Peer Review File

Article information: https://dx.doi.org/10.21037/tau-22-702

Reviewer A:

Comment 1: First of all, I would like to congratulate the authors for their interesting study on the characterization of the biofilm of AUS. There are a number of explanations and questions that are necessary for a better understanding of their manuscript.

Abstract - The authors should summarize the last two sentences of the Conclusions, as they are personal opinions and are not supported by scientific evidence. It would be important to differentiate the concept of bacterial colonization (or biofilm) from infection.

Reply 1: Thank you for the feedback. We have made the changes in the conclusions of the abstract (page 3, line 62).

> ***Bacterial colonization or biofilm formation on implants may not necessarily equate to clinically infected devices. Future studies with more sophisticated technology, such as next-generation sequencing or extended cultures, may evaluate microbial compositions of biofilm at a more granular level to understand its role in device infections.***

Comment 2: How can the microbiological study of prostheses be affected by the prior administration of systemic antibiotics to patients before their removal?

Reply 2**: We agree with the reviewer and have included a sentence in the limitations to describe this (page 14, line 289).**

> ***There is also a possibility that the characterization of biofilm may be affected by preoperative and intraoperative administration of antibiotics.***

Comment 3: The authors are advised to describe in more detail the diagnostic methods used for microbiological culture. How is biofilm removed from the surface of the AUS: fragments of the prosthesis; washing of the prosthesis surface; scraping of the surface, etc. This is an extremely important issue in determining the appropriate exposure of microorganisms.

Reply 3**: Thank you for the comment. We have included new information regarding the diagnostic techniques in the methods section (page 5, line 111)**

> ***Upon entering the pump or AUS cuff space, aerobic and anaerobic culture swabs are taken from any fluid surrounding the device. If a capsule or biofilm, if present, these are also dislodged from the tissue and implant and sent as a separate specimen. The surface of the explanted AUS device is vigorously scraped with a gauze and sent as a separate specimen together with cut fragments of the prosthesis.***

Comment 4: The authors use in the AUS implantation some kind of technique aimed at the reduction of possible contamination, such as: washout of the implant; antibiotic-impregnated. It would be very useful to know the authors' implantation protocol in depth.

Reply 4**: We thank the reviewer for this comment. We have included more information regarding our implantation protocol in the methods section (page 5, line 103).**

In order to decrease the risk of prosthetic infection, our institutional protocol when implanting AUS includes the use of the AMS 800 implant which has an antibiotic-impregnated coating (InhibiZone), with rifampin and minocycline. We also use one liter of saline irrigation mixed with vancomycin and gentamicin to the surgical bed for primary implants and two liters for revision cases. Also, all operative personnel (surgeon, assistant, scrub nurse) are instructed to change sterile gloves intraoperatively prior to handling of the implant.

Comment 5: It would be of great interest for the authors to go more deeply into the relationship between "radiotherapy" and "device erosion".

Reply 5**: Thank you for the comment. We have included a paragraph discuss the relationship between radiotherapy and device erosion and quoted several recent studies supporting this (page 13, line 263)**

Radiation causes small vessel endarteritis that results in localized changes such as tissue necrosis, fibrosis and atrophy, leading to compromise of the urethral blood supply and tissue healing. This may portend an increased risk for device erosion or urethral atrophy. A recent meta-analysis of 18 studies on AUS outcomes indicated that radiation therapy not only reduces the odds of achieving complete continence after AUS placement, but also significantly increases the risk for revision surgeries, urethral erosions and subsequently, explantations. Recent multicenter studies echo the same conclusions. Mann et al. demonstrated that the "fragile" urethra (history of urethroplasty, radiation, prior AUS) were strong predictors for earlier erosion with radiation history providing the highest hazard ratio when compared to the other risk factors (HR 2.36, 95% CI 1.52-3.64, p<0.01). AUS survival rates for "fragile" urethras were also much shorter at 1- (76.5% vs 44.1%) and 5-year (50.0% vs 14.8%) survival when compared to "non-fragile" urethras (p<0.0001). In patients with first replacement AUS, Huang et al. also found that a history of pelvic radiation was associated with a shorter time to device failure and was specifically associated with a seven-fold increase risk of device erosion. They suggest that in order to allow for optimal tissue healing, AUS replacement should be delayed after removal of an eroded device, which can leave patients incontinent for extended periods of time. Hence, adequate patient counseling is necessary in light of these risk factors to set reasonable postoperative outcomes regarding continence and complication rates.

Comment 6: As the authors comment, the techniques for assessing micro-organisms are too standard to be able to obtain all the information useful to their interesting study. This should be highlighted in the manuscript.

Reply 6**: We thank the reviewer for this comment. We have included a statement in the conclusions section to reflect this (page 15, line 306).**

Future studies should be aimed at evaluating the microbial composition of biofilm using more sophisticated technology, such as next-generation sequencing or extended cultures, to better understand its role in AUS device infections.

Comment 7: Where does author find the highest presence of micro-organisms? In which type

of sample: in the AUS; in the capsule, etc.
Reply 7**: We thank the reviewer for this comment. Unfortunately, we do not have the granularity of data to describe this question in detail. Although in some situations a single component was evaluation, the majority of patients underwent complete removal of the implant. During complete removal, each available part of the implant was swabbed in aggregate; therefore, information on single components is not available. We believe this study will help to lay the foundation to a more detailed study.**

Comment 8: The use of the word microbiome relates not only to micro-organisms, but also to their genes and metabolites. The latter part has not been evaluated, which may lead to confusion with the use of the word microbiome. Possibly it would be more useful to use "biofilm characterization".
Reply 8**: We agree with this comment and have made the changes accordingly within the text and the title of the manuscript. We have changed all instances of microbiome to biofilm (page 4, line 81 and page 14, line 287).**

Title: Characterizing the biofilm of artificial urinary sphincters (AUS)

Running Head: Biofilm of artificial urinary sphincters

When extrapolating data from penile prosthesis (PP), another common genitourinary implant device, one can also obtain insights on the biofilm composition of explanted AUS devices.

Techniques for sampling of biofilms on explanted AUS devices are still under development as mere swabbing of implants may not be enough to dislodge all microbes and its associated biofilms.

Comment 9: The implications should be summarized in the last two sentences.
Reply 9**: Thank you for the comment. We have summarized the implications of our study in the last two sentence of our manuscript in the conclusions section (page 15, line 303).**

It is important to differentiate between the concept of bacterial colonization or biofilm formation on AUS devices and true clinically infected implants as the management for these two may differ. Future studies should be aimed at evaluating the microbial composition of biofilm using more sophisticated technology, such as next-generation sequencing or extended cultures, to better understand its role in AUS device infections.

Reviewer B:
This study seeks to evaluate the microbial composition of 23 explanted artificial urinary sphincters on standard culture. Although the reported data appears to achieve this purpose, the reviewers of this study have some major concerns.

Comment 1: Lack of details of methodology - There is an obvious lack of methodology regarding the manner in which the reported microbes were detected. Although the study

mentions use of standard culture, it does not go into enough detail with how these microbes were detected. For example, the reader is not given a threshold for a positive urine culture. The reader also does not know how organisms grown on standard urine culture were identified. Was it by MALDI- ToF or by biochemical means? Although this may be due to the retrospective nature and not knowing what methods were done in the past, this does not excuse lack of knowledge on the matter and needs to be clarified.

Reply 1**: We thank the reviewer for this thoughtful comment. We have included more details in the methods section to clarify the questions at hand (page 5, line 99 and page 6, line 126).**

> ***A threshold of >10,000 organisms/mL was the threshold to be considered as a positive urine culture.***
>
> ***The organisms were identified by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry analysis which was developed, and its performance characteristics determined by Thomas Jefferson University Hospital Microbiology Laboratory.***

Comment 2: Interpretation bias – Although coagulase negative Staph appears to be correctly interpreted, we have concerns regarding the conclusions made about E. coli and Candida albicans. Figure 1 demonstrates percentage data suggesting they could not have been detected more than once given small cohort, yet there are major conclusions made about their impact on infected AUS explants. The study fails to highlight Cutibacterium (9.1%) and Propionibacterium (13.6%) entirely even though they are much more prevalent. If this is due to a belief they are not as virulent as E coli or Candida albicans, this is a major source of bias and needs to be mitigated.

Reply 2**: We agree with the reviewer regarding the concerns for interpretation bias and have addressed this throughout the manuscript.**

> ***Abstract (page 2, line 52): Among two of the four infected/eroded implants, more virulent organisms such as Escherichia coli and fungal species such as Candida albicans were identified.***
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> ***Results (page 9, line 165): There was a total of 44 occurrences of identified microbial species identified in these 20 devices. Figure 1 highlights the prevalence of microbial culture species identified on explanted AUS devices. Coagulase-negative staphylococci species, including Staphylococcus lugdunensis and Staphylococcus epidermidis, were the most identified bacteria among explanted AUS devices (n=16, 80%, 24 occurrences). This is followed by common commensal skin flora such as Cutibacterium species, including Cutibacterium acnes and Propionibacterium species (n=10, 50%, 11 occurrences). Although sample size is small, there was evidence of more virulent organisms such as Escherichia coli and fungal species such as Candida albicans identified once among two of the four infected/eroded implants.***
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> ***Discussion (page 10, line 182): The most common species identified was the coagulase-negative staphylococci species (n=16, 80%, 24 occurrences), followed by***

common commensal skin flora such as Cutibacterium species (n=10, 50%, 11 occurrences).

Discussion (page 11, line 213): Although our sample size of infected explants was small, we found that the only devices that speciated E. coli and Candida albicans were two of the four clinically infected devices.

Discussion (page 11, line 221): Further comprehensive evaluation in a larger cohort is required to describe this finding.

Conclusions: (page 15, line 296): The most commonly identified bacteria in this setting is coagulase-negative staphylococci and commensal skin flora such as Cutibacterium species, which may be a result of bacterial colonization introduced at the time of implant. Conversely, infected implants may harbor microorganisms with higher virulence including fungal elements, however larger cohort studies from infected devices are necessarily to confirm this.

Comment 3: Lack of clinical impact - Although this seems to be a pilot study investigating the microbial composition of removed AUS implants, the microbial composition of other removed urologic implants has already been well-studied. In addition, both infectious and non-infectious indications for AUS removal were analyzed together which likely confounds the data prohibiting any meaningful conclusions about microbial composition. There are also no clinical takeaways in the study with regards to role of antimicrobials in the composition of these devices and the impact of urinary infection prior to surgery. These are the answers urologists need answered to improve practice. Even less can be gathered when considering the poor methodology previously discussed since the results cannot be reliably interpreted. This study may provide preliminary direction for future studies with more consequence on clinical practice but it does not fill a useful clinical niche as it is presented.

Reply 3**: We thank the reviewer for this thoughtful review. We agree that the literature available for biofilm composition on penile prosthesis is richer than that of AUS, however, these results are not necessarily interchangeable. There may be inaccuracies when we extrapolate penile prosthesis data into AUS and assume that they share the same microbial data. As such, studies and research should be done on AUS devices to describe its biofilm before the conclusion that all urologic implants share the same microbiome can be made.**

We also recognize some limitations and lack of clear implications or conclusions in our study. We have included updated information regarding this throughout the manuscript (page 14, line 283 and page 15, line 303).

First, the study design is based on a small sample size from a tertiary referral center and analysis for AUS removal for infectious and non-infectious etiologies were analyzed together.

It is important to differentiate between the concept of bacterial colonization or biofilm formation on AUS devices and true clinically infected implants as the management for these two may differ. Future studies should be aimed at evaluating the microbial composition of biofilm using more sophisticated technology, such as next-generation sequencing or extended cultures, to better understand its role in AUS device infections.

Similar to comment 1, we have updated the methods section to improve and clarify the methodology of our study (page 5, line 99 and page 6, line 126).

A threshold of >10,000 organisms/mL was the threshold to be considered as a positive urine culture.

The organisms were identified by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry analysis which was developed and its performance characteristics determined by Thomas Jefferson University Hospital Microbiology Laboratory.

We agree that this study can provide preliminary data for future studies and build the foundation for studies with more consequence on clinical practice. In our groups experience, we have previously described the biofilm composition in PP prosthesis with the use of next-generation sequencing technology and were able to demonstrate the distinct bacterial composition for PP explanted for different indications. We also reviewed the common antibiotic regimens and prosthetic coatings and described the best coverage for bacterial identified for different explant indications. Our next step is to replicate our methodology for AUS explants in hopes to enlighten urologists with the knowledge and information that can change clinical practice and improve patient outcomes (page 15, line 301).

We hope that these findings can expand the literature on microbial composition among AUS devices which will subsequently allow for appropriate tailoring of culture-specific antibiotics in this era of increasing bacterial resistance, subsequently improving patient outcomes.