Abnormal vaginal microbiome associated with vaginal mesh complications

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Abnormal vaginal microbiome associated with vaginal mesh complications

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Abstract

Aims: To identify differences in the vaginal microbiomes of women after transvaginal mesh (TVM) surgery for pelvic organ prolapse with and without mesh-associated complications.

Methods: Patients with complications were eligible as cases, patients without as controls. DNA was isolated and the V1-2 region of the 16S ribosomal RNA gene was amplified and sequenced. Overall richness was quantified using Chao1. Overall diversity was expressed as Shannon diversity and screened for group differences using analysis of variance. Multivariate differences among groups were evaluated with functions from *R*.

Results: We recruited 14 patients after mesh exposure, 5 after contraction, and 21 as controls. The average number of operational taxonomic unit was 74.79 (SD ± 63.91) for controls, 57.13 (SD ± 58.74) after exposures, and 92.42 (SD ± 50.01) after contractions. Total 89.6% of bacteria in controls, 86.4% in previous exposures, and 81.3% in contractions were classified as either *Firmicutes*, *Proteobacteria*, or *Actinobacteria* ($P < .001$). *Veillonella* spp. was more abundant in patients after contraction ($P = .045$). The individual microbiomes varied, and we did not detect any significant differences in richness but a trend towards higher diversity with complications.

Conclusions: The presence of *Veillonella* spp. could be associated with mesh contraction. Our study did not identify vaginal microbiotic dysbiosis as a factor associated with exposure. Larger cohort studies would be needed to distinguish the vaginal microbiome of women predisposed to mesh-related complications for targeted phenotyping of patients who could benefit from TVM surgery.

KEYWORDS
contraction, exposure, microbiome, transvaginal mesh

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1 | INTRODUCTION

The use of polypropylene mesh for the treatment of pelvic organ prolapse (POP) is controversial. The initial development of vaginally placed mesh was spurred by reported reoperation rates following native tissue repair of POP being as high as 30%, although more recent publications have reported much lower rates and differences between same-site recurrences and different-site recurrences are not always properly addressed.[1-3] The main rationale for mesh use was the potential for improved anatomical restoration of pelvic floor structures and a hypothetical reduction in prolapse recurrence compared with native tissue POP surgery.

Poor biological compatibility of available mesh materials contributes to a significant risk of serious complications including vaginal extrusion (passage gradually out of a body structure or tissue), exposure (displaying, revealing, exhibiting, or making accessible), and mesh contraction (shrinkage or reduction in size).[2,4,5] Many of these complications require further surgical intervention.[6] In April 2019, the FDA ordered manufacturers transvaginal meshes (TVMs) for POP repair to stop selling all devices.[7] Before this, the use of synthetic material in vaginal POP surgery had already dramatically decreased.[8] In countries like France, with strong traditions of vaginal surgical techniques, mesh kits are still used with comparably low complication rates and acceptable outcome and patient satisfaction.[9,10] It is not clear why in most women some of these vaginal mesh kits do not cause any problems whereas in others it leads to severe complications which can lead to the mesh needing to be removed.

Among the few studies which have analyzed the risk factors for mesh exposure or contraction, one publication clearly identified tobacco use as a risk factor.[11] Other potential risk factors are diabetes mellitus, obesity, age, associated total hysterectomy, and surgical experience. However, little is known about the exact role and the reaction of the host during and after mesh surgery. The inflammatory response, as well as the microbiological vaginal environment, may be determining factors in the outcome after mesh implantation.[12] The microbiome is the totality of microbes, their genetic elements (genome), and environmental interactions, the term microbiota refers to the microorganisms themselves.[13] It is estimated that trillions of microorganisms inhabit the average healthy human body in a symbiotic existence with human cells; most of these microorganisms are of low virulence, others may be highly pathogenic. Recent publications about the vaginal microbiome have led to better understanding of vaginal dysbiosis or imbalance.[14,15] Culture-dependent microbiological procedures historically represent the standard for the assessment of vaginal microbiota. However, with such a technique, a majority of species remains undetectable, providing only a partial picture of the overall microbiome. With the analysis of 16S ribosomal RNA (rRNA) gene sequences,[16] the limitations of traditional culture techniques can be overcome by providing species-level classification [15] of all bacteria in the vagina. These analyses can increase not only our understanding of the vaginal ecosystem in general, but also the complex interrelation between microbiota and their host.[17]

Previous work demonstrates that braided suture for cervical cerclage induces a dysbiotic shift in the vaginal microbiome characterized by reduced *Lactobacillus* spp. and enrichment of pathobionts, whereas monofilament cervical cerclage suture had comparatively minimal impact on the interaction with the host.[18] This led to our hypothesis of a principle interaction of foreign material with the vaginal after mesh placement and its association with potential complications. In our case-control study we aimed to identify the vaginal microbiome of women after TVM surgery for POP with and without mesh-associated complications.

2 | MATERIALS AND METHODS

2.1 | Study population

This study was conducted at a tertiary referral center was approved by the local ethics committee. We searched the operations register of the computerized medical records established on 1 October, 2006. Patients who had a record of TVM-related complications were eligible as cases, patients who were treated with TVM without mesh-related complications were eligible as controls during routine follow-up visits. We recorded demographics (age, body mass index [BMI], hormonal status, and smoking status), type of the primary mesh repair, indication for reoperation (type of complication and symptoms), and details of the reoperation (time from primary repair to reoperation, type of surgery performed, and perioperative complications). We invited patients after mesh complications to a clinic visit and we performed routine vaginal examination and obtained vaginal specimens which were placed in commercially available DNA protectant tubes (Copan® Swabs; Copan Diagnostics, Murrieta, CA).

2.2 | DNA extraction

Total 16S rRNA sequencing DNA was isolated in a laminar flow hood to avoid contamination. Genomic DNA was extracted using the EZ1 Advanced XL® (Qiagen Inc, Valencia, CA) with the EZ1® DNA Tissue Kit (Qiagen Inc) following manufacturer recommendations.

2.3 | DNA sequencing

Samples were amplified for sequencing variable regions 1 to 2 (V1-V2) at RTL Genomics (Lubbock, TX) in process consisting of two steps. The forward primer was constructed with the Illumina i5 sequencing. The reverse primer was constructed with the Illumina i7 sequencing primer. The laboratory performed amplifications with HotStar Taq Master Mix (Qiagen Inc) in reactions on ABI Veriti thermal cycler (Applied Biosytems, Carlsbad, CA). First stage amplification products were added to a second polymerase chain reaction (Nextera PCR primers; Illumina, Inc, San Diego, CA) based on qualitatively determined concentrations. EGel (Life Technologies, Grand Island, NY) were used to visualize amplification products. They were pooled equimolar and selected in two rounds using SPRIselect (Beckman Coulter, Indianapolis, IN). Size selected pools were then run on a Fragment Analyzer (Advanced Analytical, Ankeny, IA) to assess the size distribution, quantified using the Qubit 2.0 fluorometer (Life Technologies), and loaded on an Illumina MiSeq (Illumina, Inc) and sequenced. Sequence data were processed for denoising and chimera checking using a research and testing pipeline that is described together with details about used primers in http://www.researchandtesting.com/docs/Data_Analysis_Methodology.pdf.

2.4 | Data analysis

All analyses were conducted in R statistical software and all figures were created with the ggplot2 package.[19,20] We compared demographic characteristics between the groups. To verify that sequencing effort was sufficient to characterize bacterial communities, rarefaction curves were generated by subsampling community matrices between 500 and 20 000 reads at a step size of 500 reads and the mean of 10 iterations at each step was calculated. Alpha diversity metrics included observed operational taxonomic unit (OTU—an operational definition used to classify groups of closely related individuals, simply the group of organisms currently being studied, Chao1 richness, Chao1 richness, and Shannon diversity (both indices used to summarize the diversity of a population in which each member belongs to a unique group). They were calculated using the phyloseq package.[21] Differences in alpha diversity metrics were assessed by analysis of variance (ANOVA). Bar plots were generated using OTU relative abundances. We did not scale biological read counts because rarefying biological count data reduces the reproducibility of results and is an opportunity to introduce bias.[21] Weighted and unweighted UniFrac distances (accessing the abundance and a phylogenetic tree data within an experiment-level object) were calculated using the phyloseq package. Multivariate differences among groups were evaluated with "permutational multivariate analysis of variance using distance matrices," function ADONIS (a function for the analysis and partitioning sums of squares using semimetric and metric distance matrices) in the vegan package.[22] For ADONIS (default parameter: 999 permutations), distances among samples first were calculated using weighted and unweighted UniFrac. Then, principal coordinates analyses (PCoA) and heatmaps were constructed from UniFrac distance matrices.

2.5 | Study outcomes

Study outcomes were the composition of bacterial communities in women after TVM surgery with (cases) and without (controls) mesh-related complications identified by DNA sequencing using the described 16S rRNA techniques. The cases were defined as mesh exposure or mesh contraction.

3 | RESULTS

All patients had transvaginal placement of a monofilament macroporous polypropylene mesh material for primary prolapse repair. None had local estrogen treatment before the surgery. A total of 19 out of 49 contacted patients identified with TVM-related complications agreed to participate and were recruited as cases, 14 with mesh exposure, and 5 with mesh contraction. Local estrogen treatment was initiated in each patient at the time of the complication was discovered and no patient was under treatment during sampling. Mean interval between sampling and complication discovery was 53.4 months. Partial resection of the mesh was carried out in the event of conservative treatment failure, which was the case for all patients in the exposure group with a mean interval between the intitial operation and reoperation of 17.4 months. A total of 21 patients were approached during routine follow-up after TVM surgery without any mesh related complication as controls.

The women in both groups were similar in BMI, history of prolapse surgery, menopausal status, and preoperative hormonal replacement treatment (Table 1). ANOVA showed no significant differences for groups and residuals for Shannon diversity, Chao1 richness, and observed number of OTUs. Total DNA was extracted from all vaginal samples to quantify bacterial DNA and to characterize the microbial community of the vaginal by16S rRNA gene sequencing. The amount of bacterial DNA varied from 4 to 38 ng/μL, with no difference between cases and controls (mean, 11.18 ng/μL; SD,

TABLE 1 Demographics of cases and controls

	Controls	Mesh erosion		Mesh retraction	
	n = 20	n = 14	*P* value	n = 5	*P* value
Age (mean, ±SD)[a]	64.20 (±8.98)	56.67 (±9.29)	.021	57.50 (±8.98)	.196
Body mass index (mean, ±SD)[a]	24.90 (±2.66)	26.10 (±4.31)	.310	24.59 (±3.24)	.856
Hormonal replacement treatment before surgery (%)[b]	4.80%	0%	.391	0%	.391
Diabetes (%)[b]	4.80%	13.30%	.359	0%	.656
Tobacco use (%)[b]	4.80%	0%	.656	0%	.656
Prior hysterectomy (%)[b]	14.30%	20%	.650	50%	.102
Time between sampling and primary surgery in months (mean, ±SD)[a]	24.76 (±23.50)	62.13 (±37.85)	.001	107.00 (±30.00)	<.001
DNA sample concentration in/mL (mean, ±SD)[a]	11.18 (±7.08)	13.28 (±8.98)	.435	14.02 (±5.17)	.409

[a]Calculated with the independent *t* test.
[b]Calculated with the χ^2 test.

±7.08 in 21 control vaginal samples; mean, 13.28 ng/μL; SD, ±8.89; $P = .112$ in 15 exposure case vaginal samples; mean, 14.02 ng/μL; SD, ±5.17 in 5 contraction case vaginal samples; $P = .732$). The average number of OTUs per sample was 74.79 (SD, ±63.91 for controls), 57.13 (SD, ±58.74 for exposure cases), and 92.42 (SD, ±50.01 for contraction cases). We identified these OTUs as belonging to 30 different genera from all vaginal specimens. At the phyla level, 89.6% of the bacteria in the control group, 86.4% of the bacteria in the exposure group, and 81.3% of the bacteria in the contraction group were respectively classified as either *Firmicutes*, *Proteobacteria* or *Actinobacteria*. The average relative abundances for cases and controls are listed in Table 2. The most abundantly detected genus in the control group was *Lactobacillus* spp. (29.2%), followed by *Streptococcus* spp. (11.19%) and *Staphylococcus* spp. (10.45%). The most abundantly detected genus in the exposure group was also *Lactobacillus* spp. (47.89%), followed by *Gardnerella* spp. (9.80%) and *Staphylococcus* spp. (6.78%). The most abundantly detected genus in the contraction group was again *Lactobacillus* spp. (17.87%), followed by *Staphylococcus* spp. (13.95%) and *Gardnerella* spp. (12.15%) (Figure 1).

The composition of each individual's vaginal microbiome varied greatly, with anywhere from 8 to 225 different bacterial genera detected per sample in the control group, 5 to 190 in the exposure group, and 16 to 160 in the contraction group. Total 14 controls, 11 exposure cases, and 2 contraction cases had a microbiota that was dominated by a single bacterial genus (a genus dominating at least 45% of the microbiome sample), while all other samples were diverse. The number of different bacterial species in each individual's vaginal microbiome also varied widely (Figure 2).

We did not detect any significant differences in alpha diversity measures, that are the established measures of species richness and distribution within a sample (as measured by the Chao 1 or Shannon index between cases and controls (Figure 3A and 3B).

The ADONIS and the PCoA did not detect significant multivariate differences among groups.

4 | COMMENTS

In this pilot case-control study designed to assess the vaginal microbiome in a clinically well-defined patient population, we have characterized the vaginal microbiome of women with and without mesh-related complications (exposure and contraction) after TVM surgery for POP using Illumina MiSeq sequencing of the bacterial 16S rRNA gene. We performed a thorough literature review using the following search terms: "mesh

TABLE 2 Mean relative abundance of bacteria (as percent) in cases and controls; reported by species and family

	Control n = 21	Erosion n = 15	Retraction n = 5	*P* value
Firmicutes				
Lactobacillus crispatus	15.36%	13.68%	4.79%	.375
Lactobacillus delbrueckii	1.40%	7.14%	0.00%	.825
Lactobacillus gasseri	4.78%	10.23%	11.63%	.307
Lactobacillus iners	4.94%	10.97%	1.44%	.269
Lactobacillus jensenii	2.61%	5.87%	0.01%	.202
Total *Lactobacillus spp.*	29.09%	47.89%	17.87%	<.001[a]
Anaerococcus spp.	0.68%	0.40%	0.10%	.522
Enterococcus spp.	1.20%	0.01%	0.07%	.608
Finegoldia spp.	1.56%	0.69%	2.07%	.434
Peptoniphilus spp.	0.42%	0.48%	0.06%	.854
Veillonella spp.	0.07%	0.03%	2.46%	.025
Clostridiales spp.	0.56%	0.06%	0.42%	.600
Firmicutes spp.	2.46%	1.16%	0.78%	.366
Enterococcus faecalis	1.13%	0.00%	0.03%	.448
Staphylococcus aureus	10.45%	6.78%	13.95%	.619
Streptococcus anginosus	5.85%	2.18%	1.26%	.299
Streptococcus pasteurianus	2.65%	0.00%	0.00%	.634
Streptococcus spp.	2.70%	0.12%	0.20%	.661
Total Firmicutes	58.83%	59.82%	39.26%	<.001[a]
Proteobacteria				
Enterobacter spp.	0.07%	0.62%	2.36%	.045
Acinetobacter spp.	4.35%	2.99%	7.05%	.032
Escherichia coli	5.30%	0.27%	4.27%	.699
Morganella morganii	0.58%	1.27%	0.05%	.766
Proteus mirabilis	2.84%	1.88%	0.01%	.317
Pseudomonas aeruginosa	1.90%	0.00%	0.03%	.545
Pseudomonas spp.	0.30%	1.62%	0.79%	.500
Total Proteobacteria	15.34%	8.66%	14.56%	<.001[a]
Actinobacteria				
Actinomyces spp.	0.45%	0.36%	0.05%	.770
Actinotignum spp.	0.56%	0.24%	0.12%	.711
Alloscardovia spp.	0.61%	0.20%	0.00%	.595
Actinobaculum massiliense	1.01%	0.22%	0.46%	.726
Bifidobacterium breve	0.40%	3.67%	8.40%	.709
Bifidobacterium dentium	0.00%	1.19%	0.00%	.697
Bifidobacterium longum	4.04%	0.00%	0.03%	.292
Corynebacterium spp.	0.89%	0.05%	1.74%	.568
Gardnerella vaginalis	5.34%	9.80%	12.15%	.797
Propionibacterium acnes	1.51%	1.60%	4.47%	.650
Propionimicrobium lymphophilum	0.63%	0.54%	0.00%	.523
Total Actinobacteria	15.45%	17.88%	27.43%	<.001[a]
Other				
Prevotella timonensis	1.33%	0.63%	0.06%	.629

(Continues)

TABLE 2 (Continued)

	Control n = 21	Erosion n = 15	Retraction n = 5	*P* value
Fusobacterium nucleatum	0.14%	0.06%	3.95%	.728
Other	8.91%	12.95%	14.74%	···
Total other	10.38%	13.64%	18.75%	···

[a]Calculated with Pearson χ^2.

complications" OR "erosion" OR "exposure" OR "extrusion" OR "contraction" OR "retraction" AND "vaginal microbiome" and could not find any relevant publication. This study is the first of its kind aiming for the identification of microbial DNA in the vagina after mesh augmentation surgery. Our results demonstrate significant differences among cases and controls concerning the vaginal microbiome. Our findings revealed that after mesh implantation, the vagina harbors a polymicrobial composition with substantial individual variability in diversity and richness from each sample. We were able to detect bacteria from all of our samples, which is consistent with the findings of other studies.[17]

Previous analyses of the vaginal microbiome have identified *Lactobacillus* spp. as the predominant genus with age-dependent differences in abundance. *Lactobacilli* inhibit the growth of other microorganisms by competitive exclusion through adherence to vaginal epithelial cells and production of antimicrobials.[14] Some studies reported that *Lactobacillus* spp. was dominant in 83% of precompared with only 54% of postmenopausal women.[14,23] Our results confirmed results from previous studies where one or two predominant *Lactobacillus* species were isolated, the most common being of these included *Lactobacillus crispatus*, *Lactobacillus iners*, *Lactobacillus jensenii*, and *Lactobacillus gasseri*.[24,25] We found a modified distribution of these species in favour of *Lactobacillus gasseri* in the contraction group without statistical significance though (Table 2). However, recent studies have found that in a certain proportion of healthy women of reproductive age, other species than *Lactobacillus* spp. are predominant in the vaginal microbiome. These earlier data suggest the existence of multiple "vagitype" clusters with no evidence so far whether or not these microbial profiles are causal indicators for health or disease.[14] Moreover, numerous vaginally

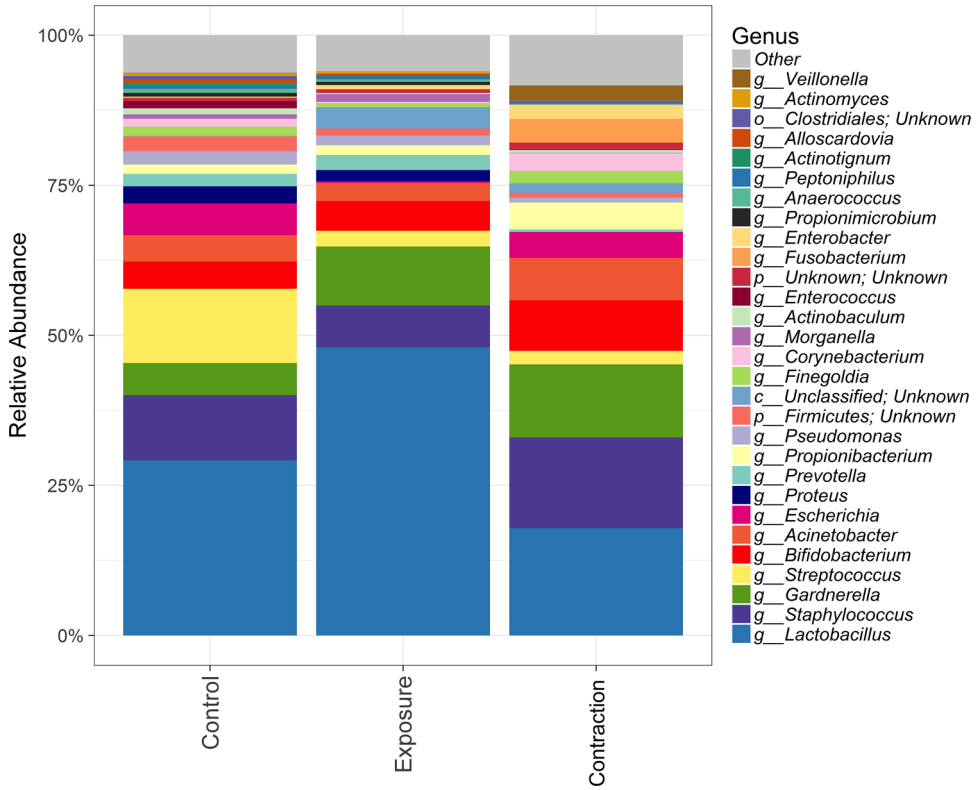

FIGURE 1 Relative abundance of the top 30 genera in all groups

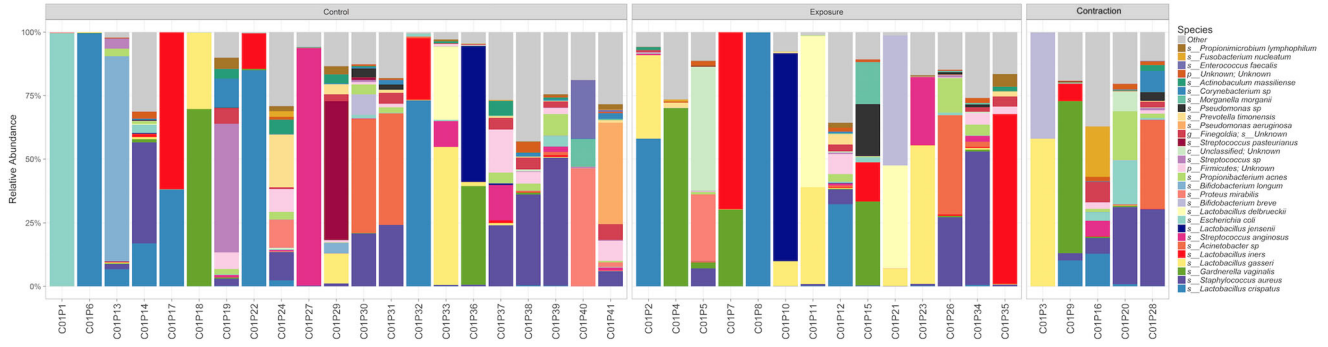

FIGURE 2 Relative abundance of the top 30 species in all samples, faceted by group

relevant bacterial species have yet to be characterized. Beside estrogen, environmental factors, such as smoking, douching, and some sexual practices can decrease vaginal *Lactobacilli* levels.[26] Estrogen deficiency during menopause often leads to a depletion in *Lactobacilli* whereas estrogen substitution generally leads to a reestablishment of the same bacteria.[27,28] Throughout all our study groups, a large majority of women were nonsmokers. Only 30% (6 out of 20) of samples in the control group, 57.14% (8 out of 14) of samples in the exposure group, and 20% (1 out of 5) of samples in the contraction group demonstrated a dominance of *Lactobacillus* spp. This may be an indicator for the impairment by the mere presence of prosthetic material caused on the vaginal microbiome and a positive result of the consequent continuous estrogen treatment in patients with diagnosed mesh exposure. The latter could also explain the higher relative abundance of the phyla *Firmicutes* (including the genus *Lactobacillus*) in the exposure group. The higher abundance of *Actinobacter* in the contraction group compared with controls may represent a risk factor for the occurrence of this mesh related complication although these findings again lack statistical significance.

Overall, we did not detect any statistically significant differences in the diversity or richness measures between cases and controls at the genera and species level, except for the species *Enterobacter spp, Acinetobacter spp.*, and *Veillonella* spp., which were more abundant in patients after mesh contraction ($P = .045$, $P = .032$, and $P = .025$, respectively). Other studies have shown that *Veillonella* spp. are associated with bacterial vaginosis, we believe that they could represent a risk factor for mesh contraction group.[29,30] Moreover, we detected a trend of an association between higher bacterial diversity and mesh complications when compared with controls, although these findings were not statistically significant. According to the literature, the rate of mesh exposure when placed via the vaginal route varies from 5% to 30%.[31] Surgical treatment of mesh-related complications accounted for 7% of TVM procedures in our center.[32] Risk factors reported to be associated with complications included: operative technique, surgeon experience, previous prolapse repair, concomitant hysterectomy, inverted T colpotomy during concomitant hysterectomy, total vaginal mesh repair, mesh properties, younger age at the time of surgery, sexual activity, and smoking.[33] Our study was not able to identify vaginal microbiotic dysbiosis as a factor associated with mesh exposure. It rather confirmed that the microbiota of women after vaginal mesh surgery are comparable whether or not an exposure had occurred. The recommendations for mesh complication management involve two steps: First, local healing treatment can be further enhanced with an antiseptic or estrogens. Second, partial resection of the mesh may be required if local treatment proves inadequate. We followed these recommendations for the patients included in our cohort and we can conclude, that our management of mesh related complications does not seem to have had a significant impact on the vaginal microbiome.

Although this study is the first of its kind to investigate the relationship between mesh complication and the vaginal microbiome, there are some limitations. Its retrospective design, the high number of patients lost to follow-up, the differences in time of follow-up represent significant sources of potential bias. Moreover, we cannot exclude the possibility that some patients in the control group will go on to develop complications and/or require reoperation. Another limitation is certainly that the microbiome was impacted by different antibiotic exposure between cases and controls. With a larger clinically well-characterized cohort and a prospective collection of DNA preoperatively and at predefined points in time postoperatively, we may well identify a subset of patients for whom the diversity, presence, or absence of particular bacteria influences the outcome of TVM surgery.

In conclusion, *Veillonella* spp., *Actinobacteria* spp. may contribute to the development of mesh related complications, such as exposure, extrusion or contraction.[30] Future research should include a focus on the state of the vaginal microbiome before surgery and whether or not the predominance of some organisms is expected, or not, after surgery. Large, cross-sectional studies defining the vaginal microbiome

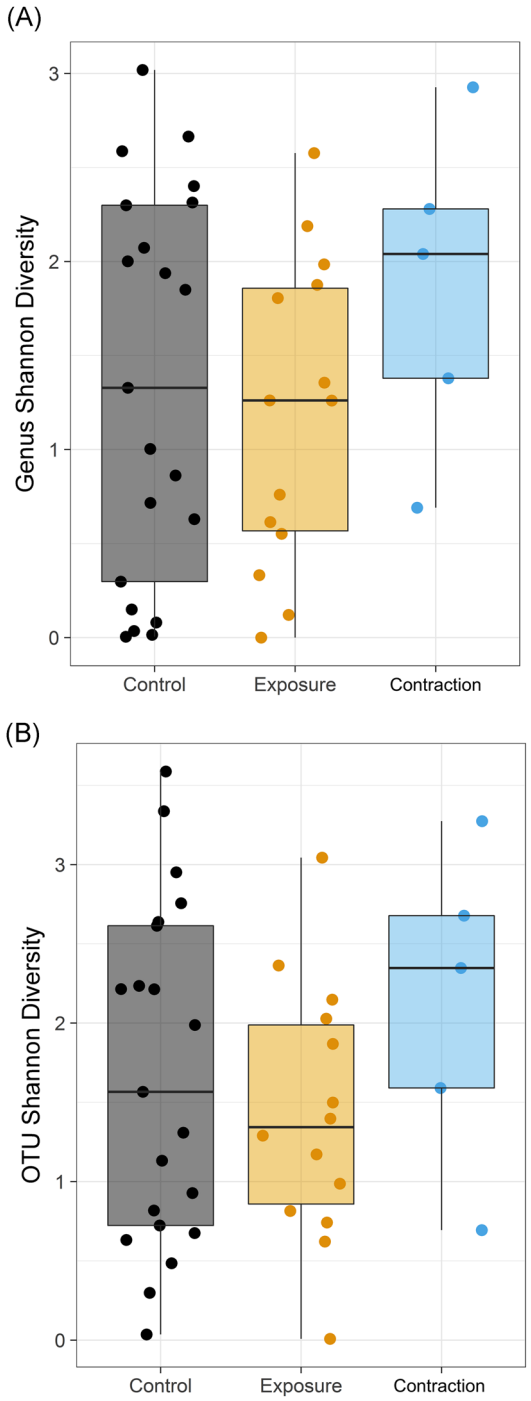

FIGURE 3 Boxplots showing Shannon diversity distribution at genus (A) and OTU (B) level. OTU, operational taxonomic unit

preoperatively and postoperatively would be needed to distinguish vaginal microbiome profiles of healthy, asymptomatic women from women predisposing to mesh-related complications. With targeted phenotyping of patients, it may be possible to identify those patients who would benefit from the advantages of TVM augmented POP surgery with an associated low-risk profile.

CONFLICT OF INTERESTS

Renaud de Tayrac is a consultant for Boston Scientific and Coloplast. The other authors do not report any conflict of interest.

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