Characterizing the Microbiome of the Contracted Breast Capsule Using Next Generation Sequencing

Jonathan Cook, MD; Casey J. Holmes, MD; Roger Wixtrom, PhD; Martin I. Newman, MD; and Jason N. Pozner, MD

Abstract

Background: Recent work suggests that bacterial biofilms play a role in capsular contracture (CC). However, traditional culture techniques provide only a limited understanding of the bacterial communities present within the contracted breast. Next generation sequencing (NGS) represents an evolution of polymerase chain reaction technology that can sequence all DNA present in a given sample.

Objectives: The aim of this study was to utilize NGS to characterize the bacterial microbiome of the capsule in patients with CC following cosmetic breast augmentation.

Methods: We evaluated 32 consecutive patients with Baker grade III or IV CC following augmentation mammoplasty. Specimens were obtained from all contracted breasts (n = 53) during capsulectomy. Tissue specimens from contracted capsules as well as intraoperative swabs of the breast capsule and implant surfaces were obtained. Samples were sent to MicroGenDX Laboratories (Lubbock, TX) for NGS.

Results: Specimens collected from 18 of 32 patients (56%) revealed the presence of microbial DNA. The total number of positive samples was 22 of 53 (42%). Sequencing identified a total of 120 unique bacterial species and 6 unique fungal species. Specimens with microbial DNA yielded a mean [standard deviation] of 8.27 [4.8] microbial species per patient. The most frequently isolated species were *Escherichia coli* (25% of all isolates), *Diaphorobacter nitroreducens* (12%), *Cutibacterium acnes* (12%), *Staphylococcus epidermidis* (11%), fungal species (7%), and *Staphylococcus aureus* (6%).

Conclusions: NGS enables characterization of the bacterial ecosystem surrounding breast implants in unprecedented detail. This is a critical step towards understanding the role this microbiome plays in the development of CC.

Level of Evidence: 4

Breast augmentation is the most frequently performed cosmetic surgical procedure in the United States, and capsular contracture (CC) continues to be the most common complication. Recent work has shown convincingly that bacteria play a role in the formation of CC. Numerous studies, based on traditional culture techniques, have over the past 20 years identified bacteria on or around breast implants explanted from patients with CC. Review of this literature indicates that the most common bacterial species isolated are *Staphylococcus epidermidis*, *Cutibacterium acnes*, and coagulase-negative staphylococci (Figure 1).
Bacterial contamination of implanted devices has been shown to frequently result in the formation of biofilms. These complex systems of bacteria encased within an extracellular polymer matrix are resistant to antibiotics and evade host defenses. This protective structure and state of bacterial existence renders traditional culture techniques insufficient for detecting bacteria present in biofilms, and for adequately characterizing their constituent species. More recent studies have employed techniques such as sonication, fluorescent in situ hybridization, and quantitative polymerase chain reaction (PCR), which are designed to detect and sequence bacterial DNA and RNA within biofilms. Although these methods are time-consuming and expensive, their improved diagnostic accuracy highlights the benefit of combining multiple diagnostic methods to understand the microbial ecosystem of breast implants and CC.

Next generation sequencing (NGS) represents an evolution of PCR technology that can sequence all DNA present in a given sample, thereby providing a more complete picture of the microbial community present within biofilms. Microbial identification is achieved by performing 2 methods in parallel: quantitative PCR and amplicon sequencing. In this specific assay, the 16S rRNA gene is amplified and sequenced. These are highly conserved and variable regions of the rRNA gene, allowing for specific microbial identification. In microbiology, NGS serves to replace or augment traditional culture methods, where microbes are characterized by morphology, staining properties, and metabolic criteria, with a genomic definition of pathogens and their antibiotic susceptibility. This experimental approach was recently used to study the breast microbiome in patients with anaplastic large-cell lymphoma (ALCL). MicroGenDX (Lubbock, TX) is a commercial laboratory that performs NGS for the molecular diagnosis of biofilms. Their technology has been utilized across a range of specialties, including orthopedic surgery, otolaryngology, urology, and wound care.

We sought to determine the practical application of NGS technology in our clinical practice. Our goal in this pilot study was to use NGS to examine the microbiome residing within contracted capsule tissue and on the surface of breast implants in patients with CC after cosmetic breast augmentation. We hypothesized that characterization of the breast microbiota in patients with CC could provide new clues about the etiology of CC following cosmetic breast augmentation.

**METHODS**

We evaluated 32 consecutive patients who underwent capsulectomy for Baker grade III or IV CC following cosmetic augmentation mammoplasty, who desired surgical correction, from April 2018 to May 2019. The study was conducted in compliance with the Declaration of Helsinki and written informed consent was obtained from all patients. All procedures were performed by the senior author (J.N.P.). Tissue specimens of the contracted capsules as well as intraoperative swabs of the breast capsule and implant surface were obtained at the time of implant exchange and capsulectomy. All samples were sent to MicroGenDX Laboratories for NGS.

The MicroGenDX NGS process (Figure 2) begins with sample preparation, including tissue manipulation and lysis. This is followed by DNA extraction with a Roche High Pure PCR Template Preparation kit (Hoffman-La Roche, Basel, Switzerland). Conventional PCR to amplify microbial DNA is then performed with an ABI Veriti Thermal Cycler (ThermoFisher Scientific, Grand Island, NY). Forward and reverse primers homologous to the regions flanking the 16S rRNA gene and the ITS2 gene are used to identify bacteria and fungi. The V1-V2 regions of the 16S rRNA gene are then sequenced. These are highly conserved regions of the bacterial and fungal genomes, enabling their accurate identification. A negative PCR control of molecular-grade water is run on every PCR plate and added to the sample pool stage, which then follows samples through every stage of library preparation, sequencing, and the bioinformatics pipeline. Three positive PCR controls (ATCC, Manassas, VA) for bacteria and fungi detection at high, medium, and low
concentrations are also run on every PCR plate, and these are added to the sample pool stage as well. The controls are known positives but are not known human pathogens, and consist of *Marinobacter hydrocarbonoclasticus* for bacteria and *Vanderwaltozyma polyspora* for fungi. This process helps verify true positive signals and ensures that patient samples are not contaminated during processing. The amplified DNA is given unique tags, in order to differentiate them when being run on the sequencer. The amplified DNA is then pooled based on the strength of the amplification. Sample DNA is loaded into the flow cell for bridge PCR which generates sufficiently high levels of DNA in the sample to enable NGS. Positive samples are then sequenced on the Illumina MiSeq platform (Illumina Inc, San Diego, CA). These data are then denoised to remove short sequences that may interfere with data interpretation and to eliminate other artifacts (such as chimeric sequences). USearch7 is then used to compare the sequences against a curated database containing sequences from the National Center for Biotechnology Information (NCBI) Nucleotide database; an agreement of at least 90% between the sequences and the database is necessary. A report is then published providing qualitative details of the bacteria and fungi in the sample, as well as antibiotic susceptibility based on known resistance genes. (Supplemental Figure 1, MicroGenDX NGS report)

**RESULTS**

All 32 patients were female, and their average age was 48 years (range, 22-71 years). Specimens collected from 18 of 32 patients (56%) revealed the presence of microbial DNA. Among patients who had bilateral procedures, there were 5 bilateral CC cases with bilateral positive NGS results, 2 bilateral contracture cases with 1 side positive, 2 unilateral contractures with the contracted side positive, and 3 unilateral contracture cases with the contralateral side positive. The total number of positive samples was 22 of 53 (42%). Sequencing identified a total of 120 unique bacterial species and 6 unique fungal species (Figure 3 and Supplemental Figure 2). Specimens with microbial DNA yielded a mean [standard deviation] of 8.27 [4.79] microbial species per patient. The most frequently isolated species were *Escherichia coli* (25% of all isolates), *Diaphorobacter nitroreducens* (12%), *C. acnes* (12%), *S. epidermidis* (11%), fungal species (7%), and *Staphylococcus aureus* (6%) (Figure 4).
In our pilot study, we identified a diverse group of microbial species in patients with pathologic CC after cosmetic breast augmentation. When compared with the aggregate isolates of bacterial species recovered from patients reported in the literature,\textsuperscript{5-11} we found a significantly different profile of microorganisms (Figures 1 and 4). Because this was an observational pilot study, we were unable to compare the microbiota recovered from CC patients with that from patients without CC. However, it is interesting to theorize that, in patients with CC, a shift in the composition...
of the microbiome may be responsible for an augmented host response that leads to increased inflammation and ultimately CC.

One particularly interesting finding of our study was the identification of *E. coli* more frequently than any other species in patients with CC. The question that comes to mind is: where does this *E. coli* come from? Is it part of an altered endogenous microbiome, or is it the result of implant contamination from the urinary tract, “leaky gut,” or other sources? Further examination of the *E. coli* found in breast capsules by other sequencing techniques could help determine its source. The identification of a “capsulogenic” microbe (or group of microbes) strongly associated with CC could lead to the development of diagnostic tools to determine a patient’s risk of CC prior to cosmetic breast augmentation. It may also be possible to identify “helpful” species, whose presence is theoretically protective against CC. The relative proportions of bacteria in a patient’s breast could therefore provide a biomarker of their risk for CC following breast augmentation.

Conceivably, by selectively reducing the numbers of these “capsulogenic” bacteria with preoperative antibiotics or probiotics, it may be possible to reduce the incidence of CC in an otherwise susceptible population. This may initially take the form of pocket irrigation with narrow-spectrum antibiotics, and could eventually lead to a personalized antibiotic or probiotic strategy for prophylaxis based on an individual's breast microbiome.

This pilot study has several limitations that must be considered. The purpose of our study was only to examine the feasibility of substituting traditional culture methods with NGS to study patients with CC following cosmetic breast augmentation. For this reason, we did not include a control arm. Although we included all patients in our practice with pathologic CC who desired correction within the date range of our study, the small numbers of patients and samples obtained do not permit statistical analysis. The inherent imprecision of data obtained from small samples does not allow for meaningful correlation.

The number of negative results (31 of 53 samples, 58%) in this pilot study was significant and warrants further consideration. First, it should be noted that negative results are fairly common in studies applying traditional culture results as well as those utilizing newer sampling techniques to evaluate breast implants in human subjects. One explanation for this is that bacterial biofilms may contribute to a proportion of, but not all, diagnosed CCs. It is conceivable that “nonmicrobial” CC is a distinct entity, resulting from noninfectious inflammatory triggers (such as a ruptured silicone implant, radiation therapy, trauma, or other alterations in the immune system). These etiologies could account for some of the negative results seen here and in other studies.

Alternatively, it is possible that our sampling methodology resulted in low microbial yield from specimens that did, in fact, contain biofilm. The threshold for detection is approximately 10^3 CFU/mL, meaning that the negative results in this study may have contained bacteria below this concentration. The opportunity to improve upon our experimental design is one of the benefits of a pilot investigation, and we recognize that sample collection and analysis must be optimized before conducting a large-scale study.

One final hypothesis is that a certain microbial load (or microbial variety) may be necessary to induce capsule formation. Testing during this high-load “capsule formation phase” would yield positive NGS results above this
threshold, as shown in Figure 5. Over time the body may eradicate much of the capsulogenic microbes, leaving behind the thickened capsule. Capsulectomy and testing during this “post cleared” time period would show a thickened capsule, but a lower concentration of microbes than needed for detection by NGS (Figure 5).

Concerns have been raised about whether organisms detected by NGS truly represent the active microbiome because PCR-based techniques cannot distinguish between DNA from viable or dead cells. However, Kaplan et al found that DNA from heat-killed bacterial biofilms was undetectable by PCR just 48 hours after implantation in an animal model. If the time frame for in vivo degradation of bacterial DNA is measured in days following cell death, then this refutes the notion that NGS is an oversensitive tool which identifies both current and historically present microbes. Rather than cataloging the DNA of every organism to ever reside in the breast, positive NGS results instead reflect the active microbiome (or one that is no more than a few days old). It is therefore possible that the contracted capsule signifies the residual effect of a biofilm-host interaction which remains long after the bacteria has been eliminated. This could also account for some of our negative results.

NGS shows great promise as a tool for understanding complex microbial ecosystems, but it is important to recognize its limitations. Although NGS is extremely sensitive, it is not possible to determine whether positive results originate from within the biofilm, or from colonies on the implant surface and surrounding capsular tissue. As noted by Poppler et al, detection of biofilms remains challenging and prohibitively expensive for routine clinical practice. However, the polymicrobial nature of positive results in this study (mean of 8.27 species per patient) is consistent with the “cooperative diversity” of microbiota found within biofilms. Nonetheless, we agree that it is important to corroborate the findings of NGS with imaging and/or culture techniques. Another limitation of NGS is that it does not provide information on the actual amount of bacteria and fungi contained within a specimen (ie, the “microbial load”), but instead reports the “relative abundance” of each microbe as a percentage of the total DNA recovered (Supplemental Figure 1). These limitations underscore the importance of undertaking multimodal analysis to validate and interpret the results of NGS. Nonetheless, we find the positive results of this study to be encouraging, and believe that NGS could play a role in sampling a much larger cross section of patients with CC after cosmetic augmentation, where other, more specific techniques are too costly.

NGS is certainly not the most sophisticated analysis tool available for evaluating microbiomes. Newer methodologies, such as whole-exome sequencing and fluorescent in situ sequencing, may provide more accurate and more sensitive analysis. However, these newer methods are currently prohibitively expensive for large-scale studies. In this preliminary study, our patients were charged the retail price of $199 per specimen for their analysis. Patients were willing to pay for this information, and patients with positive sequencing results were prescribed appropriate antibiotics or antifungals for a 4-week period. We concede that this regimen was not based upon society guidelines, systematic reviews, or published data, but rather clinical judgment, and therefore represents an opportunity for future study. We believe that data from NGS and other diagnostic techniques may ultimately provide clinical guidance for treating or preventing biofilm-related complications.

Finally, we are also beginning to study unaffected (noncontracted) breasts by applying NGS to specimens obtained during breast reduction or mastopexy, in order to understand the breast microbiome in patients without breast implants. We agree with previous researchers that it is of primary importance to understand the bacterial community in clinically normal breasts in order to determine how changes in these communities relate to the formation of CC. However, establishing the “normal” microbiome is a significant challenge; breast reduction patients may have an altered microbiome, as could women undergoing a prophylactic contralateral mastectomy. The best control may be the individual; patients undergoing primary augmentation could be swabbed or have a small tissue specimen taken of the breast pocket prior to implant placement. This would allow evaluation of the shift of an individual’s breast microbiome following breast augmentation. Ultimately, we hope to create a database of breast microbiota from patients with clinically normal breasts, patients with clinically benign breast implants, and patients with CC. We believe this will help further our understanding of the etiology of CC and provide insight into its treatment and prevention.

CONCLUSIONS

Accurately categorizing the microbiome of the contracted breast capsule is a critical step towards understanding the role that alterations of the breast microbiome play in the development of CC. We speculate that dysbiosis of the breast microbiome may have local and systemic consequences on the immune and inflammatory response, and could potentially underlie the formation of CC, ALCL, or breast implant–associated illness. By understanding and ultimately controlling changes in the breast microbiome after breast augmentation, it may be possible to reduce, prevent, or treat these complications.

Supplementary Material
This article contains supplementary material located online at www.aestheticsurgeryjournal.com.
Disclosures
The authors declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

Funding
The authors received no financial support for the research, authorship, and publication of this article.

REFERENCES
29. Khot PD, Ko DL, Fredricks DN. Sequencing and analysis of fungal rRNA operons for development of


