

Assessing the prevalence of bacterial vaginosis among infertile women of Qom city

Mahdieh Ghiasi, Hoda Fazaeli, Naser Kalhor, Mohsen Sheykh-Hasan, Reza Tabatabaei-Qomi*

Highly specialized Jihad Daneshgahi Infertility Center, Stem cell laboratory,
Qom Branch (ACECR), Qom, Iran.

Received: July 2014, Accepted: November 2014

ABSTRACT

Background and Objectives: Bacterial vaginosis (BV) is a common disorder which happens when the balance of bacterial flora in vagina is disrupted by a shift in concentration of lactobacillus and pathogenic bacteria. It has significant sequelae including increased rates of late miscarriage when diagnosed in early pregnancy, premature rupture of the membranes, endometritis, preterm labour and delivery and tubal factor infertility. So it seems to be necessary to evaluate the prevalence of BV among women with primary infertility.

Material and Methods: All specimens were collected during vagina examination by use of a speculum and swabbing. A sampling swab was introduced into vaginal canal and rotated for at least 8 seconds before withdrawal. The vaginal swabs were examined in standard microbiological analysis including of microscopy, culture and sensitivity examination.

Results and Conclusion: Totally identified Gram positive bacteria were significantly higher in number than the Gram negative bacteria. We found that the prevalence of bacterial vaginosis as 70.34% among infertile women of Qom city. *Staphylococcus aureus* was the most prevalent vaginal pathogen (57.33%) followed by *E. coli* (25.33%). *S. aureus* showed maximum sensitivity to penicillin and gentamicin. It means that fortunately in Qom, this bacterium has not acquired resistance against penicillin yet. So, all physicians must have a high index of suspicion and use readily available screening methods to recognize and treat the patients with infectious vaginitis adequately.

Keywords: Bacterial vaginosis, Microbial culture, *Staphylococcus aureus*, Antibiotic therapy

INTRODUCTION

Bacterial vaginosis occurs by an imbalance in the vaginal bacterial flora but some associated pathogenic effects are evidenced such as increased rates of late miscarriage when diagnosed in early pregnancy, premature rupture of the membranes, endometritis, preterm labour and delivery have been reported (1). It is the most common lower genital tract disorder

among women of reproductive age (pregnant and non-pregnant) and the most prevalent cause of vaginal discharge and malodour (2).

Both aerobic and anaerobic bacteria form the normal vaginal flora, with *Lactobacillus* species being the predominant microorganisms and accounting for greater than 95% of all bacteria present (3). It is proven that lactobacilli provide support against infection, in part by maintaining an acidic pH in the vagina and ensuring hydrogen peroxide is present in the genital environment. In contrast, bacterial vaginosis is a polymicrobial syndrome leading to a decreased concentration of lactobacilli and an increase in pathogenic bacteria, mainly anaerobic or microaerophiles. These organisms include *Gardnerella vaginalis*, *Mobiluncus* species, *Bacteroides* and *Prevotella* species, and *Mycoplasma*

*Corresponding author: Reza Tabatabaei-Qomi
Address: Highly specialized Jihad Daneshgahi Infertility Center, Stem cell laboratory, Qom Branch (ACECR), Qom, Iran.
Tel: +98-25-32700155
Email: taba_1359@yahoo.com

species (4).

Bacterial vaginosis is very common, but its exact prevalence varying widely depending on the patient population and this syndrome can be diagnosed both clinically and microbiologically (5).

There are several factors which increase the possibility of bacterial vaginosis acquisition. It has been associated with smoking, racial origin, vaginal douching and sexual activity while bacterial vaginosis is more common in black women (6), women who smoke (7), women who are sexually active compared with virginal women (8), and those who use vaginal douches (9).

Infertility is the subject of worldwide interest and importance in both clinical practice and research, affecting both men and women in reproductive age. It is defined as failure to conceive within one year despite normal cohabitation (10) or within two years according to the European society for human reproduction and embryology (11), which is proved in 10-15% of all couples.

In-vitro fertilization has basically changed the treatment of infertile couples, as well as profoundly increasing the understanding of human reproduction. Today treatment options and results are promising in most subgroups of infertility, including unexplained infertility, where IVF is used both as a diagnostic and therapeutic tool (12).

Tubal factor infertility is primarily the result of pelvic inflammatory disease (PID) and the main causes of PID, and hence tubal factor, is bacterial vaginosis (13). Generally, infectious vaginitis is a common disorder with significant clinical consequences if left untreated. It seems that screening is a reasonable approach to improve these consequences and may be cost effective. However, all physicians must have a high index of suspicion and use readily available screening methods to recognize and treat the patients with infectious vaginitis adequately (14).

In this study women with primary infertility history were examined in terms of existence of bacteria and candida. Then the cultured bacteria were assessed by use of different antibiotic discs to determine their resistance and sensitivity against antibiotics.

MATERIALS AND METHODS

The study population consisted of 73 samples of high vaginal swab (HVS), collected and analyzed by standard microbiological methods with different

bacterial stains. Couples with explained infertility were excluded from the study. The maximum and minimum ages of patients who participated in this study were 38.5 and 23.1 respectively. Most of the women attending in this study were undergoing IUI (78.1 %) while for rest of them IVF was applied (21.9 %).

Sample collection. After obtaining written consent from patients, all specimens were collected during vaginal examination using speculum. The speculum may be dampened with normal saline before use, but antiseptic cream was not used, since this action may have lethal effect on bacteria and yeasts. After inserting the speculum in vaginal mucus, it should be wiped off with a swab. A sampling swab should then be introduced into vaginal canal and rotated for at least 8 seconds before withdrawal. The vaginal swabs were examined in standard microbiological analysis including microscopy, culture and antimicrobial susceptibility testing.

Isolation and identification. Micro aerophilic and aerobic bacteria and yeast isolated from specimens, were subjected to standard microbiological methods to identify the members of the Enterobacteriaceae family, *Staphylococcus aureus*, *Enterococcus* and yeast. Preparation of Gram stained smear was the selected method for diagnosis of bacterial vaginosis. All specimens were cultured on the EMB agar, blood agar and Muller Hinton agar plates by streaking. Then the plates were incubated aerobically at 37°C for 24-48 hours. Growth characteristics of the isolates were recorded. In many plates, no growth was observed. The cultures were purified by sub culturing and were refrigerated for additional studies. The aerobically isolated bacteria from vaginitis were Gram stained and confirmed by standard biochemical tests such as catalase, oxidase, MSA and hemolysis. Types of *Streptococcus* and *Entrococcus* were determined based on the hemolysis reaction on human blood agar medium. To determine the susceptibility of isolates to antibiotics, all isolated colonies were suspended in nutrient broth and then incubated for overnight. The sterile swab was dipped in the bacterial suspension and then rolled over the surface of the Muller-Hinton agar medium and covered evenly with the bacterial suspension. Different antibiotic discs including ampicillin, ceftriaxon, imipenem, cephalosin, gentamicin, nitrofurantoin, penicillin,

Table 1. The frequency of negative and positive samples of different bacteria spices.

Name of bacteria	Count of positive samples	Count of negative samples
<i>Staphylococcus aureus</i>	43	30
<i>Entrococcus</i>	5	68
<i>Group B Streptococci</i>	2	71
<i>E. coli</i>	19	54
<i>Klebsiella</i>	4	69
<i>Proteus vulgaris</i>	1	72
<i>Pseudomonas aerogenosa</i>	1	72
<i>Serratia</i>	1	72
<i>Candida</i>	1	72

ceftriaxon, streptomycin, vancomycin, clindamycin, erythromycin and oxacillin with definitive distance were placed over the surface of the agar plates.

For candida, amphotricin B, caspofungin, ketoconazol, itraconazol, voriconazole and nystatin antibiotic discs were placed over the surface of the Muller Hilton agar plate.

RESULTS

Totally the identified Gram positive bacteria were significantly higher in number than the Gram negative bacteria. We found that the prevalence of bacterial vaginosis as 70.34% in infertile women of Qom city. The bacterial species isolated from these samples are shown in Table 1.

After applying IVF and IUI procedure, 25.8% of infertile women got pregnant, while 74.2 % of them didn't get pregnant.

E. coli was sensitive to ceftriaxon, imipenem, gentamicin but it is resistant to nitrofurantoin (Table 2); *Staphylococcus aureus* was sensitive to penicilin and gentamicin but it is not resistant to any of the tested antibiotic discs (Table 3).

Proteus vulgaris was sensitive to ceftriaxon, gentamicin but it was resistant to nitrofurantoin, imipenem, cephalosolin and ampicillin. *Serratia* was sensitive to ceftriaxon, imipenem, gentamicin but showed resistance to nitrofurantoin and cephalosolin. *Klebsiella* was sensitive to ceftriaxon, cephalosolin, gentamicin but it was resistant to nitrofurantoin and ampicillin. *Entrococcus* was sensitive to vancomycin and penicilin. *Streptococcus* group B was sensitive to Penicillin, but it was resistant to nitrofurantoin and gentamicin, streptomycin, ceftriaxon and vancomycin.

Candida was sensitive to all the applied antibiotics.

DISCUSSION

Bacterial vaginosis is the most common lower genital tract disorder among women of reproductive age. It is a common cause of malodorous vaginal discharge. It is also associated with sexually transmitted infections and adverse pregnancy outcomes (15). In this study we evaluated the frequency of bacterial vaginosis in women with primary infertility to show the importance of diagnosis and treatment of infectious vaginitis. Also we isolated the bacteria with sub-culturing and assessed their sensitivity and resistance to different antibiotic to find the most effective drug against BV depend on isolated bacteria.

We found that the prevalence of bacterial vaginosis is 70.34% in infertile women in Qom city. It is shown that the prevalence of BV is varying widely depending on the patient population. For instance in studies on private office patients, the prevalence has ranged from 4% to 17%, while in gynaecology clinics (with a higher proportion of low income and uninsured women) it has been 23% (16, 17). In college students, the prevalence has ranged from 4% to 25%, while it has been as high as 61% in women attending sexually transmitted disease clinics (3, 18, 19). In pregnant women, studies have documented similar prevalence rates to those seen in non-pregnant populations, ranging from 6% to 32% (20). A Canadian study of maternity patients reported an overall prevalence of bacterial vaginosis of 14% (21). The environment factor may be main reason for the high rate of vaginosis in our study sine the high temperature or tropical zone is probably the major

Table 2. Resistance and sensitivity of *E. coli* to different antibiotics.

Antibiotic	Resistance	Sensitive	Intermediate
Ampicillin 10	0	0	100%
Ceftriaxon 30	0	100%	0
Imipenem 20	0	100%	0
Cephazolin 30	0	94.8%	5.2%
Gentamicin 10	0	100%	0
Nitrofurantoin 300	94.8%	0	5.2%

Table 3. Resistance and sensitivity of *Staphylococcus aureus* to different antibiotics.

Antibiotic	Resistance	Sensitive	Intermediate
Penicillin 10	0	100%	0
Oxacillin	0	72.1%	27.9%
Vancomycin 30	0	88.4%	11.6%
Erytromycin	0	9.3%	90.7%
Clindamicin	0	83.7%	16.3%
Gentamicin 10	0	100%	0

cause of vaginosis.

In a study by Liversedge *et al.* which the possible association of BV with infertility in women undergoing IVF treatment was assessed, the prevalence of BV was 25.6% which is lower than our finding (22).

The isolated bacteria in this study were as follow: *Enterococcus*, *Staphylococcus aureus*, *Streptococcus B*, *E. coli*, *Klebsiella*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Serratia* and *Candida*. Among these identified bacteria prevalence of *Staphylococcus aureus* and *E. coli* was more than others: 57.33 % and 25.33% respectively. Our results are consistent with a study by Shamim Mumtaz *et al.* (23) which presented *Staphylococcus aureus* and *E. coli* as the most prevalent vaginal pathogens at the ages ranging from 11–60 years. Also in another study (24) the most common pathogens in vaginal infections were *Enterococcus* (31%), *E. coli* (21%) and *Streptococcus pyogenes* (22%).

Lactobacillus belongs to the dominant microbiota of the normal and balanced vaginal ecosystem (25, 26). On the other hand *Staphylococcus aureus* is part of the opportunist pathogenic bacteria that can be isolated from the vaginal microbiota of several hosts (27). It is shown that lactobacillus has an inhibitory potential on *Staphylococcus aureus* growth results from many different factors which compose

a complex and intricate network (27). Since BV is caused by an imbalance of naturally occurring bacterial flora, infection of *Staphylococcus aureus* is not unexpected.

It is shown that approximately 80-92% of yeast infections are caused by *Candida albicans* (28). Surprisingly, in our study infection with *Candida* was lower than expected: 1.33%. Our result is inconsistent with the reported prevalence of *Candida* infection which is approximately 33% of all vaginitis cases (29).

BV is treated with administration of proper antibiotics. In this study we applied different antibiotic discs to find the sensitivity and resistance of identified bacteria and yeast to them.

In terms of *Staphylococcus aureus* we found that the most sensitivity is against penicillin and Gentamicin which is not supported by a research carried out in Pakistan that showed *S. aureus* has gradually acquired resistance towards penicillins while only 26.3% of the *S. aureus* were sensitive to ampicillin and amoxicillin only. In most cases, resistance to penicillin is attributable to β -lactamase production. Therefore, penicillin in combination with one of the β -lactamase inhibitors gives much better results (30). It means that fortunately in Qom this bacterium has not acquired resistance to penicillin yet.

In conclusion, the high prevalence of bacterial infection among women with primary infertility demands that the all the patients attending in infertility treatment centers be investigated thoroughly.

REFERENCES

- Hay PE, Lamont RF, Taylor-Robinson D, Morgan DJ, Ison C, Pearson J. Abnormal bacterial colonization of the genital tract and subsequent preterm delivery and late miscarriage. *BMJ* 1994; 308: 295–298.
- Flery FJ. Adult vaginitis. *Clin Obstet Gynecol* 1987; 24:407–438.
- Spiegel CA, Amsel R, Eschenbach D, Schoenknecht F, Holmes KK. Anaerobic bacteria in nonspecific vaginitis. *N Engl J Med* 1980; 303:601–607.
- Hillier SL. Diagnostic microbiology of bacterial vaginosis. *Am J Obstet Gynecol* 1993;169: 455–459.
- Yudin MH, Money DM; Infectious Diseases Committee. Screening and management of bacterial vaginosis in pregnancy. *J Obstet Gynaecol Can* 2008; 30: 702- 716.
- Goldenberg R, Klebanoff M, Nugent R, Krohn M, Hillier S, Andrews W; for the Vaginal Infections and Prematurity Study Group. Bacterial colonization of the vagina during pregnancy in four ethnic groups. *Am J Obstet Gynecol* 1996; 174:1618–1621.
- Jonsson M, Karlsson R, Rylander E, Gustavsson A, Wadell G. The associations between risk behavior and reported history of sexually transmitted diseases, among young women: a population based study. *Int J STD AIDS* 1997;8: 501–505.
- Bump R, Buesching III WB. Bacterial vaginosis in virginal and sexually active adolescent females: evidence against exclusive sexual transmission. *Am J Obstet Gynecol* 1988; 158: 935–939.
- Hawes SE, Hillier SL, Benedetti J, Stevens CE, Koutsky LA, Wolner-Hanssen P, et al. Hydrogen peroxide-producing lactobacilli and acquisition of vaginal infections. *J Infect Dis* 1996; 174: 1058–1063.
- Zabrek EM. Can I get pregnant? The basic infertility workup. *Clin Obstet Gynecol* 1996 ; 39: 223-230.
- Forti G, Krausz C. Clinical review 100: Evaluation and treatment of the infertile couple. *J Clin Endocrinol Metab* 1998; 83: 4177-4188.
- Leanza V, Coco L, Grasso F, Leanza G, Zarbo G, Palumbo M. Unexplained infertility and ovulatory induction with menopausal gonadotropins. *Minerva Ginecol* 2014; 66: 303-307.
- Rasheed M, Salah, Abdelmonem M. Allam, Amin M. Magdy, Abeer Sh. Mohamed. *Eur J Obstet Gynecol Reprod Biol* 2013; 167: 59–63.
- Michael F. Rein and Bryan A. Liang. Diagnosis and treatment of infectious vaginitis. *Hospital Physician* 1999; 46 – 58.
- Jean-Pierre Menard. Antibacterial treatment of bacterial vaginosis: current and emerging therapies. *Int J Womens Health* 2011; 3: 395-305.
- Bump RC, Zuspan FP, Buesching WJ 3rd, Ayers LW, Stephens TJ. The prevalence, six-month persistence, and predictive values of laboratory indicators of bacterial vaginosis (nonspecific vaginitis) in asymptomatic women. *Am J Obstet Gynecol* 1984; 150: 917–924.
- Hill LH, Ruperalia H, Embil JA. Nonspecific vaginitis and other genital infections in three clinic populations. *Sex Transm Dis* 1983; 10:114– 118.
- Eschenbach DA, Hillier S, Critchlow C, Stevens C, DeRouen T, Holmes KK. Diagnosis and clinical manifestations of bacterial vaginosis. *Am J Obstet Gynecol* 1988; 158: 819–828.
- Embree J, Caliendo JJ, McCormack WM. Nonspecific vaginitis among women attending a sexually transmitted diseases clinic. *Sex Transm Dis* 1984; 11: 81–84.
- Meis PJ, Goldenberg RL, Mercer B, Moawad A, Das A, McNellis D, et al. The preterm prediction study: significance of vaginal infections. *Am J Obstet Gynecol* 1995; 173: 1231–1235.
- Wenman WM, Tataryn IV, Joffres MR, Pearson R, Grace MGA, Albritton WL, et al. Demographic, clinical and microbiological characteristics of maternity patients: a Canadian clinical cohort study. *Can J Infect Dis* 2002; 13: 311–318.
- Liversedge NH, Turner A, Horner PJ, Keay SD, Jenkins JM, Hull MG. The influence of bacterial vaginosis on in-vitro fertilization and embryo implantation during assisted reproduction treatment. *Hum Reprod* 1999; 14: 2411-2415.
- Mumtaz S, Ahmad M, Aftab I, Akhtar N, ul Hassan M, Hamid A. Aerobic vaginal pathogens and their sensitivity pattern. *J Ayub Med Coll Abbottabad* 2008; 20:113- 117.
- Khan I, Khan UA. A hospital based study of frequency of aerobic pathogens in vaginal infections. *J Rawal Med Coll* 2004; 29:22- 25.
- Reid, G., Bruce, A.W. Urogenital infections in women: can probiotics help? *Postgrad Med J* 2003; 79, 428– 432.
- Aslim, B., Kilic, E. Some probiotic properties of vaginal lactobacilli isolated from healthy women. *Jpn J Infect Dis* 2006; 59: 249–253.
- Charlier C, Cretenet M, Even S, Le Loir Y. Interactions between *Staphylococcus aureus* and lactic acid bacteria: an old story with new perspectives. *Int J Food Microbiol* 2009; 131:30-39.
- Mibbard LT, Snyder WJ. Subgluteal and retrosoas infections in obstetrical practice. *J Obstet Gynecol* 1972; 39: 137- 139.
- Kent HL. Epidemiology of vaginitis. *Am J Obster Gynecol* 1991; 165: 1168-1176.
- Hafiz S, Hafiz AN, Ali L, Chughtai AS, Memon B, Ahmad A, Hussain S, et al. Methicillin resistant *Staphylococcus aureus*; a multicentre study. *J Pak Med Assoc* 2002; 52: 312–315.