Objective: To determine the prevalence of Bacterial Vaginosis (BV) in pregnant women using a simple clinical diagnostic approach. Method: The clinical criteria developed by Amsel’s and colleagues were used as a reference standard for accurate diagnosis of Bacterial vaginosis (BV) infection. This is based on the presence of the following clinical signs: 1) Homogeneous thin, white vaginal discharge 2) Presence of clue cells (greater than 20%). 3) Positive amine (whiff) test. 4) Vaginal pH > than 4.5. The presence of any three of the four clinical criteria was considered diagnostic for BV. Samples were collected from 75 pregnant females, irrespective of their gestational duration, presenting with vaginal discharge at the antenatal clinic of Jinnah Hospital, Lahore. Results: BV was diagnosed in 14 females giving an incidence of 18.7%. The most common symptom was a thin white homogeneous vaginal discharge seen in 27(36%) women. The other diagnostic clinical signs observed were the presence of clue cells on wet mount 9(12%), positive “whiff” test 13(17.3%), and elevated pH>4.5 in 18(24%) women. Microscopic analysis of vaginal secretion revealed diminished polymorph epithelial ratio 19(25.3%) and loss of normal vaginal Lactobacilli 22(29.3%). The other associated findings were candidiasis seen in 16(21.3%) women, while Trichomonas infection in only one woman. These findings were confirmed on Gram staining. Gardnerella vaginalis 9(12%) and Gram variable organisms (Mobiluncus) 11(14.7%) were also identified by Gram smear of vaginal discharge. Conclusion: The clinical diagnosis of BV infection can be established by identifying three of Amsel’s four clinical criteria. In most cases it provides a rapid inexpensive and accurate diagnosis. Strategies should be planned to screen and treat women with BV so as to prevent adverse Obstetric outcome associated with it.

Keywords: Bacterial vaginosis (BV), Gardnerella vaginalis (G. vaginalis), Pelvic Inflammatory Disease (PID).

Bacterial vaginosis (BV) a non-inflammatory condition is currently the most prevalent and potentially serious vaginal infection to affect women of reproductive age. Previously known as non-specific vaginitis or Gardnerella vaginitis, BV may be responsible for up to half cases of vaginitis in all women and 10-30% of cases in pregnant women. As many as 16% of pregnant women in the United States are reported as having BV infection. About 90% of women infected with sexually transmitted diseases may also have BV.

This clinical syndrome is now recognized as a polymicrobial superficial vaginal infection involving loss of protective hydrogen per oxide producing Lactobacilli and overgrowth of anaerobic and aerobic microbes. The exact pathogenesis of BV is still unknown, however, several mechanisms have been implicated to facilitate growth of potential pathogens. Hormonal mechanism probably plays a role as this condition affects female of reproductive age. BV associated discharge contains succinate, sialidase and other substances that promote proliferation of anaerobes. Bacteria associated with BV appears to produce mucolytic enzymes that facilitates their passage through the cervical mucosal barrier into upper genital tract. Common agents of BV includes Gardnerella vaginalis, Mobiluncus, Bacteroides, Mycoplasma homis, group B Streptococcus and Enterococcus.

About half of the women with BV may be asymptomatic. Typical symptoms however, include a profuse malodorous vaginal discharge often with an unusual or fishy/foul odour. Less common symptoms include recurrent urinary tract infection, mild vulval burning and/or pruritus. When upper genital tract is involved classical symptoms of pelvic inflammatory disease (PID), dysfunction uterine bleeding and new onset painful periods may be experienced.

Although asymptomatic and elusive in origin BV is associated with significant gynaecological and obstetric complications as well as risk of contracting HIV and other sexually transmitted diseases. Observations studies have constantly shown an association between BV and adverse obstetric outcome including preterm labour, premature rupture of membranes, miscarriages, spontaneous abortions, post-partum endometritis and PID. This epidemiological evidence has been used as a rationale for screening pregnant women for BV.

Since BV may be a marker for adverse pregnancy outcome rather than a positive factor a growing number of experts recommend routine testing of vaginal discharge for identification as treatment may reduce the risk of pre-term births and its consequences. The present study was conducted to determine the prevalence of BV in pregnant women using a simple clinical diagnostic approach so as to plan strategies to reduce morbidity and mortality risks associated with it.

Materials and methods
This study was conducted on 75 pregnant women, irrespective of their gestational period presenting with vaginal discharge in the antenatal clinic of Jinnah Hospital, Lahore. Patients who had taken antimicrobial therapy in the previous 7 days or had had unprotected sexual intercourse in the preceding 24 hours were excluded from this study.
A detailed history including preterm births, premature rupture of membranes, spontaneous and dysfunctional bleeding was taken. Age, parity, urinary complaints, mild vulvar burning/itching and colour, odor, consistency of vaginal discharge was also noted.

The clinical criteria developed by Amsel’s et al.11 was used as a reference standard for clinical signs of bacterial vaginosis. These criteria listed from highest to lowest predictive value included:

- Clue cells on wet mount > 20% (clue cells are vaginal epithelial cells that have a stippled appearance due to adherent coccobacilli)
- Positive Potassium Hydroxide test for volatile amines (whiff test) A drop of 10% potassium hydroxide solution when added to the vaginal secretions results in release of amines (trimethylamine and cadaverine) by the mixed vaginal flora referred to as ‘whiff test’.
- An elevated pH > 4.5 (accurately measured with pH paper)
- Homogeneous thin coat white vaginal discharge with a malodorous fishy odour. The presence of any 3 of the four signs were considered diagnostic for BV.

Samples of discharge were collected on sterile cotton swabs from the posterior vaginal fornix using a non-lubricated Cusco’s speculum.

Results
Out of a total of 75 pregnant females evaluated using Amsel’s clinical diagnostic criteria, BV was found in 14 women giving an incidence of 18.7%.

BV was diagnosed by the presence of 3 of the four clinical and microscopic findings listed in table 1. The most common symptom was a thin homogeneous vaginal discharge seen in 27(36%) of women. Other important diagnostic signs observed were the presence of clue cells on wet mount 12% and positive amine (whiff test) 17.3%, while elevated pH > 4.5 in 24% women. (Table I)

Table I. Analysis of Vaginal Discharge by Amsel’s Criteria

<table>
<thead>
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<th>Feature</th>
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<tr>
<td>Homogenous Vaginal discharge</td>
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<td>36</td>
</tr>
<tr>
<td>pH &gt; 4.5</td>
<td>18</td>
<td>24</td>
</tr>
<tr>
<td>Whiff test</td>
<td>13</td>
<td>17.3</td>
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<tr>
<td>Clue cells</td>
<td>9</td>
<td>12</td>
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A microscopic analysis of wet smear made from vaginal discharge revealed Candidiasis in 16(21.3%) women but Trichomonas was seen in only one (1.33%) women. Loss of Lactobacilli and diminished polymorph epithelial ratio was observed in 22(29.3%) and 19(25.3%) women respectively. (Table II)

Table II. Microscopic evaluation of vaginal discharge

<table>
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<tr>
<td>Trichomonas vaginalis</td>
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<td>1.3</td>
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<tr>
<td>Yeast Cells (Candida)</td>
<td>16</td>
<td>21.3</td>
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<tr>
<td>Polymorph &lt; Epithelial cells</td>
<td>19</td>
<td>25.3</td>
</tr>
<tr>
<td>Diminished lactobacilli</td>
<td>22</td>
<td>29.3</td>
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</table>

Gram staining performed on all vaginal secretions showed G. vaginalis in 9(12%) and Mobiluncus in 11(14.5%) women. (Table III)

Table III. Gram staining analysis

<table>
<thead>
<tr>
<th>Feature</th>
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<tr>
<td>Diminished Lactobacilli</td>
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<td>Polymorph &lt; Epithelial cells</td>
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<td>Yeast Cells (Candida)</td>
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<tr>
<td>Gardnerella vaginalis</td>
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<td>12</td>
</tr>
<tr>
<td>Mobiluncus</td>
<td>11</td>
<td>14.7</td>
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</table>

Discussion
BV is potentially a serious non-inflammatory vaginal infection that describes an imbalance in the normal vaginal bacterial flora characterized by a decrease in Lactobacilli and increase in G. vaginalis, Mycoplasma and anaerobic bacteria. It is a common cause of vaginal discharge and has been associated with adverse pregnancy outcome4,5,7,10.

The true prevalence of BV in the community is unknown however, an accurate diagnosis based on Amsel’s clinical criteria has been described by different authors to identify this condition4,5,7-11. Studies in medical centers and public hospitals found that 9 – 23% of pregnant women have BV.17

In our study on pregnant women, BV was observed in 14(18.7%) of women. More or less similar results were given by other workers4,5,7,9,11. Eschenbach et al.5 reported 15% incidence whereas rates in perinatal or obstetrics clinics vary from 10-26% (Thomson unpublished data).10

Higher incidence (29.3%) of BV was reported among pregnant women by Crowley et al.4. Rates of 23-29% have been reported in other gynaecological clinics as well10.

In our study the most common sign was a thin homogeneous vaginal discharge observed in 27(36%) women. This has consistently been reported by women with BV in various studies4,5,7,11. However, the amount and physical appearance of vaginal discharge are difficult to evaluate objectively as it may appear normal and can be affected by recent intercourse and douching4,5,6,10.

Vaginal pH is considered more sensitive but least specific character of Amsel’s criteria because it can be influenced by various factors such as vaginal bleed or recent intercourse. Hence the use of pH alone cannot be used as an indicator for BV10,18. Studies of Thomson et al.10 and Colding et al.18 have shown that use of only two of four objective signs i.e., clue cells and positive whiff test also allow accurate and rapid diagnosis of BV without sacrificing sensitivity.

Clue cells are considered the most sensitive and specific sign for BV especially when seen in wet smear of vaginal secretion5,7,10,11. In our study clue cells were identified in 9(12%) patients both on wet preparation and Gram Smear. However the use of clue cells as a single diagnostic criteria is limited to skilled microbiologist only.10
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In our study whiff (amine) test was positive in 13(17.3%) women with vaginal discharge but it was also positive in the woman who had Trichomoniasis. This correlation has been observed by other workers as well. Whiff test is also reliable and in the absence of microscope provides a specific and relatively sensitive method of diagnosis of BV. According to Thomason et al, the amine test alone predicts the diagnosis of BV accurately in 94% patients.

The other associated findings on microscopic evaluation of vaginal discharge were loss of Lactobacilli in 22(29.3%) women and diminished polymorph < epithelial cell ratio seen in 19(25.3%) women in our study. This has been reported in several similar studies done on BV patients.

Candida 16(21.3%) and Trichomonas 1(1.3%) were also encountered in the vaginal discharge of our patients. Farrukh et al gave an incidence of Candida in 11.9% and Trichomonas in 2.38% patients, which is similar to our study.

Another diagnostic criterion utilizes Gram staining of vaginal secretions. The loss of Lactobacilli and increase in G. Vaginalis and curved Gram variable rods (Mobiluncus) when combined with pH, correlates well with Amsel's criteria. Gram staining of vaginal secretions in our study revealed G. vaginalis in 9(12%) and Mobiluncus in 11(14.7%) females. Gram stain may not be useful in determining eradication of the infection because of its high proportion of indeterminate results. Hence diagnosis by Gram staining has not been adopted universally. Even cultures are not recommended as a diagnostic tool because their predictive value is believed to be less than 50%.

Prevalence studies indicate that there is a potentially large reservoir of BV infection in the population. As fifty percent of patients with BV may be asymptomatic or produce few symptoms, serious infectious sequelae occurs in women who have this disease. It is not a new syndrome but rather a disease which has finally been recognized as the most common type of vaginal infection. While the pathogenesis and transmission of BV is not completely understood it is now possible to establish diagnosis on the basis of Amsel’s clinical criteria which provides a simple, rapid, inexpensive and accurate diagnosis. As BV is common and elusive, screening and effective therapies available could help to reduce the mobility and mortality risks associated with it in pregnant women.

References
8. Hillier SL. The vaginal microbial ecosystem and resistance to HIV. AIDS Res Hum Retroviruses 1995; 14(1); 17-21