Molecular testing with next-generation sequencing appears to identify biofilm on penile prostheses better than traditional cultures: The new gold standard?

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Introduction: *Traditional culture is the current standard-of-care to determine therapeutic antibiotics for patients suffering from penile prostheses (PP) infections. However, approximately 50% of PPs removed for infection are culture negative. Next-generation sequencing (NGS) compares DNA sequences to reference sequences with known microbial taxonomies to identify isolates and report relative abundances. We aim to compare the ability for standard culture and NGS techniques to identify microorganisms and biofilm composition on PPs.*

Materials and methods: *Ninety-one PPs explanted for mechanical malfunction were included in this study. Devices removed for infection or erosion were excluded. During revision surgery, two specimens were collected and sent for culture testing at institutional laboratory and for NGS testing (MicroGenDx, Lubbock, TX, USA). Species' relative abundances, sample diversity and richness, and compositional differences among samples were analyzed.*

Results: *NGS had a higher rate of microbial detection (n = 72, 79.1%) compared to culture results (n = 3, 3.3%). Some of the bacteria identified using both methods were known prosthetic infectious pathogens, with NGS producing more isolates (mean: 11) than culture (mean: 1). Escherichia coli was the most abundant and most frequently occurring bacteria detected on NGS. Coagulase-negative Staphylococci were the most common bacteria detected on traditional culture.*

Conclusions: *NGS appears to be beneficial in its thorough analysis of PP biofilm composition when compared to culture methods. We hope that further research will be able to demonstrate a clinical benefit of NGS in characterizing distinct microbiomes and biofilms of infected PP, which can aid in tailoring antimicrobial therapy and improving patient outcomes.*

Key Words: biofilm, culture, next-generation sequencing, penile implant, penile prosthesis

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Introduction

With excellent satisfaction rates among patients and their partners, the penile prosthesis (PP) has become a popular and acceptable treatment for medically refractory erectile dysfunction (ED).[1] Although complication rates are low, implant infection can be a devastating outcome, often resulting in complete removal of the PP despite aggressive antimicrobial therapy. Studies have elucidated that infections

associated with surgically implanted biomaterials may be due in part to bacterial growth and proliferation by way of their protective biofilms, comprised of bacteria and their secreted glycocalyx, adhering to the inert prosthetic surfaces.[2] Biofilm bacteria can maintain slow growth rates and remain quiescent for long periods. These factors can pose problems for conventional laboratory bacterial culture procedures and studies have reported that cultures can report non-specific to no growth in up to 33% of clinically infected cases.[3] Clinical culture techniques are often unable to detect bacteria present in a biofilm, which can make the detection of bacteria in an implanted device a diagnostic dilemma.[4]

Clinical molecular methods are increasingly being employed to investigate the microbiota of various infections as part of clinical standard of care. For example, novel techniques of microbiological sequencing, such as 16s ribosomal DNA molecular identification, has been implemented to detect bacteria in blood cultures or in previously concealed anaerobic bacteria in prosthetic joint infections.[5,6] Similarly, novel next-generation sequencing (NGS) approaches can be applied to PP, with hopes to advance the ability to detect bacterial biofilm composition on these prosthetic devices and improve targeted antimicrobial therapy.[7] NGS is playing a larger role for identifying infectious isolates in other medical fields such as wound healing and orthopedics.[8] These culture-free sequencing techniques deliver relative abundance scores which appear to provide clinicians with insight into which bacteria are most abundant in a sample; therefore, may better determine which isolate is causing infection. Characterizing biofilm on PP is also a clinical imperative since it may additionally be linked to mechanical failures potentially resulting in decreased device longevity and increased need for revision surgery.[9,10] The purpose of this study is to compare the ability for traditional culture and NGS techniques to identify microorganisms on PP.

Materials and methods

Patient population

Patients undergoing PP revision surgery at two institutions between June 2015 and June 2019 were evaluated. Only patients with complete NGS data who underwent revision surgery for mechanical malfunction were included in this analysis. Patients who underwent device removal for infection or erosion were excluded. All patients undergoing revision surgery underwent routine preoperative testing with a physical exam and urinalysis, and if positive, a urine culture. Preoperative intravenous antibiotics were administered according to the American Urological Association (AUA) Guidelines. In addition, patients were treated postoperatively with 5 days of oral antibiotics.[11]

Intraoperative sample collection and NGS testing

The protocol for sample collection has been previously described.[12] Upon entering the pump or cylinder space, two aerobic and anaerobic culture swabs were taken from the implant, capsule, fluid surrounding the device, and biofilm if present. All components usually were removed, but in some cases the reservoir was left behind. In these patients, the old reservoir was drained and retained, and a new reservoir was placed in a different location. All implant spaces were then irrigated with normal saline-based antiseptic solution. Strict sterility protocols were maintained to avoid contamination of the specimens.

Specimens for culture were sent to the hospital laboratory for routine culture evaluation. Specimens for NGS (MicroGenDx, Lubbock, TX, USA) testing were stored in sterile containers and shipped overnight at ambient temperature. Rapid molecular testing of 16s ribosomal DNA was performed by NGS using an Illumina MiSeq sequencing platform (Illumina, San Diego, CA, USA). For this, variable regions 1-2 of 16S ribosomal DNA gene were amplified and prepared into libraries for sequencing following molecular methods outlined in Tipton et al but using primers 28F and 388R as reported in Tipton et al.[13,14] Bioinformatic processing followed that reported in Cook et al and McDonald et al.[15,16]

Prior to statistical analysis, all NGS sample results were compared to their corresponding controls which were a combination of DNA extraction controls and no-template PCR controls. NGS detection for control samples were first transformed to relative abundances and then compared to matched samples. If the sample and control both had detection for a given microbe, the read counts of the sample were depleted proportional to the relative abundance in the control. Also, any detection of *Pelomonas saccharophila* and *Ralstonia pickettii* were eliminated because they are known common reagent contaminants.

Data collection and statistical analysis

Demographic data was abstracted for each patient. Both culture and NGS results were tabulated as "yes" for positive growth or result, or "no" for negative growth or result. Microorganism species identifications and relative abundances were documented. Differences in number of species detected (richness) and species

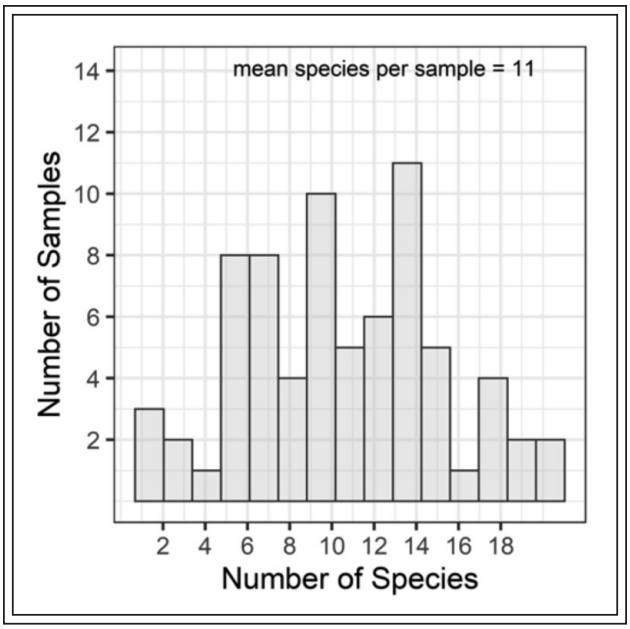

Figure 1. Histogram illustrating the distribution of species richness across 72 NGS-positive samples.

diversity (expressed as the exponential function of the Shannon diversity metric) across samples against age, ethnicity, diabetes status, implant duration, and year of implant removal were assessed using ANOVA. Differences in microbiome compositional profiles among samples were calculated as Bray-Curtis community dissimilarities and Permutational Analysis of Variance was used to test for the effect of the sample variables considered in ANOVA.[17,18] An ordination was performed by principal coordinates analysis using Bray-Curtis distances. Statistical analyses were performed using R statistical software.

Results

A total of 91 patients, with a mean age of 68 ± 10 years, underwent PP revision surgery for mechanical malfunction. The mean time from PP implant to explant was 62 ± 57 months. NGS and culture reported positive results in 72 (79.1%) and 3 (3.3%) cases, respectively. Some of the bacteria identified using both methods were known prosthetic infectious pathogens, with NGS producing more isolates (mean

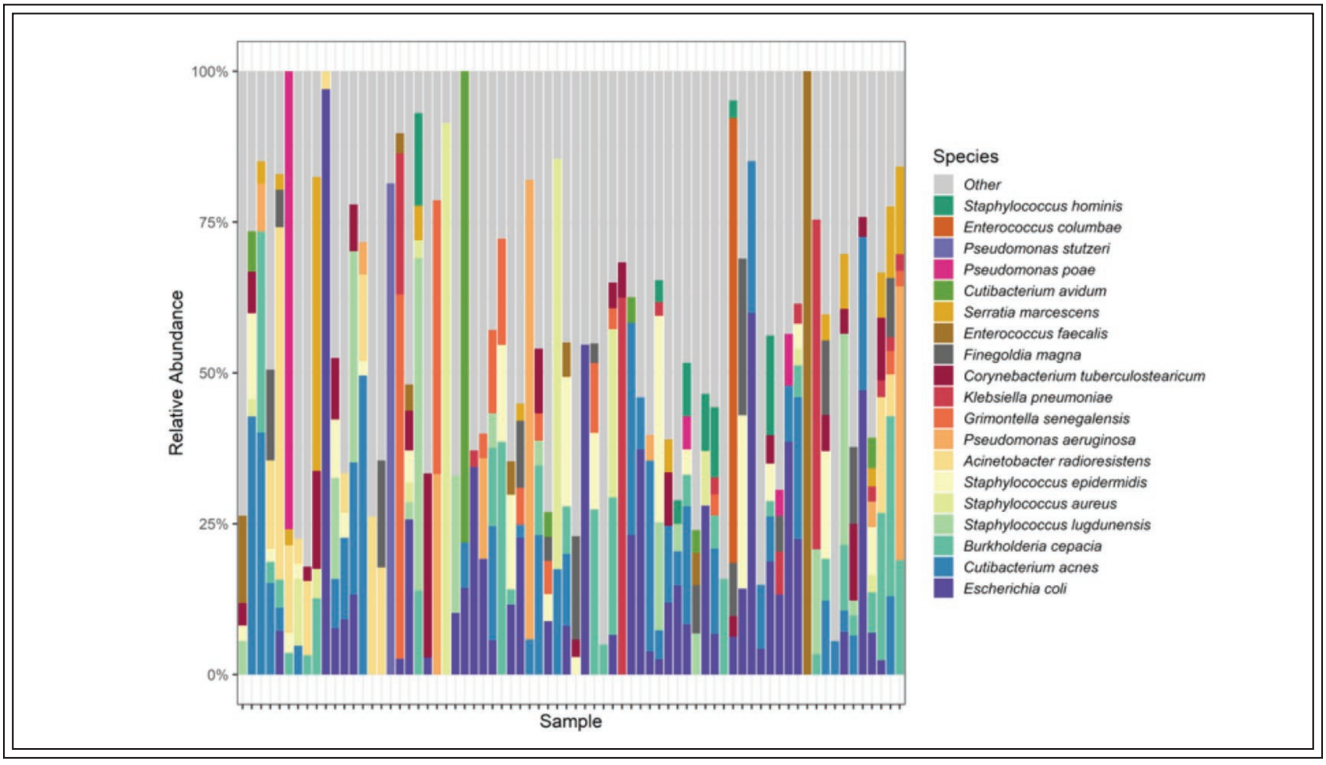

Figure 2. Stacked bar plot illustrating bacterial composition across 72 NGS-positive samples. The proportion of coloring for each species represents its relative abundance in a given sample. Gray shading for Other denotes the remaining proportion of each sample's microbiome that was composed of species not included in the list of top 20 most common.

11 species per device) than traditional culture methods (mean 1 species per device), Figure 1. Fungal elements were detected in 7 (7.7%) NGS specimens but were minimal in representation and were all less than 1% of the sample abundance. No fungal elements were detected on culture. Microbiome composition and species occurrence were diverse amongst NGS samples, Figure 2.

NGS detected organisms in all 3 devices with positive cultures. Two devices were congruently positive between NGS and culture. One device grew *Staphylococcus lugdunensis*, which was also the most abundant organism (28%) on NGS; however, an additional 9 organisms were detected on NGS. Another device grew *Enterococcus faecalis*, which was not the most abundant organism (5%) on NGS. NGS had detected an additional 9 organisms with greater abundance ranging from 5%-19%. The third positive culture returned anaerobic gram-positive cocci which was too general to compare to the 13 species detected on NGS.

NGS demonstrated precise and comprehensive results reporting with percentage of relative abundance for each detected microbe. *Escherichia coli* was the most frequently occurring and most abundant microorganism detected, Figure 3. *Cutibacterium acnes* and *Burkholderia cepacia* made up the remaining top three most abundant and frequently presenting isolates. *Staphlococcus epidermis* was the sixth most abundant and fourth most frequently presenting isolate. Richness and species diversity did not vary based on ethnicity, diabetes status, duration of implantation, sample year, or age at revision, Figure 4.

Discussion

In 1981, Costerton et al defined bacterial biofilm as "a glycocalyx matrix enclosed microbial population adherent to each other and/or surfaces or interfaces".[2] These protective biofilms allow bacteria to adhere and grow in colonies and have a major impact on temporary and permanent devices placed in the human body. For example, biofilms have been shown to form on central venous catheters, prosthetic heart valves, artificial hip prostheses, and intrauterine devices. Specific to the field of urology, biofilms have been found on urethral catheters, ureteral and prostatic stents, artificial urinary sphincters, and PP.[19-21]

Implanted prosthetic devices are at increased risk for biofilm colonization and subsequent device infection, because they lack the protective mechanisms of healthy tissue surfaces. Microorganisms that may initiate the colonization process on implanted

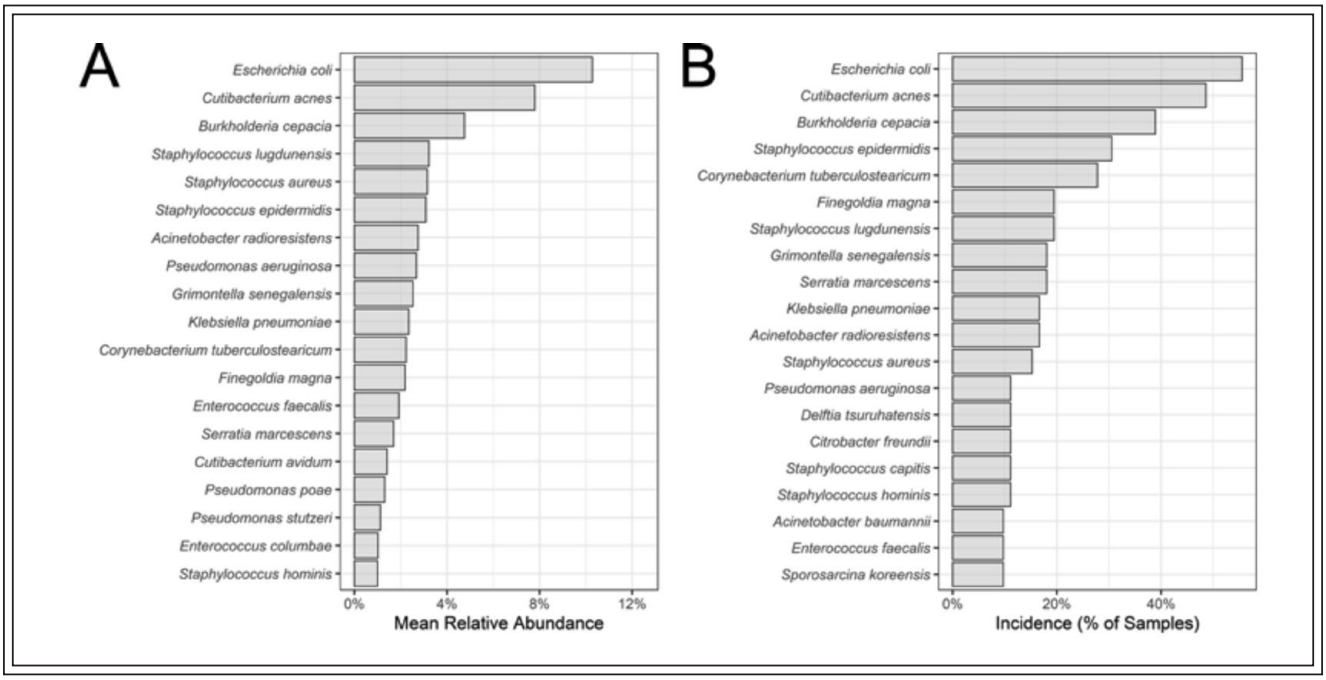

Figure 3. Bar charts of **(A)** mean relative abundance for bacteria with a mean relative abundance greater than 1% and **(B)** incidence of the 20 most common bacterial species.

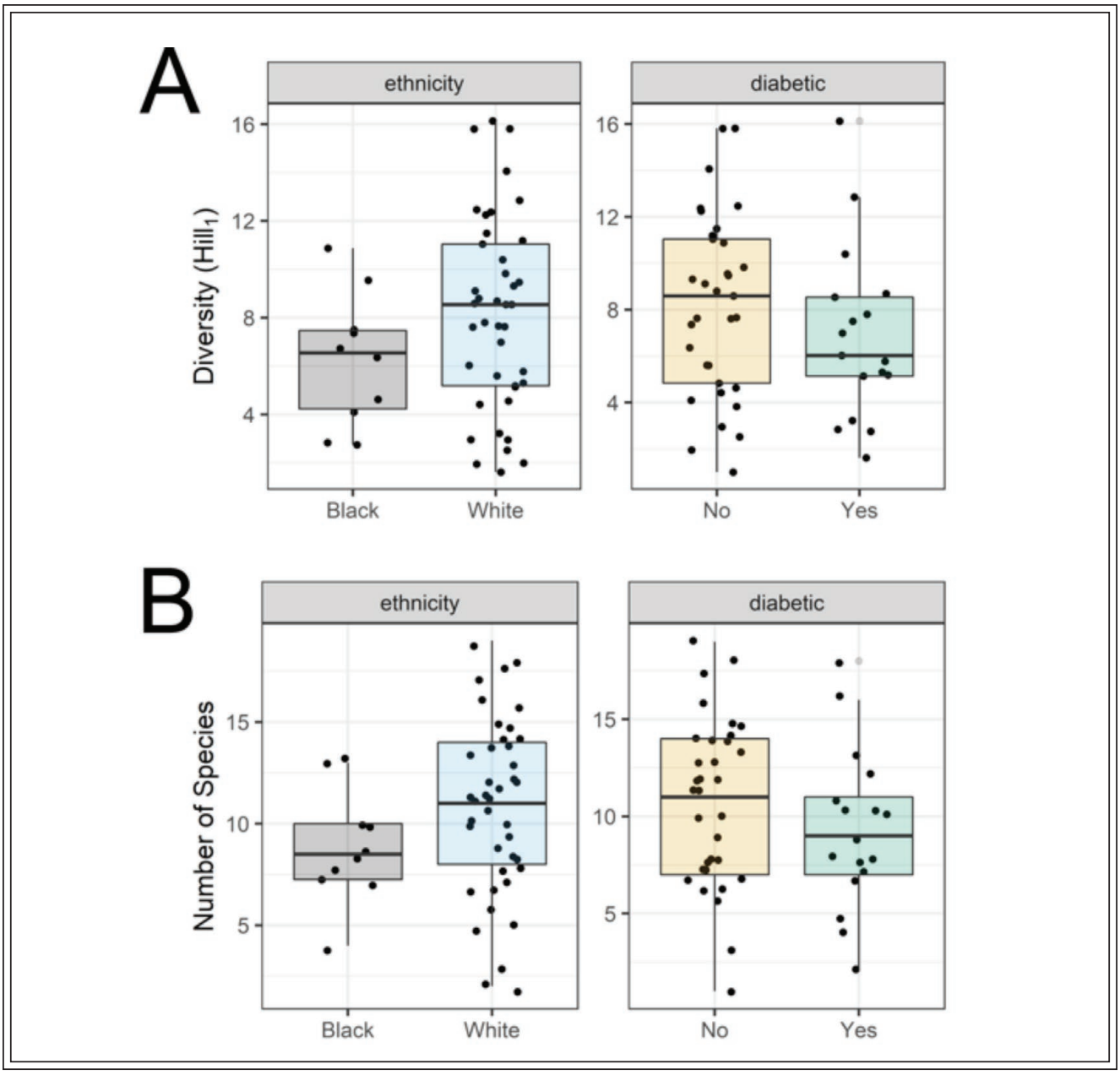

Figure 4. Boxplots illustrating **(A)** distribution of species diversity and **(B)** distribution of number of species detected (richness) with respect to race and diabetic status.

prosthesis have been hypothesized to be introduced during surgery or via hematogenous spread.[22] Glycocalyx-encapsulated bacteria are protected from the host-activated immune response, such as antibody opsonization or leukocyte phagocytosis, and externally administered antibiotics. These factors allow for the continuous proliferation of bacteria and attempts to eradicate the infection, without total device removal, may be futile.

NGS is an emerging technology which may provide a better understanding of PP biofilm and implant infection. Overall, we found that NGS (79.1%) had a higher rate of microbial detection when compared to traditional culture (3.3%) in PP removed for mechanical malfunction. The majority of bacteria identified on standard culture were also identified with molecular testing. Conversely, the vast majority of bacteria identified with molecular

methods were not necessarily identified with culture methods. Although not consistently tested for by culture, anaerobes were commonly found to be a component of the microbial population in this study. In most of the samples, culture underreported the diversity and richness of the wound microbiota and failed to detect the most abundant bacteria compared to NGS.

Previous studies have utilized traditional culture methods to direct antibiotic therapy at the time of surgery.[12,20] The advent of NGS molecular testing may allow for detection of microorganisms not routinely accounted for on culture. When analyzing individual culture and NGS reports in further detail, we found that NGS had a higher tendency to detect a polymicrobial growth, while culture results tend to report a monomicrobial growth. In fact, NGS detected a mean of 11 species, compared to standard culture which detected an average of one bacterial species per device. NGS was able to detect additional bacterial species that were underreported by traditional culture. This may in part be due to the difficulty in growing certain bacteria in the presence of their protective biofilms.

Despite detecting a higher number of microbes per device, we recognize that more bacteria may not necessarily equate to a higher likelihood of developing a clinically significant infection. In the orthopedic literature, treatment of polymicrobial periprosthetic joint infections have reported lower success rates when compared to treating monomicrobial infections.[23,24] Similarly, whether all the bacteria identified by NGS needs to be treated individually, or if certain species predominates while the other microbes are upregulated microbiota, still needs to be determined in the urologic literature in the context of PP infections.[25] Furthermore, the clinical significance of additionally detected organisms in clinically non-infected devices by NGS in this study needs further evaluation. Nevertheless, the additional data of abundance may aid in identifying the predominant microorganism needing to be targeted for infection prevention and treatment.

The historical paradigm of coagulase-negative *Staphylococcus* being the dominant species of PP biofilm needs to be reassessed. There has been a slow reduction in infections caused by coagulase-negative *Staphylococcus* species, the most commonly referenced PP pathogen.[26,27] Instead, isolates of Gram-negative bacteria are increasingly common among revised PP devices. This was evident in our cohort which identified *E. coli* as the most abundant and frequently isolated organism. *E. coli* was not a skin contaminant as it is not part of the normal skin flora; however, it may be a contaminant from the urinary tract potentially supporting the use of surgically adhesive drapes to isolate the incision, changing of gloves after catheter placement, and application of povidone-iodine solution to the catheter itself.[28] Further, the prevalence of fungi in the NGS samples appears consistently (7% vs. 12%) in line with previous studies by Gross et al, albeit a smaller sample size (91 vs. 217).[29] These findings may help to explain the increase in virulence of organisms causing PP infection and guide the use of perioperative antibiotics for systemic use, surgical-site irrigation, and hydrophilic coating dip solutions.[30]

Several limitations exist with this study. First, the study design is based on a smaller sample size precluding any powered statistical analysis and establishment of generalizable conclusions regarding the significance of the polymicrobial bacteria identified by NGS. As mentioned, the presence of these bacteria may not necessarily lead to a clinically significant infection, and studies are required to determine this phenomenon and the relevance of individual organisms examined. Best techniques for sampling the microbiota of PP and proper controls are under development as swabbing the implants may be not sufficient to dislodge all microorganisms. Samples were shipped overnight at ambient temperatures which may affect DNA integrity. Lastly, the ability of NGS to affect clinical care was not assessed in this study.

Conclusions

NGS appears to be beneficial in its thorough analysis of biofilm composition on PP and identified *E. coli* as the most abundant and frequently isolated organism. NGS appears to be more sensitive, precise, and gives relative abundance data as compared to traditional culture methods. Currently, its utility for PP revision surgeries and the significance of its polymicrobial results are still unknown. Yet, as the data continues to mature, this technology may serve as a useful modality for the treatment and management of this patient cohort.

Disclosures

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