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

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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,[1] 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.[2-4] 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.[2,5-11] Review of this literature indicates that the most common bacterial species isolated are *Staphylococcus epidermidis*, *Cutibacterium acnes*, and coagulase-negative staphylococci (Figure 1).

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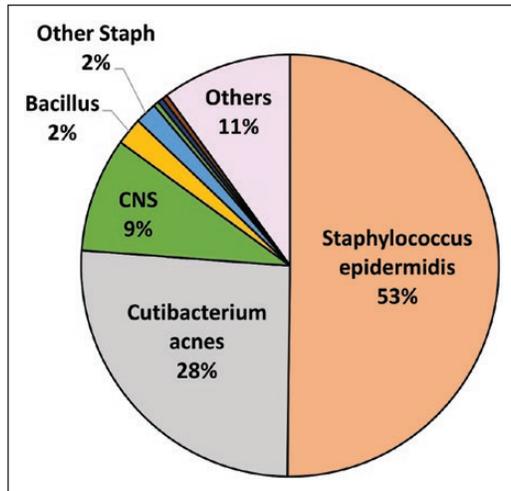
Figure 1. Aggregate species identified by traditional culture methods from human patients with capsular contracture, as reported in the literature.[5-11]

Bacterial contamination of implanted devices has been shown to frequently result in the formation of biofilms.[3] 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[12] for detecting bacteria present in biofilms, and for adequately characterizing their constituent species. More recent studies have employed techniques such as sonication,[10] fluorescent in situ hybridization,[3] and quantitative polymerase chain reaction (PCR),[13] 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.[3,13-16]

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 rRNA gene is amplified and sequenced. The 2 regions of interest for detection of bacterial and fungal species are the 16S and internal transcribed spacer sequences (ITS2), respectively.[17] These sequences are both highly conserved and variable regions of the rRNA gene, allowing for specific microbial identification.[18] 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.[19] This experimental approach was recently used to study the breast microbiome in patients with anaplastic large-cell lymphoma (ALCL).[20] 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,[21-23] otolaryngology,[24] urology,[25] and wound care.[26,27]

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 a 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.[28,29] 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
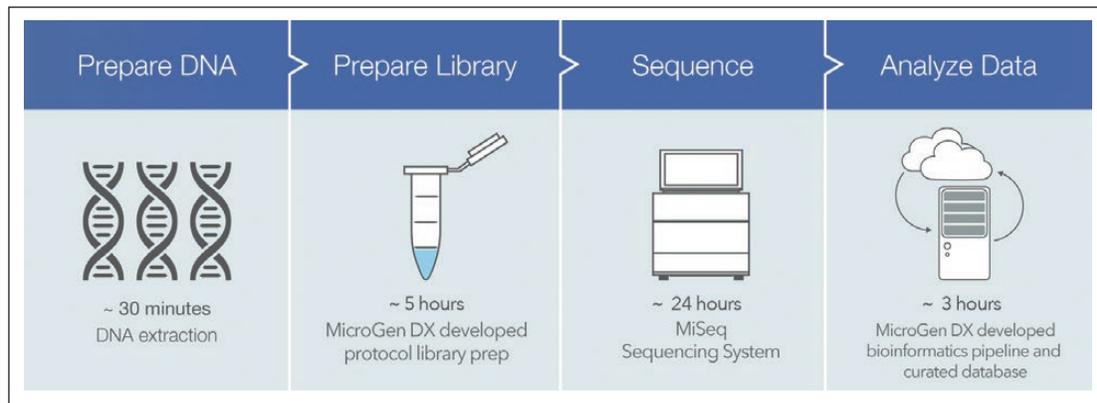
Figure 2. Overview of the MicroGenDX next generation sequencing process (adapted from Illumina[18]).

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;[30,31] 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).

DISCUSSION

Bacteria have been shown to play a causative role in CC,[2,3,5-9,11,32] but the precise way in which this occurs remains obscure. The relation between the human body and its resident bacteria has been the focus of scientific study across a range of disciplines.[20,33-39] It is now recognized that there is a community of bacteria in almost every part of the body—a "microbiome"—which likely serves a variety of helpful functions depending on the body location, the bacterial species present, and their interaction with the host immune system. Perhaps the best-known example of this is the skin flora, which helps to maintain cutaneous barrier function, regulate inflammation, prevent infection, and promote wound healing.[40-42]

The microbiome of the human breast was first posited[43] and studied[36] by Urbaniak et al, who used 16S rRNA sequencing to study breast tissue obtained from 81 Canadian and Irish women undergoing lumpectomy or mastectomy for benign or malignant breast lesions.[36] None of these patients had clinical signs or symptoms of breast infection, yet bacteria were recovered from all samples. The most abundant bacteria they found included Bacillus, Acinetobacter, Enterobacteriaceae, Staphylococcus, *Listeria welshimeri*, and Proprionibacterium. The authors concluded that their research "shows that breast tissue is not sterile but contains a diverse community of bacteria, adding to the literature that body sites once believed to be sterile do indeed have an endogenous microbiome."[36]
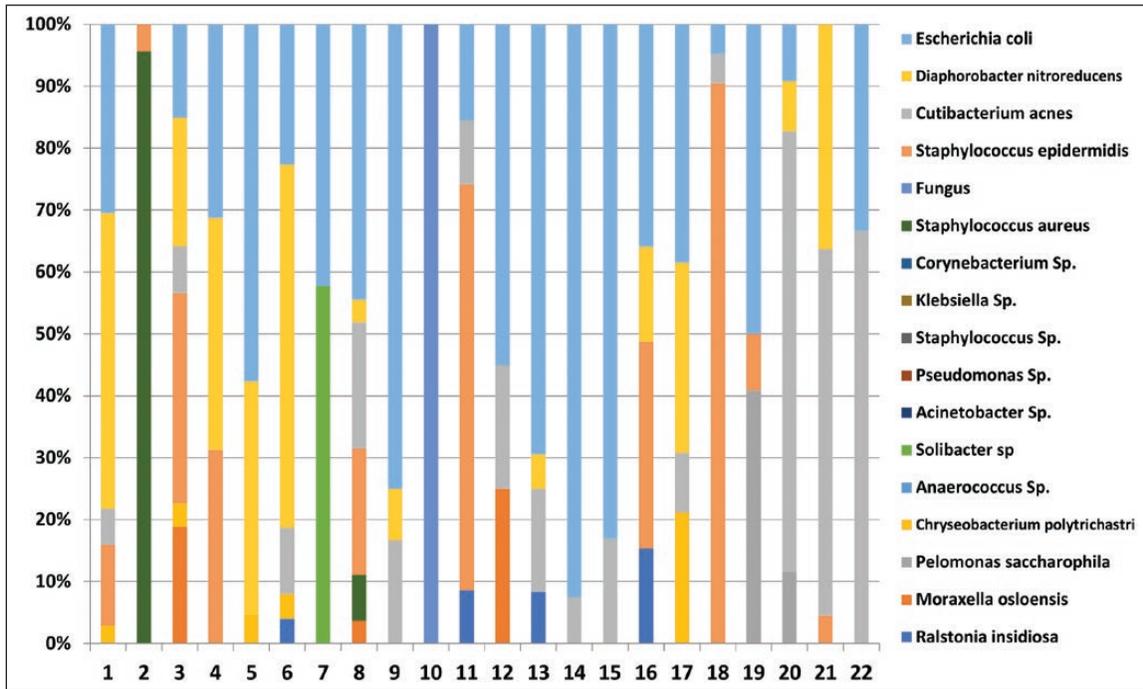
Figure 3. Microbes identified by next generation sequencing, from positive samples only (threshold for inclusion set at >5% for at least 1 sample).
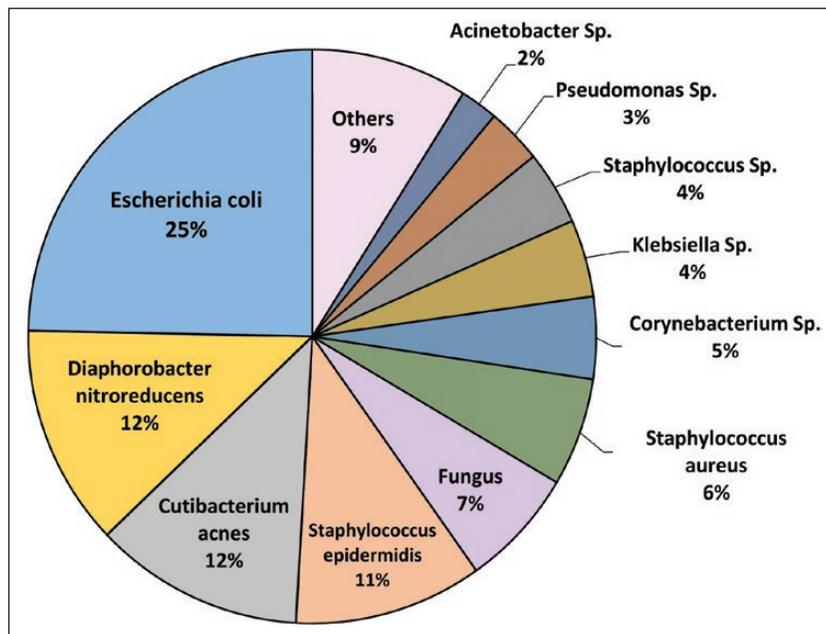

Figure 4. Cumulative microbes identified by next generation sequencing (threshold set at >5%).

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,[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
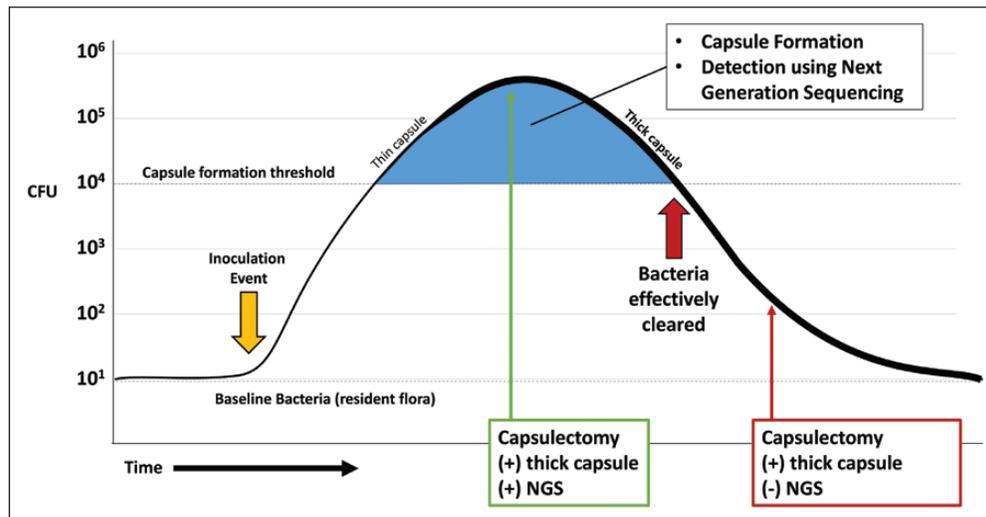
Figure 5. Bacterial clearance hypothesis for negative next generation sequencing results, despite the presence of a microbially mediated capsule.

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[11] 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[6-9,44] as well as those utilizing newer sampling techniques[2,10,45,46] 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.[45] 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[46]). 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.[47] However, Kaplan et al[48] 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).[48] 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,[45] detection of biofilms remains challenging[3] 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"[27] of microbiota found within biofilms.[3,23,49] Nonetheless, we agree that it is important to corroborate the findings of NGS with imaging and/or culture techniques.[50] 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.[45] 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[2] 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

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REFERENCES

1. The American Society for Aesthetic Plastic Surgery's cosmetic surgery national data bank: statistics 2018. *Aesthet Surg J.* 2019;39(Suppl_4):1-27.
2. Walker JN, Pinkner CL, Pinkner JS, Hultgren SJ, Myckatyn TM. The detection of bacteria and matrix proteins on clinically benign and pathologic implants. *Plast Reconstr Surg Glob Open.* 2019;7(2):e2037.
3. Deva AK, Adams WP Jr, Vickery K. The role of bacterial biofilms in device-associated infection. *Plast Reconstr Surg.* 2013;132(5):1319-1328.
4. Tamboto H, Vickery K, Deva AK. Subclinical (biofilm) infection causes capsular contracture in a porcine model following augmentation mammaplasty. *Plast Reconstr Surg.* 2010;126(3):835-842.
5. Virden CP, Dobke MK, Stein P, Parsons CL, Frank DH. Subclinical infection of the silicone breast implant surface as a possible cause of capsular contracture. *Aesthetic Plast Surg.* 1992;16(2):173-179.
6. Dobke MK, Svahn JK, Vastine VL, Landon BN, Stein PC, Parsons CL. Characterization of microbial presence at the surface of silicone mammary implants. *Ann Plast Surg.* 1995;34(6):563-569; disscusion 570.
7. Ahn CY, Ko CY, Wagar EA, Wong RS, Shaw WW. Microbial evaluation: 139 implants removed from symptomatic patients. *Plast Reconstr Surg.* 1996;98(7):1225-1229.
8. Pajkos A, Deva AK, Vickery K, Cope C, Chang L, Cossart YE. Detection of subclinical infection in significant breast implant capsules. *Plast Reconstr Surg.* 2003;111(5):1605-1611.
9. Dancey A, Nassimizadeh A, Levick P. Capsular contracture—what are the risk factors? A 14 year series of 1400 consecutive augmentations. *J Plast Reconstr Aesthet Surg.* 2012;65(2):213-218.
10. Rieger UM, Mesina J, Kalbermatten DF, et al. Bacterial biofilms and capsular contracture in patients with breast implants. *Br J Surg.* 2013;100(6):768-774.
11. Galdiero M, Larocca F, Iovene MR, et al. Microbial evaluation in capsular contracture of breast implants. *Plast Reconstr Surg.* 2018;141(1):23-30.
12. Costerton JW, DeMeo P. Discussion: The role of biofilms: are we hitting the right target? *Plast Reconstr Surg.* 2011;127(Suppl. 1S):36-37.
13. Stoodley P, Kathju S, Hu FZ, et al. Molecular and imaging techniques for bacterial biofilms in joint arthroplasty infections. *Clin Orthop Relat Res.* 2005;(437):31-40.
14. Gomez E, Cazanave C, Cunningham SA, et al. Prosthetic joint infection diagnosis using broad-range PCR of biofilms dislodged from knee and hip arthroplasty surfaces using sonication. *J Clin Microbiol.* 2012;50(11):3501-3508.
15. Tunney MM, Patrick S, Curran MD, et al. Detection of prosthetic hip infection at revision arthroplasty by immunofluorescence microscopy and PCR amplification of the bacterial 16S rRNA gene. *J Clin Microbiol.* 1999;37(10):3281-3290.
16. Gemmel F, Van den Wyngaert H, Love C, Welling MM, Gemmel P, Palestro CJ. Prosthetic joint infections: radionuclide state-of-the-art imaging. *Eur J Nucl Med Mol Imaging.* 2012;39(5):892-909.
17. Ram JL, Karim AS, Sendler ED, Kato I. Strategy for microbiome analysis using 16S rRNA gene sequence analysis on the Illumina sequencing platform. *Syst Biol Reprod Med.* 2011;57(3):162-170.
18. Illumina. 16S metagenomics sequencing with the iSeq™ 100 System. https://www.illumina.com/content/dam/illumina-marketing/documents/products/appnotes/iseq100-16s-app-note-770-2018-009.pdf. Published 2018. Accessed June 14, 2019.
19. Behjati S, Tarpey PS. What is next generation sequencing? *Arch Dis Child Educ Pract Ed.* 2013;98(6):236-238.
20. Walker JN, Hanson BM, Pinkner CL, et al. Insights into the microbiome of breast implants and periprosthetic tissue in breast implant-associated anaplastic large cell lymphoma. *Sci Rep.* 2019;9(1):10393.
21. Williams DL, Bloebaum RD. Biofilm-related periprosthetic joint infections. In: Springer BD, Parvizi J, eds. *Periprosthetic Joint Infection of the Hip and Knee*. New York, NY: Springer New York; 2014:85-96.
22. Tarabichi M, Alvand A, Shohat N, Goswami K, Parvizi J. Diagnosis of *Streptococcus canis* periprosthetic joint infection: the utility of next-generation sequencing. *Arthroplast Today.* 2018;4(1):20-23.
23. Tarabichi M, Shohat N, Goswami K, et al. Diagnosis of periprosthetic joint infection. *J Bone Jt Surg.* 2018;100(2):147-154.
24. Kilty SJ, Desrosiers MY. The role of bacterial biofilms and the pathophysiology of chronic rhinosinusitis. *Curr Allergy Asthma Rep.* 2008;8(3):227-233.
25. McDonald M, Kameh D, Johnson ME, Johansen TEB, Albala D, Mouraviev V. A Head-to-head comparative phase II study of standard urine culture and sensitivity versus DNA next-generation sequencing testing for urinary tract infections. *Rev Urol.* 2017;19(4):213-220.
26. Attinger C, Wolcott R. Clinically addressing biofilm in chronic wounds. *Adv Wound Care (New Rochelle).* 2012;1(3):127-132.
27. Wolcott R. Economic aspects of biofilm-based wound care in diabetic foot ulcers. *J Wound Care.* 2015;24(5):189-190, 192-194.
28. Clarridge JE 3rd. Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clin Microbiol Rev.* 2004;17(4):840-862, table of contents.
29. Khot PD, Ko DL, Fredricks DN. Sequencing and analysis of fungal rRNA operons for development of

broad-range fungal PCR assays. *Appl Environ Microbiol.* 2009;75(6):1559-1565.

30. Tarabichi M, Shohat N, Goswami K, Parvizi J. Can next generation sequencing play a role in detecting pathogens in synovial fluid? *Bone Joint J.* 2018;100-B(2):127-133.

31. Edgar RC. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics.* 2010;26(19):2460-2461.

32. Wixtrom RN, Stutman RL, Burke RM, Mahoney AK, Codner MA. Risk of breast implant bacterial contamination from endogenous breast flora, prevention with nipple shields, and implications for biofilm formation. *Aesthet Surg J.* 2012;32(8):956-963.

33. Holmes CJ, Plichta JK, Gamelli RL, Radek KA. Dynamic role of host stress responses in modulating the cutaneous microbiome: implications for wound healing and infection. *Adv Wound Care (New Rochelle).* 2015;4(1):24-37.

34. Plichta JK, Holmes CJ, Nienhouse V, et al. Cutaneous burn injury modulates urinary antimicrobial peptide responses and the urinary microbiome. *Crit Care Med.* 2017;45(6):e543-e551.

35. Plichta JK, Gao X, Lin H, et al. Cutaneous burn injury promotes shifts in the bacterial microbiome in autologous donor skin: implications for skin grafting outcomes. *Shock.* 2017;48(4):441-448.

36. Urbaniak C, Cummins J, Brackstone M, et al. Microbiota of human breast tissue. *Appl Environ Microbiol.* 2014;80(10):3007-3014.

37. Hieken TJ, Chen J, Hoskin TL, et al. The microbiome of aseptically collected human breast tissue in benign and malignant disease. *Sci Rep.* 2016;6:30751.

38. Chan AA, Bashir M, Rivas MN, et al. Characterization of the microbiome of nipple aspirate fluid of breast cancer survivors. *Sci Rep.* 2016;6:28061.

39. Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. Bacterial community variation in human body habitats across space and time. *Science.* 2009;326(5960):1694-1697.

40. Chehoud C, Rafail S, Tyldsley AS, Seykora JT, Lambris JD, Grice EA. Complement modulates the cutaneous microbiome and inflammatory milieu. *Proc Natl Acad Sci U S A.* 2013;110(37):15061-15066.

41. Grice EA. The skin microbiome: potential for novel diagnostic and therapeutic approaches to cutaneous disease. *Semin Cutan Med Surg.* 2014;33(2):98-103.

42. Grice EA, Segre JA. The skin microbiome. *Nat Rev Microbiol.* 2011;9(4):244-253.

43. Urbaniak C, Burton JP, Reid G. Breast, milk and microbes: a complex relationship that does not end with lactation. *Womens Health (Lond).* 2012;8(4):385-398.

44. Netscher DT, Weizer G, Wigoda P, Walker LE, Thornby J, Bowen D. Clinical relevance of positive breast periprosthetic cultures without overt infection. *Plast Reconstr Surg.* 1995;96(5):1125-1129.

45. Poppler L, Cohen J, Dolen UC, et al. Histologic, molecular, and clinical evaluation of explanted breast prostheses, capsules, and acellular dermal matrices for bacteria. *Aesthet Surg J.* 2015;35(6):653-668.

46. Bachour Y, Poort L, Verweij SP, et al. PCR characterization of microbiota on contracted and non-contracted breast capsules. *Aesthetic Plast Surg.* 2019;43(4):918-926.

47. Young G, Turner S, Davies JK, Sundqvist G, Figdor D. Bacterial DNA persists for extended periods after cell death. *J Endod.* 2007;33(12):1417-1420.

48. Kaplan HB, Miranda JA, Gogola GR, Gomez K, Ambrose CG. Persistence of bacterial DNA in orthopedic infections. *Diagn Microbiol Infect Dis.* 2018;91(2):136-140.

49. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science.* 1999;284(5418):1318-1322.

50. Hu H, Johani K, Almatroudi A, et al. Bacterial biofilm infection detected in breast implant-associated anaplastic large-cell lymphoma. *Plast Reconstr Surg.* 2016;137(6):1659-1669.