Review

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Mammary Gland Microbiota in Benign Breast Diseases

Nikita I. Ukraincev [1,*], Maria I. Kashutina [1,2,3], Larisa I. Kasatkina [1], Adkhamzhon B. Abduraimov [1] and Yury V. Zhernov [3,4,5,6,7]

1 Loginov Moscow Clinical Scientific and Practical Center, Moscow, Russia
2 National Research Centre for Therapy and Preventive Medicine, Moscow, Russia
3 Department of Prophylactic Medicine, Reaviz Moscow Medical University, 117418 Moscow, Russia
4 A.N. Sysin Research Institute of Human Ecology and Environmental Hygiene, Centre for Strategic Planning and Management of Biomedical Health Risks of the Federal Medical and Biological Agency, 119121 Moscow, Russia
5 Department of General Hygiene, F. Erismann Institute of Public Health, I.M. Sechenov First Moscow State Medical University (Sechenov University), 119435 Moscow, Russia
6 Fomin Clinic, 119192 Moscow, Russia
7 Center for Medical Anthropology, N.N. Miklukho-Maclay Institute of Ethnology and Anthropology of the Russian Academy of Sciences, 119017 Moscow, Russia
* Correspondence: ukraincev.nikita@mail.ru; Tel.: +7-(996)-656-81-57

Abstract

The human microbiome is a critical factor in health and disease, including breast pathology. While its association with breast cancer (BC) is increasingly studied, the specificity of the microbiome and its role in benign breast diseases (BBD) remain poorly understood. This review synthesizes current evidence on the origins of the mammary gland (MG) microbiota, which is distinct from the skin and formed via exogenous (e.g., cutaneous, retrograde via ducts) and endogenous (e.g., enteromammary, hematogenous) pathways. We detail the mechanisms of host-microbiota interaction, such as regulation of estrogen metabolism, immunomodulation, and epigenetic modifications, which can influence disease pathogenesis. The analysis reveals that while taxonomic profiles of tissue and gut microbiota share similarities between BBD and BC, key differences exist in the abundance of specific taxa (e.g., *Pseudomonadota*, *Bacillota*) and associated metabolic pathways. The review summarizes microbiota alterations associated with specific BBDs, including fibroadenomas, cysts, lactational and non-lactational mastitis (e.g., linked to *Corynebacterium kroppenstedtii*), and purulent-septic complications. Major limitations in the field are identified, such as the low microbial biomass of breast tissue, a lack of data on the virome and mycobiome, and the inability of current studies to establish causality. We conclude that microbial dysbiosis is implicated in BBD. However, further research is essential to elucidate cause-effect relationships. Understanding the microbiome's role holds significant promise for developing novel diagnostic, preventive, and personalized therapeutic strategies for benign breast conditions.

Keywords: breast disease; benign breast disease; breast tissue; mammary gland microbiota; dysbiosis; microbiome

1. Introduction

Microorganisms play a crucial role as biological agents in both the external and internal environments of the human body, existing predominantly not as individual cultures but as complex communities known as biofilms [1,2]. These polymicrobial associations, composed of various types of microorganisms such as bacteria, viruses, archae, micromycete, and eukarya, colonize different human organs and tissues, forming what is known as the 'microbiome' [3]. The microbiome

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constitutes a complex ecosystem that can have a wide range of impacts on human health. It can be neutral, beneficial, such as vitamin synthesis and fiber degradation, or detrimental, such as infections and carcinogenesis [4–6]. Bacterial communities have the highest diversity of species and microbial abundance [7].

The alteration in the composition of microbiota that occurs during dysbiosis frequently involves a reduction in microbial diversity, an increased prevalence of pathogenic microbes, and disruption of normal polymicrobial relationships [8]. This disruption in polymicrobial interaction can lead to a variety of health problems, including acute and chronic inflammation, infections, the development of tumors, both benign and malignant [8,9]. Scientific research has explored the role of microbes in tumor formation at various anatomical sites such as the stomach, intestines, liver, lung, skin and others [10,11].

The results of the Human Microbiome Project's sequencing efforts revealed significant interindividual variability in microbial community diversity and abundance [4]. A multitude of factors were implicated in the formation of microbiomes and the incidence of dysbiosis [12], encompassing previous infections, inflammatory disorders [8], dietary habits, chemical exposures [13], and genetic predispositions [4,14].

Breast pathology is one of the most urgent public health problems, and BC, the most common type of cancer among women, is a significant socio-economic issue. According to the World Health Organization (WHO), 2.3 million women were diagnosed with breast cancer in 2022 [15]. Despite advances in diagnosis and treatment, the burden of this disease is difficult to underestimate. It completely changes the lives of patients and their families, often leading to a decrease in quality of life [16]. Research shows that breast cancer is third in the list of top five cancers with the highest costs worldwide, projected for 2020–2050 [17].

However, studies show that breast disease is mostly benign, although accurate data on its prevalence is not available [18,19]. In the presence of breast symptoms, breast cancer is detected only in 3-6% of cases. Despite the high prevalence of benign breast pathologies, there is a lack of evidence-based approaches to managing such patients, since the main focus has been on optimizing diagnosis and treatment for breast cancer [20].

New researches suggest a link between certain microorganisms in breast tissue and the development of benign breast dysplasia. This finding is promising for the advancement of diagnostics and treatment methods.

Research on the microbiome's role in breast pathology is still in its early stages. Most studies focus on dysbiosis in breast tissues, such as the skin, nipple-areola complex secretions, ducts, and glandular fibrous tissues. Additionally, intestinal flora is also being investigated. This review aims to synthesize the current understanding of how human microbiomes might contribute to the development of benign breast diseases.

2. Pathways of Mammary Gland Microbiota Formation and Mechanisms of Its Interaction with the Host Organism

Breast tissue obtained under aseptic conditions from patients without clinical signs of a local infectious process possesses its own unique microbiota [21–23]. A significant diversity of microorganisms is specific to breast tissue and distinct from the microbiota of the skin and other body sites [10,21,23,24]. Furthermore, the MG is considered an organ with a low microbial biomass compared to other body areas [9].

2.1. Origin of the Mammary Gland Microbiota

The microbiota of breast tissue is formed through the influx of microorganisms via the nipple-areolar complex [22,25], translocation from the gastrointestinal tract (GIT) and oral cavity [26], the urinary [27]. and reproductive systems [21], from the skin surface, and other sources [28]. The unique

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MG microbiota is established via distinct exogenous and endogenous pathways, each characterized by specific mechanisms and influenced by key factors (Figure 1).

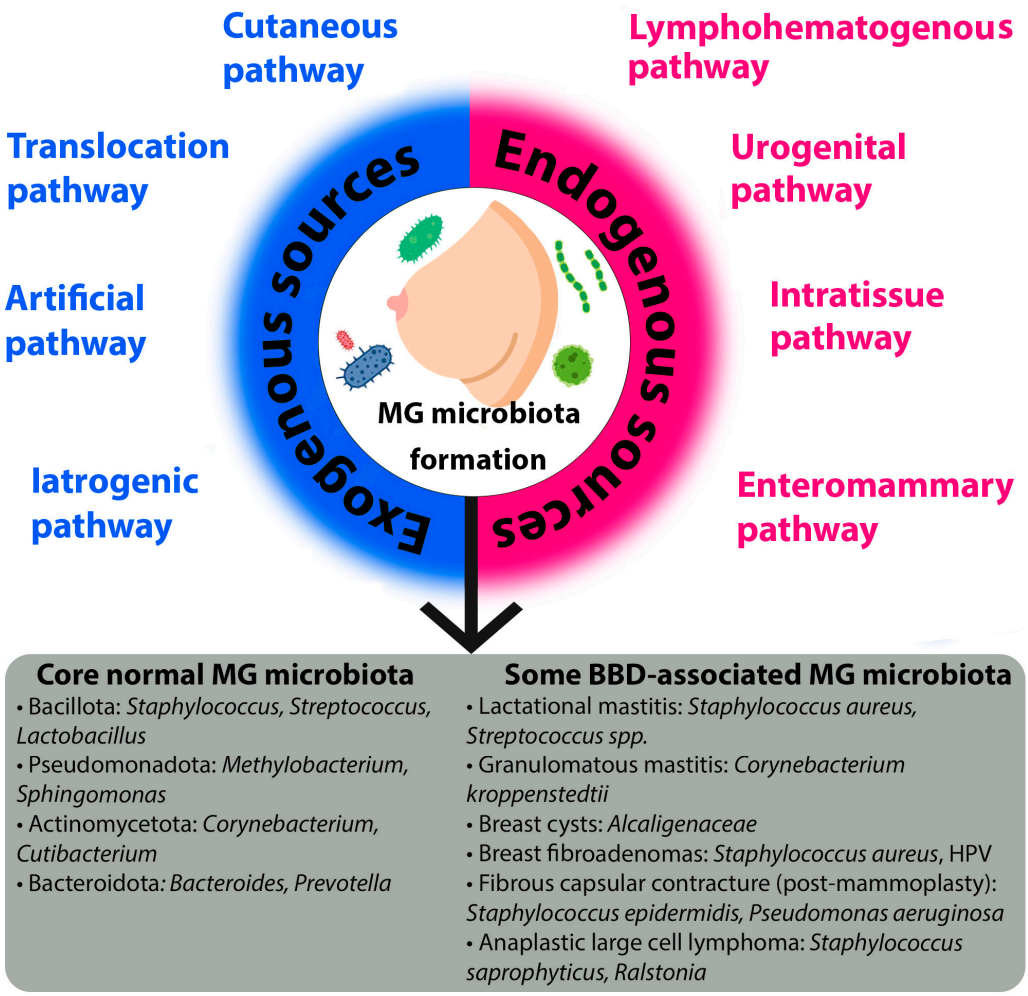

Figure 1. Exogenous and endogenous sources of mammary gland microbiota formation (MG – mammary gland; BBD – benign breast diseases; HPV – human papillomavirus).

Exogenous sources refer to the introduction of microorganisms from the external environment. The cutaneous pathway's a primary route, where microbes from the skin surface translocate to the breast tissue. Although the microbiota of the MG and the overlying skin share species diversity, they remain distinct communities [23,28]. This pathway is significantly influenced by environmental factors (e.g., climate, geography, occupation, living conditions), lifestyle habits, particularly hygiene practices, and host physiology, such as a compromised skin barrier function.

Another critical exogenous route is the translocation pathway, which involves the retrograde inoculation of microbes via the lactiferous ducts [29,30,32,35]. This pathway is highly dependent on breastfeeding practices, including the type of feeding (direct breastfeeding, pumping, or formula), the mode of delivery (if breastfeeding), and the sex of the infant.

Medical interventions constitute two additional exogenous pathways. The artificial pathway describes the direct introduction of microbiota during procedures such as breast biopsies or surgery. Conversely, the iatrogenic pathway refers to alterations in the existing microbiota induced by medical treatments, most notably the use of antibiotics or probiotics.

Endogenous sources involve the translocation of microorganisms from within the host organism. The most well-studied is the enteromammary pathway, a sophisticated mechanism where gut and oral microbiota are transported to the MG via immune cells, such as CD18+ cells and

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dendritic cells [26,28,32,36–39]. This pathway creates a direct link between the gut and the breast, and its efficiency is influenced by the composition of the gut microbiota (e.g., states of dysbiosis associated with high body mass index (BMI)) and host diet.

The urogenital pathway proposes that microbiota from the urogenital tract may serve as a source for MG colonization [9], although the specific mechanisms are less defined. Furthermore, the local tissue environment, or intratissue pathway, dictated by the MG tissue structure (specifically the ratio of glandular/fibrous to adipose tissue) [40], shapes a unique ecological niche that determines which microbial communities can survive and thrive. Finally, the lymphohematogenous pathway involves the systemic dissemination of microbes to the breast through the lymphatic system and bloodstream [35], representing a potential route for bacteria from distant sites of infection or colonization.

In conclusion, the establishment of the mammary gland microbiota is a dynamic process governed by a multitude of exogenous and endogenous pathways. Understanding the intricate interplay between these routes and their modulating factors is crucial for elucidating the role of the microbiome in breast health and disease.

2.2. Microbiota of Unaltered Mammary Gland Tissues

Alpha-diversity analysis indicates a greater number of bacterial species in breast tissue compared to skin tissue. However, beta-diversity analysis reveals a significant difference in the community composition of breast and skin microbiota, though the difference is not substantial in weighted analysis, suggesting that the distinctions lie in rare or less abundant species [23,41].

The microbial community currently identified in unaltered breast tissue is dominated by the species listed in Table 1 and Figure 1. The higher prevalence of the phyla *Pseudomonadota* (Proteobacteria) and *Bacillota* (Firmicutes) compared to other taxonomic groups may be due to the affinity of these microorganisms for the fatty acid-rich environment of breast tissues [4]. Disruption of polymicrobial interactions leading to dysbiosis may contribute to the development of breast diseases [42].

Table 1. Representatives of the bacterial community identified in unaltered female breast tissue.

Phylum	Family	Genus	Species	References
Pseudomonadota (Proteobacteria)	*Sphingomonadaceae*	-	-	[43]
	Methylobacteriaceae	*Methylobacterium*	-	[4,9]
	Burkholderiaceae	*Ralstonia*	-	[4,43,44]
	Sphingomonadaceae	*Sphingomonas*	*yanoikuyae*	[4]
			-	[21,23,43]
	Pseudomonadaceae	*Pseudomonas*	-	[21,43,45]
	Comamonadaceae	-	-	[21]
	Enterobacteriaceae	-	-	[21]
	Moraxellaceae	*Acinetobacter*	-	[21]
	Pasteurellaceae	*Haemophilus*	-	[45]
	Neisseriaceae	*Neisseria*	-	[45]
	-	-	-	[4,9,21,25,44]
Bacillota (Firmicutes)	*Veillonellaceae*	*Veillonella*	-	[45]
	Staphylococcaceae	*Staphylococcus*	-	[21,43]
	Streptococcaceae	*Lactococcus*	-	[43]
		Streptococcus	-	[43]
	Clostridiaceae	*Clostridium*	-	[43]
	Lactobacillaceae	*Lactobacillus*	-	[43]
	Listeriaceae	*Listeria*	*welshimeri*	[21]
	-	-	-	[4,9,21,25,44,46]

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Actinomycetota (Actinobacteria)	*Corynebacteriaceae*	*Corynebacterium*	-	[43]
	Micrococcaceae	*Micrococcus*	-	[4]
	Propionibacteriaceae	*Propionibacterium*	-	[21,43]
	-	-	-	[4,25]
Bacteroidota (Bacteroidetes)	*Bacteroidaceae*	*Bacteroides*	-	[9]
	Prevotellaceae	*Prevotella*	-	[21,43]
	-	-	-	[9,25,43]

In recent years, several studies have clarified the role of viruses and fungi (micromycetes) in the human microbiome [47]. For instance, DNA from human papillomavirus (HPV), Epstein-Barr virus (EBV), human cytomegalovirus (HCMV), herpes simplex virus (HSV), and human herpesvirus type 8 (HHV-8) has been detected in breast tissue both under normal conditions and with pathological changes [48,49]. Given that viruses are obligate parasites, it is difficult to distinguish a separate infectious process in the MG from long-term viral persistence in breast tissue cells with potential carcinogenicity—in this case, one can refer to[4,35,40,50–53 the MG virome.

The predominant focus on the bacterial fraction of the human microbiome in most studies has led to an incomplete understanding of the role of micromycetes in polymicrobial interactions. Research into the role of fungal communities (the mycobiome) is complicated by the low fungal biomass in breast tissues compared to the bacterial component. According to published studies, *C. albicans* and *Saccharomyces* are identified in normal breast tissue structure, while *Malassezia, Davidiella, Sistotrema*, and *Penicillium* are found in breast milk during lactation [6,29].

2.3. The Microbiota-Human Organism Interaction

The MG microbiota interacts with the host organism by regulating immune, metabolic, transcriptional, and epigenetic processes through the production of enzymes and other biologically active substances [4,35,40,50–53].

Bacterial metabolites (e.g., short-chain fatty acids, acetate, butyrate, pyruvate, formate, amines (cadaverine), bile acid derivatives (lithocholic acid, deoxycholic acid), indole, etc.) can influence processes of cell growth, apoptosis, epithelial-mesenchymal transition, anti-tumor immunological surveillance, and also exert cytotoxic and genotoxic effects [54,55]. Dysbiosis leads to changes in the bacterial metabolite profile [55,56]. Some bacterial metabolites and their mediated effects are presented in Table 2.

Table 2. Some significant bacterial metabolites and their mediated metabolic effects.

Bacterial metabolite	Metabolic effect	References
Cytolethal distending toxin (CDT) and colibactin	Promotes DNA double strand breaks (DSB)	[35]
Rho GTPase family proteins	Reorganizing actin cytoskeleton	[35]
Cadaverine	Endothelial to mesenchymal transition modulation	[4]
Lithocholic acid (LCA)	Increases oxidative stress. Regulates KEAP1, NRF2, TGR5, GPX3 expression	[4]
Lipopolysaccharides (LPS)	Associated with S100A7 expression – regulates mammary cell proliferation	[4]
Trimethylamine N-oxide (TMAO)	Effects cell proliferation by α-casein	[4]
β-glucuronidase and/or β-glucosidase	Promote recirculation of estrogen and estrogen-like metabolites	[30]
Short chain fatty acids, folates, biotin	Activate epigenetically silenced genes in cells such as p21, BAK etc.	[12,30]

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Furthermore, microorganisms inhabiting remote areas of the body can influence each other through metabolites and immune factors [11,39]. These interactions can impact the onset and development of pathological conditions, particularly breast diseases [57–59].

2.4. Mechanisms of Microbiota-Induced Breast Pathogenesis

Several hypotheses have been proposed regarding the ability of microorganisms to cause DNA damage, modulate estrogen metabolism, shape the immune microenvironment of the breast—inducing chronic inflammation and local immunosuppression—and potentiate proliferative processes (Figure 2) [10,60–62].

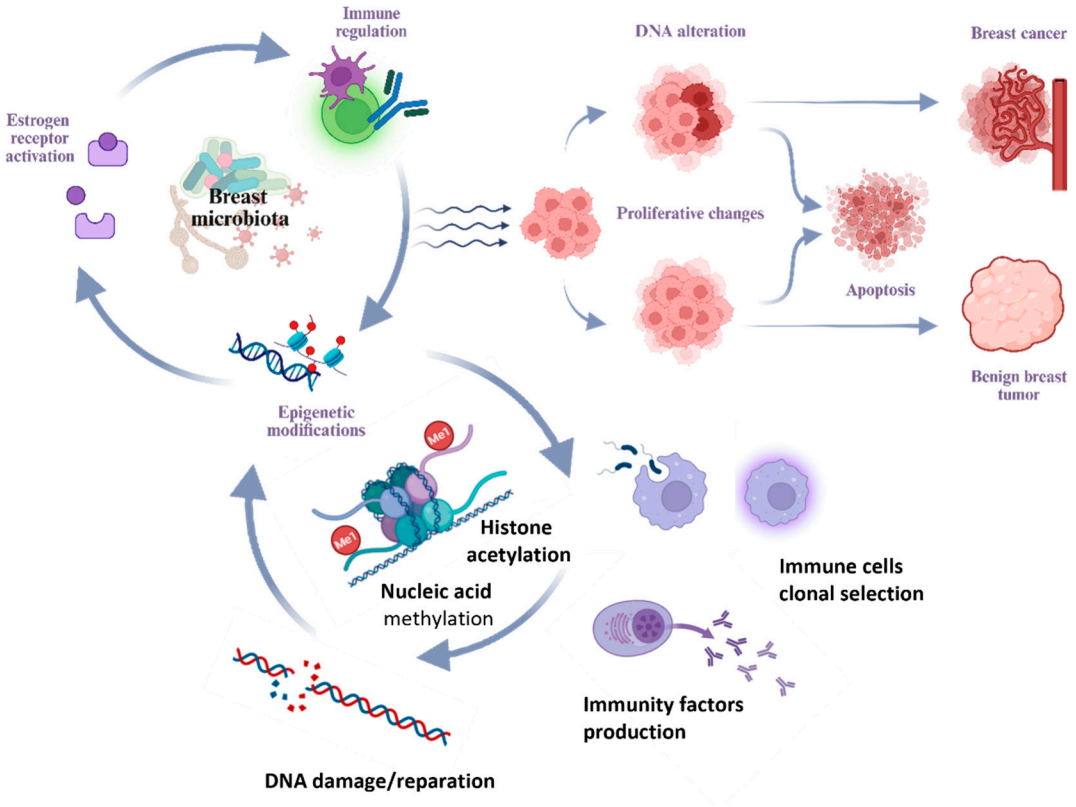

Figure 2. Main mechanisms of microbiota influence on the development of breast pathology (Me1 – monomethylation site).

One of the primary mechanisms involves the regulation of estrogen metabolism. Elevated estrogen levels are closely associated with the activation of estrogen receptors, promoting proliferative processes in breast tissue in BBD and BC [10,63,64]. The gut microbiota regulates processes of enterohepatic circulation of active estrogen forms [65,66]. and the synthesis of estrogen-like substances [56,59,60]. The collection of microorganisms influencing systemic levels of estrogen and its metabolites constitutes the concept of the estrobolome [67,68]. The role of local estrogen levels in breast tissue in BC should also be noted [9].

Beyond hormonal influence, the microbiota exerts a significant effect through local immunomodulation and the maintenance of chronic inflammation. Available research data indicate that commensal microflora of the gastrointestinal tract possesses immunomodulatory properties. The gut microbiota can maintain chronic inflammation by altering the balance between proliferation and apoptosis and triggering unregulated innate and adaptive immune responses. Immunoglobulin A (IgA), which maintains mucosal barrier integrity, is involved in recognizing and regulating the composition of the gut microbiota [22,56,69].

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A further mechanism by which the microbiota may influence breast tissue is through epigenetic modifications. Previous studies have shown that the microbiota can influence processes of epigenetic regulation [70]. Epigenetic mechanisms include DNA methylation, acetylation and deacetylation of histone proteins, and modification of non-coding RNAs and microRNAs. Epigenetic modifications lead to enhanced or attenuated cell growth and regulation of cellular signaling pathways. Therefore, studying epigenetics as one of the mechanisms by which the microbiota influences pathological states of the breast appears promising [39].

3. Microbiota Composition and Diversity in BBD

3.1. General Characteristics of BBD

BBD is among the most common breast diseases and includes conditions varying by clinical, morphological, and etiological criteria. These conditions are based on an imbalance between the epithelial and connective tissue components due to specific features of proliferation and regression processes in breast tissue. Given the heterogeneity of benign pathology, a number of terms are used to denote it: BBD, mastopathy, fibrocystic mastopathy, fibrocystic changes, fibrocystic disease, dyshormonal hyperplasia of the mammary glands, fibroadenomatosis, etc. The prevalence of benign breast changes in the female population is 50% or higher. Considering the prevalence and the increased risk of developing BC with certain types of benign changes, the issues of improving diagnosis, differential diagnosis, and treatment are relevant [71,72].

BBD is most often classified by clinical manifestations, degree of proliferative changes, type of pathomorphological changes on biopsy, etc. (Figures 3 and 4) [20,71,73–76]. Breast tissue changes on histological examination are classified from B1 to B5 depending on the degree of suspicion for BC [20,77].

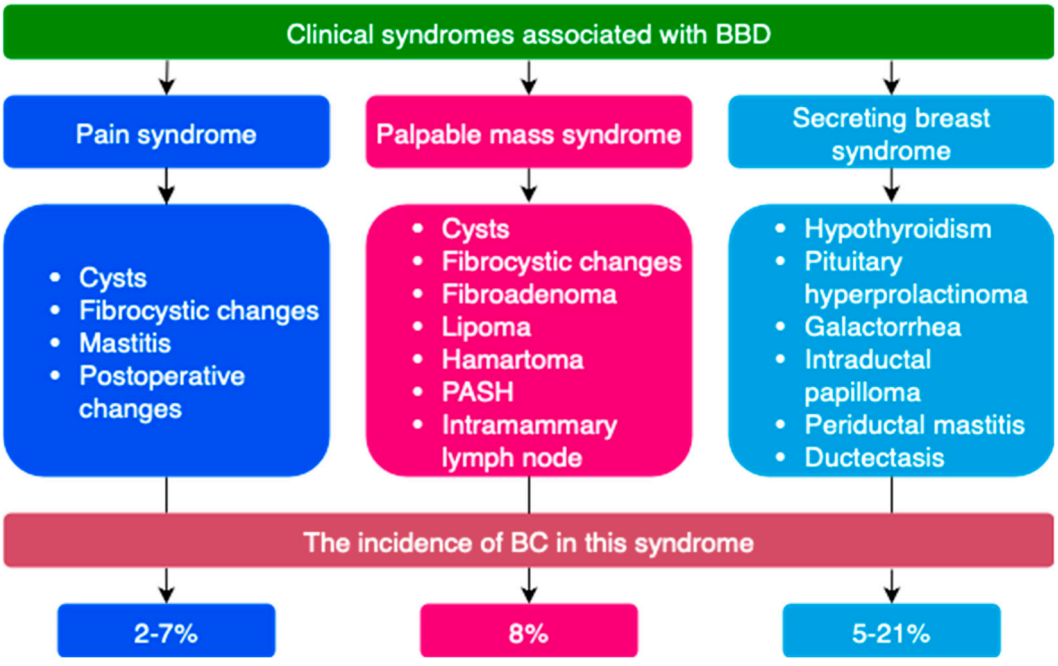

Figure 3. Classification of benign breast tissue changes by dominant clinical syndrome (Adapted with permission from Stachs A., et al. [20]. Copyright 2019, Deutscher Ärzteverlag GmbH).

A special group of diseases consists of lesions of uncertain malignant potential (B3). Some proliferative breast conditions may be associated with the presence of DCIS, invasive BC, or be markers of an increased risk of developing BC [56,78,79]. Such conditions are classified as lesions of uncertain malignant potential (B3) on histological examination [20]. These include atypical ductal

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hyperplasia (ADH), flat epithelial atypia (FEA), classical lobular neoplasia (LN, ALH, LCIS), as well as lesions of heterogeneous structure with a risk of incomplete sampling during biopsy—intraductal papilloma with/without atypia, radial scar, and complex sclerosing lesion [20].

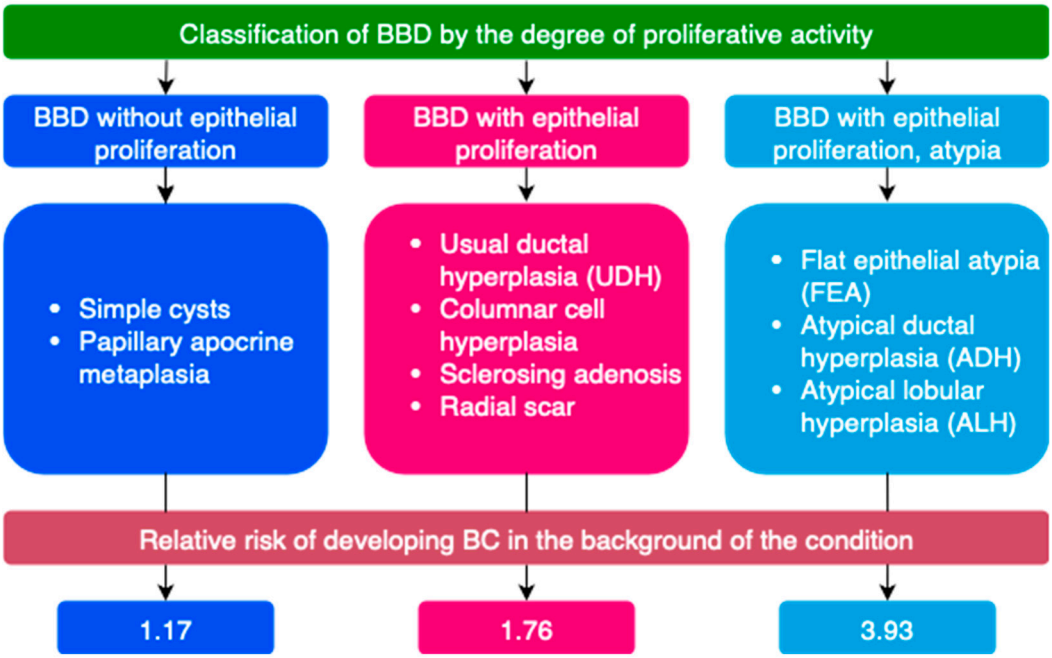

Figure 4. Classification of benign breast tissue changes by degree of proliferative activity (Adapted with permission from Stachs A., et al. [20]. Copyright 2019, Deutscher Ärzteverlag GmbH).

3.2. General Characteristics of Microbiota in BBD

The main task of clinical mammology is the differential diagnosis of benign and malignant breast neoplasms. Consequently, research is primarily aimed at identifying the association between changes in the microbiota of breast tissues and gut flora in BC, while studies assessing the features of the microbiota in BBD have been published in limited numbers to date. Most of them are aimed precisely at comparing and identifying similarities and differences in the tissue microbiota of unaltered breast tissues and tumor formations (if present) and gut microflora in the absence of breast pathology, BC, and BBD [23,42,59].

Studies of taxonomic profiles show that the bacterial microbiota of breast tissue in BC and BBD is similar, dominated by representatives of the phyla *Bacteroidota, Bacillota* (Firmicutes), *Pseudomonadota* (Proteobacteria), and the phylum *Actinomycetota* (Actinobacteria) [10,23,42]. In unaltered adjacent tissues in BC, a significantly greater abundance of certain bacterial taxa was found, including the genus *Bacillus, Staphylococcus*, and members of the family *Enterobacteriaceae*, compared to patients with benign breast tumors and healthy women [42]. Moreover, the relative abundance of *Pseudomonadota* (Proteobacteria) in benign diseases was found to be significantly lower than in malignant ones [10]. In BBD, a low level of bacteria influencing DNA damage processes is noted, which may be a possible factor preventing malignant transformation [42]. Taxonomic profiles in benign tumors are more similar to profiles of normal adjacent tissue in women with malignant tumors than to profiles of tissues from healthy patients [42]. Beta-diversity assessment shows that the microbial community in unaltered breast tissue adjacent to invasive cancer differs from that in women with benign diseases, mainly due to rare and/or less abundant species. Alpha-diversity analysis revealed no significant differences [4,23].

Metabolic pathways mediated by the tissue microbiota also differ between BC and BBD. According to KEGG (Kyoto Encyclopedia of Genes and Genomes) PATHWAY, benign tissues exhibit elevated metabolism of cysteine and methionine, glycosyltransferases, and fatty acid biosynthesis,

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while the microbiota of malignant tissues demonstrates reduced inositol phosphate metabolism [23]. Bacterial lipopolysaccharides are often present in many human solid tumors [20,59].

A number of viruses possess oncogenic potential by initiating malignant transformation of epithelial cells, prolonging the cell cycle, activating cell proliferation, and preventing apoptosis [35]. The phenomenon of the influence of androgens and estrogens on the replicative activity of some viruses makes viruses a possible etiological factor in the development of a number of hormone-sensitive tumors and conditions, particularly of the breast.

For example, HPV can integrate into the cell genome and mediate oncogenic transformation via E6 and E7 proteins. HPV DNA was identified in 20.3% of malignant neoplasms and 35% of benign neoplasms of the breast [48]. It was also detected in 27.3% of biopsy samples of unaltered breast tissue. It was not found in biopsy material of in situ neoplasms or borderline lesions. CMV can modulate the tumor immune microenvironment and stimulate tumor cell growth. In breast tissues, CMV genetic material is more frequently detected in malignant neoplasms than in normal tissue. Very low levels of EBV DNA are detected in breast tissues in BC by quantitative PCR. Thus, the role of EBV and CMV in the development of breast pathology, primarily breast cancer, remains debatable [20].

Colonization of epithelial tissues and cells by some species (tropism) of micromycetes may indicate a potential role of fungi in breast diseases [50]. Available studies only highlight the association of micromycetes with malignant neoplasms. Thus, micromycetes have been associated with 35 cancer types, yet their role in carcinogenesis remains unexplored [47,50]. Nevertheless, the most significant fungal-bacterial-immune associations in BC have been identified, particularly involving representatives of the genera *Aspergillus*, *Malassezia*, and *Cladosporium* (*Cladosporium sphaerospermum*) [80].

Fungi remain understudied but important commensals/opportunistic pathogens that shape unique host immune responses. Given the possibility of symbiotic and antagonistic interactions (physical, biochemical) between bacteria and fungi, further research is required to assess the role of fungi in the polymicrobial interaction of the tumor environment.

3.3. Features of Gut Microbiota in Breast Pathology

The role of gut dysbiosis in the development of BBD is also currently debatable. In patients with malignant breast tumors, the species diversity of the gut microbiota is lower but more homogeneous than in patients with benign tumors [58]. It should be noted that in a number of studies on the composition of gut microbiota in BBD and BC, no statistical differences were observed in the assessment of α- and β-diversity [59].

When comparing patients with BBD and healthy individuals, no differences in α-diversity indices were found [56].

Furthermore, assessment by beta-diversity noticeably differed among the three groups (BC/BBD/normal). These results indicate an altered composition of the gut microbiota in healthy women, BC patients, and BBD patients [56].

An increase in the number of representatives of the genera Clostridium, Faecalibacterium, Lachnospira, Romboutsia, Fusicatenibacter, Xylophilus, Arcanobacterium, Escherichia, Peptoniphilus, Coprobacillus, Lactobacillus, Porphyromonas, and the family Erysipelotrichaceae in the gut microbiota of patients with benign tumors has been reported, along with a decrease in the abundance of the genera Collinsella, Alistipes, Megamonas, Butyricimonas, Acidaminococcus, Asaccharobacter, Tissierella, and Cloacibacil[59,81lus [56,57,59].

The metabolic pathways of the gut microbiota in patients with malignant tumors differed significantly from those in patients with benign neoplasms [59]. In patients with malignant tumors, unlike BBD, marked activation of lipopolysaccharide biosynthesis pathways was noted. Conversely, KEGG analysis revealed significant activation of sporulation in patients with benign tumors [59,81].

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3.4. Mammary Gland Microbiota in Specific Types of BBD

Current scientific data do not cover the entire spectrum of microbiota changes across all types of BBD. Research is focused mainly, as noted earlier, on fundamental differences in microbiota (gut and breast tissue) in malignant and benign breast conditions in general. Below, a number of specific benign breast pathologies and their association with the composition of tissue and gut microbiota are considered. Summary data on associated and protective microorganisms for various BBDs are presented in Table 3 and Figure 1.

3.4.1. Breast Cysts

Among representatives of the gut microbiota, the family *Alcaligenaceae* is associated with an increased risk of developing breast cysts, while the *genus Eubacterium ruminantium* and *Lactococcus* are associated with a reduced risk [22,56]. Cysts can be complicated by secondary infection with clinical and radiological signs of a complicated cyst (cyst with inflammation); see the section '3.4.7. Purulent-septic changes of the breast'.

HPV was detected in 40.0% of biopsies taken from patients with fibrocystic mastopathy [82].

3.4.2. Breast Fibroadenomas

Staphylococcus aureus is an important factor causing mutation of the MED12 gene, which may contribute to the development of breast fibroadenoma and uterine leiomyoma [56,83]. Among benign neoplasms, HPV DNA was identified in 38.9% of histological material from fibroadenomas [48].

3.4.3. Lactational Mastitis

Lactational mastitis is the most common type of mastitis, accounting for 33% of all breast diseases [84]. In 90% of cases, the etiological agent is Staphylococcus aureus, less frequently coagulase-negative *Staphylococcus, Streptococcus, Pseudomonas aeruginosa*, and *Escherichia coli* [20]. The presence of representatives of the genera *Anaerofilum* and *Anaerotruncus* in the gut microbiota is associated with cases of lactational mastitis, while the genus *Butyricimonas* and the orders *Coriobacteriales, Pasteurellales*, and *Verrucomicrobiales* had a negative association [22]. In this regard, probiotics are used in the treatment of lactational mastitis and are potentially effective for chronic and subclinical forms of mastitis as an alternative to antibacterial therapy [56].

3.4.4. Non-Lactational Mastitis

Non-lactational mastitis accounts for up to 3% of all benign breast diseases [84]. Given the clinical picture and difficulties in diagnosis and treatment, non-lactational mastitis can cause distress in some women. Non-lactational mastitis occurs at any age, although it more often affects young and middle-aged women [84]. The pathological forms of this disease are diverse, including duct ectasia (MDE), periductal mastitis (PDM), and granulomatous lobular mastitis (GLM) [84].

The influence of the MG microbiota on the development of inflammatory breast diseases has been studied [28,85,86]. Representatives of the gut flora, for example the family *Prevotellaceae*, are associated with inflammatory breast changes [22]. The breast tissue microbiota in patients with non-lactational mastitis demonstrates differences in the composition of microbial communities compared to healthy patients. In patients with non-lactational mastitis, representatives of bacterial communities inhabiting the gut, particularly the genera *Ruminococcus, Coprococcus*, and *Clostridium*, were identified in breast tissue [84]. Non-lactational mastitis can also be associated with autoimmune reactions [84].

3.4.5. Granulomatous Mastitis

Granulomatous mastitis is a rare inflammatory breast disease in women of reproductive age [28,87]. The etiology of this condition is unknown; nevertheless, a significant role is assigned to local dyshormonal changes, hyperprolactinemia, autoimmune reactions, and infectious agents [20,84]. A

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role for the genus Corynebacterium, particularly the species Corynebacterium kroppenstedtii, has been identified in the pathogenesis of granulomatous inflammation [84,86]. Other taxa encountered in granulomatous mastitis include representatives of the genera *Pseudomonas*, *Brevundimonas*, *Stenotrophomonas*, *Acinetobacter*, and *Aspergillus* [28].

3.4.6. Ductal Changes

Periductal mastitis is an inflammatory disease of the subareolar lactiferous ducts, with a prevalence of up to 9% outside the lactation period [88]. In nipple discharge, bacterial flora is detected in 50% of cases, while against the background of duct ectasia, it is detected in 62% of cases [84]. This may be associated with structural changes in the duct wall during a persistent inflammatory process against the background of combined infections caused by representatives of the genera *Enterococcus*, *Streptococcus*, and *Bacteroides* [84,89].

3.4.7. Purulent-Septic Changes of the Breast

In abscesses associated with non-lactational mastitis, the most frequent bacterial strains were coagulase-negative *Staphylococci* and *Peptostreptococci*, *Staphylococcus aureus* (50% of cases MRSA) [90], including as part of combined bacterial infection [20,84]. Certain diseases associated with impaired skin barrier function, such as atopic dermatitis, promote contamination of deep skin layers and underlying tissues, causing the development of an infectious process, including breast abscesses [28]. Representatives of the genera *Corynebacterium* and *Pseudomonas* (*Pseudomonas aeruginosa*) are often associated with infections of the skin and underlying soft tissues (abscesses, phlegmons, fistulas, etc.) [91,92]. These skin microorganisms are capable of metabolizing fatty acids and are considered potential pathobionts in breast tissues [28].

3.4.8. Fibrous Capsular Contracture

The transfer of microorganisms during surgery via surgical instruments can lead to opportunistic subclinical infection. Staphylococci, Cutibacterium acnes, Pseudomonas aeruginosa, Staphylococcus lugdunensis, Staphylococcus hominis, Staphylococcus epidermidis, Sphingomonas paucimobilis, and Aeromonas salmonicida, which are found on the skin of healthy individuals, are also frequently identified in infections associated with surgical interventions [34,93,94]. The frequency of infectious-inflammatory complications in breast surgeries ranges from 3 to 15%. Despite prophylactic antibiotic use and adherence to aseptic and antiseptic principles, positive culture results (breast tissue) were found in 20.4% of cases [95].

Augmentation mammoplasty is a common breast surgery and is associated with the development of capsular contracture [96]. Up to 56% of capsular contracture cases are associated with the detection of bacterial flora on the surface of implants or in the fibrous capsule, particularly *Staphylococcus epidermidis* [27,97]. Furthermore, species isolated from the structure of the fibrous capsule included *Escherichia coli*, *Diaphorobacter nitroreducens*, *Cutibacterium acnes*, *Staphylococcus aureus*, and *Staphylococcus spp* [96,98]. The formation of bacterial biofilms on the implant surface promotes resistance to antibiotic therapy and allows microorganisms to escape immune surveillance, also complicating the assessment of the species composition of the bacterial flora [96].

3.4.9. Anaplastic Large Cell Lymphoma

In addition to capsular contracture, bacterial colonization and biofilm formation are linked to breast implant-associated anaplastic large cell lymphoma (BIA-ALCL) [99]. The species *Staphylococcus saprophyticus* and representatives of the genus *Ralstonia* are the most frequently detected microorganisms in BIA-ALCL, both on the side of interest and in the contralateral breast [96,100].

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Table 3. Microbiota in benign female breast diseases.

Disease/Condition	Microorganisms/Taxa (Associated)	Microorganisms/Taxa (Protective/Risk-Reducing)	Location/Notes	References
Breast cysts	Family *Alcaligenaceae* (gut)	Genus *Eubacterium ruminantium*, *Lactococcus* (gut)	Association is based on analysis of gut microbiota. HPV is detected in 40% of cases.	[22,56,82]
Breast fibroadenomas	*Staphylococcus aureus*, HPV (DNA detected in 38.9% of cases)	Not specified	*S. aureus* is considered a factor contributing to MED12 gene mutation.	[48,56,83]
Lactational mastitis	*Staphylococcus aureus* (main pathogen), coagulase-negative staphylococci, *Streptococcus*, *Pseudomonas aeruginosa*, *Escherichia coli*. Genera *Anaerofilum*, *Anaerotruncus* (gut)	Genus *Butyricimonas*, orders *Coriobacteriales*, *Pasteurellales*, *Verrucomicrobiales* (gut)	Lactational mastitis accounts for 33% of all breast diseases.	[20,22,56,84]
Non-lactational mastitis (including duct ectasia, periductal mastitis)	Family *Prevotellaceae* (gut). Genera *Ruminococcus*, *Coprococcus*, *Clostridium* (breast tissue).	Not specified	The breast tissue microbiota composition differs from that of healthy patients. May be associated with autoimmune reactions.	[22,28,84–86]
Granulomatous mastitis	*Corynebacterium kroppenstedtii* (key pathogen), genera *Pseudomonas*, *Brevundimonas*, *Stenotrophomonas*, *Acinetobacter*, fungi of the genus *Aspergillus*.	Not specified	Rare disease. Etiology is unknown; possible roles include dyshormonal changes and autoimmune reactions.	[20,28,84,86,87]
Periductal mastitis (duct changes)	Genera *Enterococcus*, *Streptococcus*, *Bacteroides*.	Not specified	Bacterial flora is detected in 50-62% of cases, often against the background of duct ectasia.	[84,88,89]
Purulent-septic changes (abscesses)	Coagulase-negative staphylococci, *Peptostreptococci*, *Staphylococcus aureus* (including MRSA), *Corynebacterium*, *Pseudomonas aeruginosa*.	Not specified	Often occur as a complication of non-lactational mastitis. May be associated with impaired skin barrier function (e.g., dermatitis).	[20,28,84,90–92]

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Fibrous capsular contracture (post-mammoplasty)	*Staphylococcus epidermidis* (most common), *Escherichia coli*, *Diaphorobacter nitroreducens*, *Cutibacterium acnes*, *Staphylococcus aureus*, *Staphylococcus* spp., *Pseudomonas aeruginosa*, *Sphingomonas paucimobilis*.	Not specified	Associated with bacterial colonization of the implant and biofilm formation.	[27,34,93–98]
Anaplastic large cell lymphoma (BIA-ALCL)	*Staphylococcus saprophyticus*, representatives of the genus *Ralstonia*.	Not specified	Associated with bacterial colonization and biofilms on breast implants.	[96,99,100]

4. Breast Microbiota in Men

Before puberty, breast tissue is identical in both sexes. During puberty, boys experience transient proliferation of the milk ducts and stroma (due to estrogen stimulation), followed by involution of these structures (with increasing testosterone levels) [45,101]. The male breast does not develop terminal ductal lobular units due to the absence of progesterone [101]. The spectrum of pathology in the male breast is limited and related to the gender-specific histological structure of the organ [45].

Most hyperplastic processes in the male breast are benign, etiologically and pathogenetically similar to changes in the female breast. Male breast cancer (1 per 100,000 in Europe with a peak at 71 years) is rare, accounting for approximately [45,1011% of breast pathologies [45,101].

Benign Conditions of the Male Breast [45,101]:

1. Developmental anomalies (amastia, polymastia, nipple inversion, athelia, polythelia, etc.)
2. Inflammatory and reactive changes (mastitis, abscess, Mondor's disease, etc.)
3. Ductal changes (duct ectasia, intraductal papilloma, etc.)
4. Systemic diseases/symptoms of systemic diseases (diabetic mastopathy, gynecomastia)
5. Benign neoplasms (lipoma, angiolipoma, cavernous hemangioma, myofibroblastoma, epidermal cysts, pseudoangiomatous stromal hyperplasia (PASH), hamartoma, etc.)
6. Traumatic and post-traumatic changes (hematoma, fat necrosis)

Gynecomastia is the most common pathological benign condition of the male breast. The prevalence of gynecomastia is high, especially in the neonatal period (60-90%), during puberty (48-64%), in the reproductive period (up to 30%), and in the elderly (60% after 70 years). The development of gynecomastia is often associated with transient physiological changes, endocrine disorders, systemic diseases, drug therapy, and can also develop idiopathically [102]. The development of glandular tissue creates a morphological substrate for the development of other breast pathologies.

Studies on the microbiota of the male breast are extremely limited. There is data on sexual dimorphism of the breast microbiota, with the concept of the breast microgenderome being identified in both BC and unaltered tissues. In the structure of unaltered male breast tissue, greater microbial richness by alpha-diversity was found compared to female breast tissue. Furthermore, samples of unaltered male breast tissue differed in beta-diversity. In unaltered male tissue, a predominance of representatives of the families *Bacteroidaceae, Caulobacteraceae, Comamonadaceae*, Enterococcaceae, *Microbacteriaceae, Peptoniphilaceae* and the genera *Brevundimonas, Clavibacter, Comamonas*, and *Rhodococcus* was noted [45].

The principles of diagnosis and approaches to the treatment of breast diseases in men are still based on knowledge gained from the diagnosis and treatment of women with similar pathology. Microbiota research will help identify differences in the development of breast diseases in men and women.

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5. Limitations in Studying the Microbiota in BBD

This review of available scientific data on the relationship between microbiota and the development of BBD has allowed for the generalization and identification of the main limitations of conducted studies, which can serve as a basis for designing future research:

1. Insufficient study of the microbiota of breast tissue and other areas in the normal state.
2. Low biomass of the MG microbiota in normal and pathological conditions.
3. Lack of quality, representative data on the breast virome and micromycetes.
4. Most research is focused on malignant breast neoplasms.
5. Studies are conducted on small patient groups without a unified research protocol [20]. Different methods for detecting microorganisms were used in existing studies of breast tissue microbiota, making comparative evaluation of results difficult [30].
6. Presence of intracellular forms of microorganisms complicates their detection [78].
7. Inability to establish cause-and-effect relationships when studying the influence of the microbiome on breast pathology. Available data do not answer the question of whether microbial dysbiosis is a consequence or a cause of breast disease development [45].
8. Individual variability of the microbiota and the multifactorial nature of BBD development.
9. Presence of borderline states – 'lesions of uncertain malignant potential (B3)'.
10. Inaccessibility of tissue samples: unaltered tissues and tissues with benign changes are often unavailable for research, as biopsies are usually performed when malignancy is suspected. Using adjacent unaltered tissue from cancer cases as a reference can distort research data.
11. Evaluation of the microbial community as potential biomarkers for diagnosis, treatment, and prognosis of diseases, which will contribute to the implementation of personalized medicine principles [59,103].

Strengthening the scientific base regarding the influence of the MG microbiota on the development of BBD and addressing the issues outlined above will contribute to the development of new directions in the prevention, diagnosis, and treatment of breast pathology:

- Creation of diagnostic test systems for the differential diagnosis of benign and malignant neoplasms.
- Differential diagnosis of the secreting breast syndrome.
- Assessment of BC development risks.
- Investigation of etiopathogenetic aspects of BBD development.
- Treatment and prevention of BBD.

6. Materials and Methods

To prepare this review, the available data from studies devoted to the study and assessment of the microbiome features in patients with benign breast pathology were analyzed. The search was carried out in the electronic bibliographic databases PubMed, Scopus, and Google Scholar. The selection of publications for subsequent analysis was carried out using such keywords and their combinations as 'breast microbiota', 'mammary gland microbiota', 'breast tumor microbiota', 'benign breast diseases', 'benign breast lesions', 'breast tissue microbiota', 'human mammary gland microbiota'. All relevant open access full-text articles published in English within the last 25 years were included in the analysis.

7. Conclusions

Accumulating evidence underscores a compelling association between microbial dysbiosis and the pathogenesis of breast diseases. This review synthesizes current understanding of the MG microbiota—a distinct community formed through exogenous (cutaneous, retrograde translocation)

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and endogenous (enteromammary, hematogenous) pathways—and its intricate interactions with the host via regulation of estrogen metabolism, immunomodulation, and epigenetic mechanisms.

Notably, while taxonomic profiles show similarities between benign and malignant breast conditions, specific dysbiotic patterns emerge in BBD. These include altered abundances of taxa such as *Pseudomonadota* and *Bacillota*, differential microbial metabolic pathways, and the prominent role of particular microorganisms like *Corynebacterium kroppenstedtii* in granulomatous mastitis and *Staphylococcus aureus* in fibroadenomas and lactational mastitis. Furthermore, viral agents, particularly HPV, have been detected in a significant subset of benign lesions. Despite these associations, current evidence remains largely correlative, and the low microbial biomass of breast tissue, alongside a scarcity of data on the virome and mycobiome, precludes definitive establishment of causality.

Elucidating the precise microbial agents and mechanisms driving dysbiosis in BBD represents a critical research frontier. Success in this endeavor holds transformative potential for clinical practice, paving the way for microbiota-based strategies in prevention, diagnostics, and personalized treatment. Future studies must prioritize longitudinal designs, multi-omics integration, and robust mechanistic models to transcend current limitations. Ultimately, advancing this field necessitates strengthened interdisciplinary collaboration across microbiology, oncology, and immunology to translate these fundamental insights into tangible benefits for patients with benign breast diseases.

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