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## Etiological Analysis and Antibiotic Sensitivity of Vaginal Infections

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### Abstract

Bacterial vaginosis is a common condition affecting women of reproductive age, caused by an imbalance in the vaginal microbiome. This study identifies the pathogens causing bacterial vaginosis in Nineveh and evaluates their antibiotic sensitivity to determine effective treatments. Key pathogens include *Staphylococcus aureus*, *Escherichia coli*, *Gardnerella vaginalis*, *Klebsiella* spp., and *Candida* spp. The results highlight significant antibiotic resistance, particularly to Ampicillin and Amoxicillin, and high sensitivity to Meropenem, Ciprofloxacin, Gentamycin, and Amikacin. The vaginal microbiome plays a protective role, dominated by *Lactobacillus*. Bacterial vaginosis disrupts this balance, leading to overgrowth of anaerobic or facultative bacteria. It is associated with abnormal discharge, discomfort, and health complications, such as increased susceptibility to sexually transmitted infections, pregnancy-related issues, and neonatal infections. 40 vaginal swabs from women aged 20-42 years in Mosul. Swabs were analyzed microscopically and cultured on various media (e.g., blood agar). Gram staining and biochemical tests identified microbial species, confirmed with the Vitek2 system. The Kirby-Bauer disc diffusion method tested 22 antibiotics for effectiveness against isolated bacteria. *Staphylococcus aureus* (42.5%) dominant isolate. *Escherichia coli* (12.5%), *Gardnerella vaginalis* (5%), and *Klebsiella* spp. (2.5%). *Candida* spp. (37.5%) often found in mixed infections. High resistance to Ampicillin, Amoxicillin, and Ceftazideme. Effective antibiotics include Meropenem, Ciprofloxacin, Gentamycin, and Amikacin. The study emphasizes the necessity of tailored antibiotic treatments to combat resistance and prevent complications.

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### Introduction

Bacterial vaginosis is a condition characterized by a change in the bacterial ecosystem of the vagina characterized by a decrease in the number of lactobacilli (the normal bacterial flora) and an overgrowth of many anaerobic or facultative bacteria and is the most common infection in women of reproductive age <sup>[1]</sup>.

The vaginal environment is a unique, complex and delicate environment consisting of different types of microorganisms in varying quantities and proportions, and it can change in response to a large number of internal and external factors. Just as there are many studies discussing ethnicity and genetic differences that contribute to inter-individual differences in the vaginal microbiome, it is reasonable to state that there is still much to be explored in understanding how women's vaginal microbiomes function throughout their lives <sup>[2, 3]</sup>.

The normal vaginal environment consists of a wide variety of anaerobic and aerobic bacterial genera and species dominated by the facultative anaerobic genus *Lactobacillus*. These bacteria play a protective role in protecting the urogenital system from infection with pathogens <sup>[4]</sup>.

Bacterial vaginitis (BV) occurs in one third of women worldwide at different ages but is more prevalent among females of reproductive age and its prevalence varies from country to country within the same region and even within similar population groups, due to the complex balance in the environment vaginal bacteria. It is a common cause of abnormal vaginal discharge and is associated with other health problems. The anaerobic microbes associated with bacterial vaginosis such as *Gardnerella vaginalis* were first described in the 1950s [5, 2].

Vaginal infections can be caused by bacteria, fungi, parasites or viruses, and the most important microorganisms that mainly cause vaginitis that have been isolated from cultures of vaginal swabs are *Escherichia coli*, *Pseudomonas* spp, *Staphylococcus aureus*, *Mycoplasma hominis*, Group B streptococcus and *Ureaplasma urealyticum* and *Gardnerella vaginalis* and *Mobilunchus* species [1].

Clinically, symptoms of vaginitis are red and inflamed vagina, yellowish vaginal discharge with a foul smell with burning sensation, dyspareunia, itching and irritation, which if not treated leads to serious complications [6, 7].

For many years, bacterial vaginosis received scant attention as a non-risk disease. However, several studies have shown that it has several complications as it increases the risk of acquiring HIV and other sexually transmitted diseases, such as gonorrhea, trichomoniasis, herpes simplex virus type 2 (HSV-2), infertility, cellulitis, urethral syndrome, miscarriage and premature birth. and postpartum complications such as endometriosis and wound infections in pregnant women, as well as infection of newborns with abscesses, pneumonia and meningitis [8, 9, 10, 11].

#### Aim of the study

Although bacterial vaginosis is associated with many health problems and is a major global concern, it has not been the focus of extensive study in Nineveh. Therefore, the purpose of this study was to determine the prevalence of bacterial vaginosis, identify the most important types that cause it, and conduct a sensitivity test to antibiotics to find out the appropriate treatment for the disease and reduce it.

#### Materials and methods

40 samples were collected from Al-Khansa Teaching Hospital and some private laboratories in the city of Mosul for women aged (20-42) years suffering from bacterial vaginosis. the samples were vaginal swabs, as two swabs were taken from each patient, the first one was wet from the vagina, as it was moistened with physiological saline and examined directly under the microscope for the initial detection of the content of the sample, whether it was bacteria, fungi, parasites, or others, as well as identifying its content from the epithelial cell, red blood cells and purulent cells.

As for the second swab, it was taken from the cervix without wetting (dry swab) and using a uterine dilator device (Specula) to facilitate the taking of the sample, and then these swabs were cultured on the culture media, which is blood agar, Macconkey agar and chocolate agar, in sterile conditions, and then placed these plates were kept in the incubator at (37°C) for (24) hours.

After incubation, gram staining of the developing bacterial species was carried out on the culture media to investigate their species and then some biochemical tests were performed to identify them accurately.

Mannitol Salt agar was used in the case of the growth of *Staphylococcus* bacteria in order to distinguish between its types through this medium.

#### Antibiotic sensitivity test:

After identifying the type of bacteria growing on culture media, an antibiotic sensitivity test was performed, and the standard method of Kirby and Bauer (1966) was used to test the sensitivity of bacteria to antibiotics, which is known as the method of spreading around the disc. Tables 1 and 2 show the antibiotics used for both Gram-negative and Gram-positive bacteria with their symbols and concentrations. Isolation of these organisms and testing for their sensitivity to antibiotics is fundamental to the successful treatment of vaginal infections [13].

**Table 1:** Antibiotics used for Gram-negative bacterial isolates.

No.	Antibiotic	symbol	concentration µg
1	Ampicillin	AM	10
2	Meropenem	MEM	10
3	Ceftriaxone	CRO	30
4	Norfloxacin	NOR	10
5	Ceftazidem	CAZ	30
6	Pipracillin	PRL	30
7	Amoxacillin	AMC	10
8	Ciprofloxacin	CIP	5
9	Amikacin	AK	30
10	Gentamicin	GM	10
11	Tetracycline	TE	30
12	Trimethoprim	TMP	5

**Table 2:** Antibiotics used for Gram-positive bacterial isolates.

No.	Antibiotic	symbol	concentration µg
1	Ampicillin	AM	10
2	Meropenem	MEM	10
3	Norfloxacin	NOR	10
4	Ceftazidem	CAZ	30
5	Clindamycin	DA	2
6	Azithromycin	AZM	15

7	Ciprofloxacin	CIP	5
8	Amikacin	AK	30
9	Gentamicin	GM	10
10	Tetracycline	TE	30
11	Trimethoprim	TMP	5
12	Rifampicin	RA	5

As for the bacteria *Gardenerella vaginalis*, the following antibiotics were used: Amoxicillin, Ampiclox, Trimoxazole, Cephalexin, Tetracycline, Erythromycin, Doxycycline and Metronidazole.

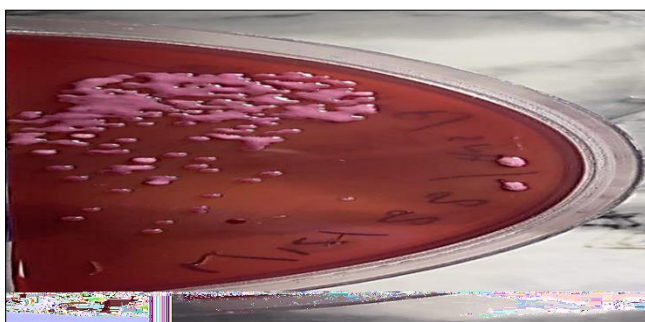
### Results and Discussion

40 developing isolates were obtained as a total number of samples taken from vaginal swabs, 10 of them (25%) showed normal bacterial flora, while (25) different bacterial isolates were obtained with a percentage of 62.5% (, as in Table 3 which shows the percentage of bacterial and fungal isolates from The grand total of samples.

**Table 3:** Numbers and percentages of the isolates under study.

Samples	Number and percentage
<b>Bacterial species</b>	
<i>Staphylococcus aureus</i>	17 (42.5%)
<i>Escherichia coli</i>	5 (12.5%)
<i>Gardenerella vaginalis</i>	2 (5%)
<i>Klebsiella spp</i>	1 (2.5%)
<b>Fungal species</b>	
<i>Candida Spp.</i>	15(37.5%)
Total Number of samples	40(100%)

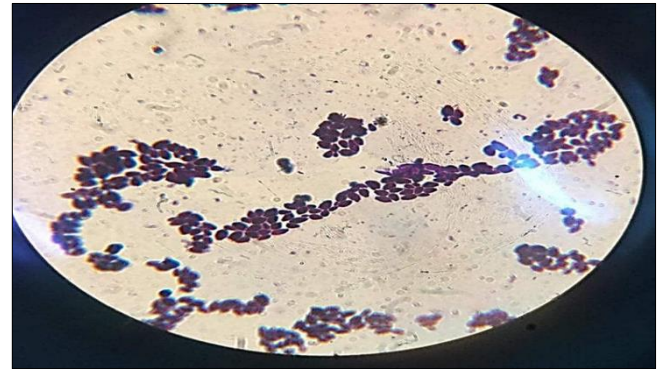
The highest percentage of the bacterial type was *Staphylococcus aureus*, as it appeared in 9 (samples individually and) 8 (samples in a mixed growth condition with *Candida*, meaning that the total was) 17 (isolates,) 42.5% (of the total samples grown, followed by *Escherichia coli* with) 4 (single bacterial isolates and one isolate in mixed growth with *Candida*, the total was five bacterial isolates at a rate of) 12.5% (,while the type *Gardnerella vaginalis* was obtained by one single bacterial isolate and another mixed with *Candida*, by two isolates of this type at a rate of 5%, and the lowest isolation rate was for the type *Klebsiella spp* at) 2.5% ( . As in the picture (1), which shows the growth of the isolate of *Klebsiella spp*. on MacConkey agar.



**Picture 1:** Colonies of *Klebsiella spp* on a medium MacConkey Agar.

As for *Candida* isolates, it appeared in five samples individually, while it appeared with the bacterium *Staph.aureus* in eight samples, as well as it appeared with each of the bacteria *E.coli* and *Gardenerella vaginalis* in one sample for each, and thus)15(isolates of *Candida* were obtained as a total of single growth and mixed with bacteria,

so the percentage is)37.5%(. As shown in figure (2), the *Candida* cells are under a light microscope at 1000x magnification.



**Picture 2:** cells of *Candida spp*. Under a light microscope at 1000X magnification.

The results were based on microscopic examination, Gram staining and biochemical tests, and the diagnosis was confirmed using the Vitek2 system.

Compared to local studies, the results of our study were higher than the results of researcher Daood *et al.*, 2020 <sup>[14]</sup> in Nineveh, in terms of bacterial growth, as their percentage was)47% (and the percentage of normal flora bacteria was higher than what appeared in our study, as it was)63%(for them compared to)25% (in our study. Also, the percentage of our study in terms of bacterial growth was higher than in the study of Kamga *et al.*, 2019 <sup>[15]</sup>, when the percentage was 26.2% <sup>[14]</sup>.

As for the growth of *Candida*, our isolation rate was)37.5% (and thus it is less than the rate of isolates in Baghdad in the study of the researcher Shaker *et al.*, 2017, when their isolation rate was) 52% (Also, our study was higher than that of researcher Baek *et al.*, 2021 and Kamga *et al.*, 2019 <sup>[6, 15]</sup> as the percentage of *Candida* isolates they had was)20% (and)27.8% (, respectively. As well as higher than the study of the researcher and Freitas *et al.*, 2020 <sup>[17]</sup> as their percentage was)31.5% ( . While the percentage was lower than the study of researcher Mulu *et al.*, 2015, as they showed a growth of *Candida* by 8.3% <sup>[13, 6, 15]</sup>.

By comparing the percentage of isolation of our study for bacterial species, the highest percentage of isolation of the bacterial type *Staph.aureus* was) 42.5% (, and thus it is higher than the percentage of isolation of researcher Al-Naqshbandi *et al.*, 2018 and researcher Shaker *et al.*, 2017 when the percentage of isolation of this type was) 4.76% (and) 32% (, respectively. As well as higher than the study of researcher Baek *et al.*, 2021 <sup>[13, 16, 6]</sup>.

While the rate of isolating *E. coli* bacteria was (12.5%), and thus it is lower than that of researcher Al-Naqshabandi *et al.*, 2018 <sup>[16]</sup>, when the rate of isolation was (40.4%). As well as less than the study of researcher Baek *et al.*, 2021 <sup>[6]</sup>. While the percentage was higher than the rate of isolation of researcher Shaker *et al.*, 2017, when they had 6% <sup>[6, 13]</sup>.

*Gardnerella vaginalis* bacteria was in the third place in the

percentage of isolation of bacterial species by (5%), which is less than the study of the researcher Baek *et al.*, 2021 and Kamga *et al.*, 2019 [6, 15], when the percentage was (64.9%) and (55%) for each of them, respectively. While our study was higher than the study of the researcher Freitas *et al.*, 2020, as the percentage was 1.25%. [6, 15, 17].

The lowest percentage of isolation of bacterial species was (2.5%), which belongs to the type. Kleb. spp. Thus, it is an approach to the study of researcher Orish *et al.*, 2016 and Baek *et al.*, 2021 [18, 6], as their percentages were (2.1%) and (2.9%), respectively. While it was less than that of the researcher Al-Naqshbandi *et al.*, 2018, Shaker *et al.*, 2017, and Bitew *et al.*, 2017, when the percentages were (14.2%), (20%) and (18.5%) respectively [18, 6, 16, 13, 19].

The reason for these high rates of the presence of the aforementioned pathogenic microorganisms, which is one of the main causes of the occurrence of recurrent vaginal infections, is the microbial imbalance between these pathogenic microorganisms and the naturally present bacteria (normal flora), by increasing the presence of these pathogens over the presence of natural bacteria, and this may be due to

the use of antibiotics wrongly and randomly without medical advice, which leads to this imbalance in the natural balance and also changes the pH of the vagina [13, 14].

#### Antibiotic susceptibility test results:

The results of sensitivity to antibiotics differed according to the different types of bacterial isolates, as shown in Table (4), as (22) antibiotics were used, and the results for each bacterial type were as follows. Vancomycine and Ciprofloxacin had a sensitivity of (82.3%), and their sensitivity to Amikacin was (76.4%), while it was less sensitive to Ampicillin and Ceftazidime, which was (29.4%) and (5.8%) for each, respectively. Thus, this type was more resistant to the last two types of antibiotics. This result was in agreement with the study of researcher Al-Naqshbandi *et al.*, 2018 in terms of high sensitivity to Vancomycine and high resistance to Ampicillin, but it was in contrast to our study in terms of resistance to Ciprofloxacin, and it was similar to the study of researcher Orish *et al.*, 2016 [18] in terms of high resistance to Ampicillin. As shown in the picture (3), the results of the sensitivity test for one of the isolates of Staph. aureus bacteria.



**Picture 3:** Results of susceptibility testing for one of the isolates of Staph. Aureus.

The results of the sensitivity of E. coli bacteria showed that it was highly sensitive to meropenem, Amikacin and Gentamycin, and it was (100%) resistant to both Ciprofloxacin and Amoxicillin. Thus, it agreed with the study of Ahmad *et al.*, 2014 and Al-Naqshbandi *et al.*, 2018 in terms of high sensitivity to E. coli. Gentamycin as well as in terms of high resistance to Ampicillin and Piperacillin. [20, 16].

As for the isolation of bacteria Kleb. spp. It was sensitive to 100% of Trimethoprim, Tetracycline, Norfloxacin, Ceftriaxone, Meropenem, Ciprofloxacin and Piperacillin, while it was (100%) resistant to Ampicillin, Ceftazidim, and Amoxicillin. Thus, our study was compatible with the study of Al-Naqshbandi *et al.*, 2018 except for its resistance to all antibiotics. Piperacillin antagonist that showed (100%) resistance in this study [16].

As for Gardnerella vaginalis bacteria, it showed (100%) sensitivity to each of Gentamycin, Ciprofloxacin, and Metronidazole, while it was (100%) resistant to each of Ampicillin, Amoxicillin, Trimethoprim, Tetracycline, CO-Trimoxazole, Amiplox, Cephalexin, Doxycylin and Ampicillin. Thus, our study agreed with the study of

researcher Abdelaziz *et al.*, 2014 and researcher Singh *et al.*, 2016, except for the difference in resistance to metronidazole, which was (100%) in the study of Singh *et al.*, 2016 [21, 22].

In general, most of the isolates shared their high resistance against the antibiotics Ampicillin and Amoxicillin, which may be due to the repeated and indiscriminate use of these antibiotics, which leads to the killing of the natural flora present in the vagina, which plays a major role in protecting it from infection with external bacterial and fungal causes, as well as the patient taking antibiotics. Wrongly and without medical advice or doing a sensitivity test or misdiagnosing the pathogen and thus increasing the resistance of bacteria and the emergence of resistant strains of them [23].

All isolates also showed a high sensitivity to the antibiotics Meropenem, Ciprofloxacin, Gentamycin and Amikacin, as well as the antibiotic Norfloxacin, but with lower sensitivity rates, which means the efficiency of these antibiotics in treating bacterial infections caused by the bacterial species isolated in our study, that is, these isolates do not possess Bacterial resistance genes or plasmids against these antibiotics (1).

**Table 4:** Antibiotic susceptibility test results for bacterial isolates under study and their percentages.

microbial species	number of isolates	Antibiotics									
		Amikacin (%)	Gentamycin (%)	Ciprofloxacin (%)	Amoxicillin (%)	Pipracillin (%)	Ceftazidim (%)	Norfloxacin (%)	Ceftioxone (%)	Meropenem (%)	Ampicillin (%)
Staph. aureus	17	13S (76.4) 4R (23.5)	11S (64.7) 6R (35.2)	14S (82.3) 2R (11.7) 1MS (5.8)	NO	NO	1S (5.8) 16R (94.1)	9S (52.9) 8R (47)	NO	16S (94.1) 1R (5.8)	5S (29.4) 11R (64.7) 1MS (5.8)
E. coli	5	4S (80) 1R (20)	4S (80) 1MS (20)	3S (60) 2R (40)	5R (100)	1S (20) 3R (60) 1MS (20)	1S (20) 4R (80)	2S (40) 3R (60)	5R (100)	5S (100)	2S (40) 3R (60)
Kleb. spp	1	S (100)	MS (100)	S (100)	9R (100)	S (100)	R (100)	S (100)	S (100)	S (100)	R (100)
Gardnerella vaginalis	2	NO	2S (100)	2S (100)	2R (100)	NO	NO	NO	NO	NO	2R (100)

**Supplement Table 4:** Antibiotic susceptibility test results for the bacterial isolates under study and their percentages.

microbial species	number of isolates	Antibiotics										
		Metronidazole (%)	CO - Trimoxazole (%)	Ampiclox (%)	Cephalexin (%)	Doxylin (%)	Vancomycin (%)	Rifampicin (%)	Azithromycin (%)	Clindamycin (%)	Trimethoprim (%)	Tetracycline (%)
Staph. aureus	17	NO	NO	NO	NO	NO	14S (82.3)	9S (52.9)	7S (41.1)	7S (41.1)	9S (52.9)	11S (64.7)
							3R (17.6)	8R (47.0)	8R (47.0)	10R (58.8)	7R (41.1)	5R (29.4)
									2MS (11.7)		1MS (5.8)	1MS (5.8)
E. coli	5	NO	NO	NO	NO	NO	NO	NO	NO	NO	2S (40)	1S (20)
											3R (60)	4R (80)
Kleb. spp	1	NO	NO	NO	NO	NO	NO	NO	NO	NO	S (100)	S (100)
Gardnerella vaginalis	2	2S (100)	2R (100)	2R (100)	2R (100)	2R (100)	NO	NO	NO	NO	2R (100)	2R (100)

### Conclusion

Our current study showed the main pathogens causing bacterial vaginosis for women of childbearing age, which are Staph aureus, E. coli, Gardnerella vaginalis, Klebsiella spp. and Candida spp. It also showed the high rate of resistance of these pathogens against a number of antibiotics, and showed the efficiency of some antibiotics by 100% in treating these vaginal infections such as Meropenem, Ciprofloxacin, Gentamycin and Amikacin. As a result of this, women who have clinical symptoms of bacterial vaginosis are advised to undergo an antibiotic sensitivity test to find out the main pathogen causing the infection, as well as the appropriate and

correct treatment to avoid complications of this vaginal infection.

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