World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 5.088

THE COMMON BACTERIAL CAUSES OF VAGINAL INFECTION IN ERBIL CITY

Sahar Muhammad Zaki Abdulla*[1], Sewgil Saaduldeen Anwer[2], Sanaria Kamaran Mahmood[3], Jwan Mohamed Mahmod[4], Sarmed Shahid Abbas[4], Rozhgar Aziz Mahmod[4] and Diana Tarriq Raaof[4]

[1]M.B. Ch.B, Lecturer in Medical Microbiology and Immunology /Hawler Medical University/ College of Health Sciences/Erbil/Iraq.
[2]Assistant Professor in Medical Microbiology/ Hawler Medical University/ College of Health Sciences/Erbil/Iraq.
[3]Biologist.
[4]Microbiologist.

***Corresponding Author: Dr. Sahar Muhammad Zaki Abdulla**
M.B. Ch.B, Lecturer in Medical Microbiology and Immunology /Hawler Medical University/ College of Health Sciences/Erbil/Iraq.

Article Received on 01/04/2019 **Article Revised on 23/04/2019** **Article Accepted on 13/05/2019**

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 and to determine the antibiotic profile of each bacteria isolated in high vaginal swab. **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 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 and antibacterial susceptibility profile determined for each bacterium either by VITEK® 2 PC or by disk method. **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* in*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 and the Antibiotic susceptibility profile showed that most of the pathogens were resistant to more than one Antibiotics. **Conclusions:** The incidence of gram positive was higher than gram negative bacteria and and the result of bacterial culture and the most of gram positive and gram negative were resistant to Ampicillin, Amoxicillin and most of these pathogens were sensitive to Amikacin, Gentamicin and Tetracycline.

KEYWORDS: Vaginitis, gram positive bacteria, gram negative bacteria, antibiotics susceptibility.

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,[5] while Candida is a frequent cause in pregnant women and diabetics, and occasionally oral contraceptives increase susceptibility for infection.[10] Another cause is viral infections such as the human papillomavirus (HPV) and herpes simplex.[6]

The most common symptom of vaginitis is 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] Acute candida infection has several known predisposing factors including antibiotic and high estrogen dose oral contraceptive usage, hormone replacement therapy, pregnancy, and poorly controlled diabetes mellitus.[9]

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).[10] 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.[11]

Antibiotics have saved millions of lives from deadly infectious diseases in the past 60 years since their introduction to clinical medicine. Despite this tremendous success, antibiotics are facing the growing problem of bacterial resistance. Antibiotic resistance was actually recognized very soon after the discovery of antibiotics. Penicillin, for an instance, was effective against virtually all strains of staphylococcus aureus was first introduced clinically in the early 1940s, but by 1946 antibiotic resistant strain of S. aureus were already identified. These strains were capable of modifying penicillin through B-lactamase activity.[12]

It was reported that that by 1987, 95% of S. aureus worldwide was penicillin resistant.[13] Since the 1980s, vancomycin has become the last weapon against drug resistance S. aureus; however, vancomycin resistant S. aureus was identified in 1996,[14] accompanied by the wide use of antibiotics, both the frequency and spectrum of resistance increased dramatically in the past few decades.[15]

Today, despite the wide variety of antibiotics, natural or synthetic, naming one that has not been associated with resistance is extremely difficult if not impossible.[13]

The inevitable emergence of antibiotic resistance is a natural evolutionary response of bacteria. Upon treatment, tremendous selection pressure screens out cells bearing innate resistance determinants or mutants that are less susceptible. One wide accepted theory for the rapid development and spread of resistance elements already exist in the antibiotic-producing organisms,[15] and bacteria possess well-known machineries by which they can acquire these resistance elements.[14]

Aims of the study

The aims of this study are:

1. To isolate the common bacterial causes of vaginal infection.
2. To determine the antibiotic profile of each bacteria isolated in high vaginal swab.

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 cover slip 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 of T. vaginalis with typical movement, budding yeasts, and clue cells. C. albicans may form pseudomycelia, which may be observed occasionally in vaginal material, pus cells, Epithelial cells and bacteria.[16]

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[12] on the following agars:

Muller Hinton Agar
It is based on the use of an antimicrobial impregnated filter paper disk. The impregnated disk is placed on agar surface, resulting in diffusion of the surrounding medium. Effectiveness of the antimicrobial can be shown by measuring the zone of inhibition for a pure culture of an organism. Zone diameters established for each antimicrobial determining resistant, intermediate, and sensitive for pathogenic microorganisms are listed in the clinical and laboratory standards institute.[16]

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

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.[18] BAPs also contain meat extract, tryptone, sodium chloride, and agar.

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.[19]

VITEK® 2 PC
Today's global healthcare challenges like Multi-Drug Resistant Organisms (MDRO) mean that microbiology labs need to be flexible and responsive to provide the right information at the right time. The efficiency of the VITEK® 2 Compact instrument and VITEK® 2 PC software offers the capacity to help improve reliable microbial identification (ID) and antibiotic susceptibility testing (AST). The instrument also helps rapid reporting capabilities and it is cost-effective (20).

Microbial identification (ID) and antibiotic susceptibility testing (AST) are key to providing the right information for targeted clinical responses and better patient-care outcomes (21). Combining an innovative, automated platform with an expansive database, the VITEK® 2 microbial ID/AST testing system offers the confidence of fast, accurate results. Its smart design helps ensure better overall laboratory workflow with fewer repetitive tasks, higher safety, improved standardization, and rapid time-to-results and reporting.[22]

Antimicrobial sensitivity test
The Kirby Bauer standardized single disk method was carried out.[23] As the following:

1. Mueller-Hinton Agar was employed. The medium was cooled to 45-50 C and poured in petri dishes on a level surface to a depth of 4 ml.
2. When the medium was hardened, the petri dishes were placed in the incubator at 35-37 C for 15-30 minutes to let the excess moisture evaporate.
3. A sterilize cotton was dipped into the standardized bacterial suspension. The excess fluid was removed by rotating the swab firmly against the inside of the tube above fluid level. The swab was then streaked onto the dried surface on Mueller-Hinton Agar in 3 different plates to obtain an even distribution of the inoculum. The plate lids were replaced and the inoculum plate were allowed to remain on a flat surface undisturbed for 3-5 minutes to allow absorption of excess moisture.
4. With the sterile forceps, the selected discs were placed on the inoculated plate and pressed gently into the agar with sterile forceps. Within 15 minutes the inoculated plates were incubated at 37 C for 24 hours in an inverted position. By using a ruler, the diameter of the inhibition zone was measured.[23]

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 from52 patients (42%), whereas Gram positive bacteria were isolated from 72(58%) patients (figure1).

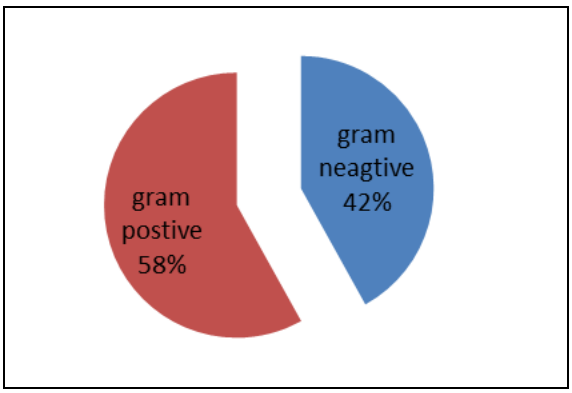

Figure 1: Pie chart shows distribution of microorganism isolates from 31 positive cases.

Distribution of bacterial isolates from vaginal discharge

In table (1) and figure (2) 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 gonorrhea* and *Staphylococcus saprophytics* 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	*E.coli*	28	22.5
	Klebsiella pneumonia	16	12.9
	Serratia marcescens	4	3.3
	Neisseria gonorrhea	4	3.3
Gram positive	*Streptococcus agalactiae*	28	22.5
	Staphylococcus haemolyticus	16	12.9
	Staphylococcus aureus	12	9.8
	Staphylococcus saprophytics	4	3.3
	Enterococcus faecalis	12	9.8

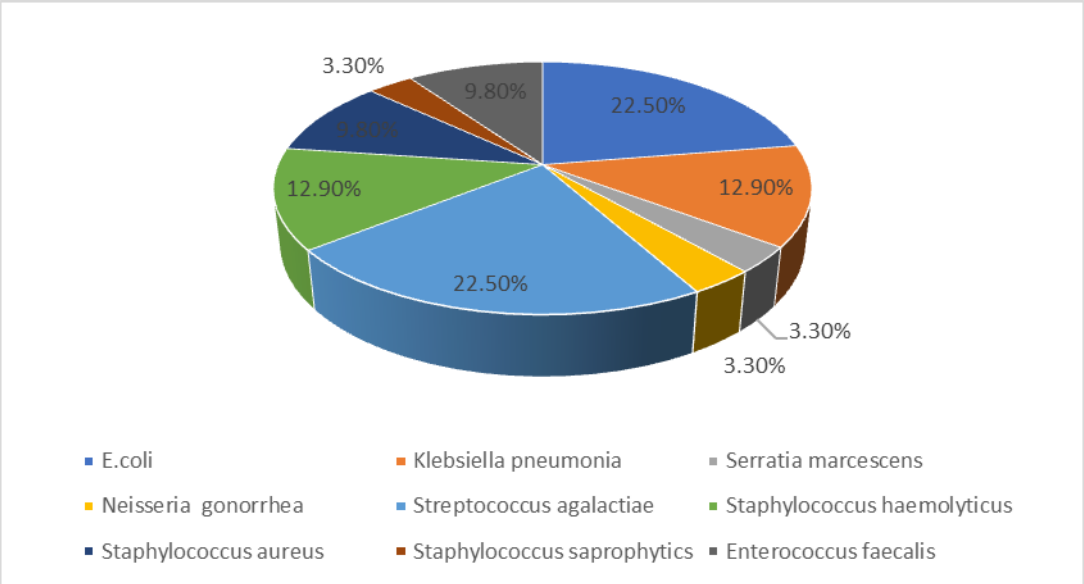

Figure 2: Distribution of bacterial isolates from high vaginal swab.

Antibiotic susceptibility profiles of gram positive bacteria

In table (2) show the antibiotic profile of garm positive bacteria which revealed that *Streptococcus agalactiae* highly sensitive to Amikacin (71.1%) and it was highly resistant to amoxicillin(85.8%) Levofloxacillin(78.6%) and Ampicillin (71.1%).

Streptococcus haemolyticus was highly sensitive to Trimethoprim/sulfamethaxole (100%), Tetracycline (93.7%) and Amikacin(81.2%) while it was100% resistant to (Ampicillin, Amoxicillin) and it was highly resistant to Amoxicillin/ clavulanic acid(75%).

Regarding *Staphylococcus aureus*, it was 100% sensitive to Ciprofloxacin and 11(91.7%) sensitive to Amikacin and 10(83.4 %) sensitive to Amoxicillin/ clavulanic acid, Gentamicin and Tetracycline while *Staphylococcus aureus* was 16(100%) resistant to Ampicillin and 11(91.7%) resistant to Trimethoprim/sulfamethaxole.

Staphylococcus saprophyticus was 4(100%) sensitive to Trimethoprim/sulfamethaxole and Ciprofloxacin and 3(75%) sensitive to Gentamicin and Amikacin while it was 4(100%) resistant to Ampicillin, Amoxicillin, Ciprofloxacin and 3(75%) resistant to Ceftriaxone and Levofloxacin.

Regarding *Entrococcus Faecalis* it was 12(100%) sensitive to Levofloxacin and 9(75%) sensitive to Gentamicin and Tetracycline while it was highly 12(100%) resistant to Ampicillin, Amoxacillin and Trimethoprim/sulfamethaxole.

Table 2: Antibiotics susceptibility profiles of gram positive bacteria isolates.

Antibiotics discs	Pathogens									
	Streptococcus agalactiae (NO=28)		S. haemolyticus (No.=16)		S. aureus (No.=12)		S. saprophytic (No.=4)		E. Faecalis (No.=12)	
	S No. (%)	R No. (%)	S No. (%)	R No. (%)	S No. (%)	R No. (%)	S No. (%)	R No. (%)	S No. (%)	R No. (%)
Ampicillin	8 (28.9)	20 (71.1)	0 (0)	16 (100)	3 (25)	9 (75)	0 (0)	4 (100)	0 (0)	12 (100)
Amoxicillin/ clavulanic acid	12 (42.8)	16 (57.2)	4 (25)	12 (75)	10 (83.4)	2 (16.6)	2 (50)	2 (50)	7 (58.4)	5 (41.6)
Amoxicillin	4 (14.2)	24 (85.8)	0 (0)	16 (100)	4 (33.3)	8 (66.7)	0 (0)	4 (100)	0 (0)	12 (100)
Ceftriaxone	12 (42.8)	16 (57.2)	5 (31.3)	11 (68.7)	4 (33.7)	8 (66.7)	1 (25)	3 (75)	8 (66.7)	4 (33.3)
Gentamycin	12 (42.8)	16 (57.2)	10 (62.5)	6 (37.5)	10 (83.4)	2 (16.6)	3 (75)	1 (25)	9 (75)	3 (25)
Ciprofloxacin	18 (64.2)	10 (35.8)	8 (50)	8 (50)	12 (100)	0 (0)	0 (0)	4 (100)	8 (66.7)	4 (33.3)
Levofloxacin	6(21.4)	22 (78.6)	12 (75)	4 (25)	8 (66.7)	4 (33.3)	1 (25)	3 (75)	12 (100)	0 0%
Trimethoprim/ sulfamethaxole	18 (64.2)	10 (35.8)	16 (100)	0 (0)	1 (8.3)	11 (91.7)	4 (100)	0 (0)	0 (0)	12 (100)
Amikacin	20 (71.1)	8 (28.9)	13 (81.2)	3 (18.8)	11 (91.7)	1 (8.3)	3 (75)	1 (25)	4 (33.3)	8 (66.7)
Tetracycline	24 (85.8)	4 (14.2)	15 (93.7)	1 (6.3)	10 (83.4)	2 (16.6)	4 (100)	0 (0)	9 (75)	3 (25)

S: sensitive R: resistant

Antimicrobial susceptibility profiles of negative isolates

Table (3) shows the antibiotic susceptibility profile of gram negative isolated bacteria from high vaginal swab.

As revealed in the table below*: E. coli* was 28(100%) sensitive to tetracycline and it was highly sensitive to gentamicine and Amikacin 24(85.7%), while it was 27(96.5%) resistant to Ampicillin.

Klebsiella pneumonia was 16(100%) sensitive to Amikacin and 12(75%) sensitive to ciprofloxacillin, while it was 12(75%) resistant to Ampicillin and Amoxicillin/clavulanic acid.

Serratia marcescens was 4(100%) sensitive to gentamicin, Amikacin and Levofloxacin in contrast it was 4(100%) resistant to Amoxicillin, Ciprofloxacin and Trimethoprim/sulfamethaxole.

Serratia marcescens was 3(75%) resistant to Ampicillin and Amoxicillin/clavulanic acid.

Regarding Neisseria gonorrhea, it was 4(100%) resistant to Gentamycin, Levofloxacin and 3(75%) sensitive to Amoxicillin/clavulanic acid and Amikacin while it was 4(100%) resistant to Ampicillin, Amoxacillin, ciprofloxacillin and Trimethoprim/sulfamethaxole.

Neisseria gonorrhea was 3(75%) resistant to Amoxicillin and Ceftriaxone.

Table 3: Antimicrobial susceptibility profiles of negative isolates.

Antibiotics discs	Pathogens							
	E.coli (No.=28)		K.pneumonia (No.=16)		S. marcescens (No.=4)		N. gonorrhea (No.=4)	
	S No.(%)	R No.(%)	S No.(%)	R No.(%)	S No.(%)	R No.(%)	S No.(%)	R No.(%)
Ampicillin	1 (3.5)	27 (96.5)	4 (25)	12 (75)	1 (25)	3 (75)	0 (0)	4 (100)
Amoxicillin/clavulanic acid	12 (42.8)	16 (57.2)	8 (50)	8 (50)	1 (25)	3 (75)	3 (75)	1 (25)
Amoxicillin	11 (39.3)	17 (60.7)	4 (25)	12 (75)	0 (0)	4 (100)	0 (0)	4 (100)
Ceftriaxone	19 (67.8)	9 (32.2)	5 (31.3)	11 (68.7)	2 (50)	2 (50)	1 (25)	3 (75)

Gentamycin	22 (87.8)	6 (12.2)	13 (81.3)	3 (18.7)	4 (100)	0 (0)	4 (100)	0 (0)
Ciprofloxacin	17 (60.7)	11 (39.3)	12 (75)	4 (25)	0 (0)	1 (100)	0 (0)	4 (100)
Levofloxacin	16 (57.2)	12 (42.8)	13 (81.3)	3 (18.7)	4 (100)	0 0%	4 (100)	0 (0)
Trimethoprim/sulfamethaxole	12 (42.8)	16 (57.2)	8 (50)	8 (50)	0 (0)	4 (100)	0 (0)	4 (100)
Amikacin	24 (85.7)	4 (14.3)	16 (100)	0 (0)	4 (100)	0 (0)	3 (75)	1 (25)
Tetracycline	28 (100)	0 (0)	9 (56.3)	7 (43.7)	3 (75)	1 (25)	2 (50)	2 (50)

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 from52 patients (42%), whereas Gram positive bacteria were isolated from 72(58%) patients (figure 1).

The result of this study was in agreement with a study done by (khamees)[24] who revealed that Gram positive bacteria were isolated from167 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[25] from Erbil (Iraq) reported the rate was (68.3%) and the same result reported by (Al- Muk and Hasony)[26] 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 *etal*) who found that *E. coli* to be more prevalent organisms isolated from patient with vaginitis.[27]

The result of this study was in agreement with a study done by (Holand *etal*) who reported that the most common isolated bacteria from HVS from the vaginal women with vaginitis was *Escherichia coli*[28] (15.96%). Other results was lower than our result which reported by (Mumtaz *et al*.) from Pakistan who reported (13.7%)[29] and (Alli *et al*.) from Nigeria who reported (12.1%).[30]

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.[31]

In this study *E. coli* was the most predominant pathogene among gram negative bacteria and this was in agreement with (Khamees, 2012) who recorded the same finding.[24]

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.[32]

Streptococcus agalactiae was isolated in 28(22.5%) and this lower than recorded by (MANIATIS, *et al*) who recorded only (5.7%).[33]

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.[34]

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 were used exclusively as culture media.[35]

Neisseria gonorrhea and *Staphylococcus saprophytics* 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 to *Staphylococcus aureus* and *Enterococcus faecalis* were positive in 12(9.8%) of the isolated bacteria.

The highest percent of the isolates belonged to Gram-positive bacteria were *Streptoococcus 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*)[36] 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[37] 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, yellowish discharge will occur.[38]

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.[39]

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.[24]

In this study *Streptococcus agalactica* was highly sensitive to Amikacin (71.1%) and it was highly resistant to amoxicillin(85.8%) Levofloxacillin(78.6%) and Ampicillin (71.1%).

This result is in agreement with (Karou *etal*) which revealed that the highest resistance rates (>25%) were recorded by S. agalactiae with tetracycline, trimethoprim-sulfametoxazole and gentamicin(119). Some strains of this bacteria resisted to all tested antibiotics, however relative low resistant rates (<5%) were recorded with ciprofloxacin, while in another research done by (Narayana *et al*) reported the that *Streptococcus agalactiae* showed sensitivity to meropenem (100%), amoxicillin (95.7%), ampicillin (91.7%) and cefixime (91.3%).[40]

Streptococcus haemolyticus was highly sensitive to Trimethoprim/sulfamethaxole (100%), Tetracycline (93.7%) and Amikacin (81.2%) while it was100% resistant to (Ampicillin, Amoxicillin) and it was highly resistant to Amoxicillin/ clavulanic acid(75%).

Regarding *Staphylococcus aureus*, it was 100% sensitive to Ciprofloxacin and 11(91.7%) sensitive to Amikacin and 10(83.4 %) sensitive to Amoxicillin/ clavulanic acid, Gentamicin and Tetracycline while Staphylococcus aureus was 16(100%) resistant to Ampicillin and 11(91.7%) resistant to Trimethoprim/sulfamethaxole and this is in agreement with (Karou *etal*) who recorded the highest resistant rates were against trimethoprim-sulfametoxazole, amoxicillin/clavulanic acid, gentamicin and ampicillin[40] and also with a study done by (Bibi, *et al*) reported that *Stphylococcus* is highly sensitive to Cefotaxime, Amikacin and Cefoprazone.[41]

Staphylococcus saprophyticus was 4(100%) sensitive to Trimethoprim/sulfamethaxole and 3(75%) sensitive to Gentamicin and Amikacin while it was 4(100%) resistant to Ampicillin, Amoxicillin, Ciprofloxacin and 4(100%) resistant to Ceftriaxone and Levofloxacin.

Regarding *Entrococcus Faecalis* it was 12(100%) sensitive to Levofloxacin and 9(75%) sensitive to Gentamicin and Tetracycline while it was highly 12(100%) resistant to Ampicillin, Amoxacillin and Trimethoprim/sulfamethaxole.

In a study done by (Sreeja *etal*) showed that 47% isolates were resistant to penicillin, 45% to ampicillin, 50% to ciprofloxacin and 47% to gentamicin.[42]

The result of our study was similar to a study done by (Mulu, *etal*) which showed high resistant (80%) to Ampicillin, Amoxacillin and rimethoprim/ sulfamethaxole and also it was highly sensitive (80%) to Gentamicin and ciprofloxacin.[43]

The *Enterococcus* species have now emerged as nosocomial pathogens. Hence, it is important to know the changing patterns of the *Enterococcus* infections and the antimicrobial susceptibility patterns of the isolates.[44]

According to (Smith *et al*) Staphylococcus aureus is one of the most common causes of infection, incidence of which has been steadily increasing and the vaginal mucosa of females is colonized by this organism.[45]

Staphylococcus aureus is one of the most persistent pathogen of humans and has remained one of the most common causes of infection, incidence of which has been steadily increasing.[46]

In a study done by (Shahina *etal*) Observation of susceptibility test of gram positive bacteria indicate that ampicillin, amoxycillin and co-trimoxazole showed increase rate of resistance and on the other hand, cefuroxime and ceftriaxone showed significantly sensitive which belongs to cephalosporin second and third generation group.[47]

In current study*: E. coli* was 28(100%) sensitive to tetracycline and it was highly sensitive to Gentamicine and Amikacin 24(85.7%), while it was 27(96.5%) resistant to Ampicillin and the result of this study is similar to a study done by (Mulu *etal*) which revealed high resistant to Amoxicillin and Ampicillin and highly sensitivity to Gentamicin.[43]

Also in a study done by(Narayana, *et al*) showed that Isolated *E.coli* highly sensitive to Amikacin and

Ciprofloxacin while it was highly resistant to Ampicillin and Amoxicillin.[48]

Klebsiella pneumonia was 16(100%) sensitive to Amikacin and 12(75%) sensitive to ciprofloxacillin, while it was 12(75%) resistant to Ampicillin and Amoxicillin/clavulanic acid.

The result of this study is similar to a study done by (Akerele *etal*) which showed highly resistant (100%) to Ampicillin and highly sensitive to Ciprofloxacillin.[49]

Serratia marcescens was 4(100%) sensitive to Gentamicin, Amikacin and Levofloxacin in contrast it was 4(100%) resistant to Amoxicillin, Ciprofloxacin and Trimethoprim/sulfamethaxole and it was 3(75%) resistant to Ampicillin and Amoxicillin/clavulanic acid.

In a study done by (NGEOW)[50] showed that high percentage of resistance of *S marcescens* to ampicillin, cephaloridine, penicillin respond to previous reports concerning the inefficacy of these ' Even the new cephalosporins have not shown great promise while the Aminoglycosides, (kanamycin and gentamicin) have been the drugs of choice for many years because of in vitro sensitivity and therapeutic effectiveness. However, Gentamicin resistance rates of 20 to 50% have been reported and Amikacin shows potential sensitivity against *S. marcescens* but already warnings of resistant strains have appeared and any further increase in Aminoglycoside resistance will seriously jeopardise the effective treatment of *Serratia* infections.[50]

S. marcescens can become resistant to Pencillin via two methods. First, *S. marcescens* has been shown to decrease its outer-membrane permeability and second, this bacterium uses beta lactamase to cleave the beta lactam ring of Pencillin which inhibits the entry of the antibiotic.[51]

Rgarding Neisseria gonorrhea, it was 3(75%) sensitive to Amoxicillin/clavulanic acid and Amikacin while it was 4(100%) resistant to Ampicillin, Amoxacillin, ciprofloxacillin and Trimethoprim/sulfamethaxole.

In a study done by (Govender *etal*) revealed that the high percentage *N. gonorrhea* isolates that showed resistance to ciprofloxacin is of concern as ciprofloxacin is used as first-line therapy for *N. gonorrhoeae* since the introduction of this antibiotic, furthermore, ciprofloxacin resistance in *N. gonorrhoeae* has been reported in some parts of the world.[52]

In a study done by (Azizmohammadi & Azizmohammadi)[39] showed that the highest levels of resistance was observed for ciprofloxacin, penicillin , ceftriaxone and tetracycline and also in a study done by (Duplessis *etal*) found that *N. gonorrhea* were resistant to ciprofloxacin, penicillin and tetracycline.[53]

In much of the world, penicillin, Ampicillin and Amoxicillin resistance is very frequent in gonococci, the usefulness of penicillin is increasingly compromised by chromosomally mediated resistance, while penicillinase mediated resistance remains a major problem.[54]

The most of gram positive and gram negative were resistant to Ampicillin, Amoxicillin and most of these pathogens were sensitive to Amikacin, Gentamicin and Tetracycline and this is agreement with a study done by (Okiki Pius *et al*) showed that Gram-positive organisms were more resistant to Ampicillin and Amoxicillin. The Gram-negative organisms were highly susceptible to Gentamicine (91.67% each).[55]

A study done by (Abdulaziz *etal*) showed High resistance rates were observed among GNB to Ampicillin, Amoxycillin-clavulanic acid, Tetracycline and trimethoprim-sulfamethoxazole. On the other hand, low resistance rates were detected for Amikacin and Colistin.[56]

This assertion can further be strengthened by the high level of antibiotic abuse in our locality, arising from self-medication which are often associated with inadequate dosage and failure to response to treatment and availability of antibiotics to consumers across the counters with or without prescription.[57]

CONCLUSION

1. The incidence of gram positive was (58%) and it was higher than gram negative bacteria which was (42%).
2. The isolated bacteria were as follow: *E.coli* and *S.agalacctiae*(22.5%), *K. pneumonae,*and S. *hemolyticus*(12.9%), *S. aureus* and *E. fecalis* were(9.8%) while *S. marcescens*, *N. gonorrhea* and *S. saprophyticus* (3.3%).
3. The most of gram positive and gram negative were resistant to Ampicillin, Amoxicillin and most of these pathogens were sensitive to Amikacin, Gentamicin and Tetracycline

REFERENCES

1. Berkow R. The Merck Manual of Diagnosis and Therapy. 16th ed. Merck Research Laboratories; 1992.
2. Holst E, Wathne B, Hovelius B, Mardh PA. Bacterial vaginosis: microbiological and clinical findings. Eur J Clin Microbiol, 1987; 6: 536-41.
3. Keane FE, Thomas BJ, Gilroy CB, Renton A, Taylor-Robinson D. The association of Mycoplasma hominis, Ureaplasma urealyticum and Mycoplasma genitalium with bacterial vaginosis: observations on heterosexual women and their male partners. Int J STD AIDS, 2000; 11: 356-60.
4. Edwards L. The diagnosis and treatment of infectious vaginitis. Dermatol Ther, 2004; 17: 102-10.

5. Sobel JD, Faro S, Force RW, Foxman B, Ledger WJ, Nyirjesy PR et al. Vulvovaginal candidiasis: epidemiologic, diagnostic, and therapeutic considerations. Am J Obstet Gynecol, 1998; 178: 203-11.
6. Mitchell H. Vaginal discharge--causes, diagnosis, and treatment. BMJ, 2004; 328: 1306-8.
7. Egan ME, Lipsky MS. Diagnosis of vaginitis. American family physician, 2000; 62: 1095-104.
8. Sobel JD. Pathogenesis and treatment of recurrent vulvovaginal candidiasis. Clin Infect Dis, 1992; 14(Suppl 1): S148-53.
9. Fidel PL. Immunity in vaginal candidiasis. Curr Opin Infect Dis., 2005; 18: 107-11.
10. Paavonen J, Stamm WE. Lower genital tract infections in women. Infect Dis Clin North Am, 1987; 1: 179–98.
11. Haggerty CL, Hillier SL, Bass DC, Ness RB. Bacterial vaginosis and anaerobicbacteria are associated with endometritis. Clin Infect Dis., 2004; 39: 990-5.
12. Sellors J, Mahony J, Goldsmith C. The accuracy of clinical findings and laparoscopy in pelvic inflammatory disease. Am J Obstet Gynecol, 1991; 164: 113–20.
13. Walsh C. antibiotic – action Origins resistance. ASM press, Washington, D.C., 2003b; 13-17 pp.
14. Lancini G F, Parenti, Gallo GG. Antibiotics – A Multidisciplinary Approach. Plenum Press, New York. 1995; 2-3.
15. Bently SD, KF, Chater, AM, Cerdeno-Tarraga, GL, Challis, NR, Thomson, KD, James, DE, Harris MA, Quail H K, and Harper, et al. Complete genome sequence of model actinomycete Streptomyces coelicolor A3(2). Nature. 2002; 417: 141-7.
16. Ikeda HJ, Ishikawa A, Hannamato M, Shinose H, Kikuchi T, Shiba Y, et al. complete genome.
17. Steven L. Modern Trial advocacy. New York, 2004; 45.
18. Segen. "Chocolate agar: Definition". The Free Dictionary. Retrieved 28 September 2012.
19. Gunn B A. "Chocolate agar: A differential medium for gram positive cocci". Journal of Clinical Microbiology, 1984; 20(4): 822–823.
20. Cheesbrough M. Medical laboratory manual for tropical countries, 11 ed. Microbiology, 1984.
21. Sanders C C, P Michel, Moland E S, Cavalieri S J, Shubert C, Thomson K S, et al . Potential Impact of the VITEK 2 System and the Advanced Expert System on the Clinical Laboratory of a University-Based Hospital. Journal of clinical microbiology (JCM), 2001; 39(7): 2379–2385
22. Washington C, Stephen DA, William MJ, Elmer WK, Gary WP, Paul CS, et al. Konemans color atlas and textbook of diagnostic microbiology, 6th ed. Baltiomore, 2006.
23. Bauer AW, Kirby WM, Sherris JC, and Truck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol, Apr; 1966; 45(4): 493-6.
24. Khamees S S. Characterization of vaginal discharge among women complaining of genital tract infection. Int. J. of Pharm. & Life Sci. (IJPLS), 2012; 3(10): 1997- 2002.
25. Jarjees R K. Bacteriological study of the incidence of genitourinary tract infection in diabetic women in Erbil.M.Sc. Thesis. Erbil: University of Salahaddin. College of Science. Iraq, 2006.
26. Al-Muk J M, Hasony H J. Isolation of Gardnerella vaginalis from Pregnant Women with Bacterial Vaginosis in Basrah, Iraq. Bahrain Medical Bulletin Journal, 2001; 23(3): 124-126
27. Lawrence U C, Achi OK, Obeagu E I, Elemchukwu Q. Prevalence Of Bacterial Vaginosis Among Female Students Of Michael Okpara University Of Agriculture, Umudike, Abia State. Nigeria. Journal of Pharmacy and Biological Sciences, 2014; 9(5): 39-52.
28. Holland J, Young ML, Lee O, Chen S C A. Vulvovaginal carriage of yeasts other than Candida albicans. Sexually Transmitted Infection Journal, 2003; 79: 249-250.
29. Mumtaz S, Ahmad M, Aftab I, Aktar N, Hassan M, Abdul Hamid. Aerobic vaginal pathogens and their sensitivity pattern. Journal Ayub Medical Collage Abbottabad. 2008; 20 (1): 113-117.
30. Alli J A O, Okonko I O, Odu N N and Kolade A F. Detection and Prevalence of Genital Pathogens among Attendees of Sti Clinic of a Tertiary Care Hospital in Ibadan, Southwestern Nigeria. World Journal of Medical Sciences, 2011; 6(3): 152-161.
31. Shilan S., Ahmad A, Ali AF. Microbiological Study of Pathogens Isolated From Women with Genital Tract Infection. Int.J. MediPharm Res, 2015; 1(1): 01-12.
32. Mohamed B J, Al- Thwani A N. Evaluation the effect of different factors on urogenital infection and the associations between urinary tract infection and vaginitis. Iraqi Journal of Reaserch, 2009; 1(2): 188-197.
33. Maniatis A N, Palermos J, Kantzanou M, Christodoulou C, Legak1s N J. Streptococcus agalactiae: a vaginal pathogen?. J. Med Microbiol, 1996; 44: 199-202.
34. Kontnick CM, Edberg SC. Direct detection of group B streptococci from vaginal specimens compared with quantitative culture. J Clin Microbiol, 1990; 28: 336-339.
35. Saini S, Gupta N, Aparna, Batra G, Arora D R. Role of Anaerobes in acute pelvic inflammatory disease. Indian Journal of Medical Microbiology, 2003; 21(3): 189-192.
36. Al-Mousawi J K N, Tarish H R, Al-Saadi M K. Microbiological Study of Bacterial Vaginosis Among Pregnant Women in Al- Diwaniya City. The Medical Journal of Basrah University (MJBU), 2006; 24(1&2): 45-49.
37. Al-Jammaly M M H and Abdulla B A. Prevalence of Microorganisms in Genitourinary Tract Infections of

Females from Mosul City. Tikrit Science Journal, 2008; 13(1): 46-51.
38. Donders GGG, Vereecken A, Bosmans E, Dekeersmaecker A, Salembier G, Spitz B. Definition of a type of abnormal vaginal flora that is distinct from bacterial vaginosis. Aerobic vaginitis Br. J. Obstet. Gynecol, 2002; 109: 34-43.
39. Azizmohammadi S, Azizmohammadi S. Antimicrobial susceptibility pattern of Neisseria gonorrhoeae isolated from fertile and infertile women. Tropical Journal of Pharmaceutical Research, 2016; 15(12): 2653-2657.
40. Karou SD, Djigma F, Sagna T, Nadembega C, Zeba M, Kabre A, Simpore J. "Antimicrobial Resistance of Abnormal Vaginal Discharges Microorganisms in Ouagadougou, Burkina Faso." Asian Pacific Journal of Tropical Bio medicine, 2012; 5: 294–297.
41. Bibi A, Sana J, Muhammad I, Sayyed S, Sana U, Zeeshan N, et al. isolation ,identification and antibiotic susceptibility testing of microorganisms from female patients of Ayub medical complex through high vaginal swab. Sci.Int.(Lahore), 2014; 26(4): 1581-1586.
42. Sreeja S, SreenivaSaa BP, Pratha BG. The Prevalence and the Characterization of the Enterococcus Species from Various Clinical Samples in a Tertiary Care Hospital. Journal of Clinical and Diagnostic Research. November, 2012; 6(9): 1486-148.
43. Mulu W, Yimer M, Zenebe Y, Abera B. Common causes of vaginal infections and antibiotic susceptibility of aerobic bacterial isolates in women of reproductive age attending at Felegehiwot referral Hospital, Ethiopia: a cross sectional study. BMC women, s Health, 2015; 15: 42-51.
44. Desai PJ, Pandit D, Mathur M, Gogate A. The prevalence, identification and the distribution of various species of Enterococci which were isolated from clinical samples, with special reference to the urinary tract infections in catheterized patients. India J Med Microbial, 2001; 19: 132-37.
45. Smith TL, Pearson ML, Wilcox KR, Cruz C, Lancaster ML, Robinson – Dunn B. Emergence of vancomycin resistance in Staphylococcus aureus: epidemiology and clinical significance N Engl Med, 1999; 340: 493–501.
46. Schlievert PM, Case LC, Strandberg KL, Timothy L, Tripp TL, Linn YC, et al. Vaginal staphylococcus aureus superantigen profile – shift from 1980 and 1981 to 2003, 2004 and 2005. J. Clin Microbial. 2007; 45: 2704 – 2707
47. Shahina Z, Chowdhury AHMI, Arifuzzaman M. Prevalence of Antimicrobial Sensitivity and resistant Pattern of Gram Positive Cluster Forming Cocci in Clinical Samples, 2014; 13(1): 53-57.
48. Narayana S N, Ramalingappa P, Bhatara U. Antimicrobial Sensitivity Pattern of Microorganisms Isolated from Vaginal Infections at a Tertiary Hospital in Bangalore, India. Int J Med Students, 2015; 3(1): 34-9.
49. Akerele J, Abhulimen P, Okonofua F. Prevalence of Asymptomatic Genital Infection among Pregnant Women in Benin City, Nigeria. African Journal of Reproductive Health, 2002; 6(3): 93-97.
50. Ngeow YF. Antimicrobial sensitivity and clinical significance. Malaysian J Path, 1981; 4: 35-41.
51. Hejazi A, Falkiner FR. Serratia marcescens. Medical Microbiology, 1997; 46: 903-912.
52. Govender S, Lebani T, Nell R. Antibiotic susceptibility patterns of Neisseria gonorrhoeae isolates in Port Elizabeth. South African medical journal, 2006; 96(3): 225-226.
53. Duplessis C , Puplampu N, Nyarko E, Carroll J, Dela H, Mensah A, Amponsa A. Gonorrhea Surveillance in Ghana. Military medicine, 2015; 180(1): 1.
54. John W, Tapsall W. Antibiotic Resistance in Neisseria gonorrhoeae. Clin Infect Dis, 2005; 41(Suppl4): 263-268.
55. Okiki PA, Nosazena AE, Josiah OM. Evaluation of microorganisms associated with vaginal infection in Owe, Nigeria. Advances in Applied Research, 2015; 6(9): 79-83.
56. Abdulaziz A, Zorgani L, Zuhair B, Hisham Z, Khalifa S G. Amimicrobial Susceptability profiles of Gram Negative Bacilli and Gram Positive cocci isolated from cancer patients in Libya. ARCHIVIES OF CLINICAL MICROBIOLOGY, 2002; 3(3): 3-8.
57. Odegbemi T. The use and abuse of antibiotics. Nig Med Pract, 1981; 1(1): 5-8.