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Microorganism Profiles of Penile Prosthesis Removed for Infection, Erosion, and Mechanical Malfunction Based on Next-Generation Sequencing.

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BACKGROUND: Next-generation sequencing (NGS) is an emerging technology that may allow for more sensitive and sophisticated microbial testing of the microbiota of penile prostheses (PP).

AIM: To describe the microorganism profiles of PP explanted for infection, erosion, and mechanical malfunction using NGS.

METHODS: All patients who underwent PP removal by two physicians at two institutions were identified. Differences in alpha diversity (i.e., number of species detected, species diversity across samples) and microbiome compositional profiles (Bray-Curtis community dissimilarities) across samples were assessed using ANOVA and PERMANOVA, respectively.

OUTCOMES: Number of species detected, species diversity across samples, and microbiome compositional profiles.

RESULTS: A total of 83 patients who underwent device removal for infection (n=8, 10%), erosion (n=5, 6%), and mechanical malfunction (n=70, 84%) were included. When considering all studies, 56% (n=48) of NGS and 29% (n=24) of standard cultures resulted positive for presence of microorganisms. Culture only detected the most abundant NGS species in 62.5% (n=5) of infected devices. Species richness and microbiome compositional profiles varied by surgical indication, but not by age, race, diabetes status, or implant duration. Most frequent organisms by surgical indication were *Pseudomonas aeruginosa* (infection), *Staphylococcus epidermidis* (erosion), and *Escherichia coli* (mechanical malfunction). The highest relative abundance organisms were *P. aeruginosa* (infection),

Corynebacterium jeikeium (erosion), and *E. coli* (mechanical malfunction). ~~GS Vancomycin and gentamicin provide the most comprehensive coverage against these organisms. Minocycline and rifampin do not cover the most abundant organisms for infection and erosion.~~

CLINICAL IMPLICATION: Identifying microbiome profiles of PP removed for infection, erosion, and mechanical malfunction may guide the selection of peri-operative antibiotics and PP antibiotic coatings or hydrophilic dip solutions for each individual scenario.

STREGTHS AND LIMITATIONS: While this is the first study to utilize next-generation sequencing to evaluate penile prosthesis biofilm, the clinical significance of these findings has yet to be determined. A prospective, randomized trial aimed at evaluating the clinical significance of NGS in patients with PP infection is currently underway.

CONCLUSIONS: NGS testing identified distinct microbiome profiles of PP removed for infection, erosion, and mechanical malfunction.

Keywords: Penile prosthesis; penile implant; infection; culture; next-generation sequencing~~; polymerase chain reaction~~

INTRODUCTION

Penile prosthesis (PP) implantation has emerged as the mainstay surgical treatment for medically refractory erectile dysfunction (ED). Substantial improvements in the efficacy and durability of PP over past decades have allowed a large and growing volume of patients to undergo PP surgery.(1) However, PP infection remains one of the most feared postoperative complications and places a significant economic burden on the healthcare system, with reported cost of management being six times that of the initial placement.(2) Significant efforts, such as infection retardant coatings on implants, better skin prep techniques, revision washout, and implementation of the "no-touch" surgical technique, have been utilized to optimize the management and reduce the risk of this complication.(3)

As evidence has shown that traditional infection rates of revision surgeries have a much higher rate of infection at 10.0 – 13.3% when compared to virgin cases at 0.5 – 2.0%, surgeons have attempted to use standard culture of the devices to help guide antibiotic therapy for revisions patients.(4) However, recent multi-institutional data evaluating clinically infected device explantations have reported device cultures showing no growth or non-specific growth in up to 33% of cases.(5) This may be attributed to flaws in the culture collection technique, the difficult nature of identifying and growing certain biofilm-associated microorganisms, or the administration of antibiotics before culture acquisition.(6) Regardless, this makes tailoring of the antibiotic regimen challenging.

Emerging technology has allowed for more sensitive and sophisticated testing and may be useful in the setting of genitourinary prostheses infections. One of the most promising advances in this realm is rapid molecular sequencing. Polymerase chain reaction (PCR) is the most familiar technology, which is a fast and inexpensive technique that amplifies small segments of DNA targets and may detect a comprehensive group of microorganisms and even resistance genes. This technology is already clinically available for blood cultures,

respiratory panels, pneumonia, meningitis, and may play a role in improving patient outcomes and decrease surgical complications in patients with infected PP.(7) Next-generation sequencing (NGS), also referred to as high throughput sequencing, is a technology which allows for hundreds to thousands of strands of DNA to be sequenced in parallel. Unlike PCR, which is limited to evaluating pre-determined targets, NGS uses bioinformatics to piece DNA fragments together and compare the sequences to reference genome databanks.(8) NGS may help to provide a more global understanding of biofilms and microorganisms found on PP. Herein, we aim to describe the microorganism profiles of PP explanted for infection, erosion, and mechanical malfunction using ~~PCR and~~ NGS molecular techniques.

MATERIALS AND METHODS

Study Design and Patient Population

Institutional review board approval was obtained to perform a retrospective review of consecutive patients undergoing PP explant procedures from January 2015 to January 2019 by two physicians at two institutions (IRB# TJU 20E.509 & WK 18.0002). Patients undergoing PP explantations were included regardless of indication for surgery (infection, erosion, and mechanical malfunction). Patients undergoing planned device explantation with or without replacement underwent routine preoperative testing, including a urinalysis and if positive, a urine culture. Positive cultures were treated with a seven-day course of culture-specific antibiotics preoperatively. Perioperative antibiotics were administered in accordance with the American Urological Association (AUA) Guidelines using vancomycin and gentamicin unless clinically contraindicated.(9, 10) Postoperatively, patients were given 5-7 days of trimethoprim/sulfamethoxazole or culture-specific antibiotics based on preoperative

urine cultures. Revision surgery was performed using either the penoscrotal or infrapubic method, but was not always performed by the same surgeon who performed the initial placement.(11) The antibiotic impregnated outer layer, InhibiZone™, was utilized for the AMS 700™ inflatable PP, while vancomycin and gentamicin were the hydrophilic solution of choice for the Titan® Touch inflatable PP. Vancomycin and gentamicin mixed in normal saline was also the irrigation fluid of choice at the time of implantation.

Intraoperative Sample Collection and Molecular Testing

At the time of explantation, surgeons minimized device contact with neighboring skin to decrease the potential risk of contamination by normal skin flora. Sterile gauze was used to swab the removed devices. The swabs were stored in sterile containers and shipped overnight at ambient temperature for NGS testing (MicroGenDX, Lubbock, TX). A second specimen or the explanted device was sent to the respective institutional microbiology laboratory for routine aerobic and anaerobic culture.

NGS of 16s ribosomal RNA was performed using an Illumina MiSeq sequencing platform (Illumina, San Diego, CA). For this, variable regions 1-2 of 16S rDNA gene were amplified and prepared into libraries for sequencing following molecular methods outlined in Tipton et al. but using primers 28F and 388R.(12, 13) Bioinformatic processing followed that reported by Cook et al. and McDonald et al.(8, 14) Generated sequences of microorganisms were compared with an in-house curated species database and an agreement of over 90% between the database and the sequence results as necessary to report a positive result. Bacteria and fungi were reported as relative abundances within each specimen (with 2% being the minimum threshold of reporting).

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 and Statistical Analysis

Patient demographics and etiologies for device explantation were abstracted. Etiologies were broadly classified by infection (e.g. gross infection), erosion (e.g. urethral erosion, tubing or pump extrusion), or mechanical malfunction (e.g. fluid leak, floppy glans, impending erosion, cylinder resizing). Standard culture and NGS results were documented as "yes" for presence of microorganisms, or "no" for absence of organisms. Microorganism species identifications and relative abundances were documented.

Differences in number of species detected (richness) and species diversity (expressed as the exponential function of the Shannon diversity metric, i.e., $Hill_1$ numbers) across samples explained by age, ethnicity, diabetes status, implant duration, and year of implant removal were assessed using ANOVA with Type III sum of squares and backward stepwise selection. Differences in microbiome compositional profiles among samples were calculated with Bray-Curtis community dissimilarities, and Permutational Analysis of Variance (PERMANOVA) was used to test for the effect of the sample variables mentioned above for ANOVA.(15, 16) An ordination was performed by principal coordinates analysis using Bray-Curtis distances. Tests for differences in the relative abundance of species depending on indication classification were conducted using Analysis of Compositions of Microbiomes

with Bias Correction (ANCOM-BC).(17) Chi-squared tests were used to assess relationships between etiologies and microorganism detection rate. Statistical analyses were performed using R statistical software.

RESULTS

Patient Demographics

From a total of 110 patients, 83 patients, with a median age of 69 (interquartile range: 17) years with both NGS and culture results, were included in this study. Indication for device removal included infection (n=8, 10%), erosion (n=5, 6%), and mechanical malfunction (n=70, 84%). The median time from PP implant to explant was 28 (interquartile range: 43.5) months. Of the devices removed, only one was a malleable PP, while the other 82 were inflatable PP. At the time of explant, 68 (82%) underwent device replacement, four of which were malleable PP, while the other 64 were inflatable PP. Eight (9.6%) patients had a concomitant artificial urinary sphincter device that was explanted at the time of revision surgery.

Standard Culture and Rapid Molecular Testing Results

Of the 83 devices, 48 (56%) NGS studies and 24 (29%) standard cultures resulted positive for detection of microorganisms ($p<0.001$). Among the 8 infected cases, all NGS and culture studies tested positive. Focusing specifically on the 8 infected samples there were 14 culture positive microorganisms, and 6 (42%) of these culture detections were also found in the corresponding patient's NGS result. Making the same comparison at the genus level resulted in 8 (57%) of these culture detections (8 of 14) also occurring in the NGS results. When culture did detect a species and genus reported by NGS, culture detected the most abundant NGS species and genus in 5 (63%) of 8 patients.

Among the 5 erosion cases, 4 (80%) NGS studies and 4 (80%) cultures studies tested positive (with one instance of NGS being negative and another instance of culture being negative). Among the 70 mechanical malfunction cases, 36 (51%) NGS studies and 12 (17%) culture studies tested positive. Considering patients with only mechanical malfunction, NGS and culture were both negative in 29 studies (41%) and both positive in 8 cases (11%).

Time for ~~PCR,~~ NGS and standard culture finalized reporting times were assessed specifically in infection and erosion cases from one institution. ~~PCR studies returned at a mean of 1 day, which was significantly faster than NGS and culture (both p<0.01).~~ NGS results returned at a mean 5 days compared to 7 days for finalized conventional culture results (p=0.12). ~~Quantitative PCR also assessed for antibiotic resistance genes, which were detected in 2 cases. Tetracycline resistance was identified in an infected device and methicillin resistance was identified in an eroded device. No resistance genes were detected in devices removed for mechanical malfunction.~~

Microorganism Profiles

Richness, defined as the number of species present in a sample, was calculated for all NGS positive samples and effect of surgical indication, age, race, diabetes status, and implant duration on observed richness values was assessed (Figure 1). Only surgical indication was significant ($F=16.01$, $p<0.01$), but time to surgery was the next most influential, albeit not significant ($F=3.04$, $p=0.09$). For $Hill_1$, which is an alpha measure that encapsulates both richness and evenness of species relative abundances of microbiota profiles, results were similar to those for observed richness values only with surgical indication being the only significant variable ($F=23.98$, $p<0.01$), and diabetes being the next most influential, albeit not significant ($F=2.08$, $p=0.16$).

The most frequent organisms by surgical indication (infection, erosion, and mechanical malfunction) were *Pseudomonas aeruginosa, Staphylococcus epidermidis,* and *Escherichia coli,* respectively (Figure 2). The highest relative abundant organisms by infection, erosion, and mechanical indications for revision surgery were *P. aeruginosa*, *Corynebacterium jeikeium*, and *E. coli*, respectively (Figure 3). Common implant antibiotic coatings and dip combinations against the most frequent and abundant organisms were broadly reviewed (Table 1).

Fungal elements were identified in two NGS specimens that were not identified in corresponding standard cultures. *Verticillium sp.* was identified by NGS in one mechanically failed implant from a diabetic patient, while *Malassezia restricta* was identified by NGS in an infected implant from a non-diabetic patient. *Candida parapsilosis* was identified by standard culture in one infected implant from a non-diabetic patient that was not detected by NGS.

Microbiome compositional profiles varied by surgical indication ($F=2.03$, $p<0.01$), but not by age, race, or diabetic status. Each surgical indication was then compared post hoc, revealing that infection and erosion samples did not significantly differ compositionally ($p=0.58$) but did significantly differ from mechanical malfunction samples (both $p<0.01$) (Figure 4). Relative abundances of species that were significant through differential abundance testing comparing indication types are exhibited in Figure 5.

DISCUSSION

Rapid molecular testing is an emerging technology that may allow for more sensitive and sophisticated microbial testing compared to standard techniques. We found that NGS may best play a role in identifying microorganisms in devices explanted due to infection or erosion rather than a mechanical malfunction etiology due to its higher positivity rates for those surgical indications. While it may not seem surprising that infected explants were more

likely to yield positive results, Henry et al. previously demonstrated that up to 70% of patients with clinically uninfected penile prosthesis can grow positive bacteria cultures at the time of reoperation.(18) Likewise, we found that 51% of NGS and 17% of standard culture studies of uninfected devices in our cohort detected microorganisms. However, this was a much lower rate when compared to NGS and standard cultures results of their infected counterparts (both 100%).

When comparing the timings to results reporting in one institution, we found that NGS testing results were reported at a slightly faster rate than culture results, although this difference was not statistically significant. ~~Most notably, PCR results could be returned within hours of receipt of the specimen.~~ Clinically, this may allow for prompt tailoring of antibiotics or antifungals and earlier targeted antimicrobial therapy~~, and identification of resistance genes~~. Furthermore, NGS assesses for both bacterial and fungal organisms simultaneously, eliminating the long wait for fungal culture results to finalize. Although immediate versus delayed salvage of an implant has not been shown to make a difference, perhaps earlier detection of the causative organisms and assurance that the correct antibiotics are being administered may allow urologists to more confidently proceed with salvage treatments.(19)

NGS provides an opportunity to better understand the microbiota on PP and may better described how specific groups may be at risk for infection. The presence of biofilm formation on the implanted device is thought to be a predisposing factor for infection in patients undergoing revision surgery.(18, 20-22) Removal of the primary device may disrupt biofilm and allow previously sequestered bacteria to be released and adhere to the new implant causing clinical infection.(23) In this present study, *E. coli* was the most frequent and abundant organism on devices replaced for mechanical malfunction, contrary to the historical paradigm of coagulase-negative *Staphylococcus* being the dominant species of PP

biofilms.(3, 20, 24, 25) The use of antibiotic coated devices and antibiotic irrigation may have reduced the coagulase-negative *Staphylococcus*, but allowed more virulent organism to become predominant as a result. Characterizing biofilm on PP is also a clinical imperative since it may additionally be linked to mechanical failures resulting in decreased device longevity and increased need for revision surgery.(26)

The most conservative treatment of an infected or eroded PP is complete removal with delayed implantation, which may result in corporal fibrosis, decreased penile length, and a potentially challenging replacement surgery. The challenge is that not all eroded devices are infected, even though they may be treated as such. Isolated single component removal and replacement has been described for eroded tubing and pumps in small cases series.(27, 28) Salvage washout with malleable or inflatable PP replacement is also feasible with potential infection-free rates of 93%; however, its use is currently limited to only 17.3% of infected cases.(19, 29)

An improved characterization of microbiota profiles of PPs may help to increase utilization and success of salvage treatments by guiding the selection of peri-operative antibiotics for systemic use and PP antibiotic and antifungal coatings or hydrophilic dip solutions. For example, in this study, virulent, Gram-negative *Pseudomonas aeruginosa* was the most frequent and abundant organisms on infected implants, while skin flora, *Staphylococcus epidermidis* (most frequent) and *Corynebacterium jeikeium* (most abundant), were identified predominantly on eroded implants. When common implant antibiotic coatings and dip combinations were broadly reviewed, vancomycin and gentamicin would provide the best coverage for infected and eroded implants which is consistent with a recent large multi-center review.(30) ~~Interestingly, the most abundant organisms for infection and erosion were not covered by the combination of minocycline and rifampin, a common infection retardant~~

~~coating.~~ Fungal elements were also detected in our study which reiterates the call to identify the clinical value of incorporating antifungals in PP surgery.(5)

NGS detected additional microorganisms not detected on standard cultures and may be more informative. For example, when focusing specifically on infected devices, culture only detected the most abundant NGS species in 62.5% (n=5). Additionally, when looking at the individual result reports, we found that the overall trend was that NGS tended to detect a polymicrobial profile, while the results demonstrated by cultures were mostly monomicrobial. Gross et al. similarly identified that 25% of culture-positive infection also showed polymicrobial growth.(5) Interestingly, while this has yet to be established in the urologic literature, treatment of polymicrobial infections has been shown to have lower success rates compared with monomicrobial infections in infected periprosthetic joints within the orthopedic literature.(31, 32) NGS may help to provide a better understanding of the predominant organism (abundance data) on PP and help to established whether these profiles are truly polymicrobial and require treatment, or rather an infection with a dominant species with the other organisms acting in concert.(33)

Our study is not without limitations. Best techniques for sampling the microbiota of PP and proper controls are still in development. Swabbing the implants may be not sufficient to dislodge microorganisms and may be an incomplete assessment of the microbial microenvironment. Controls were not utilized at the time of surgery to ensure microbes on the gauze were not contaminants from the field. Therefore, detected organisms may not represent a clinically significant infection and may reflect a contamination during device removal. Direct susceptibility testing was also not performed for NGS results and this made it difficult to compare the rates of bacterial composition and antibiotic resistance in our study. Samples were shipped overnight at ambient temperatures which may affect DNA integrity, and factoring was not performed for varying biomass between specimens during molecular

testing. In addition, no clinical studies have yet been performed showing that treating organisms identified in this study will have a positive clinical effect. A prospective, randomized trial aimed at evaluating the clinical significance of NGS in patients with PP infection and erosion is being performed. Despite these limitations, NGS was utilized to further characterize the microbiome profiles of PP removed for infection, erosion, and mechanical malfunction.

CONCLUSIONS

NGS helped to further characterize distinct microbiomes of PP removed for infection, erosion, and mechanical malfunction. The clinical potential of NGS is most useful in patients with infected and eroded devices, compared to devices removed for mechanical malfunction. ~~PCR shows promise in rapidly detecting clinically significant organisms and resistance genes in PP infections. A prospective, randomized trial aimed at evaluating the clinical significance of NGS in patients with PP infection is being performed.~~

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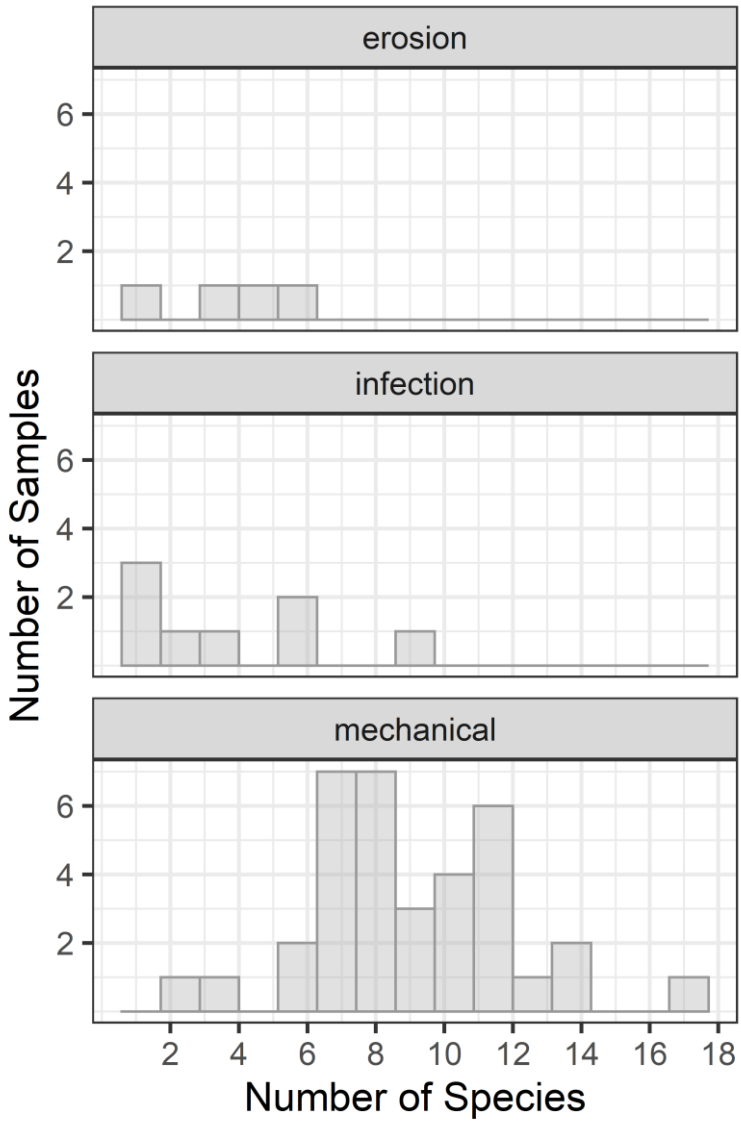

Figure 1. Histogram of species richness by surgical indication for penile prosthesis removal.

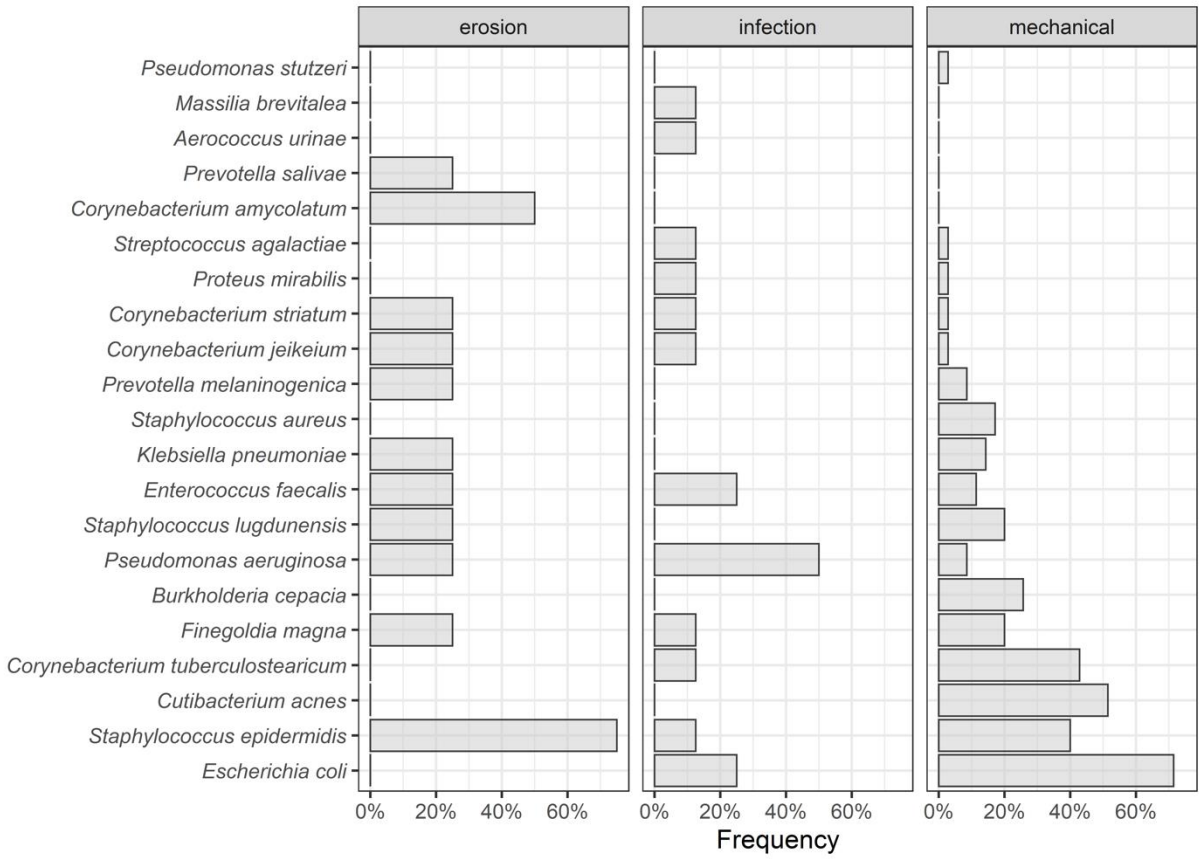

Figure 2. Bar chart of frequency of the most common species within each surgical indication for penile prosthesis removal. For this illustration the top 10 most common species within infection, erosion, and mechanical malfunction indications were identified, which resulted in 21 unique species.

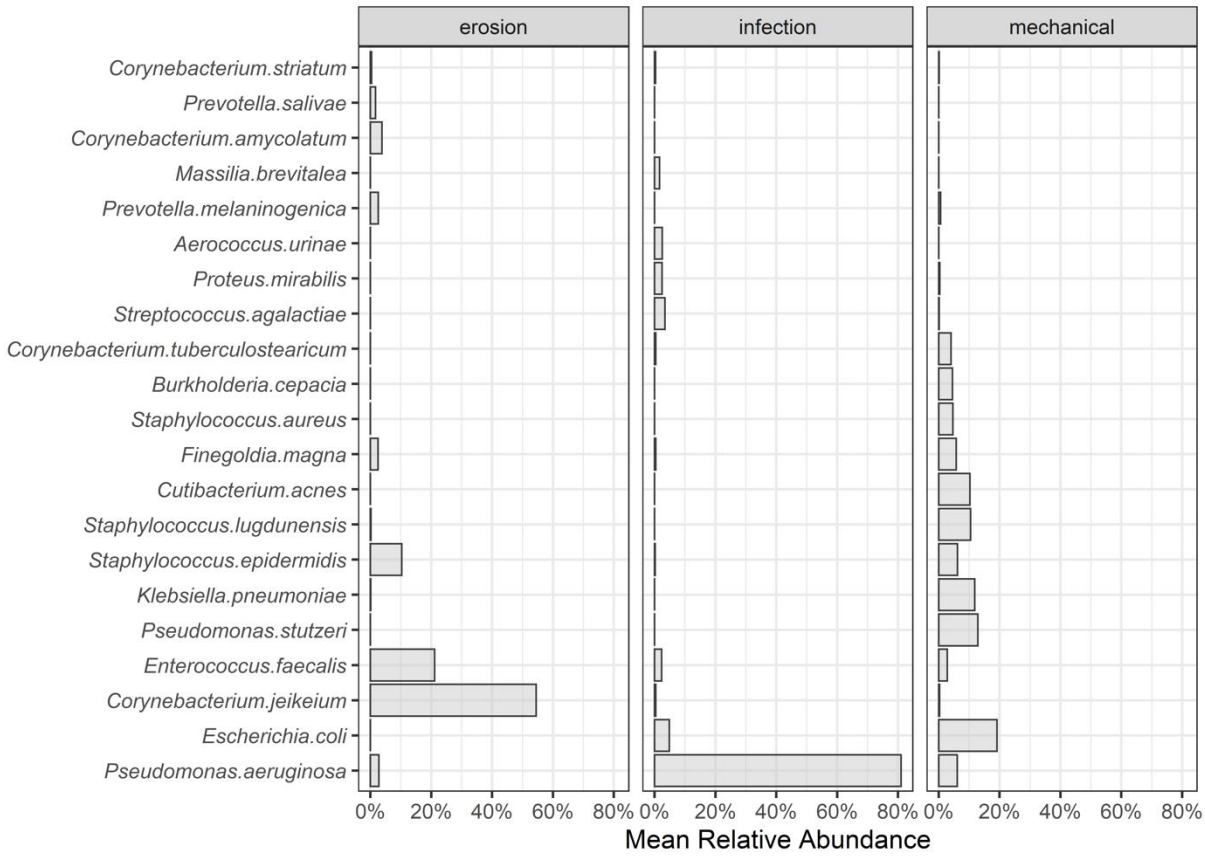

Figure 3. Bar chart of mean relative abundances of the most common species within each surgical indication for penile prosthesis removal. For this illustration the top 10 most common species within infection, erosion, and mechanical malfunction indications were identified, which resulted in 21 unique species.

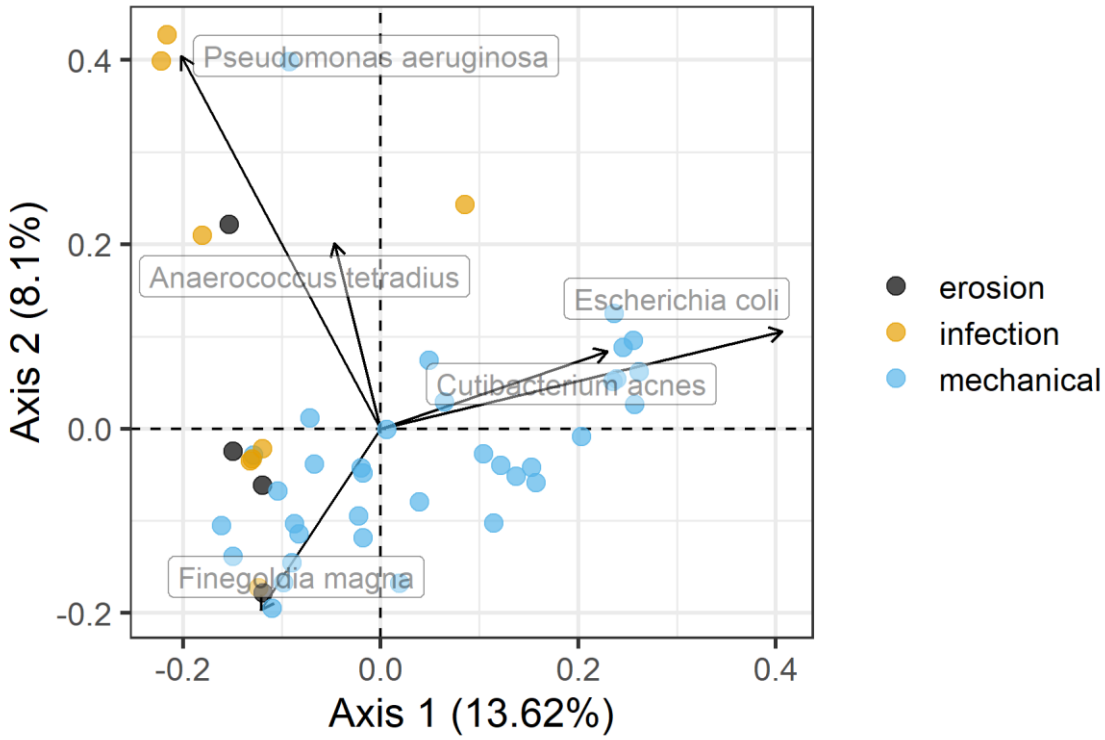

Figure 4. Principal coordinates analysis based on Bray-Curtis Dissimilarities. Species that had the strongest correlation with the first two axes are illustrated. The species evaluated for plotting were those previously identified to be the most common in the study. The direction and length of the arrow for each species represents increasing relative abundance of the corresponding species for samples in that area of the plot.

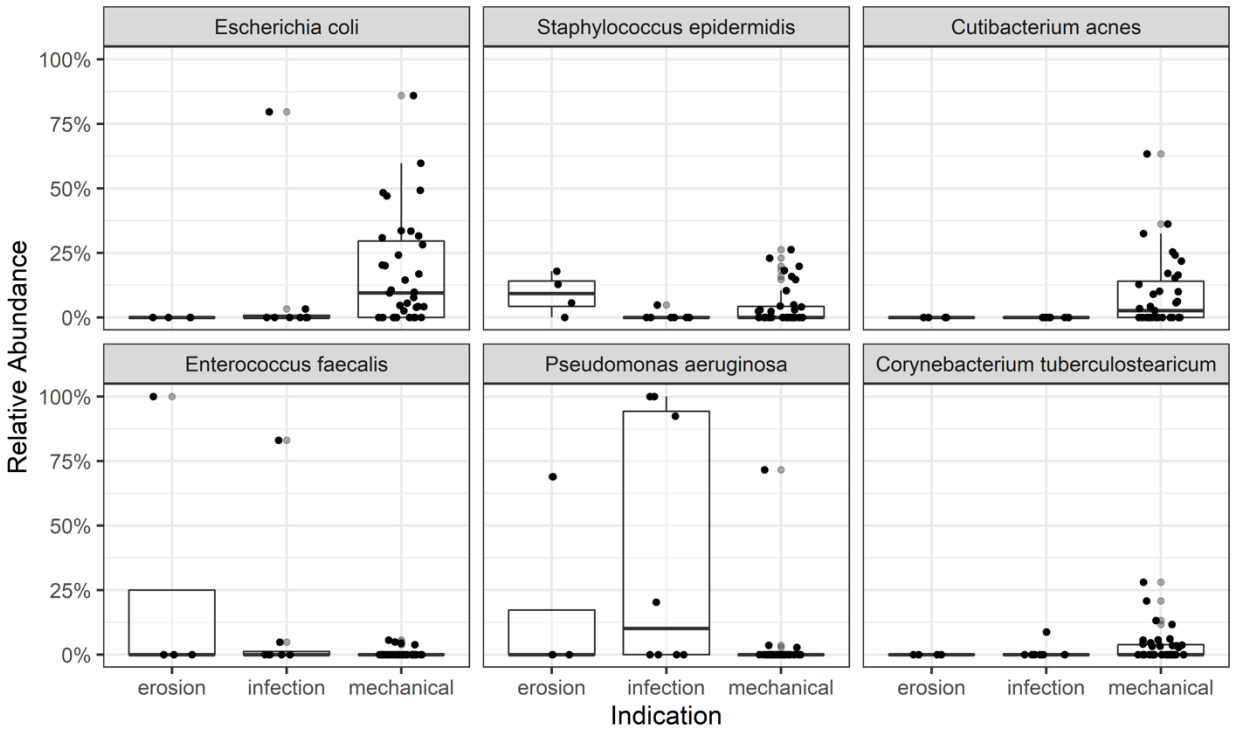

Figure 5. Box and dot plots of relative abundances of species that were significant through differential abundance testing comparing indication types. Each dot represents values for individual samples. The boxes of the boxplots are defined by 25th and 75th quartiles, the horizonal lines within boxes are medians, and whiskers calculated as 1.5 times the interquartile range.

469 Table 1. Coverage of common antibiotic coatings/dips against the most abundant and
470 frequent organisms based on surgical indication.
471

	Infected	**Eroded**		**Mechanical Malfunction**
	P. aeruginosa	*S. epidermidis*	*C. jeikeium*	*E. coli*
	Most abundant and frequent organism	*Most frequent organism*	*Most abundant organism*	*Most abundant and frequent organism*
Coverage of Common Antibiotic Coatings/Dips				
Minocycline/Rifampin		X		X
Gentamicin/Rifampin	X	X		X
Gentamicin/Vancomycin	X	X	X	X
Gentamicin/Bacitracin	X			X
Rifampin/trimethoprim/sulfamethoxazole		X		X

472