from wounds were cultured (**C**), and individual isolates were identified and tested for antibiotic susceptibility (**D**) in the clinical microbiology laboratory as part of routine care. Isolates from species identified as SSI pathogens by treatment teams were obtained, genomic DNA was extracted for WGS (**E**), and strain-specific capture probes were generated using the GenCap-Seq protocol (**F**). Cryopreserved preoperative microbiome samples for each affected patient were reflexively retrieved (**G**) and then subjected to routine DNA extraction (**H**) and metagenomic library preparation (**I**). Hybridization enrichment (**J**) was then performed using a capture-probe set derived from clinical wound culture isolate(s) from the same patient, encompassing the chromosomal and accessory genome content of the SSI pathogen. Standard Illumina sequencing of the enriched metagenomic (**K**) and SSI pathogen whole-genome (**L**) libraries was performed. WGS reads were aligned to the closest-matching public reference genome (**M**), and "strain-informative" variants and AMR gene content were cataloged. After filtering for species specificity, metagenomic reads were aligned to the same reference genome and the sequences of the postoperative SSI pathogen and preoperative microbiome were compared (**N**). Patients undergoing spine surgery in the same operative environment during the study period, but not enrolled in the prospective microbiome sampling arm, were additionally monitored for SSI, and clinical isolates were sequenced for expanded molecular epidemiology analysis (**O**).

An anatomic gradient exists along the skin microbiome of the back

To assess anatomic differences in the composition of the preoperative skin microbiome along the length of the back, we collected skin swabs on the day of surgery (immediately before topical antiseptic application) from the region directly overlying the planned incision and characterized the microbiome by 16*S* ribosomal RNA (rRNA) amplicon sequencing. For this analysis, we used a subset of samples from 124 cases with procedures focused within cervical (18.5%), thoracic (12.9%), or lumbosacral (68.5%) operative regions to limit the influence of sampling for multilevel procedures spanning several adjacent spinal anatomic regions, which would be less informative in defining regional variation in skin microbiome composition. We further stratified samples within each anatomic region for inclusion to achieve sex-balanced patient representation.

The relative abundance of opportunistically pathogenic Gram-positive organisms (for example, *Staphylococcus* and *Cutibacterium* sp.; see the "16*S* rRNA amplicon sequence data analysis" section in Supplementary Materials and Methods) was greater in cervical and thoracic skin regions, whereas opportunistic Gram-negative and anaerobic organisms (such as *Escherichia*, *Enterobacter*, and *Bacteroides* sp.) were overrepresented in lumbosacral skin regions (Fig. 2A; $P = 10^{-16.1}$ for three anatomic regions by Kruskal-Wallis test; cervical versus lumbosacral $P = 10^{-10.7}$, thoracic versus lumbosacral $P < 0.0002$, and cervical versus thoracic $P = 0.171$ by post hoc unpaired, two-sided Wilcoxon rank-sum tests). Even within spinal anatomic regions, a high degree of interpatient variability in the relative abundance of potentially pathogenic skin microbiota was observed (Fig. 2A).

Analysis of skin sampling site as a continuous (median surgical anatomic location) rather than a categorical (cervical, thoracic, or lumbosacral spine) variable demonstrated that the anatomic stratification of microorganisms is a graded phenomenon with an inflection point occurring at about the mid-thoracic region (Fig. 2B). This microbial gradient was also observed within individual patients (Fig. 2C), suggesting that it represents a common spatial anatomic feature of the human skin microbiome rather than an epiphenomenon. Consistent with the hypothesis that the skin of the back represents a mixture of microbiota from adjacent reservoirs, principal components analysis of unweighted UniFrac distances showed that cervical, thoracic, and lumbosacral skin microbiomes occupy intermediate spaces between nasal and rectal "poles" of the human microbiome (fig. S1). The overall composition and anatomic distribution of taxa in the preoperative skin microbiomes did not vary significantly by sex, with the exception of *Lactobacillus* species, which were enriched in the skin of women ($P = 0.0006$ by unpaired, two-sided Wilcoxon rank-sum test for fraction of total reads from skin samples classified as *Lactobacillus* between male and female patients).

Beyond differences in the distribution of bacterial groups clinically targeted by different prophylactic antibiotic classes (Gram-positive, Gram-negative, or anaerobic) (*30*), anatomic stratification in the relative abundance of several specific taxa was also observed along the skin of the back. Staphylococci demonstrated the greatest correlation with cephalad skin regions, whereas *Finegoldia*, *Bacteroides*, *Escherichia*, *Pseudomonas*, *Enterobacter*, and other *Enterobacterales* species exhibited an opposite correlation, showing a relative predominance in lumbosacral skin regions (Fig. 3).

The regional skin microbiome correlates with the clinical microbiology of SSI

Prior work has described an anatomic gradient in the causative organisms of spine SSI, which transitions from Gram positive to Gram negative and anaerobic pathogens down the length of the back (*26*, *27*). Although Gram stain classification and anaerobic metabolism are not traditionally used to describe taxonomic groups in microbiome studies, these categories have practical clinical relevance in predicting functional susceptibility to particular classes of antimicrobials used for surgical prophylaxis, including in spine surgery (*30*). To assess the correlation of the microbiome composition of the surgical site with the microbiology of SSI, we compared the spatial distributions of bacteria in skin microbiome samples from this study with wound culture results from spine SSIs from the same health system over a preceding 8-year period. This analysis indicated significant anatomic correlation between the predicted preoperative microbiome of the surgical site and the microbiology of postoperative SSI [coefficient of determination (R^2) = 0.74, $P = 0.014$ by Pearson correlation; Fig. 4].

Strains causing postoperative wound infection are present in the preoperative microbiome

Given the correlation between regional, preoperative skin microbiome composition and the microbiology of subsequent wound infection, we next determined whether individual SSIs could be attributed to endogenous bacterial strains present in the patient microbiome before surgery or whether they were more consistent with exogenous strains introduced from external sources.

In each case of SSI, targeted whole-genome enrichment of preoperative microbiome samples for the SSI strain was performed using a technique recently developed by our laboratory (GenCap-Seq; Fig. 1, F to K) (*28*). Interim analysis of the first 15 specimens using the standard GenCap-Seq protocol demonstrated limited and inconsistent enrichment, but specimens could be sufficiently interrogated using relatively large numbers of sequencing reads. Modifications to the library preparation and capture-probe protocol were therefore adopted to improve efficiency for low biomass swabs. After these adjustments, 41 of the 48 samples (85%) achieved enrichment for the taxon of interest. Enrichment was greatest for low-abundance taxa (fig. S3) and ranged from 1.1- to 20,030-fold (median, 30-fold) in successful samples.

Taxon-specific reads from preoperative swabs generated using the GenCap-Seq protocol were then compared with the whole-genome sequences of postoperative SSI isolates (Fig. 1N). GenCap-Seq reads at all positions discriminating the SSI strain from its most similar public reference genome were examined to determine whether samples of preexisting, endogenous strains from the patient carried the same polymorphisms. We first established a threshold for classifying sequences from preoperative strains as genomically similar or dissimilar to SSI isolates. Considering all specimen pairs, a range of 0 to 100% of covered, SSI strain-informative polymorphisms were identified in preoperative microbiome samples from the same individual. Mixture modeling of this distribution identified a threshold of ≥80% concordance at informative sites (fig. S4) for classification of SSI isolates as genomically similar to preoperative strains and thereby likely being endogenous to a patient.

Using this threshold, 19 of the 22 (86%) SSI isolates closely matched a strain present in one or more preoperative samples (fecal, nasal, or rectal), consistent with endogenous infection arising from

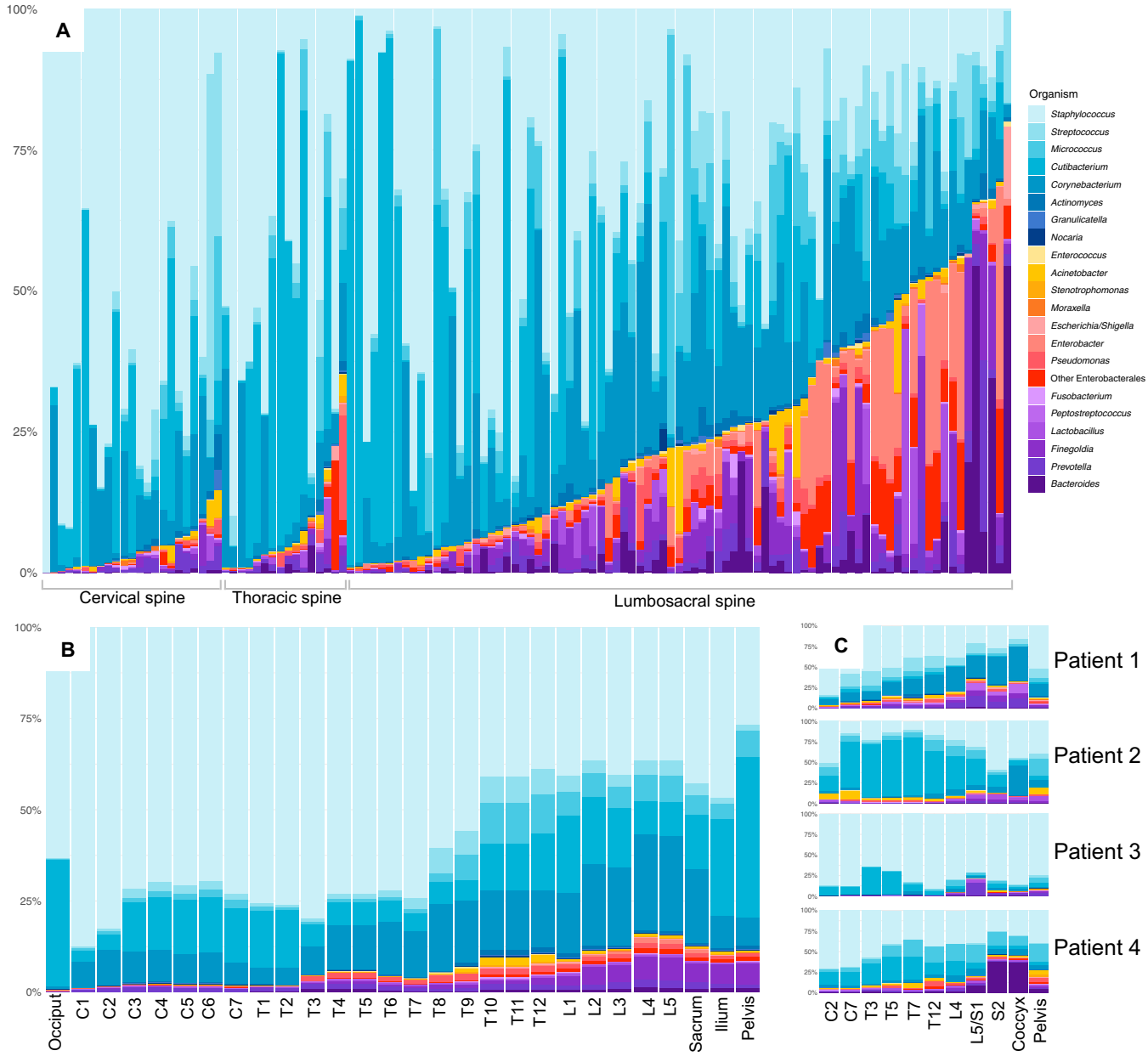

Fig. 2. Anatomic gradients and individual variability in the composition of the preoperative skin microbiome. (**A**) Variation in the skin microbiome overlying surgical sites across anatomic regions and individuals. The relative abundance of opportunistically pathogenic taxa for individual patients (bars) is grouped by operative region and sorted by the total fraction comprising Gram-negative and anaerobic taxa. (**B**) Relative taxonomic abundance when stratified by median surgical anatomic location as a continuous measure. Interquartile ranges for each taxon are provided in data file S3. (**C**) Preoperative skin microbiome composition of four individual patients at 11 sampling sites from C2 to pelvis. Temporal comparisons of surgical site microbiome composition for the same patients over the period spanning outpatient clinic evaluation to the day of surgery (range 2 to 6 weeks) are depicted in fig. S2. Spinal anatomic locations on horizontal axes of (B) and (C) are indicated according to vertebral level [cervical vertebrae (C1 to C7), thoracic vertebrae (T1 to T12), and lumbar vertebrae (L1 to L5)].

the preexisting host microbiome. Classification, summary statistics, comments from manual review of reads for each case (data file S1), and representative sequence alignments (figs. S5 and S6) are provided.

In general, when an SSI species was detected in more than one preoperative reservoir from a patient, the sequences of those strains were similar, with little allelic variation seen among aligned reads, indicating the predominance of a single member of that species across multiple body sites. However, in four cases (data file S1; "case review comments"), SSI-concordant polymorphisms occurred at low variant allele frequencies, indicating that the SSI pathogen appeared as a low prevalence strain in the preoperative microbiome and rose to predominance during infection. For example, one cefazolin-resistant strain of *Escherichia coli* SSI supported by ~10% of total *E. coli* reads in the preoperative microbiome

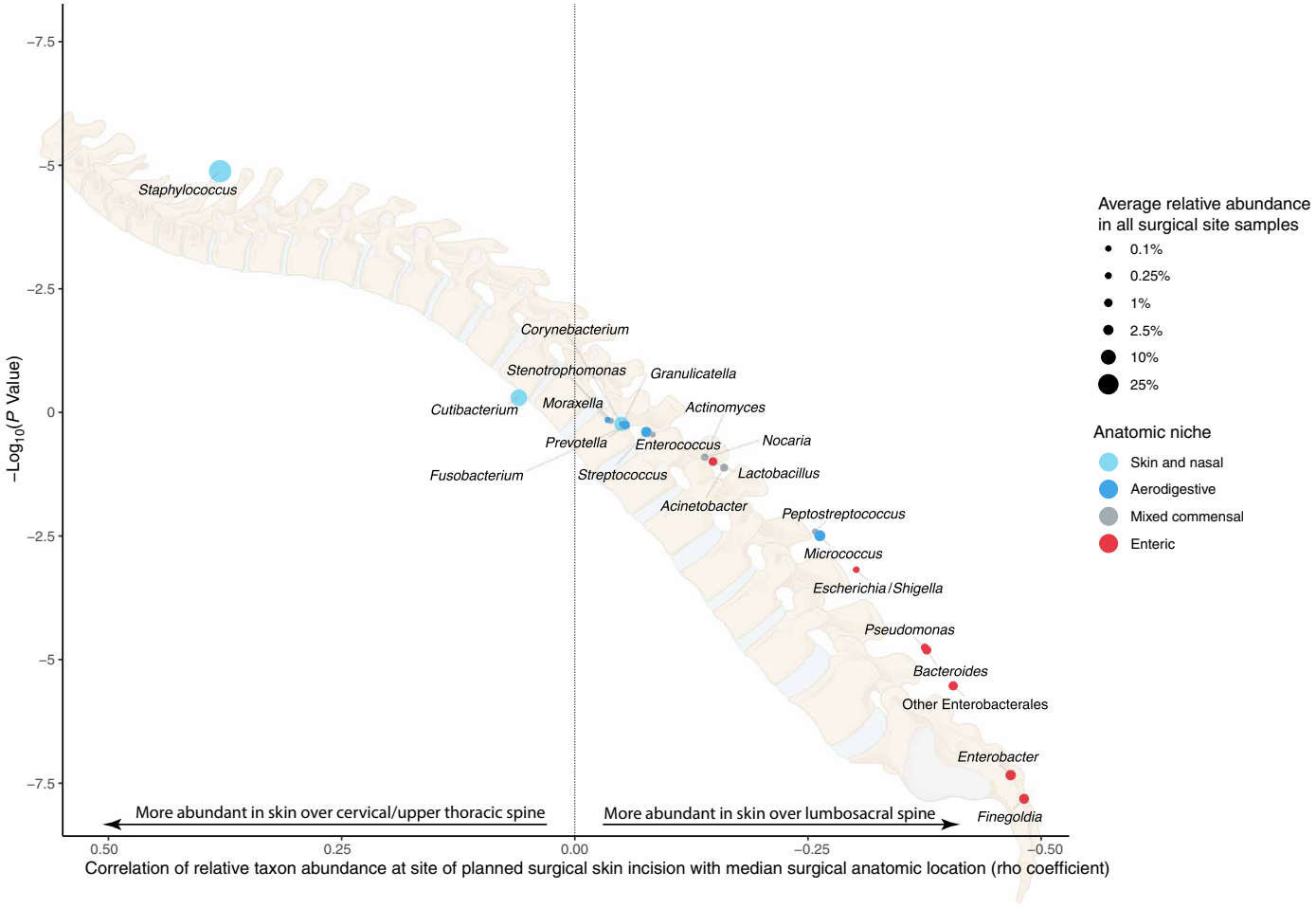

Fig. 3. Relative distribution of potential pathogens colonizing preoperative skin along the length of the back. Modified volcano plot (vertically mirrored at the midpoint of the horizontal axis to visually convey anatomic distribution) illustrating the correlation between the relative abundance of taxa (0 to 100%) in preoperative samples of skin overlying the surgical site and the anatomic location of the surgical site as measured by median vertebral level (C5, T4, L4, and so on). Correlation was assessed by the Spearman rank correlation test, with positive rho coefficient values on the horizontal axis indicating correlation with higher (cervicothoracic) skin regions and negative values indicating correlation with lower (lumbosacral) skin regions.

became predominant in the wound and in the postoperative fecal microbiome by the time of SSI diagnosis (fig. S7).

For the three SSI cases involving pathogens classified as exogenous by this approach, clinical risk factors for nosocomial acquisition were present in each. In one patient, delayed superinfection of the wound with an extended-spectrum β-lactamase–producing *Enterobacter hormaechei* strain occurred after initial treatment of an endogenous *E. coli* SSI with irrigation, debridement, and ceftriaxone. In the second patient, methicillin-resistant *S. aureus* (MRSA) SSI was concurrent with new nosocomial acquisition of MRSA detected on routine, interval inpatient surveillance. The last patient, infected with an exogenous Panton-Valentine leukocidin–positive, methicillin-susceptible *S. aureus* strain, was immunosuppressed with a history of opportunistic infections.

We used orthologous approaches to validate results of these analyses in four patients with *E. coli* SSI, given that this was the largest group of endogenous, same-species infection in our cohort. We cross-examined the genomic similarity of strains in preoperative fecal swabs (the sample type most reliably yielding *E. coli* reads) recovered by GenCap-Seq and whole-genome sequencing (WGS) of SSI isolates. A network diagram of genomic relatedness across *E. coli* strains from these four patients (Fig. 5A) indicated that, in each case, the sequence of the SSI pathogen was most similar to strains from the preoperative microbiome of the same patient and was accordingly divergent from strains originating from other patients. Separately, *E. coli* reads from GenCap-Seq were compared with WGS data from three *E. coli* strains isolated from stool culture of a single patient by matrix-assisted laser desorption/ionization–time-of-flight coliform screening (Fig. 5B), demonstrating intrapatient concordance between these methods. This focused analysis of the largest same-species subgroup provides complementary evidence that SSIs in our cohort commonly originated from strains endogenous to each patient.

The preoperative resistome is associated with prophylaxis-resistant infection

Given our observation that most SSIs arose from strains present in the patient microbiome before surgery, we sought to determine

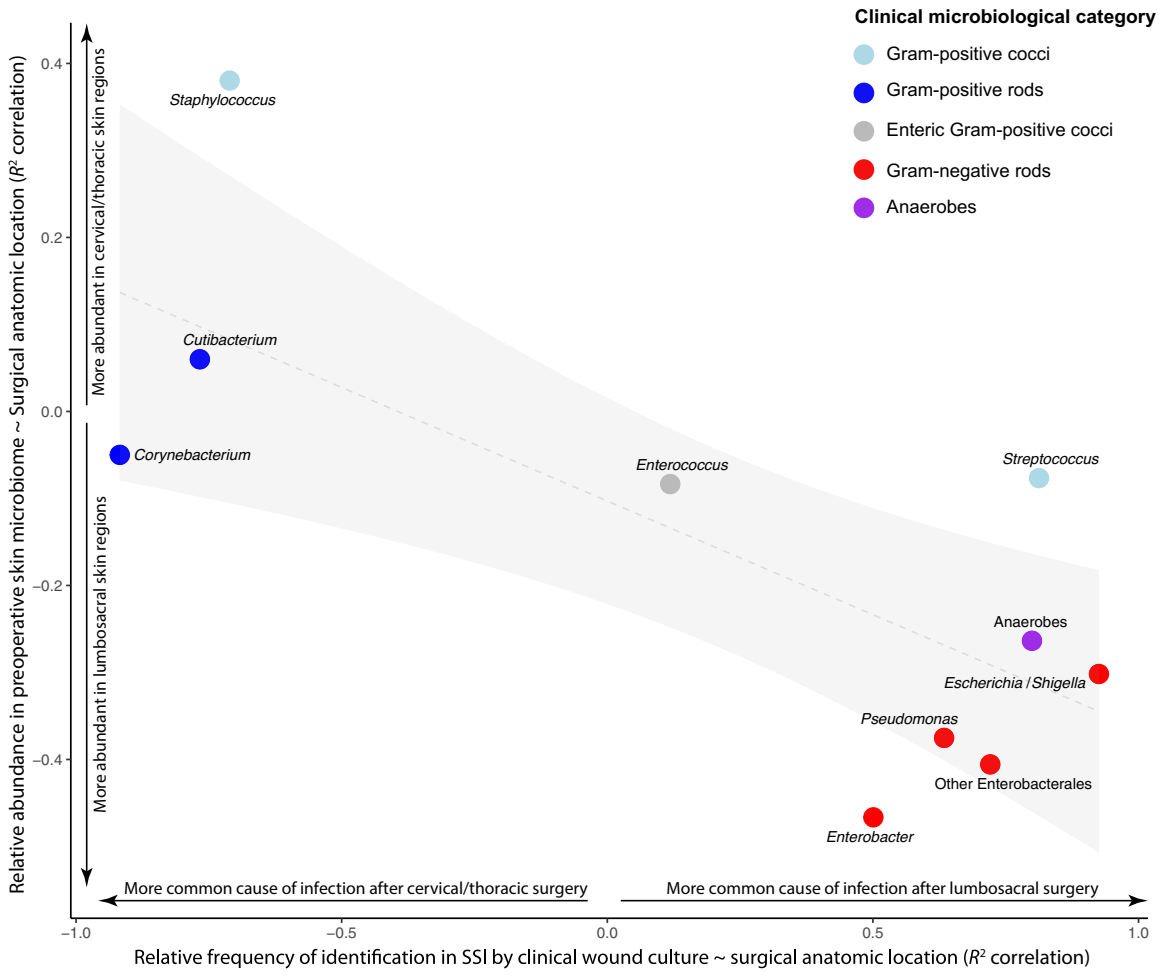

Fig. 4. Correlation between preoperative skin microbiome composition and postoperative SSI pathogens as a function of surgical anatomic location. The relative anatomic distribution of organisms in the preoperative back-skin microbiome as measured by 16*S* rRNA amplicon sequencing (vertical axis: cephalad versus caudad enrichment above versus below *y* intercept, respectively) is significantly correlated ($R^2 = 0.74$, $P = 0.014$ by Pearson correlation test) with the distribution of pathogens postoperatively isolated from these regions by wound culture in clinical cases of SSI (horizontal axis: cephalad versus caudad enrichment to the left versus right of *x* intercept, respectively). Dashed line and shaded area show the linear correlation and 95% confidence interval, respectively.

whether infections showing resistance to surgical antibiotic prophylaxis could be attributed to preexisting features of the preoperative patient microbiome. Thirteen of the 22 SSI pathogens (59%) were resistant to the antibiotic prophylaxis administered at the time of surgery. Sequence reads corresponding to relevant antimicrobial resistance (AMR) genes (prophylactic agents, resistance phenotypes, and genotypes provided in data file S1) were identified from metagenomic analysis of preoperative patient specimens using an empirically derived BLAST *E* value threshold to exclude low-specificity hits (Fig. 6A). For each patient, AMR gene read counts defining the resistome of the preoperative microbial population were correlated with gene presence or absence in whole-genome sequences of subsequent postoperative SSI isolates (Fig. 6B). Genes that contributed to the resistance phenotypes of SSI pathogens were significantly enriched in the preoperative resistomes of corresponding patients (bootstrapped *P* value = 0.0002; Fig. 6C). Moreover, normalized read counts from specific AMR genes in preoperative samples positively correlated with SSI AMR gene content in a dose-dependent fashion ($P < 0.0002$ by Spearman rank correlation; Fig. 6D). Skin samples provided the greatest sensitivity for preoperative detection of AMR genes involved in resistant infection (84.2%), followed by rectal (68.4%) and nasal (42.1%) specimens. However, integrating data from all three sampling sites yielded the highest overall sensitivity (94.7%).

No evidence of exogenous infection from shared nosocomial reservoirs

To assess the possibility that some SSIs among unrelated patients originated from common, potentially nosocomial, sources of infection, we analyzed the molecular epidemiology of all spine SSIs occurring within this shared perioperative environment during the study period. In addition to the 14 SSIs occurring among patients for whom preoperative microbiome samples were obtained (Fig. 1, "microbiome arm"), 59 SSIs also occurred among 1406 patients (Fig. 1, "genomic surveillance only arm") undergoing spine surgery in the same environment during the study period who were not enrolled in the preoperative

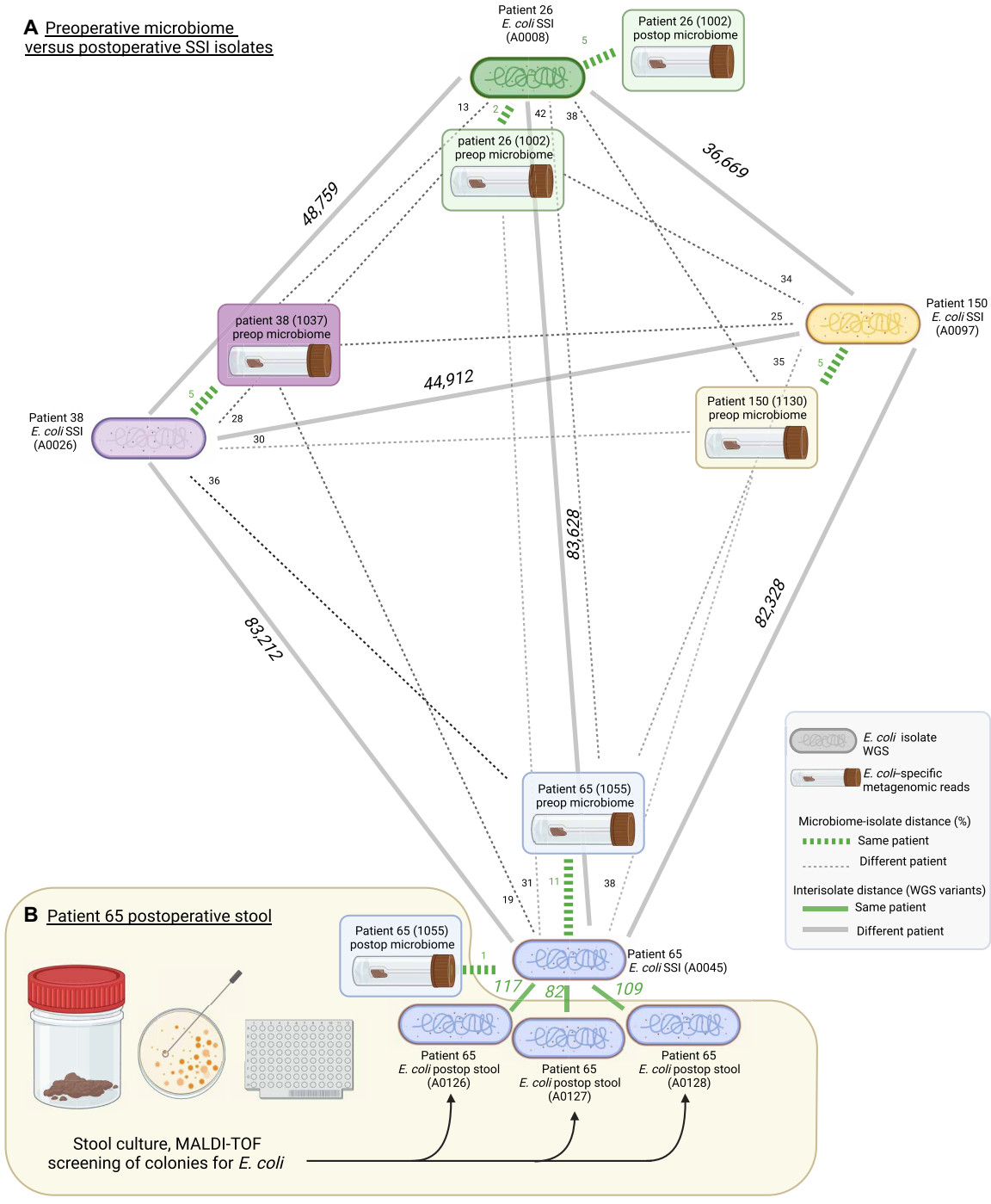

Fig. 5. Comparison of inter- and intrapatient sequence similarity among cases of *E. coli* SSI. (**A**) *E. coli* SSI isolates from four patients [labeled with the schema "patient # (isolate #)"] are highly divergent (pairwise WGS genetic distance shown as solid lines). In each case, the WGS of the *E. coli* SSI isolate was more similar to sequences from *E. coli* strains present in that patient's preoperative microbiome [labeled with the schema "patient # (swab #)"] than to those from the three other patients affected by *E. coli* SSI (proportion of allelic divergence between WGS and GenCap-Seq reads at nonreference sites shown as dashed lines). Comparison of metagenomic reads from a postoperative stool sample from patient 26 with the SSI isolate from that patient confirms reproducible concordance of sequences from the SSI strain and independently collected microbiome samples from the same patient (top). (**B**) Analysis of *E. coli* isolates recovered from a postoperative stool sample of one individual (patient 65) confirms concordance between WGS and GenCap-Seq measures of strain similarity. MALDI-TOF, matrix-assisted laser desorption/ionization–time-of-flight.

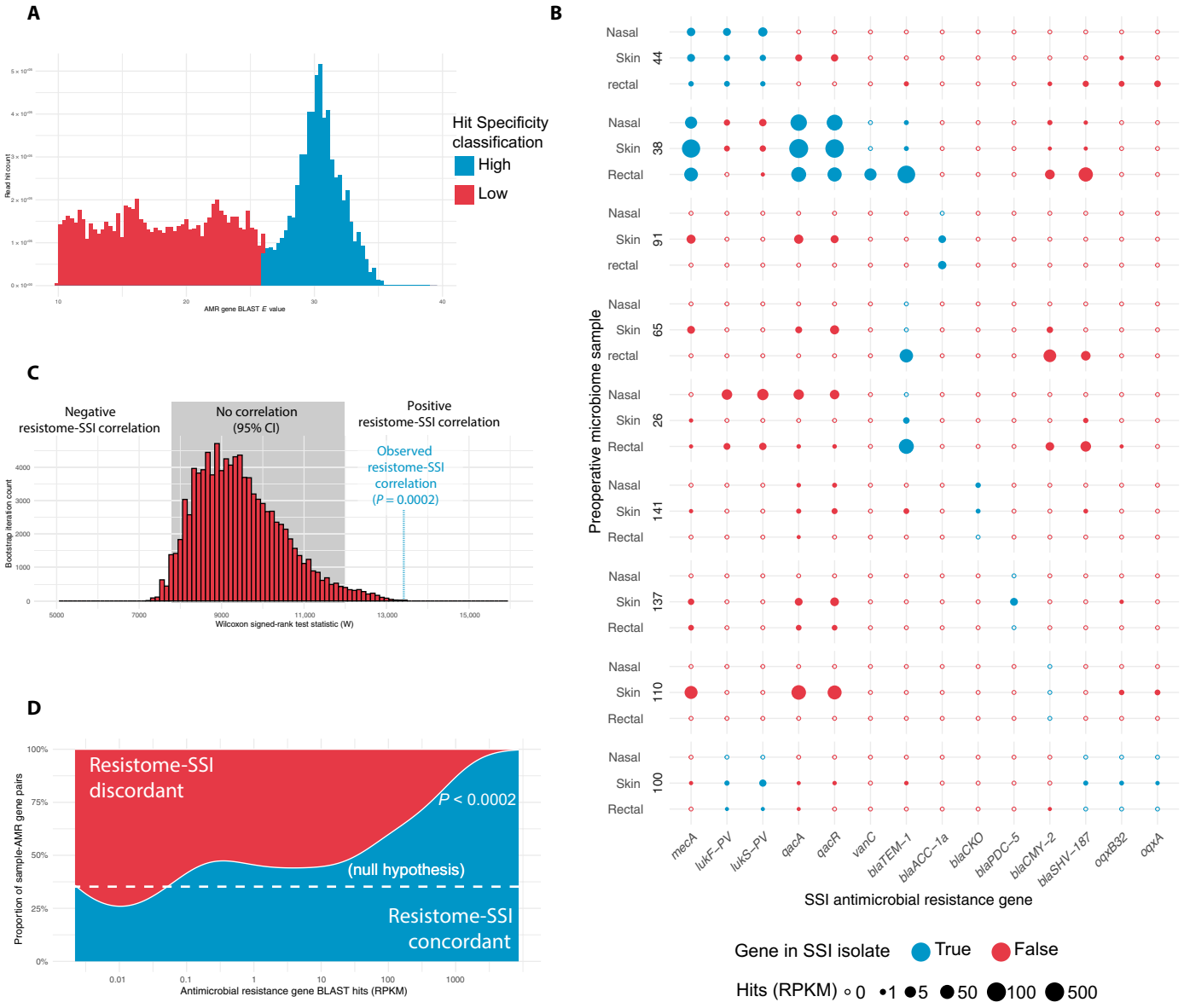

Fig. 6. Patient-level correlation of preoperative resistance genes and genotypes of postoperative, prophylaxis-resistant SSI. (**A**) Gaussian mixture modeling was used to select a BLAST *E* value threshold for classifying high-specificity hits for AMR genes. (**B**) Distribution of normalized AMR gene read counts [reads per kilobase per million reads mapped (RPKM)] in preoperative microbiome samples in relation to their presence or absence in the de novo assembled genomes of postoperative SSI culture isolates from corresponding patients. (**C**) Observed correlation between preoperative resistomes and SSI AMR genotypes (blue line) is significantly different ($P = 0.0002$) than the bootstrapped distribution of Wilcoxon rank-sum test values expected by chance in 100,000 iterations of random shuffling of preoperative samples between patients (null hypothesis, red bars). (**D**) AMR gene abundance in the preoperative microbiome (horizontal axis) is positively associated with gene presence in the genomes of subsequent SSI pathogens. Null hypothesis (no association between preoperative gene abundance and presence/absence in postoperative SSI) is shown as a dashed white line.

microbiome sampling arm. Eleven different organisms were found to infect multiple patients across the superset of 73 SSIs and could therefore potentially reflect common source infection. Isolates were available from 67 of the 73 (92%) involved patients and were subjected to WGS to enable epidemiological analysis. Speciation, SRA BioSample identifiers, and AMR gene content profiles are provided in data file S2 ("study aim: universal surveillance"). Based on (*31*–*33*), no SSI cases arose from a common strain infecting multiple patients (Fig. 7). Whereas genetic distances between intrapatient SSI isolate pairs (reflecting strains cultured from multiply sampled individuals) clustered around accepted measures of clonality by WGS (*31*–*33*), no interpatient same-species SSI pairs approached a level of genomic similarity that would be consistent with common source infection. This finding indicates that spine SSIs in our population were not caused by exogenous strains originating from shared reservoirs within the hospital environment at any measurable frequency.

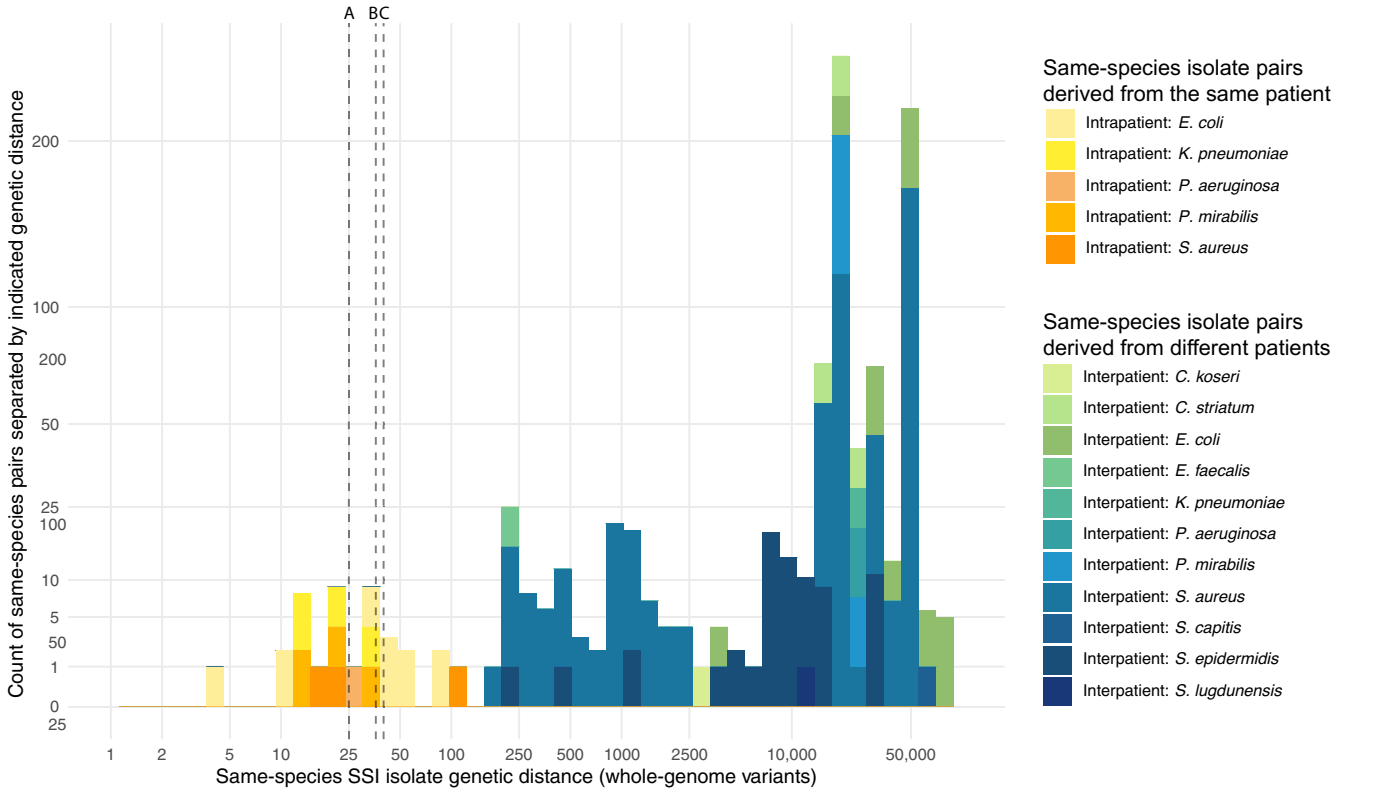

Fig. 7. Genomic surveillance of clinical SSI isolates among all patients undergoing spine surgery during study period. Pairwise genetic distances between same-species SSIs are displayed on the horizontal axis. Same-species SSI isolate pairs derived from different patients are indicated in blue-green shades ("interpatient" legend prefixes), whereas pairs derived from the same patient (for example, a single wound sampled at multiple time points or multiple specimen types such as fluid and tissue culture recovering the same species) are indicated in yellow-orange shades ("intrapatient" legend prefixes). Dashed vertical lines A (*31*), B (*32*), and C (*33*) indicate published thresholds for discriminating unrelated pairs (right of the lines) from those with degrees of genetic similarity consistent with descent from a recent common source (left of the lines).

DISCUSSION

In this prospective study, we used multiple forms of bacterial genomic analysis to assess the contributions of the preoperative patient microbiome to subsequent SSI among 204 patients undergoing a commonly performed surgical procedure—instrumented spine surgery. We observed a strong correlation between the preoperative patient microbiome and both the microbiology and antibiotic resistance phenotypes of subsequent infection. Among the superset of 1610 patients undergoing spine surgery in the same operative environment during the study period, no cases of SSI were caused by a bacterial strain that was shared among patients. Collectively, these results indicate that, in current surgical practice with the use of standard infection prevention measures, both the pathogens causing SSI after spine surgery and their AMR profiles can largely be attributed to preexisting strains from the patient's resident microbiota. We conclude that endogenous routes of infection, rather than introduction of exogenous strains originating from the hospital environment, are responsible for most such infections. The findings and methodological framework presented in this study carry practical implications for SSI prevention strategies and can inform future research on health care–associated infection.

Prior studies examining the role of endogenous bacteria in SSI have used lower-resolution methods of strain comparison [such as pulse-field gel electrophoresis (*13*, *14*) or multilocus sequence typing (*15*)] and have focused on *S. aureus*, which can readily be isolated from the anterior nares of colonized patients using selective culture media to facilitate strain-level comparison. In contrast, culture-based approaches for the isolation of other common SSI pathogens from the patient microbiome, particularly Gram-negative and anaerobic skin commensals, are comparatively complex and less reproducible (*12*, *34*). Other factors complicating the use of culture-based approaches for comparison of colonizing and infecting strains include the coexistence of multiple commensal strains of the same species (*35*, *36*), low bacterial abundance in some sample types (for example, skin swabs), and limitations in preserving viable cells from preoperative samples when the species of interest (the subsequent SSI pathogen) is not known in advance. To overcome these challenges, we used a range of complementary, culture-free techniques to characterize the contribution of the preoperative patient microbiome to SSI.

First, we leveraged established anatomic gradations in the epidemiology of SSI occurring in spine surgery to test for a correlation with the composition of the human skin microbiome across the same anatomic axis. Whereas prior studies of the skin microbiome have largely considered the back as a single region (*36*, *37*), we found that the microbiome varies measurably across that body site.

Incremental differences in the preoperative composition of skin communities along the length of the back mirrored the frequency with which the same taxa infect surgical wounds.

Second, we ascertained the epidemiological relationship between individual SSI isolates and strains present in a patient's preoperative microbiome. Previous studies have used 16*S* rRNA polymerase chain reaction (PCR) amplicon sequencing to assess changes in the microbiome during the perioperative period and their relationship with infection (*38*, *39*); however, such methods do not provide strain-level resolution, limiting inference about the dynamics of infection. In contrast, metagenomic sequencing provides the ability to distinguish individual bacterial strains and interrogate the complement of relevant AMR and virulence genes outside the 16*S* region. These advantages are offset by their own set of limitations. Because SSIs may arise from pathobionts initially present at low abundance in states of health that later rise to predominance in a wound given appropriate conditions, reads for a species of interest may be vastly overshadowed by material from the host and other microbes in samples collected in preinfection states, especially in low-biomass specimens such as skin swabs. As a result, direct metagenomic sequencing may fail to yield adequate read depth for strain-level comparisons of these taxa because of practical limitations of cost and exhaustion of available DNA from low-abundance swabs. Alternative approaches using PCR amplicon sequencing of informative sites throughout the genome (*40*), synthetic capture-probe panels (*41*), or whole-genome amplification (*42*) have been applied in other contexts but carry limitations in terms of the genomic scope, scalability, bias, and need for organism-specific primer/probe optimizations, which may not be feasible because of the limited availability of clinical sample material. In this study, we used a targeted whole-genome enrichment technique recently developed by our laboratory (GenCap-Seq) (*28*) to implement a generalized, low-cost framework for the analysis of preoperative (metagenomic) and postoperative (SSI-isolate) pairs. Using this approach, we were able to examine lineage-specific polymorphisms in the preoperative microbiome at informative sites across the genome to assess the degree of similarity between preoperative strains and those subsequently isolated by clinical wound culture in cases of SSI. We found that 86% of SSI pathogens, spanning a diverse range of Gram-positive, Gram-negative, anaerobic, and atypical organisms, matched strains carried by the same patient before surgery. This value closely corresponds with rates of endogenous infection reported in studies of *S. aureus* SSI (60 to 85%) (*13*–*15*) and generalized estimates based on inferential methods (~70 to 95%) (*9*). The remaining cases in our study were compatible with exogenous infection. Although in such cases the possibility of endogenous infection with an undetected, low-frequency strain cannot be excluded given the common coexistence of multiple same-species lineages in the human microbiome (*35*, *36*, *43*), in each case, patients had predisposing factors supporting the prior probability of a nosocomial origin.

Last, we examined the role of accessory AMR gene content in SSI, capturing an important dimension of molecular epidemiology that is independent of chromosomal (genome) similarity. Whether relevant resistance traits also originate from host reservoirs and are therefore potentially identifiable before surgery or, alternatively, are selected or acquired through exposure to prophylactic antimicrobials or horizontal transmission within hospital environments has not been thoroughly explored. Consistent with prior reports (*7*, *26*), 59% of SSI isolates in this study were resistant to the prophylactic agent administered at the time of surgery. By comparing AMR genotypes identified by whole-genome analysis of SSI isolates with antimicrobial susceptibility testing results, perioperative antimicrobial administration records, and the composition of individual preoperative resistomes, we found that the complement of resistance genes present in a patient before surgery strongly correlated with resistance traits of subsequent SSI. This observation is consistent with recent studies of urinary tract infection in which recurrent infections were found to be "seeded" from persistent host reservoirs (*44*) and in which emergence of AMR after treatment of initially susceptible infections was driven by selection of resistant strains already present in the host rather than newly evolved through de novo mutation or acquired through horizontal gene transfer (*45*). In our study, sampling of multiple patient reservoirs provided the greatest diagnostic sensitivity for preoperative detection of AMR genes that later contributed to prophylaxis-resistant infection, with the skin overlying the surgical site having the highest diagnostic yield of any single sampling location. This observation emphasizes an emerging appreciation of the skin as a central reservoir for AMR genes and pathobionts capable of causing clinical infections (*35*, *36*).

Further supporting the role of residual patient microbiota as a primary reservoir for wound infection, universal genomic surveillance of bacterial SSIs occurring in all patients undergoing spine surgery during the study period revealed no cases of common source infection. Prior studies using WGS to characterize the molecular epidemiology of health care–associated infection have either been performed in the context of case clusters (that is, having atypical patterns of occurrence prompting suspicion for a common source) (*46*) or in less-selective cohorts having limited inclusion of SSI-associated samples (*33*). This aspect of our study is unique in that it captures the molecular epidemiology of SSI among all patients in a high-risk procedural group undergoing surgery within a shared environment during routine operating conditions. Although case reports of common source SSI continue to draw attention to important avenues of nosocomial transmission (*47*, *48*), our findings suggest that such events are unlikely to be representative of the factors and modes of transmission most commonly contributing to SSI. Thus, whereas efforts to identify and disrupt environmental reservoirs of infection within the hospital remain critical to maintaining progress in SSI prevention, future innovations targeting residual patient microbiota are expected to have greater potential for achieving further improvements.

This study has several limitations, arising from both design constraints and the technical methodology. First, use of a single-procedure cohort facilitated a high rate of SSI culture acquisition, universal sampling of SSIs from a defined cohort for genomic surveillance, and correlation with the well-defined local epidemiology of SSIs based on U.S. Centers for Disease Control and Prevention National Healthcare Safety Network (NHSN) records but limits the generalizability of the findings. Even so, prevention measures and risk factors for SSI in spine surgery are similar to those in other procedural groups, making it likely that our findings will be reproducible in other procedures involving clean skin incisions. Second, even with optimizations to the GenCap-Seq protocol for specimens with low bacterial abundance, sequence depth for the SSI taxon remained low for some pairs, limiting the scope of analysis for sequence similarity and AMR gene content in those cases and precluding general use of metagenome-assembled genomes for strain-level comparison

(*49*). Similarly, our approach also did not allow for linkage of AMR gene sequences to specific bacterial genomes, which could potentially be overcome in the future with use of Hi-C or long-read sequencing technologies (*50*, *51*). In addition to AMR genetic complement, accessory virulence factors may also play important roles in SSI pathogenesis. Virulence factors were not analyzed in this study because of species- and procedure-specific considerations but could be evaluated in future work using similar designs. It is also not possible to infer on the basis of these results how or when wounds may have become seeded with commensal microbiota. Although present in the patient microbiome immediately before the start of the procedure, it remains possible that endogenous translocation of these strains occurred after the time of surgery (for example, fecal wound contamination during the early recovery period) or by more remote mechanisms such as hematogenous seeding of the wound (the "Trojan horse hypothesis of SSI") (*10*). Last, background contamination from reagents or environmental sources has the potential to confound microbiome studies at multiple stages. Although the expected impact of these factors is reduced in our work by the use of automation, process controls, internal comparison of study specimens, and multiple, independent forms of genomic analysis, they cannot be entirely eliminated.

Despite these caveats, our study provides compelling evidence that spine SSI in the era of modern surgical antisepsis is principally an infectious disease of endogenous origin and highlights the potential role of residual skin commensals as a proximal reservoir for wound inoculation. In the resulting conceptual model (fig. S8), most SSIs—not only those caused by *S. aureus*—originate from resident microbiota pre existing in the patient before the time of surgery, and resistance to surgical antibiotic prophylaxis similarly results from bacterial genetic reservoirs already established in the host. If these findings are replicated in other procedural cohorts, this model of SSI pathogenesis could drive important shifts in infection prevention strategy and enable more individualized and patient-centered approaches. One potentially high-yield implication of this model is the personalized selection of surgical antibiotic prophylaxis. The marked individual differences in microbiome and resistome composition observed in this and most other human microbiome studies are not considered by current guidelines (*52*, *53*) but could be routinely characterized before surgery. Such a strategy might guide a more sustainable framework that preserves the efficacy of surgical prophylaxis while limiting the trend of escalation to more broadly acting agents in the face of increasing population-level resistance (*7*). A conceptually similar, culture-based strategy has been explored in a limited fashion in patients undergoing colorectal surgery and been shown to be effective (*54*) without selecting for resistance (*55*) but has not been widely embraced or studied in other procedure types (for example, extra-abdominal procedures involving skin incisions such as orthopedic, neurological, cardiac, obstetric, or plastic surgery). Similarly, the detection of *qac* family genes in some SSI strains, conferring resistance to the common surgical antiseptic chlorhexidine (*56*), suggests that alternative agents such as povidone-iodine could be leveraged in a targeted or complementary fashion. Other priority areas for future research suggested by our study include improved standards for preoperative decolonization and novel methods of achieving more penetrant and durable skin antisepsis. Last, although our results identify the patient microbiome as the primary reservoir for SSI in this study, we assert that this understanding does not discharge health care systems from responsibility for infection prevention and should instead motivate the development of new clinical protocols and technologies that protect patients from their own microbiota during procedure-induced disruption of microbiological communities.

MATERIALS AND METHODS

Study design

The overall objective of this study was to assess the contribution of bacterial strains and AMR genes present in the preoperative patient microbiome to postoperative wound infection and prophylaxis resistance in spine surgery. Adult patients undergoing posterior, instrumented spine surgery at a single, high-volume academic medical center (Harborview Medical Center, Seattle, WA) between September 2019 and November 2020 were eligible for inclusion. Both elective and nonelective procedures were eligible for inclusion. Cases were identified by screening of the surgical schedule. Patients with preexisting infection were excluded on the basis of review of the electronic medical record and discussion with the performing surgical teams. The study was approved by the University of Washington Human Subjects Division (STUDY00006880). Written informed consent was obtained for elective patients enrolled in preoperative clinic, and a waiver of informed consent was provided for limited day-of-surgery sampling because the procedures were determined to be minimal risk, aligned with hospital infection control procedures with the collective benefit including diverse populations and those with limited ability to provide written informed consent (sedation or distracting injuries related to traumatic spinal cord injury, non–English speaking, or limited literacy status).

Institutional infection prevention measures

Standard infection prevention procedures were maintained during the study period and were minimally affected by COVID-19 pandemic response. Further details on these measures are provided in the Supplementary Materials.

Perioperative data collection

Patient demographics, procedural indications, operative details, and surgical antibiotic prophylaxis administration events were extracted from electronic health records.

Sample collection

Preoperative swabs from the nares, rectum, and skin overlying the intended surgical site were obtained on the day of surgery immediately before surgical skin preparation and administration of intravenous antibiotic prophylaxis. Collection was performed using swabs with enlarged tips (25-1607 1PF SC, Puritan) to maximize yield and using SCF-1 moistening buffer for skin samples as described in Human Microbiome Project Core Microbiome Sampling Protocol A (*57*). Day-of-surgery skin swabs were collected from the region of skin directly overlying the surgical site as indicated by the operating team. These swabs were rubbed longitudinally along the length of the planned incision, rotated 180° to expose the opposite side of the swab head, and rubbed again in the same fashion. The included anatomic region of the spine for each case was recorded using standard nomenclature based on vertebral levels [cervical vertebrae (C1 to C7), thoracic vertebrae (T1 to T12), and lumbar vertebrae (L1 to L5)]. For four patients, 11 preoperative samples spanning defined positions from C2 to pelvis were also collected in preoperative clinic,

in addition to day-of-surgery samples. These samples were collected using circular motions (~2.54 cm in diameter) over the midline skin at the positions indicated in fig. S9, with 180° rotation halfway through collection to expose the opposite side of the swab head. Sample processing, 16S rRNA V3-V4 amplicon sequencing, and data analysis are detailed in the Supplementary Materials.

Postoperative surveillance and collection of isolates from clinical wound cultures

Patients were monitored over 90 postoperative days for culture-positive SSI according to NHSN guidelines for instrumented spine procedures (*4*). Because treatment of SSI in cases of instrumented spine surgery commonly requires 1 to 3 months of systemic antibiotic therapy, surgical incision and drainage are routinely performed before initiation of antibiotics, both to facilitate source control and to maximize the diagnostic yield of deep wound and tissue cultures in guiding tailored antimicrobial selection. Bacterial culture, identification, and susceptibility testing of these SSI specimens were performed by the clinical microbiology laboratory as part of routine care (see the Supplementary Materials).

In parallel, positive spine SSI wound culture isolates were collected from all patients undergoing spine surgery without preexisting infection during the study period, regardless of enrollment in the prospective arm of the study. This ancillary approach enabled unbiased genomic surveillance for any common source infection among all patients sharing the same procedural teams and operating room environments, which would suggest an exogenous source of SSI (for example, transmission of a common strain from the hospital environment or between individuals). The expanded surveillance cohort also comprised noninstrumented and minimally invasive spine procedures, which were included to increase sensitivity for detection of nosocomial transmission.

WGS and genomic analysis

WGS of clinical isolates was performed on the Illumina NextSeq 500 and NextSeq 2000 platforms. Alignment and variant calling were performed using public reference genomes selected on the basis of sequence similarity and analytic approach (see the Supplementary Materials for details). Potential relatedness of strains by WGS distance was interpreted according to published distance values previously used by our group and others (*31*, *33*, *58*).

Metagenomic library preparation and targeted enrichment using GenCap-Seq

For the most informative subset of patients who completed preoperative microbiome sampling and later developed SSI, we performed targeted enrichment of sequence reads from the SSI pathogen using GenCap-Seq (*28*). Briefly, metagenomic libraries were prepared using a Illumina DNA Prep with Enrichment kit (*59*). Separately, genomic DNA was extracted directly from the pathogen(s) of interest, sheared, and converted to biotinylated capture probes covering the complete genetic complement of the strain, including plasmids and accessory gene content. Target enrichment of metagenomic libraries for the organism of interest was then performed by hybridization capture of genomic DNA–derived probes using xGen capture reagents (IDT). For polymicrobial infections, probes for each organism were combined on an equimolar basis into a single hybridization reaction to maximize mass-action effects. After interim analysis of initial samples, minor modifications to optimize performance for low-abundance swab samples were implemented. Metagenomic reads were then compared with whole-genome sequences to determine strain similarity and correspondence of AMR gene content (further details are available in the Supplementary Materials).

Statistical analysis

Statistical analyses were performed in R v.4.2.2 (*60*). Nonparametric tests (Kruskal-Wallis rank sum test for three-group comparisons, Wilcoxon rank-sum test for two-group comparisons, and Spearman rank correlation coefficient for correlation) were used for all comparisons of microbiome composition to avoid assumptions of normality when multiple taxonomic groups with potentially different distributions were considered or sample sizes were insufficient to test for normality. Application of specific statistical tests is described in corresponding sections of the methods and results. Bonferroni correction was applied on the basis of the number of taxonomic groups compared with an alpha value of 0.05.

Supplementary Materials

This PDF file includes:

Materials and Methods
Figs. S1 to S9
Tables S1 and S2
References (*61*–*79*)

Other Supplementary Material for this manuscript includes the following:

Data files S1 to S3
MDAR Reproducibility Checklist

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