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The Relation between the Result of Direct Examination of High Vaginal Swab versus the Result of Culture in Erbil City / Iraq

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**Sahar Muhammad Zaki*¹, Warkaa Faraj Alsaeed²,
Sanarya Kamaran Mahmmod³, Dilan Abdulah
khalo⁴, Chro Rasul⁴, Zina Sdeeq⁴**

¹*M.B. Ch.B, MSC Medical Microbiology/ College of
Health Sciences/ Hawler Medical University, Iraq*

²*M.B.Ch.B; F.I.C.M.S./ Pathology, Rizgary teaching
hospital/ laboratory department/ Erbil-Iraq*

³*Microbiologist, Bachelors in biology/ Cihan university
/Erbil-Iraq*

⁴*Microbiologist, Bachelors in Medical
Microbiology/College of health Sciences/Hawler
Medical University/ Erbil-Iraq*

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ABSTRACT

Background and objectives: Vaginitis is a term used to describe infectious diseases and other inflammatory conditions affecting the vaginal mucosa, bacterial vaginitis appears to be associated with pelvic inflammatory disease, infectious complications after abortion or gynecological invasive procedures. The study aimed to isolate the common bacterial causes of vaginal infection, to determine any relationship between age and type of pathogenic bacteria that cause vaginal infection. **Methods and materials:** High vaginal swabs were collected from two hundred (200) women patients with vaginal infection symptoms who attend the Rizgary Hospital, Maternity Teaching Hospital and PAR Hospital in Erbil city in the period from (September 2016-February 2017). All vaginal swabs were taken from married non-pregnant patients. The age of these patients ranged between (18-55) years, Swabs were transported to the lab, the samples were directly examined and specimens, where inoculated to several culture media after incubation overnight at 37°C, the bacterial colonies, were identified on the following medias: Muller Hinton Agar, MacConkey agar, Blood agar plate, Chocolate agar **Results:** gram positive isolated from (58%) while gram negative isolated from (42%) of patients complaining from vaginitis, the number, and percentage of isolated bacteria was as follow: *Escherichia coli*, *Streptococcus agalactiae* 28(22.5%), *Klebsiella pneumoniae*, *Staphylococcus haemolyticus* 16(12.9%), *Staphylococcus aureus*, *Enterococcus faecalis* in 12 (9.8%) while *Neisseria gonorrhoeae*, *Serratia marcescens* and *Staphylococcus saprophyticus* in 4(3.3%) and the positive bacterial growth was most prevalent in the age groups of (25-34) years which was 55(44.2%) with the mean age (29.68 ±8.11) years with maximum and minimum age (48,18) years respectively. The prevalence of pus cells in vaginal discharge was significantly related to the type of microorganism (P<0.05). **Conclusions:** The incidence of gram-positive was higher than gram-negative bacteria and the highest occurrence of vaginal infection and bacterial growth in the age group (25-34) years and followed by the age group (35-44) years and there was a significant relationship between the presence of pus cells in direct examination of the high vaginal swab.

INTRODUCTION

Vaginitis is a term used to describe infectious diseases and other inflammatory conditions affecting the vaginal mucosa and sometimes secondarily involving the vulva (1).

These conditions can result from an infection caused by bacteria, usually *Gardnerella vaginalis* and *Mycoplasma hominis* in combination with various anaerobes (2, 3). Other less commonly encountered bacteria are *Chlamydia trachomatis*, *Escherichia coli*, *Neisseria gonorrhoeae*, *Streptococci* and *Staphylococci* (4). Protozoa like (*Trichomonas vaginalis*) cause 1/3 of all cases (4), while *Candida* is a frequent cause in pregnant women and diabetics, and occasionally oral contraceptives increase susceptibility to infection (5). Another cause is viral infections such as the human papillomavirus (HPV) and herpes simplex (6).

The most common symptom of vaginitis is the vaginal discharge that is different from the normal secretions, accompanied by pruritus, erythema, and sometimes burning, pain, or mild bleeding, with or without vulvar irritation. Discomfort during urination or dyspareunia may also occur (7).

Although symptoms vary among particular types of vaginitis, there is much overlap. Trichomonal vaginitis is marked by a profuse, malodorous, yellow-green discharge and the patients may have dysuria, dyspareunia, erythema and severe itching. *Candida* vaginitis is suggested by moderate to severe vaginal and sometimes vulvar pruritus with or without burning. Dyspareunia, redness, edema and possibly secretion are common as is a thick, white, cottage cheese-like vaginal discharge that tends to adhere to the vaginal walls (8).

Various diagnostic methods are available to identify the etiology of an abnormal vaginal discharge (6). Laboratory testing fails to identify the cause of vaginitis in a minority of women. The cause of vaginal symptoms usually can be determined by pH and microscopic examination of fresh samples of the discharge (9).

Non-specific vaginitis or BV was first reported in 1955 by Gardner and Dukes, who described the unique clinical signs and symptoms and the distinctive nature of the vaginal discharge associated with it. They also described a “new” causative organism, which has variously been named *Haemophilus vaginalis* (10), *Corynebacterium vaginale* (11), and subsequently renamed and reclassified as the only member of a new genus, *Gardnerella* (12).

BV is vaginitis due to a complex alteration of vaginal flora in which normal hydrogen peroxide (H₂O₂) producing Lactobacillus species decrease and anaerobic pathogens (e.g., Prevotella sp., Peptostreptococcus sp. and Mobiluncus sp.), *Gardnerella vaginalis*, and *Mycoplasma hominis* overgrow (13,14). The cause of the microbial alteration is not fully understood. BV is associated with having multiple sex partners, a new sex partner, douching, and lack of vaginal lactobacilli (15); whether BV results from the acquisition of a sexually transmitted pathogen is unclear. Women who have never been sexually active are rarely affected (16).

BV is the most prevalent cause of vaginal discharge or malodor; BV tends to produce a white, gray, or yellowish turbid discharge with a foul or 'fishy' odor that becomes stronger when the discharge becomes alkaline (e.g., after coitus or washing with soap) (17). Vulvar pruritus or irritation may be present, but redness or edema is not usually marked. BV, once considered, appears to be associated with pelvic inflammatory disease (PID), infectious complications after abortion or gynecological invasive procedures, and increased risk of HIV transmission and acquisition (18,19).

Bacterial vaginitis during pregnancy is associated with significant reproductive morbidity, including premature rupture of membranes, preterm delivery, intra-amniotic infection, and postpartum endometritis (20).

Aims of the study

The aims of this study are:

1. To isolate the common bacterial causes of vaginal infection
2. To determine any relationship between age and type of pathogenic bacteria that causes vaginal infection
3. To know if there is any relation between direct high vaginal infection and bacterial culture

MATERIALS AND METHODS

Sample collection:

High vaginal swabs were collected from two hundred (200) women patients with vaginal infection symptoms who attend the Rizgary Hospital, Maternity Teaching Hospital and PAR

Hospital in Erbil city in the period from (September 2016-February 2017). All vaginal swabs taken from married non pregnant patients. The age of these patients ranged between (18-55) years

High vaginal swabs were taken from women patients suffering from abnormal vaginal discharge, itching, burning and lower abdominal pain. The samples were taken from patients by the gynecological specialist by putting speculum and using sterile swabs stick. Swabs were transported to the lab, the samples were directly examined.

Questioner prepared for each patient and some information was taken directly from patients. The information included: patients name, age signs and symptoms of vaginal infection, date of swabbing, history of taking antibiotics.

Those patients on antibiotics and pregnant were excluded from the research.

Identification of bacteria

Microscopic examination (direct examination):

Direct examination of vaginal secretions is the method of choice for the etiological diagnosis of vaginitis but is much less useful for the diagnosis of cervicitis.

A wet mount is prepared by mixing the vaginal sample with saline on a glass slide, after which a coverslip is added. A diluted preparation is preferred to ensure the separation of the cells, which may otherwise be clumped together. Examine at a magnification of x 400 for the presence pus cells, Epithelial cells and bacteria (21)

Culture medium examination:

In order to obtain maximal yield, specimens where inoculated to several culture media after incubation overnight at 37°C, the bacterial colonies were identified (22) on the following agars:

1 MacConkey agar:

Is a selective and differential culture medium for bacteria designed to selectively isolate Gram-negative and enteric bacilli and differentiate them based on lactose fermentation (24)

The crystal violet and bile salts inhibit the growth of gram-positive organisms which allows for the selection and isolation of gram-negative bacteria.

2 Blood agar plate:

Differential media used to isolate fastidious organisms and detect hemolytic activity. β -Hemolytic activity will show lysis and complete digestion of red blood cell contents surrounding a colony. Examples include *Streptococcus haemolyticus*. α -Hemolysis will only cause partial lysis of the red blood cells and will appear green or brown, due to the conversion of hemoglobin to methemoglobin. An example of this would be *Streptococcus viridans*. γ -Hemolysis (or nonhemolytic) is the term referring to a lack of hemolytic activity (25) BAPs also contain meat extract, tryptone, sodium chloride, and agar.

3 Chocolate agar (CHOC) or chocolate blood agar (CBA):

Is a nonselective, enriched growth medium used for isolation of pathogenic bacteria, it is a variant of the blood agar plate, containing red blood cells that have been lysed by slowly heating to 80 °C. Chocolate agar is used for growing fastidious respiratory bacteria, such as *Haemophilus influenzae* and *Neisseria spp.* (24).

RESULTS

A total of two hundred (200) high vaginal swabs were collected from women patients suspected of having vaginitis (We exclude the patients who are pregnant). The bacterial culture results showed that among 200 high vaginal swabs only 124 (62%) showed culture positive while 76(38%) samples showed no growth.

Gram-negative bacteria were isolated from 52 patients (42%), whereas Gram-positive bacteria were isolated from 72 (58%) patients (figure 1).

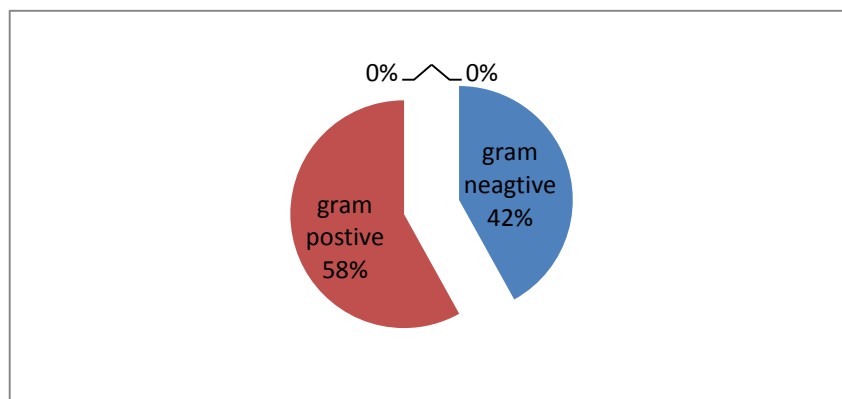


Figure 1: Pie chart shows the distribution of microorganism isolates from 31 positive cases

1 Distribution of bacterial isolates from vaginal discharge

In table (1) and figure (1) shows the distribution of bacterial isolates from vaginal discharge which was as follow:

E.coli appears to be the predominant gram-negative bacteria isolated from examined specimens, it was isolated from 28 (22.5%) and the same finding with *Streptococcus agalactiae* which was also 28(22.5%) in contrast to *Serratia marcescens*, *Neisseria gonorrhoea* and *Staphylococcus saprophyticus* which were isolated from only 4 patients (3.3%) while *Klebsiella pneumonia* and *Staphylococcus haemolyticus* were positive in 16 (12.9%) and regarding to *Staphylococcus aureus* and *Enterococcus faecalis* were positive in 12(9.8%) of the isolated bacteria

Table 1: Distribution of bacterial isolates from high vaginal swab

	Bacterial species	No. of isolates	Percentage
Gram-negative bacteria	<i>E.coli</i>	28	22.5
	<i>Klebsiella pneumonia</i>	16	12.9
	<i>Serratia marcescens</i>	4	3.3
	<i>Neisseria gonorrhoea</i>	4	3.3
Gram-positive bacteria	<i>Streptococcus agalactiae</i>	28	22.5
	<i>Staphylococcus haemolyticus</i>	16	12.9
	<i>Staphylococcus aureus</i>	12	9.8
	<i>Staphylococcus saprophyticus</i>	4	3.3
	<i>Enterococcus faecalis</i>	12	9.8

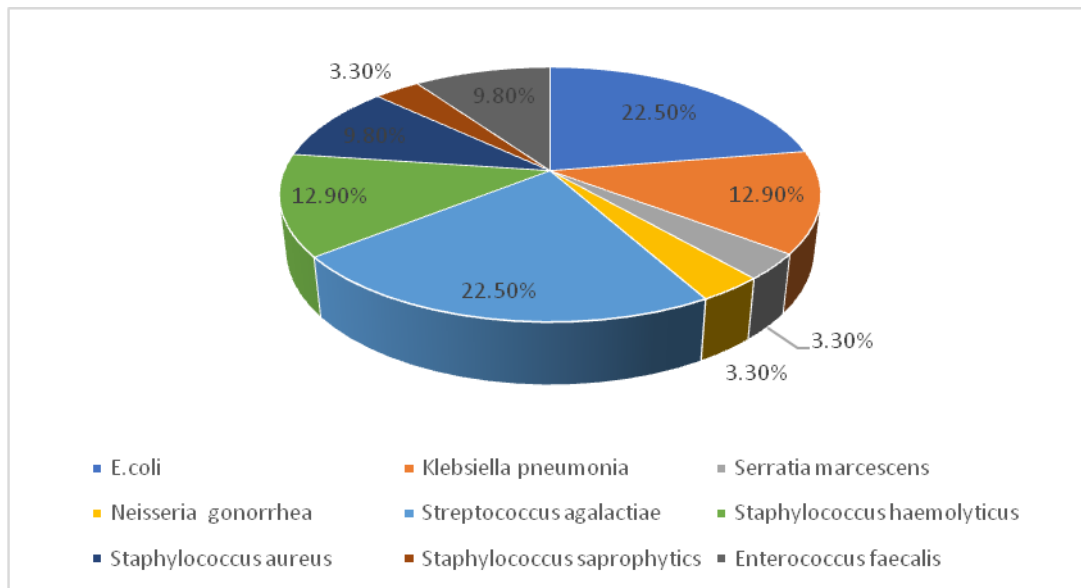


Figure 2: Pie chart shows the percentage of isolated bacteria from High vaginal discharge

2 Effect of age on the prevalence of Vaginitis

In table (2) showed that positive bacterial growth was most prevalent in the age groups of (25-34) years which was 55(44.2%) followed by the age group (35-44) year which was 32(25.8%) with the mean age (29.68 ± 8.11) years with maximum and minimum age (48,18) years respectively .

Table 2: Effect of age on the prevalence of Vaginitis

Pathogen	Age group (year)								Total	
	15-24		25-34		35-44		≥ 45		Frequency	Percentage
<i>E. coli</i>	6	4.8	8	6.4	12	9.7	2	1.6	28	22.5
<i>Str. Agalactiae</i>	5	4.1	10	8	11	8.8	2	1.6	28	22.5
<i>Staph. haemolyticus</i>	4	3.3	11	8.8	1	0.8	0	0	16	12.9
<i>Klebsiella pneumonia</i>	6	4.8	8	6.5	1	0.8	1	0.8	16	12.9
<i>Staphylococcus aureus</i>	1	0.8	6	4.8	5	4.1	0	0	12	9.8
<i>Enterococcus faecalis</i>	4	3.3	7	5.6	0	0	1	0.8	12	9.8
<i>Serratia marcescens</i>	1	0.8	2	1.6	1	0.8	0	0	4	3.2
<i>Staph. saprophyticus</i>	1	0.8	2	1.6	0	0	1	0.8	4	3.2
<i>Neisseria gonorrhoea</i>	2	1.6	1	0.8	1	0.8	0	0	4	3.2
Total	30	24.2	55	44.2	32	25.8	7	5.6	124	100

Prevalence of pus cells in relation to the bacterial growth in wet mount preparation vaginal discharge smears

In table (3) shows that microscopic examination of patient’s vaginal discharge revealed that pus cells were present in 105 (84.7%) cases, while 19 (15.3%) showed absence of pus cells (Table 7) and the prevalence of pus cells in vaginal discharge was significantly related to the type of microorganism ($P < 0.05$).

Table 3: Prevalence of pus cells in relation to the bacterial growth in wet mount preparation vaginal discharge smears

Bacterial pathogens	Pus cells present		Pus cell absent		Total
	number	%	Number	%	
<i>E.coli</i>	20	71.5	8	28.5	28
<i>Streptococcus agalactiae</i>	24	85.7	4	14.3	28
<i>Klebsiella pneumonia</i>	15	93.8	1	6.3	16
<i>Staphylococcus haemolyticus</i>	14	87.5	2	12.5	16
<i>Staphylococcus aureus</i>	10	83.4	2	16.6	12
<i>Enterococcus faecalis</i>	11	91.7	1	8.3	12
<i>Staphylococcus saprophyticus</i>	4	100	0	0	4
<i>Serratia marcescens</i>	4	100	0	0	4
<i>Neisseria gonorrhoea</i>	3	66.7	1	33.3	4
Total	105	84.7	19	15.3	124

DISCUSSION

A total of two hundred (200) high vaginal swabs were collected from women patients suspected of having vaginitis (We exclude the patients who are pregnant). The bacterial culture results showed that among 200 high vaginal swabs only 124 (62%) showed culture positive while 76(38%) samples showed no growth and Gram-negative bacteria were isolated from 52 patients (42%), whereas Gram-positive bacteria were isolated from 72(58%) patients (figure 2).

The result of this study was in agreement with a study done by (khamees) (26) who revealed that Gram-positive bacteria were isolated from 167 individuals (59.2%), whereas Gram-negative bacteria were isolated from 68 (24.1%) individuals.

The result of this study was the same percentage of infections were reported by other investigators, Jarjees(27) from Erbil (Iraq) reported the rate was (68.3%) and the same result reported by (Al- Muk and Hasony) (28) from Basrah (Iraq) (67.6%).

E.coli appear the most common among Gram-negative bacteria which was the predominant organisms isolated from examined specimens, it was isolated from 28(22.5%) and the same finding with *Streptococcus agalactiae* which was also 28(22.5%).

In a study done by (Lawrence et al) who found that *E. coli* to be more prevalent organisms isolated from the patient with vaginitis (29).

The result of this study was in agreement with a study done by (Holand et al) who reported that the most commonly isolated bacteria from HVS from the vaginal women with vaginitis was *Escherichia coli* (30) (15.96%). Other results are lower than our result which reported by (Mumtaz et al.) from Pakistan who reported (13.7%) (31) and (Alli et al.) from Nigeria who reported (12.1%) (32).

The presence of this bacteria in large percent in urinary tract and bacterial vaginosis might be attributed to the fact that this bacterium is part of the normal fecal flora and different virulence factors contributing to their pathogenicity and the difference in the result might be attributed to the number of sample taken and the difference in the time (year) of the study (33).

In this study, *E. coli* was the most predominant pathogen among gram-negative bacteria and this was in agreement with (Khamees, 2012) who recorded the same finding(26)

The result of this study regarding *E.coli* was higher than reported by (Mohamed and Al-Thwani,) who reported (10.93%) of *E.coli* isolated from HVS (34).

Streptococcus agalactiae was isolated in 28(22.5%) and this lower than recorded by (MANIATIS, et al) who recorded only (5.7%) (35)

Until now, *S. agalactiae* has been regarded as a normal component of the vaginal flora, associated with premature birth, amnionitis, neonatal meningitis and sepsis, but recent studies have concluded that invasive infections caused by *S. agalactiae* are not uncommon and that they pose a major problem not only in pregnant women and neonates but also in non-pregnant adults, especially the elderly and patients with chronic diseases (36)

Differences in the prevalence of *S. agalactiae* internationally may be attributed to different methods of specimen collection, transport media, and isolation methods. In the present study chocolate and blood, agar was used exclusively as culture media (37).

Neisseria gonorrhoea and *Staphylococcus saprophyticus* 1(3.3%) which were isolated from only 4 patients (3.3%) while *Klebsiella pneumonia* and *Staphylococcus haemolyticus* were positive in 16(12.9%) and regarding *Staphylococcus aureus* and *Enterococcus faecalis* was positive in 12(9.8%) of the isolated bacteria.

The highest percentage of the isolates belonged to Gram-positive bacteria were *Streptococcus agalactiae* 28 (22.5%), *Staphylococcus haemolyticus* 16 (12.9%), followed by *Staphylococcus aureus* 12(9.8%), *Enterococcus fecalis* 12 (9.8%) and *Staphylococcus saprophyticus* 4(3.3%) and similar finding have been reported by (Al- Musawi et al) (38) from Al- Diwaniya (Iraq), who reported a prevalence of isolated *Staphylococcus aureus* was (5.6%) and *Staphylococcus saprophyticus* was (4.8%) and agree with Al- Jammaly and Abdulla (39) from Mosul (Iraq), who reported the rate of infection by *Staphylococcus saprophyticus* was (1.9%).

Staphylococcus aureus belongs to pathogenic bacteria not commonly present in the vagina but however, have been implicated in vaginitis. Infection of the vagina by intestinal flora is quite common due to the close proximity of the anus to the vagina. Furthermore, it was also

reported that whenever Lactobacillus species is displaced by an overgrowth of pathogens like Escherichia coli, Group B Streptococcus, S. aureus and Enterococcus faecalis, clinical signs such as itching/burning sensation, dyspareunia, the yellowish discharge will occur(40)

In a study done (Azizmohammadi & Azizmohammadi) in Iran showed that 32 out of 320 high vaginal swab samples (10 %) were positive for N. gonorrhoeae and this is higher than that recorded in our study (41).

Factors influencing the change in the microbiologic flora include hormonal changes (during menstruation: relapse of bacterial vaginosis around the first 7 days of menstruation and resolved bacterial vaginosis during midcycle), pregnancy or antibiotic administration (26).

The highest percentage of occurrence of vaginal infection was 55 (44.2%) in the age group (25-34) year followed by the age group (35-44) year which was 32 (25.8%) and this in agreement with many studies which reported the high incidence in the same age group.

(Shilan et al) revealed that the highest percentage of infection detected at age group (26- 35) years 113 (48.5%) (42) and similar results were reported by (Hassan et al.) (43) from Basrah (Iraq) who showed that the highest percentage of infection occurred (47.2%) at the same age group.

Bhalla et al (44) from India showed the highest prevalence of infection in age (> 30) years and also study by (Sewankambo et al) showed a strong association between the presence of bacterial vaginosis with age (> 25) years (45).

In another study by Khamees (26) the highest prevalence of sexually transmitted infection (32%) has been found in the age group of (29 – 33) years that attributed this to higher sexual activities in this age group

4.2 The frequency of pus cells in relation to the type of bacteria isolated from women complaining of vaginal infection:

The highest percentage of pus cells associated with S. agalactiae (24) followed by E.coli which was 20 samples and this in agreement with a study done by MANIATIS et al. who evaluated the possible pathogenic role of S. agalactiae based on the criterion of the presence of > 10 leucocytes/ High power field (H.p.f) in the vaginal secretions to denote infection of the vagina and not only on the presence of clinical symptoms. We strongly believe that S.

agalactiae is as pathogenic as the other established pathogens and should be considered a vaginal pathogen (35)

Leukorrhea means increased vaginal discharge; however, the term is often used loosely to include any abnormal vaginal discharge (46).

Vaginal discharge constitutes a considerable problem for many women causing discomfort, anxiety affecting women. Some vaginal discharges are normal and can vary with age, use of contraceptives and menstrual cycle (47).

There are several risk factors for the bacterial vaginosis. Women with BV also have an elevated risk especially after surgical abortion in the first trimester (48).

Many diseases of the genitourinary system are still common due to sexual activity, availability of contraception. Many bacteria are associated with urogenital tract infection especially women with the intrauterine contraceptive device (49).

Leukorrhea is one of the most common complaints for which a patient seeks help, particularly if the discharge is accompanied by itching and burning.

Microscopic examination revealing pus cells (>10 White Blood Cells/ High power field) can confirm the diagnosis of leukorrhea (50).

Pathologic leukorrhea: is usually due to infections of the upper and lower female genital tract. The most common sexually transmitted pathogens associated with leukorrhea are Chlamydia trachomatis, Neisseria gonorrhoeae, and Trichomonas vaginalis), leukorrhea may be the only presenting sign in women infected with these pathogens (51)

CONCLUSION

1. This study revealed the growth of both gram-positive bacteria and gram-negative bacteria.
2. The incidence of gram-positive was (58%) and it was higher than gram-negative bacteria which was (42%).
3. The isolated bacteria were as follow: *E.coli* and *S.agalactiae*(22.5%), *K. pneumoniae*, and *S. haemolyticus* (12.9%), *S. aureus* and *E. faecalis* were(9.8%) while *S. marcescens*, *N. gonorrhoea* and *S. saprophyticus* (3.3%).

4. The highest occurrence of vaginal infection and bacterial growth in the age group(25-34)years and followed by the age group (35-44) years.
5. There was a significant relationship between the presence of pus cells in direct examination of high vaginal swab and the result of bacterial culture.

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