Characterization and Identification of Microorganisms Associated with Vaginal Infections in Pregnant Women attending the Ribat University Hospital, Sudan

Rania A Ahmed[1*], Wafa I Elhag[1], Khalid A Abdelhalim[2]

[1]Department of Microbiology, Faculty of Medical Laboratory Sciences, Al-Neelain University
[2]Department of Microbiology, Faculty of Medical Laboratory Sciences, The National Ribat University
Corresponding author: Rania A Ahmed
Email: rania-alser@hotmail.com

Abstract

Background: In pregnancy, vaginal infections can be associated with drastic complications both to the mother and the neonate. It can be easily treated with antibiotics; however, the situation is complicated by the absence of symptoms since patients will not seek medical attention. Identifying the size of infection among antenatal attendees would provide valuable information to health care authorities. Further, unveiling the identity of associated microbes by applying methodologies and criteria that are unusual in clinical practice would point out the importance of following standard protocols in the routine work-up of vaginal specimens. This study aims at characterizing and identifying some microorganisms that are associated with vaginal infections in pregnant women attending the Ribat University Hospital. Accordingly, the prevalence of bacterial vaginosis and vaginal candidacies among these women will be estimated.

Materials and Methods: This is a laboratory based descriptive study. It included 100 pregnant ladies. Two high vaginal swabs were collected from each lady and conventional microbiological assays were applied. Three international standard criteria were used to evaluate the pathogenic role of the isolates. Ethical clearance was obtained from the Ethical Committee Board of Al-Neleen University. Data were analyzed by the statistical package program SPSS version 20.

Results: The prevalence of bacterial vaginosis and vaginal candidiasis in the 100 pregnant women was to 14%, 73% respectively. *Gardnerella vaginalis* was isolated in 14 (14%) samples, *Candida albicans* in 73 (73%) samples, combined infection with *Gardnerella vaginalis* and *Candida albicans* was detected in 3 (3%) samples.

Conclusion: The prevalence of vaginal candidiasis prevails among our population, followed by bacterial vaginosis. Our microbial isolates showed typical biological features, however, the

clinical features did not always concur. Applying international standard clinical criteria supports the diagnosis.

Key words: bacterial vaginosis, vaginal candidiasis, pregnant women.

{**Citation:** Rania A Ahmed, Wafa I Elhag, Khalid A Abdelhalim. Characterization and identification of microorganisms associated with vaginal infections in pregnant women attending the Ribat University Hospital, Sudan. American Journal of Research Communication, 2014, 2(10): 301-309} www.usa-journals.com, ISSN: 2325-4076.

Introduction

The most frequent cause of genital discharge in women is an infection or colonization with different microorganisms; the commonest are *Gardnerella vaginalis* with a combination of mixed anaerobes, *Candida albicans*, and *Trichomonas vaginalis*. *Gardnerella vaginalis*, in combination with mixed anaerobic bacteria, is associated with the unpleasant condition bacterial vaginosis. In the developed world, it is the commonest cause of vaginal discharge, but the prevalence is unknown in the developing area. Its significance arises from being associated with pregnancy complications, preterm labour and post caesarean endometritis.

The vaginal microflora of healthy asymptomatic women consists of a wide variety of anaerobic and aerobic bacterial genera and species dominated by the facultative, microaerophilic anaerobic genus *Lactobacillus* (Witkin, 2007). This role is particularly important during pregnancy because vaginal infection has been claimed as one of the most important mechanisms responsible for preterm birth and perinatal complications (Lidbeck, 1993). There are many different strains of lactobacilli present in the vagina, the most frequent being *L. jensenii*, *L. gasseri*, *L. iners* and *L. crispatus* and there is a wide variation in species and relative numbers of species according to the population studied (Witkin, 2007). During physiological pregnancy, the higher levels of estrogens induce not only a better epithelial tropism but also a positive effect on lactobacillary activity and proliferation due to increased glycogen availability. As pregnancy advances, the genital tract flora becomes progressively more benign, until term. It is particularly difficult to define abnormal genital tract flora in pregnancy (Aroutcheva *et al*., 2001).

No single organism causes bacterial vaginosis; however, there appears to be an independent association between bacterial vaginosis and *Gardnerella vaginalis*, *Mobiluncus spp*., anaerobic Gram-negative rods, and *Mycoplasma hominis*. The exact role and significance that *Mycoplasma hominis* and *Ureaplasma spp*. play in bacterial vaginosis remain uncertain (Aroutcheva *et al*., 2001).

The reason why bacterial vaginosis causes preterm birth or labor in some women, and remains practically asymptomatic and without any complications for pregnancy in others, is still poorly understood (Aroutcheva *et al*., 2001).

Vaginal infection represents one of the most important risks factors for complications of pregnancy such as premature rupture of membrane, preterm labor and birth and perinatal infection. Bacterial vaginosis occurs in up to 20% of pregnant women and has been associated with premature birth and spontaneous abortion (Waites *et al*., 2005). This condition is characterized by a watery discharge with a fishy odor, but half of the women with this infection may be asymptomatic or experience only mild symptoms. Bacterial vaginosis may spontaneously resolve without treatment although most women identified as having bacterial vaginosis in early pregnancy are likely to not have persistent infection later in pregnancy (Larsson *et al*., 1990). Bacterial vaginosis is relatively common, even in populations of women at low risk of adverse events, and as it is amenable to appropriate therapy (McDonald *et al*., 1994).

This study aims at characterizing and identifying some microorganisms that are associated with vaginal infections in pregnant women attending the AL-Ribat University Hospital. Accordingly, the prevalence of bacterial vaginosis and vaginal candidiasis among these women will be estimated.

Materials and Methods

This is a laboratory based descriptive study. It included 100 pregnant ladies. Two high vaginal swabs were collected from each lady and conventional microbiological assays were applied. One was immersed immediately in Amies transport medium and used later for culture and the conventional isolation and identification of *Gardnerella vaginalis* and *Candida albicans*. The second sample was placed in sterile peptone water and used for the measurement of vaginal pH, KOH testing for amine odour, wet mount examination for the presence of clue cells, and/or yeast or hyphae, and direct Gram stain preparations for the evaluation of microbial flora. Three international standard criteria were used to evaluate the pathogenic role of the isolates. Ethical

clearance was obtained from the Ethical Committee Board of Al-Neleen University. Data were analyzed by the statistical package program SPSS version 20.

Results

A total of 100 vaginal swabs were collected from women on labour attending the aforementioned hospital. Two vaginal swabs were obtained simultaneously from each woman. Their age ranged from 18 to 40 years.

Gardnerella vaginalis was isolated in 14 (14%) samples by conventional culture. All the 14 isolates that were identified as *Gardnerella vaginalis* were catalase and hydrolysed sodium hippurate positive. They all grew on the Gardnerella Selective Media producing white, small and smooth colonies. The indirect Gram staining showed small and large Gram negative and Gram variable bacilli. Growth on Blood Agar was masked by different commensal flora, therefore, was not considered.

When applying the criteria of Eschenbach *et al* (1984) for the diagnosis of bacterial vaginosis, 12 (12%) samples fulfilled the 4 criteria (vaginal discharge, fishy odour, alkaline pH, clue cells). 2 (2%) samples fulfilled only 2 criteria and, therefore, findings are considered to be within normal limits.

When applying the scoring system of Nugent *et al* (1991) to determine a normal or abnormal appearance of vaginal flora using Gram stain on all of the 100 samples that were used in this study, 69 (69%) scored 7 to 9 indicating an abnormal flora and therefore meeting the criterion for bacterial vaginosis. However, out of these, *Gardnerella vaginalis* was isolated from 14 (14%).

Candida species was isolated in 73 (73%) samples by conventional culture. Epithelial cells and yeasts were detected on direct wet preparation from all the 73 samples. On direct Gram stain, 55 (55%) samples showed Gram positive round yeast cells and 18 (18%) showed Gram positive elongated yeast cells.

Culture from all 73 samples produced visible growth on Sabourauds agar; 73 (73%) of these were wet, creamy and small colonies. Indirect Gram stain showed Gram positive round and budding yeast cells for all of the isolates. Growth on Blood Agar was masked by different commensal flora, therefore, was not considered.

The germ tube test was positive in 70 (70%) isolates and these were identified as *Candida albicans*, the test was negative in 3 (3%) isolates and these were identified as other *Candida* spp.

To determine whether *Candida* species are becoming pathogenic, 3 criteria were considered; the presence of vaginal discharge, a luxuriant growth on primary plating and the presence of budding yeast cells or pseudohyphae in direct microscopy.

Out of the 73 *Candida* isolates, 40 (40%) fulfilled the 3 criteria; 35 (35%) of these were *Candida albicans*. Out of the 73 isolates; 23 (23%) fulfilled 2 criteria (vaginal discharge, budding yeasts) and it was *Candida albicans.* 16 (16%) fulfilled 2 criteria (vaginal discharge, luxuriant growth) and 10 (10%) of these were *Candida albicans*. Possibly pathogenic *Candida albicans* and other *Candida* spp were isolated from 35% and 2% of apparently healthy women attending AL-Ribat University Hospital care clinics for routine check-up or delivery.

Discussion

Vaginal discharge is one of the most common healthcare problems in women (Kent, 1991). The three leading agents that cause 90% of infectious vaginitis are bacterial vaginosis (mixture of *Gardnerella vaginalis* and anaerobes); fungal infections (*Candida* species); and parasitic infections (*Trichomonas vaginalis*) (Kent, 1991).

Bacterial vaginosis has been repeatedly considered to be the most common vaginal infection in industrialized countries, causing 15 to 50% of all episodes of infectious vaginitis (Sobel, 1990). In addition to the industrialized countries, the studies of Mead *et al* (1993) and Govender *et al* (1996) from the developing world identified 10 to 30% and 52%, respectively, to be the prevalence of bacterial vaginosis in pregnant women. Findings in this study, however, did not concur; we identified 11 to 19% as having bacterial vaginosis. Of the many possible reasons that were reported by Fonck *et al* (2000) for the increased incidence in industrialized countries and the less privileged countries of the developing world is sexual behavior.

We did not find high estimates of bacterial vaginosis in Islamic developed or developing countries, including this country. Therefore, multiple sexual partners and the general sexual behavior may be the major cause. On the other hand, if we considered that most of our population were presenting mild symptoms and therefore our estimates are less, the study of (Mead and colleagues., 1993) identified up to 75% of their reported cases to be asymptomatic. Another interesting study reported 38% as having symptomatic infection among the 206 consecutive subjects surveyed, however, on speculum examination, an abnormal discharge was seen in 91%; 63% of these had microbiologically confirmed infection (Klufio *et al.*, 1995).

Studies performed in developed countries leave no doubt that bacterial vaginosis plays an important role in pregnancy complications and *Gardnerella vaginalis* is still considered to be one of the major bacteria causing this infection (Sullivan *et al*., 2000).

In the present study, success in cultivating *Gardnerella vaginalis* was the major marker that differentiated doubtful from definite findings obtained by the standard criteria. For instance, by applying Nugent *et al* (1991) scoring system we identified 69% out of our total population of 100 as having abnormal vaginal flora and therefore meeting the criterion for bacterial vaginosis. However, *Gardnerella vaginalis* was detectable and cultivable in only 14 (14%).

Having said that, the earlier criteria is of equal importance as it assists in objectively interpreting confusing vaginal smears, though for better assumption, it should be used in conjunction with the culture of the ultimate biological marker *Gardnerella vaginalis*. Modak *et al* (2011), however, reported the presence of clue cells to best correlates with a positive diagnosis by Nugent's score.

Relative to the other causes of infectious vaginitis, episodes of vaginal candidiasis were more common in this population; we identified a prevalence of 73%. This is probably explained by the fact that all participating women were pregnant, and it is known that pregnancy represents a risk factor in the occurrence of vaginal candidiasis (Fonck *et al*., 2000). Candidiasis is associated with an increased risk of delivery complications. In some studies, pregnant women are found more symptomatic than non-pregnant women, but in others a higher prevalence of asymptomatic infections is described during pregnancy. Of course, most our participants were not admitted with clear complains of symptoms.

In a study conducted by Moaiedmohseni *et al* (2012), 36% of women attending antenatal clinics were found to have vaginal infections and Shrestha *et al* (2011) reported 39%. These findings are in full agreement with ours; we identified 70%. Among those having vaginal infections, Moaiedmohseni and colleagues detected candidiasis in 26.4% and bacterial vaginosis in 8%. We also detected candidiasis in 40%, bacterial vaginosis in 14%.

Proper diagnosis and treatment of infectious vaginitis can be difficult if based on clinical symptoms and character of the vaginal discharge alone, nevertheless, it is a common practice in Sudan. Globally, the clinical diagnosis of infectious vaginitis, in addition to the physical exam and the appearance of vaginal discharge, has traditionally relied on tests performed at point of care, such as a microscopic exam using wet mount, a potassium hydroxide amine odour test, and a pH test of vaginal fluid, as well as laboratory diagnosis via cultures and scored Gram stains (Hillier, 1994). In the current study, we intended to apply these internationally known, but locally unknown, standard techniques to demonstrate their usefulness in the routine work-up of vaginal specimens in Sudan.

Conclusion

According to the findings in the present study, we may conclude that; Typical biological features were observed when isolating and identifying, *Gardnerella vaginalis* and *Candida albicans*. Atypical clinical features for diagnosing vaginal infections were common. Combining and applying international clinical criteria and scoring systems support the final diagnosis. Vaginal candidiasis is the top frequent cause of vaginal infections among pregnant women in this study, followed by bacterial vaginosis. Therefore screening for vaginal infections in the routine check-up during pregnancy should be included and large trials are needed to clarify whether screening and treatment of vaginal infections improves maternal and neonatal outcomes. Also further research is needed to improve the sensitivity of existing methods and find reliable low-cost and easy-to-use ones.

References

1. Lidbeck A, Nord CE, (1993). Lactobacilli and the normal human anaerobic microflora. *Clin Infect Dis*. 1993;16(Suppl 4):181–187.
2. Witkin SS, Linhares IM, Giraldo P (2007). Bacterial flora of the female genital tract. Function and immune regulation 21(3):347–354.
3. Aroutcheva A. A. et al (2001). Defense factors of vaginal lactobacilli. Am J Obstet Gynecol. 185, 375–379.
4. Waites KB et al (2005). Mycoplasmas and Ureaplasmas as Neonatal Pathogens. Clin. Microbiol. Rev.;18(4):757-789.
5. Larsson PG, Bergman B, Forsum U, Platz-Christensen J, Pahlson C (1989). Mobiluncus and clue cells as predictors of PID after first-trimester abortion. *Acta obstet Gynecol Scand*.;68:217–229.
6. McDonaldHM, O'Loughlin JA, Vigneswaran R, Jolley PT,McDon- ald PJ (1994). Bacterial vaginosis in pregnancy and efficacy of short course oral metronidazole treatment: a randomized controlled trial. *Obstet-rics & Gynecology*;84:343–8.

7. Eschenbach D A, Bekassy S, Blackwell A, Ekgren J, Hallén A, Wathne B. (1985). The diagnosis of bacterial vaginosis (Working group recommendations). *Scand J Urol Nephrol*:260-261.

8. Nugent RP, Krohn MA, Hillier SL (1991). Reliability of diagnosing bacterial vaginosis is improved by a standardized method of Gram stain interpretation. *J Clinical Microbiol*.;29:297–301.

9. Kent HL (1991). Epidemiology of vaginitis. *Am J Obstet Gynecol*; 165:1168 – 76.

10. Sobel JD (1990). Vaginal infections in adult women. Sexually transmitted diseases. *Med Clin North Am*; 74:1573 – 602.

11. Govender L, Hoosen AA, Moodley J, Moodley P, Sturm AW (1996). Bacterial vaginosis and associated infections in pregnancy. *Int J Gynaecol Obstet*.; 55:23-8.

12. Fonck K, Kidula N, Jaoko W, Estambale B, Claeys P, Ndinya-Achola J (2000). Validity of the vaginal discharge algorithm among pregnant women and nonpregnant women in Nairobi,Kenya. *Sex Transm Infect*; 76:33–8.

13. Klufio CA, Amoa AB, Delamare O, Hombhanje M, Kariwiga G, Igo J (1995). Prevalence of vaginal infections with bacterial vaginosis, Trichomonas vaginalis and Candida albicans among pregnant women at the Port Moresby General Hospital Antenatal Clinic.Faculty of Medicine, University of Papua New Guinea, Port Moresby, Papua New Guinea. Sep; 38 (3):163-71.

14. Sullivan EA, Abel M, Tabrizi S, Garland S, Grice A, Poumerol G. (2003). Prevalence of sexually transmittedinfections among antenatal women in Vanuatu, 1999–2000. *Sex Transm Dis* ; 30:362–6.

15. Mead PB (1993). Epidemiology of bacterial vaginosis. *Am J Obstet Gynecol*; 169:446–9.

16. Modak T, Arora P, Agnes C, Ray R, Goswami S, Ghosh P, Das NK (2011). Diagnosis of bacterial vaginosis in cases of abnormal vaginal discharge: comparison of clinical and microbiological criteria. Department of Dermatology, Medical College and Hospital, Kolkata, West Bengal, India.*J Infect Dev Ctries*. May 28; 5(5):353-60.

17. Sakine Moaiedmohseni; M.D., MPH, Laleh Bashardoost; M.D., Maryam Abbasi (2012). Cervicovaginal Infections during Third Trimester of Pregnancy. Department of Gynecology and Obstetrics, Shahed University of Medical Sciences, Tehran, Iran. Vol. 6, No. 1.

18. Shrestha S, Tuladhar NR, Basnyat S, Acharya GP, Shrestha P, and Kumar P (2011). Prevalence of vaginitis among pregnant women attending Paropakar Maternity and Women's Hospital, Thapathali, Kathmandu, Nepal. *Nepal Med Coll J*; 13(4): 293-296.

19. Hillier SL (1994). Evaluation of Affirm VP microbial identification test for Gardnerella vaginalis and trichomonas vaginalis. *J Clin Microbiol* ; January: 148 – 52.