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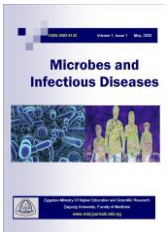

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Review article

Probiotics: Mitigating antibiotic resistance in the vaginal microbiome

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ABSTRACT

Background: Studies have shown a strong correlation between probiotics and incidence of antibiotic resistance. Many studies have reported antibiotic resistance in the vaginal microbiome especially in women with dysbiosis condition, and it is possible that the antibiotic resistance plays a role in the alteration of the vaginal microbiome and disease complications. Consequently, this study aims to investigate the vaginal microbiome with antibiotic resistance in healthy, menopausal and post-menopausal women and the mitigating effect of probiotics. In this narrative review, a comprehensive review of web of science, PubMed, Scientific information database, Google scholar and Scopus with no time limit. The keyword "Probiotics", "Antibiotic resistance", "Vaginal Microbiome", "Vaginal Microbiota" were used to provide a synopsis of mitigating antibiotic resistance in the vaginal microbiome. Among the 917 sources identified, 12 articles were found most appropriate for the study. Our findings recommend administration of probiotics to alleviate antibiotic resistance. The study revealed that probiotics may mitigate antibiotic resistance in the vaginal microbiome and possibly improve the female reproductive health at all levels including menopausal and post- menopausal.

Introduction

Since the introduction of antibiotics, there have been a persistent rise in resistance so alternative approaches are been considered where probiotics could play a role. Recently, researchers are beginning to look into the broader ecological aspect of an infection whereby the entire microbial community is studied to know the effect of the antibiotics on the host microbiota [1]. Rapid advances in high-throughput sequencing technologies have led to a realization that the resident human microbiota and its corresponding genes, or microbiome, play an integral role in human health [2]. Many of the organisms that make up the human microbiome have not been successfully cultured, identified, or otherwise characterized.

Probiotics involve diverse microbes. The administration of probiotics are mostly strain-specific thus, in mitigating the global challenge of antibiotic resistance, the use of probiotics as a substitute for antibiotics in the treatment of reproductive diseases in women have been investigated [3]. Probiotics mitigate antibiotic resistance in the vaginal microbiome by inhibiting

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pathogenic or antibiotic resistant microbes by commensal and sometimes mutualism relationship, consequently; combating the risk for vaginal infection [4]. It is noteworthy that the phenomenon of a balanced vaginal microbiome or "normal" microbiota could prevent reproductive disease conditions either of infectious etiology or microbial imbalance thus, improving the vaginal health [5]. In women with antibiotic-resistant strains, an alteration of the vaginal microbiota could be observed [6]. Therefore, with the known beneficial effects of probiotics on maintaining microbial balance in the vagina, its application to combat antibiotic-resistant infections seems critical.

In the microbial homeostatic milieu, the human vaginal microbiome plays a crucial role keeping the vaginal health. Understandably, a lot of research work has focused on characterizing the diverse microbial communities residing in the vaginal environment, with a growing area of interest is the vaginal resistome [7]. Vaginal resistome refers to the collection of antibiotic resistance genes (ARGs) present in the vaginal microbial milieu [8]. Understanding the vaginal resistome in healthy females is essential, as it sheds light on the dynamic, complex and sophisticated interplay between the vaginal microbiome and antibiotic resistance [9].

This review article aims to explore the current knowledge about the use of probiotics to mitigate antibiotic resistance and also explore the vaginal resistome in healthy females and its implications for women's health.

In this narrative review article, the keyword "Probiotics", "Antibiotic resistance", "Vaginal Microbiome", "Vaginal Microbiota" was searched in Web of science, PubMed, Google scholar, Scopus and Scientific information database with no time limit. Published articles in duplicates and also not appropriate for this study were expunged. Among the 917 sources identified, 12 articles were found most appropriate for the study.

The vaginal microbiome: A vibrant ecosystem

The vaginal microbiome is a dynamic and complex ecosystem predominantly inhabited by *Lactobacillus* species in healthy individuals. These beneficial bacteria maintain a slightly acidic pH, produce lactic acid, and play a pivotal role in preventing the overgrowth of pathogenic microbes [10]. However, the composition of the vaginal microbiome can differ considerably between individuals. Four main vaginal community types have been identified: *Lactobacillus*-dominant, diverse *Lactobacillus*, mixed anaerobes, and high diversity [11]. These variations have important implications for reproductive health, including bacterial vaginosis (BV) susceptibility to infections and complications during pregnancy [12].

The pathogenic bacteria found in the lower female genital tract include: *Eubacterium, Atopobium, Mycoplasma, Bacteroides, Gardnerella vaginalis, Streptococcus, Propionibacterium, Prevotella, Ureaplasma,* among others. [13]. When the lactobacilli concentration in the vagina reduces, these bacteria may overgrow to the extent that they dominate the environment as opportunistic pathogens.

Probiotics

Probiotics are living organisms, which, when given to a host in adequate amount, can exert beneficial health on them by improving their microbial balance. Probiotics can also be referred to as; friendly, healthy, good or beneficial microbes and can be supplied through foods, beverages, and dietary supplements [14]. They have also been described as bacterial species that may bring benefits to the host through a symbiotic relationship.

Probiotics play an essential role in promoting and alleviating infectious diseases. Among the most common groups of probiotics are bacteria in the genera *Lactobacilli, Bifidobacteria, Streptococci*, some strains of genera like Bacillus, *Enterococcus, Escherichia, Propionibacterium* and the fungus of genera *Saccharomyces* [15].

The general knowledge about probiotics is that the health benefits they confer are usually strain specific and cannot be extrapolated to other strains; not even of the same species [16]. These microorganisms have characteristics that give them the ability to withstand adverse conditions in the host, such as enzymatic action and acidity. They readily colonize the host thereby regulating the microbiome and performing vital biological functions [17].

Most drugs administered orally, influence the survival of probiotics which subsequently affect their colonization and efficacy. Though probiotics have been proven effective in the treatment of many diseases, it is essential to update and enhance their controlled testing, adverse interactions, therapeutic outcomes, approval, and administration [18]. Indistinct guidelines for the production and administration of probiotics will provide a backbone

for improved regulation for probiotics thus promoting safe and effective use.

Vaginal microbiome alteration by antibiotic resistant genes

Table 1 summarizes antibiotic resistance studies in the vaginal microbiome among women of child bearing age. In 2008, Nagaraja examined 321 high vaginal swab samples for resistance to clindamycin and metronidazole [19]. In this study, resistance to metronidazole was detected with increase in the relative abundance of *G. vaginalis* [19]. Likewise, **Li *et al.*** examined the vaginal microbiome of 10 reproductive aged women between the ages of 18 – 50 years using 16S rRNA molecular methods [20]. The percentage of metronidazole resistant genes was 58.84% with a corresponding relative increase in *Gardnerella vaginalis (G. vaginalis)*. Overall, these data suggest that the presence of metronidazole resistant genes (rdxA and frxA) alters the vaginal microbiome by resulting in relative abundance of *G. vaginalis* [19,20].

In 2016, **Bostwick *et al.*** evaluated the vaginal microbiome of 289 women of child bearing age in a case-control study using next-generation sequencing (NGS). The aim was to determine the frequency of fourteen (14) anti-microbial resistance (AMR) genes [21]. A total of 58.2% of antibiotic resistant genes were detected. There were varied distribution of the genes: lincosamides resistance (58.9%), and tetracycline resistance (35.6%) ($p < 0.001$). The *ermTR* (clindamycin resistance gene) had the highest frequency (61.8%) and *nim* (metronidazole resistance genes) had the lowest frequency of 1.4%. However, the main limitation of this study was that AMR gene findings were not linked to the alteration of vaginal microbiome. Furthermore, **Petrina *et al.*** and **Deng *et al.*** subsequently evaluated the presence of AMR genes in the vaginal microbiota with clindamycin and metronidazole resistance genes respectively [22,23]. They reported the presence of *Prevotella* spp. with 108 isolates of *Lactobacilli* and a relative abundance of *G. vaginalis* respectively.

More recently, whole-genome sequencing (WGS) has also been used to investigate the presence of antibiotic resistant genes such as metronidazole resistance genes in the vaginal microbiota [24]. The WGS revealed *Gardnerella* spp. as the most highly abundant bacterial spp. in the vaginal microbiota. There was a decline in the relative abundance of *Lactobacillus* spp. and *Prevotella* spp. The metagenome of the AMR genes revealed the prevalent genes was tetracycline resistance (*tetM*), clindamycin resistance (*IsaC*), and the least common was metronidazole resistance gene, (*nimJ*) [24].

The key resistance mechanism among clinically relevant bacteria detected in the vaginal microbiota against clindamycin involves changes in the antibiotic binding site by ribosomal methylation [25]. This is coded by the erythromycin methylase (*erm*) genes. Genes coding for nitroimidazole resistance are referred to as *nim* genes [26]. The *nim* genes encode a 5-nitroimidazole reductase enzyme which converts 4- or 5-nitroimidazole to 4- or 5-aminoimidazole, resulting nitroimidazole resistance. This leads to the formation of toxic nitro radicals [27].

Vaginal microbiota and antibiotic resistance

The composition of the vaginal microbiota can have implications for antibiotic resistance in menopausal women. The vaginal microbiota may harbor antibiotic resistance genes, which can be transferred to pathogenic bacteria [28]. This reservoir of ARGs could contribute to antibiotic resistance in vaginal infections. Antibiotic use can disrupt the balance of the vaginal microbiota in menopausal women, potentially leading to dysbiosis and an increased risk of infections [9]. Treatment challenges may also be an issue as alterations in the vaginal microbiota composition may affect the effectiveness of antibiotic treatments commonly prescribed for urinary tract infections and vaginal infections, presenting challenges in managing these conditions in menopausal women [29-32].

The vaginal resistome in health

Several studies have revealed the presence of ARGs in the vaginal microbiomes of healthy females. While the clinical significance of these ARGs in the absence of antibiotic exposure remains a subject of debate, their presence underscores the need for further investigation [33]. Some ARGs may naturally exist in commensal bacteria of the vaginal microbiome. These genes may seem a benign threat, but could serve as a reservoir for antibiotic resistance transfer to pathogenic bacteria [34]. The transfer of ARGs between bacteria, known as horizontal gene transfer, can occur within the vaginal microbiome. This phenomenon may facilitate the spread of antibiotic resistance among microbial species [33]. Lifestyle factors, such as

sexual activity and contraceptive methods, may also influence the constitution of the vaginal resistome. Understanding these associations could provide insights into strategies for minimizing the spread of antibiotic resistance [35].

Obviously, it is beyond doubt that the vaginal resistome is a relatively new and evolving field of research that has significant and direct implications for women's health. While the presence of antibiotic resistance genes in the vaginal microbiome of healthy females is well-established, the clinical relevance and impact of these genes are still not fully understood [36]. Further research is needed to unravel the complex interactions between the vaginal microbiome [37], resistome, and women's health.

Vaginal microbiota composition and its implications for antibiotic resistance in menopausal health.

It is now well understood that the vaginal microbiota is a complex ecosystem that plays a pivotal role in maintaining female reproductive health. Extensive research has been conducted on the vaginal microbiome in women of reproductive age [38]. However, there is also growing need to understand its composition and antibiotic resistance implications in menopausal women. The menopause is associated with various physiological changes, including alterations in the vaginal microbiota [39]. This article also aims to explore the current state of knowledge regarding vaginal microbiota composition and its relevance to antibiotic resistance in menopausal health.

The female resistome in post-menopausal women (PMW)

The resistome is composed of ARGs within the female microbial milieu. It adds a layer of microbial complexity to post-menopausal health. These resistomes may be influenced by a variety of factors such as: Exposure to antibiotics, horizontal gene transfer and Hormonal changes [40].

Women may be exposed to antibiotics for various reasons throughout their lives, this could be as a result of treating infections, hospitalization or surgical procedures. These exposures can lead to the acquisition and accumulation of ARGs within the female microbiota [41, 42]. Antibiotic resistance genes in the female microbiome may undergo horizontal gene transfer, allowing for their spread to pathogenic bacteria. This can lead to antibiotic resistance in infections and complicate treatment [33]. The dynamics of hormonal changes during the transition to menopause may also influence the resistome, by potentially lowering the *Lactobacillus* population, promoting the transfer of ARGs and effecting susceptibility to antibiotics [32, 43].

The vaginal resistome- a group of overall microbiome which consists of ARGs that confers resistance mechanisms against antibiotics of different spectra. This genetic attribute provides microorganisms with the ability to withstand the effects of antibiotics, rendering them ineffectual [44]. Understanding the presence and diversity of ARGs in the vaginal microbiome is important for certain reasons: comprehend the dangers of antibiotic resistance, understand the effect on treatment with antibiotics and role on reproductive health. The rise in antibiotic resistance poses a significant threat to public health in the global sphere [45]. The discovery of ARGs in the vaginal microbiota, highlights the potential reservoirs for these resistance genes therein, which could contribute to the dissemination of antibiotic resistance and portend remarkable threat for the global health community [46]. Antibiotics are commonly recommended for vaginal infections such as bacterial vaginosis, sexually transmitted infections and urinary tract infections [8]. The presence of ARGs in the vaginal microbiome may negatively affect the course of these treatments, and lead to treatment failures and recurrent infections in urogenital health management [47]. The vaginal microbiome plays an important role in maintaining a balanced milieu in healthy reproductive tract [48]. The presence of ARGs may alter the microbial balance, displace probiotics and effectively increase the risk of infections and complications during pregnancy such as preterm births [8].

Menopause, marked by the end of menstruation, typically occurs in the late 40s to early 50s. Hormonal changes during this period result in a down-regulation in estrogen levels, which can impact the vaginal environment [49]. Estrogen plays a crucial role in maintaining the vaginal epithelium, downregulating pro-inflammatory cytokines and promoting the growth of *Lactobacillus* species, which are predominant in a healthy vaginal microbiome [49].

Some of the changes experienced during menopause are: Vaginal dryness and shift in microbial composition. Estrogen decline can result in vaginal atrophy and dryness, which may lead to

discomfort and an increased risk of vaginal infections. Menopause is often associated with a shift in the vaginal microbiota from a Lactobacillus-dominant state to a more diverse composition. This change can lead to a decrease in lactic acid production and a higher pH in the vaginal environment, making it more susceptible to colonization by pathogenic bacteria [50].

Given the importance of the vaginal microbiota in menopausal health and its potential implications for antibiotic resistance, healthcare intervention strategies should tailor antibiotic treatments based on the individual's vaginal microbiota composition in order to improve treatment outcomes and reduce the risk of antibiotic resistance. Also this review has shown that rational antibiotic use, compliance to the prescribed antibiotic schedule should be encouraged.

As established, the human microbiome-a dynamic community of microorganisms living within the human body, plays an important role in the maintenance of health and prevention of diseases. The female microbiome, which includes the vaginal and gut microbiota, undergoes significant changes during the post-menopausal period [36]. Moreover, novel knowledge on antibiotic resistance genes (ARGs) within the female microbiome (resistome) have raised concerns on the impact on post-menopausal health. Exploration of the current state of knowledge regarding the female microbiome and resistome in post-menopausal women and their potential implications for health is thereby imperative. Post-menopause is a natural phase in a woman's reproductive lifetime, typically occurring around the age of 50 when ovarian function stops physiologically. This period is marked by a decrease in estrogen levels, which has remarkable and significant effects on the female microbiome, effects have also shown a dependent interplay with inflammatory cytokines [49].

Post-menopause often leads to alterations in the composition of the vaginal microbiota. The decline in estrogen can result in reduced dominance of *Lactobacillus* spp, leading to a shift toward a more diverse microbiome, which may increase the risk of vaginal infections and discomfort. Fluctuations in the hormonal system can also have a significant impact on the gut microbiology, and this could have a direct bearing on weight gain, digestive issues, inflammation and metabolic changes [50].

Understanding the dynamics between the female microbiome and resistome in post-menopausal women is crucial for health management. Post-menopausal women (PMW) are susceptible to a variety of conditions such as urinary tract infections (UTI), vaginal health issues, digestive health complications and systemic health implications [32]. Post-menopausal women are at an increased risk of UTIs, and the composition of their vaginal microbiome may change remarkably. These changes may influence the vaginal structure and functions, contributing to the onset of genitourinary syndrome in PMW. A dysbiosis of the urinary microbiome is linked with urinary incontinence in PMWs [32]. Additionally, alterations in the vaginal microbiota composition can lead to vaginal atrophy and infections. These issues may be further aggravated by antibiotic use, which may cause disruption of the microbiota and promote the growth of antibiotic-resistant pathogens. Furthermore, alterations in the gut microbiome may influence digestive health and metabolic outcomes. The presence of ARGs in the gut microbiota could also constrain treatment options for gastrointestinal infections [51].

Lastly, emerging research studies suggests that the microbiome, including the resistome, may have systemic effects, impacting overall health and promote inflammation in post-menopausal women [52]. Understanding the dynamic changes in the microbiome and resistome during this life stage is essential for providing comprehensive healthcare to post-menopausal women [53-55].

In conclusion, the vaginal microbiome, a multifaceted subject with associations with antibiotic resistance and overall reproductive health. The vaginal resistome remains an important area of study that links medical microbiology to clinical practice. It underscores the need for a holistic approach to women's reproductive health that considers the complex balance of microbial communities and antibiotic resistance genes within the vaginal environment. Future research is needed with focus on the elucidation of specific mechanisms which connects the vaginal microbiota, antibiotic resistance, and probiotics leading to the development of targeted interventions that will optimize women’s health (pre-menopausal and post-menopausal) while minimizing the risk of antibiotic resistance emergence. An understanding of these intricate connections, healthcare providers can better support women in maintaining a healthy

vaginal microbiome and overall reproductive well-being given the beneficial effects of probiotics.

Table 1. Antibiotic resistance studies in the vaginal microbiome of reproductive aged women.

First Author, year	Study population	Antibiotic resistant gene	Laboratory analysis	Vaginal microbiome alteration
Nagaraja 2008 [19]	321 reproductive aged women	34 metronidazole genes (*rdxA* and *frxA*)	Polymerase chain reaction	Increase in the relative abundance of *G. vaginalis*
Petrina 2017 [22]	713 women of reproductive age	58 clindamycin genes (*ermA, ermB, ermC* and *ermF*)	16S rDNA for restriction fragment length polymorphism (RFLP) analysis	*Prevotella* spp. 108 isolates of Lactobacilli
Li *et al.* 2020 [20]	10 women of reproductive age of 18 – 50 years.	58.85% of metronidazole (*rdxA* and *frxA*) genes	16S rRNA gene hypervariable V1–V3 region	*G. vaginalis* relative abundance.
Bostwick *et al.* 2016 [21]	289 reproductive aged women	The genes: *ermA, ermB, ermC, ermM, ermTR* and *mefA*; tetracyclines, β-lactams, streptomycin, gentamicin and/or tobramycin - *acrA, acrB, mecA, tet, tetA, tolC* and *aac2*; 5-nitroimadazoles - *nim* and *nimB*; and triazoles - *cdr1* and *mdr1*.	Next-generation sequencing used to describe the complete vaginal microbiota and identify bacterial genes associated with resistance	Upsurge of *Gardnerella vaginalis* *Atopobium vaginae* *Atopobium parvulum* *Dialister micraerophilus* *Prevotella timonensis* *Megasphaera micronuciformis* *Sneathia sanguinegens*
Deng *et al.* 2018 [23]	37 women	7 of 8 clustered regularly interspaced short palindromic repeat (CRISPR)-associated (Cas) genes	Meta-transcriptomic analysis of the vaginal microbiota	*G. vaginalis* were highly upregulated. The CRISPR-Cas system may protect the vaginal microbiota against the DNA damaging effect of metronidazole.
Ruiz-Perez 2021 [24]	5 African American women ages 19–22 sexually active	Resistance genes: *tetM* and *lscA* were detected.	Whole-genome sequencing was used to determine changes in the vaginal microbiota	Decline in the relative abundance of *Lactobacillus* and *Prevotella* spp. and an upsurge in the relative abundance of *Gardnerella* spp.

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Conflict of interest

The authors declare that there is no conflict of interest.

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