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Bacterial Vaginosis in Recent Abortion: An Observational Study in the Department of Obstetrics & Gynaecology, SMS Medical College, Jaipur

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Abstract: ***Background: Bacterial Vaginosis (BV) is the most common cause of abnormal vaginal discharge among women of reproductive age, accounting for 40 to 50 per cent of all cases of vaginal discharge.Bacterial Vaginosis (BV)is a special public health concern in India because of the high burden of reproductive and pregnancy-related morbidity.The purpose of the study is to lookfor the cost-effective, less time-consuming method that can be added to the routine screening for the women who are pregnant and having persistent vaginal discharge to help in reducing adverse pregnancy outcome. Also, screening of BV in fertile women is necessary to prevent spontaneous abortion. Material & Methods: This is a hospital based observational study done on women between the age of 18-39 years, attending Gynaecological Out-Patient Department, with a history of spontaneous abortion 2 to 6 weeks back in SMS Medical College, Jaipur, Rajasthan.For sample collection, a nonlubricated bivalve speculum was inserted into the vagina, and vaginal swabs were taken from the vaginal walls and posterior fornix with four sterile cotton swabs for pH examination, whiff test, pap's smear, Gram's staining and culture. Results: Our study showed that no significant association was found between age, religion, residence & socio-economic status and BV. Maximum proportion (36%) of BV observed among women who had history of 2nd trimester pregnancy loss.While classifying Bacterial vaginosis positive cases of recent spontaneous abortion according to Nugent score (Gram staining), 52.17% cases were positive on both scores and remaining all 47.82% cases showed intermediate results. Conclusion: We concluded that the screening of Bacterial Vaginosis in pregnant women may be used for evaluating women who are predisposed for abortion. However, to be able to use BV screening for routine purpose in determining the poor reproductive outcome, further work and research are needed.***

Keywords: Bacterial Vaginosis, Pregnant women, Nugent Score, Recent abortion

1. Introduction

Bacterial Vaginosis (BV) is the most common cause of abnormal vaginal discharge among women of reproductive age, accounting for 40 to 50 per cent of all cases of vaginal discharge. BV is a polymicrobial disorder. It does not follow Koch's postulate that a single pathogen is responsible for a specific disease. BV is referred to as "one of the most prevalent enigmas in the field of medicine."[1]

BV is a special public health concern in India because of the high burden of reproductive and pregnancy-related morbidity. In India, an estimated prevalence of BV is around 19 per cent. BV is the most common lower genital tract disorder among women of reproductive age, either pregnant or nonpregnant. Prevalence rates vary 13% to 31% in pregnant women.[2]

Gardner and Dukes were first to link bacterial vaginosis to Gardnerella vaginalis in 1955. Initially, it was called Haemophilus vaginalis due to Gardnerella appearance like the Haemophilus genus.[3] Until this time, clinicians called the infection "nonspecific vaginitis". When the organism resembled the genus Corynebacterium rather than Haemophilus, the name changed to Corynebacterium vaginalis.[4] Anaerobic vaginitis was another name given due to high levels of gram-negative anaerobes.[5] Probably the most recognized name is Gardnerella vaginalis (named for its discoverer, Gardner).[3] In 1983, current term Bacterial Vaginosis was coined, with the acceptance that there are many bacterial species are responsible for the condition.

A healthy vaginal environment is characterized by an abundance of Lactobacilli species accounting for more than 95% of all bacteria present.

The most common healthy vaginal communities are dominated by only a few species Lactobacillus iners, Lactobacillus crispatus, Lactobacillus jensenii and Lactobacillus gasseri.[6] Lactobacilli has several mechanisms to inhibit colonization by other microorganisms. Vaginal epithelial cells produce glycogen and lactobacilli ferment that glycogen to produces D- and L-lactic acid.[7]Lactobacilli maintain vaginal pH at the acidic level by producing lactic acid.

Lactobacilli inhibit pathogen colonization by competing for host cell receptors used by urogenital pathogens such as Gardnerella vaginalis, Neisseria gonorrhoeae, Candida albicans, Staphylococcus aureus, group B Streptococcus species, Pseudomonas aeruginosa, Streptococcus agalactiae, Escherichia coli and Prevotella bivia.[8,9] So in this way, Lactobacilli show a higher affinity for host cell receptors and it can displace adherent G vaginalis and N gonorrhoeae.[10] Some Lactobacilli co-aggregate with pathogens (e.g. G vaginalis, C albicans and E Coli), hence inhibiting them from binding to host cells and allowing more effective clearance.[11] The combined activities of the host and different inhibitory mechanisms of Lactobacillus contribute to the maintenance of a healthy vaginal ecosystem.

Many factors influence the composition of the vaginal microbiota. The composition of vaginal flora depends on age, menarche, menses, pregnancy, infections, birth control

behaviour and sexual behaviour. Exposure to spermicides or β-lactam or other antimicrobials can decrease the prevalence of Lactobacilli and therefore increase susceptibility to vaginal infections.[12]

Risk factors for BV include black race, ethnicity,[13] douching,[14] smoking,[15] menses,[16] lack of male circumcision,[17] low socioeconomic status, previous pregnancy, sexual activity,[18] low vitamin-D levels,[19] other dietary factors,[20] chronic stress,[21]genetic variants of a wide range of host genes[22] and the intrauterine device.[23] The use of hormonal contraception is associated with a decreased incidence of BV.[23] The early prevalence study of Gardner et al found an increased rate of Bacterial Vaginosis in black women compared with white women (29% vs 19%).[24]

BV is not a sexually transmitted disease but sexual activity is a risk factor for BV.[25] It is a sexually enhanced disease. The other sexually transmitted infections appear to be associated with an increased prevalence of BV.[26] In a systematic review and meta-analysis of studies evaluating the association between BV infection and herpes simplex virus (HSV)-2 infection, women infected with HSV-2 had a 55 per cent higher risk of BV infection compared with women who were HSV-2 uninfected.[27] Similarly, a five-year prospective cohort study reported that BV was both more prevalent and more persistent among HIV-infected women compared withthose without HIV. BV may also be a risk factor for HIV acquisition and transmission.[28] Bacterial Vaginosis also renders women vulnerable to the acquisition of sexually transmitted diseases that include gonorrhoea, chlamydiosis etc.[26]

Some studies found an increased risk for miscarriage in the mid-trimester when Bacterial Vaginosis was diagnosed before 16 weeks of gestation.[25] The findings of these studies support the pathological role of Bacterial Vaginosis in early pregnancy. Bacterial Vaginosis may have adverse effects on early pregnancy and results in various outcomes.[29]

Bacterial Vaginosis can cause subclinical Chorio-amniotic infection with bacteria ascending from the lower genital tract. Vaginal microorganisms found in BV may first ascend into the choriodecidual space. Then spontaneous abortion or preterm labour occurs by a maternal and foetal response to choriodecidual bacterial colonization. Sometimes, bacteria cross the intact Chorio-amniotic membranes and entered into the amniotic fluid, and some of the embryo-foetuses ultimately become infected. Chronic BV may result inintrauterine infection early in pregnancy that becomes symptomatic with contractions and leads to spontaneous abortion.[30]

Despite the belief that Bacterial Vaginosis is a non-inflammatory condition, shreds of evidence explain altered levels of certain pro-inflammatory cytokines in women with Bacterial Vaginosis.[31] Local immune response is responsible for these alterations.Pregnant women with bacterial vaginosis have elevated levels of endotoxin, mucinase, sialidase, and interleukin-1β, suggesting that Bacterial Vaginosis associated microorganisms stimulate the production of cytokines.[32]

The high prevalence of BV during pregnancy attracts the attention to find out its correlation with adverse pregnancy outcome. The purpose of the study is to lookfor the cost-effective, less time-consuming method that can be added to the routine screening for the women who are pregnant and having persistent vaginal discharge to help in reducing adverse pregnancy outcome. Also, screening of BV in fertile women is necessary to prevent spontaneous abortion.

2. Material & Methods

This is a hospital based observational study done on women between the age of 18-39 years, attending Gynaecological Out-Patient Department, with a history of spontaneous abortion 2 to 6 weeks back in SMS Medical College, Jaipur, Rajasthan.

Inclusion criteria

1) Woman with history of spontaneous abortion 2 to 6 weeks back.
2) Participants were chosen between the age of 18-39 years.
3) Participants who were willing to participate in the study.

Exclusion criteria-

1) Women who were pregnant.
2) Woman with history of induced abortion either by medical or surgical method.
3) Women who were taking hormonal contraceptives.

Procedure of Vaginal Sample Collection

Written or informed consent was taken from each patient before subjecting them for sample collection. Ethical clearance was obtained from institutional ethics committee.

For collection, a nonlubricated bivalve speculum was inserted into the vagina, and vaginal swabs were taken from the vaginal walls and posterior fornix with four sterile cotton swabs for pH examination, whiff test, pap's smear, Gram's staining and culture.

Per-speculum examination- Vaginal discharge was examined for colour, form, consistency, amount and appearance. Presence of scanty to profuse grey, white, thin and sticky to vaginal walls, discharge was considered a positive finding for bacterial vaginosis.

pH examination – pH was measured by putting a drop of vaginal sample on a pH strip. pH greater than 4.5 was considered as positive.

Whiff Test – Vaginal sample was smeared on slide and then one drop of 10% potassium hydroxide (KOH) was added to it. Smear with fishy odour were accepted as Whiff (+).

Cytological Examination - The vaginal sample was smeared on slide in one direction, fixed with 90% ethanol. Smears were stained using Papanicolaou (PAP) method & examined by light microscope. Smears satisfying all three of following criteria were diagnosed as Bacterial Vaginosis on pap's smear.

1) Presence of coccobacilli flora.
2) Presence of significant clue cells [At least 20% of the epithelial cells should be clue cells].

3) Absence of lactobacilli flora.

The clinical diagnosis of Bacterial Vaginosis was made by Amsel's criteria. The criteria are as following –

1) Homogeneous, thin, grey or off-white discharge.
2) vaginal pH > 4.5
3) Fish like amine odor when the preparation was mixed with a 10% solution of potassium hydroxide.
4) Presence of significant clue cells in wet-mount preparation(Figure -1)
   (At least 3 criteria must be present for diagnosing Bacterial Vaginosis).[33]

Gram staining-The swab was smeared on to a glass slide then air-dried and later Gram's stained. The Gram-stained slides were examined under oil immersion objective (1000x magnification). The amount of three morphotypes was quantified and scored.[34] (Figure-2-4)

Gardnerella vaginalis is a facultatively anaerobic Gram-variable bacilli. It has a Gram-positive cell wall but, because the cell wall is so thin, it can appear either gram positive or gram negative under the microscope, so it is gram variable. Lactobacillus is gram positive bacilli. In gram staining it appeared as purple blue rods. Other mobiluncus and anaerobic species are gram variable curved bacilli.

We took Nugent scoring system.[34] This is a standardized 0–10points scoring system for evaluation of Gram stained vaginal smears based on three morphotypes: large Gram-positive rods (Lactobacilli), small gram negative/variable rods (G. vaginalis and anaerobic rods) and curved gram variable rod (Mobiluncus species). (Figure-2-4)

Culture- The vaginal sample was obtained and transferred to microbiology laboratory and then cultured on blood agar & Gardnerella selective agar with 5% human blood. These plates were incubated at 37c for 48 hours in 5 to 10% CO2. After 4 days of incubation, the plates were examined for Lactobacillus and Gardnerella, which were identified by assessment of colony.[35]

3. Results

Our study showed that maximum number of recent abortion cases (30) were in the age group of 23- 26 years, no significant association was found between age and BV. 52 women were Hindu and 29 were from Muslim community. Though there was no significant association found between BV and religion as P value is >>0.05, a high ratio of BV women belonged to Muslim community. Mostly women from urban area showed high proportion ofBV.Bacterial vaginosis proportion increases as their socio-economic status decreases. However, the association between occurrence of bacterial vaginosis and socio-economic status found to be statistically insignificant (p>0.05) (table 1).

In our study we didn't find any statistically significant association. Maximum proportion (36%) of BV observed among women who had history of 2nd trimester pregnancy loss (table 2).

In present study, among recent abortion cases who had Bacterial vaginosis, maximum proportion of women had Gardnerella predominant culture report (table 3).While classifying Bacterial vaginosis positive cases of recent spontaneous abortion according to Nugent score (Gram staining), 52.17% cases were positive on both scores and remaining all 47.82% cases showed intermediate results. Similarly, among cases with negative AMSEL score, none was showing AMSEL–ve and Nugent +ve score. 86.2% of AMSEL negative were also negative on Nugent score (table 4).

4. Discussion

The mean age of present study population was 25.53±3.71 years whereas Pippa Oakeshott et al (2002)[36] included pregnant women with mean age 31. The age range of present study cases was 18-39 years whereas Mengistie et al (2014)[37] included the pregnant women between 18 to 40 years age for study.

Bacterial vaginosis (BV) is an extremely prevalent vaginal microbial disorder. BV is the most common cause of vaginitis among both pregnant and nonpregnant women. Around 28.3% of women with a history of recent spontaneous abortion visiting the outpatient department showed Bacterial Vaginosis. The proportion of BV in the present study was 28.3%. Studies have found the prevalence of BV among nonpregnant women ranged from 15 per cent to 30 per cent, and up to 50 per cent of pregnant women have found to have BV. Studies conducted by Jacobsson (2006),[38] Svare,[39] and McGregor et al[40] also found that the prevalence of BV was between 15.6% and 32.5% among pregnant women. According to the United States Public Health Service, the incidence of BV is nearly 30% in females of reproductive age. In the US, BV is a common condition among women, with prevalence varying by race and ethnicity.[12]

Majority of recent abortion cases were Hindu in the present study population (64.2%). There was no statistically significant association observed between religion and BV. But the maximum proportion of Muslim women had BV in the present study; this may be due to poor hygienic behaviour.

Majority 58.02% of women resided in the urban area in the present study. Deborah B Nelson et al (2007)[36] did their study among urban women. Other studies by Klebanoff et al (2008)[14] and Marconi et al (2015)[41] have reported that personal hygienic behaviour such as vaginal douching is associated with BV. Many findings show that education and socioeconomic status of female plays an important role in knowledge regarding vaginal hygiene, adoption of protective measures and to overcome the social stigma in treatment-seeking behaviour. In a study by Bhalla et al (1994)[42] BV showed a positive correlation with low socioeconomic status. Thakur et al (1986)[43] found that colonization with G. vaginalis was more common among women of low socioeconomic status. According to Gravett et al (1986),[44] patient with and without BV did not differ significantly for demographic factors. In the present study, the author also did not find a statistically significant association between

socio- demographic factor and BV. In an article by Jenifer E Allsworth (2007)[12] the prevalence of BV in women between ages 14-49 years in the USA was 24% in women who were above the poverty line, 34% in women at the poverty line or 37% in women below the povertyline.

In the present study, the age of the women did not correlate with BV (p = 0.23). Ranjit et al (2018)[45] revealed that BV was high among women of age group 30–40 years (8.8%) and least for 10–20 and 50–60 years age groups (1.3%). However, the difference between them was not statistically significant as found in the present study. Like the present study, Mengistie et al (2014)[37] recorded the highest prevalence in women aged 21–29 years. Individuals in these age groups are most sexually active and thus at the highest risk of BV. Contrary, Bhattarai (2012)[45] in Nepal observed the highest prevalence of BV among the age group of 31–40 years (60.16%) and least among those below 20 years of age and 51–60 years age group (33.33%). Also, Gravett et al[44] in Nigeria found BV to be most prevalent among 26– 30 years age group (35.8%) and least in >40 years age (10.5%).

Mean Nugent score in the study population was 3.0617±2.2658. Women who had BV according to Gram's staining were 14.82% in the present study. These women had Nugent score 7-10. Women with 4-6 Nugent score were 25.92%. These 25.96% of women had intermediate flora. Jorge E. Tolosa et al (2006)[46] found 12.3% of women had bacterial vaginosis according to Nugent's criteria. S G Ralph et al (1999)[47] found that among study population 29.6% were with normal vaginal flora, 34% with intermediate vaginal flora, and 32.1% with bacterial vaginosis.

Gravett et al (1986)[44] studied the relationship of obstetrical history to BV. Patient with BV did not differ significantly for the past reproductive sequel. But the history of prior first-trimester abortion was more significantly associated with the presence of BV ($p < 0.05$). months. GozdeIsik et al (2016)[48] concluded that BV is more frequent in fertile women with the history of spontaneous abortion in the last 6 months ($P < 0.05$) than the women with recurrent pregnancy losses ($P > 0.05$). But in the present study author observed that the proportion of BV was more in women who had a history of previous 2 abortion and same proportion was also observed in women ≥abortions.

Among the various methods available for diagnosis of bacterial vaginosis, Amsel's criteria are easy to perform and often used by clinicians for establishing clinical diagnosis As India is a developing country so Amsel's criteria seem to be most useful for the low re-source country. In Nugent's method, we require a microscope and microbiologist which may not always be available especially in rural areas. Nugent score is considered as the gold standard method and culture is a specific method where etiological agent G. vaginalis is isolated but has its disadvantages like time, cost and requirement of perfection. In Amsel's criteria, its components are subjective and dependent upon the acuity of the physician. In our study, Amsel's criteria diagnosed 28.3% of females as having bacterial vaginosis. This is different to a study done by Ranjit et al[45] where Amsel's criteria were significant in 40% of subjects.

Nugent score categorized nearly 59.25% women as having normal flora, 25.92% as having intermediate flora and 14.61% as bacterial vaginosis. A study conducted by Madhivanan et al (2008)[49]showed 65.4% had normal flora, 15.4% had intermediate flora and 19.1% had bacterial vaginosis. These proportion of three categories were compared with the results of the present study.About 19.15% of samples grew etiological agent G. vaginalis in the present study, which correlates well with the study by Ranjit et al[45] where culture positivity was 17.42%.

In the present study, among the three methods, Amsel's criteria identified more positives than the other two methods. Detection of positivity by Amsel's criteria was more than a culture which was followed by Nugent scoring. A study by Ranjit et al[45] showed a difference in the order of positives with the same three methods where culture detected more positives followed by Amsel's criteria and Nugent score. That was one among the few studies where culture positivity outnumbered the positives by Nugent methods. Initial difficulties faced in isolation of anaerobes could be the reason for the lower percentage of culture positivity in the present study. Another study by Udayalaxmi et al (2011)[50] which involved a comparison of Amsel and culture with Nugent as gold standard showed culture as the least sensitive method.

5. Conclusion

We concluded that the screening of Bacterial Vaginosis in pregnant women may be used for evaluating women who are predisposed for abortion. However, to be able to use BV screening for routine purpose in determining the poor reproductive outcome, further work and research areneeded.

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Table 1: Association of socio-demographic profile with Bacterial Vaginosis (AMSEL criteria) in recent abortion cases

Parameters	BV Present No. (23)	BV Absent No. (58)	Total	P-value
Age (yrs)				
18-22 years	3 (23.07%)	10 (76.92%)	13	0.7842
23-26 years	7 (23.22%)	23 (76.66%)	30	
26-30 years	9 (36%)	16 (64%)	25	
31-34 years	3 (27.7%)	8 (72.2%)	11	
35-39 years	1 (50%)	1 (50%)	2	
Religion				
Hindus	14 (26.92 %)	38 (73.07 %)	52	0.891
Muslims	9 (31.03 %)	20 (68.96 %)	29	
Residential address				
Urban	15 (31.91%)	32 (68.08%)	47	0.7070
Peri urban	6 (23.07%)	20 (76.92%)	26	
Rural	2 (25%)	6 (75%)	8	
Socio-economic status				
Upper middle	0 (0.00%)	4 (100%)	4	0.342
Lower middle	1 (16.66%)	5 (83.33%)	6	
Upper lower	5 (20.83%)	19 (79.16%)	24	
Lower	17 (36.17%)	30 (63.82%)	47	
History of previous abortion				
1	15 (24.59%)	46 (75.40%)	61	0.4150
2	6 (40%)	9 (60%)	15	
≥3	2 (40%)	3 (60%)	5	

Table 2: Association of Bacterial Vaginosis (AMSEL criteria) according to gestational age in recent abortion cases

Gest. Age	Bacterial Vaginosis according to AMSEL criteria		Total	p-value
	BV Present	BV Absent		
Abortion <12weeks POG	14 (25%)	42 (75 %)	56	0.455
Abortion >12weeks POG	9 (36 %)	16 (64 %)	25	
Total	23 (21.39 %)	58 (71.61 %)	81	

Table 3: Comparison of Bacterial Vaginosis (AMSEL criteria) to culture report

Gest. Age	Bacterial Vaginosis according to AMSEL criteria		Total	p-value
	BV Present	BV Absent		
Lactobacillus predominant	4 (17.39%)	55 (94.82%)	59 (72.83%)	0.0001
Gardnerella predominant	16 (69.56%)	0 (0.00%)	16 (19.75%)	
Mixed	3 (13.05%)	3 (5.18%)	6 (33.32%)	
Total	23 (21.39 %)	58 (71.61 %)	81	

Table 4: Comparison of Bacterial Vaginosis (AMSEL criteria) to Nugent Score in recent abortion cases

Nugent Score * Bacterial Vaginosis according to AMSEL criteria				
Nugent Score	BV Present	BV Absent	Total	P-value
0-3 (normal)	0 (0.00%)	48 (86.20%)	48 (61.73%)	0.0001
4-6 (Intermediate)	11 (47.82%)	10 (13.80%)	21 (23.45%)	
7-10 (Positive)	12 (52.17%)	0 (0.00%)	12 (14.82%)	
Total	23 (100.0%)	58 (100.0%)	81 (100.0%)	

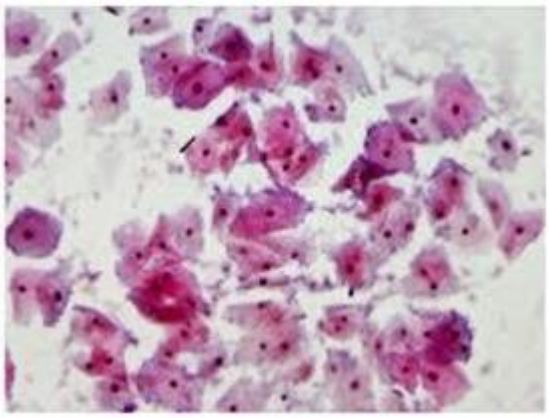

Figure 1: Showing Clue cells inpapsmear

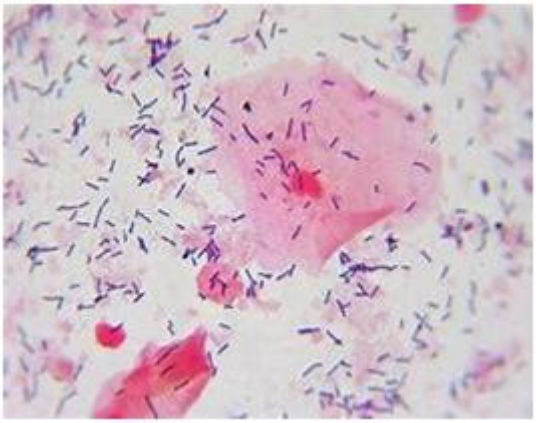

Figure 2: Showing Normalvaginal flora on Gram's staining

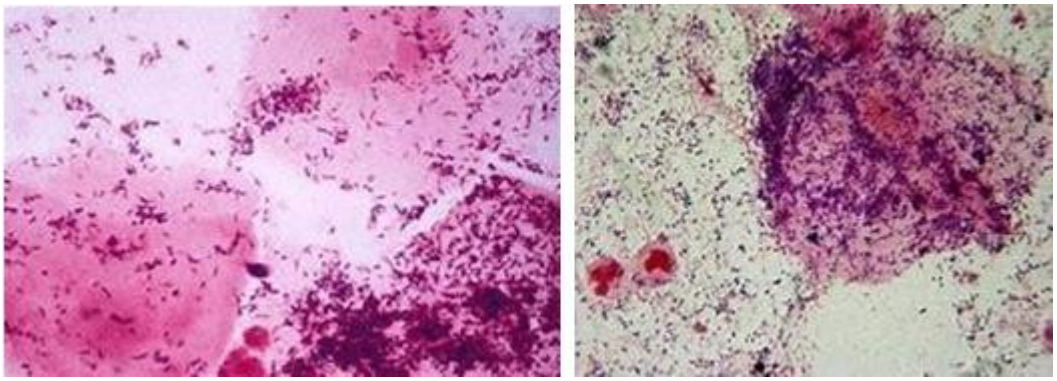

Figure 3 & 4: Showing Bacterial Vaginosis on Gram's staining