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Aerobic bacterial pathogens causing vaginitis in third trimester of pregnancy

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Abstract

Introduction: Aerobic vaginitis is defined as a disruption of the lactobacillary flora, accompanied by signs of inflammation and the presence of predominantly aerobic microflora, composed of enteric commensals or pathogens. The Lactobacilli are replaced by aerobic facultative pathogens like E.coli, Staphylococcus aureus, group B Streptococci, Klebsiella pneumonia, and Enterococcus species which lead to ascending vaginal infections and various complications of pregnancy.

Aim and Objectives: To analyze the prevalence of aerobic vaginitis in third trimester of pregnancy and to study different aerobic bacterial vaginal pathogens and their antibiogram.

Materials and Method: One hundred and sixty six pregnant women in the third trimester of pregnancy were studied for aerobic pathogens by gram staining and culture- sensitivity. High vaginal swab was taken of all the women and sent for culture and sensitivity. Diagnosis of aerobic vaginitis was made on microscopy and culture report.

Results: Out of total 166 women, 88 were asymptomatic and 78 were symptomatic. Significant aerobic growth was seen in 29 women. Seventeen (21.79%) symptomatic women had positive vaginal culture and 12 (13.64%) asymptomatic women showed positive aerobic vaginal cultures. Six in the asymptomatic group had candidiasis. Commonest aerobic isolate was Enterococcus followed by E. coli and Staphylococcus aureus. We found that the overall prevalence of aerobic vaginitis was17.47%.

Conclusion: High prevalence of aerobic isolates (21.79% in symptomatic and 13.64% in asymptomatic group) may have detrimental implications on the maternal and neonatal wellbeing. We recommend that all women in third trimester should be screened for the presence of vaginal pathogens.

Keywords: Aerobic Bacteria, Aerobic Vaginitis (AV), High vaginal swab, Escherichia coli (E. coli), Staphylococcus aureus (Staph aureus).

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Introduction

In healthy women, normal pH of 3.5 to 4.8 in vagina is maintained by presence of organisms belonging to Lactobacillus species.(1) Staphylococcus epidermidis (coagulase negative staphylococcus), micrococcus and diphtheroids are the other microorganisms which are also present in human vagina.(2) If abnormal, the flora can be disturbed by anaerobic overgrowth (bacterial vaginosis) or by aerobic microorganisms such as E. coli, group B Streptococcus, Enterococci, Staphylococci spp. etc (aerobic vaginitis) or can be a mixture of both.(3) Aerobic vaginitis (AV) is defined as a disruption of the lactobacillary flora, accompanied by signs of inflammation and the presence of a rather scarce, predominantly aerobic microflora, composed of enteric commensals or pathogens.(4) Aerobic vaginitis corresponds to a type of disturbed microflora, in which the Lactobacilli are replaced by aerobic facultative pathogens like E.coli, Staphylococcus aureus, group B Streptococci, Klebsiella pneumonia and Enterococcus species. Disturbances in the vaginal ecosystem during aerobic vaginitis cause an increase in pH to more than 6, a decrease in lactate concentration and an increase in leucocytes and pro-inflammatory cytokines concentration in the vaginal discharge. The increased local production of IL-1, IL -6 and IL -8 associated with aerobic vaginitis is responsible for the increased risk of preterm delivery, premature rupture of membranes and chorioamnionitis during pregnancy. Vaginitis with aerobic microbes like group B streptococci and E coli has been hypothesized to be accountable for complications of pregnancy.(5) Ascending infection during gestation may result in maternal complications such as sepsis, septic arthritis and maternal respiratory distress.(6) The consequences of intrauterine infection for the fetus is not limited to the complications and squeal of preterm birth, but are also related to inflammation-induced neurologic injury resulting in intra or peri-ventricular haemorrhage and cerebral palsy.(7)

Therefore this study was designed to assess the prevalence of various aerobic bacterial vaginal pathogens in the third trimester of pregnancy in symptomatic as well as asymptomatic pregnant women.

Materials and Method

This prospective cross sectional study including 166 pregnant women was done in the Department of Obstetrics and Gynecology at Christian Medical

College and Hospital Ludhiana, Punjab, India over a period of one year. Informed consent was taken from all the women after explaining them about the study in the language they best understood.

Inclusion criteria: Pregnant women in the last trimester of pregnancy (28 to 40 weeks) coming for routine antenatal care (asymptomatic). Symptomatic women presented with the symptoms of abnormal vaginal discharge, dysuria, lower abdominal pain, vaginal dyspareunia, vulvo-vaginal itching and foul smell.

Exclusion criteria: Women who were taking antibiotics in last two weeks of gestation before coming to the hospital.

Detailed socio demographic and obstetric history, past obstetric history, any previous illness and history of taking any antibiotics in the last two weeks was taken. All women underwent thorough general physical, systemic and obstetrical examinations and later two high vaginal swabs were collected from each woman for gram staining and culture- sensitivity to the antibiotics. Samples from posterior fornix of vagina were taken with two sterilized cotton swabs under direct vision using Cusco / Sims speculum prior to first vaginal examination. The first swab was used for Gram stain examination under 400x (for determining AV score) and 1000x magnification (for identification of organism). The diagnosis of aerobic vaginitis was made according to the Lactobacillary grades and were the basis for a composite score to which any of the four following variables were added: leucocytes, presence of toxic leucocytes, presence of parabasal cells (no parabasal cells: score = 0; parabasal cells representing <10% of the epithelial cells: score = 1; parabasal cells representing >10% of the epithelial cells: score = 2), and background flora. Background flora was allocated score 0 if the background flora was unremarkable or showed debris and bare nuclei from lysed epithelial cells (cytolysis), score 1 if the lactobacillary morpho types were very coarse or resembled small bacilli (other than lactobacilli) and 2 if there were prominent cocci or chained cocci visible. A composite score of 1 to 2 represents normality. A score of 3 to 4 corresponds to mild aerobic vaginitis, a score of 5 to 6 to moderate vaginitis, and a score above 6 (to maximum 10) to severe aerobic vaginitis.[5] The second swab was inoculated onto MacConkey's agar, blood agar and chocolate agar.[8] These petri-plates were incubated aerobically and bacterial growth was identified by standard biochemical reactions.[9] The antibiotic sensitivity testing of these aerobic bacterial isolates was performed. Women with positive cultures for aerobic pathogens were treated with appropriate antimicrobials according to the sensitivity pattern. Routine antenatal care was given to all the women under study.

The results were expressed as percentages for the analysis of various data. Microsoft excel was used for the interpretation of these results.

Results

A total of 166 high vaginal swab samples were collected from the pregnant women in the last trimester of pregnancy. Overall high vaginal swab culture showed presence of aerobic bacterial infection in 29 out of 166 women with the prevalence rate of 17.47%. On microscopy mild aerobic vaginitis was seen in 12, moderate in 14 and severe in 3 women. In our study 80 participants were primigravida and rests were multigravida. Out of these 80 primigravida 13 (16.25%) were positive for aerobic vaginitis and the positivity was 18.60% (16/86) in multigravida. Out of total 166 women 87.95% were housewives not working outside, most of them were in the age group of 20 to 30 years (135) and maximum positive aerobic cultures (24) were in this age group. Out of 166 women, 129 (77.71%) had normal body mass index, 58 (39.94%) were anaemic and 8 (4.82%) were diabetic. Twelve out of 29 (41.38%) participants with positive culture were anaemic [Table 1].

Of the 78 symptomatic women we found significant aerobic bacterial growth in 17 (21.79%) and Candida spp. growth in one. The commonest aerobic pathogens in symptomatic women were Enterococcus in (7) followed by E. coli (4), Methicillin sensitive Staph aureus (MSSA) (4), Enterobacter species (2), Pseudomonas aeruginosa (2) and non-hemolytic Streptococcus (1). Three women (10.34%) showed polymicrobial growth. Of the 88 asymptomatic women, we found positive aerobic bacterial growth in 12 (13.6%) and 6 (6.82%) had Candida growth in high vaginal swab cultures. The commonest aerobic pathogens were E. coli (4), Enterococcus (3), MSSA (2), Methicillin resistant Staph aureus (MRSA) in (1), Gardnerella vaginalis (1) and Proteus vulgaris (1) [Table 2]. Drug sensitivity was done and the positive women were treated with the antibiotics that were sensitive to the grown aerobic pathogen while considering the safety profile during the pregnancy [Table 3]. The treatment was given for two weeks.

Table 1: Socio-demographic & obstetric profile

			%
Gravida	Primigravida	80	48.19
	Multigravida	86	51.81
Age (years)	< 20	4	2.41
	20-30	135	81.33
	>30	27	16.26
Antenatal visits	Booked	129	77.71
	Unbooked	37	22.29
Modified Kuppuswamy's SES Scale (Education, Occupation, Family income per month in Rupees)	Upper	40	24.10
	Upper Middle	48	28.92
	Lower Middle	51	30.72
	Upper Lower	15	9.04
	Lower	12	7.23
Anemia (Hb < 10.5 gm%)	Present	58	34.94
	Not Present	108	65.06
BMI	Normal	129	77.71
	Obese	10	6.02
	Overweight	22	13.25
	Underweight	5	3.02
Work	Working	20	12.05
	Not working	146	87.95
Diabetes Mellitus	Gestational	6	3.62
	Overt	2	1.20
	Normal	158	95.18
Past obstetric history in multigravida	LSCS	16	18.60
	Abortion	31	36.05
	Preterm birth	15	17.44
	None	24	27.91

Table 2: Prevalence of high vaginal swab culture positivity

Presentation to hospital	Number of women	%age
Symptomatic (n=78)	17	21.79
Asymptomatic (n=88)	12	13.64

Table 3: Details of microorganisms isolated in 29 women and their sensitivity to the antibiotics

Microorganism	Symptomatic		Asymptomatic	
	n	Antibiotic Sensitivity	n	Antibiotic Sensitivity
E. coli	4	Cefoperazone + Sulbactum; Piperacillin + Tazobactum	4	Cefoperazone + Sulbactum; Amoxycillin+ Clavulanic acid; Cefotaxime
Enterococcus	7	Cefoperazone + Sulbactum; Linezolid; Ceftriaxone	3	Ampicillin; Linezolid;
Staph aureus (MSSA)	4	Amoxycillin + Clavulanic acid; Cefoperazone + Sulbactum; Linezolid;	2	Amoxycillin + Clavulinic acid;
Staph aureus (MRSA)	0	-	1	Penicillin; Erythr mycin
Enterobacter specisis	2	Linezolid;	0	-
Pseudomonas	2	Cefoperazone + Sulbactum	0	-
Nonhaemolytic streptococcus	1	Linezolid	0	-
Gardinella	0	-	1	Cefixime
Proteus vulgaris	0	-	1	Ceftriaxone

Discussion

The overall prevalence of aerobic vaginal pathogens in our study was 17.47% which was similar to one reported by Donders et al (15.53%, 98/631)[5] and Sangeetha et al (20.8%, 26/125)[10] but Zarbo et al reported a very high incidence (60.49%) in their study

and probably this was because the participants in their study had different sexual habits and multiple partners.[11] In our study 80 women were primigravida and rest was multigravida. Out of 80 primigravida, incidence of aerobic vaginitis was 16.25% (13/80) and was slightly higher 18.60% (16/86) in multigravida. Majority of the women were in the age group of 20 to 30 years (135) and maximum positive cultures (24) were in this age group. On microscopy mild aerobic vaginitis was seen in 12 (7.22%), moderate in 14 (8.43%) and severe in 3 (1.81%) women which corresponds to the observations made by Donders et al (7.61% mild, moderate and severe 7.92%)[5] and Sangeetha et al (7.14%, 3/42).[10] Zarbo et al studied 243 pregnant women between 16 to 39 weeks of gestation and found aerobic vaginitis in 147 (60.49%) women.[11] In our study the positive high vaginal swab cultures showed 89.66% monomicrobial and 10.34% polymicrobial growth which is similar as reported by Sangeetha et al (single infection in 80.77% and mixed in 19.23%).[10] Razzak et al (2011) observed polymicrobial infections in 50 out of 105 cases (47.62%) which was significantly higher than our observations.[12]

We found that in the symptomatic group of women, the commonest organism isolated was Enterococcus (7) followed by E. coli (4), MSSA (4), Enterobacter species (2), Pseudomonas aeruginosa (2) and Non-haemolytic Streptococcus (1). One positive culture (E. coli) also had growth of Candida spp. In asymptomatic group of women the commonest organism isolated was E. coli (4) followed by Enterococcus (3), MSSA (2), MRSA (1), Gardnerella (1) and Proteus vulgaris (1). Five women showed growth of only Candida species and one had Enterococcus plus Candida species. In our study Entercoccus faecalis was the commonest pathogen isolated amounting to 34.48% (10/29) followed by E coli 27.59% (8/29), Staphylococcus aureus 24.14% (7/29), Enterobacter 6.9% (2/29), Pseudomonas aeruginosa 6.9% (2/29), Nonhaemolytic Streptococcus (1), Gardnerella (1) and Proteus vulgaris (1) 3.45% each. In a similar study done by Zarbo et al, they studied aerobic vaginitis during pregnancy in 243 women between the 16th and the 39th week of gestation who all underwent a vaginal swab culture sensitivity and found that 147 (60.49%) were symptomatic and rest 96 (39.51%) were asymptomatic. In their study the presence of aerobic vaginitis was found in 147 pregnant women who then underwent pharmacological therapy. Of the 147 symptomatic women they found 49 (33.3%) with infection from E. coli, 41 (27.9%) from Staphylococci, 27 (18.37%) from Enterococci, 22 (15%) from Streptococcus agalactiae and 8 (5.43%) from Gardnerella.[11]

In 2002, Donders et al studied 631 pregnant women to define abnormal vaginal flora i.e. aerobic vaginitis and found that 50 of 631 women (7.92%) had moderate to severe and 48 (7.6%) showed mild aerobic vaginitis. In this study the overall prevalence of aerobic vaginitis was 15.53%. Commonest pathogens found in their study were Gardnerella vaginalis, Enterococcus, E. coli, group B Streptococcus and Candida species.[5] Sujata et al reported 100 pregnant women between 28 to 37 weeks of gestation and their high vaginal swab culture showed the most common organism to be E. coli amounting 34% followed by Candida spp 21%, Enterococci 10%, Staphylococci 8%, Gardenerella vaginalis 7% and Group B Streptococcus 5%.[13]

We observed in our study that E. coli was highly sensitive to Cefoperazone + Sulbactum, Piperacillin + Tazobactum, Amoxycillin+ Clavulinic acid, Cefotaxime; Enterococcus was sensitive to Cefoperazone + Sulbactum, Linezolid, Ceftriaxone, Ampicillin; MSSA to Amoxycillin + Clavulinic acid, Cefoperazone + Sulbactum, Linezolid and Pseudomonas to Cefoperazone + Sulbactum. Singh et al[5] reported in their study that E. coli was sensitive to Linezolid, Vancomycin, Cefoperazone and Sulbactum. Enterococcus was sensitive to Vancomycin, Amoxicillin, Clavunilic acid and Amikacin. Staph aureus was sensitive to Linezolid, Vancomycin, Cefotaxime and Clindamycin. Group B Streptococcus was sensitive to Gentamycin. Gardnerella vaginalis was sensitive to Ampicillin and Metronidazole.[13] Mumtaz et al did vaginal sampling to study aerobic vaginal isolates in the reproductive age group. The most prevalent organisms found by them were Staphylococcus aureus (46%), E. coli (13.7%), Klebsiella pneumoniae (8.8%), Pseudomonas (7,3%) and Candida species (1%). Sensitivity was shown to antibiotics imipenem, vancomycin, piperacillin/ tazobactum.[14] Zarbo et al (2013) reported that on high vaginal swab culture, Staphylococcus was sensitive to Amoxicillin+ Clauvulinic acid, Neomycin, Cefotaxime; E.coli + Enterococcus to Ofloxacin, Norfloxacin, Ciprofloxacin, Ampicillin; Streptococcus beta hemolyticus to Netilmicin, Cefalotin, Gentamicin, Clindamycin, Ampicillin, Amoxacillin and Gardnerella spp to Metronidazole, Clindamycin, Gentamicin and Ampicillin.[11]

Conclusion

Overall prevalence of aerobic vaginal pathogens and aerobic vaginitis in pregnant women in our study was 17.46%. High prevalence of aerobic isolates (21.79%) in symptomatic and 13.64% in asymptomatic pregnant women in third trimester of pregnancy was observed in the total 166 women studied. Most of the vaginal aerobic pathogens are enteric commensals. The presence of aerobic pathogens even in asymptomatic women can be a cause of neonatal sepsis, maternal sepsis, endometritis and puerperal infections. This can have detrimental implication on maternal and neonatal wellbeing. The highest prevalence of positive high vaginal swab culture (24) was seen in the age group of

20 to 30 years (135) of age. Women in this age group are most sexually active, so we strongly recommend that for prevention of acquiring aerobic infection the usage of barrier contraception during pregnancy, maintenance of good personal hygiene, avoidance of washing the perineum using jet water nozzle from anal to vagina direction after defecation as this can disseminate anal bacteria to the vagina. We also recommend that all pregnant women in third trimester be screened for presence of aerobic vaginal pathogens, treat the women according to antibiotic sensitivity pattern to drugs and the treatment of aerobic vaginitis should be specific. Restoring the vaginal flora to normal can prevent ascending infections during pregnancy, neonatal sepsis, preterm birth, chorioamnitis and peripartum infections.

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