REVIEW

Modulation effect of vaginal mucosal microflora and susceptibility to *Neisseria gonorrhoeae* infections: a systematic review and meta-analysis

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Abstract

Objective The vaginal microbiota may modulate susceptibility to *Neisseria gonorrhea* (NG) infections. The objective of this meta-analysis was to evaluate the association between these NG infections and the vaginal microbiota.

Method A systematic review and meta-analysis was conducted to investigate the correlation of vaginal microbiota and NG risk. Primary sources of the reviewed studies were from inception through December 2018. Vaginal mucosa microflora were dichotomized into high-*Lactobacillus* vaginal microbiota and low-*Lactobacillus* vaginal microbiota (LL-VMB), using either Nugent score, Amsel's criteria, presence of clue cells or 16S rRNA gene sequencing.

Results A total of 8 studies qualified for inclusion in this meta-analysis. LL-VMB could be regarded as worse prognostic factor, and the pooled OR was 1.33 (95% CI 1.02, 1.73; $P = 0.04$, $I^2 = 44\%$). LL-VMB was associated with a significantly higher susceptibility of NG. Trend for the sensitive analysis was consistence with the primary outcome. Significant publication bias was not detected by the funnel plot.

Conclusion In conclusion, the systematic review and meta-analysis has demonstrated that LL-VMB was significantly associated with a high NG susceptibility.

Keywords Neisseria gonorrhea · Vaginal microbiota · Susceptibility · Meta-analysis

Introduction

There are a lot of microorganisms in the body surface and cavity. These microorganisms including bacteria, fungi, viruses and so on, form a "Super-organism" with their hosts. They have established an inseparable symbiotic relationship with an impact on human health and disease status [1]. Vaginal microecology, as one of the four major micro-ecosystems of human body, has attracted more and more attention. Vaginal microecology is a part of human microecology. It is an important part of life to study the structure and function of microorganisms and their relationship with their hosts in women's vaginal health and disease [2, 3]. The clinical evaluation system of vaginal microecology is to evaluate the vaginal microecology from the aspects of morphology and function, including

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dominant bacteria, diversity and density of vaginal microflora, pathogenic bacteria (such as trichomonas and fungi), Lactobacillus classification, white blood cell number, epithelial cells and some functional indicators (such as pH value, sialidase, leucocyte esterase, beta-glucuronidase, acetylglucosaminidase, etc). Nugent score and Donders score can be used to evaluate the content of the evaluation, which can also reflect the functions of Lactobacillus and white blood cells [4–6].

In addition, it is worth noting that the vagina, as an open human cavity, is vulnerable to external environment and other disturbances, which makes the dynamic balance of the vagina vulnerable to disturbance. Physiological activities such as age, menstrual cycle, pregnancy, childbirth, breastfeeding, contraception and sexual life can affect the vaginal micro-ecological environment [7]. Some pathological conditions such as infectious diseases, endocrine diseases and the use of antibiotics, immunosuppressants and chemotherapeutic drugs can also break the balance of vaginal micro-ecological environment. Once this balance is broken, it will cause vaginal-related infections and lead to pathological changes. Any factor affecting vaginal microecological balance will cause discomfort to the host, i.e. the clinical manifestations of vaginal microecological abnormalities. The most typical vaginal microecological disorders are bacterial vaginosis (BV), and aerobic vaginitis (AV) proposed in 2002. Other abnormal vaginal microecology include *Vulvovaginal candidiasis* (VVC), *Trichomonas vaginitis* (TV) and *Cytolytic vaginosis* (CV) [8–10].

Gonorrhea is a sexually transmitted disease (STD) caused by *Neisseria gonorrhoeae* (NG). The columnar epithelial cells of the human urogenital tract are vulnerable to NG invasion and destroy the mucosal epithelium. In view of the complexity of the vaginal flora in women, it is recommended to culture and identify the bacteria directly instead of smear examination. The primary site of NG infection in female genital tract is mainly in the cervix. By means of pili protein II and its released IgA, decomposing enzymes adhere to epithelium, and NG is swallowed by columnar epithelial cells and multiplied in large quantities after entering cells [11, 12]. If the genital tract is not treated in time after NG infection, or improper treatment, pelvic inflammation and other diseases are more likely to occur. Repeated chronic episodes will lead to tubal stenosis or occlusion, resulting in infertility. Therefore, timely diagnosis and treatment can avoid the development and aggravation of the disease [13]. Therefore, it is very important for patients and medical workers to understand the clinical application value of STD experimental detection methods, and to carry out extensive early screening, early diagnosis and early treatment of STD. This study is to study the relationship between vaginal microflora change and susceptibility to NG, so as to provide new ideas and means for the prevention and treatment of NG.

Materials and methods

This study was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [14].

Literature search

Retrieval of all clinical studies published before December 2018 evaluating the correlation of vaginal microflora change and susceptibility to NG. Retrieval databases include: PubMed, Embase, Cochrane Library, and Web of Science. The searched combination of keywords include: *Neisseria gonorrhoeae/gonorrhea* or NG or sexually transmitted disease or STD and vaginal/genital microflora/microbiota/microbial or colonization/organisms or vaginosis or *Lactobacillus*. Language is limited to English. References were consulted for enlarged retrieval.

Inclusion and exclusion criteria of literature

Included studies met the following eligibility criteria: (1) the association of NG and vaginal microflora must be investigated; (2) Nugent score/Amsel's criteria and/or 16S rRNA gene amplicon sequencing were applied to identification and classification of vaginosis; (3) studies should be cohort, cross sectional or interventional designed on the human female population.

Exclusion criteria: (1) Unable to extract data or failed to find the full text of the literature. (2) Select high-quality or the most recently published literature from the same author or team when duplicate publications were found. (3) Reviews, editorials, correspondences, comments, letters, practice guidelines, case report or editorials.

Data collection and quality evaluation

Two reviewers independently read the title, abstract and full text of the literature. Bias risk of data extraction and evaluation included in the study was cross-checked. In case of disagreement, the two reviewers discussed or seek for a third-party solution. The lack of important data should be supplemented by email and telephone contact with the original author. The contents of extraction include: (1) basic information included in the literature, including the first author, year of publication, sample size, age and so on. (2) Baseline characteristics and intervention measures of the subjects. (3) Research types and key elements of bias analysis and evaluation. (4) Outcome indicators and outcome measurement data of concern. Newcastle–Ottawa

Scale was applied to evaluate the quality of included studies [15].

The clinical indicators for extraction and analysis included: Authors, countries, study design/population, number of patients and age information, method for microbiota characterization and NG diagnosis, follow-up duration and study quality.

Outcome measures

To better present the relations of vaginal microflora and NG infection, the former was classified into low-*Lactobacillus* and high-*Lactobacillus* vaginal microbiota (LL-VMB and HL-VMB). LL-VMB is characterized by a lack of Lactobacillus and a large number of strictly facultative anaerobes. This definition has a good correlation with a high Nugent score (7–10), positive Amsel test, and the existence of clue cells. HL-VMB corresponds to the types of vaginal communities dominated by either *L. crispatus*, *L. iners*, *L.gasseri* and *L. jensenii*, Nugent score is low (0–6) and Amsel test is negative.

Statistical methods

Heterogeneity test and effect value were analyzed by Comprehensive Meta-Analysis (CMA) [Computer program], Version 2.0 [16]. Firstly, the heterogeneity was tested by 2 test (test level: $a = 0.1$). If P (> 0.1), $I^2 < 50\%$, it showed that the included studies were homogeneous. Meta-analysis was carried out using fixed-effect model. If there is heterogeneity among the studies, after excluding the influence of obvious heterogeneity, the random effect model is used for meta-analysis [17]. Subgroup analysis or sensitivity analysis or descriptive analysis was used for the studies with obvious heterogeneity. Odds ratio (OR) and the corresponding 95% confidence interval (CI) were selected as combined statistics for the continuous variables. The test level of Meta-analysis was alpha = 0.05. Sensitivity analysis: The results of $P < 0.10$ and I^2 ($> 50\%$) in heterogeneity test were analyzed by the method of conversion between fixed effect model and random effect model [18, 19].

Results

Collection and selection of literatures

Initially, we retrieved 298 literatures in various databases through relevant search terms and strategies, of which 7 were manually retrieved. After deleting duplicated 163 literatures among the major databases, we screened the title and abstract of the remaining 142 literatures. Since then, 107 literatures with unrelated or incomplete data have been excluded. We downloaded and reviewed the remaining 35 literatures in full text. 27 literatures that did not meet the inclusion criteria were subsequently excluded. Finally, 8 studies [20-27] were included in the meta-analysis (Fig. 1).

Baseline characteristics and study quality assessment

In the included 8 studies published from 2002 to 2015, LL-VMB was characterized by the positive Amsel test, a higher Nugent score between 7–10 and the presence of clue cells (whether or not there were 16S rRNA gene amplification subsequences). In most studies, the relationship between vaginal microflora and NG was not always the main option of choice, therefore, we must extract relevant data from subgroup analysis.

For each study in detail, 1200 women at high risk of sexually transmitted diseases were enrolled from five clinical research sites in the United States (Ness et al.) to explore how the frequency, recentness and causes of irrigation affect the occurrence of vaginal microflora and cervical pathogens associated with bacterial vaginosis [27]. Abbai et al. evaluated the point-of-care test for NG detection in patients at the KwaZulu Natal Public Health Clinic in South Africa [20]. Kapiga et al. attempted to identify risk factors for sexually transmitted diseases among 958 women in South Africa, Tanzania and Zambia in a 1-year prospective study [23]. In a cohort of 966 women in northwestern Tanzania, Francis et al. investigated the prevalence, trends and other related factors of with NG at the sites of food and entertainment facilities near bars, hotels and other large mines [21]. In a cross-sectional study of 1938 women conducted by Yen et al., Nugent-Gram staining was used to assess the prevalence of bacterial vaginosis and to test the clinical correlation with bacterial vaginosis [26]. Kaul et al. studied the relationship between prevalent HSV-2 infection and the incidence of sexually transmitted infections in a prospective randomized trials among female sex workers in Kenya [24]. In a longitudinal study involving 3620 non-pregnant women aged 15–44, the association of bacterial vaginosis and NG infection was explored by Brotman et al. [22].

Newcastle–Ottawa Scale was applied to evaluate the quality of included studies [15]. Selection (4 stars), comparability (2 stars), and outcome assessment (3 stars) were the three key points of scoring for the included studies. All study qualities were scored as medium–high.

Meta-analysis

Figure 2, meta-analysis on the correlation of vaginal microbiota and NG susceptibility demonstrated that LL-VMB could be regarded as worse prognostic factor, and the pooled OR was 1.33 (95% CI 1.02, 1.73; $P = 0.04$,

Fig. 1 Flow chart of publication
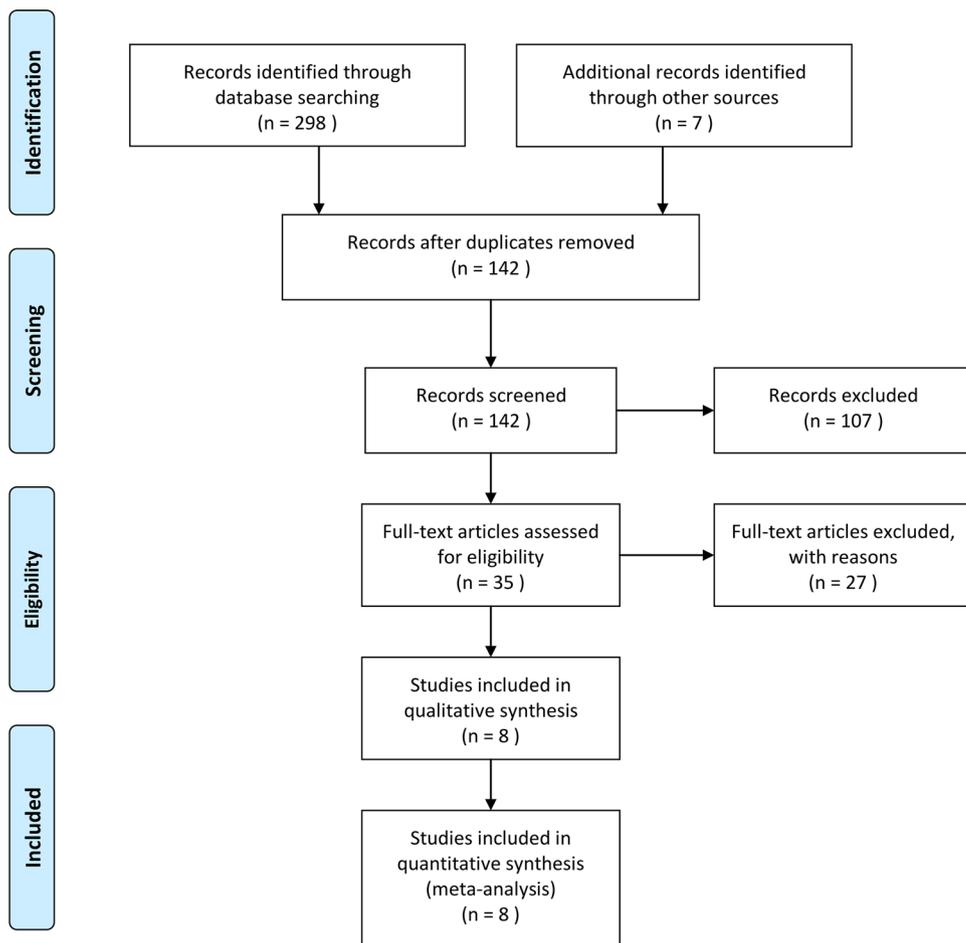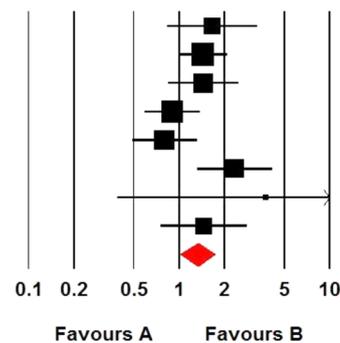search and selection

Fig. 2 Forest plot depicting the
meta-analysis of *Neisseria gon-
orrhoeae* susceptibility between
low-Lactobacillus vaginal
microbiota and high-Lactobacil-
lus vaginal microbiota

$I^2 = 44\%$). LL-VMB was associated with a significantly higher susceptibility of NG. Furthermore, we investigated the robust of the meta-analysis by sensitive analysis with the one-leave out method. Figure 3, only when Ness et al.'s [27] and Brotman et al.'s [22] studies were excluded the final analysis, the pooled outcomes were non-significant, with the pooled ORs were 1.24 (95% CI 1.00, 1.55; $P = 0.054$) and 1.20 (95% CI 0.98, 1.46; $P = 0.079$) respectively. However the trend towards a high risk for NG infection was still very obvious.

Fig. 3 Forest plot depicting the sensitive meta-analysis of *Neisseria gonorrhoeae* susceptibility between low-Lactobacillus vaginal microbiota and high-Lactobacillus vaginal microbiota by the leave-one out method
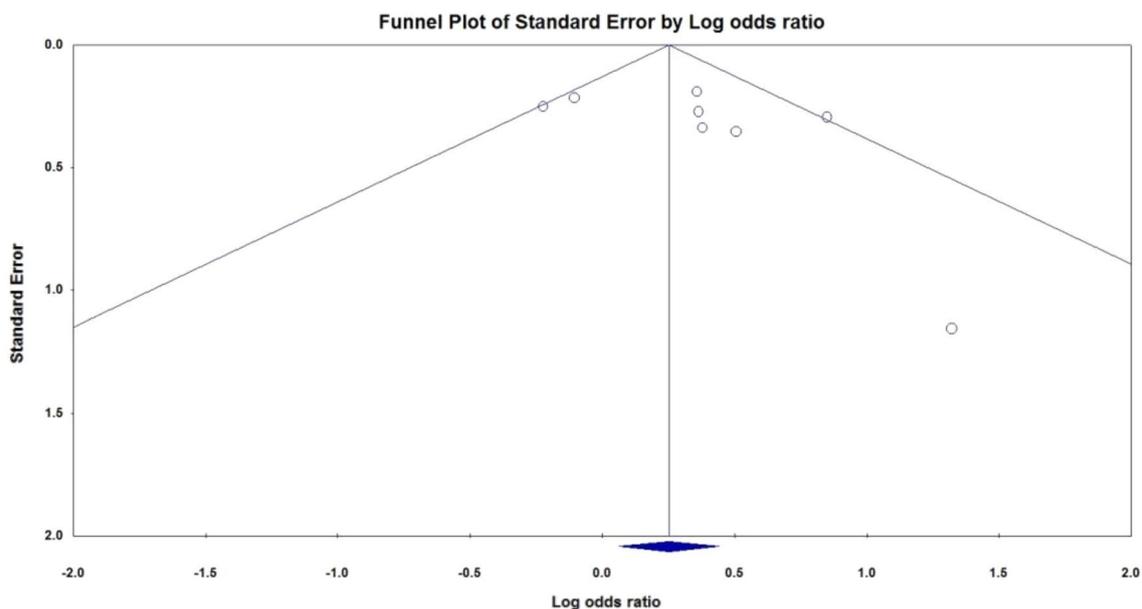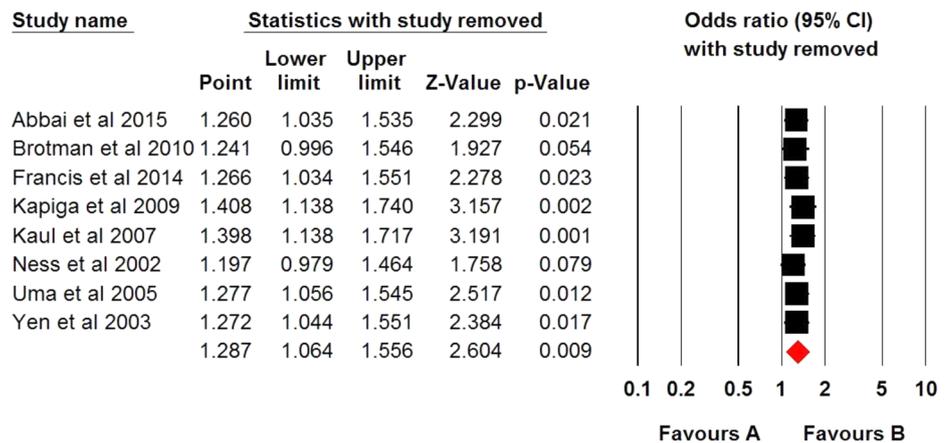
Study name	Statistics with study removed					Odds ratio (95% CI) with study removed
	Point	Lower limit	Upper limit	Z-Value	p-Value	
Abbai et al 2015	1.260	1.035	1.535	2.299	0.021	
Brotman et al 2010	1.241	0.996	1.546	1.927	0.054	
Francis et al 2014	1.266	1.034	1.551	2.278	0.023	
Kapiga et al 2009	1.408	1.138	1.740	3.157	0.002	
Kaul et al 2007	1.398	1.138	1.717	3.191	0.001	
Ness et al 2002	1.197	0.979	1.464	1.758	0.079	
Uma et al 2005	1.277	1.056	1.545	2.517	0.012	
Yen et al 2003	1.272	1.044	1.551	2.384	0.017	
	1.287	1.064	1.556	2.604	0.009	

0.1 0.2 0.5 1 2 5 10

Favours A   Favours B

Fig. 4 Funnel plot depicting the publication of the included 8 studiess

Publication bias was not significantly detected, as shown by Fig. 4, the funnel plot was overall symmetry.

Discussions

Gonorrhea is one of the STD with a high incidence. It accounts for about 3/4 of STD cases in the world and is spreading gradually in China. The incidence of gonorrhea accounts for the first place in the total number of STDs reported in China and is on the rise. NG is the pathogen of gonorrhea. It exists in purulent secretions of acute and chronic urethritis and vaginitis and conjunctival secretions of newborns. Gonorrhea can be divided into acute and chronic diseases according to the duration of the disease, which seriously threatens people's health. In addition, in recent years, due to the diversification of sexual life style, NG infection sites are also increasing, clinical gonorrhea in oral, anal and rectal areas. Generally, the number of male patients infected with NG is higher than that of female patients. Among them, male patients mostly show symptoms such as stinging pain of urinary tract, frequent urination, yellow and white pus flowing from the urethral orifice, and have sharp characteristics, accompanied by fever and fatigue symptoms, which have a greater impact on the normal life of patients. Compared with male patients, female patients have fewer symptoms and are easily mistaken for increased leucorrhea, leading to missed diagnosis and misdiagnosis. However, female gonorrhea patients can be clinically asymptomatic and neglected, but as far as the physiological

anatomy of female genital organs is concerned, female cervix is more susceptible to infection than male urethra, therefore, the prevalence may be higher.

In healthy women of reproductive age, the vagina is a microenvironment with hydrogen peroxide-producing *Lactobacillus* as dominant bacteria, symbiosis of several microorganisms, fungus-free sporogenesis or hyphae, *Trichomonas vaginalis* and other pathogenic bacteria, and pH value of 3.8–4.5. *Lactobacillus* is a probiotic bacteria in the vagina. It can produce hydrogen peroxide, lactic acid and lactobacillin, and competently adhere to the vaginal epithelium, occupy binding sites, and consume vaginal nutrition to obtain its dominant position in the vagina and inhibit the excessive proliferation of other bacteria. *Lactobacillus*, as a dominant genus of bacteria in the vagina of normal women, can maintain the stability of vaginal microecology through a variety of mechanisms. On the one hand, it can form biofilm. On the other hand, it can maintain the vaginal acidic environment (normal pH 3.8–4.4) by secreting lactic acid and hydrogen peroxide, and then inhibit the growth of other miscellaneous bacteria. There are more than ten kinds of *Lactobacillus* in the vagina of women of childbearing age, but one or two of them are the dominant bacteria in the vagina of a single woman. The most common ones are *Lactobacillus curvulus*, *Lactobacillus inertia*, *Lactobacillus Calmette* and so on. Ravel et al. analyzed the vaginal secretions of healthy women of childbearing age of many races (white, Asian, African, Hispanic) in the United States by 16S DNA sequencing technology. It was found that *L. crispatus* was the dominant group of vaginal bacteria in white women, while *L. iners* was the dominant group in Asian women. According to the research results, Ravel proposed for the first time to divide the microflora of female vagina into five types (CST), i.e. type I (*L. crispatus* as dominant flora), type II (*L. gasseri* as dominant flora), type III (*L. iners* as dominant flora), type IV (anaerobic bacteria as dominant flora), type V (*L. jensenii* as dominant flora). Bacteria are dominant flora, which has gradually become the consensus of vaginal flora researchers. However, more and more studies have shown that, similar to intestinal microecology, the composition of vaginal microorganisms in women of different regions and races is different. In addition, as an integral part of the reproductive tract, vaginal flora is different from intestinal microecology, which is affected by menstrual cycle and sexual intercourse. Brotman et al. and Romero et al. have studied the composition of vaginal microorganisms in different periods (adolescence, pregnancy, perimenopause) of women, and found that a variety of factors (hormones, pregnancy, immunity, sexual behavior, etc.) can make the vaginal flora change.

In this meta-analysis, we found that vaginal flora is indeed closely related to women's reproductive health in general. In LL-VMB status, women's risk of NG infection

was significantly increased. Therefore, vaginal microecology is a very sensitive system. When influenced by endogenous and exogenous factors, it is easy to change, and then lead to the occurrence of diseases. For women with LL-VMB vaginal presentation, the most commonly used and effective means of improvement is to fill with *Lactobacillus* vaginalis capsules. It contains live *Lactobacillus* bacteria, which are normal bacteria in the vagina of healthy women. It can be colonized in the vagina and grow and reproduce. Its metabolites, such as lactic acid and hydrogen peroxide, can maintain the normal acidic vaginal environment and inhibit and eliminate the growth of harmful bacteria.

Of note was the observation that except for NG infection risk, other STIs such as trichomonal, and chlamydial infection incidence could also be affected by the status of vaginal microflora. In Brotman et al.'s pooled logistic regression estimating the hazard ratios for the comparison of trichomonal, gonococcal, and chlamydial infection incidence in participants by Nugent score at the prior visit, bacterial vaginosis microbiota as gauged by Gram stain was associated with a significantly elevated risk for acquisition of all these genital infections [22]. Moreover, the associations between douching and *Gardnerella vaginalis*, *Mycoplasma hominis*, and lack of hydrogen peroxide-producing lactobacilli were similar to those between douching and bacterial vaginosis [27]. Therefore, the imbalance of vaginal flora may increase the susceptibility risk of various STIs. Of course, more detailed studies are needed to confirm this.

Several limitations should be addressed. A majority of the included studies were retrospective designed, which might inevitably introduce confounding factors. The diagnoses of STIs were different in methods with various sensitivity and specificity. Also, definitions of HL-VMB and LL-VMB were not consistent in the included studies. Although we used Nugent score, Amsel test, clue cells and 16S rRNA gene sequencing to characterize the status of the vaginal microbiota, the four detect methods did not overlap perfectly and with low resolution in some cases. Nonetheless, strict inclusion criteria and exclusion criteria were established, data were extracted from the univariate and/or multivariate analyses. To the best of our knowledge, our work was the first to investigate the correlation of vaginal microbiota and NG susceptibility. The trend for a higher NG risk in LL-VMB was clear, and preventative strategies should be highlighted to modulate the vaginal microbiota to its normal status.

In conclusion, the systematic review and meta-analysis has demonstrated that LL-VMB was significantly associated with a high NG susceptibility. Due to the potential drawbacks of the present analysis, future well-designed and large studies are warranted to further confirm our conclusion and the other STIs risks.

Compliance with ethical standards

Conflict of interest The author declares that there is no competing interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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