Vaginal Ecological Profile and Characteristic of Vaginal Infection of the Genital Tract of Pregnant Women followed at the Laboratory of the Inter-Army Medical Center Senegal

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Female genital tract infections are a public health problem. Initial treatment of vaginal infections is most often probabilistic. The benignity of the condition and the safety of topical medications allow for immediate management, even empirical. Hence this study, which aims to evaluate the vaginal microecological profile and vaginal infections in pregnant women followed at the laboratory of the Inter Army Medical Center.

Methodology: We carried out a descriptive retrospective study over a period of 8 years, from 2012 to 2020, conducted by the laboratory of the Inter Army Medical Center., on pregnant women. After the collection of sociodemographic data, each woman was subjected to a vaginal swab in accordance with good laboratory practices. A macroscopic and microscopic examination and culture were carried out on each sample.

Results: During the study period, 3221 pregnant women were included with an average age of 29.91years. 59.05% of these had normal vaginal cleanliness. 1634 women had type IV flora. The direct examination showed that 210 pregnant women were carriers of *Gardnerella vaginalis.* The prevalence of *Candida albicans* and *Trichomonas vaginalis* infections was 10.90% and 1.06%. There was statistically significant difference of the type of flora, bacterial vaginosis in pregnant women of different age groups.

Conclusion: The microecological state of the pregnant women was mostly normal. Low rates of vaginal trichomoniasis infections were found in our study. There was a significant difference between the type of flora and the incidence of bacterial vaginosis in pregnant women of different age groups.

1. INTRODUCTION

The vagina is a sensitive and complicated micro-ecosystem, composed of anatomical structures, microorganisms, local immunity and endocrine regulation functions (Onderdonk et al. 2016). Bacterial colonization of the vagina is generally a mixed population, with a predominance of anaerobic bacteria. Vaginal microbiomes are mutually antagonistic and interdependent, maintaining a dynamic balance, regulated by the endocrine system and the immune system of the vagina, and affected by the internal environment of the vagina. The level of estrogenic hormones, Lactobacillus, local immunity and the vaginal pH play an important role in maintaining the micro-ecological balance of the vagina (Bulanda et al. 1996). During pregnancy, the vagina is susceptible to infection with many microorganisms. In addition, the immunosuppression that occurs during pregnancy reduced immunity, increasing the risk of opportunistic infections such as vulvovaginal candidiasis and Trichomonas vaginitis infection, thus contributing to the fragility of the vaginal micro-ecosystem (Dan et al. 2020).

In Senegal, few studies have been conducted to assess the characteristics of the vaginal micro-ecosystem and genital tract infection (GTI) in pregnant women. Most women, especially pregnant women, have a normal vaginal pH (3.8 to 4.5) and Lactobacillus spp. Various types of vaginitis can lead to abortion, intrauterine infection, fetal intrauterine growth restriction, premature rupture of membranes, preterm labor, low birth weight, and other adverse pregnancy outcomes. Severe vaginal infection and its rapid progression can cause cervical cancer and other diseases, which can negatively impact the health of the mother and fetus (Ghosh et al. 2017). Thus, the evaluation of the micro-ecological profile of the pregnant women and the detection of bacteria and yeasts are key elements in the management of vaginitis and/or vaginosis. Therefore in this study, we evaluated the vaginal microecological profile and infections in a group of pregnant women received at the laboratory of

the Inter-Army Medical Center Dakar, Senegal for a prenatal check-up.

2. METHODOLOGY

2.1 Type, location and duration of study

This is a descriptive retrospective study, covering a period of 8 years (from 2012 to 2020) conducted in the bacteriology departments of the laboratory of the Inter-Army Medical Center (CMIA) which is the third health structure of the Senegalese Armed Forces.

2.2 Study Population

All pregnant women underwent an external or internal basis who had received a vaginal swab for a prenatal check-up were included. Informed consents were obtained from all women prior to collection and analysis of vaginal swabs. Consenting women were administered questionnairies asking about their age, the number of pregnancies, the state of their pregnancy, their marital status, the nature of their vaginal discharge (color, level of secretions, itching and perineal dysuria), and their symptoms. The inclusion criteria were as follows: Pregnancy proven by ultrasound and/or positive urine pregnancy test (G test), no sexual intercourse or intimate toileting in the last 24 hours and no antibiotic treatment in the last week.

2.3 Vaginal Swab

Before starting this collection, the name written on the collection swabs and the patient's analysis report were checked for consistency. The quality of the samples and clinical information collected were used to determine the relevance of the bacteriological results. The samples were taken after any local or general antibiotic therapy had been stopped and in the absence of a local toilet on the day of the examination. The patient must not have urinated for at least two hours. Swabs were only taken outside of menstrual periods and away from sexual intercourse (12 hours). On inspection, we note the macroscopic appearance, namely the presence of leucorrhoea, their color, odor, and the appearance of the cervix. The sites of the sample are dictated by clinical signs and include the vagina-exocervix and the endocervix, depending on the context.

2.4 Vaginal Micro-Ecological Observation

Pap smears were subjected to direct examination and Gram staining. The micro-ecological examination included enumerations of white blood cells, red blood cells, epithelial cells, lactobacilli, bacterial flora and the identification of *candida*, *Trichomonas vaginalis*, types of bacteria (bacilli and cocci).

2.5 Diagnostic Criteria

Bacterial density is defined by the number and distribution of bacteria in a sample. In this study, based on the microscopic reading, we classified the bacterial density into three groups: Group I: 1-9/field, Group II: 10-99/field, and Group III: >100/field.

The diversity of the flora represents the distribution of bacteria during direct microscopic examination and/or after Gram staining. This divWzersity has also been divided into 4 types of flora: Type I: Exclusive presence of Döderlein bacilli, Type II: Clear predominance of Döderlein bacilli associated with another flora, Type III: Presence of Döderlein bacilli, but associated with a predominance of another flora, Type IV: Absence of Döderlein bacilli and presence of another mono or poly microbial flora (Gram (+) or Gram (-).

In this study we define a normal micro-ecological profile by vaginal pH 4-5, flora type I and II. On the other hand, the micro-ecological profile is abnormal if and only if pH>5, and type of flora greater than III.

Vaginal cleanliness was stratified into 4 grades, Grade I: Presence of lactobacilli, vaginal epithelial cells, absence of bacteria and leukocyte count between 0 and 10; Grade II: Presence of lactobacilli, vaginal epithelial cells, bacteria and leukocyte count between 10-15/field; Grade III: Presence of a low amount of lactobacilli, epithelial cells, a significant number of bacteria and leukocytes between 15-30/field and Grade IV: Absence of lactobacilli with the presence of epithelial cells and other bacteria and white blood cell count greater than 30 field. The presence of grades I and II indicates normal vaginal cleanliness, while that of III and IV indicates abnormal vaginosis with inflammation (increase in white blood cells) or without inflammation (absence of leukocytes). Bacterial vaginosis was diagnosed based on the presence of Gardnerella vaginalis, homogeneous vaginal

discharge, and a pH greater than 4.5. Trichomoniasis was defined by the presence of a flagellated single-celled parasite called *Trichomonas vaginalis* in its fresh state. Vaginal candidiasis has been diagnosed by the presence of *Candida albicans* or spp on direct microscopy or after culture on Sabouraud's medium. *Candida albicans* infection is characterized by a positive filamentation test (1 ml of rabbit blood + 2 colonies on Sabouraud medium and incubation at 37°C for 30 minutes and presence of filaments on microscopy). If this test is negative, the infection is due to *Candia Spp.*

2.6 Statistical Analyses

The data was exported from the File Maker and saved to Excel. They were analysed by the EPI info software version 7.0. The K2 test was used to analyze the different factors of vaginal micro-ecological flora such as vaginal cleanliness, Candida albicans and spp., Trichomonas vaginalis, Gardnerella vaginalis, bacterial density, flora type, dominant bacteria, pH value. The difference was statistically significant if $P<0.05$.

3. RESULTS

3.1 General Characteristics of the Study Population

We included in this study 3221 pregnant women. Among the 3193 women whose age was provided, their average age was 29.91±6.36 (15 to 45 years). The distribution by age is shown in Table 1. Women aged between 25 and 34 years accounted for 52.1% (n=577) of the study population . The average week of amenorrhea of the women included in this study was 12 weeks, or 3 months of pregnancy.

Regarding the marital status of pregnant women, 19 or 0.59% (95% CI [0.38 – 0.93]) were single.

Married women were the most represented with a prevalence of 98.87% (n=3160, 95% CI [98.44 -99.18]), Table (1).

The mean of the weeks of amenorrhea, the gestality and the parity at the time of the cytobacteriological examinations of the vaginal swabs were 12, 2.33 and 1.60, respectively, with statistically significant differences. Almost half of the study population 42.40% (n=1323; 95% CI [40.68-44.15]) were in their first trimester of pregnancy (Table 2). The number of pregnant women who had never had a baby was 722 or 27.90%. (Table 2).

3.2 Analyses of Vaginal Micro-Ecological Factors

The frequency of pregnant women with normal vaginal cleanliness was 59.05% (n=1902; 95% CI[57.34 – 60.74] (Table 3). More than half of pregnant women (n=1767 (54.86%; 95% CI[53,14-56,57] had a *lactobacillus* count greater than 10 per field (Table 3). The diversity of type I and II flora represented 1.30% and 25.18% of cases, respectively. The number of women who had an acidic pH of less than 4 (23.15%) was twice as large as that of women with a basic pH (11.25%) (Table 3).

The direct examination showed that 210 pregnant women, or 6.51%, were carriers of *Gardnerella vaginalis*. For bacterial vaginosis diagnosed on the basis of the presence of *Gardnerella vaginalis*, homogeneous vaginal discharge and a pH greater than 4.5, its frequency was 2.08% (n=66, 95% CI [1.64 – 2.64]). One thousand three hundred and sixty-nine pregnant women (42.50%) had vaginal candidiasis. The prevalence of *Candida albicans, candida spp* and *Trichomonas vaginalis* infections was 10.90%, 31.61% and 1.06%, respectively. (Table 4)

Table 1. Socio-demographic characteristics

Average age	Deviation Standard	Age range		
29.91 years	6.36	18 – 45 years old		
Age range (years)	**Number (n)**	**Prevalence (%)**	**IC95%**	**P-Value**
18- 24	705	22.2	20.8 – 23.7	
25 – 34	1653	52.1	50.4 – 53.8	
>35	815	27.7	24.2 – 27.2	
Marital status	**Number (n)**	**Prevalence (%)**	**CI95%**	
Bachelor	19	0.59	0.38 – 0.93	**P = 0.744**
Divorcee	12	0.38	0.21 – 0.66	
Bride	3160	98.87	98.44- 99.17	
Widows	5	0.16	0.07 – 0.77	

Table 2. Characteristics of the study population according to weeks of amenorrhea (WA), gestationity, parity

	Average	Deviation Standard	Intervals	P-Value
Weeks of amenorrhea	12	10.91	4 – 36	
Gesturity	2.33	1.59	1 – 10	
Parity	1.60	1.53	0 – 9	
Number of Deposits	**Number (n)**	**Prevalence (%)**	**CI95%**	
1	1323	42.40	40.68 – 44.15	
2	672	21.54	20.13 -23.02	0.0000
4	483	15.48	14.25 – 16.79	
+4	642	20.58	19.19 – 22.03	
Number of parities	**Number (n)**	**Prevalence (%)**	**CI95%**	
0	722	27.90	26.20 – 29.66	
1	728	28.13	26.43 -29.89	
2	515	19.90	18.41 – 21.48	0.0000
3	313	12.09	10.89 – 13.41	
+4	310	11.98	10.78 – 13.29	

Table 3. Analysis of vaginal cleanliness, lactobacilli number, flora type, and pH of the study population

	Number (n)	Prevalence (%)	CI95%
Vaginal cleanliness			
Normal	1902	59.05	57.34 – 60.74
Abnormal with inflammation	1014	34.28	32.66 – 35.93
Abnormal without inflammation	215	6.67	5.86 – 7.59
Lactobacillus Count/Field			
<1	986	30.61	29.04 – 32.23
1 – 10	468	14.53	13.35 – 15.79
>10	1767	54.86	
Flora of type			
I	42	1.30	0.97 – 1.76
II	811	25.18	23.71 – 26.71
III	734	22.79	21.37 -24.27
IV	1634	50.73	49.00- 52.45
pH			
≤4	745	23.15	21.73 – 24.64
5-6	2111	65.60	63.94 – 67.22
>6	362	11.25	10.20 – 12.39

Table 4. Analysis of bacterial vaginosis, candidiasis, and vaginal trichomoniasis in pregnant women

	Number (n)	Prevalence (%)	CI95%
Bacterial vaginosis			
Absence	3108	97.92	97.36 – 98.36
Presence	66	2.08	1.64 – 2.64
Candidiasis			
Absence	1852	57.50	55.78 – 59.19
Presence	1369	42.50	40.81 – 44.22
Types of Candida			
Candida albicans	351	10.90	9.87 – 12.02
Candida spp	1018	31.61	30.02 – 33.23
vaginal trichomoniasis			
Absence	3185	98.94	98.53 – 99.24
Presence	34	1.06	0.76 – 1.47

Table 5. Analyses of differences between vaginal cleanliness, number of Döderlein bacilli, type of flora, vaginal pH of pregnant women and ages of pregnant women

Factors		Age groups (years)			P-Value
		[16 – 24] n, %	[25-34] n, %	≥ 35 years old n, %	
Vaginal cleanliness	Normal	411 22.05	978 52.47	475 25.48	
	Abnormal with inflammation	245 22.33	567 51.69	285 25.98	0.988
	Abnormal without inflammation	49 23.11	108 50.94	55 25,94	
Lactobacillus count/field	<1	216 22.04	95 20.56	394 22.76	
	1-10	498 50.82	250 54.11	905 52.28	0.612
	> 10	266 27.10	117 25.32	432 24.96	
Flora of type	I	8 20%	21 52.50	11 27.50	
	II	188 23.53	441 55.19	170 21.28	
	III	164 22.81	374 52.02	181 25.17	0.039
	IV	345 21.36	817 50.59	453 28.05	
pH	≤4	140 19.02	409 55.57	187 25.41	
	5-6	484 23.27	1071 51.49	525 25.24	0.070
	>6	80 22.60	172 48.59	102 28.81	

Table 6. Analyses of the differences between bacterial vaginosis, vaginal trichomoniasis, vaginal candidiasis and the ages of pregnant women

Factors		Age groups (years)			P-Value
		[16 – 24] (n, %)	[25-34] (n, %)	≥ 35 years old (n, %)	
Bacterial vaginosis	Absence	665 22.07	1589 52.74	759 25.19	0.0044
	Presence	40 25	64 40	56 35	
vaginal trichomoniasis	Absence	698 22.25	1633 52.06	806 25.69	0.9724
	Presence	7 22.69	18 52.94	9 26.47	
vaginal candidiasis	Absence	313 21.63	715 53.77	333 26.60	
	Presence	188 23	441 52.5	170 24.47	0.3471

The multivariate analysis between the ages of pregnant women and micro-ecological factors showed that there was only a statistically significant difference between the type of flora and the age groups (p=0.039) (Table 5).

Table 6 shows that there was no statistically significant difference in the incidence of trichomoniasis, candidiasis in pregnant women of the different age groups. In contrast, there was a statistically significant difference (p=0.004) between the occurrence of bacterial vaginosis and the age groups of pregnant women (Table 6).

4. DISCUSSION

In our study, the population size was 3221 pregnant women, much is larger than that of Sy in Mauritania (n=200) and Sanou (n=195) in Burkina Faso. This difference could be explained by the difference in the duration of the follow-up (Sy et al. 2018; Sanou et al. 2017). The average age of the pregnant women in our study was 29.91±6.36 years. This was slightly lower than that the median age found in the study of Li's study in China which was 31.5 ± 5.5 (Dan et al. 2020). On the other hand, the average age of our study population was higher than the average ages (26.3 and 27.6 years) of pregnant women recruited in Rwanda (McMillan et al. 2018). Vaginal secretions contain 104 to 109 bacteria/g without any pathology; these are Döderlein's bacillus but also cocci and bacilli in different proportions. This ecosystem can include 4 types of bacterial flora (I, II, III and IV) which vary according to age, the period of the menstrual cycle, and pregnancy. Regarding the vaginal micro-ecological factors of pregnant women, more than half of our study population had normal vaginal cleanliness (59.05%) with grades ranging from I to II, respectively (Verhelst et al. 2005). Four hundred and forty-one (55.19%) and 374 (52.05%) pregnant women aged between 17 and 34 years had type II and III flora. Vaginal pH is a measure of the level of acidity or alkalinity in the vagina. For healthy controls, the vaginal environment is slightly acidic. This is due to the presence and activity of lactobacilli, which are the majority microorganisms in the vaginal flora. These bacteria secrete lactic acid, which leads to the natural acidification of the vaginal environment, and the lowering of the vaginal pH. At childbearing age, the normal pH varies between 3.8 and 4.5. Among the study population 65.60% had a pH normal. It has been shown by Gilbert et *al.* in that less than half of its

study population, 42.9% (145/338), had normal pH (Thunder et al. 2016). Changes in hormone concentrations in pregnant women, especially increased estrogen levels, promote the accumulation of glycogen in vaginal epithelial cells. Secondly, an increase in lactic acid due to the degradation of glycogen in epithelial cells by Lactobacillus leads to a low vaginal pH and imbalance of the vaginal microecosystem, which favours anaerobic bacteria in an acidic environment (Sun, 2011). In pregnant women, the bacteria responsible for vaginal infections can synthesize proteins and lipids that can degrade cervical mucus and digest the fetal membrane, reducing the thickness and elasticity of fetal membranes, which can lead to early abortion (Wang et al. 2015). Candida spp can alter the fetal membrane, lead to a drop in the local tension of the membrane or even an early abortion in severe cases (Wang et al. 2017). Bacterial vaginosis is a very common infection. However, it is very difficult to determine its exact prevalence because the figures vary greatly depending on the geographical location, the age of the patients, their socio-economic origin, the types of consultations and the state of pregnancy. The prevalence of bacterial vaginosis is generally estimated to be between 15% and 30% (Koumans et al. 2007). But some studies show higher prevalences (more than 30% in women of childbearing age) (Marconi et al. 2015) or sometimes much lower (from 4.9% to 20% in pregnant women) (Yalew et al. 202). In our study, the vaginosis was diagnosed on the basis of the presence of Gardnerella vaginalis, homogeneous vaginal discharge and a pH greater than 4.5 account for 2.08% of cases. A prevalence 10 times higher than that of our study was found in Cameron by Kamga et al with a rate of 26%(Kamga et al. 2019). In our study, trichomoniasis and candidiasis accounted for 1.06% and 42.5% of vaginal infections, respectively. Higher rates were reported in Ethiopia by Husen et al with a 7.7% for trichomoniasis (Aliyo et al. 2023) and in Mauritania by Sy et al. with a prevalence of 26% for candidiasis (Diongue et al. 2018).

5. CONCLUSION

Our data has shown that the microecological state of the pregnant women was mostly normal. Low rates of vaginal Trichomoniasis infections were found in our study. At the time of analysis, vulvovaginal candidiasis was strongly found in pregnant women. There was no significant

difference in vaginal microecological observations in pregnant women of different age groups, except for the type of flora and the incidence of bacterial vaginosis.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

CONSENT AND ETHICAL APPROVAL

Free and informed consent was obtained from the participants. Patient information was coded and kept confidential. All test results were sent to the patients. Those with a positive test for a pathogen were informed by the clinicians and received appropriate treatment.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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