Original Article

Vaginal infections among pregnant women at Omdurman Maternity Hospital in Khartoum, Sudan

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Abstract

Introduction: Microbial infections of the vagina in pregnant women are health problems that lead to serious medical complications and consequences. This study aimed to investigate and determine antimicrobial susceptibilities of the causative agents of vaginal infections in pregnant women.

Methodology: A cross-sectional study of pregnant women (n = 200) was conducted between August and December 2008 at Omdurman Maternity Hospital, Khartoum, Sudan. Vaginal and cervical swabs were obtained from each subject and processed for isolation and identification of pathogenic microorganisms using standard methods of wet mount preparation, direct Gram smear, Nugent scoring system, direct immunofluorescence, and cultural techniques. Antimicrobial susceptibility testing of bacterial isolates was performed using standard procedures. Statistical analysis was done using SPSS program version 12.0.1. A p value < 0.05 was considered statistically significant.

Results: Of the 200 pregnant women enrolled, BV was detected in 49.8%, followed by *Chlamydia trachomatis* (31.3%) and *Candida albicans* (16.6%), with low frequencies of *Neisseria gonorrhoeae* (1.8%) and *Trichomonas vaginalis* (0.5%). Higher infection rates were recorded among subjects in the third trimester (71.6%) than in the second trimester of gestation (28.4%). No significant association (p = 0.7) between history of abortions and *C. trachomatis* infections was found. Gentamicin was the most active agent against Gram-positive and Gram-negative bacteria. Clarythromycin was the most active against *Mycoplasma* species.

Conclusions: Pregnant women with vaginal complaints revealed various positive microbiology results. Such cases may require specific medication. Routine culture of vaginal and cervical samples should be performed on all pregnant women during prenatal visits.

Key words: vaginitis; vaginosis; pregnant women; antimicrobial susceptibility; Sudan

Introduction

Microbial infections of the vagina (vaginosis and vaginitis) among pregnant women are serious problems because they can lead to serious medical complications such as preterm labor, amniotic fluid infection, premature rupture of the fetal membranes, and low birth weight of the neonate [1], leading to high prenatal mortality [2]. However, proper identification and treatment will reduce the risk of preterm birth and its consequences [3].

The vagina could be infected by a variety of pathogens including bacteria, fungi, viruses, and parasites [1,2]. Bacterial vaginosis (BV) is the most frequent vaginal infection, characterized by the replacement of *Lactobacillus* species of normal vaginal flora by the excessive growth of a mixture of microorganisms including *Gardnerella vaginalis*, *Bacteroides* species, genital mycoplasma, and fastidious anaerobic bacteria [4].

Vaginal complaints such as BV, candidiasis, trichomoniasis, and *Chlamydia trachomatis* infections are common among women of reproductive age, with high incidences during pregnancy [5,6]. For unknown reasons, they are more prevalent in women in sub-Saharan Africa and other low-income countries than in women in developed countries [4], affecting up to 55% of women in some studies [4]. In Sudan, there is little data about vaginal infections in pregnant women [7,8,9]. BV has been found in 17.2% of subjects, candidiasis in 10.1%, trichomoniasis in 7.7%, gonorrhoeae in 1.2%, HIV in 1.2%, and syphilis in 0.9% of Sudanese women examined in the El Obeid region [8].

Vaginal conditions could be diagnosed using known Amsel's clinical criteria including vaginal

discharge appearance, pH measurement, whiff test, and clue cell on microscopy [10]; however, these requirements alone cannot identify the cause of vaginal illness [11]. According to the Centers for Disease Control and Prevention, there are various guidelines can be used for the diagnosis of vaginal infections, such as wet mount microscopy, direct Gram staining, potassium hydroxide preparation, routine culture, Papanicolaou smear, or DNA probes [12]. This study aimed to investigate the causative agents of vaginal infections and antimicrobial susceptibilities of the isolates from pregnant women at Omdurman Maternity Hospital in Khartoum, Sudan, using different laboratory techniques.

Methodology

Study design

This was a cross-sectional study carried out at Omdurman Maternity Hospital in Khartoum, Sudan, between August and December 2008. Pregnant women (n = 200) between 17 and 47 years of age suspected of having symptomatic and asymptomatic vaginal infections were enrolled during routine prenatal visits to the gynecology and obstetrics clinic. Patients' data, which included age, location, previous history of abortions, last menstrual period, and clinical signs and symptoms, were obtained from patients' referral files and laboratory request forms. Pregnant women in the third and second trimester of gestation were included in the study, while those in the first trimester were excluded to avoid abortion cases. The study was approved by the Research Council Board of the Faculty of Medical Laboratory Science, Khartoum University.

Sample collection and transportation

Vaginal and cervical swabs samples were obtained from each subject using the standard sampling technique for microbiological investigation described by Onderdonk *et al.* [13]. Three high vaginal swabs were collected from each patient by a gynecologist and placed into Amies transport medium. One cervical swab was taken to prepare the smear on a sterile glass slide for the detection of *C.trachomatis* using the immunoflourescence technique, and the remaining swab sample was placed into a mycoplasma transport medium R1 for transportation. Once the samples were obtained, they were transported within three hours to the Microbiology Laboratory Department at Faculty of Medical Laboratory Sciences, University of Khartoum.

Sample processing

After the samples were submitted to the microbiology laboratory, they were processed immediately for possible isolation and identification of pathogenic microorganisms in accordance with standard laboratory methods [14,15].

Of the three high vaginal swabs obtained from each patient, one swab sample was inoculated immediately onto blood agar (Oxoid, Basingstoke, UK), MacConkey agar (Oxoid, Basingstoke, UK) plates, Sabouraud agar (Oxoid, Basingstoke, UK) and incubated aerobically at 37ºC for 48 hours, while the inoculated chocolate blood agar plate was incubated aerobically with 20% CO_2 moist atmosphere. The second sample was placed into a sterile universal container containing 10 mL of freshly prepared cooked meat medium for anaerobic isolation. The third sample was used for direct microscopic examination of *Trichomonas vaginalis*. The cervical samples were processed for the detection of *C. trachomatis* and *Mycoplasma* species.

Diagnosis of vaginosis

BV was diagnosed based on Amsel's clinical criteria [10] and standard microbiological techniques [14,15].

In Amsel's methods, three of the four criteria should be recorded: a vaginal pH of greater than pH 4.5; the presence of clue cells in the vaginal fluid; a milky, homogeneous vaginal discharge; and the release of a fishy odor after the addition of 10% potassium hydroxide to the vaginal fluid [10].

Microscopic examination

A wet preparation of vaginal swab samples were examined microscopically for the presence of yeast cells, clue cells, and *T. vaginalis* [15]. Direct Gram stain smears were then prepared for diagnosis of BV by the Nugent scoring system [16]. The Gram smear was examined for the following morphotypes: large Gram-positive rods (*Lactobacillus* morphotypes), small Gram-variable rods (*Gardnerella vaginalis* morphotypes), small Gram-negative rods (*Bacteroides* species morphotypes), curved Gram-variable rods (*Mobiluncus* species morphotypes), and Gram-positive cocci. Increased numbers of Gram-positive cocci are not part of the pattern of the normal vaginal flora. A score of zero to three was considered to be normal, and a score of four to ten was defined as BV (Table 1). In case of *Neisseria gonorrhoeae*, intracellular Gram-negative diplococci were examined [15].

Table 1: Scoring system (0–10) for the Gram-stained vaginal smears*

Curved Gram-negative variable rods	*Gardnerella* and *Bacteroides* species morphotypes	*Lactobacillus* morphotypes	Score
0	0	4+	0
1+ or 2+	1+	3+	1
3+ or 4+	2+	2+	2
	3+	1+	3
	4+	0	4

1+ ≤ 1/1000× field; 2+ = 1–5/1000× field; 3+ = 6–30/1000× field; 4+ ≥ 30/1000× field

*adapted from Nugent *et al.* (15=1991)

Identification of genital microorganisms

Isolated bacteria were identified by conventional methods [14,15] including Gram stain, colonial morphology, germ tube test, motility, and biochemical tests such as catalase, coagulase, DNAse, Indole, lactose fermentation, citrate utilization, urease, Kligler iron, oxidase, sugar fermentation, and automated system (Vitek II, bio-Mérieux, Marcy l'étoile, France). The identification of the Lancefield antigen was done using the Streptococcal Grouping Kit (Oxoid, Basingstoke, UK).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of isolates was performed on Mueller-Hinton agar plate (HiMedia, Mumbai, India) by the Kirby-Bauer disk diffusion method following the National Committee for Clinical Laboratory Standards Interpretative (CLSI) criteria [17]. The antimicrobial agents tested were amoxicillin-clavulanic acid (30 µg), penicillin G (10 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cefuroxime (30 µg), cephalothin, co-trimoxazole (25 µg), ciprofloxacin (5 µg), gentamicin (10 µg), imipenem (10 µg), kanamycin (30 µg), metronidazole (5µg), piperacillin (100 µg), tetracycline (30 µg), and vancomycin (30 µg) (Oxoid, Basingstoke, UK). Standardized inoculum adjusted to 0.5 McFarland standard turbidity of each isolate was inoculated onto Mueller-Hinton agar plates using a sterile cotton swab by streaking the swab over the entire sterile agar surface three times. Sterile forceps were used to place the appropriate antibiotic disks on the inoculated plate. The plates were incubated aerobically at 37°C overnight, and then tests were read. The control strain was tested each time when susceptibility testing was performed. Test results were only validated in the cases where inhibition zone diameters of the control strains were within performance ranges in accordance with CLSI guidelines [17].

Identification and susceptibility of Mycoplasma species

Mycoplasma IST 2 strip diagnostic kit (bioMerieux, Marcy l'Etoile, France) was used for identification and determination of antimicrobial susceptibility of *Ureaplasma urealyticum* and *Mycoplasma hominis*. The test kit was used according to the manufacturer's instructions. Cervical swab samples were placed into R1 transport medium and mixed; 3 mL of R1 was then transferred to the mycoplasmaR2 vial to reconstitute the lyophilized growth medium. ThemycoplasmaIST 2 strip containing 22 test wells was inoculated with the reconstituted medium (mycoplasmaR1 + mycoplasma broth R2). An adjusted 55 µL volume of the medium was dispensed into each of the 22 test wells, then two drops of mineral oil were added on top of each well, and the lid was placed on the strip and incubated together with the remaining inoculum in the mycoplasmaR2 vial at 37°C. The results were interpreted after 24 and 48 hours of incubation. The strip containing 22 tests was evaluated after being divided into three sections: well 1 as growth control, wells 2 to 5 identified the presence of *M. hominis* and *U. urealyticum* and determined growth count (equals to or higher than the threshold set at 10^4 colony-forming units), and the remaining wells, 6 to 22, were used to test the susceptibility of the identified strains against nine antibiotics (in two different concentrations) including doxycycline (4 and 8 mg/L), josamycin (2 and 8 mg/L), ofloxacin (1 and 4 mg/L), erythromycin (1 and 4 mg/L), tetracycline (4 and 8 mg/L), ciprofloxacin (1 and 2 mg/L), azithromycin (0.12 and 4 mg/L), clarythromycin (1 and 4 mg/L), and pristinamycin (2 mg/L).

Direct fluorescence test for the detection of Chlamydia trachomatis

Chlamydia DFA kit (bioMérieux, Marcy l'étoile, France) was used to detect *C. trachomatis* serovars A, B, Ba, C, D, E, F, G, H, I, J, K, LGV1, LGV2, and

LGV3. The cervical swab smear was prepared on a sterile class slide. The smear was allowed to air dry, then fixed in methanol for 5 minutes. It was then drained and air dried and stained following the manufacturer's instructions. Briefly, a total volume of 25 µL of chlamydial DFA reagent was dispensed onto the fixed smear and the positive control; the slides were then incubated in a moist chamber for 30 minutes in the dark, then rinsed gently in a bath of phosphate buffer saline for approximately 1 minute. The excess moisture around the well was removed using absorbent tissue, one drop of mounting fluid was added to the well, and a glass cover was then placed gently to avoid air bubbles. The entire sample was then scanned using a fluorescence microscope after adding oil immersion at ×600 to ×1000 magnification.

Intracellular elementary bodies were detected within the cells as bright, apple-green fluorescent pinpoint, smooth-edged disk-shaped bodies against a red background of counterstained cells and were seen extracellularly as a bright apple-green fluorescence against a black background. Inclusion bodies were also seen. A fixed smear free of any chlamydial bodies but containing cells indicated negative results.

Statistical analysis

Descriptive analysis and comparison of proportions was performed using SPSS statistical software version 12.0.1 (SPSS Inc., Chicago, IL, USA). All p values < 0.05 were considered statistically significant.

Results

Of the 200 pregnant women enrolled, 176 (88%) were found to be positive for all types of vaginal and cervical infections (BV, candidiasis, *C.trachomatis* infection, gonorrhoeae, and trichomoniasis). Out of the 176 infected women, 71.6% (126/176) were in the third trimester of gestation, whereas 28.4% (50/176) were in the second trimester. Of these, the most common infected age groups were the 21-30 group, followed by the 31-40 group (Figure 1).Of the 176 vaginal infection cases, the causative agents varied from single infection caused by one type of microorganism to polymicrobial infections; a total number of 434 significant microorganisms were identified by laboratory methods. The majority of subjects were infected by three organisms (35.2%, 62/176) and two organisms (31.1%, 60/176), followed by one organism (18.2%, 32/176) and more than three organisms (12.5%, 22/176).

Figure 1. Frequency of vaginal infections by age group of pregnant women (n = 176) at Omdurman Maternity Hospital, Khartoum State, Sudan
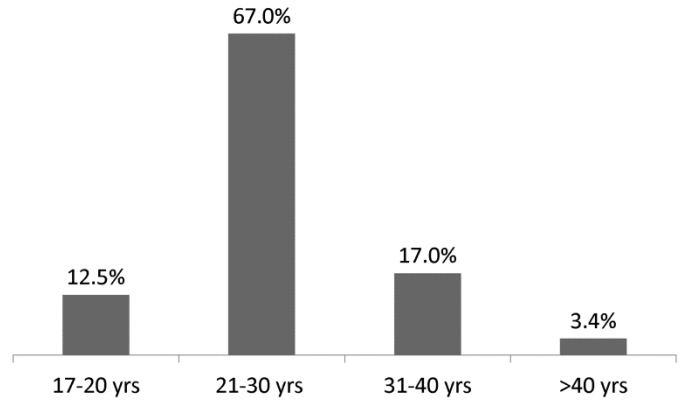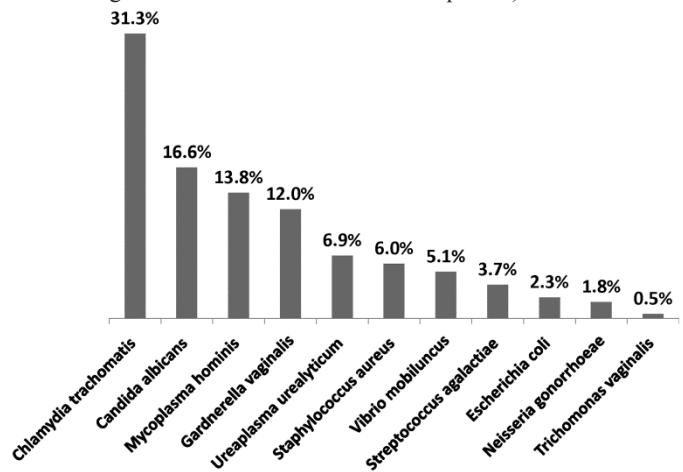

Figure 2. Types and proportions of microorganisms (n = 434)* recovered from 176 pregnant women with vaginal infections at Omdurman Maternity Hospital, Khartoum, Sudan (*more than one organism could be isolated from one patient)

Prevalence rates of various types of infections

Among the vaginal infections detected, BV was the most prevalent, found in 49.8% of samples, followed by *C. trachomatis* infection (31.3%) and candidiasis (16.6%), with low frequencies of gonorrhoeae (1.8%) and trichomoniasis (0.5%) infections.

Etiological agents of bacterial vaginosis

Figure 2 illustrates the types and proportions of microorganisms (n = 434) recovered from the 176 pregnant women with vaginal infections at Omdurman Maternity Hospital, Khartoum. As shown in Figure 2, the most common BV microorganisms were *M. hominis* (n = 60, 13.8%), followed by *G.vaginalis* (n = 52, 12%), *Ureaplasma urealyticum* (n = 30, 6.9%),

Staphylococcus aureus (n = 26, 6%), *Vibrio mobiluncus* (n = 22, 5.1%), *Streptococcus agalactiae* (n = 16, 3.7%), and *Escherichia coli* (n = 10, 2.3%).

Distribution of chlamydial infections according to history of abortion

The distribution of *C. trachomatis* infections in pregnant women at Omdurman Maternity Hospital according to their history of abortion is shown in Figure 3. No significant association (p = 0.7) was found between history of abortion and the presence of *C.trachomatis* infections in the studied group.

*Antibiotic susceptibility patterns of the bacterial isolates*

Table 2 shows the antibiotic susceptibility of different bacterial isolates from pregnant women with vaginosis cases at Omdurman Maternity Hospital. Gentamicin was the most active antibiotic against both Gram-positive and Gram-negative bacteria. Vancomycin was the most active antibiotic against Gram-positive bacteria, followed by amoxicillin-clavulanic acid. Imipenem was the most active antibiotic against anaerobic bacteria.

The most common active antibiotics against *Mycoplasma* species were clarythromycin and tetracycline. In general, *M. hominis* were more susceptible to the tested antibiotics than *U. urealyticum* (Table 3).

Discussion

Pregnant women have a twofold increase in the prevalence of vaginal microorganisms colonization compared to non-pregnant women. Increased levels of circulating estrogens and deposition of glycogen and other substrates in the vagina during pregnancy [18] influence this association. In the present study, pregnant women demonstrated various types of vaginal infections present in either one or more than one microorganism per case. Nevertheless, BV represented 49.8% of vaginal infections. This finding is similar to findings other studies in various African countries such as the 32% rate of BV recorded among women in Ethiopia [19], 47.7% in Uganda [20], and 62.6% in Tanzania [21]. Such large ratios of pregnant women with positive culture or vaginosis might necessitate medical treatments and indicate the high prevalence and variations in the causal agents associated with vaginal infections. This outcome cast doubt on whether diagnosis and management of this condition should be revised in countries such as Sudan. This is because BV increases the risk of

Figure 3. Association between *Chlamydia trachomatis* infections and numbers of abortions in pregnant women
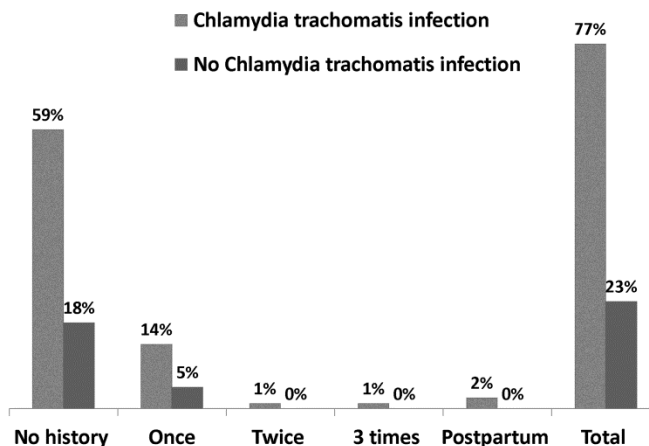
sexually transmitted infection, including HIV [4,22,23]. Failure to detect it could have serious effects on women's health.

According to the meta-analysis carried out by Leitich *et al.* [24,25], pregnant women with BV have an over twofold greater risk of preterm delivery than women without BV (odds ratio: 2.16; 95% CI: 1.56-3.00). This risk was shown to be even greater among women in whom BV was diagnosed early in pregnancy. Among women undergoing preterm labor, BV doubles the risk of delivering before 37 weeks of gestation (odds ratio: 2.38; 95% CI: 1.02-5.58) [25]. In addition, BV increases the risk of late miscarriage and maternal infection postpartum [25]. While BV has been found to be associated with preterm birth, no link has been found with neonatal infection or perinatal mortality.

Genital mycoplasma, including *M. hominis* and *M. Ureaplasma* spp., are suspected to contribute to a number of pathological conditions such as preterm birth, premature birth, low birth weight, and perinatal morbidity and mortality [26]. In this study, the higher frequency of infection among *Mycoplasma* species isolates was due to *M. hominis* (13.8%) than to *U. urealyticum* (6.9%) infection. *M. hominis* accounted for 35% of the vaginosis cases in Nigerian women and it was regarded as the most common isolate [27]. The present study can be compared with a study carried out in Poland [28], which found that *U. urealyticum* and *M. hominis* infections accounted for 29.5% and 3.7% of vaginosis cases, respectively. It is obvious that there are significant differences between our results and results from Nigeria and Poland.

Table 2. Antibiotic susceptibility patterns of the bacteria isolated from vaginosis cases at Omdurman Maternity Hospital, Khartoum, Sudan to 16 various antimicrobial agents

Microorganism	Number of isolates	Amoxicillin/clavulanic Acid	Ceftriaxone	Ceftazidime	Cefuroxime	Cephalothin	Cephotaxime	Ciprofloxacin	Co-trimoxazole	Gentamycin	Imipenem	Kanamycin	Metronidazole	Penicillin	Piperacillin	Tetracycline	Vancomycins
Gardnerellavaginalis	52	100%	100%	8076%	8%	4%	23%	-	31%	100%	96%	4%	0%	0%	4%	-	100%
Streptococcus agalactiae	16	100%	25%	25%	-	-	-	-	9%	100%	-	100%	-	100%	-	75%	100%
Neisseria gonorrhoeae	8	-	100%	75%	100%	0%	100%	100%	75%	100%	-	0%	0%	0%	0%	-	-
Escherichia coli	10	80%	20%	60%	-	-	-	100%	0%	80%	-	-	-	0%	-	60%	-
Staphylococcus aureus	26	100%	-	92%	-	-	-	-	0%	100%	92%	-	-	0%	-	-	92%
Vibrio mobiluncus	22	-	100%	100%	-	100%	100%	82%	91%	100%	100%	-	64%	-	-	-	100%

-: not done

Table 3: Antibiotic susceptibility patterns of the *Mycoplasma* species isolated from vaginosis cases at Omdurman Maternity Hospital, Khartoum, Sudan to 9 various antimicrobial agents

Species	Number of strains	Azithromycin	Ciprofloxacin	Clarythromycin	Erythromycin	Doxycycline	Josamycin	Pristinamycin	Oflaxacin	Tetracycline
Mycoplasma hominis	60	77%	73%	93%	77%	60%	70%	53%	45%	77%
Ureaplasmaurealyticum	30	87%	67%	87%	73%	67%	53%	53%	47%	80%

In the present study, significant proportions of vaginal infections were reported due to vaginal candidiasis and trichomoniasis. In comparison to a previous study conducted on a similar population in Sudan, our study reported higher rates (16.6%) of candidiasis than the 10.1% reported by Kafi *et al.* [8]. In contrast, these authors recorded 7.7% of genital infections due trichomoniasis, which was higher than the 0.5% in our study. In another study carried out in Khartoum, the prevalence of *C. trachomatis*, *T. vaginalis*, *N. gonorrhoeae*, and candidiasis among pregnant women attending an antenatal clinic was reported to be 7.3% in patients suffering multiple infections [7]. The variation of these isolation rates may be due to socio-economic status and immunity of patients, or environmental, nutritional, or cultural factors.

In the present study, *C. trachomatis* was the most prevalent microorganism (31.3%). This result is higher than the 3.9% observed in Jordan [29] and the 8% reported in pregnant woman in Botswana [30].In developing countries, including Sudan, diagnosis of cervical infections namely due to *C. trachomatis*is limited to the syndromic approach of relying on the appearance of vaginal discharge [31]. Due to the high prevalence of *C. trachomatis* infections in this study, routine diagnostic techniques such as the direct fluorescence test or polymerase chain reaction are needed in our hospitals. Though *C. trachomatis* infections can lead to spontaneous abortion in early pregnancy, the study did not demonstrate a significant association between previous abortions among studied populations and the presence of *C. trachomatis*. However, our clinical specimens were obtained from pregnant women in the second and third trimesters to avoid risk of abortion.

BV is a polymicrobial infection and asymptomatic carriage (*G. vaginalis*) is found in up to 70% of women. These factors render routine culture and antibiotic sensitivity testing requests inappropriate. However, a culture is appropriate to exclude or to confirm notable agents, namely *Trichomonas* species, *C. trachomatis*,and *N. gonorrhoeae* [32,33]. The standard treatment for BV, vulvovaginal candidiasis, and trichomoniasis is oral metronidazole at a dosage of 500 mg twice daily for seven days.

The present study demonstrated that 64% of the isolates of *Vibrio mobiluncus* were sensitive to metronidazole, whereas *G. vaginalis* and *N. gonorrhoeae* isolates were resistant.One of the limitations of this study was gaps in the *in vitro*antimicrobial testing for some isolated organisms

(Table 2), but a good number of antimicrobial agents were tested against *Mycoplasma* species. The antimicrobial susceptibility testing of isolated bacteria showed that clarythromycin was active against both *M. hominis* and *U. urealyticum*. Gentamicin was the most active antibiotic against both Gram-positive and Gram-negative bacteria. Vancomycin was active against Gram-positive bacteria. Ciprofloxacin was the most active drug against Gram-negative, and imipenem was most active against anaerobic organisms. Shopova *et al.* [34] reported *G. vaginalis* as the main microorganisms related to recurrent BV; it was sensitive in 34% of cases to metronidazole and in 82% of cases to clindamycin. Such results show that the general administration of metronidazole is a standard treatment, regardless of its *in vitro* results.

Conclusions

In conclusion, this study revealed the polymicrobial nature and high prevalence of vaginal complaints such as BV (49.8%) and *C. trachomatis* infections among pregnant women. Such cases may require specific medical treatment. Routine vaginal and cervical swab sample cultures should be performed on all pregnant women during prenatal visits, especially during the second and third trimesters. Generally, the administration of metronidazole as a standard treatment against vaginal infections is not in line with the *in vitro* antimicrobial sensitivity results.

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