An unexpected link: The role of mammary and gut microbiota on breast cancer development and management (Review)

DANIELA TOUMAZI, SANDRA EL DACCACHE and CONSTANTINA CONSTANTINOU

Department of Basic and Clinical Sciences, University of Nicosia Medical School, CY-1700 Nicosia, Cyprus

Received November 26, 2020; Accepted March 4, 2021

DOI: 10.3892/or.2021.8031

Abstract. Breast cancer (BC) impacts 2.3 million women each year, making it the most frequent cancer diagnosed among the female population. An unexpected link has been discovered between BC and alterations in the mammary and gut microbiota, suggesting their possible role in BC development, prevention and management. Studies suggest a distinct microbiome in healthy breast tissue compared to BC tissue. The healthy breast tissue has been found to be mostly enriched with bacteria of the phyla *Proteobacteria, Firmicutes* and *Actinobacteria*. However, certain bacteria are more abundant in cancerous tissues compared to adjacent non-cancerous tissues in BC women or compared to the breast tissues of healthy women. On the other hand, bacteria such as *Lactococcus* spp. are increased in the breast tissues of healthy women compared to the cancerous tissues of BC women and may therefore have potential protective effects against BC. Additionally, preliminary studies propose that the mammary microbiota is distinct in the different subtypes of BC, proposing a specific role of microbes in the development of BC and suggesting their possible use as biomarkers. Similarly, dysbiosis in the gut microbiota has been further linked to BC since certain gut bacteria can alter the production of beneficial metabolites and disrupt estrogen metabolism in the gut. While still at its infancy, such unexpected links between breast and gut microbiota and BC propose possible alternatives with regards to the prevention but also management of BC such as through the use of probiotics. The current review is focused on evaluating the recent evidence regarding the association between mammary and gut microbiota and BC and discusses the most important bacteria involved.

Correspondence to: Dr Constantina Constantinou, Department of Basic and Clinical Sciences, University of Nicosia Medical School, 21 Ilia Papakyriakou, 2414 Engomi, P.O. Box 24005, CY-1700 Nicosia, Cyprus
E-mail: constantinou.co@unic.ac.cy

Key words: breast cancer, gut microbiota, mammary microbiota, prevention, biomarkers, probiotics

Contents

1. Introduction

Breast cancer (BC) affects 2.3 million women per year and is responsible for the highest number of cancer-related deaths among women globally (1) BC contributes to 30% of female cancers in the USA and it is estimated that 276,480 new cases of BC and 42,170 deaths will be attributed to BC for the year 2020 (2).

Since BC is a multifactorial disease, incidence, survival and mortality rates differ across countries. The latter is due to the involvement of a multitude of modifiable and non-modifiable risk factors such as ethnicity, mutations in the *BRCA1/2* genes, diet, alcohol, sedentary lifestyle, radiation and lifetime exposure to estrogens (3-5). For example, the 5-year survival rate for BC is 90% in the USA (2) whereas in certain underdeveloped countries the 5-year survival rate can be as low as 57% (6). Incidence and survival rates not only differ between different countries, but also vary among the different types of BC. The best survival rates are seen in women with the subtype estrogen receptor $(ER)^+$ and/or progesterone $(PR)^+$ and HER-2, while the worst survival rates are observed in women with the triple-negative subtype (ER^- PR^-, $HER\text{-}2^-$) (7).

The microbiota found in our bodies is estimated to outnumber our cells by a factor of 10 and it is considered crucial for the correct functioning of our physiology, mostly due to the production of metabolites by the microbes (8). Humans have therefore evolved to be dependent on these bacteria and have cultivated a fruitful symbiotic relationship between them. Resulting from this, the Human Microbiome Project was created to further document the effect that the microbial genomes have on our physiology (8). Initially, this project aimed to investigate the microbiota of only the skin, mouth, nose, colon and vagina and to examine how the bacteria can manipulate the function of the human body to shift from a healthy state to a diseased one. Interestingly, body

sites that have been previously considered as sterile such as the pancreas, the prostate, the lungs and the breast have been found to harvest unique microbial environments (9). Given the fact that the breast has a naturally occurring nutrient rich and fatty environment and knowing that bacteria found in the skin have direct access to the mammary ducts through the nipple, it is not surprising that the breast contains a vast array of bacteria in its tissues (10).

When in a healthy state, the microbiota and our body exist in perfect balance i.e. symbiosis. However, when this delicate balance is disrupted, a microbial imbalance develops known as dysbiosis, potentially leading to numerous malignancies such as colorectal, skin, liver, stomach, and lung cancers (4,11). In fact, studies suggest that the microbial flora is responsible for at least 18% of all malignancies worldwide (4). In the past few years, an increasingly well-established relationship has been recognised between gut microbiota and colorectal cancer (11). The mechanisms implicated in the carcinogenesis depends vastly on the different types of microbiota involved. Some bacteria alter immunological functions while others synthesize genotoxins or change the regulation of circulating steroid hormones (10).

Since BC is linked to many modifiable risk factors and affects many women worldwide, it has become a growing priority to identify new biomarkers to facilitate the screening and management of BC. Recently, emerging evidence in the literature supports that alterations in the microbiota of the mammary and gut tissue are associated with the development of BC (10). It is therefore important for more research to be carried out to identify the bacteria and molecular mechanisms involved in breast carcinogenesis. The aim of the current review is to provide recent evidence regarding the role of the mammary and gut microbiota in the development, prevention and management of BC.

2. Microbiota in normal vs. malignant breast tissues

The highly diverse microbiota in normal breast tissue. Research in the past few years has provided evidence that the breast tissue harbors a diverse and unique community of microbes (4). There are many theories regarding the origins of the breast microbiota, ranging from translocation of microbiota from the skin through the nipple, to the translocation of microbiota during lactation (4). Whatever the origin, the breast tissue contains a high diversity of bacteria. Using Shannon's diversity index (SDI), an index used to characterize species diversity, studies have concluded that the mammary microbiota has an average SDI of 3.6 (12). This is a relatively high diversity since the gut and oral cavity, which are considered to have a variety of bacterial communities, have an SDI of 3.9 and 6.5 respectively, while the vagina which is known to have a low diversity of bacteria has an SDI between 0.46 and 2.9 (10).

The knowledge that breast tissue has its own unique microbiome has sparked interest as to its involvement in the development of BC (4). Even though this area of research has yet to be fully explored, there have been various studies that have proved to be fruitful.

A study conducted by Urbaniak *et al* (10) was conducted to investigate if healthy mammary tissues from women in Canada and Ireland contained their own microbiome. In this study, breast tissue samples were collected from women undergoing breast surgery to either remove a cancerous or benign tumor or to have breast reductions. Interestingly, the results showed that the breast tissues have their own unique microbiome. By using 16S rRNA sequencing and culture, the researchers identified various bacteria and found the most abundant phyla in normal breast tissues to be *Proteobacteria, Firmicutes* and *Actinobacteria* (10) (Table I). Interestingly, while *Proteobacteria* were seen in abundance in breast tissue, they are normally known to be a minority in other body sites, such as the vagina, bladder and GI tract. This could be because *Proteobacteria* have adapted to the fatty acid environment of the breast tissue (10). More specifically, the Canadian samples showed that the most abundant taxa were from the genera *Bacillus*, *Acinetobacter* Enterobacteriaceae, *Pseudomonas, Staphylococcus, Propionibacterium,* Comamonadaceae, *Gammaproteobacteria* and *Prevotella* (Table I). On the other hand, the Irish samples demonstrated that the most abundant taxa were Enterobacteriaceae, *Staphylococcus Listeria welshimeri*, *Propionibacterium* and *Pseudomonas* (10) (Table I).

Bacteria in non-cancerous adjacent tissue and cancer tissue of BC women compared to breast tissue of healthy women. Xuan *et al* (13) reported that in addition to the presence of different species between tumor and normal breast tissues, tumor tissues seem to have a dramatic reduction in bacterial load compared to normal tissues. In addition, the inverse correlation between bacterial load and tumor stage implies that bacterial load could be used in conjunction with current methods to monitor the progression of BC and to facilitate staging of the disease. However, further research is warranted to evaluate and determine a possible role of the bacterial load in the diagnosis or staging of BC. Furthermore, in the study by Xuan *et al* (13) it was observed that one third of antibacterial genes were downregulated in tumor tissues compared to healthy tissues. These results suggest that bacteria may play a role in regulating immune responses within healthy breast tissue and these responses may be disrupted during tumorigenesis (13).

Following on from previous research, Urbaniak *et al* (14) used 16S rRNA amplicon sequencing and showed that the bacterial profiles differ between tissues from healthy controls (women undergoing breast reduction or breast enhancement surgeries), normal adjacent tissue from women with BC and tumor tissue from women with BC. The researchers found that women with BC had higher relative abundances of *Bacillus,* Enterobacteriaceae and *Staphylococcus,* among others. The researchers also reported that *Escherichia coli* (a member of the Enterobacteriaceae family) and *Staphylococcus epidermidis*, isolated from BC patients, were shown to induce DNA double-stranded breaks in HeLa cells using the histone-2AX (H2AX) phosphorylation assay. They also found that microbial profiles are similar between normal adjacent tissue and tissue sampled directly from the tumor in BC women. Interestingly they also found that *Lactococccus*, *Streptococcus* and *Prevotella* were higher in the tissues of healthy women compared to BC patients (Table I). Interestingly, all of the aforementioned bacteria have shown to confer some protective properties against BC. *Lactococcus* spp. is known to have anti-carcinogenic properties through potentially activating

Table I. Summary of the main bacteria found in healthy breast tissue, non-cancerous adjacent tissue and breast cancer (BC) tissue.

Main bacteria in normal breast tissue	Study participants	Authors (Refs.)
Most abundant Phyla: *Proteobacteria* *Firmicutes* *Actinobacteria* Most abundant Taxa in Canadian samples: *Bacillus* (Phylum: *Firmicutes*) (11.4%) *Acinectobacter* (Phylum: *Proteobacteria*) (10%) Enterobacteriaceae (Phylum: *Proteobacteria*) (8.3%) *Pseudomonas* (Phylum: *Proteobacteria*) (6.5%) *Staphylococcus* (Phylum: *Firmicutes*) (6.5%) *Propionibacterium* (Phylum: *Actinobacteria*) (5.8%) Comamonadaceae (Phylum: *Proteobacteria*) (5.7%) *Gammaproteobacteria* (Phylum: *Proteobacteria*) (5.0%) *Prevotella* (Phylum: *Bacteroidetes*) (5.0%) Most abundant Taxa in Irish samples: Enterobacteriaceae (Phylum: *Proteobacteria*) (30.8%) *Staphylococcus* (Phylum: *Firmicutes*) (12.7%) *Listeria welshimeri* (Phylum: *Firmicutes*) (12.1%) *Propionibacterium* (Phylum: *Actinobacteria*) (10.1%) *Pseudomonas* (Phylum: *Proteobacteria*) (5.3%)	Total number of participants: 81 women Canadian participants: 43 women • 38 underwent resection for benign (n=11) or cancerous tumors (n=27) • 5 women had no history of BC and underwent breast reduction surgery Irish participants: 38 women • 33 underwent lumpectomies or mastectomies for cancerous tumors • 5 had no history of BC and underwent breast reduction surgery	Urbaniak *et al* (10)

Bacteria in healthy breast tissue or non-cancerous adjacent tissue or tumor tissue of BC patients	Study participants	Authors (Refs.)
Increased levels of bacteria in breast tissue of healthy women: *Lactococcus* (Phylum*: Firmicutes*) *Streptococcus* (Phylum: *Firmicutes*) *Prevotella* (Phylum: *Bacteroidetes*) Increased levels of bacteria in non-cancerous adjacent tissue and tumor tissue in BC patients: *Bacillus* (Phylum: *Firmicutes*) Enterobacteriaceae (Phylum: *Proteobacteria*) (e.g., *Escherichia coli*) *Staphylococcus* (Phylum: *Firmicutes*)	Total number of participants: 81 women Tissue samples were obtained from: • 58 women who had lumpectomies or mastectomies for benign (n=13) or cancerous (n=45) tumors • 23 women who underwent breast reductions or breast enhancement surgeries (healthy control)	Urbaniak *et al* (14)
Increased levels in nipple aspirate fluid (NAF) of healthy women: Sphingomonadaceae (Phylum: *Proteobacteria*) Increased levels of nipple aspirate fluid (NAF) of BC survivors: *Alistipes* (Phylum: *Bacteroidetes*)	Total number of participants: 48 women undergoing skin sampling Tissue samples were obtained from: • 25 women with a history of ductal breast carcinoma with at least one intact nipple (metastatic breast cancer patients were excluded) • 23 healthy control	Chan *et al* (15)
Bacteria found at increased levels in malignant vs. benign disease: *Fusobacterium* (Phylum: *Fusobacteria*) *Atopobium* (Phylum: *Actinobacteria*) *Gluconacetobacter* (Phylum: *Proteobacteria*) *Hydrogenophaga* (Phylum: *Proteobacteria*) *Lactobacillus* (Phylum: *Firmicutes*)	Total number of participants: 33 women undergoing non-mastectomy breast surgery for benign disease (n=16) or cancer (n=17) Tissue samples were obtained from 33 women with benign disease (n=16) and cancer (n=17) and were compared to 28 adjacent normal tissues from the patients with benign disease without atypia (n=13) and from patients with invasive breast cancer (n=15)	Hieken *et al* (17)

Table I. Continued.

Bacteria in healthy breast tissue or non-cancerous adjacent tissue or tumor tissue of BC patients	Study participants	Authors (Refs.)
Increased levels of bacteria in normal breast tissue: *Actinobacteria* Increased levels of bacteria in BC tissue samples: *Proteobacteria*	Total number of participants: Tissue samples were obtained from 668 women and included in the TCGA data portal • 668 tumor tissues were derived from the TCGA data portal • 72 non-cancerous adjacent tissues were derived from the TCGA data portal	Thompson *et al* (18)
No significant difference either in the overall diversity or in the number of microbes between cancerous and healthy simples Increased levels of *Methylobacterium* (Phylum: *Proteobacteria*) in tissues of healthy individuals compared to BC patients	Total number of participants: 78 women Tissue samples were obtained from: • 57 women who underwent mastectomy due to invasive breast carcinoma (samples of 15 women could not be obtained) • 21 women underwent cosmetic procedures such as bilateral reduction mammoplasty and mastopexy (sample of 1 woman could not be obtained)	Wang *et al* (20)
Increased levels of bacteria in tumor tissues of BC women compared to tissues of healthy women: *Escherichia coli* (Phylum: *Proteobacteria*) *Bacillus cereus* (Phylum: *Firmicutes*)	Total number of participants: 8 women undergoing breast surgery Tissue samples were collected from: • 58 women who had lumpectomies or mastectomies for benign (n=13) or cancerous (n=45) tumors • 23 were healthy controls who underwent breast reductions or enhancements	Urbaniak *et al* (14)

natural killer (NK) cells and enhancing cellular immunity, thus being potentially protective against BC. *Streptococcus*, especially *S. thermophilus* has also been shown to have protective properties against cancer by producing anti-oxidant metabolites that neutralize reactive oxygen species and thus protect the host from DNA damage. In addition, *Prevotella* is able to produce the short-chain fatty acid (SCFA) propionate, which has shown to have anti-inflammatory properties (14).

In another study, Chan *et al* (15) investigated the composition of nipple aspirate fluid (NAF) on BC survivors and reported a higher level of β-glucuronidase levels as well as a higher abundance of *Alistipes* in the NAF of BC survivors compared to healthy controls. On the other hand, they found an unclassified genus of the Sphingomonadaceae family in the NAF of healthy individuals. Additionally, they also found *Sphingobium yanoikuyae* to be more abundant in normal breast tissue when compared to ER^+ breast tumor tissue (Table I). While the benefits of Sphingomonadaceae are not well known, this family has also been known to break down aromatic hydrocarbons as well as polycyclic aromatic hydrocarbons, both of which are known to be associated with BC (15,16). Due to their ability to metabolize aromatic hydrocarbons, they may confer protective properties against BC, as they were only observed in NAF of healthy breast tissue. However, more research is warranted to confirm this association (15).

Hieken *et al* (17) explored the different microbiota composition between benign vs. malignant breast tumor tissue. The study reported significant differences in the breast tissue microbiome between women with benign vs. women with malignant disease. Specific genus-level taxa that were significantly enriched in breast tissue from women with malignant disease included *Fusobacterium*, *Atopobium*, *Gluconacetobacter, Hydrogenophaga* and *Lactobacillus* (17) (Table I).

In a study by Thompson *et al* (18), the researchers aimed to compare the microbiota in BC tissues compared to non-cancerous adjacent tissues. They found *Proteobacteria* to be enriched in breast tumor tissue samples while *Actinobacteria* were found in abundance in non-cancerous adjacent tissues. In addition, Thompson *et al* (18) found *E. coli* to be prevalent in cancerous and adjacent non-cancerous tissues but with a higher abundance in non-cancerous adjacent tissue of BC women (Table I). This was inconsistent with the observations by Urbaniak *et al* (14) who reported higher levels of *E. coli* in BC compared to healthy controls. Thompson *et al* (18) also observed an increase in *Streptococcus pyogenes,* in tumor breast tissue samples among others. Other recent studies have also established a relationship between *Streptococcus* spp. and β-glucuronidase/β-glucosidase enzymes that are responsible for promoting the re-circulation of estrogen by breaking down the estrogen-glucoronate conjugate (18,19). Therefore,

S. pyogenes may increase systemic estrogen levels which have been continuously proven to increase the risk for BC (18) (Table I).

In another study, Wang *et al* (20) observed the microbiome of breast tissue, urine and oral rinse from BC patients and compared them with those of healthy women (women who underwent cosmetic procedures such as bilateral reduction mammoplasty and mastopexy). Unlike previous studies, they found no significant differences either in the overall diversity or in the number of microbes between cancerous and healthy samples. Additionally, tissues from healthy participants were shown to have an increased abundance of *Methylobacterium* while in BC patients the same bacterium was observed to be significantly decreased (20) (Table I).

In addition, Parida and Sharma (16) reported that Enterobacteriaceae (specifically *E. coli*), and *Bacillus cereus* were more abundant in tumor tissues than in normal breast tissues. *E. coli*, (B2 phylotype), has cancer-promoting properties as it has the ability to produce colibacin, a known genotoxin. Colibacin is known to induce double-stranded DNA breaks and has not only been implicated in colorectal cancer but has now also been linked to BC (16). DNA damage induced by microbes may not be enough for tumorigenesis to occur, but if coupled with other molecular errors the result could be detrimental to host health (14). On the other hand, even though *B. cereus* does not necessarily induce DNA damage, it still possesses pro-carcinogenic properties. For example, *B. cereus* is able to metabolize progesterone into 5-alpha-3,20-dione (5aP), which has been seen to be much higher in BC and has been observed *in vitro* to induce BC cell proliferation (14,16).

Some of the limitations of the studies investigating the microbiota in normal and malignant breast tissue are the low number of participants as well as the fact that the microbiota from BC tissues was commonly compared to that of women with benign disease or women that underwent breast reduction or breast augmentation surgeries (Table I). It is questionable whether the tissues assessed by women undergoing breast reduction should be considered as 'healthy.' The potential absence of epithelium or stroma in these fatty tissues could render these not directly comparable to a breast cancer which is comprised predominantly of epithelium and stroma, or healthy breast tissue that has a varying abundance of these cell types.

3. Bacteria found in different BC subtypes

The microbiota of ER$^+$ malignant tumors compared to healthy tissues. Xuan *et al* (13) investigated the potential role of breast microbiota on ER$^+$ BC by comparing the DNA of the microbiota found in the breast tumor tissue compared to the normal adjacent tissue from the same patients and healthy women that underwent reduction mammoplasty. *Sphingomonas yanoikuyae* was the most abundant bacterium found in normal adjacent breast tissue, while the bacterium *Methylobacterium radiotolerans* was abundant in ER$^+$ breast tumor tissues (13). Interestingly, while *S. yanoikuyae* was detected in almost half of normal adjacent tissue samples, it was not detected at all in the paired corresponding tumor tissues, whereas *M. radiotolerans* was detected in both the normal adjacent and the tumor tissue samples. This observation suggests that *S. yanoikuyae* may have a protective function in the breast, potentially due to its ability to express certain ligands that activate important cancer immuno-surveillance mediators (13) (Table II).

The microbiota in ER$^+$ and PR$^+$ malignant tumors compared to ER$^-$ PR$^-$ tumors. Wang *et al* (20) observed that the microbial diversity and abundance was higher in hormone-positive BC than in hormone-negative BC. This could be due to the ability of some microbes to metabolize and regulate the bioavailability of steroid hormones in the former (16,20). Additionally, they found *Methylbacterium* to be significantly decreased in hormone-positive BC when compared to hormone-negative BC (20) (Table II).

The microbiota of HER-2-positive (HER-2$^+$), hormone receptor-positive (PR$^+$/ER$^+$), triple-negative (PR$^-$, ER$^-$, HER-2$^-$) and triple-positive (PR$^+$, ER$^+$, HER-2$^+$) BC. A study by Banerjee *et al* (21) was carried out to investigate the specific microbial signatures in 4 different BC subtypes i.e., HER-2-positive (HER-2$^+$), hormone receptor-positive (PR$^+$/ER$^+$), triple-negative (PR$^-$, ER$^-$, HER-2$^-$) and triple-positive (PR$^+$, ER$^+$, HER-2$^+$) BC. In addition, women who had undergone breast reduction surgery were used as a control. The researchers observed that, among others, *Sphyngomonas* and *Mycobacterium,* were present in all four BC subtypes. Additionally, they found that each subtype had a unique microbial signature; Triple-negative had the least complex microbial signature, while PR$^+$/ER$^+$ was associated with the most complex microbial signature. Additionally, higher levels of *Brevundimonas* were noted in PR$^+$/ER$^+$ and in triple-positive compared to PR$^-$/ER$^-$ and triple-negative subtypes. In hormone-negative BC (PR$^-$ and ER$^-$) an increased abundance of *Mycobacterium* was observed (21) (Table II). Identifying BC subtype signatures through microbiota composition could pave way for potential BC screening techniques. Nevertheless, it is evident that investigations regarding microbiota residing in normal breast tissue and its role in BC development is still in its infancy, but can potentially have a huge role in developing screening and therapy techniques for BC.

Overall, the limitations of the studies investigating the microbiota composition in different BC subtypes were similar to the studies comparing the microbiota in BC vs. normal tissue; i.e. the number of participants in these studies was low and they used women that underwent cosmetic surgeries as healthy controls (Table II).

4. Microbiota in the gut and its association with BC

Even though microbiota can reside in multiple body habitats, most of the microbial biomass is located in the GI tract and as such it plays a huge role in the maintenance of the host's health as well as in the development of various diseases (22). Therefore, the role that the gut microbiota plays in BC development should also be explored.

Differences in gut microbiota composition between healthy individuals and women with BC. The dysregulation of the gut microbiota has been associated with BC since 1990, where a study found that women with BC had an abundance of multiple different microbes in the GI tract (Table III) (23). Since then,

Table II. Summary of some of the most important bacteria found in different breast cancer (BC) subtypes.

Bacteria found in normal adjacent tissues compared to ER^+ breast tumors of BC patients	Study participants	Authors (Refs.)
Most abundant bacterium in normal adjacent tissues of BC patients: *Sphingomonas yanoikuyae* Most abundant bacterium in ER^+ breast tumor tissues: *Methylbacterium radiotolerans*	Total number of participants: 91 women Tissues samples were collected from: • 65 women with ER^+ BC • 26 healthy women that underwent reduction mammoplasty with no evidence of BC	Xuan *et al* (13)
Microbiota of ER^+ and PR^+ malignant tumors vs. ER^- PR^- tumors	**Study participants**	**Authors (Refs.)**
Higher microbial diversity and abundance in hormone-positive BC vs. hormone-negative BC Increased levels of bacterium in hormone-negative BC compared to hormone positive BC: *Methylbacterium*	Total number of participants: 78 women Tissues samples were collected from: • 57 women who underwent mastectomy due to invasive breast carcinoma (samples of 15 women could not be obtained) • 21 women who underwent cosmetic procedures such as bilateral reduction mammoplasty and mastopexy (sample of 1 woman could not be obtained)	Wang *et al* (20)
Microbiota of HER-2-positive ($HER\text{-}2^+$), hormone receptor-positive (PR^+/ER^+), triple-negative (PR^-, ER^-, $HER\text{-}2^-$) and triple-positive (PR^+, ER^+, $HER\text{-}2^+$) BC	**Study participants**	**Authors (Refs.)**
Microbiota found in all four BC subtypes: *Sphyngomonas* *Mycobacterium* Higher levels in PR^+/ER^+ and triple-positive compared to PR^-/ER^- and triple-negative subtypes: *Brevundiomanas* Increased levels of bacterium in PR^- and ER^- BC compared to ER^+PR^+ tissues: *Mycobacterium*	Total number of participants: 168 women Tissue samples were collected from: • 50 women with hormone receptor-positive breast cancer • 34 women with $HER\text{-}2^+$ breast cancer samples • 24 women with triple-positive breast cancer samples • 40 women triple-negative breast cancer samples • 20 women who underwent breast reduction surgery Due to HIPAA regulations that the study was subjected to, there was no information on the type of treatment that the individuals with BC received.	Banerjee *et al* (21)

more recent studies have continuously showed that the gut microbiome composition does indeed change in women with BC compared to those without (22).

A more recent study by Zhu *et al* (24) showed that the composition and functions of the gut microbial community differ between post-menopausal BC patients and post-menopausal healthy controls. In this study, the researchers performed a comprehensive shotgun metagenomic analysis of 18 pre-menopausal BC patients, 25 pre-menopausal healthy controls, 44 post-menopausal BC patients and 46 post-menopausal healthy controls. The results of the study showed that the microbial diversity was higher in BC patients than in controls. Relative species abundance in gut microbiota did not differ significantly between pre-menopausal BC patients and pre-menopausal controls. However, the relative abundance of 45 species differed significantly between post-menopausal BC patients and post-menopausal controls: 38 species were enriched in post-menopausal BC patients

Table III. Summary of the intestinal microbiota found in women with breast cancer (BC), post-menopausal women with BC, and healthy post-menopausal women.

Bacteria found in the gut of women with BC	Study participants	Authors (Refs.)
Escherichia coli *Clostridium* *Enterobacterium* *Lactobacillus* *Bacteroides*	Total number of participants: 66 women Faeces samples were collected from: • 18 women with BC • 18 women with uterine myoma • 30 healthy women	Minelli *et al* (23)
Bacteria found in the gut of healthy post-menopausal women vs. post-menopausal women with BC	**Study participants**	**Authors (Refs.)**
Bacteria found at increased levels in the gut of post-menopausal women with BC compared to healthy post-menopausal women: *Escherichia coli* *Klebsiella sp_1_1_55* *Prevotella amnii* *Enterococcus gallinarum* *Actinomyces* sp. *HPA0247* *Shewanella putrefaciens* *Erwinia amylovora* Bacteria found at decreased levels in the gut of post-menopausal women with BC compared to healthy post-menopausal women: *Eubacterium eligens* *Lactobacillus vaginalis* Bacteria found at increased levels in the gut of healthy post-menopausal women compared to post-menopausal women with BC: *Eubacterium eligens* *Roseburia inulinivor*ans	Total number of participants: 133 women Faeces samples were collected from: • 18 pre-menopausal women with breast cancer • 25 pre-menopausal healthy controls 44 post-menopausal women with breast cancer • 46 post-menopausal healthy controls None of the BC patients received any form of chemotherapy, radiation or surgery before fecal sample collection	Zhu *et al* (24)

including *Escherichia coli, Klebsiella sp_1_1_55, Prevotella amnii, Enterococcus gallinarum, Actinomyces* sp. *HPA0247, Shewanella putrefaciens*, and *Erwinia amylovora*. On the other hand, 7 species were less abundant in post-menopausal BC patients compared to healthy controls including *Eubacterium eligens* and *Lactobacillus vaginalis* (24).

Interestingly Zhu *et al* (24) reported that *Erwina amylovora* and *Shewanella putrefaciens* were shown to have a positive correlation, albeit a weak one, with estradiol, suggesting potential involvement of both microbes in the metabolism of estrogen. Due to their potential involvement in estrogen metabolism, such gut microbiota could be used in the future as novel biomarkers for BC (24) (Table III).

In the same study, Zhu *et al* (24) also observed that *Eubacterium eligens* and *Roseburia inulinivorans* were more abundant in post-menopausal healthy controls than in post-menopausal women with BC. Interestingly, *R. inulinivorans* is a bacterium with known anticarcinogenic properties; i.e. it produces butyrate which has anti-inflammatory properties mainly by inhibiting the activation of nuclear factor (NF)-κB in intestinal epithelial cells through tumor necrosis factor (TNF)-α (24,25). Therefore, a reduction in butyrate in the colon caused by decreased levels of *R. inulinivorans* in post-menopausal women can increase inflammation and therefore increase their risk of BC (24). It was also observed that in both pre-menopausal women and post-menopausal women, genes of the iron transport system were increased, however, in post-menopausal women genes involved in lipopolysaccharide (LPS) biosynthesis were also seen to be increased. Both the iron transport system and LPS biosynthesis increase systemic inflammation and therefore increase the risk for BC (24) (Table III).

Microbial metabolites with anticancer potency in the gut microbiota. It has recently been proposed that the microbiota in the GI tract may act as an endocrine organ, due to the gut's ability to produce various metabolites such as SCFAs and to regulate hormone metabolism, all of which affect distal organs through the blood stream (26). Some of these microbial metabolites have been shown to influence the progression of BC (27). A well-documented microbe metabolite linked to BC is cadaverine, a biogenic amine whose biosynthesis is

decreased in early stage BC (27,28). Cadaverine is considered an anticancer bacterial metabolite because of its ability to suppress the aggressiveness, metastasis and progression of tumor cells. Cadaverine has also been shown to induce the metabolism of tumor cells that have begun glycolysis by decreasing cellular oxygen consumption (27,28). Additionally, other bacterial metabolites such as lithocholic acid (LCA), a secondary bile acid, have been observed to inhibit BC progression by creating oxidative and nitrosative stress (27). More specifically, LCA inhibits epithelial-mesenchymal transition, metastasis and BC progression by activating nuclear factor erythroid 2-related factor 2 (NRF2) and has pro-apoptotic effects on BC tumor cells (27,28).

SCFAs are products of fibre being fermented in the colon by certain bacteria (27). The most predominant SCFAs are butyrate, propionate, and acetate, which are well known for their anticancer effects (27). These metabolites have shown to be protective against cancer as they are involved in mediating cell cycle arrest, and inducing apoptosis (28). Therefore, changing the composition of the gut microbiome will inevitably also change the anti-carcinogenic metabolites being produced, thereby preventing or promoting BC progression (27).

The role of the gut microbiome in estrogen metabolism. As previously discussed, the gut microbiome may be considered an endocrine organ, due partly to its role in estrogen metabolism. The microbiota in the GI tract is known to be one of the most important regulators in estrogen circulation (27). This is especially relevant in hormone-positive BC, as there is an undisputable relationship between endogenous estrogen burden and the development of BC in post-menopausal women (there is a similar relationship with pre-menopausal women but the correlation has not proven to be as strong) (28,29).

Estrogen metabolism in the GI tract is attributed to what is currently being referred to as the estrobalome. The estrobalome is defined as the aggregate of bacterial genes that result in enzymes that can metabolize estrogens (28,30). Free-circulating estrogens are subjected to hepatic first-pass metabolism where they are conjugated and then eliminated via urine or feces (30). However, a significant portion of the conjugated estrogen is reabsorbed back into circulation before it gets excreted. This suggests that bacteria in the gut possess β-glucuronidase/β-glucosidase activity allowing them to deconjugate estrogens, permitting them to become biologically active again and re-enter the circulation (30). The re-circulated estrogens then interact with breast tissue facilitating cellular growth and thus contributing to the initiation and progression of BC, as well as increasing the risk of ER-positive BC in post-menopausal women (27,28). This is especially relevant as certain bacteria that have been previously shown to be abundant in BC are coincidentally also related to an increase in activity of β-glucuronidase/β-glucosidase activity. Such bacteria include *S. pyogenes, Clostridia* spp., *Baccilus* spp. and *E. coli* (18,31). It was also further seen that the relationship between microbiota and estrogen metabolism can be used to predict the risk of BC in post-menopausal women due to the diversity and composition of gut microbiota being associated with patterns of estrogen metabolism (32). Therefore, a change in microbiota composition can alter normal host function, favoring BC development.

The role of the gut microbiome in breast cancer metastasis. A study by Rosean *et al* (33) used a mouse model for hormone receptor-positive (HR^+) mammary cancer in order to investigate whether commensal dysbiosis, more specifically, pre-existing dysbiosis in the microbiome can influence the progression and outcome in HR^+ BC. The researchers demonstrated that a pre-established disruption of commensal homeostasis results in enhanced circulating tumor cells and subsequent dissemination to the tumor-draining lymph nodes and in distant sites such as the lungs; all of which promote the poor outcomes seen in HR^+ BC. Due to the results being so promising, Buchta Rosean *et al* (33) concluded that commensal dysbiosis may have therapeutic implications and could potentially be used as a biomarker for HR^+ BC (33). The study by Buchta Rosean *et al* (33) currently presents one of the strongest evidence to date that gut dysbiosis may promote breast cancer metastasis, and further studies should be conducted to investigate this association.

Diet and microbiota in BC. Diet is a well-established modifiable risk factor for BC, as changes in diet have been linked with increasing overall health and survival rates in patients that already have BC (34). A diet rich in fat has been shown to affect β-glucuronidase activity by increasing bile acid secretion, thus promoting the growth of *E. coli* and *Enterobacter,* both of which are potent β-glucuronidases and thus contribute to the estrogen burden (30,35). However, a fibre-rich diet can potentially be onco-protective; soluble fibre is fermented by SCFAs and creates a favorable environment for beneficial bacteria such as *Bifidobacterium* (30).

5. Probiotic therapies for BC

Due to the newly established role that the microbiota potentially plays in the development of BC, it is important to explore how interventions that affect the composition of the microbiome (e.g., via the use of probiotics) may affect the process of breast carcinogenesis. The most common microorganisms used in probiotics are lactic acid bacteria (LAB) which have been shown to have multiple health benefits for the host (36). Various other studies have been conducted both *in vitro* and *in vivo* demonstrating the effects of probiotics on BC (Table IV).

Studies in cell lines. Hassan *et al* (37) investigated the role of *Enterococcus feacalis* and *Staphylococcus hominis in vitro*. They isolated these bacteria from the breast milk of healthy women and conducted the rest of the investigation in various antibiotic-free media. They used three different cell forms of the bacteria (live, heat-killed and cytoplasmic fractions) and found that all three forms of the bacteria caused a significant decrease in cell proliferation via induction of both cell cycle arrest and apoptosis in BC cells such as in MCF-7 cells (37).

Another study by Esfandiary *et al* (38) investigated the effect of the probiotic strains *Lactobacillus crispatus SJ-3C-US* and *Lactobacillus rhamnosus GG* on MDA-MB-231 cell lines. They found that both strains induced cytotoxic effects on triple-negative BC cells and that *L. rhamnosus* especially, downregulated genes associated with hypoxia, such as *SLCA2A1* (38), which is known to code for the production

Table IV. Summary of *in vitro* and animal studies investigating the role of probiotics in breast cancer (BC).

In vitro studies

Probiotic strain	Cell lines	Effect on BC	Authors (Refs.)
Enterococcus feacalis *Staphylococcus hominis*	*In vitro* (MCF-7 cell line)	↑Apoptosis ↓Cell proliferation	Hassan *et al* (37)
Lactobacillus crispatus SJ-3C-US *Lactobacillus rhamnosus GG*	*In vitro* (MDA-MB-231 cell line)	↑Cytotoxic effects on triple-negative BC cells *L. rhamnosus* showed a greater downregulation of hypoxia-associated genes (*HF1α*, *HSP90*, *SLCA2A1*)	Esfandiary *et al* (38)
Lactobacillus plantarum *Lactobacillus acidophilus*	*In vitro* (MCF-7 cell line)	Inhibition of growth of cell lines *L. plantarum* showed the highest cytotoxic effect on the MCF-7 cell line	Bharti *et al* (39)
Lactobacillus plantarum	*In vitro* (MDA-MB-231 cell line)	Downregulation of the NF-κB pathway leading to apoptosis of ER-negative BC cell lines	Kadirareddy *et al* (40)

Animal studies

Probiotic strain	Animal model	Effect on BC	Authors (Refs.)
Lactobacillus helveticus R389	*In vivo* BALB/c mouse model	↑IL-10 ↓IL-6 Induction of cellular apoptosis Delayed BC development	de Moreno de LeBlanc *et al* (41)
Lactobacillus reuteri ATCC-PTA-6475	*In vivo* mice models: Swiss models fed 'Westernized chow' and erbB2 (HER2) mice supplemented with *L. reuteri*	Delay in onset of pre-neoplastic features Activates $CD4^+$ and $CD25^+$ cells	Lakritz *et al* (42)
Lactobacillus casei	*In vivo* orally administered, BALB/c mouse model	↓Growth rate of tumors ↑Survival compared with the control ↑IFN-γ and IL-12 Affects the stimulation of Th1 cytokine production ↑NK cell cytotoxicity in spleen cells of mice with invasive ductal carcinoma.	Soltan Dallal *et al* (45)
Lactobacillus brevis (enriched with selenium nanoparticles)	*In vivo* orally administered, BALB/c mouse model	Improved disease prognosis in mice with highly metastatic breast tumors ↑NK cytotoxicity ↑IFN-γ and IL-17 ↓Metastasis to the liver	Yazdi *et al* (46)
Lactobacillus acidophilus	*In vivo* orally administered, BALB/c mouse model	↑Increased survival time of BALB/c mice ↑IFN-γ ↓IL-4	Imani Fooladi *et al* (36)
Lactobacillus acidophilus *Lactobacillus casei* *Lactococcus lactis*	*In vivo* BALB/c mouse model with triple-negative 4T1 BC cells fed Kefir water	↓Cell mitosis ↑Cancer cell apoptosis Inhibition of inflammation in tumor environment Modulation of immune system Regulation of angiogenesis proteins and genes	Zamberi *et al* (47)

of GLUT 1. By downregulating the production of GLUT 1, metastasis can be prevented through the MMP-2 and JNK pathways (9).

Lactobacillus plantarum has also shown promise in various studies, such as in the study conducted by Bharti *et al* (2015), where the highest cytotoxic effect on MCF-7 cancer cell lines was seen with the administration of *L. plantarum* (39). Kadirareddy *et al* (40) demonstrated that *L. plantarum* induced apoptosis in MDA-MB-231 cells by the production of conjugated linoleic acid which downregulated the NF-κB pathway causing proteasome degradation of IκBα, inhibition of p65 nuclear translocation, release of cytochrome *c* from the mitochondria and finally overexpression of Bax protein (40). These are but a few examples of *in vitro* studies in which probiotics strains have been used to investigate their potential role in breast cancer prevention.

Studies in animal models. The efficacy of orally administered probiotics in tumor cell inhibition was also confirmed by animal studies. For example, in an *in vivo* mouse model, the intake of milk fermented with *Lactobacillus helveticus R389* played a role in delaying breast tumor cell development. The latter was associated with increased regulatory cytokines such as interleukin (IL)-10 and decreased IL-6 levels in serum and mammary mouse cells, as well as by induction of cellular apoptosis. IL-10 is known to induce the expression of TNF-α related apoptosis-inducing ligand (TRAIL) and death receptor 4 (DR4), as well as recruiting lymphocytes, all of which contribute to the apoptosis of mammary cells (41).

Additionally, Lakritz *et al* (42) observed the effect that *Lactobacillus reuteri* has on the development of mammary cancer in two different mouse models. In the first model, Swiss mice were fed Westernized chow (mimicking a westernized diet) thereby putting the mice at increased risk for the development of mammary tumors. The second animal model used was FVB strain erbB2 (HER2) mutant mice which are genetically susceptible to mammary tumors. Both of these mouse models were exposed to *L. reuteri* through their water supplies and compared to controls. Interestingly, exposure to *L. reuteri* in the experimental groups was found to be sufficient in delaying the onset of pre-neoplastic features in both models. This hypothesis is consistent with other research studies supporting that lactic acid-producing bacteria decrease the risk of BC in women (42-44). In addition to inhibiting early stage tumorigenesis, *L. reuteri* has also been reported to increase the sensitivity of BC cells to apoptosis (27,42). This study demonstrates that by modulating the tumor microenvironment through the use of probiotics, one can potentially regulate systemic immune cell responses impacting cancer progression. Such findings may have future implications in the public health sector since such administrations could be used to decrease BC risk (42).

Additionally, a study conducted by Soltan Dallal *et al* (45) investigated the effect that orally administrated *Lactobacillus casei* has on NK cytotoxicity and production of cytokines in the spleen cells of BALB/C mice that have invasive ductal carcinoma. The results showed that *L. casei* was able to decrease the growth rate of tumors, prolong survival, stimulate Th1 cytokine production and increase NK cell cytotoxicity in the spleen cells of mice with invasive ductal carcinoma demonstrating its potential in BC therapy (45).

Yazdi *et al* (46) conducted a unique study in which BALB/c mice were administered with *Lactobacillus brevis* that was enriched with selenium nanoparticles. The results showed that this not only improved the prognosis of mice with highly metastatic BC, but it also increased NK cytotoxicity, IFN-γ and IL-17 as well as decreased tumor metastasis to the liver (46). Subsequently, another study confirmed that the oral administration of *Lactobacillus acidophilus* significantly increased the survival time of BALB/c mice with mammary tumors. *L. acidophilus* promoted the proliferation of immune cells and increased the production of IFN-γ and decreased the production of IL-4. IFN-γ is crucial in the activation of NK cells, which are known to be the first line defence against tumor cells (36). High levels of IFN-γ not only increase NK cytotoxicity but may also increase tumor cell visibility [through its role in major histocompatability complex (MHC) expression], as well as inhibiting intra-tumoral angiogenesis (36).

In another study by Zamberi *et al* (47), BALB/c mice were injected with triple-negative 4T1 BC cells, and were treated with Kefir water that is naturally enriched with *L. acidophilus, L. casei,* and *Lactobacillus lactis*. The results showed a decrease in cell mitosis and an increase in apoptosis in mice that drank the water enriched with the specific probiotic strains. In addition, they found that the various strains also inhibited the inflammation in the tumor environment, modulated the immune system and regulated genes and proteins of angiogenesis (47). Moreover, Zamberi *et al* (47) and the Soltan Dallal *et al* (45) demonstrate how especially *L. casei*, not only decreased the growth of tumors, but also increased apoptosis and inhibited inflammation, further illustrating the emerging importance of this microbe in BC prevention and possibly treatment.

Limitations and future directions of studies with probiotics. While the aforementioned studies presented promising results, it should be noted that some of the studies had limitations. For example, most of the studies were either conducted *in vitro* or using mouse models and therefore could not investigate the different factors that affect the diversity of the microbiome in humans, such as diet, age or genetic background (48).

In their very comprehensive perspective review Suez *et al* (48), reports that even though probiotics are commonly used by the general public there are still conflicting clinical results for many probiotic strains and formulations. Furthermore, the researchers stress the importance of how large scale randomized blind clinical trials should be designed to assess a number of questions such as gut colonization by probiotics, the importance of specific bacterial strains and their interactions with the indigenous microbiome and the safety and impact on the host.

Evidence from the literature discusses how the safety and adverse effects of probiotics have neither been extensively researched nor correctly documented in most of the clinical trials. In fact, it has been observed that the use of probiotics in critically ill adults in intensive care units, young infants, or neonates with a very low birth weight is associated with an increased risk of infection. In addition, patients that underwent antibiotic treatments showed increased colonization by probiotic strains, which was linked to persistent dysbiosis induced by long term probiotics (48). A systematic review by

Table V. Summary of ongoing clinical trials regarding breast cancer (BC) and microbiota.

Study ID	Type of study	Year study started	Expected year of completion	Status	Aim	Probiotic/Drug used	(Refs.)
NCT03358511	Interventional study	2017	2020	Completed	To investigate if probiotics are able to affect the body's immune system on BC. All subjects that had operable stage I-III breast adenocarcinoma were given Primal Defense Ultra® Probiotic formula* for 2-4 weeks before surgery	*Saccharomyces boulardii, Lactobacillus plantarum, Bacillus subtilis, Bifidobacterium lactis, Bifidobacterium bifidum, Lactobacillus rhamnosus, Bifidobacterium breve, lactobacillus casei, Lactobacillus salivarius, Lactobacillus acidophilus, Lactobacillus brevis, Bifidobacterium longum*, and *Lactobacillus paracasei*	Clinicaltrials.org (50)
NCT03586297	Observational prospective cohort study	2017	2022	Recruiting	To observe the gut and intratumoral micro biome composition as well as antitumor immune responses in women with triple-negative BC receiving standard of care neoadjuvant therapy.	No probiotics used	Clinicaltrials.org (52)
NCT03885648	Observational case-control, cross sectional study with 200 participants	2018	2022	Recruiting	To investigate if the risk of breast cancer in women with stage I and II is associated with the composition of the gut/mammary microbiota and if exposure to environ mental contaminants could contribute to the alteration of the microbiota.	No probiotic was used in this study	Clinicaltrials.org (51)
NCT04138979	Observational cross-sectional case control study	2019	2020	Recruiting	To explore the link between gut microbiota and chemotherapy used in BC	Cyclophosphamide	Clinicaltials.org (53)
NCT03290651	Interventional, double blind randomized, placebo controlled pilot trial	2019	2021	Recruiting	To test the hypothesis that by taking an oral probiotic, it can lead to the displacement of harmful bacteria in the breast and to reduce inflammation linked to BC	Probiotic contains *Lactobacillus rhamnosus GR-1* and *Lactobacillus reuteri RC-14*	Clinicaltrials.org (54)

Table V. Continued.

Study ID	Type of study	Year study started	Expected year of completion	Status	Aim	Probiotic/Drug used	(Refs.)
NCT04139993	Interventional non-randomized study	2020	2025	Not yet recruiting	To evaluate the safety and tolerability of a novel oral Microbiome Restoration Therapy™, RBX7455 and to evaluate intratumoral immunomodulatory effects in patients with stage I-III BC before undergoing definitive surgery	Novel oral Microbiome Restoration Therapy™: RBX7455	Clinicaltrials.org (55)

Befeta *et al* (49), reported that most clinical trials did not report the adverse effects accurately and support how the increasing use of probiotics both in clinical practice and over-the-counter increases the urgency to design and conduct clinical trials that adequately report and document any adverse effects caused by probiotics (49).

Therefore, it is important to note that research on the effects of probiotics on BC is still at its infancy and larger and more elaborate studies should be conducted to conclude if they have a protective long-term anticancer effects and to find the optimum dosage, strain and regimen for a treatment with probiotics to be effective.

6. Conclusion

In recent years, the microbiota has proven to play an important role in diseases and has sparked interest regarding its involvement in the development, management and screening of BC. Preliminary studies have provided evidence showing diverse microbiota belonging to the phyla *Proteobacteria, Firmicutes* and *Actinobacteria* being present in normal breast tissue (10,14,15). Some bacteria were found to be more abundant in malignant breast tissue compared to benign breast tissue and these include, among others, *Fusobaterium, Atopodium, Gluconacetobacter, Hydrogenophaga, Lactobacillus* (17), *E. coli* and *B. cereus* (16) (Table I). On the other hand, *Lactococcus, Streptococcus, Prevotella and S. yanoikuyae* were more abundant in healthy breast tissue compared to malignant tissue, suggesting that they may confer various protective effects against BC (13,14) (Tables I and II).

In addition, differences in the microbiota were reported between different types of BC. *Sphyngomona* and *Mycobacterium* were reported in all four BC subtypes (HER-2-positive (HER-2$^+$), hormone receptor-positive (PR$^+$/ER$^+$), triple-negative (PR$^-$, ER$^-$, HER-2$^-$) and triple-positive (PR$^+$, ER$^+$, HER-2$^+$) BC), *Brevudioomonas* was present at higher levels in PR$^+$/ER$^+$ and triple-positive BC compared to PR$^-$/ER$^-$ and triple-negative subtypes, whereas *Mycobacterium* was found at increased levels in PR$^-$ and ER$^-$ BC compared to ER$^+$PR$^+$ tissues (21). In addition, a study has reported that *Methylobabacterium radiotolerans* was the most abundant bacterium in ER$^+$ tissues (13), whereas another study supported that *Methylbacterium* was found at increased levels in hormone-negative BC compared to hormone-positive BC (20) (Table II).

Overall, evidence in the literature has suggested an association between bacterial species and breast cancer pathogenesis. However, it should be noted that these studies were association studies and in addition suffered from a few limitations such as the low participant number and the use of tissues from women that underwent breast reduction or augmentation surgeries as normal tissue controls in some of the studies (Tables I and II). Therefore, care should be taken in interpreting the findings from these studies.

Other studies have also provided evidence for an association between gut microbiota and BC. Certain bacteria such as *Escherichia coli, Klebsiella sp_1_1_55, Prevotella amnii, Enterococcus gallinarum, Actinomyces* sp. *HPA0247, Shewanella putrefaciens, Erwinia amylovora* were seen to be present at higher levels in post-menopausal women with

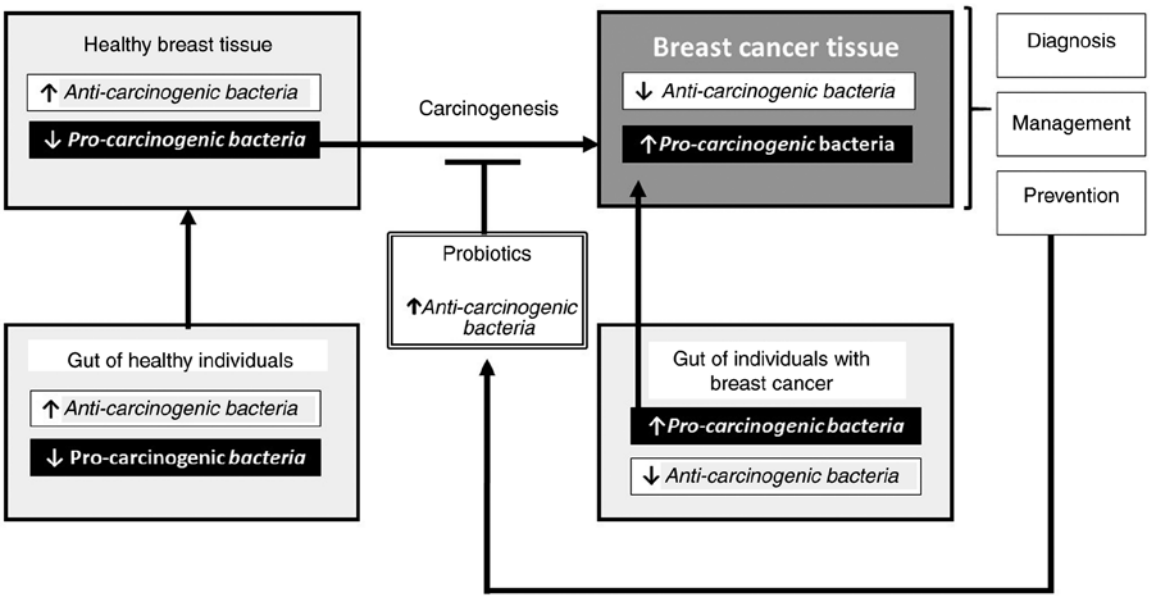

Figure 1. Evidence in the literature supports an association between dysbiosis and breast cancer development. This dysbiosis may be the result of increased levels of pro-carcinogenic bacteria and lower levels of anti-carcinogenic bacteria in the breast cancer tissue contributed by the gut microbiome. Understanding of the identity of the pro-carcinogenic microbes and the molecular mechanisms involved in the dysbiosis of breast cancer may contribute to improved diagnosis (e.g., via the use of biomarkers), management (via the targeting of specific pathways) and prevention (e.g., via the use of anti-carcinogenic microbes in probiotics).

BC compared to healthy post-menopausal women (24). On the other hand, bacteria such as *Eubacterium eligens* and *Lactobacillus vaginalis* were found to be at decreased levels in the gut of post-menopausal women with BC compared to healthy menopausal women (24) (Table III). Similarly, to the studies investigating the microbiota in normal and BC tissue, only a few studies investigated the association between gut microbiota and BC and these studies used a small number of participants (Table III) again supporting the notion that caution should be taken in interpreting the results of these studies.

Evidence from the literature also suggests an association between gut microbiota and BC progression possibly through metabolites that certain bacteria produce, as well as their role in dysregulating estrogen metabolism. Currently, we have enough knowledge to confirm that bacterial metabolites are crucial to the molecular pathways of carcinogenesis. These metabolites can either promote carcinogenesis through genotoxins and other metabolites or can be protective, through the production of SCFAs.

At the present time, the situation is still complex and there are discrepancies between studies with regards to the direct association of specific bacteria to BC (13,20), supporting the need for larger randomised controlled studies to be carried out to derive more conclusive results. Furthermore, specific bacteria used in probiotics mainly *Lactobacillus spp* but also *Enterococcus feacalis* and *Staphylococcus hominis* may confer protective advantages against BC as shown in preliminary studies in cell lines and animal models and larger randomised controlled trials will have to be conducted to derive conclusive results (Table IV).

By understanding the identity and the molecular mechanisms regulated by pro-carcinogenic and anti-carcinogenic bacteria, as well as the association between the microbiota in the gut and the breast it will easier to explore new management routes that may be more effective and produce less toxic side effects than current treatments. Additionally, by knowing how bacterial load changes in BC and which specific microbial signatures are present in the different BC subtypes, new methods will be developed to improve screening (i.e. by the use of biomarkers) and consequently prevention of BC (e.g. by the use of probiotics consisting of anti-carcinogenic bacteria) (Fig. 1). This will therefore lead to the development of less invasive and more effective ways to detect BC and as a result, a visible decrease in morbidity and mortality associated with BC should be expected to be seen.

Currently there are various clinical trials ongoing investigating various intervention strategies. For example, an interventional study was created to observe the effect that probiotics have on the immune system of patients suffering from breast adenocarcinoma stage I-III. This study used a probiotic formula that included 13 species of bacteria. Some of these beneficial bacteria include *Lactobacillus plantarum Lactobacillus rhamnosus* and *Lactobacillus casei* (50). Additionally, an observational case-control, cross sectional study has been developed to observe the relationship between BC risk in women with stage I and II and the gut/mammary microbiota, as well as investigating the possible contribution of environmental contaminants in the alteration of the microbiota (51). These are only a few examples of the many clinical trials that are currently in progress. The remaining (52-55) are documented in Table V to investigate the link between BC and the microbiota.

Overall, more research in the field of the association between the microbiota and cancer development will significantly improve the prevention of BC since particular metabolites of the microbiome could be used as biomarkers for screening. In addition, probiotics (especially those of the *Lactobacillus spp.*) could be used in the management of women diagnosed

with BC in order to prevent BC metastasis or relapse (13). Even though such research is still at its infancy, the microbiota both in the mammary tissue and in the gut seem to have a promising future in the management and prevention of BC.

Acknowledgements

Not applicable.

Funding

No funding was used for the development of the current manuscript.

Availability of data and materials

All information included in this review has been documented by relevant references.

Authors' contributions

DT, SED and CC were all involved in the conceptualization of the current manuscript. DT and SED were involved in the literature search and review of the resources that were used in the current manuscript. All authors were involved in the writing and revision of the manuscript and they all approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Authors' information

Dr Constantina Constantinou: Orcid ID: 0000-0001-6167-4023.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F: Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin: Feb 4, 2021 (Epub ahead of print). doi: 10.3322/caac.21660.
2. Siegel RL, Miller KD and Jemal A: Cancer statistics, 2020. CA Cancer J Clin 70: 7-30, 2020.
3. Brown SB and Hankinson SE: Endogenous estrogens and the risk of breast, endometrial, and ovarian cancers. Steroids 99: 8-10, 2015.
4. Fernández MF, Reina-Pérez I, Astorga JM, Rodríguez-Carrillo A, Plaza-Díaz J and Fontana L: Breast cancer and its relationship with the microbiota. Int J Environ Res Public Health 15: 1747, 2018.
5. Momenimovahed Z and Salehiniya H: Epidemiological characteristics of and risk factors for breast cancer in the world. Breast Cancer (Dove Med Press) 11: 151-164, 2019.
6. da Costa Vieira RA, Biller G, Uermura G, Ruiz CA and Curado MP: Breast cancer screening in developing countries. Clinics (Sao Paulo) 72: 244-253, 2017.
7. Howlader N, Cronin KA, Kurian AW and Andridge R: Differences in breast cancer survival by molecular subtypes in the United States. Cancer Epidemiol Biomarkers Prev 27: 619-626, 2018.
8. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R and Gordon JI: The human microbiome project. Nature 449: 804-810, 2007.
9. Parida S and Sharma D: Microbial alterations and risk factors of breast cancer: Connections and mechanistic insights. Cells 9: 1091, 2020.
10. Urbaniak C, Cummins J, Brackstone M, Macklaim JM, Gloor GB, Baban CK, Scott L, O'Hanlon DM, Burton JP, Francis KP, *et al*: Microbiota of human breast tissue. Appl Environ Microbiol 80: 3007-3014, 2014.
11. Toumazi D and Constantinou C: A fragile balance: The important role of the intestinal microbiota in the prevention and management of colorectal cancer. Oncology 98: 593-602, 2020.
12. Chung YR, Kim HJ, Kim YA, Chang MS, Hwang KT and Park SY: Diversity index as a novel prognostic factor in breast cancer. Oncotarget 8: 97114-97126, 2017.
13. Xuan C, Shamonki JM, Chung A, Dinome ML, Chung M, Sieling PA and Lee DJ: Microbial dysbiosis is associated with human breast cancer. PLoS One 9: e83744, 2014.
14. Urbaniak C, Gloor GB, Brackstone M, Scott L, Tangney M and Reid G: The microbiota of breast tissue and its association with breast cancer. Appl Environ Microbiol 82: 5039-5048, 2016.
15. Chan AA, Bashir M, Rivas MN, Duvall K, Sieling PA, Pieber TR, Vaishampayan PA, Love SM and Lee DJ: Characterization of the microbiome of nipple aspirate fluid of breast cancer survivors. Sci Rep 6: 28061, 2016.
16. Parida S and Sharma D: The power of small changes: Comprehensive analyses of microbial dysbiosis in breast cancer. Biochim Biophys Acta Rev Cancer 1871: 392-405, 2019.
17. Hieken TJ, Chen J, Hoskin TL, Walther-Antonio M, Johnson S, Ramaker S, Xiao J, Radisky DC, Knutson KL, Kalari KR, *et al*: The microbiome of aseptically collected human breast tissue in benign and malignant disease. Sci Rep 6: 30751, 2016.
18. Thompson KJ, Ingle JN, Tang X, Chia N, Jeraldo PR, Walther-Antonio MR, Kandimalla KK, Johnson S, Yao JZ, Harrington SC, *et al*: A comprehensive analysis of breast cancer microbiota and host gene expression. PLoS One 12: e0188873, 2017.
19. Flores R. Shi J, Fuhrman B, Xu X, Veenstra TD, Gail MH, Gajer P, Ravel J and Goedert JJ: Fecal microbial determinants of fecal and systemic estrogens and estrogen metabolites: A cross-sectional study. J Transl Med 10: 253, 2012.
20. Wang H, Altemus J, Niazi F, Green H, Calhoun BC, Sturgis C, Grobmyer SR and Eng C: Breast tissue, oral and urinary microbiomes in breast cancer. Oncotarget 8: 88122-88138, 2017.
21. Banerjee S, Tian T, Wei Z, Shih N, Feldman MD, Peck KN, DeMichele AM, Alwine JC and Robertson ES: Distinct microbial signatures associated with different breast cancer types. Front Microbiol 9: 951, 2018.
22. Parida S and Sharma D: The microbiome-estrogen connection and breast cancer risk. Cells 8: 1642, 2019.
23. Minelli EB, Beghini AM, Vesentini S, Marchiori L, Nardo G, Cerutti R and Mortani E: Intestinal microflora as an alternative metabolic source of estrogens in women with uterine leiomyoma and breast cancer. Ann N Y Acad Sci 595: 473-479, 1990.
24. Zhu J, Liao M, Yao Z, Liang W, Li Q, Liu J, Yang H, Ji Y, Wei W, Tan A, *et al*: Breast cancer in postmenopausal women is associated with an altered gut metagenome. Microbiome 6: 136, 2018.
25. Inan MS, Rasoulpour RJ, Yin L, Hubbard AK, Rosenberg DW and Giardina C: The luminal short-chain fatty acid butyrate modulates NF-kappaB activity in a human colonic epithelial cell line. Gastroenterology 118: 724-734, 2000.
26. Heinken A and Thiele I: Systematic prediction of health-relevant human-microbial co-metabolism through a computational framework. Gut Microbes 6: 120-130, 2015.
27. Eslami-S Z, Majidzadeh-A K, Halvaei S, Babapirali F and Esmaeili R: Microbiome and breast cancer: New role for an ancient population. Front Oncol 10: 120, 2020.
28. Zhang Z, Tang H, Chen P, Xie H and Tao Y: Demystifying the manipulation of host immunity, metabolism, and extraintestinal tumors by the gut microbiome. Signal Transduct Target Ther 4: 41, 2019.
29. Mani S: Microbiota and breast cancer. Prog Mol Biol Transl Sci 151: 217-229, 2017.
30. Komorowski AS and Pezo RC: Untapped '-omics': The microbial metagenome, estrobolome, and their influence on the development of breast cancer and response to treatment. Breast Cancer Res Treat 179: 287-300, 2020.

31. Flores R, Shi J, Gail MH, Gajer P, Ravel J and Goedert JJ: Association of fecal microbial diversity and taxonomy with selected enzymatic functions. PLoS One 7: e39745, 2012.
32. Fuhrman BJ, Feigelson HS, Flores R, Gail MH, Xu X, Ravel J and Goedert JJ: Associations of the fecal microbiome with urinary estrogens and estrogen metabolites in postmenopausal women. J Clin Endocrinol Metab 99: 4632-4640, 2014.
33. Buchta Rosean C, Bostic RR, Ferey JCM, Feng TY, Azar FN, Tung KS, Dozmorov MG, Sirmnova E, Bos PD and Rutlowski MR: Preexisting commensal dysbiosis is a host-intrinsic regulator of tissue inflammation and tumour cell dissemination in hormone receptor-positive breast cancer. Cancer Res 79: 3662-3675, 2019.
34. Malik SS, Saeed A, Baig M, Asif N, Masood N and Yasmin A: Anticarcinogenecity of microbiota and probiotics in breast cancer. Int J Food Prop 21: 655-666, 2018.
35. Yang J, Tan Q, Fu Q, Zhou Y, Hu Y, Tang S, Zhou Y, Zhang J, Qiu J and Lv Q: Gastrointestinal microbiome and breast cancer: Correlations, mechanisms and potential clinical implications. Breast Cancer 24: 220-228, 2017.
36. Imani Fooladi AA, Yazdi MH, Pourmand MR, Mirshafiey A, Hassan ZM, Azizi T, Mahdavi M and Soltan Dallal MM: Th1 cytokine production induced by *Lactobacillus acidophilus* in BALB/c Mice bearing transplanted breast tumor. Jundishapur J Microbiol 8: e17354, 2015.
37. Hassan Z, Mustafa S, Rahim RA and Isa NM: Anti-breast cancer effects of live, heat-killed and cytoplasmic fractions of Enterococcus faecalis and *Staphylococcus hominis* isolated from human breast milk. In Vitro Cell Dev Biol Anim 52: 337-348, 2016.
38. Esfandiary A, Taherian-Esfahani Z, Abedin-Do A, Mirfakhraie R, Shirzad M, Ghafouri-Fard S and Motevaseli E: Lactobacilli modulate hypoxia-inducible factor (HIF)-1 regulatory pathway in triple negative breast cancer cell line. Cell J 18: 237-244, 2016.
39. Bharti V, Singh S, Ahirwal L, Mehta A and Jain N: Cytotoxicity of live whole cell, heat killed cell and cell free extract of lactobacillus strain in u-87 human glioblastoma cell line and mcf-7 breast cancer cell line. Int J Probiotics Prebiotics 10: 153-158, 2015.
40. Kadirareddy RH, Vemuri SG and Palempalli UM: Probiotic conjugated linoleic acid mediated apoptosis in breast cancer cells by downregulation of NFκB. Asian Pac J Cancer Prev 17: 3395-3403, 2016.
41. De Moreno de LeBlanc A, Matar C, Thériault C and Perdigón G: Effects of milk fermented by *Lactobacillus helveticus* R389 on immune cells associated to mammary glands in normal and a breast cancer model. Immunobiology 210: 349-358, 2005.
42. Lakritz JR, Poutahidis T, Levkovich T, Varian BJ, Ibrahim YM, Chatzigiagkos A, Mirabal S, Alm EJ and Erdman SE: Beneficial bacteria stimulate host immune cells to counteract dietary and genetic predisposition to mammary cancer in mice. Int J Cancer 135: 529-540, 2014.
43. Van't Veer P, Dekker JM, Lamers JW, Kok FJ, Schouten EG, Brants HA, Sturmans F and Hermus RJ: Consumption of fermented milk products and breast cancer: A case-control study in The Netherlands. Cancer Res 49: 4020-4023, 1989.
44. Chagas CE, Rogero MM and Martini LA: Evaluating the links between intake of milk/dairy products and cancer. Nutr Rev 70: 294-300, 2012.
45. Soltan Dallal MM, Yazdi MH, Holakuyee M, Hassan ZM, Abolhassani M and Mahdavi M: *Lactobacillus casei* ssp.casei induced Th1 cytokine profile and natural killer cells activity in invasive ductal carcinoma bearing mice. Iran J Allergy Asthma Immunol 11: 183-189, 2012.
46. Yazdi MH, Mahdavi M, Setayesh N, Esfandyar M and Shahverdi AR: Selenium nanoparticle-enriched *Lactobacillus brevis* causes more efficient immune responses in vivo and reduces the liver metastasis in metastatic form of mouse breast cancer. DARU 21: 33, 2013.
47. Zamberi NR, Abu N, Mohamed NE, Nordin N, Keong YS, Beh BK, Zakaria ZA, Nik Abdul Rahman NM and Alitheen NB: The antimetastatic and antiangiogenesis effects of kefir water on murine breast cancer cells. Integr Cancer Ther 15: NP53-NP66, 2016.
48. Suez J, Zmora N, Segal E and Elinav E: The pros, cons and many knowns of probiotics. Nat Med 25: 716-729, 2019.
49. Bafeta A, Koh M, Riveros C and Ravaud P: Harms reporting in randomized controlled trials of interventions aimed at modifying microbiota: A systematic review. Ann Intern Med 169: 240-247, 2018.
50. Mayo Clinic: Engineering Gut Microbiome to Target Breast Cancer. https://clinicaltrials.gov/ct2/show/NCT03358511. NLM identifier: NCT03358511 Accessed October 28, 2020.
51. Universidad de Granada. Breast Cancer and Its Relationship With the Microbiota (MICROMA). https://clinicaltrials.gov/ct2/show/NCT03885648. NLM identifier: NCT03885648. Accessed October 28, 2020.
52. Hackensack Meridian Health: Gut and Intratumoral Microbiome Effect on the Neoadjuvant Chemotherapy-induced Immunosurveillance in Triple Negative Breast Cancer. https://clinicaltrials.gov/ct2/show/NCT03586297. NLM identifier: NCT03586297. Accessed October 28, 2020).
53. First Affiliated Hospital of Harbin Medical University: Intestinal Microbiota of Breast Cancer Patients Undergoing Chemotherapy. https://clinicaltrials.gov/ct2/show/NCT04138979. NLM identifier: ID: NCT04138979. Accessed October 28, 2020.
54. Lawson Health Research Institute: Probiotics and Breast Health. https://clinicaltrials.gov/ct2/show/NCT03290651. NLM identifier: NCT03290651. Accessed October 28, 2020.
55. Mayo Clinic: A Pilot Trial of Preoperative Oral Microbiota-based Investigational New Drug (RBX7455). https://clinicaltrials.gov/ct2/show/NCT04139993. NLM identifier: NCT04139993. Accessed October 28, 2020.