The role of microbiota in epithelial ovarian cancer: a scoping review

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Objective: The objective of this review was to examine the comprehensive role of microbiota in epithelial ovarian carcinogenesis. Methods: A scoping review method was used, and relevant databases were searched using combinations of key terms. Human and animal studies were selected that met inclusion criteria and critical appraisal tools were used to assess study quality. Results: A total of 10 international studies (human n = 8; animal n = 2) were included with total samples sizes varying from 16 to 580. Mean/median ages of women with epithelial ovarian cancer (EOC) were 50.5 to 66 years, and controls were 47.3 to 56 years. Compared to the ovaries and fallopian tubes of women without disease, tissue collected from women with EOC were characterized by differing proportions of bacterial phyla including Actinobacteria, Bacteroidetes, Chlamydiae, Firmicutes, and Proteobacteria. Intestinal depletions and reduced diversity of genera Lactobacillus accelerated ovarian tumor growth in animal models. Cytophagovirus and human papillomavirus types 6, 16, 18, and 45 had a significantly higher prevalence in women with disease and represented up to 70% of cases with high-grade serous ovarian carcinoma. Colonized bacteria were detected in fallopian tubes, peritoneal fluid, and ovarian tissue similar to that of commensal GI tract and vaginal microbiota. Conclusion: The EOC microenvironment harbors diverse microbes. Due to the heterogeneity of microbiota identified between studies, additional research is needed to reconcile findings and ascertain clinical applicability. Future investigations should also examine potential associations between EOC tumor, gut, and vaginal microbiota, patient symptoms throughout disease, chemotherapy response, recurrence, and survival.

Keywords
Microbiota; Epithelial ovarian cancer

1. Introduction

Annually, 23,000 women are diagnosed in the United States with ovarian cancer, and 14,000 women die of the disease, contributing to the staggering 185,000 global ovarian cancer deaths [1, 2]. Ovarian cancer is the fifth leading cause of cancer deaths among women following lung, breast, colorectal, and pancreatic cancers [3]. The high national fatality rate has been attributed to failure to identify the disease at an early stage due to inadequate screening methods [4]. Women with ovarian cancer are often asymptomatic in the early stage of disease and are undiagnosed until a more advanced stage [5]. Epithelial tumors account for 90% of ovarian cancers [6]. The high-grade serous ovarian carcinoma (HGSOC) epithelial subtype is characterized by bilateral ovarian involvement, aggressive behavior, late-stage diagnosis, and high mortality [7]. The urgency for timely and effective treatment strategies has led researchers to test and compare therapeutic agents that prove most beneficial for increasing survival rates [8]. Meanwhile, the biological processes surrounding fallopian tube and ovarian susceptibility in early carcinogenesis remains under investigation [9, 10]. Genomic studies have shown heterogeneity within HGSOC tumors, although there are still gaps in definitive regulatory mechanisms that initiate and drive disease [11–13]. Long noncoding RNAs and microRNAs (miRNAs), such as extravascular-derived circulating miRNAs, have emerged as promising biomarkers in early EOC detection [14, 15]. The ongoing search for discovery of new genomic markers that could signal early tumorigenesis has redirected scientific attention to the role of the microbiome in ovarian cancer development and progression.

The human microbiome is the collective genomes of microbes living in the human body [16]. Since the launch of the National Institutes of Health (NIH) Human Microbiome Project (HMP) over a decade ago, scientists have utilized advances in DNA sequencing technology to identify and characterize unique communities of microorganisms residing in the oral cavity, nasal passages, skin, urogenital tract, and gastrointestinal (GI) tract to determine their influence on health and disease [17]. Bacterial microbiota compositional changes have correlated with cancers [18, 19]. The identification of potential molecular pathways linked to microbial signatures has heightened the interest of researchers and clinician scientists. Microbiome research has become an emerging field to address important inquiries concerning the tumor microenvironment; consequently, novel studies have surfaced in the literature that explore both virulent and protective contributions of microbiota in ovarian cancer development [20].
Several ovarian cancer risk factors are well documented such as advancing age, nulliparity, and most importantly genetic mutations that affect homologous recombination and microsatellite instability [21]. Yet this highly heterogeneous disease poses multifactorial challenges for establishing definitive carcinogenic pathways among varying phenotypes. Nonetheless, the immune system is considered to have a valuable role in tumor activity, and inflammation is a key factor in immune response [22]. Microbial invasion promotes inflammatory reactions that mediate immune cells as a defense mechanism, and chronic inflammation is a risk factor for epithelial ovarian cancer (EOC) [23]. Fallopian tube inflammation is believed to contribute to ovarian cancer development, and pelvic inflammatory disease (PID) has been associated with increased disease risk [24]. Some investigators further hypothesize associations of Chlamydia trachomatis, cytomegalovirus (CMV), and human papilloma virus (HPV) with EOC while others have found no such relationship [25–33]. In the lower reproductive tract, the vagina is a non-sterile environment dominated primarily by aerobic bacterial colonies (e.g., Lactobacillus species) [34]. Consequently, vaginal microbial imbalances trigger immune responses, degrade the host mucosa, and increase vulnerability to the overgrowth of anaerobes causing bacterial vaginosis infection associated with PID [35]. However, only limited research has explored possible contributions of vaginal microbiota in ovarian cancer development [36].

In the absence of infection, microbial communities have been identified within the upper female reproductive tract, once assumed to be a sterile environment [37]. Microbiota composition within these areas may provide an important link between inflammation and ovarian carcinogenesis. Because HGSC derives in the mucosal epithelium of the fallopian tubes, microbial activity in this region has generated growing interest [38]. Although studies in various literature have concentrated on the role of single microbial pathogens in ovarian cancer development, gaining knowledge of how microbes can coexist in the tumor microenvironment is valuable. While the etiology of ovarian cancer remains inconclusive, discovery of possible microbiota influences can inform research and practice. The objective of this paper is to examine existing evidence on the comprehensive role of microbiota in epithelial ovarian carcinogenesis.

2. Methods

Scoping reviews are undertaken to examine the extent of the evidence surrounding a topic, identify research gaps in the literature, and draw conclusions regarding the overall state of research activity [39]. This review is guided by the Arksey and O’Malley methodological framework for conducting a scoping review that was further refined by Levac and colleagues [39, 40]. The stages of this framework are: (1) identify the research question; (2) identify relevant studies; (3) select studies; (4) chart to data; and (5) summarize and report the results. Data reporting was based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) Checklist to ensure accuracy, completeness, and transparency.

2.1 Search strategy for relevant studies

The search strategy was developed and conducted in consultation with an experienced librarian. A search was conducted to identify any publications up to April 2021 using electronic databases: PubMed (yielded 141 articles); Cumulative Index of Nursing and Allied Health Literature (CINAHL) (yielded 25 articles); and Embase (yielded 5 articles). Combinations of the following relevant terms were used: “microbiota”, “microbiome”, “GI tract microbiome”, “gastrointestinal microbiome”, “GI tract microbiota”, “vaginal microbiota”, “vaginal microbiome”, or “oncobiome”, combined with “ovarian cancer”, “dysplasia”, “ovarian neoplasms”, “ovary tumor”, and “ovary cancer”. Citation searching and reference lists were used to locate any additional primary studies that were not indexed in the original electronic database search. This method yielded six additional studies.

2.2 Study selection

Included were cross-sectional, prospective, retrospective, observational, and experimental human and animal studies that were published in the English language and involved investigation of the relationship between the microbiome consortium and ovarian cancer. Human study inclusion criteria were: (1) subjects ages 18 years and older; (2) EOC histologic subtypes confirmed by tissue biopsy; and (3) EOC tumor stage of any International Federation of Gynecology and Obstetrics (FIGO) classification. Animal study inclusion criteria were EOC mouse model studies (i.e., EOC cell lines, patient-derived xenografts, or genetically engineered models) that examined the relationship between the microbiota and ovarian cancer. Studies were excluded that concentrated on single microbial pathogens. The PRISMA-ScR literature search flow diagram is provided in Fig. 1.

2.3 Data charting

A customized data extraction instrument was developed to investigate the scope of the available literature. Data extraction elements included author(s) year of publication, design, and study location, purpose, study population, human versus animal study, type of tissue sample assessed, microbiota evaluation, and microbial features unique to ovarian cancer that were agreed upon by two independent reviewers (DM and SP). The data extraction tool summarizes study elements, offers expansion of sections pertinent to each study under review, and allows for comparisons across studies. A summary of the extracted data is presented in Supplementary Table 1.

2.4 Study quality assessment

The Joanna Briggs Institute (JBI) Critical Appraisal Tools Checklist for Case Control Studies and the Systematic Review Center for Laboratory Animal Experimentation (SYRCLE) Risk of Bias Tool for Animal Studies were used to determine the quality of the selected studies (see Supplementary Ta-
bles 2,3). The JBI checklist is a critical appraisal tool assesses methodological quality of the extent to which investigators address the likelihood of bias in study design, conduct, and analysis in their case control studies [41]. The SYRCLE’s Risk of Bias Tool was developed and adjusted based on the Cochrane Collaboration Risk of Bias Tool to address varying aspects of bias in animal intervention studies. This includes (1) selection bias; (2) performance bias; (3) detection bias; (4) attrition bias; (5) reporting bias; and (6) other biases [42]. The two authors (DM and SP) who extracted data elements also completed the quality assessment.

3. Results

A total of 10 studies were included with samples sizes varying from 16 to 580 cases. One study was conducted in Denmark [30], Poland [43], and India [26]. Three were conducted in China [44–46] and the United States [47–49]. The remaining study recruited subjects from the Czech Republic, Germany, Italy, Norway, and the United Kingdom [50]. All investigations were cross-sectional, observational, and case control studies involving human tissue samples with the exception of two animal experiments [45, 49]. Technologies used for microbiota evaluation comprised 16S rRNA sequencing, PCR-based assay, PathoChip array, and bacterial culture. Ovarian, fallopian tube, peritoneal, fecal, and cervicovaginal tissues were assessed for microbial expression. Human studies incorporated all EOC histologic subtypes (serous, n = 415; endometroid, n = 38; mucinous, n = 32; clear cell, n = 26; and undifferentiated, n = 1) at varying FIGO Stages of I through IV. A subtotal of 33 cases were reported as Stage I; 26 cases were Stage II; 218 cases were Stage III; and 35 cases were Stage IV. Additionally, some cases were combined as Stage I–II (n = 66), Stage III–IV (n = 108), and not staged (n = 2). One study [47] did not report on the number of cases per histologic subtype, and two studies [26, 47] did not report on FIGO staging. A summary of sample demographics in the human studies is described in Table 1 (Ref. [26, 30, 43, 44, 46–48, 50]).

Although not all studies provided age demographics, reported mean/median ages of women with EOC ranged from 50.5 to 66 years and controls ranged from 47.3 to 56 years. Two studies [48, 50] reported subjects as primarily white although race/ethnicity data were not described in the other studies. Most studies included women who were newly diagnosed with EOC and chemotherapy naïve, and those with recurrent disease or undergoing neoadjuvant therapy were excluded. While one study [47] made microbiota comparisons between EOC tissue samples and non-tumor ovarian tissue from the same cases, others compared EOC case samples to separate ovarian tissue control groups comprised of normal ovarian tissue, [26, 44] benign ovarian conditions [30, 43], and those of positive BRCA mutations [47]. In addition, one
Table 1. Summary of sample demographics in human studies.

<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Location</th>
<th>Mean age</th>
<th>Epithelial Ovarian Cancer (EOC) case types</th>
<th>Control case types</th>
<th>EOC cases of recurrence?</th>
<th>EOC cases chemotherapy naïve?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miao et al. (2020) [48]</td>
<td>United States</td>
<td>Cancer group: 66.1 years; Control group: 56 years</td>
<td>Total n = 10; serous n = 9; endometroid n = 1</td>
<td>Benign ovarian mass n = 20</td>
<td>No. Per exclusion criteria</td>
<td>Yes</td>
</tr>
<tr>
<td>Wang et al. (2020) [44]</td>
<td>China</td>
<td>Cancer group: 57.3 years; Control group: 51.6 years</td>
<td>Total n = 6; serous n = 6</td>
<td>Benign conditions n = 10; Uterine adenomyosis n = 7; Uterine myoma n = 3</td>
<td>No. Per inclusion criteria of new EOC diagnosis</td>
<td>Yes</td>
</tr>
<tr>
<td>Ingerslev et al. (2019) [30]</td>
<td>Denmark</td>
<td>Cancer group: *55 years; Control group: *64 years</td>
<td>Total n = 198; serous n = 163; endometroid n = 15; mucinous n = 11; clear cell n = 9</td>
<td>Benign mucinous cystadenoma tissue n = 176</td>
<td>No. Per inclusion criteria</td>
<td>Yes</td>
</tr>
<tr>
<td>Nené et al. (2019) [50]</td>
<td>Czech Republic, Germany, Italy, and United Kingdom</td>
<td>UC Reported by ages &lt; and ≥ 50 years</td>
<td>Total of cancer set n = 176; high grade serous; n = 119; low grade serous n &gt;= 13; endometroid n = 16; mucinous n = 13; clear cell n = 15</td>
<td>Benign conditions n = 69; healthy controls n = 115</td>
<td>No. Per inclusion criteria of suspicion for new EOC diagnosis</td>
<td>Yes</td>
</tr>
<tr>
<td>Paradowska et al. (2019) [43]</td>
<td>Poland</td>
<td>Cancer group: *53.5 years; Control group: *66 years</td>
<td>Total n = 27; high grade serous n = 20; borderline serous n = 2; mucinous n = 2; clear cell n = 2; undifferentiated n = 1</td>
<td>Benign ovarian tumor tissue n = 8</td>
<td>No. Per inclusion criteria of suspicion for new EOC diagnosis</td>
<td>Yes</td>
</tr>
<tr>
<td>Zhou et al. (2019) [46]</td>
<td>China</td>
<td>Cancer group: 54.5 years; Control group: 48.2 years</td>
<td>Total of discovery phase n = 25; high-grade serous n = 25</td>
<td>Benign adenomyoma or myoma of uterus n = 25</td>
<td>UC</td>
<td>UC</td>
</tr>
<tr>
<td>Banerjee et al. (2017) [47]</td>
<td>United States</td>
<td>UC Not reported</td>
<td>Total n = 99; serous, endometrioid, mucinous; clear cell; transitional cell; mixed types; and carcinosarcoma (number per type not reported)</td>
<td>Matched n = 20 non-tumor tissue from the ipsilateral or contralateral ovary; Unmatched n = 20 benign ovarian tissue of women with BRCA mutations</td>
<td>Yes. Three subjects had recurrent disease</td>
<td>UC</td>
</tr>
<tr>
<td>Shanmughapriya et al. (2012) [26]</td>
<td>India</td>
<td>Combined sample: *55 years</td>
<td>Total n = 24; serous n = 12; endometroid n = 6; mucinous n = 6</td>
<td>Benign ovarian lesion n = 6; healthy controls n = 9</td>
<td>UC</td>
<td>UC</td>
</tr>
</tbody>
</table>

*, Median; EOC, Epithelial Ovarian Cancer; UC, Unclear.
study [43] assessed for the presence of microbes in fallopian tube tissue of EOC cases, while another study [46] evaluated the normal distal fallopian tube fimbria tissue of uterine adenomyoma and myoma controls. One study [50] compared the proportions of lactobacilli species found in the cervicovaginal smears of women from two study sets. The ovarian cancer study set consisted of tissue samples of women with EOC, benign gynecological conditions, and healthy controls. The BRCA set included women with BRCA1 mutations without EOC and women with wildtype BRCA1 and BRCA2 mutations who had benign gynecological conditions or were healthy controls. In another study, researchers [48] compared the microbial profile in peritoneal fluid collected from surgical peritoneal washings of women with EOC and those with benign ovarian masses. The animal studies used ovarian cancer (cell line) mouse models to investigate either induced GI bacteria dysbiosis [45] or GI commensal bacterial [49] influences on EOC initiation, progression, and immune response.

3.1 Bacteria and epithelial ovarian cancer

3.1.1 Phylum

Unique proportions of Proteobacteria, Actinobacteria, Bacteroidetes, Chlamydiae, Firmicutes, Fusobacteria, Spirochaetes and Tenericutes were found in EOC tissue compared to controls [47]. In one study [46], Proteobacteria was upregulated while Firmicutes, Candida, and Acidobacteria were downregulated in EOC tissue. Wang and colleagues [44] found no differences in the relative abundance of Proteobacteria, Bacteroidetes, or Firmicutes among groups. However, the authors detected an increase in prevalence of Aquificae and Planctomycetes and a decreased prevalence of Crenarchaeota exclusively in the serous epithelial tissue subtype compared to controls. Eighteen bacterial signature combinations were found in the peritoneal fluid of women with EOC belonging to phyla Bacteroidetes, Firmicutes, Proteobacteria, or Verrucomicrobia [48]. A summary of bacteria shared between studies is provided in Fig. 2 and key findings are described in Table 2 (Ref. [26, 46–48, 50–53]).

3.1.2 Genus

Acinetobacter, Sphingomonas, and Methyllobacterium were significantly increased and Lactococcus was significantly decreased in the EOC group in one study [46]. Miao et al. [48] reported different genera associated with epithelial ovarian tumors consisting of Prevotella, Odoribacter Roseburia, Oscillospira, Clastridium, Eubacterium, Faecalibacterium, Sutterella, Bradyrhizobium, and Akkermansia. Differences were discovered between ovarian cancer and control groups in the relative abundance of Paenibacillus, Halaferula, Zavarzinella, Photobacter, Volucrribacter, Blastococcus, Mesotoga, Defluviitoga, and Dorea [44]. Furthermore, intestinal depletions Lactobacillus significantly accelerated growth of ovarian tumors compared to control groups in tumor xenograft models [45].

3.1.3 Species

The prevalence of C. trachomatis was significantly higher [26], Acinetobacter lwoffi were more enriched, and Lactococcus piscium were less enriched in invasive EOC sample cases [46]. Of 24 EOC cases with Chlamydia trachomatis detection, 12 were classified as serous subtype [26]. The relative abundance of Anoxynatronum sibiricum and Methanosarcina vacuolata was significantly reduced in EOC cases compared to con-
In another study, the prevalence of Lactobacillus crispatus, Lactobacillus iners, Lactobacillus gasseri, and Lactobacillus jensenii was lower in women with ovarian cancer and those with BRCA1 gene mutations compared to those without the disease [50]. A variety of additional species were also associated with EOC, including, Prevotella stercorua, Bacteroides ovatus, Clostridium colinum, Eubacterium dolidum, and Akkermania mucinaphila [48].

### 3.2 Viruses and epithelial ovarian cancer

Banerjee et al. [47] reported that among the viral signatures identified for all cases, 23% were characterized as tumorigenic, yielding a prevalence of greater than 50% in EOC tissue. These viruses included Retroviridae, Hepadnaviridae, Papillomaviridae, Flaviviridae, Polyomaviridae, and Herpesviridae. Although common viral groups were also detected in control tissue samples, specific genetic signatures differed between EOC and control cases. For instance, within the Papillomaviridae family, unique molecular properties of high risk types HPV 16 and HPV 18 were detected in the ovarian cancer samples, whereas molecular signatures of other low risk HPV types were detected in the controls. In another study [43], HPV prevalence was significantly higher in ovarian cancer where HPV 6, HPV 16, and HPV 45 were detected in 74% of all EOC tissues and HPV 16 was detected in 70% of HGSOC subtypes. Moreover, HPV was also observed in the fallopian tube samples of cases with EOC (HPV 6 and HPV 16) and in cases with ovarian metastatic cancer (HPV 16) [43]. To the contrary, Shannughapriya et al. [26] identified HPV 6 in both EOC and control cases with no significant differences in prevalence between groups. Cytomegalovirus (CMV) was detectable in 50% to 70% of EOC case samples representing a significantly higher prevalence in cases compared to controls [26, 43]; among those with HGSOC, CMV was present in 70% of cases [43]. CMV was present in all metastatic ovarian cancer cases [43]. Nonetheless, in a different study, the prevalence of CMV in EOC tissue was insufficient for analysis although Epstein-Barr Virus (EBV) was detected in 5% of EOC cases compared to 0.5% of control cases [30]. A summary of viruses shared between studies is provided in Fig. 3.

### 3.3 Other microbes and epithelial ovarian cancer

Only one study evaluated additional microbes. Banerjee and colleagues [47] identified distinctive fungi signatures that were significantly detected in either all EOC tissues samples (Cladosporium, Pneumocytis, Acremonium, Cladophialophora, Malassezia, and Pheistophora) or in 95% of EOC cases (Rhizomucor, Rhodotorula, Alternaria, Geotrichum). A parasitic signature (Trichinella, Ascaris, and Trichomonas) was also identified in greater than 95% of the EOC cases.

### 3.4 Immune system response, inflammation, and gastrointestinal microbiota

When comparing human antibacterial-response gene expression profiles among a subset of EOC and normal distal fallopian tube tissues, Zhou et al. [46] identified the activation of inflammation-associated signaling pathways (cytokine-cytokine receptor interaction, chemokine signaling, and NF-kappa B signaling) in EOC tissues. In one animal study, TLR5 signaling at regions of bacterial colonization in ovarian tumor-bearing hosts initiated tumor-promoting systemic inflammation and the deployment of myeloid derived suppressor cells (MDSCs) and immunosuppressive gamma delta (γδ) T cells [49]. Moreover, tumors that induced TLR5-dependent systemic interleukin 6 (IL-6) up-regulation responded to exogenous IL-6 by producing additional levels. In the remaining animal study, the presence of macrophages promoted ovarian cancer growth in the xenograft model [45]. Ovarian tumor size and weight assessed in intestinal microbiota dysbiosis mice progressed significantly faster than in the control group, yet this situation resolved with macrophage depletion. Macrophages collected from intestinal microbiota dysbiosis mice were more likely to promote inflammatory cytokine (tumor necrosis factor alpha [TNF-α] and IL-6) production. Additionally, the cytokines secreted by macrophages isolated from intestinal microbiota...
dysbiosis mice were favorable to epithelial-mesenchymal transition (EMT), epithelial cadherin (E-cadherin) suppression, mesenchymal neural cadherin (N-cadherin) and vimentin overexpression, and proliferation of ovarian cancer cells compared to controls.

4. Discussion

This review examined the association between EOC and a consortium of microbiota harbored within and beyond the female reproductive tract and identified microbial differences with ovarian cancer. While there were commonalities among viral and bacterial phyla classifications, genera and species differed in bacteria groupings between studies. These overall findings suggest a rather convoluted yet dynamic tumor microenvironment involving bacteria and viruses. Although human microbiota encompass the entire genome of microbes, one of 10 studies assessed and used detected widespread signatures across microorganisms including protozoans (parasites) and fungi [47]. However, the few parasites (i.e., Opisthorchis viverrini, Clonorchis sinensis) known to contribute to cancer were not identified in this review [54]. It is important to acknowledge that Banerjee and colleagues [47] used pan-pathogen array technology to capture parasites and fungi, which would not be detected with methods used in the other studies. While investigation of parasitic and fungi influences on ovarian cancer has received less attention, scientists are exploring the benefit of anti-parasitic therapeutics to suppress ovarian tumors [55].

Infectious microorganisms are known to initiate inflammatory mechanisms and cellular degradation. Several virulent pathogens (i.e., Helicobacter pylori, Hepatitis B and C, HPV, and Epstein-Barr virus) are considered global attributes to gastric, liver, cervical, and nasopharyngeal cancers [54]. An important challenge will be differentiating common bacterial species attributable to ovarian tumorigenic states across studies. For example, four of ten studies reported on bacteria at the species level although none of them shared commonalities. Still, reduced amounts of Lactobacillus in the GI tract of female mice and Lactobacillus species in human cervical smear samples were associated with EOC. Chlamydia trachomatis was detected in 80% of EOC cases, which is consistent with prior research [26, 31, 56]. In fact, researchers have found C. trachomatis in 84% of high-grade fallopian tube serous cancers and in 17% of HGSOCs [57]. This evidence corroborates the negative impact of chlamydial infection on fallopian tube health particularly as HGSOC, the most lethal EOC histotype, originates within the fallopian tube [38].

Similar to that of the GI tract and vagina, bacteria of Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria phyla were detected in the ovaries, fallopian tubes, and peritoneal fluid of women with EOC [52, 58, 59]. Acinetobacter and Sphingomonas genera belonging to the Proteobacteria phylum have been detected in the fallopian tubes and peritoneal fluid of cancer-free reproductive-aged women who were without any known infections [37]. Approximately 150 to 400 bacterial species reside in the GI tract and primarily belong to Bacteroidetes, Bacteroidetes, Firmicutes, and Proteobacteria phyla [60]. While exact proportions of GI tract bacteria do vary among individuals, scientists have observed influences of GI microbiota composition shifts in the development of several cancers. With colorectal (CRC) cancer for example, chronic inflammation, intestinal barrier breakage, signaling pathways, and DNA damage are associated with certain bacterial strains [61]. Specifically, Bacteroidetes and Fusobacterium enrichment are well observed in colorectal cancer tissue and fecal samples [62]. In addition, researchers [63] have observed increases in the richness of Bacteroidetes and Proteobacteria and reductions of Actinobacteria and Firmicutes in the stool specimens of patients with preneoplastic colon lesions; other investigators [64] have observed the opposite in CRC tissue samples. While mounting evidence supports the
prevalence of GI tract microbiota dysbiosis in CRC disease, microbial mechanistic pattern associations have not been determined in ovarian cancer research [65]. Unique ecosystem shifts and impaired cell membrane permeability could be important factors and a plausible explanation for bacterial peritoneal migration during ovarian tumorigenesis which may also have consequences in malignant ascites observed in both primary ovarian cancer and recurrent disease [66]. Potential contributory mechanisms could involve disruption of intestinal epithelial adhesion molecule signaling events that compromise normal immune response and cause widespread bacterial expansion [67]. Data from this review substantiate the presence of differing GI commensal bacteria groupings in fallopian tube, peritoneal, and ovarian tissue of women with EOC. Interestingly, primary cancers of the fallopian tubes and peritoneum are staged and managed in the same fashion as EOCs because they are histologically similar [68]. Thus, if initial HGSOc cells that originate in the fallopian tubes also displace to the peritoneum, detection of specific GI tract microbes may signal the presence of early precursor lesions.

Based solely on anatomical location, GI tract microbiota would seem less likely to reside in the reproductive tract than vaginal microbiota. Indeed, one study in this review validated the importance of vaginal commensals by reporting that women with EOC or BRCA mutations had reduced quantities of vaginal Lactobacillus species compared to women without disease [46]. Increasing literature has demonstrated probiotic effects of Lactobacillus against opposing vaginal and GI tract pathogens [69, 70]. Vaginal flora diversity has been associated with several gynecological cancers [36]. For instance, a greater abundance of Porphyromonas species was detected in cervicovaginal swabs collected from women with endometrial cancer [71]. Gardnerella vaginalis, Atopobium vaginae, and Chlamydia trachomatis have been enriched in cervical tissue samples of women with cervical intraepithelial lesion abnormalities and cervical cancer [72–75]. Researchers have also observed reductions in vaginal Lactobacillus species during HPV infection [76]. Consistent with some existing literature, HPV types 6, 16, 18, 45, and CMV were the most common viruses observed with EOC cases in this review [32]. These findings suggest possible synergistic actions of combined bacterial and viral pathogens during early transformation of epithelial ovarian cells. Yet the correlation between HPV and EOC oncogenesis remains controversial as several studies have reported conflicting findings, which are not seen with other cancers associated with HPV [33, 77]. For example, HPV 16, 18, and 45 types demonstrate the highest invasive cervical carcinogenic potential compared to all other types, and HPV 16 presents the highest risk in the in development of anal cancer [78, 79]. One study in this review reported that CMV was more prevalent in HGSOc cases [45]. In breast carcinogenesis, CMV gene products are proposed to instigate oncogenic infection of macrophages in breast epithelial cells favoring the appearance of tumor-associated macrophages (TAMs) [80]. Although specific pathways linking CMV to EOC are unclear, higher expressions of CMV protein in EOC tissue are linked with shorter survival rates compared to those without infection [81]. Pathogenic viral and bacterial infiltration stimulates inflammatory and immunological responses within the host [82]. Thus identification of microbial modulated immune checkpoints could prove most beneficial for early detection during cellular transformation and for more enhanced treatment targeting.

One animal study in this review reported that GI tract microbiota dysbiosis triggered inflammation, accelerated ovarian tumor growth, and activated TAMs infiltration causing induction of epithelial-mesenchymal transition (EMT) [45]. A hallmark of EMT is the downregulation of E-cadherin to reinforce the deterioration of adherens junctions contributing to the demise of the epithelial barrier function [83]. Investigators also observed microbiota mediated tumor-promoting inflammation locally [45, 46, 49] and systemically [45] through the production of proinflammatory cytokines including IL-6 and TNF-α. IL-6 signaling is regulated to facilitate proliferation, adhesion and invasion in human ovarian cancer cells [84]. TNF-α is an important mediator of tumor promotion correlated with elevated ovarian cancer risk and advanced EOC tumor grade [85]. Microbiota-proinflammatory interactions have shown cytokine- and stimulus-specific patterns that influence immune response [86]. Thus GI microbiota dysbiosis could be linked to precise immune signaling throughout the tumorigenesis process. MicroRNAs have an important role in tumor cell metabolism and have emerged as key gene regulators to control inflammation [87, 88]. Existing data confirms a relationship between ovarian cancer, vaginal microbiota, miRNAs, and immune response, however, the regulatory mechanisms explaining gut microbiota activity in EOC are much less understood [89].

While chemotherapy influences on gut microbiota and the bidirectional relationship on cancer outcomes was not examined in the studies from this review, GI microbial activity during treatment is of particular importance in gynecologic cancer research [90, 91]. Specifically in women with EOC, fecal samples have shown increases in abundances of Bacteroidetes and Firmicutes and decreases in Proteobacteria after chemotherapy compared to prechemotherapy [92]. Yet, it is uncertain how these fluctuations correlate with overall treatment outcomes. Human investigations are lacking that evaluate tumor and gut microbiota influences on the survival rates of women with EOC. However, in women with cervical cancer who undergo chemoradiation, fecal enrichment of Escherichia, Shigella, Enterobacteriaceae, and Enterobacteriales are found to be an independent predictor of long term survival and Porphyromonas, Porphyromonadaceae, and Dialister enrichment are shown to be a predictor of short term survival [93]. Additionally, bacterial induced chemotherapy resistance is now broadly recognized across several cancers [94, 95]. Thus probiotics are promising modalities to eliminate the colonization of opposing bacterial species and re-
store healthy microbiota [96, 97]. Immunotherapy has become an exciting option in cancer treatment which includes the administration of immune checkpoint inhibitors that enhance T cell-mediated immune responses to counter tumor activity an improve overall survival of patients [98, 99]. Microbiota are shown to impact the efficacy of immunotherapy in kidney, lung, and melanoma cancers although the role of immunotherapy in EOC treatment remains controversial [100–104]. Consequently, several EOC clinical trials are underway evaluating immunotherapy although new strategies should also incorporate microbial targeted checkpoints.

4.1 Advantages and limitations of study techniques used for microbe detection

This scoping review summarized known associations between microbes. Yet only 10 studies were included that used different techniques of microbiota evaluation with variable sample sizes that could limit the generalizability of findings. Furthermore, none of the studies expanded testing methods to assess for associations between intratumor microbiota functional profiles and subsequent treatment outcomes. Investigators in one study [47] used a pan-pathogen functional gene array (Patho Chip) to capture a broader range of microbes including protozoan and fungi [105], which are not designed for detection with 16S rRNA sequencing. Quantitative PCR (qPCR) assays and 16S rRNA sequencing were the primary methods used to measure microbial genetic expressions in varying tissue samples. Each of these methods has advantages and drawbacks. Although qPCR is highly valid and valuable for detecting specific pathogens, it is not sufficient for large scale genomic detection and quantification. Thus, qPCR methods have been suggested as a validation tool for quantification of gene expression [106]. While advanced genome technology allows high throughput applications for analysis of microbial DNA and RNA, sequencing errors are possible [106]. Included studies varied in the reporting of bacterial taxonomy by phylum, genus, and species levels and used differing quantifiable terms of magnitude (i.e., up/down-regulated, abundance, diversity, proportion, and signatures). Ideally, consistency of species reporting would provide a means to robust interpretations across studies that could be further validated with qPCR. For instance, Zhou et al. [46] used qPCR validation in an attempt to recover the bacterial 16S rRNA sequencing results. Meanwhile, the qPCR platform was used for detection of HPV, EBV, and CMV in all the viral studies [26, 30, 43]. While qPCR tests are the traditional approach, a recent meta-analysis [77] reported the prevalence of HPV higher in ovarian tissues when using two different techniques such as qPCR combined with immunohistochemistry or in situ hybridization. Moreover, expansions in genomic viral sequencing databases are also allowing opportunities to better refine associations between viral genomes and EOC in future investigations.

4.2 Implications for practice and future research

Researchers in each human study in the present review assessed microbial characterization in cervical, ovarian, fallopian tube, and/or peritoneal tissue samples whereas GI tract microbiota was assessed in fecal specimens in the animal studies. Forthcoming animal experiments should integrate analysis of local and systemic host immune response, intestinal epithelial integrity, and tumor tissue microbial markers in the presence of GI tract microbiota dysbiosis. Distinguishing possible direct and indirect microbial-mediated molecular interactions in the tumor microenvironment will prove paramount. Future research should also address chronic inflammation and fecal microbiota profiles in differing ovarian cancer histologic subtypes. Although early lesions are undetectable with current screening methods, women with ovarian cancer often report GI-related symptoms. It is possible that the GI tract commensal microbes signal peritoneal inflammation and GI microbiota dysbiosis becomes a co-factor to many symptoms that women experience. Human investigations are needed to incorporate patient reported symptoms with microbial migration, inflammation, tumor progression, and disease recurrence among ethnically diverse populations for clinical application. Furthermore, research is needed to examine associations between microbiota signatures, treatment tolerance, and tumor response to chemotherapy, and patient survival rates.

5. Conclusions

In this review, we identified a variety of microbes associated with ovarian cancers of epithelial histology. While exact mechanisms remain unknown, the EOC tumor microenvironment harbors diverse microbiota that vary among studies. Based on these findings, we conclude that additional research is needed to replicate study findings and reconcile the literature. The clinical applicability of tumor, GI tract, and vaginal microbiota biosignatures remains indeterminate in this population of women. The detection of distinctive microbial candidates that correlate with existing disease markers could have important implications in the practice setting. While identification of valid and reliable ovarian cancer diagnostic and prognostic biomarkers remains a research priority, differentiating microbiota types that signify early disease would be groundbreaking in clinical gynecologic oncology. EOC research is situated at a pivotal time when investigations are lacking that examine symptom biology and symptom burden on patients. If GI microbiota imbalances are associated with symptom burden, this opens new possibilities for the development of new probiotics that promote healthy GI bacteria and symptom relief. Furthermore, if microbiota profiles unique to EOC could be routinely detected in fecal or vaginal samples, a simple microbial-based test could aid with symptom management, earlier diagnosis, and inform on precise treatment strategies. Nonetheless, important issues must still be considered including uniformity of testing methods and tissue sampling and, most importantly, the practicality of specimen collection in a clinic setting. In particular, the activity of commensal GI tract and vaginal microbiota warrants additional examination in the scientific pursuit of
novel biomarkers that detect early disease, aid prognosis, improve symptom management during treatment, predict disease recurrence, and guide development of therapeutic interventions.

Author contributions
DEM and SP contributed to the conception of the manuscript. BML provided search strategy assistance. DEM and SP wrote the manuscript. LS and JDP reviewed and edited the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

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