Next-generation DNA sequencing for infected genitourinary implants: How I do it

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Infection of artificial urinary sphincters or inflatable penile prostheses is one of the most devastating complications after prosthetic surgery and can have a significant impact on a quality of life. Patients undergoing revision surgery with or without device replacement may have increased risk for infection when compared to initial primary surgery. As such, surgeons may utilize traditional culture results to direct antimicrobial therapy for these patients. Unfortunately, culture results can be inconclusive in up to one-third of the time even in the setting of active device infection. Next-generation sequencing (NGS) of DNA is an emerging technology capable of sequencing entire bacterial genomes and has the potential to identify microbial composition in explanted devices. Herein, we describe our institutional experience on NGS utilization in patients with genitourinary prostheses. We also highlight our methods and techniques to inform readers on the potential practices that can enhance the utility and diagnostic yield of this new and upcoming technology.

Key Words: inflatable penile prosthesis, IPP, artificial urinary sphincter, AUS, next-generation sequencing, NGS, infection

Introduction

Two of the most commonly implanted genitourinary devices include the artificial urinary sphincter (AUS) for patients with urinary incontinence and the inflatable penile prosthesis (IPP) for patients with medically refractory erectile dysfunction. While satisfaction rates remain high, complications associated with implant surgery can have significant impact on quality of life. Device infection remains one of the most feared complication of any prosthetic implant, as this not only requires device removal, but can also lead to subsequent cicatrization and atrophy of the surrounding tissue, potentially making any future AUS or IPP reimplantations more challenging.

As infection rates of revision surgeries are typically higher than that of virgin cases, surgeons may benefit from the use of traditional culture of the infected device to guide antimicrobial therapy. However, these results can be influenced by the number of specimen acquired, anatomic locations where samples are obtained, intraoperative specimen handling, as well as laboratory interpretation and analysis. Recent studies have also reported that device culture can show non-specific to no growth in up to 33% of clinically infected cases.[1] Regardless, these factors complicate the tailoring of antibiotic regimen.

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Next-generation sequencing (NGS) of DNA is a promising technology that has allowed for more sophisticated and sensitive testing for microorganisms that may play a role in the setting of genitourinary prosthesis infections. Its use in the urologic literature is expanding, with promising results being described to analyze the microbiome in patients with prostatitis, urinary tract infections, and to see if specific microbes portends an increased risk for prostate cancer development.[2-4] Unfortunately, there is still paucity of data evaluating the utility of NGS in genitourinary prosthetic infections.

Herein, we aim to describe our institutional experience with NGS utilization in patients with genitourinary prosthetic infections. We further highlight our methods and techniques in this "How I Do It" article to inform readers on the potential practices that can enhance the utility and diagnostic yield of this new and emerging technology.

Our institutional experience

Patient demographics

A total of 33 men, with a mean age of 67 ± 11 years, underwent 35 device explantations at our institution: 21 (60.0%) AUS and 14 (40.0%) IPP, Table 1. One patient underwent two AUS explantations on separate occasions, while another underwent simultaneous IPP and AUS explant. Among the 35 devices, 24 (68.6%) were due to device malfunction or a mechanical failure, and the remaining 11 (31.4%) were removed due to device infection. The average time from device implant to explant was 47 ± 45.9 months. Of the 35 devices, only 26 (74.3%) were concomitantly replaced. With regards to device replacement, patients with an infected device (n = 3, 27.3%) were less likely to receive a replacement at the time of revision surgery, when compared to a malfunctioning device (n = 23, 95.8%; $p < 0.001$).

TABLE 1. **Patient demographics and device characteristics**

Variables	**N (%)**
Age	67 ± 11 years
Race	
White	20 (57)
Black	14 (40)
Hispanic	1 (3)
History of diabetes mellitus	
Yes	15 (43)
No	20 (57)
Device explanted	
AUS	21 (60)
IPP	14 (40)
Etiology of IPP revision	
Mechanical failure/device malfunction	24 (69)
Device erosion/infection	11 (31)
Device replacement during revision	
Yes	26 (74)
No	9 (26)
Culture results	
Positive	27 (77)
Negative	8 (23)
Culture results reporting time	7.8 ± 4.8 days
NGS results	
Positive	18 (51)
Negative	17 (49)
NGS results reporting time	4.5 ± 1.7 days

NGS = next-generation sequencing; AUS = artificial urinary sphincter; IPP = inflatable penile prosthesis

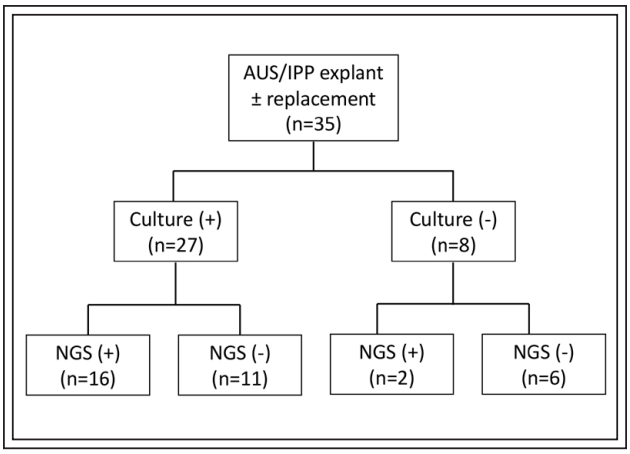

Figure 1. Outcomes of microbiology culture and next-generation sequencing (NGS) results for patients who underwent artificial urinary sphincter (AUS) and inflatable penile prosthesis (IPP) explantation with or without replacement.

Microbiology culture and next-generation sequencing results

Among the 35 devices, 27 (77.1%) standard cultures and 18 (51.4%) NGS reports resulted positive ($p = 0.025$) for microorganism growth, Figure 1. Among the 11 infected devices, cultures and NGS resulted positive in 11 (100.0%) and 8 (72.7%) devices, respectively. Of the 29 total positive results, NGS and cultures were congruently positive in 15 (51.7%) cases. While traditional cultures appeared to be able to detect microorganisms at a slightly higher rate than NGS, overall, we found that NGS was able to detect additional microorganisms not detected by standard culture in 14 (48.3%) devices ($p = 0.018$). This was also true among the infected explant cohort ($n = 5$, 45.5%; $p = 0.182$) although not significantly so.

Cultures and NGS were both more effective in detecting microorganisms in devices explanted for infection ($n = 11$, 100.0% and $n = 8$, 72.7%, respectively) than for mechanical failure ($n = 16$, 66.7% and $n = 10$, 41.7%, respectively; $p = 0.037$ and $p = 0.146$, respectively).

When comparing reporting times, NGS results returned at an average of 4.5 ± 1.7 days compared to 7.8 ± 4.8 days for conventional culture ($p = 0.001$). The rapid sequence PCR was processed at an average of 2.3 hours, which was significantly faster than NGS and culture reporting times (both $p < 0.001$); however, it only reported a total of three positive results among the entire cohort, all of which were patients who suffered from grossly infected devices.

Methods and technique

Patient preparation and perioperative antimicrobial management

There are many reasons why a patient with genitourinary implants may warrant a device explantation. The main etiologies for explantation can be broadly classified into either device malfunction/mechanical failure (e.g. worsening incontinence, fluid leak, urethral atrophy) or infected implant (e.g. gross infection, urethral erosion, cylinder extrusion).

Regardless of the etiology, all patients undergoing planned device explantations with or without replacement should ideally undergo routine preoperative testing, including a urinalysis and urine culture. Positive cultures should be treated with a course of culture-specific antibiotics preoperatively in accordance to the American Urological Association (AUA) guidelines.[5] Postoperatively, patients are commonly given 5-7 days of trimethoprim/sulfamethoxazole or culture-specific antibiotics based on preoperative urine cultures. Some penile implant patients may benefit from concomitant device replacement during revision surgery for infected cases. This requires an individualized, shared decision-making to determine whether this is the ideal treatment option.

Intraoperative sample collection and NGS

During the time of explantation, devices are immediately scrubbed with sterile gauze, stored in sterile containers, and shipped overnight at ambient temperature for NGS testing (MicroGen Diagnostics, Lubbock, TX, USA) laboratories. Surgeons are especially mindful to minimize explant contact with neighboring skin during device removal. Subsequently, the explanted device is sent to institutional microbiology laboratories for routine aerobic and anaerobic culture.

Details of NGS techniques have been previously described by Tarabichi et al and testings are performed in two separate stages.[6] Firstly, the DNA sample is extracted from the gauze and quantitative PCR is performed to determine the bacterial burden. The PCR functioned as a rapid screening test to identify any of the 25 most common bacteria and eight known resistance genes. These initial results can be returned within a few hours of receiving the swab. Next, the NGS assay is performed. The DNA sample is first amplified and pooled before undergoing sequencing using the Illumina MiSeq sequencing platform (Illumina, San Diego, CA, USA). Lastly, generated sequences were compared with a an in-house curated species database (MicroGen Diagnostics, Lubbock, TX, USA) and an

agreement of over 90% between the database and the sequence results is necessary to report a positive result.

What worked and what did not

In our experience, we found that both standard culture and NGS were more likely to identify microorganisms in devices explanted due to an infectious etiology (100.0% and 72.7%, respectively) rather than a malfunction etiology (66.7% and 41.7%, respectively). Although at a much lower rate when compared to their infected counterparts, the results seen in the malfunctioning devices may suggest a false positive due to subclinical microorganism growth or biofilm formation on these genitourinary implants. As such, our experience suggests that the utility of NGS is better suited for patients with a significantly evident infection, as these results can assist with targeted antimicrobial therapy in the clinically infected implant.

Other trends that we observed to increase diagnostic yield of NGS were the use of sterile 4"x4" gauze to rigorously swab the implant immediately after device removal. While we noticed that wiping the devices with moist gauze did not provide additional benefit to NGS results when compared to dry gauze, we realized that the use of cotton swabs was insufficient to collect an adequate sample for NGS testing. Furthermore, although cotton swabs are sterilized, DNA may survive and cause NGS contamination. Thus, at minimum, a dry sterile gauze should be used when collecting samples for NGS testing. Lastly, surgeons should also ensure minimal explant contact with neighboring skin during removal to decrease the potential risk of normal skin flora contamination.

Management of difficult situations

From our cohort, we had a larger than expected number of patients without detected organisms on NGS results and this made us question the best method of specimen collection for NGS testing. While we suggest the use of sterile gauze, we will continue innovating our approach to see if we can achieve the optimal technique to increase diagnostic yield and minimize contamination, e.g. attempting to send parts of the explant for NGS testing and the remainder for microbiology cultures.

Next, while NGS provides a comprehensive report of the device microbiome, it does not provide sensitivity and antibiotic resistance data for individual or even the predominant bacteria. This can make tailoring of targeted antimicrobial therapy challenging. For this, we recommend using individualized institutional antibiograms for the management of clinically evident device infections. Furthermore, it may be difficult to interpret the results when due to the number and lack of familiarity with the bacteria that may result, making the clinical use of these results challenging. Companies performing NGS may also provide the services of a consulting infection disease specialist to help interpret these results.

Additionally, studies have demonstrated the presence of microorganisms or biofilms in surgical locations previously thought to be sterile. The lack of adequate sampling in these areas may be mitigated with NGS technology, which can allow for appropriate empiric antimicrobial coverage even prior to revision surgery, which may help to increase salvage rates and decrease patient morbidity. Currently, a multi-institutional, prospective, randomized study is underway to determine the utility of NGS in detecting microbiome in the oral mucosa, preoperative urine samples, inguinal ring, scrotal septum and intracoporal regions prior to revision surgery. Their preliminary study has shown promising results and has demonstrated that NGS may be more sensitive and faster than cultures for the detection of microbacteria, even as early as the preoperative stages when the patients are evaluated in the clinics.

Pearls and tricks of the trade

We believe that adequate and proper sample collection is imperative to unveil the full potential and benefits of NGS testing in patients with infected genitourinary implants necessitating targeted antimicrobial therapy. At minimum, a dry sterile gauze should be used when swabbing DNA samples for NGS analysis and surgeons should respect the sterile field when retrieving the explanted device, regardless of whether or not a replacement device is being implanted into the patient. Lastly, if pus or biofilms are present on the device during revision surgery or able to be obtained during the initial evaluation for infection, additional specimens should be sent for NGS testing as well.

Discussion

Patients with infected devices in our cohort were less likely to receive a device replacement at the time of revision due to presence of gross infection, urethral erosion, or after careful patient discussion. An individualized approach, with potential risks and benefits explained, is essential to determine if concurrent device replacement during revision surgery of the infected patient should be performed. Studies have shown the risk of infections in virgin IPP/AUS implant cases to range between 0.5%-5.0%. However, the risk of postoperative infection in revision surgeries

can approach 18%.[7] The formation of biofilm on implanted devices is thought to be a nidus for bacterial growth and proliferation in patients undergoing revision surgery. Removing the initial implant may disrupt this biofilm and allows previously sequestered bacteria to cause clinical infection and adhere to the new device.[8] Additional work by Henry and Dawn et al has shown that copious irrigation and revision washout with antiseptic solution to the implant space may be a means of mitigation for future infection and biofilm formation.[9-11] NGS may help to further characterize biofilm and guide the developments of techniques to minimize its formation.

We found that both culture and NGS were able to identify microorganism growth at significantly higher rates in devices explanted due to an infectious etiology rather than a malfunction etiology. While it may not seem surprising that infected explants are more likely to yield positive results, an analysis of 87 clinically uninfected devices by Licht et al found that 36% of AUS and 40% of IPP had a positive bacterial culture during revision surgery.[12] Yet another study by Henry et al also demonstrated that patients with clinically uninfected penile prosthesis can grow positive bacteria cultures in up to 70% of cases.[13] Consistent with the literature, we found that 16 (66.7%) and 10 (41.7%) cases of mechanical failure devices in our cohort also detected microorganisms among cultures and NGS, respectively. These results suggest that the presence of low virulence organisms may not necessarily cause device infections, and NGS may have the potential to differentiate between these significant versus non-significant infections.

Notably, we found that cultures had a slightly higher microorganism detection rate than NGS. However, of the 29 positive results, NGS was able to detect additional microorganisms not detected on traditional culture in 14 (48.3%) cases. When analyzing individual reports in detail, we found the overall trend to be that cultures tended to detect a monomicrobial infection, while results demonstrated by NGS were mostly polymicrobial. Historically, gram positive bacteria, specifically coagulase-negative *Staphylococcus*, are found to be the most common microorganism seen in device infections, followed by the gram-negative rod, *Escherichia coli*.[1,12,14] Our culture results demonstrated coagulase-negative *Staphylococcus* spp growth, specifically *Staphylococcus epidermidis* or *Staphylococcus lugdunensis*, in 21 (77.7%) devices. However, of the 11 cases of infection, respectively, only 5 (45.5%) cases grew coagulase-negative *Staphylococcus*, further suggesting this species as a low virulence organism on implanted devices. Conversely, in addition to being more comprehensive and quantitatively specific, NGS detected additional bacteria including, *Corynebacterium* spp., *Finegoldia magna*, *Aerococcus urinae*, *Pelomonas saccarophila*, *Telluria mixta*, and even one fungus, *Verticillium* spp., that were not detected or tested for on standard cultures alone. While this has yet to be established in the urologic literature, the significance of monomicrobial versus polymicrobial results in genitourinary device infections remain unclear. Whether all identified bacteria should be individually treated or whether certain species predominates while the others are upregulated microbiota is a topic worthy of further research.

When comparing timings of result reporting, we found that NGS results (4.5 days) were reported at a significantly faster rate than culture results (7.8 days). Furthermore, rapid sequence PCR, the first-stage to NGS reporting, were resulting at an average of 2.3 hours of specimen analysis. Although not as sensitive, these preliminary reports may save significant amounts of time and allow for early targeted antimicrobial therapy. To date, the BioFire Film Array Blood Culture Identification (BCID) Panel is an exemplary model of the advantages of NGS utilization.[15] Currently in our institution, we have been treating revision patients based off their microbiology culture results and are reserving the NGS results as supplementary data in cases whereby patient's clinical conditions do not improve after culture-specific treatment. With faster, more accurate and detailed results reporting, this technology can hopefully decrease surgical complications and ultimately, improve overall outcomes in patients with infected genitourinary prosthesis. Further utility of NGS versus standard culture will be compared and assessed in the upcoming, multi-institutional, prospective trial.

Lastly, as the data on NGS for device infections continue to mature, its utility is not restricted to these scenarios in our institution. Although anecdotal and requires further assessment, we have found NGS to be helpful in patients with painful bladder syndrome, recurrent urinary tract infections, persistent urethral discharge despite empiric antibiotic treatment and negative gonorrhea/chlamydia screening, persistent dysuria or lower urinary tract symptoms despite negative cultures and patients with a suprapubic tube which often grows mixed organisms on standard culture. Moreover, NGS has the added benefit of saving time and healthcare cost, as it can detect bacteria tested on standard culture, sexually transmitted infections panel and even fungal species, all in one testing, potentially decreasing the number of samples required from the patient.

Conclusions

While there is good correlation between NGS and standard culture results, NGS may help further characterize the microbiome of genitourinary devices by identifying bacteria not routinely detected on cultures. From our experience, NGS is most likely useful in patients with infected devices rather than malfunctioning devices. While it may offer faster, more sensitive and precise results, randomized, prospective studies are required to confirm the advantages and utility of NGS. □

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