PIPELLE AND ENDOBRUSH CATHETERS DO NOT PREVENT CONTAMINATION OF ENDOMETRIAL SAMPLES BY CERVICAL MICROBIOTA

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The risk of contamination by cervical microbiota during transcervical sampling represents a fundamental methodological challenge in endometrial microbiome research. This study aimed to experimentally evaluate the efficacy of Pipelle and Endobrush endometrial sampling catheters in preventing this contamination. An *in vitro* cervix model with two anatomically distinct canal types (cylindrical and slit-like) was developed and filled with a synthetic cervical mucus containing a defined quantity of bacterial DNA. After catheter passage through the model cervical canal, a simulated ‘endometrial’ sample (sterile air) was collected and subjected to quantitative PCR analysis. Both catheter types facilitated substantial transfer of bacterial DNA from the cervical mucus into the endometrial sample. The median transfer of total bacterial DNA was 81.6% [54.4–107] for the Pipelle catheter and 29.8% [14.8–56.3] for the Endobrush catheter (p = 0.009), indicating that neither device provided sufficient protection for reliable characterization of the endometrial microbiota. Catheter efficacy was further dependent on cervical canal morphology and the specific microbial group analyzed. These findings demonstrate that transcervical sampling with either catheter type introduces a significant and variable degree of cervical contamination, thereby confounding the interpretation of endometrial microbiota data and underscoring the need to conceptualize and study a combined cervico-endometrial microbiota.

Keywords: endometrial microbiota, cervical canal, contamination, Pipelle catheter, Endobrush catheter, real-time PCR, molecular diagnostics

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Compliance with ethical standards: the study was approved by the Ethics Committee of Ural State Medical University (Protocol № 3 dated June 20, 2025).

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УРОГЕНИТАЛЬНЫЕ ЗОНДЫ ТИПА ПАЙПЕЛЬ-С НЕ ПРЕДОТВРАЩАЮТ КОНТАМИНАЦИЮ ЭНДОМЕТРИАЛЬНЫХ ОБРАЗЦОВ ЦЕРВИКАЛЬНОЙ МИКРОБИОТОЙ

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Существенным методологическим ограничением в изучении микробиома эндометрия выступает риск контаминации проб цервикальной микробиотой в ходе трансцервикального взятия материала. Целью работы было экспериментально оценить эффективность урогенитальных зондов типов Пайпель-С1 (Пайпель-биопсия) и Пайпель-С2 (Эндобраш) в предотвращении данного вида контаминации. Для этого была разработана *in vitro* модель шейки матки двух типов (с цилиндрическим и щелевидным каналом), заполненная модельной цервикальной слизью с известным количеством бактериальной ДНК. После прохождения зондами через модельный цервикальный канал выполняли забор «эндометриальной» пробы (стерильный воздух) с последующим количественным ПЦР-анализом. Оба типа зондов продемонстрировали значительный перенос бактериальной ДНК из цервикальной слизи в эндометриальную пробу. Медианный перенос общей бактериальной ДНК составил 81,6% [54,4–107] для Пайпель-С1 и 29,8% [14,8–56,3] для Пайпель-С2 (p = 0,009). Ни один из зондов не обеспечивал защиты от контаминации на уровне, позволяющем достоверно интерпретировать состав микробиоты эндометрия. Эффективность зондов в предотвращении контаминации зависела от анатомической формы канала и конкретной группы микроорганизмов. Полученные результаты свидетельствуют, что ни один из исследованных зондов не обеспечивает надежной защиты от контаминации, что затрудняет интерпретацию данных о составе микробиоты эндометрия в трансцервикально полученных образцах и указывает на целесообразность изучения совокупной цервико-эндометриальной микробиоты.

Ключевые слова: микробиота эндометрия, цервикальный канал, контаминация, зонд Пайпель, ПЦР в реальном времени, молекулярная диагностика

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Since the discovery of resident microbiota in the uterine cavity by molecular genetic methods [1], the study of endometrial microbiota has become a priority area in human microbiome research [2, 3]. That foundational study by Mitchell et al. [1] was performed on hysterectomy specimens, which guarantees the absence of vaginal and cervical microbiota contamination and confirms the presence of resident microorganisms specifically in the endometrium. Subsequent similar studies on excised uteri have shown that the total amount of bacterial DNA in the endometrium is approximately 10 times lower than [4], or comparable to, that in cervical mucus [5].

In clinical practice, transcervical sample collection from the uterine cavity is almost universally used for endometrial microbiome research: using intrauterine insemination catheters or embryo transfer catheters [6, 7], hysteroscopically [8–10], or with Pipelle and Endobrush catheters [11–20], or similar devices with outer and inner sheaths analogous to the Endobrush catheter [21, 22]. In most studies, additional measures are taken to reduce contamination risk, including preliminary washing of the vagina/cervix with saline or antiseptics and comparative analysis of microbiota from different reproductive tract compartments [6, 7, 9–22]. However, the reliability of these approaches, particularly in the context of molecular genetic diagnostics, remains debatable. Studies using transcervically collected endometrial samples reported associations between endometrial microbiota and pregnancy loss [23], chronic endometritis [24], endometrial polyps [10, 18], endometrial hyperplasia [20], endometriosis [25], and polycystic ovary syndrome [26].

Any transcervical sampling method risks contaminating the uterine sample with vaginal, and particularly cervical, microbiota, given the anatomy of the female reproductive tract. This is particularly relevant given that endometrial samples inherently contain fewer microorganisms than cervical samples [4].

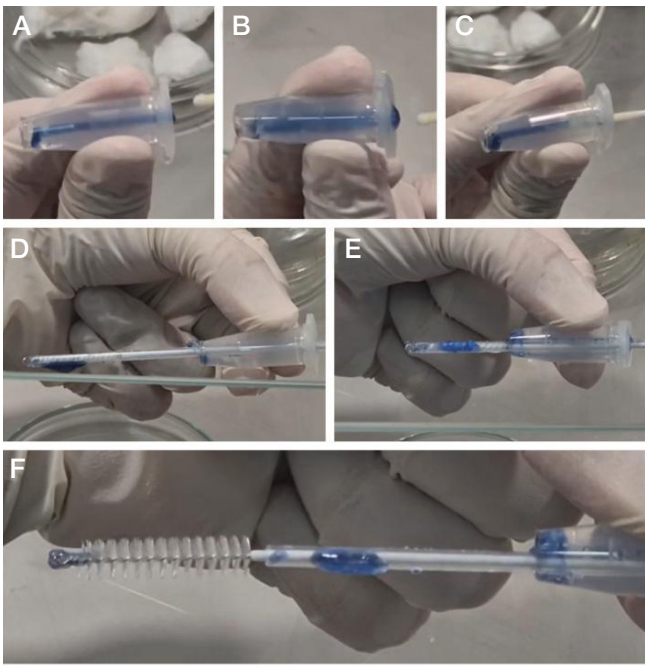

Fig. 1. Anatomical cervical models with canals filled with synthetic gelatin-alginate mucus. For better visualization, the mucus is stained with an aqueous methylene blue solution. **A**. Model with a cylindrical canal. **B**. Model with a slit-like canal. **C**. Sample collection from the cervical canal using a universal A2 catheter. **D**. 'Successful' exit of the Endobrush catheter beyond the cervical canal; the catheter is in the closed position, with minimal mucus on the protective sheath. **E**. 'Unsuccessful' exit of the Endobrush catheter beyond the cervical canal; the catheter is in the closed position, with abundant mucus on the protective sheath. **F**. Transfer of cervical mucus onto the brush of the catheter from the previous panel after the brush was extended

Most of the published studies do not adequately address the potential impact of this contamination on their results, raising questions about the true origin of the reported microbiota. This methodological gap calls into question whether the 'endometrial microbiome' reported in such studies is a true endometrial signal or either a mixture of cervical and endometrial microbiota or an artifact of cervical contamination. Furthermore, transcervical sampling is invasive and risks iatrogenically introducing pathogens from the lower tract into the uterine cavity.

Considering these risks, it is essential to thoroughly evaluate the capabilities and limitations of studying endometrial microbiota in transcervically collected samples, including the use of Pipelle and Endobrush catheters as the most popular tools for this purpose [11–20]. To assess these capabilities and limitations, we need to understand the true efficacy of the catheters in preventing contamination of endometrial samples by cervical microbiota.

The aim of the study was to experimentally evaluate the efficacy of Pipelle and Endobrush intrauterine catheters in preventing cervical microbiota contamination of endometrial samples intended for microbiota analysis.

METHODS

Development of an Experimental Cervical Model

For the experimental evaluation of the catheters efficacy, an *in vitro* model was developed to simulate passage through a cervical canal containing bacterially contaminated mucus.

Fabrication of Anatomical Cervical Models

Two types of cervical models were created using 2% agarose: a model with a cylindrical canal (n = 18), simulating the cervix of a nulliparous woman; and a model with a slit-like canal (n = 18), simulating the cervix of a parous woman.

Sterile Eppendorf tubes were modified by removing the bottom and creating a 5 mm diameter opening in the lid. Forming elements were placed inside: for the cylindrical canal — a 1.5 mm diameter rod (the inner part of the Endobrush catheter), for the slit-like canal — a plastic prism strip measuring 3 × 0.5 mm. The tubes were filled with agarose, and after polymerization, the forming elements were removed. Both openings of the tube (bottom and lid) were sealed with layers of sterile paraffin tape. Immediately before the experiment, the cervical canals of the models were filled with prepared synthetic mucus and incubated at 37°C. Photographs of the completed models are presented in Fig 1.

Preparation of Model (Synthetic) Cervical Mucus

Immediately before the experiment, model cervical mucus was prepared using a base of non-sterile gelatin (instant food-grade gelatin granules 220 bloom, Gold Gello, Tajikistan) and sodium alginate (food-grade sodium alginate powder viscosity 300–400, Qingdao Nanshan Yuanquan Seaweed Co., Ltd., China). Two separate solutions were prepared first: a 4% gelatin solution (40 mg in 1 ml of sterile water with 20 µl of 10% $CaCl_2$) and a 3% sodium alginate solution (30 mg in 1 ml of sterile water). Both initial solutions were incubated at 60°C for 30 minutes in a 'Gnome' thermostat (DNA-Technology LLC, Russia) and thoroughly mixed on a vortex mixer.

Equal volumes (2 ml each) of the prepared solutions were transferred to separate syringes, connected with a Luer Lock adapter, and carefully mixed manually for 2 minutes. The resulting final gel contained 2% gelatin and 1.5% sodium alginate and

demonstrated spinnbarkeit of 1–2 cm. For intentional mucus contamination, 200 µl of a mixture of bacterial cultures containing equal volumes of clinical isolates of *Lactobacillus acidophilus*, *Escherichia coli*, and *Staphylococcus aureus* (optical density 0.5 McFarland for each) was aspirated into the syringe with the final gel. After adding bacteria, the gel was mixed again through the adapter to ensure uniform microorganism distribution. For demonstration models aimed at better visualization of the cervical canal, 0.3% aqueous methylene blue solution was additionally added to the mucus.

Catheters Used for Sampling

Three types of catheters were used for sampling (Fig. 2): a universal urogenital catheter A2 (Meditsinskie Izdeliya, Russia), Pipelle catheter (Unicornmed, China), Endobrush catheter (Unicornmed, China). The A2 catheter was used to collect samples of cervical mucus from the model cervical canal, while the Pipelle and Endobrush catheters were used to collect air samples after passing through the model cervical canal (simulating a sterile uterine cavity).

Study Design and Sampling Protocol

A total of 36 cervical models were used: 18 with cylindrical and 18 with slit-like canals. For each experimental run, 3 models with cylindrical and 3 models with slit-like canals were used. A total of 3 independent replicate experiments were conducted for both Pipelle and Endobrush catheters. The sampling protocol consisted of two steps.

At the first step, a universal A2 catheter was used to collect a mucus sample from the cervical canal at a depth of 1–1.5 cm, which was then transferred to sterile saline.

At the second step, after cervical sampling, a Pipelle or Endobrush catheter was completely passed through the cervical canal to collect a sample beyond the internal os (simulating the uterine cavity). When using the Pipelle catheter, after exiting 3 cm beyond the canal, an air sample was aspirated. When using the Endobrush catheter, the brush was deployed, several rotational movements were performed, and then it was closed.

After collection, the catheter was removed (with the Endobrush kept in its closed state). Its external surface was wiped with 96% ethanol to remove any adherent cervical mucus and prevent contamination of the sample by cervical microbiota. The sample was then transferred to saline. As a negative control sample at the end of each experiment, an air sample was collected using Pipelle/Endobrush catheters without prior passage through the model system.

Molecular Genetic Analysis

Total DNA extraction from all samples was performed using the 'Proba-NK-PLUS' kit (DNA-Technology LLC, Russia). Quantitative microbiota analysis was performed using the 'Androflor' PCR kit (DNA-Technology LLC, Russia) with detection of the following targets: total bacterial load (TBL), *Lactobacillus* spp., *Staphylococcus* spp., and the *Enterobacteriaceae/Enterococcus group* (EE group). The minimum detection threshold for TBL and all target microorganism groups was 10^3 genome equivalents per sample (GE/sample). For each collected sample, one PCR reaction was performed. Target DNA amplification and amplicon detection were performed in DT-Prime 5M thermocyclers using the manufacturer's standard software (DNA-Technology LLC, Russia). Results are presented as medians across all experimental samples with 1st and 3rd quartile values.

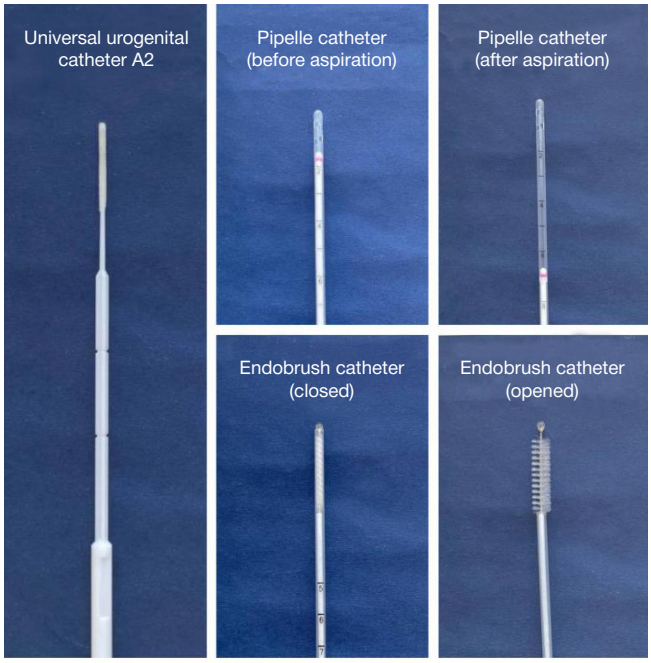

Fig. 2. Catheters used for biomaterial collection. Universal urogenital catheter type A2 for collecting model mucus from the cervical canal. Pipelle and Endobrush catheters used for sampling from the uterine cavity. The Pipelle and Endobrush catheters are shown in two states: before passing through the cervical canal *(central panel)* and after passing through the cervical canal — at the moment of biomaterial collection from the uterine cavity (*right panel*)

Evaluation of Catheter Efficacy in Preventing Contamination

To evaluate catheter efficacy in preventing contamination by cervical microbiota, the percentage transfer of bacterial DNA from 'cervical mucus' to the bacterial DNA-free 'uterine cavity' sample was calculated for each model using the formula:

$$\%\ \text{transfer} = \frac{DNA_{MO,\ \text{uterine cavity (Pipelle/Endobrush)}}}{DNA_{MO,\ \text{cervical mucus (A2)}}} \times 100\%$$

where % transfer — percentage of transferred DNA matrix; $DNA_{MO,\ \text{uterine cavity (Pipelle/Endobrush)}}$ — amount of target microorganism DNA in the 'sterile uterine cavity' sample collected by the investigated Pipelle or Endobrush catheter; $DNA_{MO,\ \text{cervical mucus (A2)}}$ — amount of target microorganism DNA in the cervical mucus of the same model collected by the universal A2 catheter.

Statistical Analysis

Statistical processing and data visualization were performed in R environment, version 4.5.2 (R Foundation for Statistical Computing; Vienna, Austria). Quantitative indicators are presented as median with 1st and 3rd quartile values. For comparison of two independent groups, the non-parametric Mann–Whitney *U*-test was applied. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Initial Microbiota Composition of Models

To assess initial contamination of cervical mucus, samples were collected from all 36 anatomical cervical models (18 with cylindrical and 18 with slit-like canals) using a universal A2

Table 1. Microbial composition of cervical mucus after collection with universal urogenital catheter A2 in models with cylindrical and slit-like canals. Amount of microbial DNA in GE/sample (median, Q_1–Q_3)

Parameter	Cylindrical canal	Slit-like canal	*p*
Total Bacterial Load	$10^{4.9}$ ($10^{4.8}$–10^{5})	10^{5} ($10^{4.9}$–$10^{5.1}$)	0.128
Lactobacillus spp.	$10^{3.5}$ ($10^{3.2}$–$10^{3.9}$)	$10^{3.7}$ ($10^{3.4}$–$10^{3.9}$)	0.375
Staphylococcus spp.	$10^{3.4}$ ($10^{3.1}$–$10^{3.7}$)	$10^{3.6}$ ($10^{3.2}$–$10^{3.9}$)	0.41
EE group	$10^{4.3}$ ($10^{4.1}$–$10^{4.6}$)	$10^{4.5}$ ($10^{4.4}$–$10^{4.7}$)	0.199

catheter. No statistically significant differences were observed in the microbial composition between samples from cylindrical and slit-like canal models (Table 1). Median levels of bacterial DNA were comparable between model groups for all investigated parameters: TBL ($10^{4.9}$ and 10^{5}, $p = 0.128$), *Lactobacillus* spp. ($10^{3.5}$ and $10^{3.7}$, $p = 0.375$), *Staphylococcus* spp. ($10^{3.4}$ and $10^{3.6}$, $p = 0.410$), and EE group ($10^{4.3}$ and $10^{4.5}$, $p = 0.199$).

Efficacy of Pipelle and Endobrush Catheters

Prior to comparative analysis of Pipelle and Endobrush catheter efficacy, the comparability of the initial models intended for their testing was verified. In the models designated for testing the Pipelle catheter, the initial content of *Staphylococcus* spp. was statistically significantly lower ($10^{3.1}$ vs. $10^{3.8}$, $p < 0.001$), while the bacteria of the EE group were significantly higher ($10^{4.6}$ vs. $10^{4.4}$, $p = 0.049$), compared to the group for the Endobrush catheter. At the same time, TBL levels (10^{5} and $10^{4.9}$, $p = 0.612$) and *Lactobacillus* spp. ($10^{3.8}$ and $10^{3.6}$, $p = 0.526$) did not differ significantly between the groups (Table 2).

Pipelle catheters facilitated significant transfer of bacterial DNA from cervical mucus into sterile ‘uterine cavity’ samples. The TBL transfer level ranged from 14% to 172% (median — 81.6%, Q_1–Q_3: 54.4–107%, Table 3). In 12 of 18 models, transfer was less than 100%, while in 6 samples, the TBL amount in sterile ‘uterine cavity’ samples exceeded the initial level in cervical mucus. The DNA transfer level for specific bacterial groups was: for *Lactobacillus* spp. — 25.8% (0–38.7%), for the EE group — 27.6% (11.5–38.7%), and for *Staphylococcus* spp. — 0% (0–0%), below the detection threshold (10^{3} GE/sample) in 100% of cases.

Endobrush catheters demonstrated a statistically significant lower transfer of bacterial DNA (TBL) from cervical mucus into sterile ‘uterine cavity’ samples compared to Pipelle catheters ($p = 0.009$, Table 3). Transfer ranged from 3.9% to 131%, with a median of 29.8% (Q_1–Q_3: 14.8–56.3%). In 12 of 18 models, transfer was less than 50%, in 4 samples — from 50% to 100%, and in 2 samples, the TBL amount in sterile ‘uterine cavity’ samples exceeded the initial level in cervical mucus. Transfer levels for *Lactobacillus* spp. (36.2%; 24.8–61.2%) and the EE group (19%; 9.6–29.4%) were comparable to those observed with Pipelle catheters. In contrast, *Staphylococcus* spp. transfer was significantly higher with Endobrush catheters (14.8%; 0–27.2%) than with Pipelle catheters ($p = 0.004$).

Influence of Canal Shape on Pipelle and Endobrush Catheter Efficacy

The efficacy of Pipelle and Endobrush catheters differed depending on cervical canal shape and microorganism group (Table 4).

For the Pipelle catheter in cylindrical canals, significantly lower contamination levels were observed for *Lactobacillus* spp. (0% vs. 35.2%, $p = 0.032$) and the EE group (19.7% vs. 37.5%, $p = 0.027$). At the same time, no significant differences between canal shapes were detected for *Staphylococcus* spp. (0% vs. 0%, $p = 0.350$) and TBL (71.3% vs. 81.6%, $p = 0.730$).

For the Endobrush catheter, conversely, a trend toward better performance in slit-like canals was noted for TBL contamination level (17.2% vs. 50.8%, $p = 0.052$), while for the remaining parameters, contamination levels were comparable between models with cylindrical and slit-like canals: *Lactobacillus* spp. (41.5% vs. 33.8%, $p = 0.309$), *Staphylococcus* spp. (19.7% vs. 10%, $p = 0.413$), and EE group (19.7% vs. 16.1%, $p = 0.136$).

DISCUSSION

In most endometrial microbiota studies, measures are taken before transcervical sampling to reduce contamination risk, including visual inspection and washing with saline or antiseptics [6, 7, 9–11, 13, 15–22, 27]. However, the efficacy of antiseptics for decontamination is questionable due to short exposure time, particularly in the context of molecular genetic studies that detect DNA from both viable and non-viable microorganisms. Any antimicrobial effect is more likely attributable to mechanical removal of microbial cells rather than true sterilization. Several studies have implemented additional

Table 2. Microbial composition of cervical mucus in models used for evaluation of Pipelle and Endobrush catheter efficacy. Amount of microbial DNA in GE/sample (median, Q_1–Q_3)

Parameter	Pipelle catheter	Endobrush catheter	*p*
Total Bacterial Load	10^{5} ($10^{4.9}$–10^{5})	$10^{4.9}$ ($10^{4.8}$–$10^{5.1}$)	0.612
Lactobacillus spp.	$10^{3.8}$ ($10^{3.4}$–$10^{3.9}$)	$10^{3.6}$ ($10^{3.3}$–$10^{3.8}$)	0.526
Staphylococcus spp.	$10^{3.1}$ ($10^{2.9}$–$10^{3.4}$)	$10^{3.8}$ ($10^{3.5}$–10^{4})	< 0.001
EE group	$10^{4.6}$ ($10^{4.4}$–$10^{4.8}$)	$10^{4.4}$ ($10^{4.2}$–$10^{4.5}$)	0.049

Table 3. Efficacy of Pipelle and Endobrush catheters in preventing contamination by microorganisms of cervical mucus. Contamination level, % DNA transfer from cervical mucus (median, Q_1–Q_3)

Parameter	Pipelle catheter	Endobrush catheter	*p*
Total Bacterial Load	81.6 (54.4–107)	29.8 (14.8–56.3)	0.009
Lactobacillus spp.	25.8 (0–38.7)	36.2 (24.8–61.2)	0.078
Staphylococcus spp.	0 (0–0)	14.8 (0–27.2)	0.004
EE group	27.6 (11.5–38.7)	19 (9.6–29.4)	0.428

Table 4. Efficacy of Pipelle and Endobrush catheters depending on cervical canal shape. Contamination level, % DNA transfer from cervical mucus (median, Q_1–Q_3)

Parameter	Cylindrical canal	Slit-like canal	*p*
Pipelle catheter			
Total Bacterial Load	71.3 (54.4–107)	81.6 (54.4–107)	0.73
Lactobacillus spp.	0 (0–29.6)	35.2 (25.8–67.1)	0.032
Staphylococcus spp.	0 (0–0)	0 (0–0)	0.35
EE group	19.7 (4.7–27.6)	37.5 (32.4–51.7)	0.027
Endobrush catheter			
Total Bacterial Load	50.8 (24.1–100)	17.2 (9.3–33.8)	0.052
Lactobacillus spp.	41.5 (31.6–76.3)	33.8 (22.5–41.5)	0.309
Staphylococcus spp.	19.7 (0–44.4)	10 (0–24.1)	0.413
EE group	19.7 (18.4–62.2)	16.1 (8.2–19.7)	0.136

controls by analyzing vaginal and/or cervical canal microbiota [6, 13, 14, 16, 17, 19, 22]. While this approach may identify gross contamination from distal compartments, it does not adequately address the fundamental problem of admixing microbiota from distinct anatomical niches.

The original clinical application of Pipelle and Endobrush catheters was to obtain endometrial samples for histological or cytological examination [27, 28]. With the Pipelle catheter, sample aspiration occurs only after the device has traversed the cervical canal [29]. The Endobrush catheter incorporates a protective sheath designed to prevent the internal brush from contacting the cervical mucosa during insertion; the brush deploys only within the uterine cavity and retracts before removal. These design features were intended to minimize the inclusion of cervical cells in the sample.

When these catheters are repurposed for microbiological investigation of the endometrium, it is assumed that their design provides adequate protection against contamination by cervical microbiota. However, this assumption overlooks two critical considerations: first, the inherent bacterial load in the uterine cavity is substantially lower than in the cervical canal [4]; second, unlike histological/cytological examination where cell morphology distinguishes endometrial from cervical origin, the anatomical source of detected microorganisms cannot be differentiated.

To assess the actual risk of contamination under controlled conditions that circumvent the limitations of clinical sampling protocols, we developed an experimental *in vitro* model simulating passage through a cervical canal filled with bacterially contaminated mucus (Fig. 1). The spinnbarkeit of the model mucus (1–2 cm) approximated that of genuine cervical mucus during the proliferative phase of the menstrual cycle [29]. The total bacterial DNA load in the model mucus was 10^5 GE/sample, consistent with median levels we have observed in clinical cervical samples using the same PCR assay (unpublished data). Potential background bacterial DNA in the gel matrix did not confound our analysis, as contamination was quantified by calculating the percentage of specific microbial DNA transferred from the cervical mucus sample to the simulated uterine sample within the same model.

The median transfer of bacterial DNA from cervical mucus to simulated uterine cavity samples was 81.6% for Pipelle catheters and 29.8% for Endobrush catheters (Table 3). Given that bacterial DNA levels in genuine endometrial samples typically do not exceed — and are often lower than — those in cervical samples [4, 5], this degree of transfer represents substantial, potentially critical distortion of results. Pipelle catheter sampling, intended for the endometrium, effectively collected cervical mucus instead. In 6 of 18 Pipelle catheter samplings and 2 of 18 Endobrush catheter samplings, the bacterial DNA concentration in the simulated endometrial sample actually exceeded that in the paired cervical sample. This paradoxical finding may reflect more efficient mucus collection by the endometrial sampling catheters compared to the A2 catheter for those specific sample pairs.

DNA transfer levels for *Lactobacillus* spp. and the EE group did not differ significantly between catheter types, with median transfers of 19–36.2%. For *Staphylococcus* spp., transfer was significantly lower with Pipelle catheters (0% vs. 14.8%, $p = 0.004$). However, this difference likely reflects the initially lower *Staphylococcus* spp. concentration in models designated for Pipelle catheter testing ($10^{3.1}$ vs. $10^{3.8}$ GE/sample for Endobrush models, $p < 0.001$) (Table 2). This baseline discrepancy may be attributable to degradation of staphylococcal DNA in the bacterial inoculum, which was refrigerated for three weeks between experimental series (Pipelle catheter experiments followed Endobrush catheter experiments). With the assay's detection limit of 10^3 GE/sample, any reduction from the initial $10^{3.1}$ GE/sample concentration would yield negative results for this bacterial group.

Our results demonstrate a relationship between cervical canal morphology and bacterial DNA transfer (Table 4). With Pipelle catheters, significantly greater transfer of *Lactobacillus* spp. and EE group DNA occurred with slit-like canals (typical of parous women). Conversely, with Endobrush catheters, a trend toward greater total bacterial DNA transfer was observed with cylindrical canals (typical of nulliparous women).

We acknowledge that our model may not fully recapitulate *in vivo* physiology, such as cervical wall tone (resistance), the precise rheological and chemical properties of mucus, or potential microbial gradients along the canal. Consequently, the absolute percentages of DNA transfer should not be directly extrapolated to clinical practice. Nevertheless, our results provide compelling evidence that endometrial sampling with either Pipelle or Endobrush catheters invariably transfers a portion of cervical microbiota into the uterine sample. For the Endobrush catheter, the primary contamination appears to occur after cervical canal transit — during deployment in the uterine cavity, cervical mucus adherent to the protective sheath contacts the brush (Fig. 1, panels E, F). A similar mechanism likely operates with other transcervical sampling devices, as any instrument traversing the cervical canal will inevitably carry cervical mucus into the uterine cavity. Moreover, clinical sampling during the secretory phase may exacerbate this contamination due to increased cervical mucus spinnbarkeit [29].

The demonstration of such contamination when using Pipelle or Endobrush catheters has two major implications: it calls into question the endometrial origin of microbiota detected in such samples, and it confirms the real risk of iatrogenic introduction of microorganisms into the uterine cavity during

these procedures. Given the impossibility of distinguishing the two microbiota sources (cervical canal and endometrium) in transcervical samples, we propose a paradigm shift. Rather than studying a putative 'endometrial microbiota,' research should focus on the combined cervico-endometrial microbial profile obtained from a single sampling procedure.

CONCLUSIONS

In this study, neither Pipelle nor Endobrush intrauterine catheters provided reliable protection against contamination of endometrial samples by cervical microbiota. Both catheter types permitted substantial transfer of bacterial DNA: median transfer levels were 81.6% for Pipelle catheters and 29.8% for Endobrush catheters. Although Endobrush catheters demonstrated 2.5- to 3-fold greater efficacy than Pipelle catheters, a contamination level of 29.8% remains critically significant, particularly given that the bacterial load in the uterine cavity is inherently lower than in the cervical canal for most patients. Catheter efficacy was dependent on both the anatomical configuration of the cervical canal and the specific microbial group analyzed. For Pipelle catheters, efficacy was significantly reduced in slit-like canals for *Lactobacillus* spp. and *Enterococcus/Enterobacteriaceae*, whereas for Endobrush catheters, a trend toward poorer performance was observed in cylindrical canals regarding total bacterial contamination. These findings necessitate a critical reevaluation of studies investigating uterine cavity microbiota using samples obtained with Pipelle or Endobrush catheters, as well as other transcervical sampling methods. As a constructive alternative, we propose reorienting research focus from the analysis of purported 'endometrial microbiota' toward the investigation of the combined cervico-endometrial microbial profile obtained from a single sample collected simultaneously from the cervical canal and uterine cavity. This approach would circumvent the methodological artifacts inherent to contamination-prone sampling techniques.

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