Available online at www.elixirpublishers.com (Elixir International Journal)

Biosciences

Elixir Biosciences 128 (2019) 52852-52856

ISSN: 2229-712X

Biofilm forming Klebsiella among Chlamydia trachomatis positive pregnant women with special reference to Azeezia Medical College Hospital, Kollam, Kerala-South India

Neena A[1], Udhaya V[2,*] and Prasobh K.K.[3]

[2]Department of Microbiology, Rajah Muthiah Medical College, Annamalai University, Chidambaram, Tamil Nadu, India.

[1,3]Department of Microbiology, Azeezia medical college, Kollam, Kerala, India.

ARTICLE INFO

Article history:

Received: 8 February 2019;

Received in revised form:

8 March 2019;

Accepted: 18 March 2019;

Keywords

Biofilm,

Klebsiella,

C.trachomatis,

MDR,

BV.

ABSTRACT

Bacterial biofilms present everywhere and creating problem in different fields including medical field. Biofilm and its negative impacts have been mentioned by many authors. In this study we made an attempt to screen the biofilm producing Klebsiella in the vaginal specimens of the C.trachomatis IgM positive pregnant women, those who had symptomatic genital infection. Biofilm forming Klebsiella was identified by both microscopic study and phenotypic study on solid culture media. Significant percentage of the biofilm producing Klebsiella was recorded in our study population. It was interesting to note that all these biofilm forming Klebsiella isolated from the vaginal secretions were found as Multidrug Resistant (MDR) bacteria. From this we conclude that the occurrence of biofilm forming, multidrug resistant Klebsiella among the C.trachomatis sero positive pregnant women is possible, especially with symptomatic genital infection cases. This need special attention of the public and health care professionals. It is suggested that the need of screening these culprits during pregnancy to eradicate it in view of prevent ascending infection which may involve with the adverse pregnancy outcomes and produce negative impacts on the fetus.

Introduction

Biofilms are known as the extracellular polysaccharide secreted by Quarum sensing bacterial population and that embading the bacterial community. Bacterial vaginosis (BV) has been noticed as the leading vaginal disorder among the women of sexually active age groups and it has been recorded that more than 60% vulvovaginal infections has been contributed by BV (Sobel et al ,2000). On the whole, many authors discussed about BV, and the associated serious health complications such as preterm birth, spontaneous abortion, pelvic inflammatory diseases, endometritis and accession and transmission of many sexually transmitted infections and the responsible infectious agents (Leitich et al, 2003, Guerra et al,2006, Rothman et al,2003,Jcobsosn et al 2002 and Gallo et al,2012).

Mostly the profuse vaginal discharge with rotten fishy odour are found to be the commonest clinical symptom of the BV, while, it has been recorded that the asymptomatic cases of BV (Koumans et al, 2007). In both asymptomatic as well as symptomatic BV, microbiologically, BV is characterised by a pronounced shift of vaginal microbes, which in turn involves either with the reduction of vaginal Lactobacilli or the loss of Lactobacilli. Again this condition result in simultaneous proliferation of the anaerobic bacteria such as, Gardnerella vaginalis, Atopobium, Mobiluncus species, Bacteroides species and Prevotella species. (Verhlst et al, 2004).

Because of the diversity and complexity of microorganisms involved in the abnormal status of the vagina, BV and its etiopathogenesis is yet to be cleared and seems to be poorly understood, still BV is a matter of controversy (Schwebke et al,2014) .

Pertain to BV and the related causative agents, different authors said different statements according to their findings. It seems to be controversial since 1955 (Gardner and Ducker, 1955). The Invitro research studies performed by some authors saying that the high level resistance to the protective mechanisms of the healthy vagina enhanced by the normal microbial flora (Patterson et al,2007) and also showing the increased level of antimicrobial resistance (Swidsinski et al 2008). On the whole these above said points, it is well known that the vaginal biofilms, play a key role not only in the pathogenesis of any bacteria, but also render treatment failure and associated with recurrent BV. Our study report also supporting the previous research authors. When we correlated the microscopic study results and culture results of these biofilm producing Klebsiella, we found that the presence of vaginal biofilm was 100% matched with the culture, and we could able to isolate biofilm forming Klebsiella from all of those vaginal specimens. However the quantity as well as quality of biofilm formed by different Klebsiella strains may vary. The cultured Klebsiella phenotypes also found to be varied (Fig.2.a,b&c).

It was very interesting to study and bring out the following informations. As the earlier research authors noted and recorded, in this study we also recorded 100% drug resistance, that too multidrug resistance with the Klebsiella strains. And the pregnant women seropositive with C.trachomatis IgM antibody had shown to have recurrent BV and even after antibiotic treatment. On microbiological

the involvement of biofilm forming MDR Klebsiella were noticed. Multidrug resistant Klebsiella could be the cause for the above said condition and that may emerge as co-infectious agent along with the C.trachomatis genital infection.

One should look forward to the negative impacts on the fetus while its intrauterine life and after its delivery , since there is a possibility of ascending type of infection by which these biofilm producers could reach uterus and affect the fetus. There seems to be a high possibility of these biofilm producers / MDR bacteria.

Many literatures indicating the phenotypic difference emerge with biofilm producing and non-biofilm producing strains of the same genus and species . We also acknowledge that these multidrug resistant pathogens contribute phenotypic changes and found to produce the extracellular polysaccharide known as biofilm, again. The biofilm producers always resist host cell mechanisms as well as to the antimicrobials. Hence we had given special focus on the multiple drug resistant Klebsiella population and Biofilm producers. The relationship and the negative impacts of these Multidrug resistant Klebsiella and the biofilm producing aspects of Klebsiella are considered as being true to each other. However, we have studied the association of these biofilm producers and the multidrug resistant Klebsiella among our study population.

We proud to introduce the semi qualitative method of identifying biofilm producing bacterial population and the load of polymicrobes habitating the vagina of study population which could be either pathogenic in its nature or indicating colonization of the vagina of the pregnant women. Whatever the condition of these biofilm producers and their existence with an individual, the multidrug resistance and the biofilm formation occur with pregnant individual, indicating an alarming threat of the future negative impacts on the fetus or any types of adverse pregnancy outcomes. So, it is important to note and record these types of informations while the pregnant women attend their regular health check up, especially, the women those who are complaining with repeated abortion or recurrent BV, because, based on our study record we came to know that the MDRB and BFFB associated with recurrent BV.

Materials and methods

Study population

Totally 300 C.trachomatis sero positive pregnant women, with symptoms of genital infection aged between 18-39 years attended the department of Obstetrics & Gynecology of Azeezia medical college, Kollam, Kerala, south India was included in this study.

Ethical clearance

Ethical clearance was obtained from the Ethical committee of Azeezia medical college hospital. Prior to specimen collection, the study plan and its purpose was thoroughly explained to the patients and obtained their consent in the specified form.

Specimen of choice

Two vaginal swabs were collected from posterior fornix of the C.trachomatis sero positive pregnant women with symptomatic genital infections .Vaginal swabs were collected by the qualified Gynecologists by pervaginal speculum examination from each individual. Among the two vaginal swabs collected, one swab was used to prepare smears on microscopic slide for Gram staining. Second swab used for culture and it was placed in a tube containing 1 ml Brain Heart Infusion Broth (BHIB) (immediately after collection) and subjected to the microbiological investigations.

Microbiological Investigations

Gram stained vaginal smear examination

The collected vaginal swabs were subjected to the microbiological investigations to screen the vaginal biofilm and the bacterial population dominating the vaginal smear. The vaginal smear made on the microscopic slide was air dried and heat fixed and labelled. It was subjected to Gram stain procedure and examined under oil immersion objective. The slides were looked for thick or thin extra cellular biofilm, bacterial load mainly dominating the vaginal smear, the beneficial bacteria ie; Lactobacilli, (indicator of BV) also be noted and recorded.

Biofilm identification by microscopy

The biofilm identification was done by using V.Udhaya's method (shortly published - *corresponding author – udaviarokya3@gmail.com). Briefly, the biofilm in Gram stained vaginal smear was noted as thick or thin extra cellular polysaccharide looks like matt layers, mostly appear as pink or violet colored, with various modulations. The specified reference pictures provided by V.Udhaya were followed to identify both the vaginal biofilm as well as the biofilm formed by Klebsiella on culture media. The extracellular polysaccharide biofilm surrounding the bacterial population (in the vaginal smear) was also be noted and recorded.

Vaginal specimen culture

The modified innovative method of V.Udhaya was used in this study to identify as well as to qualitate the biofilm producing Klebsiella strains. Briefly, the vaginal swab which was placed in 1ml BHIB was manually shaken for 1 minute; a loopful of the suspension was taken and inoculated on one part of the Mac Conkey's agar plate which was divided in to four quarter parts. At a time from four different vaginal specimens were inoculated on single Mac Conkey agar plate), and incubated at 37ºC for overnight incubation. Next day the biofilm forming Klebsiella phenotypes were identified and recorded.

Biofilm identification by phenotypic characterization

The bacterial colonies on Mac Conkey's agar plate was examined for the presence of the typical biofilm forming Klebsiella phenotype, ie; highly mucoid pink colored colonies /growth, which is differing from the usual mucoid phenotypes of Klebsiella , mostly oozing mucoid growth on culture media (Fig.1) was noted and recorded.

Antibiotic sensitivity of biofilm producing Klebsiella

The usual traditional golden - agar disc diffusion method was followed to perform Antibiogram of the biofilm producing Klebsiella, isolated from the vaginal specimens of the pregnant women seropositive for C.trachomatis IgM antibody. Briefly, a Loopful of 24 hrs grown fresh culture of biofilm forming Klebsiella was picked up from the Mac Conkey agar plate care should be given while taking the biofilm producing Klebsiella. The portion at where the increased mucoid biofilm appreciated, from that site the culture should be picked up and mixed with 1 ml of Brain Heart Infusion Broth. That was adjusted to Mac Farland opacity no. 0.5 (equals to $^{10\text{-}8}$ cells/ml) and that was considered as standardized bacterial inoculum. A sterile swab was soaked with the standardized bacterial inoculum and that was equally spread on Mueller-Hinton agar.

The selective antibiotics used for the Gram negative bacteria were applied on that and it was incubated at 37ºC for overnight incubation.

The Zone of Inhibition (ZOI) around the disc was noted and recorded, (which is considered as susceptible nature of the bacteria).The absence of zone of inhibition indicative of the resistant nature of the bacteria also noted. The biofilm forming Klebsiella, which had shown resistance to more than three individual antibiotics, was considered as Multidrug resistant Klebsiella strain (MDRKS).

Results and Discussion

Biofilm forming bacteria and its negative impacts on the medical treatment is an emerging threat within the field of medicine, since it involves with drug resistance and recurrent infections. Countless publications indicating the danger of these biofilm producers and insisting the eradication of these biofilm forming bacteria from the community. Many authors discussed the association of the biofilm forming bacteria and BV, and the drug resistance (Hoiby et al; 2011, Cerca et al;2006, Xie et al;2012, Cerca et al;2005, Tobudic et al;2012, Bradshaw et al;2006).

In our study we could able to record and document both antibiotics susceptible and antibiotic resistant, even multidrug resistant biofilm producing Klebsiella strains. The biofilm forming bacteria found to differ in their phenotypic character. It was interesting to note the heavy mucoid colonies obtained from the vaginal swab culture, and the same Gram stained vaginal smear exhibits the heavy load of the Gram negative bacilli, surrounded by thick extracellular polysaccharide and heavily adhered short Gram negative bacilli (Fig 1-c). On culture we obtained the heavy growth of Klebsiella with heavy mucoid colonies (Fig 1-b) .This result confirms the association of biofilm forming Klebsiella co-infection with the C.trachomatis genital infections. . Pertain to the detail about the predominant bacteria encounted from the biofilm associated genital infection, Klebsiella was the foremost bacteria we recorded in our research study. The typical phenotypic characters of these biofilm producing Klebsiella were noted as highly mucoid and even up to oozing type. Though the Klebsiella genus found to produce mucoid colonies, the biofilm producing Klebsiella known to produce, highly mucoid colonies and well differentiated from the usual Klebsiella phenotypes (Fig.2.a,b&c).

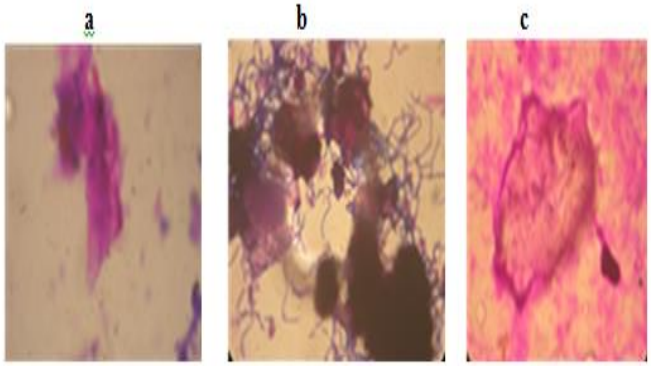


Fig.1. Gram stained vaginal smear. a & b - vaginal smear of the normal healthy women - note biofilm formed by lactobacilli covering vaginal epithelial cells indicative of natural defence mechanism

c. biofilm produced by Klebsiella covering vaginal epithelial cells - vaginal smear from BV case

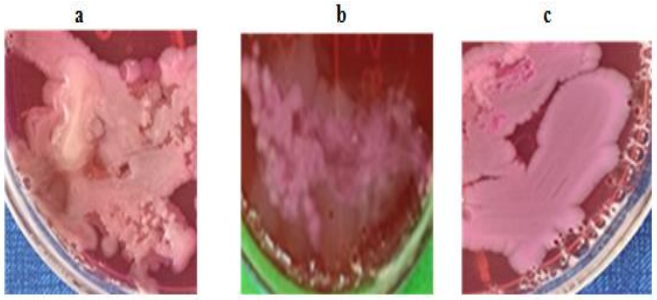


Fig 2. a - pregnant women infected with C.trachomatis genital infection co-infected with highly mucoid - Bio film forming Klebsiella vaginal specimen culture.

Fig 2. b- Non Bio film forming Klebsiella isolated from the vaginal secretion of the C.trachomatis seronegative pregnant women - isolated from BV case - note comparatively less mucoid type

Fig 2.c-The usual mucoid Klebsiella isolated (pure culture) from the vaginal specimens of normal healthy women.

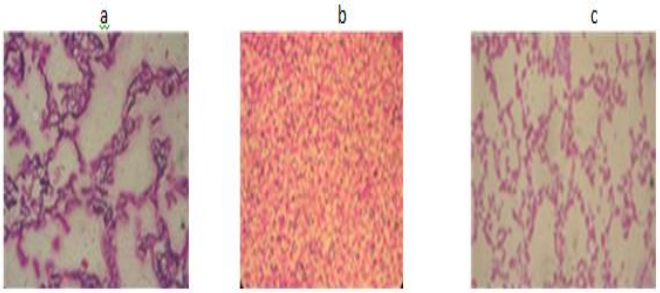


Fig.3. vaginal specimen culture smears

a. Note Gram positive lactobacilli surrounded by extra cellular polysaccharide biofilm

b. Note Gram negative Klebsiella surrounded by extra cellular polysaccharide biofilm

c. Note Gram negative Klebsiella non biofilm type. Note absence of extra cellular polysaccharide biofilm.

Fig.3. Bio film forming MDR Klebsiella

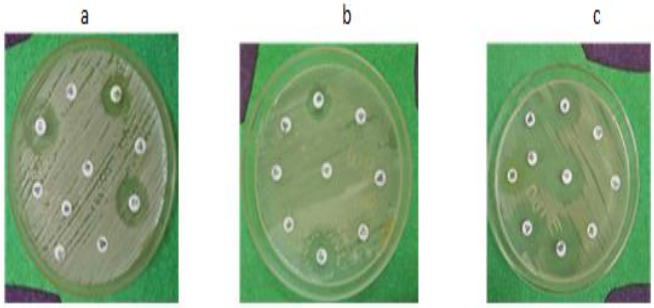


Fig 4.a &b - Bio film forming MDR Klebsiella corresponding to Fig .1 a&b

c- Non Bio film forming Klebsiella (sensitive strain) corresponding to Fig .2.c

It was interesting to note the biofilm forming Klebsiella among the C.trachomatis sero negative pregnant women with symptomatic genital infections, but there was a distinguishable difference between the quantities of the mucoid nature. The increased quantity of the biofilm producing Klebsiella was recorded with the *C.trachomatis* seropositive pregnant women with symptomatic genital infection, and all (100%) of them were found as Multidrug resistant Klebsiella, while the decreased growth pattern and both sensitive as well as resistant and MDR Klebsiella was recorded with C.trachomatis seronegative pregnant women with symptomatic genital infection.

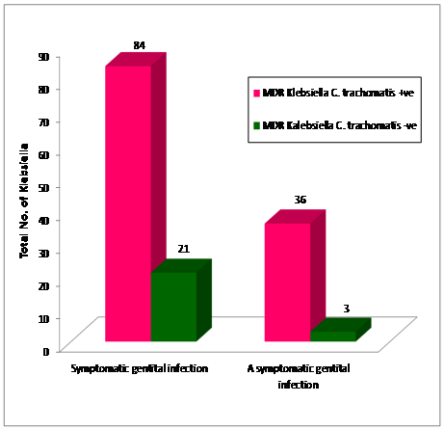
Table. Details of Biofilm positive klebsiella and its association with C.trachomatis IgM sero positive pregnant women.

S. No.	pregnant women with symptomatic genital infection n=300	Biofilm details		
		Biofilm positive Klebsiella		MDR-KLB
		Gram stained V.smear	*culture	
1	C.trachomatis IgM sero positive n= 300	100% but varied with biofilm quantity and Klebsiella strains	100% but varied with biofilm quantity and Klebsiella strains	100% resistant to even all the drugs tested
2	* C.trachomatis IgM sero negative n=450	30% but varied with biofilm quantity and Klebsiella strains	09% varied with biofilm quantity and vaginal burden of Klebsiella strains	03% resistant to maximum 5 drugs

* - Vaginal specimens culture

*-The data is presented here for comparison .Details of C.trachomatis IgM seronegative and biofilm producing Klebsiella and other bacteria will be included in PhD thesis. This paper is a bit of PhD research work-will be published shortly.

MDR-KLB = Multidrug resistant Klebsiella strain



Prevalence of MDR Biofilm Producing Klebsiella among C. trachomatis IgM Sero Positive Pregnant Women

Data- detail-Biofilm forming MDR Klebsiella prevalence with C.trachomatis seropositive pregnant women with symptomatic genital infection = 2%, with asymptomatic genital infection group=8%.

The presence of such biofilm producers in the vagina to be highly discussed and it needs the experts' opinion to predict their presence in the vagina as just colonizer, or causative agent of BV. It was very interesting to note and record the results of the direct microscopic examination of the vaginal smear made from the test group and normal healthy controls .The heavy load of Lactobacilli and thick biofilm formed by them, which covered the vaginal epithelial cells was noted with normal vaginal smear(Fig…).In case of such vaginal specimen culture smear had shown the extracellular polysaccharide biofilm surrounding each Lactobacilli that confirming the result of the direct microscopic study results of the Gram stained vaginal smear (Fig.1a&b). Present paper we are not presenting the full details of our normal healthy study population. For reference purpose we posted the vaginal smear with Lactobacilli and related culture photos.

In case of Klebsiella co-infection with C.trachomatis IgM seropositive pregnant women with symptomatic genital infections, klebsiella found to produce thick vaginal biofilm and the corresponding culture smear also confirm it (Fig1.c & Fig.3.b). From this we came to understand the presence of biofilm in the vagina could be either beneficial (when it is produced by the normal beneficial b bacteria) or harmful, when it is formed by the pathogenic bacteria. Ravel et al, 2011 studied the different species of the beneficial bacteria Lactobacilli, which is usually inhabits the vagina. Though other beneficial bacterial species such as Gardnerella vaginalis ,Enterococcus species and Prevotella species also residing at vagina, Lactobacillus species representing as the predominant beneficial bacteria inhabiting the healthy vagina, while the others comparatively in decreased numbers (Marrazzo et al,2002).

At this junction, we would like to share our views by correlating the above said points. In our study we across the presence of pathogenic biofilm forming MDR bacteria which includes Klebsiella in the vaginal specimen as well as grown in the culture of the vaginal specimens of the C.trachomatis IgM seropositive pregnant women. None of their vaginal smear or culture revealed the presence of beneficial bacteria ie; Lactobacilli. This indicates the association of biofilm forming Klebsiella co-infection with C.trachomatis genital infection.

Moreover, we are acknowledging the statement of Josey and Schwebke 2008, and saying that, though the vagina harbouring polymicrobial population, while BV, is dominated by single bacterial population and that is usually adhered to the vaginal epithelial cells. In our study we could able to record the presence of extracellular polysaccharide biofilm, dominantly, the Gram negative bacilli Klebsiella followed Pseudomonas aeruginosa strains which were strongly associated with heavily formed biofilm and total absence of vaginal epithelial cells have been noticed in the vaginal specimens of the *C.trachomatis* IgM individuals which is indicating the severe infection of the vagina with heavy burden of biofilm forming bacteria.

From our study records, thick semitransparent mucopurulent vaginal discharge with offensive odor was associated with BV associated *C.trachomatis* genital infection in which the biofilm producing bacterial infection was recorded. Hence, it is suggested that the pregnant women attending Obstetric and Gynecology division should undergo thorough pervaginal clinical examination and microbiological investigations to rule out the biofilm associated vaginal infection, for the effective healthy pregnancy outcome.

Conclusion

From our study experience, we confirm the possibility and the association of biofilm producing Klebsiella among the pregnant women with or without .trachomatis genital infection. It is an alarming threat which indicates the danger of these biofilm producers and their colonization / infection among the pregnant women of different age groups (in our study we have restricted our study population with the age group between 18-39 years).However, it was recorded that the women age ranged between 25-29 years are more prone to have biofilm related vaginal infection. The reason could be due to their decreased immunity, multiparity, personal hygienic practice and the frequency of the intercourse, above

all the condition of the sex partner, whether he is free of the sexually transmitted diseases, which may hold a major issue in the interpretive of the genital infection of the pregnant women. However, in our study we have not recorded the details of the male partner of the study population due to the personal secrecy maintained by the women and this point needs further detailed study. Hence we recommend the healthcare professionals to give special attention to these biofilm producing Multidrug resistant Klebsiella BV, especially focus on the pregnant women in view of enhancing good healthy pregnancy outcome, since BV with MDR biofilm producing Klebsiella is a dangerous culprit involved with adverse pregnancy outcomes.

Acknowledgement

We thank Dr Udhaya and her research team for helping me to carry out the bacterial biofilm identification and quantify the burden of the biofilm associated BV among the pregnant women with and without C.trachomatis genital infection.

We wholeheartedly acknowledge the timely help rendered by H.O.D. of the department of OG, RMMC&H, and Dr Lavanya kumarai to help us in the specimen collection and provided clinical photographs from the pregnant women belongs to Chidambaram, Tamilnadu and provided the clinical details about the study population. I also express my heartfelt gratitude to the H.O.D.Department of Obstetrics & Gynecology and other staffs of Azeezia medical college Hospital, Kollam, Kerala for their support in collecting samples from symptomatic pregnant women attending the OBG clinic.

References

Borges S., Silva J., Teixeira P. (2014). The role of lactobacilli and probiotics in maintaining vaginal health. Arch. Gynecol. Obstet. 289, 479–489. 10.1007/s00404-013-3064-9

Bradshaw C. S., Morton A. N., Hocking J., Garland S. M., Morris M. B., Moss L. M., et al. . (2006). High recurrence rates of bacterial vaginosis over the course of 12 months after oral metronidazole therapy and factors associated with recurrence. J. Infect. Dis. 193, 1478–1486. 10.1086/503780

Cerca N., Jefferson K. K., Oliveira R., Pier G. B., Azeredo J. (2006). Comparative antibody-mediated phagocytosis of Staphylococcus epidermidis cells grown in a biofilm or in the planktonic state. Infect. Immun. 74, 4849–4855. 10.1128/IAI.00230-06

Cerca N., Martins S., Cerca F., Jefferson K. K., Pier G. B., Oliveira R., et al. . (2005). Comparative assessment of antibiotic susceptibility of coagulase-negative staphylococci in biofilm versus planktonic culture as assessed by bacterial enumeration or rapid XTT colorimetry. J. Antimicrob. Chemother. 56, 331–336. 10.1093/jac/dki217

Gallo M. F., Macaluso M., Warner L., Fleenor M. E., Hook E. W., Brill I., et al. . (2012). Bacterial vaginosis, gonorrhea, and chlamydial infection among women attending a sexually transmitted disease clinic: a longitudinal analysis of possible causal links. Ann. Epidemiol. 22, 213–220. 10.1016/j.annepidem.2011.11.005

Gardner H. L., Dukes C. D. (1955). Haemophilus vaginalis vaginitis: a newly defined specific infection previously classified non-specific vaginitis. Am. J. Obstet. Gynecol. 69, 962–976.

Guerra B., Ghi T., Quarta S., Morselli-labate A. M., Lazzarotto T., Pilu G., et al. . (2006). Pregnancy outcome after early detection of bacterial vaginosis. Eur. J. Obstet. Gynecol. Reprod. Biol. 128, 40–45. 10.1016/j.ejogrb.2005.12.024

Høiby N., Ciofu O., Johansen H. K., Song Z. J., Moser C., Jensen P. Ø., et al. . (2011). The clinical impact of bacterial biofilms. Int. J. Oral Sci. 3, 55–65. 10.4248/IJOS11026

Jacobsson B., Pernevi P., Chidekel L., Platz-Christensen J. J. (2002). Bacterial vaginosis in early pregnancy may predispose for preterm birth and postpartum endometritis. Acta Obstet. Gynecol. Scand. 81, 1006–1010. 10.1034/j.1600-0412.2002.811103.x

Koumans E. H., Sternberg M., Bruce C., McQuillan G., Kendrick J., Sutton M., et al. . (2007). The prevalence of bacterial vaginosis in the United States, 2001-2004; associations with symptoms, sexual behaviors, and reproductive health. Sex. Transm. Dis. 34, 864–869. 10.1097/OLQ.0b013e318074e565

Leitich H., Bodner-Adler B., Brunbauer M., Kaider A., Egarter C., Husslein P. (2003). Bacterial vaginosis as a risk factor for preterm delivery: a meta-analysis. Am. J. Obstet. Gynecol. 189, 139–147. 10.1067/mob.2003.339

Marrazzo J. M., Koutsky L. A., Eschenbach D. A., Agnew K., Stine K., Hillier S. L. (2002). Characterization of vaginal flora and bacterial vaginosis in women who have sex with women. J. Infect. Dis. 185, 1307–1313. 10.1086/339884

Ravel J., Gajer P., Abdo Z., Schneider G. M., Koenig S. S. K., McCulle S. L., et al. . (2011). Vaginal microbiome of reproductive-age women. Proc. Natl. Acad. Sci. U.S.A. 108, 4680–4687. 10.1073/pnas.1002611107

Rothman K. J., Funch D. P., Alfredson T., Brady J., Dreyer N. A. (2003). Randomized field trial of vaginal douching, pelvic inflammatory disease and pregnancy. Epidemiology 14, 340–348. 10.1097/01.EDE.0000059230.67557.D3

Schwebke J. R., Muzny C. A., Josey W. E. (2014). Role of Gardnerella vaginalis in the pathogenesis of bacterial vaginosis: a conceptual model. J. Infect. Dis. 210, 338–343. 10.1093/infdis/jiu089

Sobel J. D. (20Annu. Rev. Med. 51, 349–356. 10.1146annureved.51.1.349 00). Bacterial vaginosis.

Swidsinski A., Mendling W., Loening-Baucke V., Swidsinski S., Dörffel Y., Scholze J., et al. . (2008). An adherent Gardnerella vaginalis biofilm persists on the vaginal epithelium after standard therapy with oral metronidazole. Am. J. Obstet. Gynecol. 198, 97.e1–e6. 10.1016/j.ajog.2007.06.039

Tobudic S., Kratzer C., Lassnigg A., Presterl E. (2012). Antifungal susceptibility of Candida albicans in biofilms. Mycoses 55, 199–204. 10.1111/j.1439-0507.2011.02076.x

Verhelst R., Verstraelen H., Claeys G., Verschraegen G., Delanghe J., Van Simaey L., et al. . (2004). Cloning of 16S rRNA genes amplified from normal and disturbed vaginal microflora suggests a strong association between Atopobium vaginae, Gardnerella vaginalis and bacterial vaginosis. BMC Microbiol. 4:16. 10.1186/1471-2180-4-16

Xie Z., Thompson A., Sobue T., Kashleva H., Xu H., Vasilakos J., et al. . (2012). Candida albicans biofilms do not trigger reactive oxygen species and evade neutrophil killing. J. Infect. Dis. 206, 1936–1945. 10.1093/infdis/jis607