FACULTY OF MEDICINE AND HEALTH SCIENCES

Which proportion of women with Bacterial Vaginosis experience symptoms: A systematic review and meta-analysis

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Community outreach: how many women with bacterial vaginosis experience symptoms

Bacteria live in the vagina. These bacteria, also called lactobacilli, protect the vagina from overgrowth of other bacteria that cause the flora within the vagina to become disrupted. When this happens, it is called Bacterial Vaginosis (BV).

Women with BV mainly complain of vaginal discharge. In addition, this discharge is often accompanied by an unpleasant odour that is often described as a fishy smell. This causes women to experience a sense of shame emotionally, sexually and socially.

Besides the fact that BV may or may not cause symptoms, it is associated with a lot of adverse health outcomes. It can have an adverse impact on the outcome of deliveries, including preterm birth. Since the flora of protective bacteria is disturbed, it can cause an increased risk of getting STDs.

BV is not always accompanied by symptoms but can also occur asymptomatically. However, this can still come with the adverse effects. Using lab methods, which involve looking under a microscope at the vaginal flora, one can determine whether a person has BV. That way you can diagnose BV but don’t yet know if someone is also experiencing symptoms. To know whether someone is experiencing symptoms, you need to look at the patient's clinical characteristics, including vaginal discharge and fishy odour.

Since there was not yet a good idea about how many women are now experiencing symptoms or not, a literature review was conducted in this thesis. Studies providing info on the number of women with symptoms were included and combined to arrive at a single percentage. Of all the women who were lab positive within the studies, we looked at how many were also clinically positive. Using this info we arrived at the percentage of symptomatic and asymptomatic individuals for BV.

Social added value and social impact

Bacterial vaginosis (BV) is associated with adverse effects, which can also occur when women are asymptomatic. This thesis gives a better idea about how many women being asymptomatic. This proportion does still appear to be large, especially when taking into account that BV is a common condition in women of reproductive age. It is therefore important to make women aware of this and give them info on what BV exactly means. Since we know that a large proportion of women experience BV asymptomatically, one can ensure that more attention is paid to it as well as that more studies are done on asymptomatic BV. Especially to find out whether the adverse effects are as prevalent here as in symptomatic BV. For now, treatment of asymptomatic BV is not yet recommended. However, this may possibly change when one has a better idea of what exactly asymptomatic BV entails.

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Abstract

Introduction: Bacterial vaginosis is the most commonly reported syndrome among women of childbearing age. It is mainly accompanied by symptoms of vaginal discharge and a typical fishy odor but women can also be asymptomatic. The aim of this thesis was to obtain a better picture of how many women are symptomatic or asymptomatic for BV. Both are associated with adverse health outcomes such as adverse gynecologic outcomes and increased risk for acquiring sexually transmitted diseases.

Methods: A systematic review and meta-analysis was conducted using the following databases: Pubmed, Embase, Web Of Science and Scopus. Search terms 'bacterial vaginosis', 'Nugent', and 'molecular diagnoses' were combined. Endnote reference manager was used to remove duplicates and Rayyan was used to screen the unique records. Pre-established inclusion and exclusion criteria were taken into account. Studies that indicated or compared clinical and non-clinical rates of BV were included. From these, the metaprevalence of how many women were clinically positive for BV and how many were non-clinical positive were calculated. In addition, a metaprevalence of how many of the non-clinical positive women were symptomatic or asymptomatic for BV was calculated. Lastly, the study calculated how many women with an intermediate of normal microbiome were symptomatic for BV. This was calculated using Excel and was presented graphically.

Results: A total of 106 studies were included. The metaprevalence of women with BV based on non-clinical diagnosis was 32.0% and 31.2% based on clinical diagnosis. Of all women positive based on non-clinical method, 57.2% were symptomatic and 42.8% were asymptomatic. To determine prevalence of women with a symptomatic intermediate microbiome and symptomatic normal microbiome, 11 studies were included. A total of 49.7% had a symptomatic intermediate microbiome and 41.0% had a symptomatic normal microbiome.

Conclusion: There is still a proportion of women asymptomatic for BV. More research onto the consequences of asymptomatic BV and whether it contributes as much to the global burden of BV is needed. As well as additional research into the necessary screening and treatment of asymptomatic BV is required.

Samenvatting

Inleiding: Bacteriële vaginose (BV) is het meest gerapporteerde syndroom bij vrouwen van vruchtbare leeftijd. Het gaat vooral gepaard met symptomen van vaginale afscheiding en een typische visgeur maar het kan ook volledig asymptomatisch optreden. Het doel van deze thesis was een beeld te krijgen van hoeveel vrouwen symptomatisch en asymptomatisch zijn voor BV. Beide gaan namelijk gepaard met nadelige gevolgen voor de gezondheid zoals nadelige gynaecologische uitkomsten en een verhoogd risico voor het krijgen van seksueel overdraagbare aandoeningen.

Methode: Een systematische review en meta-analysis werd opgesteld waarbij gebruik werd gemaakt van volgende databases: Pubmed, Embase, Web of Science en Scopus. Hierin werden zoektermen 'bacterial vaginosis' 'Nugent' en 'molecular diagnosis' met elkaar gecombineerd. Endnote reference manager werd gebruikt om duplicaten te verwijderen en Rayyan werd gebruikt om de unieke artikels te screenen. Hierbij werd rekening gehouden met vooraf opgestelde inclusie en exclusie criteria. Studies die de klinische en non-klinische percentages gaven van BV of deze met elkaar vergeleken werden opgenomen. Daarna werd hiervan de metaprevalentie berekend van hoeveel vrouwen nu klinisch positief zijn voor BV en hoeveel non-klinisch. Daarnaast werd er ook een metaprevalentie berekend van hoeveel van de non-klinisch positieve vrouwen nu symptomatisch of asymptomatisch zijn voor BV. Als laatste werd er nog gekeken naar hoeveel vrouwen met een intermediair of normaal microbioom symptomatisch zijn voor BV. Dit werd allemaal berekend aan de hand van Excel en grafisch voorgesteld.

Resultaten: Een totaal van 106 studies werden geïncludeerd. De metaprevalentie van vrouwen met BV op basis van een non-klinische diagnose bedroeg 32.0 % en op basis van de klinische diagnose bedroeg deze 31.2%. Van alle vrouwen die positief zijn op basis van non-klinische methode zijn er 57.2% symptomatisch en 42.8% asymptomatisch. Voor de bepaling van prevalentie van vrouwen met een symptomatisch intermediair microbioom en symptomatisch normaal microbioom werden 11 studies geïncludeerd. Een totaal van 49.7% had een symptomatisch intermediair microbioom en 41.0% had een symptomatisch normaal microbioom.

Conclusie: Er is nog een behoorlijk aandeel van de vrouwen asymptomatisch voor BV. Meer onderzoek naar de consequenties van asymptomatische BV en of dit evenveel bijdraagt aan de global burden van BV is noodzakelijk. Additioneel onderzoek naar screening en behandeling van asymptomatische BV zijn nog nodig.

Abbreviations

BV	Bacterial vaginosis
BVAB	BV-associated bacteria
CI	Confidence interval
HIV	Human immunodeficiency virus
HPV	Human papillomavirus
HSV-2	Herpes simplex virus type 2
IMS	Ion Mobility Spectrometry
IUD	Intrauterine device
NSS	Nugent scoring system
OC	Oral contraceptive
PID	Pelvic inflammatory disease
PCR	Polymerase chain reaction
PICO	Population Intervention Control Outcome
POF	Premature ovarian failure
POCT	Point-of-care test
PRISMA	Preferred Reporting items for Systematic Reviews and Meta-analysis
qPCR	Quantitative real-time PCR
RTI	Reproductive tract infection
STD	Sexually transmitted diseases
STI	Sexual transmitted infection
Atopobium	Has been renamed to *Fannyhessea*

1. Introduction

In women of childbearing age, bacterial vaginosis (BV) is the most commonly reported syndrome (1).

BV can be symptomatic or asymptomatic. The clinical symptoms associated with BV are relatively uncomplicated and easily measured (1). Women with BV can experience vaginal malodor, discharge and itching. The healthy vaginal microbiome of women of reproductive age is normally dominated by a single species of the genus *Lactobacillus,* mostly *L. crispatus* or *L. iners.* They contribute to a healthy vaginal microbiome and establish a defense against the invading pathogens by producing various antimicrobial compounds such as lactic acid, hydrogen peroxide (H_2O_2) and bacteriocins (2). BV is characterized by overgrowth of anaerobic bacteria such as *Gardnerella vaginalis*, *Mobiluncus, Fannyhessea (previously known as Atopobium* (146)), *Prevotella* and a decrease in the levels of *lactobacilli* and thus a reduction of their antimicrobial compounds (2,3).

In addition to the fact that BV causes physical and psychosocial discomfort (4), it could also trigger numerous health disorders, including adverse pregnancy outcome, pelvic inflammatory disease (PID), sexual transmitted infections (STIs) such as human immunodeficiency virus (HIV), human papillomavirus (HPV), herpes simplex virus type 2 (HSV-2), chlamydia, gonococcal and trichomonas infections (2).

BV has a prevalence ranging from 23% to 29% with racial disparities and a high global cost burden for treatment, which is estimated as $4,8 billion and with more than half of the costs due to recurrent BV (4). According to Bitew et al. the overall prevalence of bacterial vaginosis is 48,6% (131). According to the article of Achondou et al. the prevalence of bacterial vaginosis is 38% (132). Since articles find a large difference in prevalence, the purpose of this systematic review is to obtain an overview of the prevalence of BV and in addition how much of this percentage of women is symptomatic.

1.1. Pathogenesis

The vaginal microbiome is a dynamic microecosystem. During the female menstrual cycle and the woman's entire life it undergoes fluctuations (2).

BV is being assessed by a shift in vaginal microbiome. This shift is characterized by a loss of protective *Lactobacillus* species and an increase in the abundance of facultative and anaerobic

organisms in the vaginal microenvironment such as *Gardnerella vaginalis, Mobiluncus* spp*, Bacteroides* spp*, Prevotella* spp*, Fannyhessae vaginae* and *BV-associated bacteria (BVAB)* (5,6). The loss of lactobacilli is accompanied by a lactic acid depletion, leading to a higher pH than normal (6).

G. vaginalis harbors a variety of virulence factors, the most widely investigated factors being sialidase and vaginolysin. Sialidase A contributes to the BV pathogenesis because it hydrolyzes sialic acid residue from mucus sialoglycans in the vagina and then catabolizes free carbohydrate, thus contributing to the degradation of vaginal mucus barriers. Vaginolysin is a pore-forming toxic compound which facilitates the lysis of target cells, in the case of BV vaginal epithelial cells (2).

Another aspect that plays a crucial role in the pathogenesis of BV is the polymicrobial biofilm formed on the vaginal epithelium. The primary colonizer in this process is considered to be *G. vaginalis* which can establish a scaffold for the attachment of other BV-associated microbes (2). In addition, the loss of the protective mucous layer can also lead to increased adherence of the secondary colonizers such as *Fannyhessae vaginae* and other BVAB (5).

G. vaginalis biofilms have a higher tolerance against lactid acid, H_2O_2 and antibiotics (2).

Even though *G. vaginalis* is able to replace lactobacilli, adhere to epithelial cells and form a biofilm, the presence of *G. vaginalis* alone is not sufficient for developing BV. Other BVAB are also important in the pathogenesis of BV. *G. vaginalis* and *P. bivia* are seen as early colonizers. They can actively inhibit the host inflammatory response in the vaginal epithelium, allowing them to evade the immune system while forming a biofilm. Thus, they don't induce robust epithelial cell activation. Secondary colonizers of the biofilm such as *Fannyhessea vaginae* and other BVAB, on the other hand can stimulate the host immune response in the vaginal epithelial cells and thus contribute to the symptoms. They induce pro-inflammatory responses that can be observed in women with BV. The metabolites produced by BVAB may be related to the symptoms of BV (5).

1.2. Clinical features

BV is the most common cause of vaginal symptoms among women of reproductive age (3). The symptom usually seen with BV is a typical odor described as a fishy smell. This odor comes from anaerobic bacteria which produce amines. In addition, increased vaginal discharge is a common sign of BV. The discharge is often of little thickness, gray or milky in

color (7). The odor linked to BV can cause women to feel embarrassed and moreover, it can make them fear that their sexual partner will notice this odor (8). Furthermore, increased vaginal pH and vaginal itching can also occur with BV (3).

BV can be associated with diminished emotional, sexual and social health. As a result of these symptoms, it may be difficult for a person to establish a positive body image, which is vital to self-esteem and sexual health (8).

1.3. Risk factors

There are several factors that could increase the risk of BV. BV would occur more frequently among women who report new or higher numbers of male sex partners. In addition, BV almost doesn't occur in women who have not yet had sex. According to limited data, male condoms and circumcision may prevent BV and its recurrence. Another risk factor for BV acquisition is douching for hygiene (9). In the study of Shoubnikova et al. there is a significantly reduced frequency of BV when using condoms or oral contraceptive (OC) (10). In the study of E. Calzolari et al. there is a significant increase of BV with IUD users. The tail of IUD present in the endocervix or the vagina may favor the growth of vaginal anaerobic bacteria and G. vaginalis (11). The risk of BV also varies by race and ethnicity, African American women are more likely to have BV compared to non-Hispanic white women (3).

1.4. Sequelae

Women with BV have an increased risk of acquiring sexually transmitted infections (STIs) such as human immunodeficiency virus (HIV), *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis* and herpes simplex virus-2 (3).

HPV, the causal agent of cervical cancer, is the most common sexually transmitted infection among young women. The infection of HPV is significantly affected by BV. Women with BV are less likely to clear an infection than women who don't have BV (2).

BV has been suggested to be associated with pelvic inflammatory disease (PID), and it is considered a risk factor for PID. PID can cause adverse reproductive sequelae, such as infertility, chronic pelvic pain and ectopic pregnancy (2).

Furthermore, BV has rather consistently associated to preterm birth and other adverse obstetric outcomes (2). Women with a vaginal microbiome with abundant lactobacilli in the first trimester of pregnancy, have a 75% lower risk of delivering pre-term babies compared to those with vaginal microbiome characterized by colonization of *BVAB* (6).

In addition, compared to fertile women BV is more common in infertile women of the same age group and is associated with reduced rates of conception. When women have an idiopathic infertility, an abnormal vaginal microbiome is more often found. During embryo implantation and subsequent pregnancy outcomes, suggested by several in vitro studies, the vaginal microbiome plays an important role (6).

1.5. Diagnosis

To diagnose BV various diagnostic approaches can be used (2).

1.5.1. The clinical diagnosis

1.5.1.1. Amsel criteria

The Amsel criteria includes the clinical diagnosis of BV. For the diagnosis they use simple observations or procedures that could be carried out in the physician's office. BV is diagnosed when at least three of the following criteria are positive (12):

- Vaginal pH above 4.5
- Characteristic vaginal discharge: a thin homogeneous appearing vaginal discharge which has a milk-like consistency
- The presence of a fishy like-odor on addition of 10% potassium hydroxide to a drop of vaginal discharge
- Clue cells on saline wet mount examination

1.5.2. Lab-based methods: microscopy

1.5.2.1. Spiegel criteria

The Spiegel criteria (13) are used to diagnose BV on the basis of microscopy.

Gram stain of vaginal fluid is examined under the microscope. *Lactobacillus* and *Gardnerella* were counted under oil immersion and other Gram-negative and Gram-positive bacteria, such as curved rods, Gram-negative rods, fusiforms and Gram-positive cocci were categorized by

morphology only. The scheme used for categorization of *Gardnerella* and *Lactobacillus* morphotypes:

- 1+ : <1 per field
- 2+ : 1-5 per field
- 3+ : 6–30 per field
- 4+ : >30 per field

The smear is interpreted as normal when the *Lactobacillus* morphotype is present alone or in combination only with the *Gardnerella* morphotype. When a more mixed microbiome which, in addition to *Gardnerella* morphotype consists of other Gram-negative and gram-positive bacteria such as curved rods, gram-negative rods, fusiforms and gram-positive cocci and when the *lactobacillus* was absent or only present in a small amount (1+, 2+), the smear is considered as BV positive (13).

1.5.2.2. Nugent scoring system

The Nugent score is widely considered as the gold standard for the diagnosis of BV in research studies (9). Compared to Amsel, the degree of interobserver and intraobserver variability is low (14). Nugent scoring system is a scoring system that uses Gram-stained vaginal smears for diagnosing BV. It allows gradations in severity and it is a weighted combination of three morphotypes: *Lactobacillus, Gardnerella/Bacteroides* and *Mobiluncus.* Each of these three morphotypes is scored based on the amount of morphotypes that is counted on the vaginal smear. These scores are added together and give a total score of 0 to 10. This scoring system allows gradations in the severity of bacterial vaginosis (15).

- 0 - 3: normal
- 4 - 6: intermediate
- 7 – 10: Bacterial vaginosis.

According to the article of Marrazzo et al. is how to handle gram stain results with Nugent scores of 4 – 6 indicating intermediate microbiome still an unsolved problem. Some researchers are going to disregard that group and focus only on normal microbiome or BV, this could potentially result in increased reported values (9). Women with intermediate microbiome often have a mild amine odor, elevated pH and clue cells, which may be overdiagnosed as BV if only Amsel's criteria were used (16).

1.5.2.3. Molecular diagnosis

To overcome the limitations of microscopy and point-of-care tests (POCTs) in the diagnosis of BV, new techniques have been developed that use molecular markers of BV. Because molecular technologies are objective, as they can detect fastidious bacteria, allow quantitation and as they are ideal for self-sampled vaginal swabs, they are more beneficial than microscopy-based tests and point-of-care tests. Testing involves the detection of specific bacterial target genes (16).

Quantitative real-time PCR (qPCR) test is a molecular technique that can be used to diagnose BV (17).

Furthermore, traditional methods, such as reading of Nugent score for BV and in vitro culture are more labor intensive. On top of that the accuracy is sometimes questionable as the interpretation is more subjective to experience. In European and American studies, multiplex PCR has been used as a sensitive and objective platform for investigating the etiology of vaginitis (18).

To diagnose BV, a DNA probe can be applied to identify the specific sequences of targeted microbes from vaginal discharge. *Gardnerella vaginalis* has a high sensitivity and low specificity. This means that healthy or asymptomatic women may also carry *G. vaginalis* (2).

1.6. Treatment

Treatment of BV is mainly aimed at restoring a normal vaginal microbiome by stopping the proliferation of BV-associated micro-organisms. Typically, clinical therapies use broad-spectrum antibiotics against anaerobic microorganisms and protozoa. These antibiotics include clindamycin and nitroimidazoles (metronidazole and tinidazole) and/or the use of probiotics (17).

The current recommended therapy according to The Centers of Disease Control and Prevention is 500 mg of oral metronidazole twice a day for 7 days (19). They are associated with fairly good short-term cure rates (20).

Reported rates of relapses are frequent, with more than 50% relapsing within 3 – 6 months, even with successful antibiotic treatment (19). The use of antibiotics is limited due to the potential for antibiotic resistance to develop and the inability to restore the lactobacillus-dominated vaginal flora (21). One of the reasons for the high rate of BV recurrence despite

therapy, could be due to the persistence of the biofilm. Under the influence of biofilm disrupting agents such as TOL-463, BVAB detach from the biofilm, making them more sensitive to antibiotics that are given simultaneously (5).

In addition, probiotics can be used as an adjuvant therapy to antibiotics in the treatment of BV (20).

Metronidazole produces holes in the biofilm but does not eradicate the bacteria (22). Challenge with the probiotic lactobacilli *L. reuteri* RC-14 and *L. rhamnous* GR-1, however, led to extensive bacterial death in the biofilm (20). According to McMillan et al. probiotic lactobacilli cause noticeable disruption of G. vaginalis and F. vaginae biofilms, because they are able to incorporate themselves into the biofilm and thus cause disruption and cell death. In this way, lactobacilli can enhance the efficacy of metronidazole and this approach can be considered, especially with women suffering from relapses (22).

1.7. Context of this study

BV can be symptomatic or asymptomatic (2). Studies suggest that the risk of acquisition of sexually transmitted infections (STIs) could be reduced when asymptomatic BV is treated. From this, the current clinical guidelines should be reassessed and adjusted accordingly. Similar to screening for STIs, health care providers should also screen for asymptomatic BV to reduce the risk of incident STIs as well as potentially diagnose asymptomatic cases (3).

Assessing for symptoms only is not a reliable approach for excluding subjects with BV, because there are also women who experience BV asymptomatically (9). In general, the Amsel criteria underestimate the BV prevalence as diagnosed by the Nugent scoring system (NSS) by 30-40% (14).

According to the study of Klebanoff et al. (2004) vaginal discharge and odor are more prevalent among women with BV compared to women without BV. However the difference in prevalence of these symptoms is not of a great magnitude (23).

Depending on the degree of dysbiosis, virulence of colonizing pathogen and its load BV may be symptomatic or asymptomatic (6).

The aim of this systematic review is to systematically review how many women who are diagnosed with BV with laboratory tests (such as molecular diagnosis or Nugent scoring) actually have symptoms. In this study we gain a better understanding of how many women suffer from BV. This will give us a better picture about the problems previously described.

2. Methods

This systematic review and meta-analysis were conducted in accordance with a pre-established study protocol (Addendum 6.1), and adherend to the guidelines outlined in the Preferred Reporting items for Systematic Reviews and Meta-analysis (24).

2.1. Search strategy

The databases used in this study were Pubmed, Embase, Web of Science and Scopus, using a predefined search strategy. This search strategy was developed based on the PICO framework, with P (population) and I (intervention), resulting in the following search terms for the search strategy: 'bacterial vaginosis', 'nonspecific vaginitis', 'bacterial vaginitis', 'Nugent', 'molecular diagnosis', 'molecular diagnostic techniques', 'markers', 'sensitivity and specificity' and 'molecular testing'. The combinations made with these terms can be found in the protocol under search strategy (Addendum 3.3 Search strategy).

2.2. Selection process

Articles obtained from the various databases were placed in an EndNote file and duplicates were removed using the Endnote reference manager. Subsequently, they were exported to an Excel file to manually identify and eliminate any remaining duplicates. Following this, all unique records were imported into Rayyan, where an additional check for duplicates was performed.

Once the articles were added to Rayyan, the screening process started. The first step involved evaluating the article titles to determine their relevance to the review's subject. In the second stage, abstracts were examined. If they aligned with the review's topic the full-text articles were also assessed. Based on predefined data the articles were included or excluded. The reason for exclusion was documented in Rayyan. Articles where uncertainty arose about the eligibility were discussed with the promotor.

2.3. Eligibility criteria

Inclusion and exclusion criteria were defined in advance to determine whether or not to include the study.

2.3.1. Inclusion criteria

Studies were included if they reported the percentage or absolute numbers of BV diagnoses based on clinical or non-clinical methods. Additionally, studies comparing the numbers between non-clinical and clinical methods for the diagnosis of BV, were also included to determine the percentage of non-clinical diagnoses with actual symptoms. Thirdly, studies that reported the number of women being symptomatic or asymptomatic for BV after a non-clinical diagnosis was used, were also considered.

Studies were eligible if they used one of the following non-clinical diagnostic methods: Nugent Scoring system, Spiegel Criteria, molecular diagnosis, laboratory diagnosis such as wet mount, microscopy and BVBblue. Clinical diagnosis studies were eligible if they used Amsel's Criteria. They were also eligible if they used vaginal discharge in relation to BV, the Whiff test, malodor in relation to BV, or if they mentioned the number of women who were symptomatic or asymptomatic for BV.

The study population of interest consisted of women of reproductive age. There were no limitations based on geography, race or clinical settings. Articles published from 1990 to August 2023 were included and papers written in English, French, Dutch and German were reviewed.

2.3.2. Exclusion criteria

Studies were excluded from the systematic review if they did not provide information about the sample size. They were also excluded if they exclusively relied on either non-clinical or clinical methods for diagnosis. Furthermore, studies were not included when it was not specified in the results if the diagnosis of BV was based on a non-clinical diagnostic method or a clinical diagnostic method, as this made it impossible to separate or compare them individually. Additionally, studies lacking sufficient information about BV, for example whether women were symptomatic or not, or failed to provide data for calculating BV counts or percentages were not considered because they also result in a lack of necessary information. Studies that did not meet the specified article type were also excluded. This means that reviews, comments, guidelines, unpublished articles were not included, and neither were multiple reports of the same data. Studies diagnosing BV after treatment were excluded because they primarily focused on cure rates rather than the actual prevalence of BV. Lastly, studies that explicitly mentioned women being post-menopausal were not incorporated into the review.

2.4. Data collection

Using a pre-established Excel file, the following data was extracted for each study: title, first author, year of publication, country, study design, start to end date of the study, study population, total number of study participants, age, non-clinical diagnostic test method used and the positive scoring cut off for this test, clinical diagnosis (used method) and the criteria for the clinical diagnosis, non-clinical diagnosis positive, clinical diagnosis positive, non-clinical diagnosis negative and clinical diagnosis negative.

If provided in the study, the following data was also extracted: clinical positive/ non-clinical positive, clinical negative/ non-clinical positive, clinical positive/ non-clinical negative, clinical negative/ non clinical negative, non-clinical BV positive symptomatic, non-clinical BV positive asymptomatic, categorization of intermediate BV, intermediate BV (non-clinical), intermediate microbiota symptomatic, intermediate microbiota asymptomatic, categorization of normal microbiota, normal (non-clinical), normal microbiota symptomatic and normal microbiota asymptomatic.

In a second sheet within the Excel file, a legend was created providing information about the intended meaning of the data to be extracted.

2.5. Data management

The studies were added from the databases to an EndNote library. EndNote reference manager was utilized to eliminate duplicates. Subsequently, the remaining records were imported into an Excel file, providing an additional check ensuring all duplicates were removed. After this step, unique articles were added to Rayyan where another check for duplicates was performed. Once this process was completed, the screening of the articles in Rayyan started. This involved an evaluation of the inclusion and exclusion criteria. To extract data from the eligible articles an Excel file was created.

2.6. Statistics

Three analyses were conducted based on the acquired data. These were performed in Excel, where the data was processed and graphical representations were created.

The first analysis examined the percentage of BV in each study, both obtained with non-clinical and clinical diagnosis independently. The studies were displayed in a graphical representation, with the percentage of non-clinical and clinical diagnosis plotted for each study. On the Y-axis, all studies were listed one below the other. On the X-axis, the percentage of diagnosed BV was presented. In this context, 100% represented the entire sample size of the study. At the end, a meta-analysis was conducted in which the sample sizes of the various studies were aggregated to obtain the percentage of women with BV based on either non-clinical or clinical diagnosis. A confidence interval was calculated during this process.

The second analysis showed the prevalence of women with symptomatic BV in women diagnosed with BV using non-clinical diagnosis. From this, we could deduct which percentage of the non-clinical diagnosis was effectively symptomatic or asymptomatic for BV. This analysis was also presented graphically. A barplot was used for each study, the solid bar represented the non-clinical diagnosis and accounted for 100%. The blue part represented the percentage of BV which was diagnosed positive by the clinical diagnosis and the orange part was negative based on the clinical diagnosis. At the end, a meta-analysis was also conducted to determine the percentage of women who were symptomatic or asymptomatic for BV after receiving a positive non-clinical diagnosis. During this analysis the 95% confidence interval was calculated.

The third analysis was a subanalysis in which studies were included providing information on the intermediate and normal microbiome. In this analysis the percentage of symptomatic and asymptomatic women per category was obtained. This was plotted by category in the same type of graph described in analysis 2. At the end, a meta-analysis was performed per category calculating the total number of symptomatic and asymptomatic women per category.

3. Results

3.1. Study selection

After applying the search strategy in the four different databases a total of 4999 articles were obtained. Duplicates were removed and 2362 unique records were screened based on their title. Of these, 1880 articles were found to be irrelevant to the subject of this meta-analysis. After excluding these articles, 482 articles were finally screened based on their abstract and if relevant also for their full text. This involved looking at the inclusion and exclusion criteria. A total of 123 of these articles relied only on non-clinical diagnosis for the diagnosis of BV. The largest proportion of these articles used only Nugent. In addition, BVBlue, Hay/Ison criteria/molecular diagnosis, BD affirm III test were also used for the non-clinical diagnosis of BV. Another 33 articles were excluded because they only used a clinical method for diagnosing BV. The method of diagnosis was not mentioned in two studies hence also excluded. A total of 94 articles were excluded because they did not provide the necessary information regarding the prevalence of BV and/or if women were symptomatic or asymptomatic. This is because the diagnosis of BV was made based on a combination of non-clinical and clinical methods, making comparisons impossible. Exclusion occurred when no data was given on BV or because the data given is difficult to interpret or when there was too little data to calculate the prevalence of BV. A total of 21 studies were not included based on language and 20 studies were excluded based on article type. In five studies they assessed BV after treatment thus excluded. Three studies were excluded because they used a method that was not considered appropriate, namely Ion Mobility Spectrometry (IMS). Two studies were dated and fell outside the specified time frame for inclusion. Despite initial screening while reading the articles another two duplicates were identified and subsequently removed from the study. One study used the same study population as another. One study included the wrong study population, namely post-menopausal women. Finally, 69 articles were removed because the full text could not be found.

Ultimately, there were 106 articles that remained suitable to carry out the statistics.

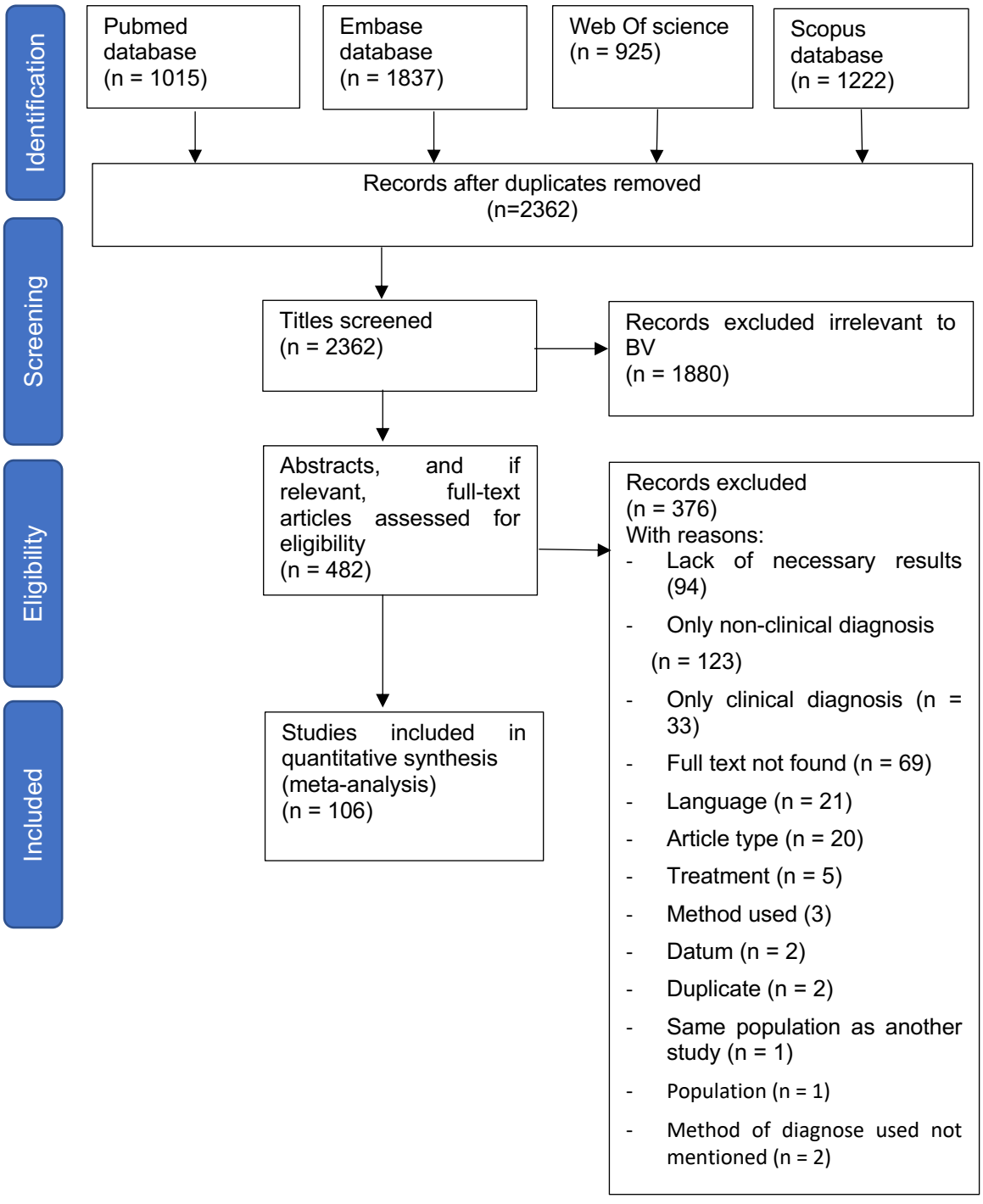

Figure 1. Flowchart of the identification of studies included in the meta-analysis.

3.2. Description of studies

A total of 106 studies were included, representing a total of 53,431 women. The studies are summarized in Table 1.

Most of the studies took place in Asia, this accounted for 41 (39%) of the studies. Twenty (19%) took place in Africa, twenty (19%) in North America, fourteen (13%) in Europe, eight (1%) in South America, one in Oceania and two articles did not state any location.

Some differences were found in the population of women included in the different studies and can be grouped into some larger categories. In 71% of the studies, the population was composed of women who present themselves at a hospital. This regarded gynecological clinics, antenatal health clinics, STI/RTI clinics and obstetrics clinics. A total of 29 studies (27%) included exclusively pregnant women. Of these, three included pregnant women who came in with complaints of vaginal discharge. The study by Bitew et al. (2021) included HIV-infected pregnant women and the study by Joyisa et al. (2019) included exclusively HIV-uninfected women. In total, eight studies explicitly included an HIV-positive population. In 18% of the studies, women reported explicitly having vaginal discharge and in five studies, women reported explicitly having symptoms. The study by Dols et al. (2016) included women with a high risk for STIs, the study by Benetti-Pinto et al. (2015) included women with premature ovarian failure (POF) and the study by Andrews et al. (2006) included women after giving birth. In 32 studies, women are not specified.

A total of 98 of the studies (92%) used Nugent Scoring System for the diagnosis of BV. The nine other studies each used a different method for the non-clinical diagnosis of BV. One study used the presence of a positive culture of *Gardnerella vaginalis*, another one the microbiological diagnosis of *Gardnerella vaginalis* and one study relied on the Hay/Ison criteria. In addition, wet mount bacterial morphotype score, qBVassay, qPCR, real-time PCR and DNA Probe laboratory standard were also used.

Most studies, 68% (72 of 106 studies), used Amsel's criteria for the clinical diagnosis of BV. Twenty studies used vaginal discharge as a clinical diagnosis of BV. Of these twenty studies, six studies combined vaginal discharge and odor. Furthermore, five studies only looked at odor alone. The study by Thulkar et al. (2010) made the clinical diagnosis based on pH $\geq$ 4.5 and a positive whiff test. Five studies used the simplified Amsel's criteria: the study by Karani et al. (2007) did not include clue cells, the study by Mengistie et al. (2014) didn't look at vaginal discharge, the study by Tosun et al. (2003) omitted pH and in the study by West et al. (2003) only three of the 4 Amsel's criteria were present to diagnose BV. Thomason et al. (1992) used

the Thomason criteria, which is similar to the Amsel's criteria but simply leaves out the vaginal discharge. Finally, three studies made the clinical diagnosis based on symptoms.

Table 1. Summary of the studies included in the meta-analysis

First author (last name, first name)	Year of publication	Country	Study population	Total number of study participants	Non-clinical diagnostic test method used	Clinical diagnosis method used	Analysis
Abdullateef, Rasheedat (25)	2017	Nigeria	non-pregnant women visting gynaecological clinic	212	nugent scoring system	malodorous discharge	1/2
Aboud, Said (26)	2023	Tanzania	patients presenting with genital discharge syndrome	883	nugent scoring system	vaginal discharge	2
Aliyi, Mohammed (27)	2022	Ethiopia	pregnant women (attending antenatal care)	248	Nugent scoring system	Amsel's criteria	1/2/3
Al-Muk, Jihan (28)	2001	Iraq	pregnant women	413	Gardnerella Vaginalis	Vaginal discharge	1/2
Andrews, William (29)	2006	England	women after birth giving	769	nugent scoring system	Amsel's criteria	1/2
Atanasievska, Sonja (30)	2023	Serbia	women of reproductive age	235	nugent scoring system	Amsel's criteria	1/2
Aubaid, Adnan (31)	2020	Iraq	women visiting outpatient clinics	158	nugent scoring system	Amsel's criteria	1
Ayenalem, Seblewongiel (32)	2010	Ethiopia	women visting an gynecological clinic	100	nugent scoring system	symptomatic	2/3
Bala, Manju (33)	2017	India	females attending STI/RTI clinics with abnormal vaginal discharge	550	nugent scoring system	Amsel's criteria	1

Bamniya, Jaishree (34)	2022	India	pregnant symptomatic and asymptomatic women visiting antenatal outpatient clinic	200	nugent scoring system	Amsel's criteria	1
Baruah, Frincy (35)	2014	India	married, nonpregnant females	200	nugent scoring system	Amsel's criteria	1
Benetti-Pinto, Cristina (36)	2015	Brazil	women with POF and controls	72	nugent scoring system	whiff test	1
Bhakta, Vidya (37)	2021	Saudi	healthy pregnant women	217	nugent scoring system	odour	1/2
Bhavana, Appikatla (38)	2019	India	pregnant women	117	nugent scoring system	Amsel's criteria	1
Bhujel, Rajshree (39)	2021	Nepal	women presenting with abnormal vaginal discharge	141	nugent scoring system	Amsel's criteria	1/2
Bitew, Abebaw (40)	2021	Ethiopia	HIV-infected pregnant women	413	nugent scoring system	type of discharge and odour	1/2
Bradshaw, Catriona (41)	2005	Australia	women with symptoms of abnormal vaginal discharge or odor	288	nugent scoring system	Amsel's criteria	1/2/3
Brown, Joelle (42)	2013	USA	sexually active women	141	nugent scoring system	unusual vaginal discharge and fishy odor over the past month	1
Buyukbayrak, Esim (43)	2010	Turkey	Premenopausal women applying to our gynecology outpatient	460	microbiological diagnosis	Amsel's criteria	1/2

			clinic with vaginal discharge complaint				
Cartwright, Charles (44)	2012	USA	women visting sexually transmitted disease and health clinics	169	nugent scoring system	Amsel's criteria	1/2/3
Castro, Erica (45)	1999	Chili	women attending family planning clinics-ranomly selected	242	nugent scoring system	Amsel's criteria	1
Challa, Apoorva (46)	2022	India	women visting Sexually transmitted diseases and obstretics and gynaecology out-patient departments	404	nugent scoring system	Amsel's criteria	1
Challa, Apoorva (47)	2022	India	non-pregnant women	283	nugent scoring system	vaginal discharge	2
Chen, Hui-Mei (48)	2018	Thaiwan	women recruited at the Department of Obstetricsand Gynecology	77	nugent scoring system	Amsel's criteria	1/2/3
Chunaifa, Leyna (49)	2015	Indonesia	sex workers	99	Hay/ison	Amsel's criteria	1
Cohen, Craig (50)	1995	Thailand	female commercial sex workers	144	nugent scoring system	Thomason criteria	1
Coppolillo, Enrique (51)	2003	Argentina	pregnant women	190	nugent scoring system	Amsel's criteria	1
Dadhwal, Vatsla (52)	2009	India	asymptomatic pregnant women	502	nugent scoring system	Amsel's criteria	1

Demba, Edward (53)	2005	Gambia/West Africa	women with VDS	227	nugent scoring system	Amsel's criteria	1
Discacciati, Michelle (54)	2006	Brazil	women visting clinic	135	nugent scoring system	Amsel's criteria	1
Djom, Gaston (55)	2016	Kenya	HIV-infected women	1063	nugent scoring system	vaginal discharge	2
Dols, Joke (56)	2016	The Netherlands	women with high risk of STI's	40	nugent scoring system	Amsel's criteria	1
Gallo, Maria (57)	2011	USA	9140 study visits by 862 HIV-infected women and421 HIV-uninfected women	9140	nugent scoring system	Amsel's criteria	1/2
Garg, Divya (58)	2020	India	HIV seropositive female patients (60 symptomatic and 60 asymptomatic for vulvovaginitis	120	nugent scoring system	Amsel's criteria	1
Ghanbari, Bahare (59)	2022	Iran	pregnant women	70	nugent scoring system	Amsel's criteria	1
Goepfert, Alice (60)	2004	/	pregnant women	83	nugent scoring system	Amsel's criteria	1
Gratacós, Eduard (61)	1999	Spain	pregnant women	492	nugent scoring system	Amsel's criteria	1
Gupta, Geeta (62)	2013	India	sexually active married women	400	nugent scoring system	Amsel's criteria	1/2
Gutman, Robert (63)	2005	/	Women visiting clinic	269	Nugent scoring system	Vaginal discharge	1/2

Haamid, Fareeda (64)	2014	USA	female patients with a vaginal complaint	100	nugent scoring system	Amsel's criteria	1
Hay, Phillip (65)	2003	United Kingdom	Women visiting clinic	644	nugent scoring system	Amsel's criteria	1/2
Hemalatha, R. (66)	2013	India	non pregnant women with complaints of white discharge, back ache and pain abdomen	270	nugent scoring system	Amsel's criteria	1/2
Hilmarsdóttir, I. (67)	2006	Iceland	women visiting Sexually Transmitted infection clinic	327	nugent scoring system	Amsel's criteria	1
Hogan, Vijaya (68)	2007	USA	pregnant women	1780	nugent scoring system	Amsel's criteria	1
Joyisa, Nkosinathi (69)	2019	South Africa	HIV uninfected pregnant women	750	nugent scoring system	vaginal discharge	1/2/3
Kamara, Paul (70)	2000	Jamaica	pregnant women	261	nugent scoring system	malodorous discharge	2
Kampan, Nirmala (71)	2011	Malaysia	non-pregnant women	151	nugent scoring system	Amsel's criteria	1
Kancheva, Nadia (72)	2018	Thailand	HIV-infected and HIV-uninfected women	137	nugent scoring system	Amsel's criteria	1
Karani, A. (73)	2007	Kenya	women visting clinic	491	nugent scoring system	simplified Amsel's criteria	1/2
Kingsley, Anukam (74)	2014	Nigeria	asymptomatic women	67	nugent scoring system	Whiff test	1/2/3

Kissinger, Patricia (75)	2005	USA	HIV infected women	216	nugent scoring system	Amsel's criteria	1
Konadu, Dennis (76)	2019	Ghana	pregnant women	589	nugent scoring system	malodour	2
Kotian, Shashidhar (77)	2011	India	women visiting a health clinic	20	nugent scoring system	Amsel's criteria	1
Ladhani, K (78)	2015	Bangladesh	pregnant women	3166	Nugent scoring system	malodorous discharge	2
Landers, Daniel (79)	2004	USA	non-pregnant women with genital complaints	598	nugent scoring system	Amsel's criteria	1/2
Lokken, Erica (80)	2022	Kenya	HIV-negative women	701	nugent scoring system	Amsel's criteria	1
Lowe, Nancy (81)	2009	USA	women presenting with vulvovaginal symptoms.	547	DNA probe laboratory standard	vaginal discharge and Whiff test	1/2
Madhivanan, Purnima (82)	2014	India	non-pregnant women	323	nugent scoring system	Amsel's criteria	1
Mala, Rajni (83)	2022	India	women visting clinic	125	nugent scoring system	Amsel's criteria	1/2
Marconi, Camila (84)	2012	Italy, Belgium, Germany	non-pregnant women	103	nugent scoring system	Amsel's criteria	1
Marconi, Camila (85)	2015	Brazil	non pregnant non HIV	1519	nugent scoring system	abnormal discharge	1/2

Mascarenhas, Rita (86)	2012	Brazil	sexually active women visting gynecology clinic	100	nugent scoring system	vaginal discharge	2
Menard, Jean-Pierre (87)	2010	France	pregnant women who reported abnormal vaginal symptomsbefore 20 weeks gestation	163	nugent scoring system	Amsel's criteria	1/2
Mengistie, Zemenu (88)	2014	Ethiopia	pregnant women, symptomatic and asymptomatic	252	nugent scoring system	symptomatic	1/2
Mengistie, Zemenu (89)	2013	Ethiopia	pregnant women	252	nugent scoring system	simplified Amsel's criteria	1/2
Mittal, Vineeta (90)	2012	India	regnant females with abnormal excessive vaginal discharge	205	nugent scoring system	Amsel's criteria	1/2
Modak, Tamonud (91)	2011	India	women with discharge	50	nugent scoring system	Amsel's criteria	1/2
Mohammadzadeh, Farnaz (92)	2014	Iran	married women with vaginal discharge	120	nugent scoring system	Amsel's criteria	1/2
Moussavi, Ziaeddine (93)	2004	Iran	with complaints of vaginal discharge	102	nugent scoring system	Amsel's criteria	1/2
Mulinganya, Guy (94)	2021	Congo	pregnant women in the second trimester of pregnancy	533	nugent scoring system	symptomatic	1/2/3
Nelson, Deborah (95)	2007	USA	pregnant women	1916	nugent scoring system	malodour	2

Numanovic, Nedzib (96)	2021	Serbia	women	67	Real Time PCR	Amsel's criteria	1
Olson, Kristin (97)	2018	USA	African-American women	564	nugent scoring system	vaginal discharge	2
Padmajakshi, Gurrapu (98)	2018	India	HIV seropositive women	156	nugent scoring system	Amsel's criteria	1
Patange, R. (99)	2022	India	pregnant women	76	nugent scoring system	Amsel's criteria	1
Poojari, Vidyashree (100)	2020	India	pregnant women	228	nugent scoring system	Amsel's criteria	2
Posner, S.F. (101)	2005	Azerbaijan	women visting clinic	200	nugent scoring system	Amsel's criteria	1
Ranjit, Eliza (102)	2018	Nepal	nonpregnant women with symp-tomatic vaginal discharge,	160	nugent scoring system	vaginal discharge (thin)	1/2
Rodrigues, Fernando (103)	2015	Brazil	women with vaginal discharge	94	nugent scoring system	Amsel's criteria	1/2
Sakwinska, Olga (104)	2016	Bangladesh	pregnant women	300	nugent scoring system	Amsel's criteria	1
Salas, Antonio (105)	2019	USA	women	110	nugent scoring system	Amsel's criteria	1/2
Sapra, Katherine (106)	2013	USA	non-pregnant women	433	nugent scoring system	Amsel's criteria	2

Schmidt, H. (107)	2000	Sweden	women	754	wet mount bacterial morphotype score	Amsel's criteria	1/2/3
Schwebke, Jane (108)	1996	USA	women visting gynecology or sexually transmitted disease clinics	617	nugent scoring system	Amsel's criteria	1/2
Sethi, Sunil (109)	2023	India	women with vaginitis	3531	nugent scoring system	vaginal discharge	1/2/3
Sha, Beverly (110)	2005	USA	HIV infected women	406	nugent scoring system	Amsel's criteria	2
Shawaky, Sherine (111)	2022	Egypt	experiencing vaginitis with one or more symptoms	516	nugent scoring system	Amsel's criteria	1/2
Shujatullah, Fatima (112)	2010	India	complaints of vaginal discharge	405	nugent scoring system	Amsel's criteria	1/2
Sihavong, Amphoy (113)	2007	Laos	women visting clinic	1125	nugent scoring system	vaginal discharge	2
Simoes, J.A. (114)	2006	Brazil	women visting family planning clinic	135	nugent scoring system	Amsel's criteria	1
Sodhani, Pushpa (115)	2005	India	women	301	nugent scoring system	thin, homogeneous discharge	1/2
Sousa, C. (116)	2019	Portugal	women	138	nugent scoring system	Amsel's criteria	1
Srinivasan, Sujatha (117)	2012	USA	women visiting Sexually transmitted diseases clinic	220	nugent scoring system	Amsel's criteria	1

Sturm, P.D. (118)	2002	South Africa	Women with pregnancy related problems	84	nugent scoring system	Amsel's criteria	1
Tam, M.T. (119)	1998	USA	pregnant women with vaginal discharge	51	nugent scoring system	Amsel's criteria	1
Thammalangsy, Sivixay (120)	2006	Laos	pregnant women	500	nugent scoring system	Amsel's criteria	1
Thomason, Jessica (121)	1992	USA	pregnant women	120	nugent scoring system	Amsel's criteria	1
Thulkar, Jyoti (122)	2010	India	women with abnormal vaginal discharge	564	nugent scoring system	pH ≥ 4.5 and positive Whiff	1/2
Tosun, I. (123)	2003	Turkey	women visiting planning clinic	86	nugent scoring system	simplified Amsel's criteria	1
Turpin, Rodman (124)	2021	USA	non-pregnant women	2956	nugent scoring system	Amsel's criteria	1
Udayalaxm (125)	2011	India	women visting clinic	527	nugent scoring system	Amsel's criteria	1/2
Van den Munckhof, Ellen (126)	2019	The Netherlands	women complaining of abnormal vaginal discharge	80	nugent scoring system	Amsel's criteria	1/2
Verwijs, Marijn (127)	2019	Rwanda	women visting clinic	705	qPCR	vaginal discharge	1
West, Beryl (128)	2003	Gambia/West Africa	symptomatic women visting clinics	219	nugent scoring system	simplified Amsel's criteria	1
Wu, Shengjun (129)	2019	Chini	women	423	qBV Assay	Amsel's criteria	1/2

Yeoman, Carl (130)	2013	USA	pre-menopausal women	36	nugent scoring system	Amsel's criteria	1/2/3

3.3. BV prevalence as defined by means of non-clinical and clinical diagnosis.

This first analysis included 92 studies, representing a total of 42,314 women (Figure 2). These studies all provided a prevalence of BV as assessed by both a clinical and non-clinical method. In 36 studies, a higher prevalence of BV was seen by the clinical diagnosis and in 55 studies, a higher BV prevalence was seen by non-clinical diagnosis. In one study, an equal prevalence was seen.

A total of 13,530 women were positive by a non-clinical method and 13,221 by a clinical method, resulting in a metaprevalence of 32.0 % (95% CI, 31.53% - 32.42%) and 31.2% (95% CI, 30.80% - 31.69%), respectively.

3.4. Prevalence of women with symptomatic BV in women diagnosed with BV using non-clinical diagnosis

The second analysis contained 60 studies (of which 46 studies were also included in analysis 1). Studies were included when they reported the proportion of women with BV by the non-clinical method that were also clinically positive. In 40 studies, more women were symptomatic for BV than asymptomatic and in 19 studies, this was the reverse (Figure 3). In one study as many were symptomatic as asymptomatic. The total population of these 60 studies was 39,204 women of which a total of 11,570 women were positive by a non-clinical method. Of these 11,570 women, 6623 were symptomatic for BV and 4947 were asymptomatic for BV. Looking at this in total, 57.2% (95% CI, 56.34% - 58.14%) of women were symptomatic for BV and 42.8% of women were asymptomatic for BV (95% CI, 41.86% - 43.66%).

3.5. Prevalence of women with symptomatic BV in women diagnosed with BV, intermediate or normal microbiome using non-clinical diagnosis.

This third analysis contained a total of 11 studies (these studies were also included in analysis 2) including information on BV microbiome as well as intermediate and normal microbiome (Figure 4a, b and c). These 11 studies represented a population of 6545 women. The number of women positive for BV by non-clinical method is 1343 of which 920 women were symptomatic for BV. This amounts to 68.5% (95% CI, 66.02% - 70.99%) of women were symptomatic and 31.5% (95% CI, 29.01% - 33.98%) of women were asymptomatic for BV

(Figure 4a). The number of women with an intermediate microbiome based on the non-clinical diagnosis was 1426, of which 709 women were symptomatic based on the clinical method. This represented 49.7% (95% CI, 47.12% - 52.31%) of symptomatic women and 50.3% (95% CI, 47.69% - 52.88%) who were asymptomatic (Figure 4b). A total of 3776 women had a normal microbiome based on non-clinical diagnosis. Of these, 1548 women did experience symptoms using the clinical method. This represented 41,0% (95% CI, 39.42% - 42.56%) of women who were symptomatic and 59,0% (95% CI, 57,44% - 60,57%) who were asymptomatic (Figure 4c).

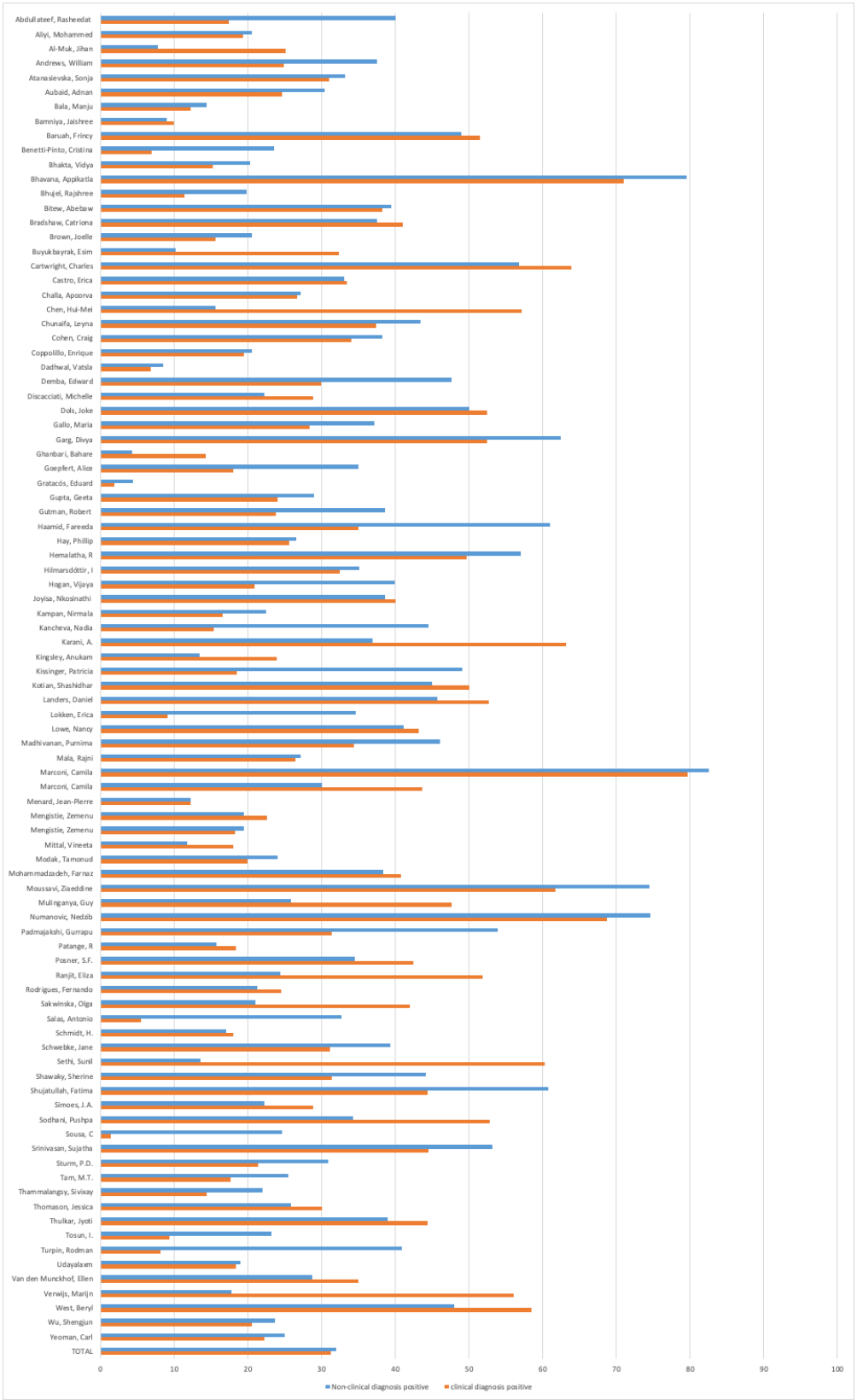

Figure 2. Prevalances of BV assessed with the clinical and non-clinical diagnosis. The figure plots the prevalences of the non-clinical diagnosis (blue) and clinical diagnosis (orange). Each study is represented by a horizontal line and are listed alphabetically according to the last name of the first author. For each study, the 95% CI has been calculated. See table 1.

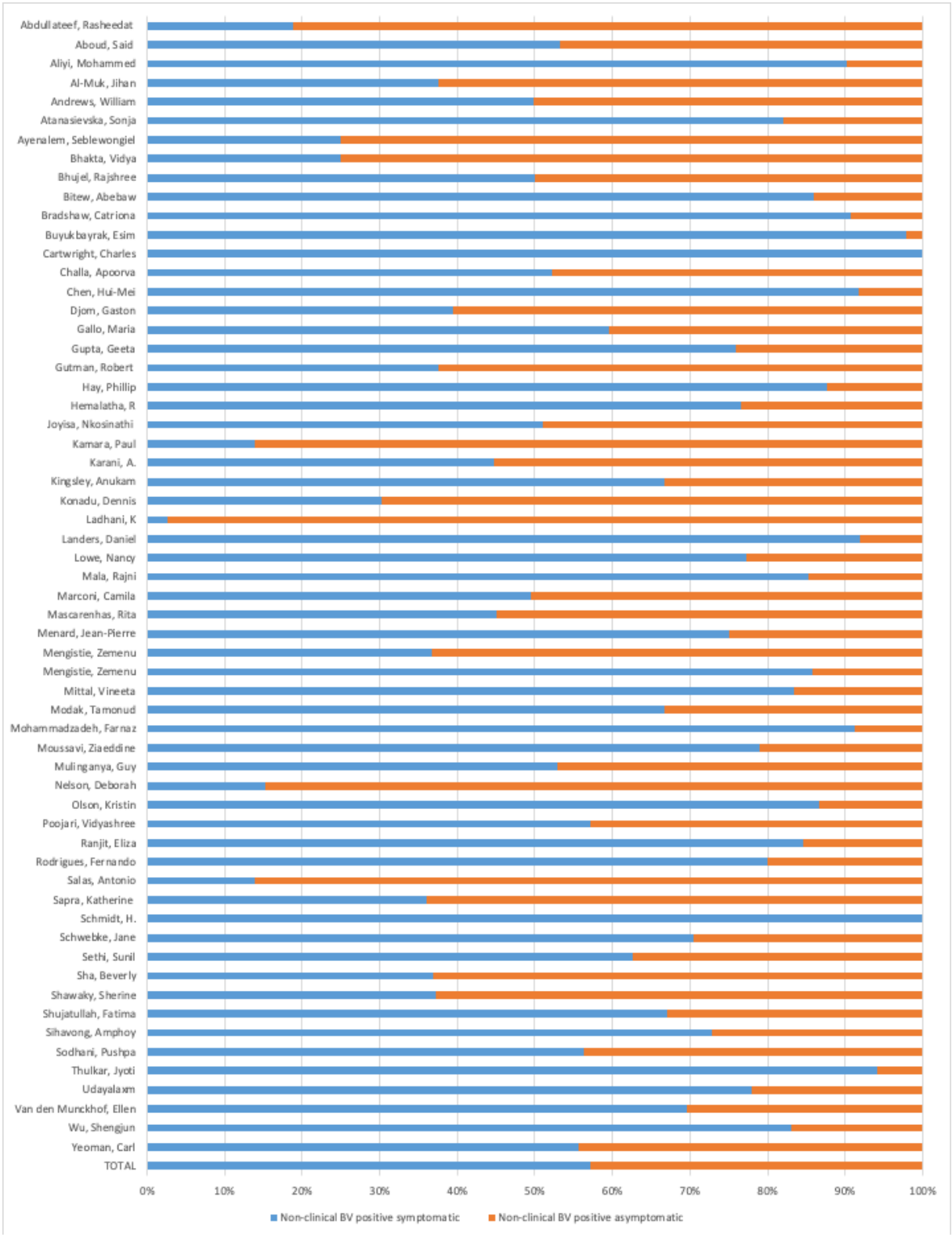

Figure 3. **Percentage of symptomatic women in women diagnosed as BV using a non-clinical diagnostic tool.** The proportion of women with symptoms is shown in blue. Studies are plotted alphabetically according the first author's last name. The sum of both values represents 100%. For each study 95% CI has been calculated. See table 1.

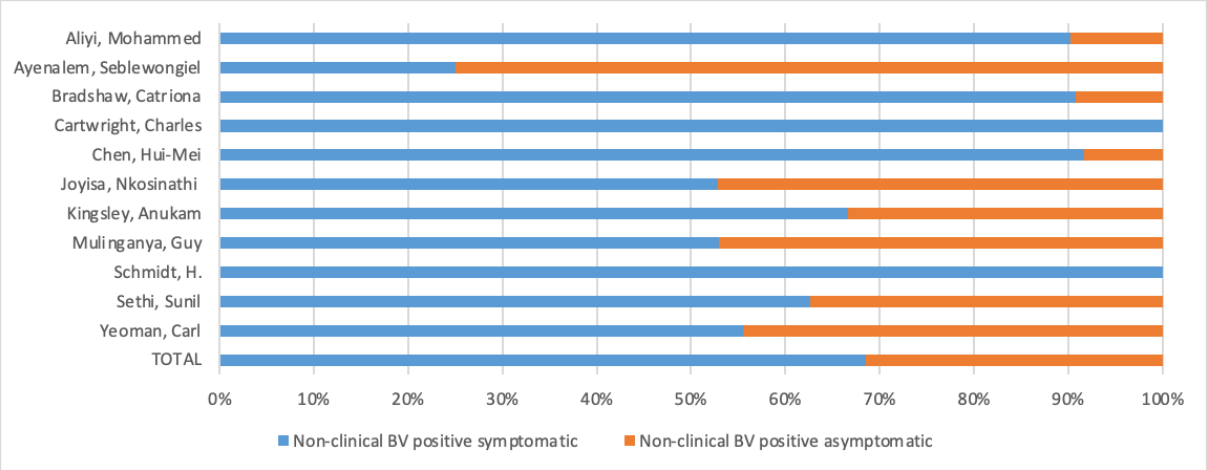

Figure 4a. **Percentage of symptomatic women in women diagnosed as BV using a non-clinical diagnostic tool.** The proportion of women with symptoms is shown in blue.

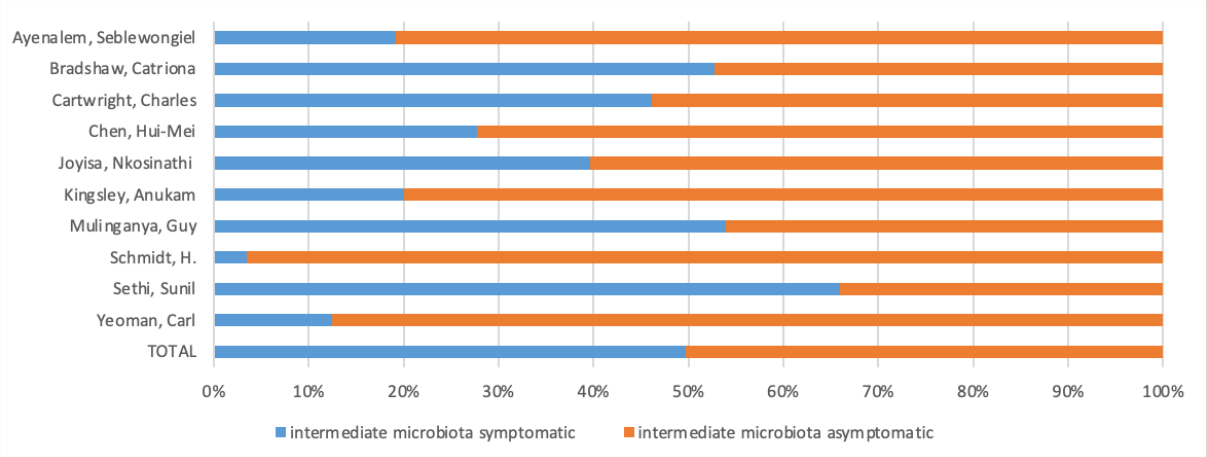

Figure 4b. Percentage of symptomatic women in women diagnosed as intermediate using a non-clinical tool. The proportion of women with symptoms is shown in blue.

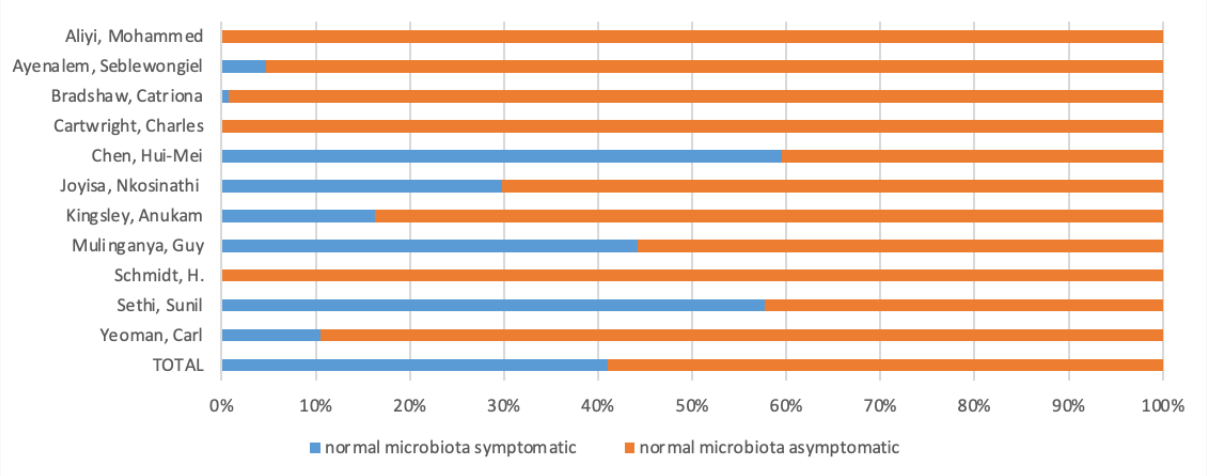

Figure 4c. Percentage of symptomatic women in women diagnosed as normal using a non-clinical tool. The proportion of women with symptoms is shown in blue.

4. Discussion

The purpose of this thesis was to determine the prevalence of BV and how many women with BV experience symptoms. This was done by a systematic review and meta-analysis. A total of 106 studies were included and three analyses were conducted.

In the first analysis where we reviewed BV prevalence as defined by means of non-clinical and clinical diagnosis 92 articles were included, with a total of 42,314 women. The results show that non-clinical diagnostic method gave a metaprevalence of BV of 32.0% (95% CI, 31.53% - 32.42%) and clinical diagnostic method a metaprevalence of 31.2% (95% CI, 30.80% - 31.69%).

The second analysis, where we reviewed the prevalence of women with symptomatic BV in women diagnosed with BV using a non-clinical diagnosis, included 60 studies, with a total of 11,570 women. According to this analysis, in women with a non-clinical BV diagnosis, 57.2% (95% CI, 56.34% – 58.14%) of the women were symptomatic for BV and 42.8% (95% CI, 41.86% – 43.66%) of the women were asymptomatic for BV.

In the third analysis, where we reviewed the prevalence of women with symptomatic BV in women diagnosed with BV, intermediate or normal microbiome using a non-clinical diagnosis 11 studies were included, with a total of 6545 women. In the category of BV positive women, 68.5% (95% CI, 66.02% - 70.99%) of the women were symptomatic, 49.7% (95% CI, 47.12% - 52.31%) of the women with an intermediate microbiome were symptomatic and 41.0% (95% CI, 38.44% - 43.55%) of the women were symptomatic with a normal microbiome.

4.1. Comparison of the metaprevalences with the literature

Based on the research conducted by Peebles et al. (4), the global prevalence of BV in the general population is ranging from 23% to 29% depending on regions. Thus, this is a lower prevalence than what we can conclude from our review. Peebles and colleagues excluded studies where women were included based on their symptomatology or BV-associated pathology such as PID, female infertility, women with or at risk of preterm birth or miscarriage, premature rupture of the membranes, diabetes, women engaging in intravaginal practices and women with intrauterine device. They also excluded studies in which women were included who deviated from the general study population, for example women with HIV, sex workers and STI clinic attendees. Thus, the prevalence represented by Peebles and colleagues focused on the general population (4). Our review did not exclude these types of studies

resulting in a broader population being reviewed. This may be a possible explanation for the slight deviation in prevalence.

The Systematic Review and Meta-analysis of Sabour et al. (133) only included women from Iran (West Asia) and also distinguished between pregnant and non-pregnant women. The overall prevalence of BV in the review of Sabour et al. was 18.5%. The prevalence of BV in non-pregnant women was 28% and in pregnant women it was 16.5% (133). Our study did not distinguish between pregnant and non-pregnant women. We also reviewed countries across different continents most of which took place in Asia, Africa and North America. This may explain why the prevalence in our study is much higher than the other studies. For example, in the review of Kenyon et al. (14), the prevalence of BV was the highest within Sub-Saharan Africa and moderate in Asia and Eastern Europe (14). Prevalence rates vary considerably among different geographic regions, within the same country and even within the same population, according to the review by Chacra et al. this depends on ethical origin and socioeconomic status. According to chacra et al. the prevalence of BV varied between 4-75% depending on the population observed in the study (17).

4.2. The influence of the criteria used to make a clinical diagnosis

The prevalence of BV obtained from the non-clinical diagnosis was 32.0% and from the clinical diagnosis 31.2%. These values hardly differ from each other. Nevertheless, BV had a higher prevalence by the clinical diagnosis in 36 out of the 92 studies. Across all studies, for the clinical diagnosis, in addition to Amsel's criteria, only the presence of vaginal discharge with or without malodour or modified Amsel's criteria was used. Of the studies not using Amsel's criteria, more than half had more clinically positive women than non-clinically positive women. According to the article by Livengood et al., increased vaginal discharge is a more frequent but less specific symptom (134).

BV, along with vulvovaginal candidiasis infection and trichomoniasis are part of vaginitis, an umbrella term to describe symptoms such as vaginal discharge, itching, burning sensation and odor. All three can have vaginal discharge as a symptom, which can differ among the three in consistency and color. In making the diagnosis, investigators should use the characteristics of vaginal discharge such as consistency, color, odor, the amount and ask for any associated symptoms such as burning and pruritis to diagnose BV, candida or trichomonas (135). Livengood et al. mentioned that a number of researchers indicate the lack of interobserver reproducibility of the characterization of vaginal discharge (134). This may be an explanation

why vaginal discharge in our review often gives a higher prevalence compared to the non-clinical diagnosis. Namely, because it could be misdiagnosed and thus it actually belongs to candida or trichomonas infection.

So, by including vaginal discharge in our study, which in the studies is related to BV and therefore seen as a clinical diagnosis of BV, it may be that the prevalence of BV was somewhat higher compared to the literature mentioned above. To see if there is a big difference in prevalence if only Amsel's criteria is taken as clinical diagnosis a subsequent analysis can be performed in a next study.

In some cases, Amsel also diagnosed a higher prevalence compared to the non-clinical method. According to the article of Forsum et al. (136) the Amsel's criteria are interdependent, according to the article of Redelinghuys et al. (137), the Amsel's criteria are based on non-quantifiable and non-reproducible clinical symptoms only. And according to Chen et al., the diagnosis with Amsel's criteria are somewhat subjective (2). This may be an explanation why Amsel's criteria gave a higher prevalence; it thus depends on the researcher's interpretation of the criteria.

4.3. Bacterial vaginosis symptomatic or asymptomatic

As mentioned earlier, the purpose of this study was to get a better idea of how many women with bacterial vaginosis as defined by a non-clinical method, actually experience symptoms.

Despite the fact that the metaprevalences of women with clinical and non-clinical diagnosis are not much different from each other, more BV was identified based on the non-clinical methods. Thus, 55 out of 92 studies gave a greater prevalence using non-clinical method than the clinical method. So, this means that from analysis 1 where we reviewed BV prevalence as defined by means of non-clinical and clinical diagnosis, some of the women were also asymptomatic. This percentage of asymptomatic women is estimated between a range of 1% to 30%. When this was addressed more specifically in analysis 2, where we reviewed the prevalence of women with symptomatic BV in women diagnosed with BV using a non-clinical diagnosis, a metaprevalence of 57.2% symptomatic women was found. In case of the second analysis, it is seen that only in the study by Cartwright et al. (44) and Schmidt et al. (107), all cases that were non-clinically positive all had symptoms.

4.3.1. Asymptomatic women: an over- or underestimation

According to the article of Muzny et al., asymptomatic BV is common although its pathogenesis remains incompletely understood (138).

A large proportion of women were also asymptomatic in our review. The question remains how reliable these values are. It could be that the percentage of asymptomatic women is overestimated. This may be because based on non-clinical diagnosis, too many women are diagnosed as BV. These women could potentially not actually have BV thus, are asymptomatic. For example, the article of Zheng et al. mentioned that the morphologic characteristic and Gram-staining proporty of *Lactobacillus iners* are clinically important. Indeed, *L. iners* is more likely to have a gram-negative morphology, this property masks the fact that it is a lactobacillus species. Therefore, the Nugent score based on gram staining of the vaginal smears might lead to the misdiagnosis of BV (139).

The percentage of asymptomatic women given in our study could also be an underestimation of the true number of asymptomatic women. Women often already present with vaginal complaints (4). In our study, more than 50% of the studies included women visiting clinics with complaints. Women without symptoms might not show up but may have bacterial vaginosis based on a non-clinical diagnosis. In case of random sampling of the population this could increase the result in percentage of asymptomatic women.

It was mentioned above that the Amsel's criteria are rather subjective (2). Thus, besides the fact that there could be an overdiagnosis with Amsel, it is also possible that there is an underdiagnosis with Amsel. In this case the number of asymptomatic women is also overestimated. According to the article by Kenyon et al., the BV prevalence is underestimated by Amsel's criteria by 30-40% comparing it to Nugent scoring system (14). Clinicians often use less microscopy or lack the skill to detect clue cells, causing cases to be missed (9).

4.3.2. Asymptomatic women: applied to clinical practice and screening

BV, whether symptomatic or asymptomatic is associated with adverse reproductive health outcomes, such as sexually transmitted infections (STIs), pelvic inflammatory disease (PID) as well as adverse obstetric outcomes such as preterm birth and low birthweight are associated with BV (140, 2). The question remains whether those asymptomatic women should be treated for BV or not.

The review of Chapman et al. recommended that pregnant women asymptomatic for BV and at low risk for preterm birth, should not be routinely screened or treated for BV. Earlier treatment of asymptomatic women has not shown a clear and consistent improvement in outcomes (141).

According to the sexually transmitted infections treatment guidelines, 2021 of Workowski et al. routine screening for pregnant women with asymptomatic BV is not recommended, either at high risk or low risk of preterm birth. At low risk of preterm birth, there is no reduction in adverse outcomes when asymptomatic BV is treated. At high risk, it is seen there is either no difference with treatment, that harm occurs or that it would be beneficial (142).

The absence of hydrogen peroxide-producing lactobacilli in the vaginal microbiome of women with BV would be the largest biological risk factor for STD (sexual transmitted diseases) acquisition (143). In the article of Schwebke et al. (2007), women with asymptomatic BV were prospectively studied to determine the effect of treatment of BV for the prevention of STD (143). Either women were treated with an intravaginal metronidazole gel or were just observed. A significant reduction in the number of chlamydia infections could be demonstrated in BV asymptomatic women who were randomly assigned prophylactic treatment with metronidazole gel compared to those who were simply observed (143). Further research should therefore consider whether intervention is required for women who are at high risk of STI and have additional BV or BV associated microbiota (144).

According to the article of Bautista et al. the role of BV in bacterial infections is an important area of research for STI control efforts. This is evidenced from randomized controlled trials showing that screening and treatment of asymptomatic women with BV reduces the risk of infection (145).

It must be considered that asymptomatic BV has not yet been adequately studied. So, it is not known whether asymptomatic BV is a milder form of infection, what the pathogenesis is behind and what the response to therapy is, as well as whether the complications are equally common in asymptomatic women (138).

4.4. Interpretation of microbiome related to symptoms

The third analysis where we reviewed the prevalence of women with symptomatic BV in women diagnosed with BV, intermediate or normal microbiome using a non-clinical diagnosis, showed that women with an intermediate microbiome or normal microbiome can be

symptomatic as well as asymptomatic for BV. The graphs (figure 4 a, b and c) revealed a much lower count of symptomatic women in comparison to those who test positive for BV by a non-clinical diagnosis. When examining these categories in terms of percentages, they remain elevated: 49.7% of the women with an intermediate microbiome are symptomatic and 41.0% of the women with a normal microbiome are symptomatic.

The fact that women with a normal microbiome can experience symptoms may also be explained by findings mentioned earlier, namely the fact that vaginal discharge can be misdiagnosed for BV and that Amsel is a subjective method. Thus, these symptoms may have been wrongly attributed to BV.

An intermediate microbiome is an understudied category and therefore challenging for the diagnosis of BV. Nugent's scoring system is considered to be the gold standard for diagnosing BV but the diagnosis can be influenced by individual skill and experience. The identification of morphotypes is subjective and technician-dependent (17).

An intermediate microbiome is characterized by a partial loss of protective lactobacilli and an increase in BV associated organisms. Thus, because they have a modification in their first line host defense against infection, women with an intermediate microbiome are also more susceptible to STIs (109). According to the article of Sethi et al (2023), an increase in abnormal vaginal microbiome is associated with an incremental increase in the likelihood of *Trichomonas vaginalis* infection (139). According to the article of Brotman et al. (2010) when women had a Nugent score of 4-6 and a score of 7-10, they were associated with a 1.4 – 1.7-fold increased risk for acquisition of an STI (144).

It can therefore be concluded that women where BV is not diagnosed but altered vaginal flora is seen may be at risk for infections with STIs (109).

As with asymptomatic BV, further research is also needed on the extent to which intermediate BV contributes to adverse outcomes and whether it is advantageous to screen and possibly treat intermediate BV. In this way, a better picture can be obtained about what the contribution is to the global burden.

4.5. Best approach for diagnosis of BV

Researchers should consider the costs, result time and accuracy of a test for diagnosing BV (16). Executing a clinical and non-clinical method to diagnose BV for each patient could be considered the best approach. The results can be compared with each other, providing a clear

diagnosis on symptomatic or asymptomatic women. As a clinical method it is best to take Amsel's criteria such that the possibility of misdiagnosis, only based on vaginal discharge, is already excluded. In addition, it is best for the investigator to use the technique he is most familiar and experienced with.

4.6. Limitations

A limitation of this study may be that no quality control was carried out on the different studies.

4.7. Further research

It would also be interesting to carry out analyses to look at the prevalences within different subpopulations. These can look at pregnant and non-pregnant women, women with HIV and without HIV, high risk women for STI and low-risk women and differentiate between different age groups. Furthermore, we could look at women who present with symptoms and women who present for other reasons, in order to get a better picture of the percentage of asymptomatic women. Furthermore, it also seems interesting to do another subanalysis only including Amsel's criteria as clinical diagnosis of BV.

5. Conclusion

The main objective of this thesis was the determine the metaprevalence of symptomatic and asymptomatic women. From this, it emerges that still a large proportion of women are asymptomatic for BV. Guidelines and studies indicate that asymptomatic BV should not be treated or screened. Yet it is seen that asymptomatic BV could also have adverse effects.

This thesis gave a better idea of how many women are asymptomatic for BV. This may perhaps result in more research on the consequences of asymptomatic BV and what its contribution is to the global burden of BV.

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6. Addendum

6.1. Protocol for a systematic review and meta-analysis of bacterial vaginosis and the prevalence of symptomatic women.

1. Administrative information

1.1 Identification

The protocol for a systematic review and meta-analysis of bacterial vaginosis and the prevalence of symptomatic and asymptomatic women is contained in this report. The Preferred Reporting Items for Systematic review and Meta-analysis (PRISMA) guidelines will be followed when reporting about this research (24).

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- Contributions

The protocol has been drafted by MD and all contributed to the final study protocol. MD will carry out the initial literature research. MD and PC will apply the predefined selection criteria to identify manuscripts to be included in the review and meta-analysis. Discrepancies in included studies will be reviewed by PC and MD. MD will

extract data from the identified manuscripts. MD will perform the statistical data-analysis. MD will draft the thesis and all authors will contribute to the final thesis.

1.3 Support

- Sources

Not applicable

- Sponsor

There is no sponsor for this study

- Role of sponsor or funder

Not applicable

2. Introduction

2.1 Rationale

Bacterial vaginosis is the most commonly reported syndrome among women of childbearing age (1). It has a prevalence ranging from 23% to 29% with racial disparities and a high global cost burden for treatment (2).

The healthy vaginal microbiome is dominated by *Lactobacillus*. They establish a defense against the invading pathogens by producing various compounds such as lactic acid, hydrogen peroxide and bacteriocins (3). BV is characterized by overgrowth of anaerobic bacteria such as *Gardnerella vaginalis*, *Mobiluncus, Fannyhessea and prevotella* and a decrease in the levels of lactobicilli. BV can be symptomatic or asymptomatic. Women who are diagnosed with BV can experience symptoms such as vaginal malodor, discharge and itching (3,4).

In addition to the fact that BV causes physical and psychosocial discomfort (2) it would also trigger numerous health disorders, including adverse pregnancy outcome, pelvic inflammatory disease (PID), sexual transmitted infections (STIs) such as human immunodeficiency virus (HIV), human papillomavirus (HPV), herpes simplex type 2 (HSV-2), chlamydia, gonococcal and trichomonas infection (3).

The women who are asymptomatic can still have adverse outcomes for their health and it’s therefore important to acquire a better insight into the total percentage of asymptomatic and symptomatic women.

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2.2 Objectives

Through systematic review of literature and performing a meta-analysis this study aims to investigate which percentage of women with BV are symptomatic.

3. Methods

3.1 Eligibility criteria

According to the inclusion and exclusion criteria outlined below the studies will be selected.

- ### Inclusion criteria

1. Studies that provide the percentage or absolute numbers of bacterial vaginosis (BV) diagnosed based on non-clinical or clinical methods. Studies that compare the numbers diagnosed with non-clinical or clinical methods. Studies that indicate which women are symptomatic or asymptomatic for BV after a non-clinical diagnosis.
2. Papers in which BV is diagnosed using at least one of the following diagnostic tools as non-clinical diagnosis: Nugent scoring system, BVBlue test, Spiegel, molecular diagnosis, laboratory diagnosis. Studies that uses Amsel's criteria, vaginal discharge in relation to BV, malodor, whiff test or mention the number of women who are symptomatic or asymptomatic as a clinical diagnosis.
3. Women of reproductive age.
4. Papers in English, French, Dutch and German.
5. No ethnical, geographic or clinical setting limitation.
6. Papers starting from the year 1990.
7. End date limitation: papers until August 2023.

- ### Exclusion criteria

1. Studies that fail to provide information of the sample size.

2. Studies that only use a non-clinical or clinical diagnosis.
3. Studies that do not provide data from women with BV who are symptomatic or asymptomatic - lack of necessary results.
4. Studies in which the diagnosis of BV is based on a non-clinical method or clinical method, but not specified in the results if the diagnosis of BV was based on a non-clinical or clinical method. This makes it impossible to provide them separately or compare them with each other.
5. Unpublished articles, reviews, comments and guidelines, reports with the same data.
6. Studies that diagnose BV after treatment, resulting in the observation of cure rates rather than the actual prevalence of BV.
7. Studies where it is explicitly stated they test on post-menopausal women.

3.2 Information sources

Databases such as Pubmed (including MEDLINE), web of science, scopus and Embase will be searched for bibliographic references.

3.3 Search strategy

Using the step by step approach specified in the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement studies will be searched and identified.

The online search strategy will include the combination of the search ‘bacterial vaginosis’ ‘nonspecific vaginitis’ ‘bacterial vaginitis’ ‘Nugent’ ‘molecular diagnosis’ ‘molecular diagnostic techniques’ ‘markers’ ‘sensitivity and specificity’ ‘molecular testing’.

After formulating the research question ‘Bacterial vaginosis positivity by Nugent or Molecular diagnosis (non-clinical diagnosis) versus clinical criteria: a systematic review and meta-analysis’ the PICO framework has been drawn.

P (population): women with bacterial vaginosis
I (intervention): Nugent – molecular diagnosis
C (control/ comparison): Clinical criteria (Amsel criteria)
O (outcome): positive for bacterial vaginosis and symptomatic or asymptomatic.

Because it is a systematic review, it is important that no relevant articles are missed out. Therefore, we only use the P and I concept from the PICO framework to search as broadly as possible. The search terms ‘bacterial vaginosis’ ‘Nugent’ and ‘molecular diagnosis’ are further elaborated.

The first database which has been examined was Pubmed. In addition to bacterial vaginosis as free text that was searched for in the title and abstract [tiab], 'vaginosis bacterial' was also searched for as MeSH term. With this MeSH term, synonyms were also mentioned, including the relevant ones such as: 'nonspecific vaginitis' and 'bacterial vaginitis'.

On top of this Nugent was examined. This was included as a free text word [tiab], as well as 'molecular diagnosis' was included as a free text word [tiab]. There are no Mesh terms in the database for Nugent. In some articles the MeSH term is used for sensitivity and specificity, which means that it is included as a MeSH term.

There is also no MeSH term for molecular diagnosis in Pubmed. However, 'molecular diagnostic techniques' is a Mesh term and is therefore included.

Furthermore, 'markers' as well as 'molecular testing' are included as free text words.

We then also applied these search terms in Embase, Web of science and Scopus, whereby the indications such as [tiab] or [MeSH] were replaced according to the syntax of the database.

This resulted in the following for all databases:

Pubmed:

("vaginosis, bacterial"[MeSH Terms] OR “Bacterial Vaginosis”[tiab] OR “Nonspecific Vaginitis”[tiab] OR “Bacterial Vaginitis”[tiab]) AND ("nugent"[Tiab] OR “molecular diagnosis” [Tiab] OR "Molecular Diagnostic Techniques"[Mesh] OR “molecular testing” [Tiab] OR “markers” [Tiab] OR "Sensitivity and Specificity"[Mesh])

Embase:

('bacterial vaginosis'/exp OR (‘Bacterial Vaginosis’ OR ‘Nonspecific Vaginitis’ OR ‘Bacterial Vaginitis’):ti,ab,kw) AND ('molecular diagnosis'/exp OR 'diagnostic test'/de OR 'sensitivity and specificity'/exp OR (‘nugent’ OR ‘molecular diagnosis’ OR ‘molecular testing’ OR ‘markers’):ti,ab,kw)

Web of science:

(TS=(“bacterial vaginosis” OR “Nonspecific vaginitis” OR “Bacterial vaginitis”)) AND (TS=(“Nugent” OR “molecular diagnosis” OR “Molecular Diagnostic Techniques” OR “molecular testing” OR “markers” OR “Sensitivity and Specificity”))

Scopus:

(TITLE-ABS-KEY ("bacterial vaginosis" OR "nonspecific vaginitis" OR "bacterial vaginitis")) AND (TITLE-ABS-KEY ("nugent" OR "molecular diagnosis" OR "Molecular Diagnostic Techniques" OR "molecular testing" OR "markers" OR "Sensitivity and Specificity"))

3.4 Study records

- Data management

Endnote reference manager will be used to remove duplicates and to create a database of the studies that are identified for review. When the duplicates are removed in endnote, the remaining ‘unique hits’ will be checked in Excel to make sure all duplicates are fully removed. Dropbox is used to upload the progress of this research. Dropbox is a file hosting service offering cloud storage, file synchronization, personal cloud and client software. It enables all co-authors to bring files together in an efficient manner as well as up-to-date at all times on different devices.

- Selection process

All references will be screened by title and abstract for full text evaluation, using Rayyan. Rayyan is a free web tool that makes it possible to screen articles in an efficient way. For each article you can indicate why it is not included and disagreements between the different authors can be clarified. The full text of the remaining studies will be evaluated to decide whether it meets the inclusion criteria. The reasons for excluding studies will be recorded.

- Data collection process

Data will be extracted from each eligible study by MD. A report on important patient outcomes will be provided along with demographic information, methodology, and analysis of the data (see 3.5). In the event that disagreements or uncertainties arise, they will be resolved by PC through discussion.

3.5 Data items

The following information will, if reported, be derived from a predefined table.

1. Title
2. First author
3. Year of publication
4. Country
5. Study design
6. Start to end date of study
7. Study population
8. Total number of study participants
9. Age
10. Non-clinical diagnostic test method used (1)
11. Positive scoring cutoff for diagnostical test method used (1)
12. Clinical diagnosis (used method)
13. Criteria clinical diagnosis
14. Non-clinical diagnosis positive
15. Clinical diagnosis positive
16. Non-clinical diagnosis negative
17. Clinical diagnosis negative
18. Clinical positive/ non-clinical positive
19. Clinical negative/ non-clinical positive
20. Clinical positive/ non-clinical negative
21. Clinical negative/ non-clinical negative
22. Non-clinical BV positive symptomatic
23. Non-clinical BV positive asymptomatic
24. Categorization of intermediate microbiota
25. Intermediate BV (non-clinical)
26. Intermediate microbiota symptomatic
27. Intermediate microbiota asymptomatic
28. Categorization of normal microbiota
29. Normal (non-clinical)
30. Normal microbiota symptomatic
31. Normal microbiota asymptomatic

A Microsoft Excel sheet will be created, with a row for each study included in the review containing all the above information. In the second Excel sheet, a legend with explanations for some data items can be found.

3.6 Outcomes and prioritization

The outcomes will be the reported symptomatic or asymptomatic cases.

3.7 Data synthesis and analysis

Three analyses will be conducted based on the acquired data. The excel data file is used for processing the data and creating the graphical representations as well as the calculation of the 95% confidence interval.

The first analysis examines the percentage of BV obtained for non-clinical and clinical diagnosis separately. The studies are displayed in a graphical representation, with the percentage of non-clinical and clinical BV plotted for each study. The Y-axis represents the studies and the X-axis indicates the percentage. In the end, a meta-analysis is performed for all included studies, and the confidence interval for this meta-analysis is determined.

The second analysis is a subanalysis including studies in which the obtained values of non-clinical and clinical diagnosis are compared. From this, the percentage of the non-clinical diagnosis which are effectively symptomatic or asymptomatic for BV can be deducted. This analysis is also presented graphically. A bar plot is used for each study, where the total bar represents the non-clinical diagnosis and accounts for 100%, the blue portion represents the percentage of BV diagnosed by the non-clinical criteria and the orange proportion represents the percentage of BV diagnosed by the clinical criteria. Again, a meta-analysis is conducted and the confidence interval is determined.

A third analysis is a subanalysis that includes studies where information is given on how many women are symptomatic and asymptomatic for intermediate and normal microbiome. Thus, if only information is given about intermediate or normal these studies are not included in the subanalysis. Here the percentage of symptomatic and asymptomatic women within BV, intermediate and normal microbiome is calculated. A barplot is made for each category as described in analysis 2, thus three bar plots are made. A meta-analysis is conducted.