



**HAL**  
open science

## Relationship between Bacterial Vaginosis and Sexually Transmitted Infections: Coincidence, Consequence or Co-Transmission?

Linda Abou Chacra, Claudia Ly, Alissa Hammoud, Rim Iwaza, Oleg Mediannikov, Florence Bretelle, Florence Fenollar

► **To cite this version:**

Linda Abou Chacra, Claudia Ly, Alissa Hammoud, Rim Iwaza, Oleg Mediannikov, et al.. Relationship between Bacterial Vaginosis and Sexually Transmitted Infections: Coincidence, Consequence or Co-Transmission?. *Microorganisms*, 2023, 11 (10), pp.2470. 10.3390/microorganisms11102470 . hal-04462886

**HAL Id: hal-04462886**

**<https://amu.hal.science/hal-04462886>**

Submitted on 16 Feb 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License



## Article

# Relationship between Bacterial Vaginosis and Sexually Transmitted Infections: Coincidence, Consequence or Co-Transmission?

Linda Abou Chacra <sup>1,2</sup>, Claudia Ly <sup>1,2</sup>, Alissa Hammoud <sup>2,3</sup>, Rim Iwaza <sup>2,3</sup>, Oleg Mediannikov <sup>2,3</sup> , Florence Bretelle <sup>3,4</sup> and Florence Fenollar <sup>1,2,\*</sup>

<sup>1</sup> Campus Santé Timone, Aix-Marseille University, IRD, AP-HM, SSA, VITROME, 13005 Marseille, France; abouchacra.linda@gmail.com (L.A.C.); claudiaks\_ly@hotmail.com (C.L.)

<sup>2</sup> IHU-Méditerranée Infection, 13005 Marseille, France; reem.iwaza@gmail.com (R.I.); olegusss1@gmail.com (O.M.)

<sup>3</sup> Campus Santé Timone, Aix-Marseille University, IRD, AP-HM, MEPHI, 13005 Marseille, France; florence.bretelle@ap-hm.fr

<sup>4</sup> Department of Gynaecology and Obstetrics, Gynépole, La Conception, AP-HM, 13005 Marseille, France

\* Correspondence: florence.fenollar@univ-amu.fr; Tel.: +33-(0)-4-13-73-24-01; Fax: + 33-(0)-4-13-73-24-02

**Abstract:** Sexually transmitted infections (STIs) are a serious global problem, causing disease, suffering, and death. Although bacterial vaginosis (BV) is not considered to be an STI, it may be associated with an increased risk of contracting a wide range of STIs. We sought to assess the link between the different microorganisms involved in STIs and BV. A total of 290 vaginal swabs from 290 women sent for diagnostic purposes to the clinical microbiology laboratory of the Marseille University Public Hospitals were tested by specific qPCR targeting STI-causing microorganisms and BV. Of these 290 swabs, 15.2% (44/290) were diagnosed with at least one STI-causing microorganism and 17.2% (50/290) with BV. The prevalence of STIs was significantly higher in women with BV (28%, 14/50) than in those without (20.4%, 51/240). The prevalence of co-infections involving two STI-causing microorganisms was significantly more frequent in women with BV than in those without (18% [8/50] vs. 2% [5/250];  $p < 0.001$ ). The prevalence of mono-infections and poly-infections with STI-causing microorganisms was lower in women without BV than in those with (8.8% [21/240] vs. 28% [14/50]),  $p < 0.001$  and 2% (5/240) vs. 8% (4/50),  $p = 0.05$ , respectively). Our data suggest that a correlation between BV and STI may exist, with a higher prevalence of both mono-infections and poly-infections involving STI-causing microorganisms in women with BV. Further research is needed to better understand BV and its links to STIs.

**Keywords:** vaginal ecosystem; bacterial vaginosis; dysbiosis; sexually transmitted infection; sexual practices



**Citation:** Abou Chacra, L.; Ly, C.; Hammoud, A.; Iwaza, R.; Mediannikov, O.; Bretelle, F.; Fenollar, F. Relationship between Bacterial Vaginosis and Sexually Transmitted Infections: Coincidence, Consequence or Co-Transmission?.

*Microorganisms* **2023**, *11*, 2470.

<https://doi.org/10.3390/microorganisms11102470>

Academic Editor: Hirokazu Kimura

Received: 26 July 2023

Revised: 23 September 2023

Accepted: 29 September 2023

Published: 1 October 2023



**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

It has long been known that the vaginal microflora plays a crucial role in maintaining a normal physiological environment for the host and that its participation is considered essential for successful reproduction [1]. In healthy women of childbearing age, the normal vaginal flora is classically dominated by *lactobacilli*. Between 1 and 4 mL of vaginal fluid can contain  $10^8$  to  $10^9$  bacterial cells, which play a key role in maintaining the physiological balance of the female genital tract and protecting the vaginal mucosa. Indeed, *lactobacilli*, with their production of antimicrobials, as well as those of lactic acid from the glycogen contained in the vaginal mucosa, making it possible to generate an acid vaginal pH (of 4.5 or less), provide an effective first-line defence against potentially pathogenic microorganisms [1]. Currently, the complex interaction between vaginal microflora and reproductive health is a major research topic in the fields of medicine and biology and continues to generate considerable interest in better understanding its impact on women's health.

Bacterial vaginosis is a disturbance of the vaginal ecosystem, characterised by a significant depletion of the *lactobacilli* due to a strong proliferation of anaerobic bacteria, which are less abundant in the normal vaginal flora, such as *Gardnerella vaginalis*, *Fannyhessea* (formerly *Atopobium*) *vaginae*, *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Prevotella*, *Peptoniphilus*, *Megasphaera*, *Mobiluncus*, and several fastidious bacteria characterised as “Bacterial Vaginosis Associated Bacteria” (BVAB-1, BVAB-2, and BVAB-3) [2]. The exact aetiology of these changes has remained elusive to date, although many trigger factors are related, such as sexual activities [3], vaginal cleansing [4], contraception [5] and antibiotic use [6]. The prevalence of bacterial vaginosis varies between countries around the world, ranging from around 10% to 30% among women who have sex with men (WSM) [7,8] and 25% to 50% among women who have sex with women (WSW) [9], with the significant majority of women with bacterial vaginosis being asymptomatic [10]. The highest prevalence is reported in sub-Saharan Africa [11]. In Europe, the prevalence of bacterial vaginosis is much lower [12]. Bacterial vaginosis occurs frequently in women of childbearing age [13].

The rise in the incidence of sexually transmitted infections by more than one million new cases every day [14] has led to an alarming major public health crisis. This increase in sexually transmitted infections is a cause for concern, as sexual contact can spread more than 30 different bacteria, viruses, and parasites [15]. There is a direct impact on sexual and reproductive health caused by sexually transmitted infections in women’s upper and lower reproductive tracts [15]. Asymptomatic sexually transmitted infections are a cause for concern, as they are difficult to identify [15]. Their non-negligible prevalence is also a worrying aspect. Indeed, these particularly insidious infections cannot be identified without specific tests and can therefore easily spread in a sexually active population [15].

Although bacterial vaginosis is not considered to be a sexually transmitted infection, this dysbiotic condition may be associated with an increased risk of contracting a wide range of sexually transmitted infections [16,17]. For example, a cohort study of 255 non-pregnant women showed that bacterial vaginosis was associated with a 3.4- and 4-fold increased risk of positive tests for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, respectively [18]. Similarly, Brotman et al. demonstrated that women with bacterial-vaginosis-associated vaginal microbiota or low levels of *lactobacilli* had the highest relative proportion of human-papillomavirus-positive samples [19].

In this paper, we sought to study the concomitance of bacterial vaginosis and sexually transmitted infections in women and compared samples from women with bacterial vaginosis versus women without, using molecular biology as a rational diagnostic tool.

## 2. Materials and Methods

### 2.1. Data Collection and Ethical Considerations

A total of 290 vaginal specimens were sampled from 290 women using Sigma Transwab® swabs (Medical Wire, Corsham, UK). These specimens were initially collected for diagnostic purposes and sent to the clinical microbiology laboratory at our University Public Hospitals (Assistance Publique—Hôpitaux de Marseille [AP-HM], Marseille, France). The data analysed in this study are thus retrospective and cover a six-month period in 2022. Before testing, these samples were meticulously stored at  $-80\text{ }^{\circ}\text{C}$  to preserve their integrity.

This is, therefore, a retrospective study of samples initially obtained during patient management, in accordance with Article L.1211-2 of the French Public Health Code. Patients were informed of the potential secondary use of their samples and personal data, collected as part of their clinical care, for research purposes. They also had the possibility of refusing this secondary use by notifying the AP-HM data protection officer of their objections. In addition, all personal data relating to patients have been anonymized to protect their privacy and their confidentiality. The study was conducted in accordance with the Declaration of Helsinki. The entire protocol has been approved by our independent ethics committee (IEC no. 2022-033).

## 2.2. Molecular Strategies for Detecting Microorganisms

The molecular strategy used in this study to detect microorganisms involved a complete set of target genes, each with its corresponding probes and primers. Eleven DNA sequences targeting ten microorganisms and one human gene were analysed using real-time quantitative PCR assay. Quantification of the human albumin gene with exon 12 as the target sequence was thus used as an internal control to provide evidence of DNA presence and quality, as well as the potential presence of PCR inhibitors. The microorganisms tested, with their target sequences in brackets, were as follows: *G. vaginalis* (*Cpn 60*), *F. vaginae* (16S rRNA), *Lactobacillus* spp. (*Tuf*), *Mycoplasma genitalium* (*FusA*), *Trichomonas vaginalis* (repeated sequence), *N. gonorrhoeae* (hypothetical protein), *C. trachomatis* (hypothetical protein), *Treponema pallidum* (*PolA*), *Haemophilus ducreyi* (*GroESL*), and Herpes simplex virus (*Pol*). The list of these target sequences and their respective probes and primers sequences used are more detailed in the Supplementary Materials (Supplementary Table S1). To ensure accuracy and reliability of results, synthetic positive controls (plasmids) and negative controls (master mixes) were also included in each PCR run. Data were systematically interpreted after validation of the quality of these positive and negative controls. This rigorous quality control strategy ensures the credibility of the data generated by the study.

DNA extraction from vaginal samples was performed using a Qiagen EZ1 bio-robot (Qiagen, Courtaboeuf, France) in conjunction with a commercial extraction kit known as the QIAamp Tissue Kit<sup>®</sup> (Qiagen), following the precise guidelines defined by the kit's manufacturer. The DNA extraction process began with the digestion of 100 µL of vaginal samples using 100 µL of G2 buffer and 10 µL of proteinase K at a controlled temperature of 56 °C for a period of 20 min. Next, the elution step yielded 100 µL of DNA extracts, which were then carefully stored at −20 °C to preserve their stability and integrity until subsequent analysis.

Specific quantitative real-time polymerase chain reaction (PCR) was performed using a CFX96 Real-Time system (Bio-Rad Laboratories, Foster City, CA, USA). DNA amplification was performed based on the following thermal profile: (1) incubation was carried out at 50 °C for two minutes (for Uracil DNA Glycosylase (UDG) activation); (2) initial DNA denaturation was performed at 95 °C for five minutes; and, (3) finally, a series of 39 cycles consisting of DNA denaturation at 95 °C for five seconds and primer annealing-probe hybridization at 60 °C for 30 s was carried out. The final reaction mix volume for quantitative real-time PCR assays contained 10 µL of Eurogentec<sup>™</sup> Probe PCR Master Mix (Eurogentec, Liege, Belgium), 3 µL of distilled water DNase and RNase free, 0.5 µL of each reverse and forward primers (50 µM), probe (50 µM), UDG, and 5 µL of extracted DNA.

For *C. trachomatis*, *M. genitalium*, *N. gonorrhoeae*, *T. vaginalis*, *H. ducreyi*, and *T. pallidum*, as well as for HSV, samples were considered positive for the targeted microorganisms if the cycle threshold (ct) value was lower than 35. In order to detect the presence of bacterial vaginosis, a quantitative real-time PCR was applied to all DNA vaginal samples to quantify both *F. vaginae* [20] and *G. vaginalis* DNA levels, as previously reported [21,22]. As mentioned in the initial articles, results were expressed as copies of microbial DNA per 1 mL of vaginal suspension. In addition, the synthetic positive control (dilution to 10<sup>4</sup> of a quantification plasmid) was used for quantification. Finally, according to the strategy presented by Ménard et al., the diagnosis of bacterial vaginosis flora was made when bacterial loads  $\geq 10^8$  DNA copies/mL for *F. vaginae* and/or  $\geq 10^9$  DNA copies/mL for *G. vaginalis* were detected in vaginal samples. This patented strategy has been routinely used as the gold standard diagnostic tool for bacterial vaginosis in our clinical microbiology laboratory for many years.

## 2.3. Statistical Analysis

IBM SPSS Statistics version 23 served as the primary tool for statistical analysis. The Chi-square test was employed to assess the potential associations between bacterial vaginosis and sexually transmitted infections among the study participants. In cases in which the effective sample sizes were low, Fischer's exact test was applied to maintain

statistical accuracy. All analyses were conducted using bilateral  $p$ -values, with statistical significance set at  $p \leq 0.05$  to ensure the robustness and reliability of our findings.

### 3. Results

#### 3.1. Main Patient Characteristics

Among the 290 women, 222 (76.6%) were pregnant and 68 (23.4%) were women of childbearing age with no pregnancies to date. Aged varied from 18 to 49 years old (median age: 28 years old, mean age: 28.7 years old). Most of the women were asymptomatic 243/290 (83.7%).

#### 3.2. Molecular Results

Bacterial vaginosis flora was diagnosed in 50 (17.2%) of the 290 women (Table 1). Forty-five of them (90%) had bacterial loads of at least  $10^8$  and  $10^9$  DNA copies/mL for *F. vaginae* and *G. vaginalis*, respectively. Three (6%) had a bacterial load of at least  $10^8$  DNA copies/mL for *F. vaginae* and two (4%) of at least  $10^9$  DNA copies/mL for *G. vaginalis*. Thirty-six women (72%) of the fifty with bacterial vaginosis flora had no complaints of vaginal discharge. The patients' ages varied between 18 and 48 years old. No age difference was observed between women with and without bacterial vaginosis flora ( $28.9 \pm 6.9$  vs.  $30.2 \pm 9.5$ , respectively;  $p = 0.35$ ).

**Table 1.** Prevalence of bacterial vaginosis flora among 290 women according to their status.

	Number of Positive/Total Number Tested	Reproductive Age without Pregnancy	Pregnancy
Bacterial Vaginosis Flora	50/290 (17.2%)	11/68 (16.2%)	39/222 (17.6%)

A total of 44 (15.2%) of the 290 women were diagnosed with at least one microorganism involved in sexually transmitted infections. Thirty-six of them (81.8%) were asymptomatic. Overall, the prevalence was 6.5% (19/290) for *C. trachomatis*, 1.4% (4/290) for *N. gonorrhoeae*, 7.9% (23/290) for *T. vaginalis*, and 2.4% (7/290) for *M. genitalium* (Table 2).

**Table 2.** Representation of the microorganism's prevalence that are involved in sexually transmitted infections, in normal flora and bacterial vaginosis flora, in pregnant and non-pregnant women.

Microorganisms	Number of Positive Samples (Percentage)			Fisher Test
	Among All Vaginal Specimens Tested	According to Vaginal Flora Type		
	Total of 290 Women	240 Normal Flora	50 Bacterial Vaginosis Flora	$p$ -value
<i>C. trachomatis</i>	19 (6.6%)	7 (2.9%)	12 (24%)	<b>&lt;0.001</b> *
<i>N. gonorrhoeae</i>	4 (1.4%)	1 (0.4%)	3 (6%)	<b>0.01</b> *
<i>M. genitalium</i>	7 (2.4%)	5 (2.1%)	2 (4%)	0.34
<i>T. vaginalis</i>	23 (7.9%)	18 (7.5%)	5 (10%)	0.56
	222 Pregnant Women	183 Normal Flora	39 Bacterial Vaginosis Flora	$p$ -value
<i>C. trachomatis</i>	16 (7.2%)	6 (3.3%)	10 (25.6%)	<b>&lt;0.001</b> *
<i>N. gonorrhoeae</i>	0	0	0	0
<i>M. genitalium</i>	7 (3.2%)	5 (2.7%)	2 (5.1%)	0.35
<i>T. vaginalis</i>	16 (7.2%)	12 (6.6%)	4 (10.3%)	0.49
	68 Non-Pregnant Women	57 Normal Flora	11 Bacterial Vaginosis Flora	$p$ -value
<i>C. trachomatis</i>	3 (4.4%)	1 (1.8%)	2 (18.2%)	0.06
<i>N. gonorrhoeae</i>	4 (5.9%)	1 (1.8%)	3 (27.3%)	<b>0.01</b> *
<i>M. genitalium</i>	0	0	0	0
<i>T. vaginalis</i>	7 (10.3%)	6 (10.5%)	1 (9.1%)	1

\* Significant  $p$ -value in bold ( $p < 0.05$ ).

The majority (35/44 [79.5%]) of patients were infected by only one sexually transmitted infection and only 9/44 (20.5%) were also co-infected by several pathogens.

### 3.3. Bacterial Vaginosis and Sexually Transmitted Microorganisms

Bacterial vaginosis flora was significantly associated with microorganisms involved in sexually transmitted infections (Table 3). Indeed, the prevalence of sexually transmitted infections in women with bacterial vaginosis flora was 36% (18/50) vs. 10.8% (26/240) in women without bacterial vaginosis flora ( $p < 0.001$ ). *C. trachomatis* and *N. gonorrhoeae* were significantly more frequently detected in the 50 women with bacterial vaginosis flora (24% [12 positive]; 6% [3]) than the 240 without (2.9% [7]; 0.4% [1]),  $p < 0.001$ ,  $p = 0.01$ , respectively. Although *T. vaginalis* and *M. genitalium* were more frequently detected in women with bacterial vaginosis flora (10% [5]; 4% [2]) than in those without (7.5% [18]; 2.1% [5]), this was not significant ( $p = 0.34$ ,  $p = 0.56$ , respectively).

**Table 3.** Prevalence of microorganisms involved in sexually transmitted infections, either as monomicrobial or polymicrobial infections, in pregnant women and in non-pregnant women. Data presented for normal flora and bacterial vaginosis flora.

	Number of Positive Samples (Percentage)			p-Value
	222 Pregnant Women	183 Normal Flora	39 Bacterial Vaginosis Flora	
Microorganisms involved in sexually transmitted infections	25 (11.3%)	15 (8.2%)	10 (25.6%)	-
<i>T. vaginalis</i>	10 (4.5%)	9 (4.9%)	1 (2.6%)	-
<i>C. trachomatis</i>	12 (5.4%)	4 (2.2%)	8 (20.5%)	-
<i>N. gonorrhoeae</i>	1 (0.4%)	0	1 (2.6%)	-
<i>M. genitalium</i>	2 (0.9%)	2 (1.1%)	0	-
2 microorganisms involved in sexually transmitted infections	7 (3.1%)	4 (2.2%)	3 (7.7%)	<0.001
<i>T. vaginalis</i> / <i>M. genitalium</i>	3 (1.3%)	2 (1.1%)	1 (2.6%)	-
<i>C. trachomatis</i> / <i>T. vaginalis</i>	3 (1.3%)	1 (0.6%)	2 (5.1%)	-
<i>C. trachomatis</i> / <i>M. genitalium</i>	1 (0.4%)	1 (0.6%)	0	-
<i>C. trachomatis</i> / <i>N. gonorrhoeae</i>	0	0	0	-
Total	32 (14.4%)	19 (10.4%)	13 (33.3%)	<0.001
	68 Non-Pregnant Women	57 Normal Flora	11 Bacterial Vaginosis Flora	p-Value
Microorganisms involved in sexually transmitted infections	10 (14.7%)	6 (10.5%)	4 (36.4%)	-
<i>T. vaginalis</i>	6 (8.8%)	6 (10.5%)	0	-
<i>C. trachomatis</i>	1 (1.5%)	0	1 (9.1%)	-
<i>N. gonorrhoeae</i>	3 (4.4%)	0	3 (27.3%)	-
<i>M. genitalium</i>	0	0	0	-
2 microorganisms involved in sexually transmitted infections	2 (2.9%)	1 (1.7%)	1 (9.1%)	<0.001
<i>T. vaginalis</i> / <i>M. genitalium</i>	0	0	0	-
<i>C. trachomatis</i> / <i>T. vaginalis</i>	1 (1.5%)	0	1 (9.1%)	-
<i>C. trachomatis</i> / <i>M. genitalium</i>	0	0	0	-
<i>C. trachomatis</i> / <i>N. gonorrhoeae</i>	1 (1.5%)	1 (1.7%)	0	-
Total	12 (17.6%)	7 (12.2%)	5 (45.5%)	<0.001

The distribution of co-infections due to sexually transmitted infection-causing microorganisms was also substantially related to the state of the vaginal flora. In women without bacterial vaginosis flora, the prevalence of mono-infections with sexually trans-

mitted infection-causing microorganisms was 8.8% (21/240) compared to 28% (14/50) in women with bacterial vaginosis flora ( $p < 0.001$ ). Meanwhile, the prevalence of polyinfections was 2% (5/240) in women without bacterial vaginosis flora versus 8% (4/50) in women with bacterial vaginosis flora ( $p = 0.05$ ) (Table 3).

#### 4. Discussion

All our data are based on rational molecular methods to evaluate the prevalence of bacterial vaginosis, as well as to study the association between this dysbiosis with four microorganisms involved in sexually transmitted infections. Until now, studies on the association between bacterial vaginosis and sexually transmitted infections have traditionally used the Nugent score or the Amsel criteria for the diagnosis of bacterial vaginosis flora [17,23,24].

However, a certain number of shortcomings of these strategies (subjective interpretation influenced by individual skills and ambiguity in the classification of the intermediate flora, etc.) led to the conclusion that these strategies were outdated [25,26]. Molecular biology is therefore increasingly practiced for the diagnosis of bacterial vaginosis flora because it is objective and rational while being able to be carried out in point-of-care laboratories [25,26].

In our series, the prevalence of bacterial vaginosis and sexually transmitted infections was 17.2% and 15.2%, respectively. The prevalence of bacterial vaginosis in our study in France is similar to that which has been observed in Western Europe, where it is generally below 20% [12]. For sexually transmitted infections, available data indicate that they are again on the rise in many EU countries and have been since the mid-1990s [27,28]. Compared with 2015, reported cases of sexually transmitted infections rose significantly in 2019. Data show an increase of 9% for *Chlamydia* infections, 55% for gonorrhoea, 25% for syphilis and 75% for lymphogranuloma venereum [28]. None of our patients had genital ulcerations, and although we looked for *H. ducreyi*, *T. pallidum* and HSV out of curiosity, as a matter of routine, no cases were detected. This is consistent with current data showing that syndromic management has reduced the prevalence of sexually transmitted microorganisms associated with genital ulcers [29]. It should be emphasised that most women diagnosed with bacterial vaginosis flora and sexually transmitted infections in our series were asymptomatic (72% and 81.5%, respectively), including those with associated polyinfections (Table 4). Moreover, the extent of clinical manifestations does not appear to be directly proportional to the number of pathogens present.

This highlights two main points:

- (1) The difficulties in preventing the circulation of microorganisms implicated in sexually transmitted infections not associated with genital ulcers [29]. Indeed, sexually transmitted infections not associated with genital ulcers present a major challenge in terms of prevention. Unlike sexually transmitted infections with visible symptoms such as genital ulcers, asymptomatic or minimally symptomatic sexually transmitted infections can spread silently, making them difficult to detect and control. This raises questions on the strategies prevention existing and suggests the necessity prevention approaches more targeted and innovative, as well as an increased awareness to encourage regular screening, in particular in high-risk populations.
- (2) The difficulty of understanding the impact of bacterial vaginosis in obstetric complications such as prematurity [30–32]. The relationship between bacterial vaginosis and obstetric complications, particularly prematurity, remains complex and incompletely understood. Bacterial vaginosis can lead to imbalances in vaginal microflora, but how this contributes to adverse obstetric outcomes requires further research. Understanding these mechanisms is essential for developing effective preventive interventions and care protocols for pregnant women with bacterial vaginosis, with the aim of reducing the risk of prematurity and other associated complications.

**Table 4.** Distribution of asymptomatic women according to vaginal flora types and microorganisms involved in sexually transmitted infections.

	Number of Asymptomatic Women According to Vaginal Flora Type (Percentage)		
	Normal Flora	Bacterial Vaginosis Flora	Total
	207/240 (86.2%)	36/50 (72%)	243/290 (83.8%)
Free of microorganisms involved in sexually transmitted infections	182/214 (75.5%)	25/32 (78.1%)	153/246 (62.1%)
Monomicrobial infections	20/21 (95.2%)	9/14 (81.8%)	29/35 (82.8%)
<i>T. vaginalis</i>	15/15	0	15/16
<i>C. trachomatis</i>	3/4	7/9	10/13
<i>N. gonorrhoeae</i>	0	1/3	1/3
<i>M. genitalium</i>	2/2	1/1	3/3
Polymicrobial infections with 2 microorganisms	5/5 (100%)	2/4 (50%)	7/9 (77.8%)
<i>C. trachomatis/T. vaginalis</i>	1/1	2/3	3/4
<i>C. trachomatis/M. genitalium</i>	1/1	0	1/1
<i>C. trachomatis/N. gonorrhoeae</i>	1/1	0	1/1
<i>T. vaginalis/M. genitalium</i>	2/2	0	2/3

Previous studies generally acknowledge that there is an increased prevalence of sexually transmitted infection-causing microorganisms in women with bacterial vaginosis [33–40]. Our data show that women with bacterial vaginosis were 1.79 times more likely to be positive for sexually transmitted infections. Overall, *C. trachomatis* and *N. gonorrhoeae* have a significantly higher prevalence in women with bacterial vaginosis than in those without bacterial vaginosis, while *T. vaginalis* and *M. genitalium* were positively but not significantly correlated with bacterial vaginosis. In their study of 95 sexually transmitted infection-positive and 91 sexually transmitted infection-negative samples, Shipitsyna et al. showed a significant age-independent association between bacterial vaginosis-associated vaginal microbiota and the presence of *C. trachomatis*, *M. genitalium*, and *T. vaginalis* [33]. In 2021, in a systematic review of 14 articles and a meta-analysis, Seña et al. demonstrated that the risk of *T. vaginalis* is almost twice as high in women with bacterial vaginosis than in women without bacterial vaginosis; however, studies note heterogeneity and potential confounding factors (age, sexual partners, etc.) [39]. Nye et al. showed that the prevalence of *M. genitalium* infection in patients with bacterial vaginosis was significantly higher than in those without bacterial vaginosis (7.0% [20/307] vs. 3.6% [42/1225]) [38].

A total of 3.2% of the women exhibited multiple infections with sexually transmitted infection-causing microorganisms. Among sexually transmitted infections, the co-infection with *C. trachomatis* was the most frequent. In women with bacterial vaginosis, co-infections have a much higher prevalence than in women without. Sexually transmitted infection-causing microorganisms commonly circulate. A much higher frequency is also observed in women with bacterial vaginosis. Although most studies suggest bacterial vaginosis as a risk factor for sexually transmitted infections, linked to local inflammation, the question that arises is whether bacterial vaginosis is a cause, a consequence, or constitutes a sexually transmitted infection in its own right. Although the lack of a known causative agent makes it difficult to classify bacterial vaginosis as a sexually transmitted infection, it is strongly linked to sexual behaviours and exhibits some of the hallmarks of a sexually transmitted infection [40].

As early as 1955, when Gardner and Dukes conducted their first exhaustive study of bacterial vaginosis, they made some intriguing findings. Indeed, they reported the possibility of women with normal vaginal flora being infected with material from the vaginas of patients with bacterial vaginosis. This observation raised vital questions about



the transmission of this condition and its potential link with sexual behaviour. In addition, Gardner and Dukes also highlighted the protective effect of condom use, suggesting that sexual practices may play a key role in the transmission of bacterial vaginosis. Another striking feature of their findings was the development of bacterial vaginosis in patients shortly after marriage, whereas their vaginal flora was normal at the time of the premarital examination [41]. Since then, most studies focusing on the risk factors for bacterial vaginosis have converged on an intriguing conclusion: the risk profile of this condition seems to strikingly mirror that of sexually transmitted infections, further underlining the complex links between bacterial vaginosis and female reproductive health. These findings are summarized in Supplementary Table S2 [7,40–78], underlining the continuing need for in-depth research to elucidate the nature of this complex association.

One of the limitations of our study is the lack of data on participants' behavioural characteristics, including their sexual practices, to analyse their potential influence. In addition, no data about partners are available, nor are follow-up data available. There is, therefore, only one time point for evaluation. In conclusion, there is a significant correlation between bacterial vaginosis flora and sexually transmitted infections. The majority of women remain asymptomatic, including those with multiple infections. Multiple sexually transmitted infections are significantly more frequent in patients with bacterial vaginosis flora than in those with normal vaginal flora. This fact sheds light on the presentation of bacterial vaginosis as a predisposing factor for sexually transmitted infections and one that is linked with inflammation may be too mechanistic. This study found the association of bacterial vaginosis with isolated monoinfections or multiple infections, aligning bacterial vaginosis with transmitted infectious diseases.

Although no specific bacteria can be directly associated with bacterial vaginosis, and bacterial vaginosis itself cannot be categorically classified as a sexually transmitted infection, there is a clear link between this condition and sexual intercourse. At present, our understanding of the mechanisms underlying this complex relationship remains limited. It is therefore imperative to pursue in-depth research to unravel this enigma and clarify the precise links between bacterial vaginosis and sexually transmitted infections. This increased knowledge is essential to guide prevention and treatment strategies, and to improve women's sexual health.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/microorganisms11102470/s1>. Supplementary Table S1. Table of primers and probes used to detect microorganisms involved in bacterial vaginosis and sexually transmitted infections. Supplementary Table S2. Review of the international literature presenting various arguments in favour or against sexual transmission of bacterial vaginosis.

**Author Contributions:** Conceptualization and methodology, F.F., O.M. and F.B.; acquisition of data, L.A.C., C.L., A.H. and R.I.; data analysis; L.A.C., C.L., A.H., R.I., O.M. and F.F.; writing—original draft preparation, L.A.C. and C.L.; writing—review and editing, F.F., O.M. and F.B. All authors have read and agreed to the published version of the manuscript.

**Funding:** This study was supported by the Institut Hospitalo-Universitaire (IHU) Méditerranée Infection (Marseille, France) and the French National Research Agency under the “Investissements d'avenir” programme, reference ANR-10-IAHU-03.

**Institutional Review Board Statement:** The overall study was carried out in accordance with the Declaration of Helsinki. The study was also approved by our independent ethics committee (IEC No. 2022-033).

**Data Availability Statement:** All data is available on reasonable request from the corresponding author.

**Acknowledgments:** We are very grateful to the Fondation Infectiopole Sud (Marseille, France) for the thesis grant awarded to Linda Abou Chacra.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Abou Chacra, L.; Fenollar, F. Exploring the global vaginal microbiome and its impact on human health. *Microb. Pathog.* **2021**, *160*, 105172. [[CrossRef](#)]
2. Abou Chacra, L.; Fenollar, F.; Diop, K. Bacterial Vaginosis: What Do We Currently Know? *Front. Cell. Infect. Microbiol.* **2022**, *11*, 1393. [[CrossRef](#)]
3. Lewis, F.M.T.; Bernstein, K.T.; Aral, S.O. Vaginal microbiome and its relationship to behavior, sexual health, and sexually transmitted diseases. *Obstet. Gynecol.* **2017**, *129*, 643. [[CrossRef](#)] [[PubMed](#)]
4. Aslan, E.; Bechelaghem, N. To ‘douche’ or not to ‘douche’: Hygiene habits may have detrimental effects on vaginal microbiota. *J. Obstet. Gynaecol.* **2018**, *38*, 678–681. [[CrossRef](#)] [[PubMed](#)]
5. Achilles, S.L.; Austin, M.N.; Meyn, L.A.; Mhlanga, F.; Chirenje, Z.M.; Hillier, S.L. Impact of contraceptive initiation on vaginal microbiota. *Am. J. Obstet. Gynecol.* **2018**, *218*, 622.e1–622.e10. [[CrossRef](#)] [[PubMed](#)]
6. Schwabke, J.R.; Richey, C.M.; Weiss, H.L. Correlation of behaviors with microbiological changes in vaginal flora. *J. Infect. Dis.* **1999**, *180*, 1632–1636. [[CrossRef](#)]
7. Bilardi, J.; Walker, S.; McNair, R.; Mooney-Somers, J.; Temple-Smith, M.; Bellhouse, C.; Fairley, C.; Chen, M.; Bradshaw, C. Women’s management of recurrent bacterial vaginosis and experiences of clinical care: A qualitative study. *PLoS ONE* **2016**, *11*, e0151794. [[CrossRef](#)]
8. Peebles, K.; Velloza, J.; Balkus, J.E.; McClelland, R.S.; Barnabas, R.V. High global burden and costs of bacterial vaginosis: A systematic review and meta-analysis. *Sex. Transm. Dis.* **2019**, *46*, 304–311. [[CrossRef](#)]
9. Bradshaw, C.S.; Brotman, R.M. Making inroads into improving treatment of bacterial vaginosis—Striving for long-term cure. *BMC Infect. Dis.* **2015**, *15*, 292. [[CrossRef](#)]
10. Klebanoff, M.A.; Schwabke, J.R.; Zhang, J.; Nansel, T.R.; Yu, K.F.; Andrews, W.W. Vulvovaginal symptoms in women with bacterial vaginosis. *Obstet. Gynecol.* **2004**, *104*, 267–272. [[CrossRef](#)]
11. Chico, R.M.; Mayaud, P.; Ariti, C.; Mabey, D.; Ronsmans, C.; Chandramohan, D. Prevalence of malaria and sexually transmitted and reproductive tract infections in pregnancy in sub-Saharan Africa: A systematic review. *JAMA* **2012**, *307*, 2079–2086. [[CrossRef](#)]
12. Kenyon, C.; Colebunders, R.; Crucitti, T. The global epidemiology of bacterial vaginosis: A systematic review. *Am. J. Obstet. Gynecol.* **2013**, *209*, 505–523. [[CrossRef](#)]
13. Lakshmi, K.; Chitrakha, S.A., Jr.; Menezes, G.A. Review on infectious vaginitis. *Res. J. Pharm. Biol. Chem. Sci.* **2013**, *4*, 679–688.
14. Eisinger, R.W.; Erbeling, E. Fauci AS Refocusing research on sexually transmitted infections. *J. Infect. Dis.* **2020**, *222*, 1432–1434. [[CrossRef](#)] [[PubMed](#)]
15. Workowski, K.A.; Bachmann, L.H. Centers for Disease Control and Prevention’s Sexually Transmitted Diseases Infection Guidelines. *Clin. Infect. Dis.* **2022**, *74*, S89–S94. [[CrossRef](#)] [[PubMed](#)]
16. Torrone, E.A.; Morrison, C.S.; Chen, P.-L.; Kwok, C.; Francis, S.C.; Hayes, R.J.; Looker, K.J.; McCormack, S.; McGrath, N.; van de Wijgert, J.H.; et al. Prevalence of sexually transmitted infections and bacterial vaginosis among women in sub-Saharan Africa: An individual participant data meta-analysis of 18 HIV prevention studies. *PLoS Med.* **2018**, *15*, e1002511. [[CrossRef](#)]
17. Gallo, M.F.; Macaluso, M.; Warner, L.; Fleenor, M.E.; Hook, E.W., III; Brill, I.; Weaver, M.A. Bacterial vaginosis, gonorrhea, and chlamydial infection among women attending a sexually transmitted disease clinic: A longitudinal analysis of possible causal links. *Ann. Epidemiol.* **2012**, *22*, 213–220. [[CrossRef](#)]
18. Wiesenfeld, H.C.; Hillier, S.L.; Krohn, M.A.; Landers, D.V.; Sweet, R.L. Bacterial vaginosis is a strong predictor of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infection. *Clin. Infect. Dis.* **2003**, *36*, 663–668. [[CrossRef](#)] [[PubMed](#)]
19. Brotman, R.M.; Shardell, M.D.; Gajer, P.; Tracy, J.K.; Zenilman, J.M.; Ravel, J.; Gravitt, P.E. Interplay between the temporal dynamics of the vaginal microbiota and human papillomavirus detection. *J. Infect. Dis.* **2014**, *210*, 1723–1733. [[CrossRef](#)]
20. Korschuh, S.; Jayaprakash, T.; Dolatabadi, A.; Dayo, E.; Ramay, H.; Sycuro, L. O02. 3 Reclassification of *Atopobium vaginae* as three novel *Fannyhessea* species: Implications for understanding their role in bacterial vaginosis. *Sex. Transm. Infect.* **2021**, *7* (Suppl. 1), A1–A186.
21. Menard, J.P.; Fenollar, F.; Henry, M.; Bretelle, F.; Raoult, D. Molecular quantification of *Gardnerella vaginalis* and *Atopobium vaginae* loads to predict bacterial vaginosis. *Clin. Infect. Dis.* **2008**, *47*, 33–43. [[CrossRef](#)] [[PubMed](#)]
22. Menard, J.-P.; Mazouni, C.; Fenollar, F.; Raoult, D.; Boubli, L.; Bretelle, F. Diagnostic accuracy of quantitative real-time PCR assay versus clinical and Gram stain identification of bacterial vaginosis. *Eur. J. Clin. Microbiol. Infect. Dis.* **2010**, *29*, 1547–1552. [[CrossRef](#)] [[PubMed](#)]
23. Zhang, Z.; Zong, X.; Bai, H.; Fan, L.; Li, T.; Liu, Z. Prevalence of *Mycoplasma genitalium* and *Chlamydia trachomatis* in Chinese female with lower reproductive tract infection: A multicenter epidemiological survey. *BMC Infect. Dis.* **2023**, *23*, 2. [[CrossRef](#)] [[PubMed](#)]
24. Qi, J.; Dai, C.; Zhang, J. Association Between Bacterial Vaginosis with HPV in US (NHANES 2003–2004). *Res. Sq.* **2023**. [[CrossRef](#)]
25. Coleman, J.S.; Gaydos, C.A. Molecular diagnosis of bacterial vaginosis: An update. *J. Clin. Microbiol.* **2018**, *56*, 10–1128. [[CrossRef](#)]
26. Muzny, C.A.; Cerca, N.; Elnaggar, J.H.; Taylor, C.M.; Sobel, J.D.; Van Der Pol, B. State of the art for diagnosis of bacterial vaginosis. *J. Clin. Microbiol.* **2023**, *61*, e00837–22. [[CrossRef](#)]
27. Fenton, K.A.; Lowndes, C.M. Recent trends in the epidemiology of sexually transmitted infections in the European Union. *Sex. Transm. Infect.* **2004**, *80*, 255–263. [[CrossRef](#)]

28. Geretti, A.M.; Mardh, O.; de Vries, H.J.C.; Winter, A.; McSorley, J.; Seguy, N.; Vuylsteke, B.; Gokengin, D. Sexual transmission of infections across Europe: Appraising the present, scoping the future. *Sex. Transm. Infect.* **2022**, *98*, 451–457. [[CrossRef](#)]
29. Loh, A.J.W.; Ting, E.L.; Wi, T.E.; Mayaud, P.; Chow, E.P.; Santesso, N.; Falconer, J.; Ofori-Asenso, R.; Ong, J.J. The diagnostic accuracy of syndromic management for genital ulcer disease: A systematic review and meta-analysis. *Front. Med.* **2022**, *8*, 2859. [[CrossRef](#)]
30. Gravett, M.G.; Nelson, H.P.; Derouen, T.; Critchlow, C.; Eschenbach, D.A.; Holmes, K.K. Independent associations of bacterial vaginosis and *Chlamydia trachomatis* infection with adverse pregnancy outcome. *JAMA* **1986**, *256*, 1899–1903. [[CrossRef](#)]
31. Capoccia, R.; Greub, G. Baud D *Ureaplasma urealyticum*, *Mycoplasma hominis* and adverse pregnancy outcomes. *Curr. Opin. Infect. Dis.* **2013**, *26*, 231–240. [[CrossRef](#)] [[PubMed](#)]
32. Fettweis, J.M.; Serrano, M.G.; Brooks, J.P.; Edwards, D.J.; Girerd, P.H.; Parikh, H.I.; Huang, B.; Arodz, T.J.; Edupuganti, L.; Glascock, A.L.; et al. The vaginal microbiome and preterm birth. *Nat. Med.* **2019**, *25*, 1012–1021. [[CrossRef](#)]
33. Shipitsyna, E.; Khusnutdinova, T.; Budilovskaya, O.; Krysanova, A.; Shalepo, K.; Savicheva, A.; Unemo, M. Bacterial vaginosis-associated vaginal microbiota is an age-independent risk factor for *Chlamydia trachomatis*, *Mycoplasma genitalium* and *Trichomonas vaginalis* infections in low-risk women, St. Petersburg, Russia. *Eur. J. Clin. Microbiol. Infect. Dis.* **2020**, *39*, 1221–1230. [[CrossRef](#)] [[PubMed](#)]
34. Bautista, C.T.; Wurapa, E.K.; Sanchez, J.L. Brief report: Associations between antecedent bacterial vaginosis and incident chlamydia and gonorrhea diagnoses, US Army females, 2006–2012. *MSMR* **2016**, *23*, 32–34.
35. Dahoud, W.; Michael, C.W.; Gokozan, H.; Nakanishi, A.K.; Harbhajanka, A. Association of bacterial vaginosis and human papilloma virus infection with cervical squamous intraepithelial lesions. *Am. J. Clin. Pathol.* **2019**, *152*, 185–189. [[CrossRef](#)] [[PubMed](#)]
36. Nava-Memije, K.; Hernández-Cortez, C.; Ruiz-González, V.; Saldaña-Juárez, C.A.; Medina-Islas, Y.; Dueñas-Domínguez, R.A.; Aguilera-Arreola, M.G. Bacterial vaginosis and sexually transmitted infections in an HIV-Positive Cohort. *Front. Reprod. Health* **2021**, *3*, 60672. [[CrossRef](#)]
37. Lokken, E.M.; Balkus, J.E.; Kiarie, J.; Hughes, J.P.; Jaoko, W.; Totten, P.A.; McClelland, R.S.; Manhart, L.E. Association of recent bacterial vaginosis with acquisition of *Mycoplasma genitalium*. *Am. J. Epidemiol.* **2017**, *186*, 194–201. [[CrossRef](#)]
38. Nye, M.B.; Harris, A.B.; Pherson, A.J.; Cartwright, C. P Prevalence of *Mycoplasma genitalium* infection in women with bacterial vaginosis. *BMC Womens Health* **2020**, *20*, 62. [[CrossRef](#)]
39. Seña, A.C.; Goldstein, L.A.; Ramirez, G.; Parish, A.J.; McClelland, R.S. Bacterial vaginosis and its association with incident trichomonas vaginalis infections: A systematic review and meta-analysis. *Sex. Transm. Dis.* **2021**, *48*, e192. [[CrossRef](#)]
40. Morris, M.C.; Rogers, P.A.; Kinghorn, G.R. Is bacterial vaginosis a sexually transmitted infection? *Sex. Transm. Infect.* **2001**, *77*, 63–68. [[CrossRef](#)]
41. Gardner, H.L.; Dukes, C.D. Haemophilus Vaginalis Vaginitis: A Newly Defined Specific Infection Previously Classified “Nonspecific” Vaginitis. *Am. J. Obs. Gynecol.* **1955**, *69*, 962–976. [[CrossRef](#)]
42. Mehta, S.D.; Agingu, W.; Nordgren, R.K.; Green, S.J.; Bhaumik, D.K.; Bailey, R.C.; Otieno, F. Characteristics of Women and Their Male Sex Partners Predict Bacterial Vaginosis among a Prospective Cohort of Kenyan Women with Nonoptimal Vaginal Microbiota. *Sex. Transm. Dis.* **2020**, *47*, 840. [[CrossRef](#)]
43. Holst, E. Reservoir of Four Organisms Associated with Bacterial Vaginosis Suggests Lack of Sexual Transmission. *J. Clin. Microbiol.* **1990**, *28*, 2035–2039. [[CrossRef](#)] [[PubMed](#)]
44. Holst, E.; Mårdh, P.A.; Thelin, I. Recovery of Anaerobic Curved Rods and Gardnerella Vaginalis from the Urethra of Men, Including Male Heterosexual Consorts of Female Carriers. *Scand. J. Urol. Nephrol. Suppl.* **1984**, *86*, 173–177. [[PubMed](#)]
45. Mngomezulu, K.; Mzobe, G.F.; Mtshali, A.; Osman, F.; Liebenberg, L.J.P.; Garrett, N.; Singh, R.; Rompalo, A.; Mindel, A.; Karim, S.S.A. Recent Semen Exposure Impacts the Cytokine Response and Bacterial Vaginosis in Women. *Front. Immunol.* **2021**, *12*, 695201. [[CrossRef](#)]
46. Mehta, S.D.; Zhao, D.; Green, S.J.; Agingu, W.; Otieno, F.; Bhaumik, R.; Bhaumik, D.; Bailey, R.C. The Microbiome Composition of a Man’s Penis Predicts Incident Bacterial Vaginosis in His Female Sex Partner with High Accuracy. *Front. Cell Infect. Microbiol.* **2020**, *10*, 433. [[CrossRef](#)]
47. Nayyar, C.; Chander, R.; Gupta, P.; Sherwal, B.L. Co-Infection of Human Immunodeficiency Virus and Sexually Transmitted Infections in Circumcised and Uncircumcised Cases in India. *Indian. J. Sex. Transm. Dis. AIDS* **2014**, *35*, 114.
48. Price, L.B.; Liu, C.M.; Johnson, K.E.; Aziz, M.; Lau, M.K.; Bowers, J.; Ravel, J.; Keim, P.S.; Serwadda, D.; Wawer, M.J. The Effects of Circumcision on the Penis Microbiome. *PLoS ONE* **2010**, *5*, e8422. [[CrossRef](#)]
49. Gray, R.H.; Kigozi, G.; Serwadda, D.; Makumbi, F.; Nalugoda, F.; Watya, S.; Moulton, L.; Chen, M.Z.; Sewankambo, N.K.; Kiwanuka, N. The Effects of Male Circumcision on Female Partners’ Genital Tract Symptoms and Vaginal Infections in a Randomized Trial in Rakai, Uganda. *Am. J. Obs. Gynecol.* **2009**, *200*, 42–e1. [[CrossRef](#)]
50. Zenilman, J.M.; Fresia, A.; Berger, B.; McCormack, W.M. Bacterial Vaginosis Is Not Associated with Circumcision Status of the Current Male Partner. *Sex. Transm. Infect.* **1999**, *75*, 347–348. [[CrossRef](#)]
51. Bradshaw, C.S.; Vodstrcil, L.A.; Hocking, J.S.; Law, M.; Pirota, M.; Garland, S.M.; De Guingand, D.; Morton, A.N.; Fairley, C.K. Recurrence of Bacterial Vaginosis Is Significantly Associated with Posttreatment Sexual Activities and Hormonal Contraceptive Use. *Clin. Infect. Dis.* **2013**, *56*, 777–786. [[CrossRef](#)] [[PubMed](#)]

52. Gallo, M.F.; Warner, L.; King, C.C.; Sobel, J.D.; Klein, R.S.; Cu-Uvin, S.; Rompalo, A.M.; Jamieson, D.J. Association between Semen Exposure and Incident Bacterial Vaginosis. *Infect. Dis. Obs. Gynecol.* **2011**, *2011*, 842652. [[CrossRef](#)] [[PubMed](#)]
53. Yotebieng, M.; Turner, A.N.; Hoke, T.H.; Van Damme, K.; Rasolofomanana, J.R.; Behets, F. Effect of Consistent Condom Use on 6-month Prevalence of Bacterial Vaginosis Varies by Baseline BV Status. *Trop. Med. Int. Health* **2009**, *14*, 480–486. [[CrossRef](#)] [[PubMed](#)]
54. Hutchinson, K.B.; Kip, K.E.; Ness, R.B. Condom Use and Its Association with Bacterial Vaginosis and Bacterial Vaginosis-Associated Vaginal Microflora. *Epidemiology* **2007**, *18*, 702–708. [[CrossRef](#)]
55. Lendamba, R.W.; Onanga, R.; Bigoumba, M.; Mouanga Ndzime, Y.; Mbehang Nguema, P.P.; Kassa-Kassa, R.F. Bacterial Vaginosis: Prevalence in Sexually Active Women Living in the City of Franceville (Gabon) and Its Surroundings. *J. Womens Health Care* **2020**, *10*, 501–509.
56. Schwebke, J.R.; Desmond, R. Risk Factors for Bacterial Vaginosis in Women at High Risk for Sexually Transmitted Diseases. *Sex. Transm. Dis.* **2005**, *32*, 654–658. [[CrossRef](#)]
57. Larsson, P.G.; Platz-Christensen, J.J.; Sundström, E. Is Bacterial Vaginosis a Sexually Transmitted Disease? *Int. J. STD AIDS* **1991**, *2*, 362–364. [[CrossRef](#)]
58. Toh, E.; Xing, Y.; Gao, X.; Jordan, S.J.; Batteiger, T.A.; Batteiger, B.E.; Van Der Pol, B.; Muzny, C.A.; Gebregziabher, N.; Williams, J.A. Sexual Behavior Shapes the Adult Male Genitourinary Microbiome. *Cell Rep. Med.* **2023**, *4*, 100981. [[CrossRef](#)]
59. Brotman, R.M.; Ravel, J.; Cone, R.A.; Zenilman, J.M. Rapid Fluctuation of the Vaginal Microbiota Measured by Gram Stain Analysis. *Sex. Transm. Infect.* **2010**, *86*, 297–302. [[CrossRef](#)]
60. Muzny, C.A.; Van Der Pol, W.J.; Lefkowitz, E.J.; Ghosh, A.; Li, M.; Redden, D.; Cui, X.; Schwebke, J. 2408 Genital Microbiomes of Women with Recurrent Bacterial Vaginosis and Their Regular Male Sexual Partner. *J Clin Transl Sci* **2018**, *2*, 13. [[CrossRef](#)]
61. Berger, B.J.; Kolton, S.; Zenilman, J.M.; Cummings, M.C.; Feldman, J.; McCormack, W.M. Bacterial Vaginosis in Lesbians: A Sexually Transmitted Disease. *Clin. Infect. Dis.* **1995**, *21*, 1402–1405. [[CrossRef](#)] [[PubMed](#)]
62. Muzny, C.A.; Lensing, S.Y.; Aaron, K.J.; Schwebke, J.R. Incubation Period and Risk Factors Support Sexual Transmission of Bacterial Vaginosis in Women Who Have Sex with Women. *Sex. Transm. Infect.* **2019**, *95*, 511–515. [[CrossRef](#)] [[PubMed](#)]
63. Plummer, E.L.; Vodstrcil, L.A.; Fairley, C.K.; Tabrizi, S.N.; Garland, S.M.; Law, M.G.; Hocking, J.S.; Fethers, K.A.; Bulach, D.M.; Murray, G.L. Sexual Practices Have a Significant Impact on the Vaginal Microbiota of Women Who Have Sex with Women. *Sci. Rep.* **2019**, *9*, 19749. [[CrossRef](#)] [[PubMed](#)]
64. Vodstrcil, L.A.; Walker, S.M.; Hocking, J.S.; Law, M.; Forcey, D.S.; Fehler, G.; Bilardi, J.E.; Chen, M.Y.; Fethers, K.A.; Fairley, C.K. Incident Bacterial Vaginosis (BV) in Women Who Have Sex with Women Is Associated with Behaviors That Suggest Sexual Transmission of BV. *Clin. Infect. Dis.* **2015**, *60*, 1042–1053. [[CrossRef](#)] [[PubMed](#)]
65. Marrazzo, J.M.; Koutsky, L.A.; Eschenbach, D.A.; Agnew, K.; Stine, K.; Hillier, S.L. Characterization of Vaginal Flora and Bacterial Vaginosis in Women Who Have Sex with Women. *J. Infect. Dis.* **2002**, *185*, 1307–1313. [[CrossRef](#)]
66. Bump, R.C.; Buesching III, W.J. Bacterial Vaginosis in Virginal and Sexually Active Adolescent Females: Evidence against Exclusive Sexual Transmission. *Am. J. Obs. Gynecol.* **1988**, *158*, 935–939. [[CrossRef](#)]
67. Vaca, M.; Guadalupe, I.; Erazo, S.; Tinizaray, K.; Chico, M.E.; Cooper, P.J.; Hay, P. High Prevalence of Bacterial Vaginosis in Adolescent Girls in a Tropical Area of Ecuador. *BJOG* **2010**, *117*, 225–228. [[CrossRef](#)]
68. McCaffrey, M.; Varney, P.; Evans, B.; Taylor-Robinson, D. Bacterial Vaginosis in Lesbians: Evidence for Lack of Sexual Transmission. *Int. J. STD AIDS* **1999**, *10*, 305–308. [[CrossRef](#)]
69. Vodstrcil, L.A.; Plummer, E.L.; Doyle, M.; Fairley, C.K.; McGuinness, C.; Bateson, D.; Hocking, J.S.; Law, M.G.; Petoumenos, K.; Donovan, B. Treating Male Partners of Women with Bacterial Vaginosis (StepUp): A Protocol for a Randomised Controlled Trial to Assess the Clinical Effectiveness of Male Partner Treatment for Reducing the Risk of BV Recurrence. *BMC Infect. Dis.* **2020**, *20*, 1–12. [[CrossRef](#)]
70. Vejtorp, M.; Bollerup, A.C.; Vejtorp, L.; Fanoë, E.; Nathan, E.V.A.; Reiter, A.; Andersen, M.E.; Stromsholt, B.; Schroder, S.S. Bacterial Vaginosis: A Double-blind Randomized Trial of the Effect of Treatment of the Sexual Partner. *BJOG* **1988**, *95*, 920–926. [[CrossRef](#)]
71. Schwebke, J.R.; Lensing, S.Y.; Lee, J.; Muzny, C.A.; Pontius, A.; Woznicki, N.; Aguin, T.; Sobel, J.D. Treatment of Male Sexual Partners of Women With Bacterial Vaginosis: A Randomized, Double-Blind, Placebo-Controlled Trial. *Clin. Infect. Dis.* **2021**, *73*, e672–e679. [[CrossRef](#)]
72. Moi, H.; Erkkola, R.; Jerve, F.; Nelleman, G.; Bymose, B.; Alaksen, K.; Tornqvist, E. Should Male Consorts of Women with Bacterial Vaginosis Be Treated? *Sex. Transm. Infect.* **1989**, *65*, 263–268. [[CrossRef](#)] [[PubMed](#)]
73. Plummer, E.L.; Vodstrcil, L.A.; Danielewski, J.A.; Murray, G.L.; Fairley, C.K.; Garland, S.M.; Hocking, J.S.; Tabrizi, S.N.; Bradshaw, C.S. Combined Oral and Topical Antimicrobial Therapy for Male Partners of Women with Bacterial Vaginosis: Acceptability, Tolerability and Impact on the Genital Microbiota of Couples—a Pilot Study. *PLoS ONE* **2018**, *13*, e0190199. [[CrossRef](#)] [[PubMed](#)]
74. Vutyavanich, T.; Pongsuthirak, P.; Vannareumol, P.; Ruangsri, R.-A.; Luangsook, P. A Randomized Double-Blind Trial of Tinidazole Treatment of the Sexual Partners of Females with Bacterial Vaginosis. *Obstet. Gynecol.* **1993**, *82*, 550–554.
75. Plummer, E.L.; Vodstrcil, L.A.; Doyle, M.; Danielewski, J.A.; Murray, G.L.; Fehler, G.; Fairley, C.K.; Bulach, D.M.; Garland, S.M.; Chow, E.P.F. A Prospective, Open-Label Pilot Study of Concurrent Male Partner Treatment for Bacterial Vaginosis. *mBio* **2021**, *12*, e02323-21. [[CrossRef](#)]

76. Colli, E.; Landoni, M.; Parazzini, F. Treatment of Male Partners and Recurrence of Bacterial Vaginosis: A Randomised Trial. *Sex. Transm. Infect.* **1997**, *73*, 267–270. [[CrossRef](#)] [[PubMed](#)]
77. Plummer, E.; Vodstrcil, L.A.; Fairley, C.K.; Danielewski, J.A.; Murray, G.L.; Doyle, M.; Fehler, G.; McGuinness, C.; Bulach, D.M.; Garland, S.M. The Impact of Partner Treatment for Bacterial Vaginosis on the Genital Microbiota of Heterosexual Couples. *Sex. Health* **2020**, *17*, xiv.
78. Mengel, M.B.; Berg, A.O.; Weaver, C.H.; Herman, D.J.; Herman, S.J.; Hughes, V.L.; Koepsell, T.D. The Effectiveness of Single-Dose Metronidazole Therapy for Patients and Their Partners with Bacterial Vaginosis. *J. Fam. Pract.* **1989**, *28*, 163–171.

**Disclaimer/Publisher’s Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.