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Socioeconomic and environmental risk factors of bacterial vaginosis in early pregnancy

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1 Introduction

Bacterial vaginosis (BV) is a well known cause of perinatal complications [6]. According to Goldenberg et al [11], BV accounted for 40 % of attributable risk for spontaneous birth at less than 32 weeks of pregnancy. Microorganisms ascending from the lower genital tract produce local inflammation, sub-clinical chorioamnionitis leading to preterm rupture of membranes (PROM) and/or preterm labor and possibly preterm birth [2,5,9,19,25]. BV is currently characterized as a change from the normal vaginal ecosystem to a reduced concentration of the aerobic bacteria normally present and Lactobacillus species, and increased concentration of anaerobic bacteria such as: *Garderella vaginalis, Mobiluncus, Bacteroides, Prevotella* and *Mycoplasma species* [8,26].

Some of the epidemiological studies have suggested that maternal urogenital tract infections are more prevalent in populations of socially underprivileged women [7,15,17]. Yet it is unclear which of the socioeconomic and environmental factors have a negative influence on the vaginal microflora before and during pregnancy. Racial and population diversity could be partly responsible for the contradictory results obtained in various studies. In view of growing evidence for the relation between abnormal microbiological flora of the lower genital tract and the risk of preterm delivery, it would be interesting to investigate the relationship between some socioeconomic and demographic characteristics of pregnant women in the Polish population and the development of bacterial vaginosis. The results of such a study would broaden our current knowledge of the obstetric risk assessment for preterm delivery by determining the characteristics of pregnant women who are susceptible to bacterial vaginosis at early pregnancy, and would contribute to an understanding of mechanisms that may translate social adversity into pathophysiology of pregnancy.

The main aim of this study was to determine the socioeconomic, demographic and environmental factors that may be associated with the occurrence of bacterial vaginosis in early pregnancy in the population of Polish women.

2 Material and methods

A group of 196 pregnant women was selected randomly from the patients of 10 district maternity units in the Lodz region, Central Poland between 01.01.1998 and 12.12.2000. Only singleton pregnancies between 8 and 16 weeks of gestation were qualified for inclusion in the survey. Women with chronic diseases diagnosed during the first prenatal visit were not considered in the study. A standard questionnaire covering medical, socioeconomic, demographic, constitutional and environmental aspects was administered to every subject and verified with medical records. This prospective cohort study was approved by Ethical Committee of Medical University in Lodz, Poland, No RNN/536/97. Each participant pro-

vided written consent for participation in the study.

For the qualitative and quantitative assessment of biocenosis in the lower genital tract, vaginal and cervical swabs were collected from the pregnant women under study. At first, bacteriological tests of cervical swabs were made to check for *Chlamydia trachomatis*, *Mycoplasma hominis* and *Ureaplasma urealyticum*. The *Ch. trachomatis* antigen was detected by direct immunofluorescence assay (Bio Merieux). For isolation, identification and differential titration of genital mycoplasmas, the commercially available Mycoplasma DUO kits (Sanofi Diagnostics Pasteur) were used. Identification was based on specific hydrolysis of urea (*U. urealyticum*) or arginine (*M. hominis*) by the species present in the specimen level of pathogenicity.

The vaginal swabs were tested for other aerobic and anaerobic bacteria. The swabs were placed in 3 ml prereduced sterile saline. Serial dilutions 1:10 from 10^{-1} to 10^{-8} were prepared. Each of the dilutions made from swabs was inoculated onto appropriate plates. Sheep blood agar, Mac Conkey, D-Coccosel agar, Gardnerella agar, Azide blood agar (Bio-Merieux) and Staphylococcus Medium 110 (Oxoid Ltd) plates were used for isolating aerobic organisms while Schaedler blood agar (Bio-Merieux) and Rogosa agar (Oxoid Ltd) were inoculated for anaerobic cultures. After the incubation period, the anaerobic and aerobic bacteria were identified by biochemical tests, API (Bio-Merieux).

Cervico-vaginal swabs were tested for bacterial vaginosis by Gram stain of vaginal smear according to Spiegel's criteria [26]. Based on microbiological results, 3 groups of pregnant women were distinguished as follows: group I, with normal cervico-vaginal flora, predominantly Lactobacillus spp., with coagulase-negative staphylococci and viridans streptococci; group II, with intermediate microbial flora, with predominant *M. hominis*, *U. urealyticum, G. vaginalis,* gram-negative anaerobic rods, *Ch. trachomatis* and few Lactobacillus spp.; and group III – bacterial vaginosis (BV).

To evaluate the risk factors, odds ratios (OR) were calculated. Statistical analysis, using EPI INFO software, was carried out, taking into account the risks ratios and their 95 % confidence intervals (CI). Logistic regression model was applied to examine the relationship between the probability of developing BV and the risk factors that were found to be significant or of borderline significance in the univariate analysis.

3 Results

3.1 Population characteristics

The mean pregnancy duration at the time of microbiological analysis was 12.3 weeks and the mean age of the subjects was 26.1 years. Bacterial vaginosis (BV) was diagnosed among 55 pregnant women (28.1 %), while grade I microflora was diagnosed among 70 (35.7 %) and grade II (intermediate) microflora among 71 women (36.2 %).

Mean pregnancy duration for women with BV diagnosed at early gestation was significantly shorter, comparing with those with grade I microflora (38,16 weeks ± 2,94 vs. 39,06 ± 1,83 weeks; p = 0,04). The pregnancy duration for women with intermediate flora (38,66 weeks ± 2,89) was not statistically different from other examined groups.

3.2 Socioeconomic and environmental factors

The role of the socioeconomic and environmental factors was examined by comparing the population of women with normal and intermediate microflora (group I and II) to pregnant women with BV (table I). The odds ratios were calculated for the combined groups I and II in relation to group III. Women below 20 years of age presented a slightly higher and those above 30 a lower risk of developing BV compared to those from the age group 21 – 25 years.

The proportion of women with a high level of education was 17.1 % in group I and 23.9 % in group II compared with only 9.1 % in group III. The risk of developing abnormal vaginal flora, although not statistically significant, was the lowest among well-educated pregnant women.

30.9 % of pregnant women from group III and 18.3 % from group II were not married, compared to 14.3 % of women from group I. Single marital status proved to be a significant risk factor for BV diagnosed at early pregnancy (OR = 2.53 95 % CI 1.14 – 5.64). Pregnant women who were divorced or widows were also at a higher risk of BV, al-

Table I. The relative risk of bacterial vaginosis in early pregnancy and selected socioeconomic, demographic and environmental risk factors

Variables	Group I (normal microflora) (n=70)		Group II (intermed. microflora) (n=71)		Group III (bacterial vaginosis) (n=55)		Odds ratio OR .95 % CI (I+II) vs. III
	n	%	N	%	n	%	
Age (years)							
≤ 20	10	14.3	5	7.0	10	18.2	1.76 (0.60–5.22)
21–25	22	31.4	25	35.2	17	30.9	reference group
26–30	25	35.7	24	33.8	19	34.5	1.07 (0.46–2.47)
> 30	13	18.6	17	23.9	9	16.4	0.83 (0.30–2.30)
Education							
primary	30	42.8	22	21.0	26	47.3	1.25 (0.61–2.57)
college	28	40.0	32	45.1	24	43.6	reference group
university	12	17.1	17	23.9	5	9.1	0.43 (0.12–1.32)
Marital status							
married	57	81.4	56	78.8	33	60.0	reference group
single	10	14.3	13	18.3	17	30.9	2.53 (1.14–5.64)
divorced/widow	3	4.3	2	2.8	5	9.1	3.42 (0.73–15.72)
Employment							
No	22	31.4	22	30.9	22	40.0	1.47 (0.73–2.95)
Yes	48	68.6	49	69.0	33	60.0	reference group
Own apartment							
Yes	50	71.4	50	70.4	32	58.2	reference group
No	20	28.6	21	29.6	23	41.8	1.75 (0.87–3.52)
Apt. standard							
high	17	24.3	16	22.5	10	18.2	0.67 (0.28–1.59)
average	44	62.9	47	66.2	41	74.5	reference group
low	4	5.7	3	4.2	3	5.5	0.95 (0.15–4.43)
no data	5	7.1	5	7.0	1	1.8	
Rest at							
pregnancy	33	47.1	26	36.6	16	29.1	0.61 (0.28–1.29)
unlimited	33	47.1	34	47.9	30	54.5	reference group
average	4	5.7	10	14.1	8	14.5	1.28 (0.43–3.70)
limited	0	0	1	1.4	1	1.8	
no data							
Household load							
very high	2	2.8	8	11.3	6	10.9	1.15 (0.31–3.97)
average	25	35.7	25	35.2	26	47.2	reference group
low	42	60.0	36	50.7	20	36.4	0.49 (0.24–1.03)
no data	1	1.4	2	2.8	3	5.5	

though the number of subjects was too small to prove statistical significance.

An excess risk of bacterial vaginosis coincided with unemployment, very high workload from household chores and low housing conditions during pregnancy. It did not, however, reach the level of statistical significance, probably due to the small number of subjects examined (table I).

The women from groups III and II were more frequently found to smoke over 5 cigarettes a day, as

Table II. Vaginal microflora grading, according to Gram stain, and cigarette smoking during pregnancy

Active smoking (cigarettes/day)	Group I (n=70)		Group II (n=71)		Group III-BV (n=55)		Odds ratio OR, 95 % CI (I+II) vs. III
	n	%	N	%	n	%	
0	53	75.7	54	76.0	35	63.6	reference group
1–5	10	14.3	6	8.5	9	16.4	1.72 (0.63–4.60)
6+	7	10.0	11	15.5	11	20.0	1.87 (0.74–4.67)

compared to the women from group I (respectively 20.0 %, 15.0 % and 10.0 %), the difference being not statistically significant (table II).

Since BV was found to be more prevalent in single women, we also evaluated the association between various microorganisms isolated from the lower genital tract of pregnant women according to their marital status during pregnancy (table III). Single pregnant women had a higher risk of positive culture of *Bacteroides spp, Mobiluncus spp., Prevotella spp.*, and *Ureaplasma urealyticum*. However, the significant risk was found only for Mycoplasma hominis OR = 3.71 95 % CI (1.27–10.84). Single marital status also constituted a risk factor for low concentrations of protective Lactobacillus spp (OR = 1,80 95 % CI: 0,84–3,87).

Since single marital status proved to be a major significant variable connected with BV at early pregnancy, we decided to focus on this factor and undertook an additional analysis to explore the socioeconomic and demographic characteristics of unmarried pregnant women in the Polish population. Compared to married women, single pregnant women were characterized by a younger age (below 20 years), lower educational level, poorer economic situation, worse housing conditions and excessive smoking during pregnancy (table IV).

To identify the socioeconomic factors independently associated with BV, a logistic regression model with three dependent variables (marital status, education and smoking) was used (table V). Only single marital status was found to be a significant risk factor of BV.

4 Discussion

Recent epidemiological studies indicate that BV appears to affect 10–30 % of pregnant women [20]. Following Gravett et al [13], it was detected in 19–30 % of women in early gestation, 19 % in mid and 14–18 % in late gestation. The authors also showed that BV detected early in the second trimester of pregnancy is strongly associated with late miscarriage and preterm birth. Therefore, women should be screened and treated for BV no later than in the early second trimester of pregnancy. Our data confirm these findings with a BV incidence of 28,1 % in the Polish population at early pregnancy. However, the lack of standardized diagnostic criteria renders the epidemiological data not readily comparable.

We postulated that there might be an association between abnormal cervico-vaginal flora and the socioeconomic, demographic or/and environmental factors which are also known to be most essential risk factors for adverse pregnancy outcome [4, 16]. The study results concerning the association between these factors and bacterial vaginosis during pregnancy are relatively scarce and inconsistent. The contradictory results might to some extent be explained by the different population characteristics, differences in BV definition and differences in gestational age at the time of investigation. There are also race/ethnicity differences in vaginal colonization with organisms reputed to be associated with bacterial vaginosis, According to Royce et al [24], black people are more likely to have pH > 4.5, no Lactobacilli, small gram-variable and gram-negative rods and Mobiluncus, compared to white race individuals. In another study, highly significant differences in vaginal colonization were observed, with the highest rates of potentially pathogenic organisms noted in black people and the lowest ones in the Asian-Pacific isles inhabitants [10].

Wessel et al [28] in a cross-sectional study observed that bacterial infection among pregnant

Table III. Association between various microorganisms isolated from lower genital tract and marital status during pregnancy

Assessment of biocenosis in the lower genital tract	Marital status				Odds ratio OR; 95 % CI
	Married (n=146)		Single (n=40)		
	Bacteroides spp.				
Culture(–)	140	95.9	36	90.0	Reference group
$< 10^5$	3	2.1	2	5.0	2.59 (0.21–23.37)
$\geq 10^5$	3	2.1	2	5.0	2.59 (0.21–23.37)
	Mobiluncus spp.				
Culture (–)	136	93.2	37	92.5	Reference group
$< 10^5$	7	4.8		0.0	Not counted
$\geq 10^5$	3	2.1	3	7.5	3.68 (0.47–28.33)
	Prevotella spp.				
Culture (–)	135	92.5	34	85.0	Reference group
$< 10^5$	2	1.4	2	5.0	3.97 (0.28–56.01)
$\geq 10^5$	9	6.2	4	10.0	1.76 (0.37–6.78)
	Gardnerella vaginalis				
Culture (–)	115	78.8	29	72.5	Reference group
$< 10^5$	12	8.2	4	10.0	1.32 (0.29–4.78)
$\geq 10^5$	19	13.0	7	17.5	1.46 (0.50–4.14)
	Mycoplasma hominis				
Culture (–)	127	87.0	28	70.0	Reference group
$< 10^4$	8	5.5	3	7.5	1.70 (0.41–5.26)
$\geq 10^4$	11	7.5	9	22.5	3.71 (1.27–10.84)
	Ureaplasma urealyticum				
Culture (–)	112	76.7	25	62.5	Reference group
$< 10^4$	14	9.6	5	12.5	1.60 (0.41–5.26)
$\geq 10^4$	19	13.0	10	25.0	2.36 (0.89–6.17)
	Chlamydia trachomatis				
Culture (–)	110	75.3	32	80.0	Reference group
Culture (+)	36	24.7	8	20.0	0.76 (0.29–1.93)
	Lactobacillus spp.				
Culture (–) or $< 10^5$	59	40.4	22	55.0	1.80 (0.84–3.87)
$\geq 10^5$	87	59.6	18	45.0	Reference group
	At least 1 pathogen				
1. Culture (–)	74	50.7	14	35.0	Reference group
2. Culture (+)	72	49.3	26	65.0	1.91 (0.87–4.21)

women was related to their young age and single marital status. In another study [3] the woman's age, marital status, number of pregnancies, smoking, and alcohol or drug abuse were not associated with the development of the genital *Chlamydia trachomatis* infection. Only such factors as the black race and older gestational age at the first prenatal visit constituted significant risk factors [3]. This study was conducted among adolescent subjects, below 19 years of age and only the chlamydial infection was evaluated, which might explain the observed differences. Also in a Spanish population, no association was found between race, parity, education, marital status, smoking and the presence of BV during pregnancy [18]. A cross-sectional study was conducted among preg-

Table IV. Comparison of selected socioeconomic, demographic and environmental characteristics of married and single pregnant women

Variables	Married (N=146)		Single (N=40)		Odds Ratio OR 95 % CI
	n	%	n	%	
Age					
< 20	11	7.5	14	35	2.80 (0.98–8.09)
21–25	44	30.1	20	50	reference group
26–30	58	39.7	3	7.5	0.11 (0.03–0.44)
> 30	33	22.6	3	7.5	0.20 (0.04–0.80)
Education					
primary	47	32.2	23	57.5	2.28 (1.02–5.14)
college	67	45.9	15	37.5	reference group
university	32	21.9	2	5.0	0.28 (0.04–1.40)
Employment					
No	39	26.7	23	57.5	3,71 (1,69–8,19)
Yes	107	73.3	17	42.5	reference group
Own apartment					
Yes	110	75.3	13	32.5	reference group
No	36	24.7	27	67.5	6.35 (2.79–14.62)
Apt. standard					
high	33	22.6	7	17.5	0.69 (0.25–1.83)
average	97	66.4	30	75.0	reference group
low	6	4.1	2	5.0	1.08 (0.14–6.40)
no data	10	6.8	1	2.5	not calculated
Active smoking (cigarettes/day)					
0	121	2.9	20	50.0	reference group
1–5	14	9.6	8	20.0	3.46 (1.15–10.30)
6+	11	7.5	12	30.0	6.60 (2.33–18.69)

Table V. Logistic regression models with BV as dependant variable

Variables	OR	95 % CI
Marital status		
married	reference group	reference group
single	1.21	1.01–4.85
divorced/widow	2.77	0.67–11.4
Education		
primary	1.00	0.49–2.08
college	reference group	reference group
university	0.50	0.17–1.46
Smoking		
Yes	1.11	0.50–2.48
No	reference group	reference group

nant women in Cote d'Ivoire to assess the factors associated with *Mycoplasma hominis* and *Ureaplasma urealyticum* colonization [7]. Young age, low educational levels and presence of BV were the factors independently associated with *M. hominis* colonization, *U. urealyticum* was more often found among unmarried women and when pregnancy was less than 20 weeks. A study conducted by Kamara et al [17] on 269 pregnant women in four prenatal clinics in Kingston, Jamaica, revealed that women who were employed were less likely to have not only BV but also trichomoniasis and candidiasis. According to Holzman et al [15], low level of education (13 years of school or less) is a strong risk factor for BV among non-pregnant women (RR = 5.0).

Smoking during pregnancy constitutes a risk factor for abnormal microbiological flora of the lower genital tract and preterm delivery. Cnattingius et al [4] underline that parous smokers are at an especially high risk for low birth weight and preterm delivery. Hellberg et al [14] investigated an association between bacterial vaginosis and smoking among 959 randomly selected healthy non-pregnant women. Before and after adjustment for confounding factors, smoking was significantly associated with BV (RR = 3.0). The results of our study on a population of pregnant women did not confirm these findings. Although we noted that 20 % of women with BV were smoking more than five cigarettes a day (10 % in reference group) the odds ratio calculated in the univariate analysis for active smokers was found to be 1.87 and thus did not reach the level of statistical significance, probably due to the limited number of cases. After adjustment for confounding factors, smoking turned out not to be a risk factor for BV at early pregnancy in our analysis. Hellberg et al [14] observed a dose-response relationship between BV and smoking, which suggests a causal link between those two factors. Also in an analysis made by Alnaif and Drutz [1], smoking independently affected the vaginal flora, thus increasing the relative risk of developing BV (RR = 2,9). The mechanism through which smoking could negatively affect the vaginal microflora still remains unclear. A possible solution is suggested in the study conducted by Pavlova and Tao [23]. The results showed that even the trace amounts of bezo(a)pyrene diol epoxide (BPDE) that can be found in the vaginal secretion of smoking women significantly increased phage induction in vaginal lactobacilli, thus reducing their number and, consequently, increasing an overgrowth of anaerobic bacteria. Szarewski et al [27], in a prospective intervention study, investigated the effect of smoking cessation on cervical Langerhans' cells and lymphocytes. The authors demonstrated a clear relationship between reduced rate of smoking and positive changes in the cervical immune system. Pastore et al [22] observed that fetal fibronectin was associated positively with BV but only among women who smoked. These data were not confirmed by Goldenberg et al [12]. The impact of cigarette smoking on BV during pregnancy should be verified in investigations based on an objective assessment of environmental tobacco smoke exposure.

The 'single marital status' variable is related to some other factors that might negatively influence the course of pregnancy, such as cigarette smoking, lower educational and economic status, higher incidence of pathological microflora of the lower genital tract and more risky sexual behavior before and during pregnancy. Psychological distress often accompanies a single mother's social situation. Comparison of selected socioeconomic, demographic and environmental characteristics of married and single pregnant women clearly showed that single women in Poland present a more risky profile e.g. they are younger, more poorly educated, often unemployed and tend to smoke actively during pregnancy.

Analysis of pathological microorganisms isolated from the lower genital tract during pregnancy revealed a higher incidence, as well as the risk of positive culture among single women as compared to married ones. The highest risk was noted for *Bacteroides spp, Mobiluncus spp; Prevotella spp; G. vaginalis, M. hominis* and *U. urealyticum*, the organisms involved in BV syndrom. Two-thirds of these women had a positive culture for at least one of the potentially pathologic bacteria. Single pregnant women also had a higher risk for reduced numbers of protective Lactobacilli.

Few studies have demonstrated that BV is associated with sexual behavior risk factors. In a cross-sectional study conducted with a Swedish population recruited from family planning and youth clinics Nilsson et al [21] determined the association between selected sexual behavior risk factors and bacterial vaginosis. Sexual factors associated with BV were as follows: a short-term relationship before and after sexual debut, high number of lifetime sexual partners, multiple partners during the last month and more frequent history of group sex, sexual abuse and rape.

As we did not analyze the impact of psychological stress, risky sexual behavior and substance abuse (except tobacco smoking) during pregnancy, we cannot consider these factors as directly responsible for BV. However, such an explanation is plausible and should be evaluated in further studies.

Our results indicate that in Poland the single marital status of pregnant women is a factor that increases the risk for pathologic vaginal microflora in pregnancy. On the other hand, we noted that

such characteristics of single mothers as young age, low level of education and smoking do not account for this finding, since these variables were controlled in the multivariate analysis. The elevated risk of BV development in this social group may result from other characteristics of single women, that were not controlled in this project, e.g. the number of sexual partners prior to pregnancy.

5 Conclusions

In the present paper, we analyzed the possible association between selected demographic and environmental factors and abnormal microbiological flora of the lower genital tract at early pregnancy in a Polish population. Since evidence has been found that abnormal cervico-vaginal flora during early pregnancy is more prevalent among women of single marital status, the former could be postulated as an important link between single motherhood and preterm delivery. Therefore, single pregnant women should be covered by more comprehensive prenatal or even pre-pregnancy surveillance to enable early detection and treatment of BV, which could reduce its negative impact on pregnancy outcome.

Abstract

The main aim of this prospective study was to determine the socioeconomic, demographic and environmental factors that may be associated with the occurrence of bacterial vaginosis at early pregnancy in an indigent population from Central Poland. A group of 196 pregnant women was selected randomly from the patients of 10 district maternity units in the Lodz region, Central Poland. Only singleton pregnancies between 8 and 16 week of gestation were qualified for inclusion in the survey. A standard questionnaire covering medical, socioeconomic, demographic, constitutional and environmental items was administered to every subject and was verified with medical records. Cervico-vaginal swabs were collected from the women under study and tested for bacterial vaginosis (BV) according to Spiegel's criteria. Based on the results of Gram stain, BV was diagnosed in 51 women (28,5 %), grade I microflora among 66 (36,9 %) and grade II among 62 women (34,6 %). In the univariate analysis, only single marital status proved to be an important risk factor associated with BV during pregnancy, this was confirmed in the multivariate analysis. Pregnant women who present risk factors for abnormal cervico-vaginal microflora should be covered by comprehensive prenatal surveillance, which enables early detection and treatment of this pathology. Research that identifies the causal pathways and mechanisms through which social disadvantage leads to a higher risk of preterm birth may help to reduce current socioeconomic and demographic disparities and improve pregnancy outcome.

Keywords: Bacterial infection, bacterial vaginosis epidemiology, pregnancy microbiology, socioeconomic risk factors.

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