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Prevalence of *Trichomonas vaginalis* and Bacterial Co-infection among Women in Holy Karbala

A Thesis

Submitted to the Council of the College of Science, University of Babylon, as a Partial Fulfillment of Requirements for the Degree of Master of Science/ Biology

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بسم الله الرحمن الرحيم

﴿ يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ ﴾

صدق الله العلي العظيم

سورة المجادلة آية (١١)

Academic Supervisor Certification

I certify that this thesis was prepared under my supervision at the Department of Biology, College of Science, University of Babylon in partial fulfillment of the requirement for the degree of Master of Science in Biology / Zoology.

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Date / /2022

In view of the available recommendation, I present this thesis for evaluation by the Examining Committee.

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Dedication

To the Prophet of mercy, **Muhammad** and his pure family, blessings of God be upon them

To my master, the present, the absent, Al-Muntadhar Imam **Muhammad Al-Mahdi**, may God hasten his honorable reappearance

To my support after God in the life of scientific and practical life and the candle that lights my life

…..my mother

To those who supported me with love and understanding

To whom all the meanings of blessings were revealed

My husband **… *Husam***

To my children who share with me the circumstances of my scientific career

…Mohammed and Laila

To my family, my ***sisters*** **and** ***brother***

Zainab

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Summary

The current study to detect the relationship of *Trichomonas vaginalis* infection with other types of bacterial and fungal pathogens in vaginal secretions and urine samples, as well as finding the relationship between demographic factors and research results. In this study the vaginal swabs and urine samples were collected from 232 patients as well as 25 samples from healthy women (control) with different ages ranged between (14-58) years. The patients attended the hospitals and medical clinics from the four district of Karbala province from the period Nov. 1, 2020 to June 5, 2021.

Direct wet mount preparation, direct Gram smear, and general urine examination results showed that the highest infection rate was 20 (8.62%) with *T. vaginalis* in direct wet mount and gram stain same number and percentage for tests while in general examination urine the number and percentage was 16 (6.89%). The results showed a significant differences between patient group and control group regarding to *T. vaginalis* in swab and urine ($P<0.001$). In addition to the presence of some microorganisms that appeared in swab cultures on MacConky and Blood agar were identified with Vitik 2 system after colonies gram stains. These are associated with parasite infection, as the high co-infection rate was *Klebsiella pneumonia* ssp *pneumonia* 5 (2.15%) (%25), *Escherichia coli* 4 (20%, 1.72%), *Enterococcus faecalis* 3 (15%, 1.3%), *Staphylococcus hominis* ssp *hominis*, *Enterobacter aerogenes*, *Staphylococcus aureus* and *Candida* spp. with lowest percentage 1(5%, 0.43%) for each one.

It was found that the highest percentage of association with microorganisms *T. vaginalis* was with *Klebsiella pneumonia* 5 (2.15%, %25). Despite the small number of women infected with *Klebsiella pneumonia*, the results showed a significant relationship between *T. vaginalis* and *Klebsiella pneumonia*, *Escherichia coli* and *Enterococcus faecalis* ($P<0.001$). All cases were associated with infection with the parasite, and there were also very low

co_ infection rates for other pathogenic bacterial species 1 (5%, 0.43%) that were associated with infection with and these bacteria were not found alone among 212 study group which were not infected with the parasite as well as among control group.

The results showed that the highest number and percentage infected women was 8 (11.3%) respectively at the age group (21-27) and that lowest at age group (35-41) was 1 (2.2%) respectively. All infected women were married with 20 (8.8%) number and percentage. The highest number and percentage infected women were with secondary educational level 6 (14.6%), while lowest occur 2 (5.1%) in illiterate. Infected women whom were pregnant had higher infected rate than non- pregnant with 7 (11.5%) number and percentage respectively. Infected women with vaginal itching was higher than infected women without vaginal itching with 13 (9.4%), and infected women whom suffer from dysuria higher than infected women without any with 14 (10.8%).

According to WBC count in urine sample with automated analyzer, the results shown a significant value ($P<0.011$) of WBC in patient and control group. The results showed that the mean for patient group 20.7, the mean for the 212 whom had alone infected with microbial without parasite infection was 14, while the mean for the 20 women with co-infection was 92, which result in highest elevated WBC count among women with co-infection.

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List of Abbreviations

AST	Antimicrobial Susceptibility Testing
AV	Aerobic Vaginosis
B cells	Bone Marrow- Or Bursa-Derived Cells
BV	Bacterial Vaginosis
CPs	Cysteine Proteases
CSTs	Community State Types
DIV	Desquamative Inflammatory Vaginitis
FRT	Female Reproductive Tract
GP	Gram Positive
HIV	Human Immunodeficiency Virus
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IL-8	Interleukin-8
LPG	Lipophosphoglycan
PCR	Polymerase Chain Reaction
SLPI	Secretory Leucocyte Protease Inhibitor
STD	Sexually Transmitted Disease
T cells	Thymus Cells
T. vaginalis	*Trichomonas vaginalis*
Tv	*Trichomonas vginalis*
VECs	Vaginal Epithelial Cells
WBCs	White Blood Cells.
GUE	General Examination Urine
IR	Infection Rate
RPM	Revolutions Per Minute

Chapter One
Introduction

Introduction

1.1. Introduction

Trichomoniasis is the most prevalent non-virus sexually transmitted disease (STD) caused worldwide (Herbst *et al*., 2016). *Trichomonas vaginalis*, the causal agent, is an anaerobic flagellate protozoan pathogenic parasite that is colorless and oval. It infects both female and male urogenital tracts (Mao and Liu, 2015).

In their life cycle, there is no cyst, so the transmission occurs via the trophozoite stage. Most infected people are asymptomatic. Patients suffering from *T. vaginalis* report various symptoms, such as purities, frothy discharge with yellowish-green color, dysuria, and the strawberry cervix recognizing by punctuates hemorrhagic lesions in women (Arbabi *et al*., 2018). There are many discomforts in urination or having sex (Itriyev, 2020). More than 280 million cases annual are reported (Mendel *et al*., 2020). *T. vaginalis* holds the distinction of being the only parasite infection transmitted sexually in humans. It is a highly effective disease, responsible for about the same number of STDs as *Chlamydia trachomatis*, the most frequent sexually transmitted bacterial infection (Al–Quraishi, 2014). Conditions with Trichomoniasis are related to an elevated human immunodeficiency virus HIV risk infections. It may result in adverse effects on pregnancy outcomes, including premature birth and birth with low weight. Additionally, *T. vaginalis* has been linked to an elevated risk of prostate and cervical cancer.

The human vagina has a naturally balanced environment with an acidic environment, where the pH ranging from 3.8 to 4.5 which is a result of the presence of natural bacteria of *Lactobacillus* spp. This genus positively protects the vagina from pathogens like *T. vaginalis* (Donne, 1836). *Trichomonadidae*, *Trichomonadida*, and vaginal bacteria (Adnan & Marjani,

2020). The "next-generation" development sequence techniques resulted in discovering five of the distinct vaginal microbiota, which referred to as “community state types (CSTs)” (Ravel *et al*., 2011). *Lactobacillus* genus bacteria dominate the population microbe in all CSTs types just one. Meanwhile, this one of the strains lacks dominating species, making a microbial community more complex.

Lactobacillus act as a protection agent by decreasing the vaginal pH by lactic acid generation for the host, competing for resources, and producing antimicrobial compounds such as hydrogen peroxide and bacteriocins to inhibit adverse microbiota growth. Pregnancy, age, antimicrobial medication, and sexual activity may interfere disrupting the vaginal microbiome dynamic equilibrium, resulting in urogenital tract dysbiosis.

Bacterial vaginosis BV is the most prevalent microbial vaginal imbalance in reproductive age women, affecting between 10% and 30% of females worldwide. BV is associated with foul-smelling and abnormal vaginal discharge, vaginal irritation, and an unusually elevated vaginal pH. As seems to be the case with *T. vaginalis*, BV is linked to preterm birth, premature rupture of membranes, and adverse reproductive outcomes. Additionally, bacterial vaginosis is a health risk for acquiring sexually transmitted diseases (STDs) (Mirmonsef *et al*., 2012).

Understanding why *Tv* is typically persistent, how *Tv* infection may lead to inflammation sequelae, and if *Tv* infection may change the microbiome in the female reproductive tract (FRT) needs determining the types of immune responses that *T. vaginalis* stimulates and establishing, and whether *T. vaginalis* cytotoxic activity can kill leukocytes. *T. vaginalis* is a pathogen that only affects humans. *T. vaginalis* commensals, symbionts, and concomitant infections are thus likely to impact the quality of the adaptive

immune response to *T. vaginalis* which may be further confounded by direct parasite destruction of effector immune cells (Mercer *et al*., 2016).

Techniques for the cultured diagnosis of *T. vaginalis* parasite, the in pouch media is a selective media, the Polymerase chain reaction (PCR) and gene sequences are the choice techniques for diagnosing and classifying *T. vaginalis* (Paxton *et al*., 2019). The direct microscopic examination of the vaginal wet mount is the most common method used to analyze vaginal trichomoniasis, which gives high specificity of fresh vaginal specimen (Al-Mamoori *et al*., 2020). The testing with swab culture media of vaginal secretions or vaginal swabs had a sensitivity which reached to (63.0–98.2%) and specificity compared to (99.4 –100%).

1.2. Aim of Study

Considering the significance of studying this disease, Iraq lacks in-depth studies on numerous aspects of the parasite's life and spread, owing to the critical conditions that Iraqi society is experiencing as a result of the deterioration of services, particularly health, in some communities, to the point where infection with parasites, including that the parasite has become widespread and known, rapidly spreading sexually among women and men, and therefore our study aims to:

1- Studying relationship *T. vaginalis* with other types of bacterial and fungal pathogens associated with infection with this parasite.
2- Finding the relationship between demographic factors and the study results.

Chapter Two
Review of Literature

2. Literature Review

2.1. Historical Background

T.vaginalis was firstly discovered in 1836 by Donne when he observed motile microorganisms in women's frothy, purulent secretions suffering from vaginal discharge and genital irritation and named as *T. vaginale*. It is considered one of the common parasitic diseases in Europe and the United States of America, which is regarded as a non-viral sexually transmitted disease. It has an estimated incidence in the world of more than 248 million cases annually (Al-Majidii *et al.,* 2020).

T. vaginalis history from its discovery to several test for detection can be abstracted in table (2-1).

Table (2-1): *T. vaginalis* history

***T. vaginalis* history**	
1836	Alfred Donne was the first to discover this parasite as a moving organism under a microscope when examining the vaginal secretions of women suffering from genital irritation (Donne, 1836)
1916	O. Hoehne was the first to call this parasite the term trichomoniasis when he identified the clinical symptoms caused by the *T. vaginalis* parasite when it colonizes the mucous membrane of the vagina (Hoehne, 1916)
(1934-1939)	L. Procaccini was the first to classify *T. vaginalis* as a sexually transmitted disease (STD) (Woike *et al*., 2015).
1940	R.E. Trussell pioneered the practice, employing culture medium to identify *T. vaginalis* infections in women's secretions (Asmah *et al*., 2018)
1960	The first scientist uses nitroimidazole as a medication for the treatment of trichomoniasis, (Jaloob Al- Janaby *et al*., 2018)

2.2: Classification of *T. vaginalis*

T. vaginalis is classified as follows (De Aquino *et al*., 2020).

Domain: Eukarya

Kingdom: Protista

Phylum: Metamonda

Sub phylum: Trichozoa

Class: Parabasaila

Order: Trichomondida

Family: Trichomonadidae

Genus: *Trichomonas*

Species: *Trichomonas vaginalis*

2.3. Morphology and Structure

T. vaginalis is flagellated protozoan extracellular parasite and lives in anaerobic conditions. *T. vaginalis* causes a vaginal infection which also causes trichomoniasis (Hinderfeld & Simoes-Barbosa, 2020). It is one of the leading causes of STD (Margarita *et al*., 2020). It infects the urogenital tract, such as the cervix, vagina, urethra, and is distinguished by its shape and size (Pekmezovic *et al*., 2019). *T. vaginalis* size and morphological shape have been shown to vary depending upon the condition under which the organism is maintained. Grown axanically in vitro, *T. vaginalis* tend to be ovoid or pear-shaped, while organisms grown in the presence of vaginal epithelial cells (VECs) appear to transform to an amoeboid shape (Mahmud *et al*., 2018).

The parasite is considered to have four major morphological characteristics (i) an anteriorly located nucleus that has been reported to contain six telocentric chromosomes, (ii) four anterior flagella, (iii) undulating membrane and, (iv) a long axostyle that protrudes through the organism posterior end and bisects the cell longitudinally. Double membrane-bound organelles called hydrogenosomes, visible under the light microscope as Paracostal and paraxostylar granules, are considered the anaerobic equivalent of mitochondria in *T.vaginalis*. This structure is considered to serve as a point of attachment for the parasite to the vaginal epithelial cells (Al-Hadraawy *et al.*, 2013).

The size of *T. vaginalis* (figure 2-1) ranges from 7-32 x 5-12µm (long x wide), it has 4 free anterior flagella and the 5th flagellum return along the edge of the undulating membrane and the end posterior of 5th flagellum located in the middle of the body (Al-Majidii *et al.*, 2020).

The movement of the undulating membrane and the flagella gives characteristic quivering motility for this parasite. The nucleus of *T. vaginalis* is located in the anterior situation and surrounded by a nuclear envelope porous. A rod-like structure, slender hyaline, called an axostyl, begins at the nucleus and runs longitudinally through the protozoan, projecting through the parasite's posterior end and culminating in a sharp tip (Muluneh, 2011). The flagella and axostyle are the main features of the diagnosis of *T. vaginalis* (Owino, 2020).

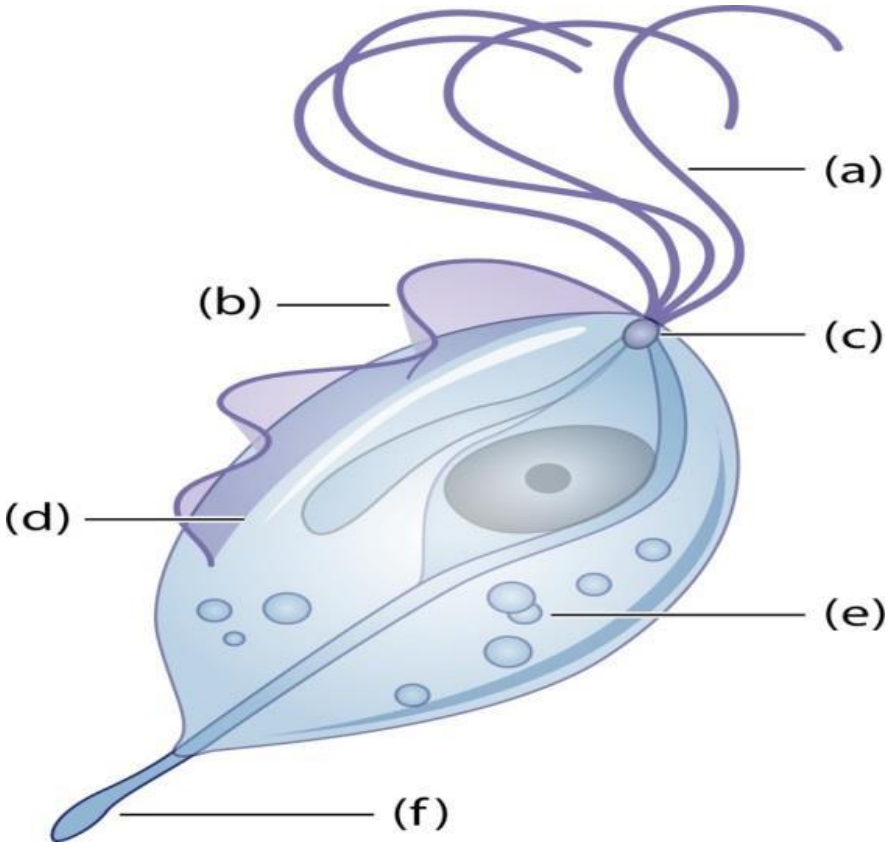


Figure (2-1): Diagram of *Trichomonas vaginalis:* (a) Anterior flagellum, (b) undulating membrane, (c) pelto, (d) costa, (e) hydrogenosomes, (f) axostyle (Bouchemal *et al.*, 2017).

2.4: Life cycle of *T. vaginalis.*

This parasite has a simple life cycle which no cysts stage. The trophozoite stage is the only stage of this parasite. It is responsible for the transmission and infection, and it is the diagnostic stage for this parasite (Beri *et al*., 2020). This parasite cannot survive for a long time outside the human body. A human being is the only host of this parasite, where the infection is transmitted through coitus and rarely by the fomites (Valenti *et al*., 2018) and humans are considered the only host for the parasite (Chinyere *et al*., 2010).

This species lives in the urinary tract and prostate in males and in the lower genital tract in females and is the most common in terms of injuries where

bilateral longitudinal fission is a method of sexual reproduction (D'Ancona *et al*., 2019).

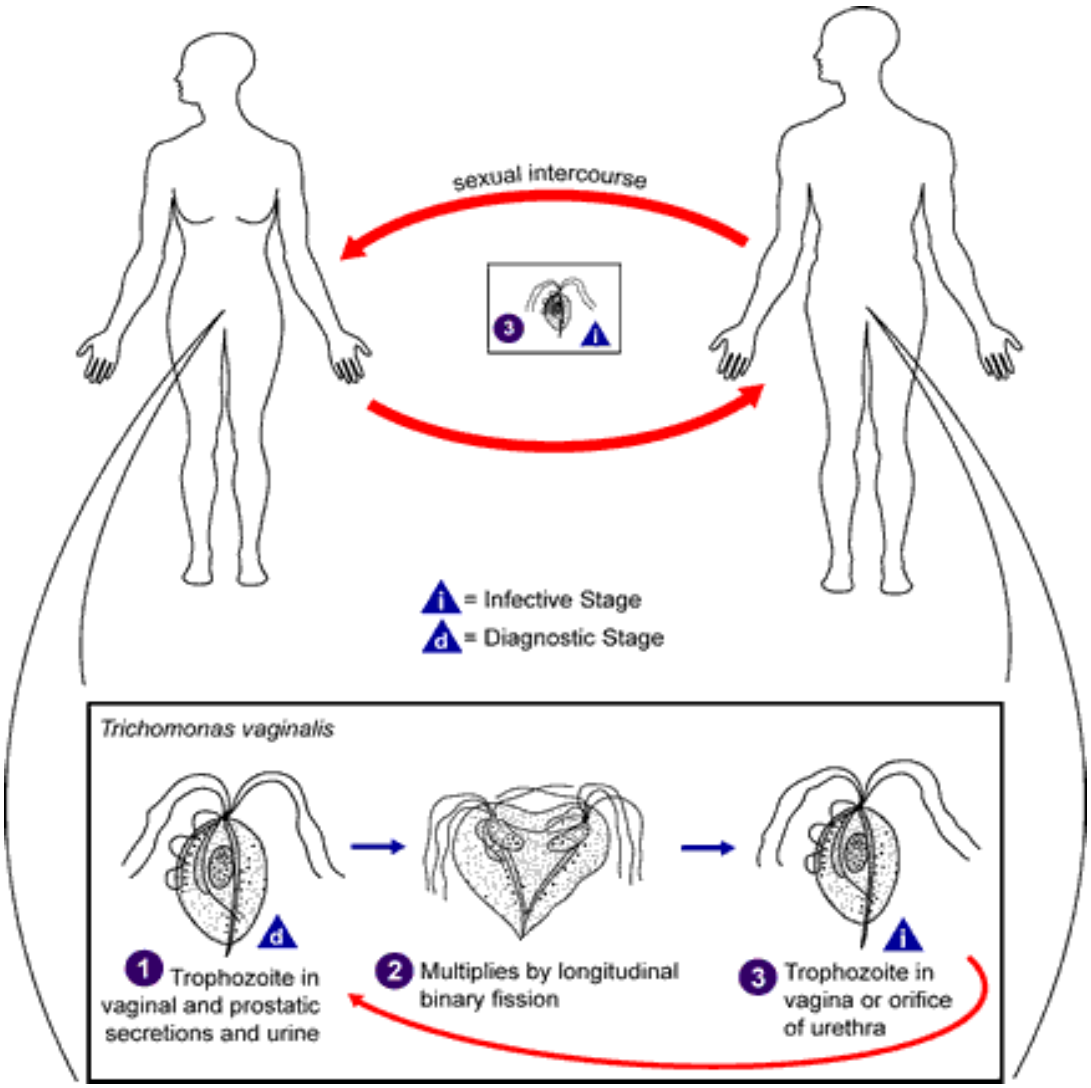


Figure (2-2): Life cycle of *Trichomonas vaginalis* (AL-Majidii *et al.*, 2020)

2.4.1 Biology of *Trichomonas vaginalis*

The vaginal Trichomonas parasite lives in the urinary, genital tract for both sexes, and the vagina is the prominent place for the parasite to exist in females. The normal acidic function of the vagina ranges from (3.8-4.2). Studies have indicated that the parasite loses its viability at an acidic condition of less than 4.9 and more than 7.5 (Al-Badri & Al-Tikriti, 2013). Although the *T.vaginal* parasite is an anaerobic primary parasite, it grows under aerobic conditions (Land & Wrischnik, 2013).

Some studies show that *T. vaginalis* parasite can survive for about 20 hours at temperatures of -10°C, and it remains for 7 hours at room temperature. It can also stay in seminal fluid for 6 hours and in urine for 24 hours as they can live in river water for five days, in a bathtub, wet towels, and sitting places on the toilet for 12 hours. From this, the parasite can be spreading outside the human body. The incubation period for this parasite ranges from (5-28) days, and symptoms of the disease may appear on the affected person, but some are without any symptoms (Tompkins *et al*., 2020).

T.vaginalis trophozoites thrive in alkaline rather than acid pH environments such as that seen in inpatients. The avidity of the average vagina (pH 3.8 to 4.4) ordinarily discourages the infection; the organism itself causes a shift toward alkalinity (pH 5.0 to 6.0), which further encourages its growth (Jawetz *et al.*, 2016).

2.5. *Trichomonas vaginalis* Infection

The vaginal Trichomonas parasite is sexually transmitted between people (Hillier, 2013). There are other cases of transmission other than sexual intercourse, such as the use of contaminated toilet seats, towels, and swimming

pools, and it may be transmitted through contaminated water hoses and a non-sterile Speculum during examination in medical clinics and other medical materials that are not sterile (Al-Badri& Al-Tikriti, 2013). In recent births, infections also occur, so that the infection is transmitted from the mother to the newborn baby (Hussein, 2010).

Trichomoniasis includes broad variation symptoms, ranging from irritation and acute inflammation with frothy malodorous discharge to asymptomatic carrier status; increased amount of iron in a menstrual fluid may contribute to the fact that various pathogenic mechanisms are active. *T. vaginalis* is one of the causative agents of vaginitis; nevertheless, more than half of end cervical infections do not generate enough inflammation to show clinical signs and symptoms. However, vaginal discharge, dysuria, dyes pareunia, pelvic discomfort or pain, and perineal itching erythema multiform and vulva or labia swelling may indicate trichomonal disease, (Al-Hadraawy *et al.*, 2013).

2.6. The Pathogenicity of *T. vaginalis*

T.vaginalis infection may lead to vaginitis, urethritis, cervicitis, infertility, post-hysterectomy disease, and pregnancy complication such as premature labour and low-weight offspring. The parasite also causes non-gonococcal urethritis, prostatitis, and perhaps other lower genitourinary tract syndromes in infected men. Trichomoniasis also has been associated with an increased risk of transmission infection with human immunodeficiency virus (HIV) and pelvic inflammatory disease (Vanissa *et al*., 2010).

Lactobacilli are essential in protecting the vagina from infection; they remain the dominant flora, but not the only flora of the vagina. They may be

decreased or absent in acute or chronic disease with trichomoniasis, (Al-Hadraawy *et al.*, 2013).

The pathogenic mechanism of this parasite was not fully clarified or is still being not clarified, where many reports show multiple interactions between the host and these parasites, which leads to the emergence of clinical symptoms of different spectra. Still, the most potent factor is the adhesion of the parasite's surface to the host cells, its hemolytic activity, and its secretion of proteins and cell separation factors proteins with pores an essential role in signs of pathogenicity (Dessì *et al*., 2019).

There is a highly complex relationship between *T. vaginalis* and its host. A wide range and clinical symptoms cannot be considered a pathogenic mechanism for this parasite since all clinical isolates material can cause infection and produce disease (Baer, 2020).

The premenopausal women's vagina is colonized by a particular microbiota that has been divided into five kinds. One *Lactobacillus* species dominates four of these community state types (CSTs). The fifth kind CST (named CST-IV) is identified as lactobacilli absence and the overgrowth of anaerobic bacteria. Although infection with *T. vaginalis* is linked to the microbiota of the CST-IV, this association does not necessarily suggest causality (Hinderfeld *et al.*,2019).

2.7: Immune system avoidance of *T. vaginalis*

Vagina is a variable and anti-microbial environment, *T. vaginalis* must synthesize many CPs that enable it to avoid the human immune system and thus activate and flourish the parasite, which is more important on the part of pathogenesis (Pekmezovic *et al*., 2019). One of the most important supplements available in the vagina is menstrual blood, whose activity is

half the blood activity in the vein, and according to experiments about a third of samples from menstrual blood is not a complementary activity at all and there is cellular toxicity towards *T. vaginalis*, but even with a low concentration of the parasite during the menstrual period, there is continuity post - menstrual infection while other organisms decrease their number in the vagina during menstruation (Leka and Moran, 2020). Increased iron levels are due to increased CP and erythrocyte degradation, which makes the iron environment high and increases expression for CP (Moonah *et al*., 2019).

Another method of disguising this parasite is the mechanism of appearance variation by expression or non-expression of protein 270 KDa at the specific region. Where *T. vaginalis* is described as negative or positive for protein 270KDa and is positive but the expression does not occur and notify the adhesion characteristic (Al-Hadraawy *et al*., 2018).

2.8. Diagnosis of *Trichomonas vaginalis*

Samples used to diagnose *T. vaginalis* are clinical samples such as urine, semen, vaginal fluid, and a vaginal swab from the cervix. Each sample is treated with a particular method for diagnosing trichomoniasis by a physician (Bruni *et al*., 2019). The clinical signs cannot be relied upon in diagnosing the Trichomoniasis vaginal parasite for its similarity with symptoms caused by other organisms (Chandrasekhar *et al*., 2013). Among the methods adopted in the diagnosis are:

2.8.1. Saline wet mount evaluation

Under microscopic examination, a wet swab is taken from the vagina to diagnose Trichomoniasis. It is done by using saline solution, where a small

amount is placed on a clean glass slide and mixed with a small amount of vaginal secretions from the infected person and then examined under a microscope lens with different enlargement forces. With this method, many motile vaginal parasites, epithelial cells, as well as white blood cells are seen (Rayan *et al.,* 2019).

The standard for the diagnosis of trichomoniasis is wet microscope smear because it is quick, cheap, and specific; therefore, wet smear has been used for more than 150 years (Delavar, 2001; Al-Hadraawy *et a*l., 2013).

Not seeing the parasite in the slide when the microscopic examination is done is not considered the absence of vaginal trichomoniasis because the sensitivity of this method is weak, but sensitivity can be increased by using saline to wash the cervix. The sensitivity rate for this method ranges from (54.7%) to (74%). (Menezes *et al*., 2016).

2.8.2. Staining method:

This method is one of the methods used to detect infection with the vaginal Trichomonas parasite, and in this way, many dyes are used, including:

-Acridine organ

-Leishman

-Gram stain

-Giemsa stain

A vaginal swab is taken, and the vaginal secretions are placed on a glass slide, then passed on to the flame to fix. Any dye is placed, as the benefit of this dye is to distinguish the flagella and cytoplasm of the parasite. It is washed with

distilled water to dilute the stain and then examined under a microscope. The sensitivity of this method has been estimated at 57% and specificity 95%, while other studies have shown the sensitivity of this method at 70%. Therefore, the accuracy of this technique depends on the efficiency of the examiner (Awadh & Al-Quraishi, 2016).

2.8.3. Culture method

This method is more sensitive than the microscopic examination method for diagnosing trichomoniasis in humans (Divakaruni *et al*., 2018). Commercial culture systems (such as InPouch TV, Biomed Diagnostics, USA) provide a more remarkable ability to diagnose this parasite than other culture media, including diamonds. *T. vaginalis* can be grown in liquid, solid, egg and tissue media, and the usual medium for use is cysteine Peptose liver maltose (CPLM) as well as plastic envelope medium (PEM). After the completion of the culture process, the shocked media are transferred with the samples to the incubator and then the results are read after five days using microscopic examination (Hassan *et al.*, 2019).

T. vaginalis growth inside the incubator at a temperature ranging between (35-37°C) and anaerobic conditions and a pH of about (5.5-6.0). This culture method confirms the results of examination and diagnosis, especially in the case of diagnosing samples that have given a negative result in a microscopic examination, where the degree of sensitivity to them is about (95%) (Adjei *et al.*, 2019).

2.8.4. The deoxyribonucleic acid probe (DNA)

This method is commercially available (Horii *et al*., 2009).

2.8. 5. Enzyme-linked Immunosorbent Assay (ELISA)

It was used to detect 82%, and the specificity is 73%. (Al-Hadraawy *et al*., 2013).

2.8.6. Indirect Immunofluorescent Antibody Test (IFAT)

It was used to detect antibodies to *T. vaginalis* (Al-Hadraawy *et al*., 2013).

2.8.7. Latex Agglutination Test

It has good sensitivity and specificity (Al-Hadraawy *et al*., 2013).

2.8.8. Polymerase chain reaction (PCR) technique

This method is considered better than the previous methods. It contributes to the knowledge of dead organisms and the detection of the molecular sequence of samples in clinical examination of patients, as well as identification of cells that are fixed or that have been destroyed (Ahady *et al.,* 2016).

The specificity and sensitivity of this test for vaginal swab samples are (100%) and (98%), respectively. As for urine samples, their specificity and sensitivity for this test was (100%) and (99.7%) compared with vaginal swab samples, so this type is considered the most sensitive and specific for diagnosing this parasite, but its low use is due to its cost, which limits its widespread use in diagnostics (Grad *et al*., 2020).

2.9.Treatment of *T. vaginalis*

Metronidazole is a treatment of choice and effective antibiotic for Trichomoniasis in the United States. The recommended dose to take orally is 2mg once per day. The cure rate for this disease was about (97%) (Sherrard, 2020). One of the essential things that must be emphasized is that the treatment is comprehensive for both sexes, meaning the patient and his sexual partner. Studies have been published showing the relationship of this treatment during pregnancy with the premature birth of the fetus. Two studies suggested that taking this treatment may increase the risk of premature labor rather than reduce its risks during pregnancy (Faught and Reyes, 2019).

Some patients suffer from an allergy to metronidazole. Allergy to metronidazole occur due to the lack of an alternative treatment that is effective against this parasite, so the only solution is desensitization (Beyaz *et al*., 2020, Bouchemal *et al*., 2017). Some estimates indicate that equivalent to 2.5–5% of all trichomoniasis treated cases with metronidazole show resistance to this treatment (Tien *et al*., 2020).

Tinidazole is a 5-nitroimidazole compound chemically linked with metronidazole and is widely used outside the United States, but more recently, it has been licensed in the United States to treat trichomoniasis. Tinidazole has a plasma half-life twice that of metronidazole. For example, if there are about 6 to 7 hours in metronidazole, tinidazole is 12 to 14 hours (Mukherjee *et al*., 2016, Cortez-Maya *et al.*, 2020). Some side effects of trichomoniasis were treated with oral metronidazole 500mg twice a day for a week, but when they were treated with tinidazole at a higher oral dose of 2-3g and a sedative of 1-1.5g, the rate of cure was 92%, and no treatment was stopped due to the emergence of side effects of this treatment (Argüello-García *et al*., 2020).

Some experiments prove the possibility of treating Trichomonas vaginal parasite infection with some medicinal plants, as the researchers Al-Quraishi and Al-Hasnawi (2010) used the hot and cold aqueous extract and the alcoholic extract of the peel of the pomegranate fruits *Punican granatum* to treat the infested rats with the mentioned parasite as they found that the hot water extract is the hands in getting rid of the parasite infection, where after six days of treatment, 90% of the affected animals recovered.

2.10. Epidemiology of *T. vaginalis*

2.10.1. Epidemiology of *T. vaginalis* In the World

T. vaginalis is widespread in all regions of the world (Masha *et al.,* 2019). It is considered one of the most common types of infectious diseases that are sexually transmitted and treatable (Sherrard, 2020). The incidence of new cases as estimated at 174 million most of occur in resource-poor settings at an average of 154 million new cases per year (Alessio & Nyirjesy, 2019).

The *T. vaginalis* prevalence and epidemiology are significantly affected by the fact that they are often asymptomatic and can be transmitted through sexual intercourse without the knowledge of both partners (Chemaitelly *et al*., 2019).

The trichomonal infection has appeared on all continents, all climates, seasonal changes. This parasite has a global distribution in all ethnic and social strata in the USA (Rayan *et al.,* 2019). The number of *T. vaginalis* infections is estimated as (22% of females, 2.2% of males), women between the ages of (14-49) years had the highest rates of infection with vaginal signs (Shahraki *et al*., 2020).

In African women, vaginal infections that include vaginal trichomonad are highly correlated with the appearance of sex at ages less than 20 years, as well as with more frequent of *T. vaginalis* infections in that the partner because of frequent traveler, Trichomoniasis infection rate among black women is higher than that of white women; this may be affected the low level of education and socioeconomic factors that impact on the prevalence of Trichomoniasis (Chetty *et al.,* 2020).

For sexually transmitted diseases (STDs), according Kenyon *et al.,* (2014), the highest incidence of it is in sub-Saharan Africa Major, in Africa found the highest incidence (20.2% of females, 2% of males) (Awadh & Al-Quraishi 2016).

According to recent "World Health Organization" (WHO) estimates based on 2020 statistics, there are more than 370 million new cases each year with one of four STIs, or 1 million individuals are acquired every day. This presents more new infections, a significant number than the combined total of the two most STIs prevalence, infections with and *Chlamydia trachomatis* estimated to number 156 million and 129 million, respectively. while *Neisseria gonorrhoeae* estimated to number 82 million. The prevalence in Europe is estimated as 5.8% of females and 0.6% of Males (WHO, 2021).

Due to the different prevalence rates of infection of this disease and according to many research and topics in the Republic of Korea, the first case of *T. vaginalis* was recorded by Lee and Yang (2020). According to one of the studies there, the infection rate among women attending women's clinics in Seoul was 17.3% (Huh, 2019).

Epidemiological research on infection with the parasite *T.vaginalis* increased in Iran. The infection rate in Zanjaan province in northwestern Iran in pregnant women is 3.3%, and the highest infection rate was in uneducated women (Nourian *et al*., 2013).

In Hamadan, western Iran found Matini *et al*. (2012) that the diagnosis rate of the parasite using the method the implants are 2.1% compared to the wet swab method 1.7%. In Ardokan, Maybod, and Yazd, the estimated infection rate of the parasite among women is 5.9%, 5.0%, and 2.0%, respectively (Bafghi *et al*., 2009).

Deivam *et al*. (2014) studied the incidence of this parasite in women in India the infection rate is 8.1%, and the highest rate of infection was in within age group 34-40 years, while in Vietnam, it was estimated that the rate of infection with parasite among women who suffer from symptoms of the disease is 19.3%, compared to women without depression symptoms, 0.7% (Ton Nu, 2010).

As it was estimated, the incidence of *T.vaginalis* among women in Ethiopia was 4.89%. The highest infection rate was in the age group 25-34 years (Eshet *et al*., 2013) as indicated by Mairiga *et al*. (2011), the rate of infection of women with this parasite in Nigeria is 11%, and the highest rate infection was confined to the age group (39-44) year.

A lot of research has been published on the epidemiology of infection with this parasite in Europe, including Sweden Pellrud *et al*. (2015) that the highest incidence of parasite infection in women is in the age group 20-24 years, while for males, it is in the (25-29) age group.

2.10.2. Epidemiology of the Parasite in Arab Countries

In Arab countries, the *T. vaginalis* infection prevalence was reported in Saudi Arabia 28.1%, in Jordan 3.2%,and in Libya 1.2%. Socioeconomic status, poverty, high-risk sexual behaviors, low educational level, and HIV infection increase the risk of suffering vaginal trichomoniasis. In Palestine, the estimated infection rate of the parasite among women is %13.6 (Houso *et al*., 2011).

Variation in the number of researches and studies related to the infection of *T. vaginalis* in countries, for example, the latest studies conducted in Lebanon on the epidemiology of diseases, the reproductive system, and this study included five types of microorganisms that cause inflammation, including *T. vaginalis*. The highest infection rate of 22.9% among women was *T.vaginalis* (Ramia *et al*., 2012).

In Libya, the infection rate of the parasite was recorded among women at 26.8%, in the age group (15- 34) was the highest infection rate (Khamees, 2012). In Sudan the highest rate of parasite infection was 14.2% for women in the age group 20-24, while socially speaking, the highest rate of infection was among married, non-pregnant women with a rate of 13.3% (Dahab *et al*., 2012).

2.10.3. Parasite epidemiology in Iraq

In Iraq, the parasite infection rate among women was estimated at 34.41% using the culture method 13% the wet swab method was 49%, and the highest rate of infection was within the age group(25-34) years (Merdaw *et al*., 2014).

In Dohuk governorate, Al-Saeed (2011) found that the highest infection with the parasite was in women aged 20-25 years with a rate of 7.6%, while the

lowest infection rate was within the age of 36-40 years at a rate of 2.2%. It was also found that the best method for diagnosis is the modified diamond farm.

In Kirkuk, Salman and Kareem (2013) found that the rate of infection with the parasite among women was 20.49%, and the highest rate of infection was within the age group (51-60) years, which is 28.57%, and the lowest infection was within the age group 41-50 years, at a rate of 15%.

In a study conducted by Al-Quraishi (2014) in the province of Babylon, the incidence of urine infection differed compared to wet swabs among women between rural and urban setting. The percentages were 12.16% and 7.38%, respectively, in the urine samples. The vaginal swab samples in urban setting were 16.23% and 11.25% in the rural setting.

As for male urine samples, the infection rate in the rural was 5.09%, and in the urban, 4.2%, and another study in Babil Governorate by (Ali, 2011), the infection rate of the parasite among postmenopausal women was 12%. Hussein (2010) also found in Babylon that the infection rate of the parasite is 21.87% and in the rural is higher than in the urban, with a rate of 24.58%, 4.47%, respectively. The parasite was isolated from the eyes of newborn children and the amniotic sac.

The parasite infection rate in Karbala governorate was 9.5% (Al-Haidari, 2011). while in Najaf governorate, infection with the parasite among women, using wet mount examination and culturing, revealed 5.6% and 14.3%, respectively. Based on culturing method age group of 25-35 had the highest rate of infection (20.3%) (Taher, 2018).

In a study conducted in Wasit, the infection rate with the parasite among women was estimated at 20%, and the age group was 14-34 years has the highest infection rate (Rahi *et al*., 2014).

In a recent study conducted in Maysan Governorate, the results of the microscopic examination for the study population among women showed that the rate of infection was 75.22%, and the age group (34-40) years has the highest (86.95%) (Al-Majidii *et al*., 2020).

2.11. Normal Flora of Vaginalis

The vagina is an environment that is highly nutrient-dense for microorganisms (Sierra *et al*., 2018). As a result, the vaginal microbiota component is influenced by a host of factors variation, including changes in hormones level (during pregnancy, menopause, menstruation, or as a result of hormone contraceptive usage), age, various genital illnesses, as well as sexual and hygienic behaviors. Although most commensal microbiotas with their human hosts are mutualistic, others are opportunistic pathogens that can lead to premature birth, persistent infections, or life-threatening fetal and maternal illnesses (WHO, 2015).

The premenopausal women's vagina is colonized by a particular microbiota that has been divided into five kinds. One *Lactobacillus* species dominates four of these “community state types” (CSTs), specifically, *Lactobacillus crispatus*, *L. gasseri*, *L iners.*, or *L. jensenii. T*he vaginal epithelial cells release glucose that degrades to produce the lactic acid that is responsible for decrease pH reproductive-age women vaginal fluid and for vaginal environment to be maintained healthy.

The fifth kind, CST (named CST-IV), is identified as lactobacilli absence and the anaerobic bacteria overgrowth, such as *Atopobium vaginae*, *Prevotella bivia*, *Gardnerella vaginalis*, and others (Ravel J *et al*., 2011). Although infection with *T. vaginalis* linked to the CST-IV microbiota, this association does not necessarily suggest causality (Hinderfeld *et al*., 2019).

2.11.1. Other Common Causes of Abnormal Vaginal Discharge

Compared to physiological (normal) vaginal discharge, the color and consistency are different in abnormal vaginal discharge (thickness, thin, frothy, gray, green, yellow, or white). It is commonly coupled with other symptoms such as fishy, foul-smelling, or itchy. Although abnormal discharge frequently results from vaginal infections, it can also be caused by several relatively common non-infectious causes (Willacy., 2021).

Sexually transmitted diseases such as *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis*, and *Ureaplasma*, as well as less often occurring HSV-2 and endogenous infections are the most prevalent infectious causes of abnormal discharge (i.e., the dysbiotic outgrowths of bacteria common commensal, such as Aerobic vaginosis (AV) and Bacterial vaginosis **(**BV) (Barad., 2020).

2.11.2. Bacterial Vaginosis

Bacterial vaginosis is defined by the existence of clinical symptoms and an elevated vaginal pH (typically 4.5), the presence of a white adherent

discharge containing Gram-variable polymorphic rod-shaped bacteria attached to the surfaces of exfoliated epithelial cells (clue cells), and the presence of a fishy odor. BV is generally polymicrobial, with the microbial communities being anaerobic, such as *Prevotella* species, *Gardnerella vaginalis* *Mobiluncus* species, and *Mycoplasma hominis* in pregnant women, BV is related to an increased risk of STIs (in addition to HIV infection), and premature birth (Hemalatha *et al.*, 2013).

2.11.3. Aerobic Vaginitis

Aerobic vaginitis was initially described in 2002 by Donders *et al*. 2002 as a vaginal disease separate from BV, requiring specific treatment and posing distinct clinical concerns. As with BV, AV is defined by a loss of *Lactobacillus* dominance but is associated with more severe inflammatory changes and the presence of predominantly aerobic enteric commensals or pathogens, such as *Enterococcus faecalis*, Group B *Streptococcus* (*S. agalactiae*), *Escherichia coli*, and *Staphylococcus aureus* (Sangeetha *et al*., 2015). AV has been detected in around 8%–11% of pregnant women and approximately 5%–24% of those experiencing vaginal symptoms (Donders *et al*., 2011).

In certain situations, AV is linked with an increase in genital inflammation, an increase in the number of leukocytes visible in vaginal smears, and an increase in pathogen-specific activity (dubbed "toxic leukocytes"). Vaginal mucosa in women with AV is thinner than women with BV, with a higher number of parabasal and intermediate cells in vaginal smears, indicating

increased desquamation and turnover of superficial epithelial cell layers (Donders *et al*., 2015).

2.11.4. Interaction *T. vaginalis* with Vaginal Flora

In the pathology of this parasite, the interaction between the vaginal flora that is found naturally in vagina and the *T. vaginalis* most to be mentioned. It is known that the natural pH in the vagina is acidic and the ideal pH of *T. vaginalis* is between (5-6.5). It has been observed that the vagina has a high pH with low lactobacilli, or its loss is final, which leads to an increase in anaerobic bacteria. In laboratory conditions and pH control, it was found that lactobacilli did not show an effect on the *T. vaginalis* parasite, while the parasite harmed lactobacilli (Green Baum *et al*., 2019).

In order to cause injury, *T. vaginalis* is crossing with the barrier in vagina. *Lactobacillus*, which is a natural barrier against microbes, because it prevents the parasite adherence to the target cell or inhibits its adhesion, so the parasite must overcome it in several ways, including the production of special materials such as proteinase, which destroys *Lactobacillus* (Hinderfeld *et al*., 2019).

T. vaginalis infection was found to be associated with other microbial infections. Because *T. vaginalis* may be considered abnormal flora, severely abnormal flora may result in either anaerobic or aerobic bacterial vaginosis. The term aerobic vaginitis is used for a condition in which disrupted pathogenic aerobic microorganisms have replaced vaginal flora, commonly, group B *Streptococci, E. coli* or *Enterobacter* and *Enterococci* of the intestine, which result in severe localized immune response inside the host, as evidenced by increased vaginal leukocytosis. Bacterial vaginosis (BV) is usually devoid of

vaginal host immune response. Still, it is associated with reduced morphotypes of lacto bacillary and also with low lactic acid and high PH (Yasin *et al*., 2021).

2.12. VITEK 2 Compact

The VITEK system is used as an automated system for the bacterial diagnosis and antimicrobial susceptibility testing (AST) since the 1970s, and 17 years since then has been developed into the VITEK 2 system, which carry out automatically all of the stages needed for the identification and AST following preparation and standardization of a primary inoculum (Funke *et al*., 1998). Various automated systems have been developed for the microbial identification and performing AST depending on the automated analysis of the findings of the biochemical tests or the use of microdilution trays after overnight incubation and photometric growth determination (Ferraro, 1995).

Emergence of new technologies that might offer fast bacterial detection and performing AST are known to have both clinical and financial benefits (Barenfanger *et al*., 1999). The Vitek system comes in three formats, which involve VITEK 2, VITEK 2 compact, and VITEK 2 XL, with varying capacities and degrees of automation. The same colorimetric reagent cards are incubated and interpreted automatically in all three systems (Pincus, 2010).

The GP diagnostic card has 64 plastic wells, 18 of which are empty and 46 of which are for inhibitory and fluorescent tests, such as pH alteration tests and derivatives for –osidases and aminopeptidases detection substrates for detection of -osidases are combined with 4-methylumbelliferone (4MU). While substrates used to detect aminopeptidases are combined with 7- amino-methylcoumarin (7AMC).

Substrates used with the 21 tests involve: 4MU-α-D-glucoside, 4MU-β-Dglucoside, 4MU-α-D-galactoside, 4MU-β-D-galactoside, 4MU-β-D-glucuronide, 4MU-α-L-arabinofuranoside, 4MU-α-D-N-acetylneuraminic acid, 4MU-n-acetyl-β-Dglucosaminide, 4MU-β-D-mannoside, arginine-7AMC, alanine-7AMC, α- glutamic acid-7AMC, 4MU-phosphate, histidine-7AMC, urease (butiloxicarbonyl- Val-Pro-Arg- AMC), phenylalanine-7AMC, lysine-7AMC, tyrosine-7AMC, threonine-7AMC, proline-7AMC, and pyroglutamic acid-7AMC.

Moreover, the card contains 16 fermentation tests for the following: D-glucose, D-galactose, Dxylose, lactose, D- mannitol, D-maltose, D-melibiose, D-trehalose, D-sorbitol, Draffinose, amygdaline, arbutin, salicin, glycerol, N-acetyl-glucosamine, and Larabinose. Likewise, the card includes two decarboxylase tests for arginine and ornithine, and six other tests for pyruvate, urease, optochin, novobiocin, NaCl (6.5%), as well as polymyxin B sulfate (Queck *et al*., 2013).

The identification card for gram-negative bacilli (ID-GNB card) for the VITEK 2 system is a 64-well plastic card containing 41 fluorescent biochemical tests, including 18 enzymatic tests for aminopeptidases and -osidases. Substrates used for detection of aminopeptidases are usually coupled with 7-amino-methylcoumarin (7AMC); substrates for detection of -osidases are usually coupled with 4-methylumbelliferone (4MU).

The 18 test substrates are as follows: 4MU-a-arabinopyranoside, 4MU-a-D-galactoside, a-Lglutamic acid-7AMC, 4MU-b-D-cellobiopyranoside, 4MU-b-D-galactoside, 4MU-b-D-glucoside, 4MU-b-D-glucuronide, 4MU-b-D-mannopyranoside, 4MU-*N*-acetylb- D-glucosaminide, 4MU-*N*-acetyl-b-D-

galactosaminide, 4MU-b-D-xyloside, glutaryl-glycyl-arginine-7AMC, g-L-glutamic acid-7AMC, 4MU-phosphate, L-proline- 7AMC, L-pyroglutamic acid-7AMC, L-lysine-7AMC, and *Z*-arginine-7AMC. Furthermore, the ID-GNB card includes 18 fermentation tests (adonitol, L-arabinose, D-cellobiose, D-galacturonate, D-glucose, glucose-1-phosphate, D-glucuronate, inositol, 5-keto-gluconate, D-maltose, D-mannitol, D-melibiose, palatinose, D-raffinose, L-rhamnose, sucrose, D-sorbitol, and D-trehalose), 2 decarboxylase tests (ornithine and lysine), and 3 miscellaneous tests (urease, utilization of malonate, and tryptophane deaminase) (Funke *et al*., 1998).

2.13. Interaction of Immunity System and Infection with *T. vaginalis*

Characterizing the type of immune response that *T. vaginalis* stimulates and determining whether *T. vaginalis* cytotoxic activity can kill leukocytes will be important to understanding why *T. vaginalis* is often persistent, how *T. vaginalis* infection may lead to inflammatory sequelae, and how *T. vaginalis* infection may affect the microbiome in the FRT, *T. vaginalis* is a human-specific pathogen.

T. vaginalis infection is a complicated illness with a wide variety of symptoms that can be attributed to several pathogenic processes caused by the parasite via contact-dependent and contact-independent mechanisms. The parasite initiates colonization of the site of the infection by secreting a number of chemicals known as cytolytic agents, which cause damage to cells in the host tissue. Elevated concentrations of proteolytic activity were related to cysteine

proteases (CPs), proteins found on the parasite surface, in *T. vaginalis*, despite the fact that only a few CPs have been identified and characterized.

The parasite's proteolytic activity caused by *T. vaginalis* CPs has been found to influence cell recognition and adherence to epithelial host cells (McGovern *et al*., 2011). These proteins not only play a significant role in colonization at the infection site, but they also help the bacteria evade host immune defenses by degrading IgA and IgG antibodies, as well as humans extracellular complement and matrix proteins (Eltzschig *et al*., 2011). Environmental variables such as iron, temperature, pH, and polyamines influence the proteolytic activity and synthesis of some CPs (Whitehouse *et al*., 2016).

In women infected with *T.vaginalis*, menstrual blood is the only source of complement available to the vagina, and despite the fact that menstrual blood has a significant complement-mediated cytotoxicity against *T. vaginalis*, and trichomonal infection persists during menses. Many investigations have shown that provoked *T. vaginalis* produces cytokines in monocytes. The local concentration of neutrophils, particularly adherent trophozoites, is critical for increasing resistance to *T. vaginalis* infection.

The immune response to *T. vaginalis* has been investigated most extensively in pregnant women. When compared to bacterial vaginosis alone, *T. vaginalis* positive women who are pregnant with bacterial vaginosis (Nugent 7–10) exhibited more vaginal IL-1 and neutrophils (Ley *et al*., 2018).

The infection is often asymptomatic and recurrent despite the presence of specific antibodies, suggesting the importance of the innate immune defense. *T. vaginalis* adhesion proteins, cysteine proteases, and the major parasite

lipophosphoglycan (LPG) play distinct roles in the pathogenesis and evasion of host immunity. LPG plays a key role in the parasite adherence and signaling to human vaginal and cervical epithelial cells, which is at least in part mediated by galectins. The epithelial cells respond to *T. vaginalis* infection and purified LPG by selective upregulation of proinflammatory mediators. At the same time, *T. vaginalis* triggers an immunosuppressive response in monocytes, macrophages, and dendritic cells (Bhakta, 2020).

Marcer *et al.,* (2016) used primary human leukocyte components to create an in vitro co-culture system to study the interaction between *T. vaginalis* and human immune system cells. They discovered that *T. vaginalis* can lyse T-cells and B-cells in vitro, with a predilection for B-cells.

T. vaginalis lysis of lymphocytes was also discovered to be mediated by contact-dependent and soluble substances. *T. vaginalis* lysis of monocytes is significantly less effective, and it is nearly entirely dependent on contact. Surprisingly, *T. vaginalis* 's common symbiont, *Mycoplasma hominis*, had little effect on the parasite's cytolytic activity but had a significant impact on cytokine responses. *T. vaginalis* strains cleared of *M. hominis* produced only IL-8 secretion from monocytes, whereas *M. hominis* facilitated more diversified inflammatory cytokine secretion in response to *T. vaginalis* which commensals, symbionts and concomitant infections are likely to impact the quality of the adaptive immune response to *T. vaginalis*, which may be complicated further by direct parasitic lysis of effector immune cells. *T. vaginalis* coexists in the FRT with a variety of other commensal microbes, and infection is frequently associated with other STIs (Brotman *et al.*, 2014).

Leukocytes are found in the FRT's mucosal tissues, where they regulate response to commensals and other STIs. Leukocytes are primarily found in the lamina propria, but they may also migrate to the luminal surface of the mucosa in response to trans-epithelial chemokine signals (Wira *et al.*, 2005). *T. vaginalis* may thus come into contact with leukocytes while sticking to epithelial cells on the luminal side of the FRT, or as the epithelial layer is penetrated by *T. vaginalis* cytolysis of epithelial cells as it approaches the lamina propria. *T. vaginalis* has been shown to phagocytoze human leukocytes, implying that direct leukocyte death could lead to *T. vaginalis* immune subversion; nevertheless, the efficacy, kinetics, and mechanisms of *T. vaginalis* immune subversion remain unknown.

2.14. White Blood Cells.

A few WBCs can be present in the vagina as a result of physiologic cervical discharge, particularly premenstrually, but the number normally does not exceed the number of vaginal epithelial cells. A large number of WBCs suggests trichomoniasis, cervicitis, or occasionally candidiasis. Women with desquamative inflammatory vaginitis (DIV) also have a large number of WBCs (Eschenbach, 2021).

Chapter Three

Methodology

3. Materials & Methods

3.1. Materials

3.1.1. The Equipment and the Tools:

Table (3-1): The Equipment and the tools that were used in the current study with the manufacturing companies and the country of the origin are as following:

NO.	The Equipment and the Tools	The company	The country
1.	Autoclave	Hiarayam	Japan
2.	Benzen Burner	Shandon	England
3.	Centrifuge	HETTICH	Japan
4.	Cover slide	HIRSHMAN	China
5.	Disposable pipettes	Plasti Lab	Lebanon
6.	Disposable syringe 5ml	ILANIMEDICAL	Jordan
7.	Filter paper	Ahlstrom	Germany
8.	Flask (50ml, 500ml)	MEMMERT	China
9.	Glass slide	J.W.R	China
10.	Hot plate	OYALITY LINE	China
11.	Incubator	JRAD	Syria
12.	Laminar flow cabinet /Hood	TIPS HOW	USA
13.	Light microscopic	UNIMEDICA	China
14.	Loop	Shandon	England
15.	Marking pen	DOMS	India
16.	McFarland meter	BioMérieux	France
17.	Medical cloves	TOP GLOVE	Malaysia
18.	Microscope camera system	ZEISS	Germany
19.	Oil emersion	Himedia	India
20.	Parafilm	Can	USA
21.	Petri dishes	Sterilin	England
22.	Plane tubes	AFMA-Dispo	China
23.	Refrigerator	CONCORD	Lebanon
24.	Sensitive balance	CAMRY	China
25.	Speculum	MAX	China

26.	Test tube	Pyrex	Italian
27.	Test tubes	Al-Hanoof	Jordan
28.	Vaginal swab	CITOSWAB	China
29.	Vitek	bio-Merieux SA	France

3.1.2. The Biological and Chemical Materials:

The biological and chemical materials used in this study are listed in the table below (3-2).

Table (3-2): The biological and chemical materials.

NO.	Type of material	Manufacturer	Origin
1.	Distil water	LAB TEACH	Korea
2.	Ethanol 70%	Aljoud	Iraq
3.	Gram Stain	BDH	UK
4.	Hand sanitizer	Aljoud	Iraq
5.	Saline solution REF v1204	BioMérieux	France
6.	Sterile Normal Saline 0.9% NaCl	PIONEER	Iraq
7.	Sterile Transport medium swap	BIOZEK MEDICAL	Netherlands

3.1.3. Materials Required for the Vitek2 Diagnosis System

Materials used in the Vitek2 test are sterile normal saline, sterile swabs, vortex, Vitek2 DensiCHEK standard, Vitek2 cassette and VIitek2 GP and GN cards.

3.2. Methods

3.2.1. Construction of Study Instrument:

There is some information that were interviewed from the study population such as: The Age (14-20, 21-27, 28-34, 35-41, 42-48 and 49-56) years age group, Occupation Status (Married, Widow, Divorced and Unmarried), Education level (illiteracy, primary, intermediate, Secondary and A graduate), Occupation (Housewife, Employee, Student), Residency (Urban and Rural), Status of Women (Pregnant and Non- pregnant), Vaginal discharge (Yes or No), Vaginal itching (Yes or No), Dysuria (Yes or No), Dyspareunia (Yes or No), Malodor (Yes or No), Contraceptives used before pregnancy (Yes or No), Type of Contraceptives used (Intrauterine device, Pills, depo-provera and Implanon), This information is saved in the questionnaire sheet in Appendix (A) and (B).

3.2.2. Study Population:

The study was carried out in accordance with the Karbala Health Statement, and all samples were taken under the direct supervision of a gynecologist in all of the hospitals and medical clinics visited. After obtaining the participants' consent, an informative questionnaire was distributed to each patient. By using the sterile metal speculum, the vaginal wet amount for 232 females were collected and urine samples from same patients as well as 25 samples from healthy females (control) were collected with different ages ranged between (14-58) years who attended the hospitals and medical clinics for Al-Hindiya General Hospital and Obstetrics and Gynecology Teaching Hospital as well as medical clinics from different regions of Karbala province in period Nov. 1, 2020 to June. 5, 2021. The methodology steps were followed the described scheme.

3.2.3: Schematic plan of the Study:

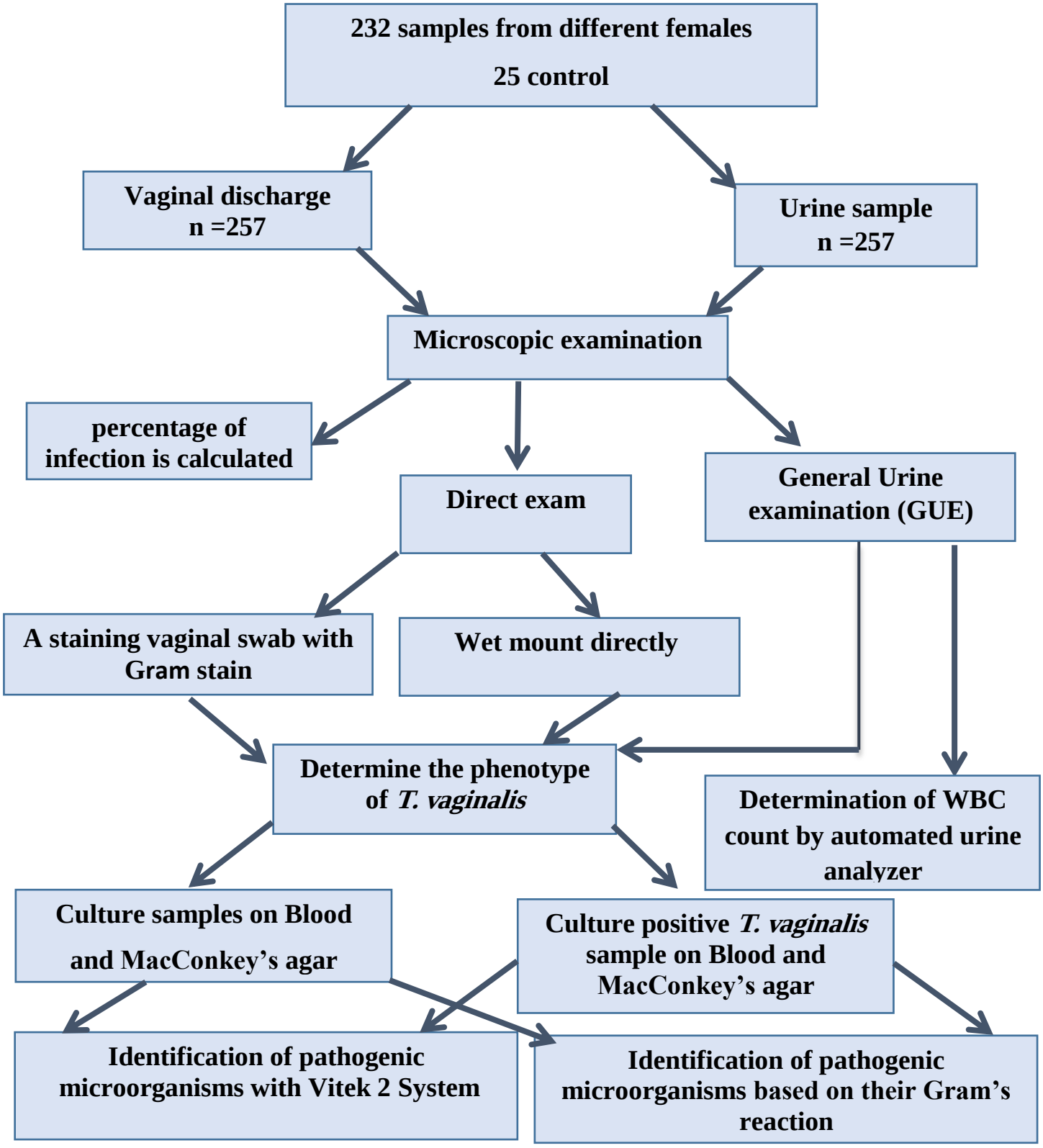


Figure (3-1)Plan of the study

3.3.1.Vaginal Discharge Specimens Collection:

After written approval obtained from the council of the college of the Science / University of Babylon to conduct the study as appendix(C) and (D). Samples were collected by taking vaginal swabs from 232 patients and 25 healthy women (control) from Obstetrics and Gynecology Teaching Hospital, Al-Hindia General Hospital and private outpatient clinics. The vaginal discharge swabs were taken by using swabs with the help of the attending physician. Swabs are wooden sticks with a cotton swab on one end sterilized and kept in their own plastic containers. The swabs were placed directly in their own container containing 0.5 mm of physiological normal saline. The second collection samples was cultured in amies transport media, Figure (3-2**),** and kept in cool box and then transferred to the parasitology laboratory in hospital and samples are cultured in appropriate media. then incubated at 37°C for 24 hours.

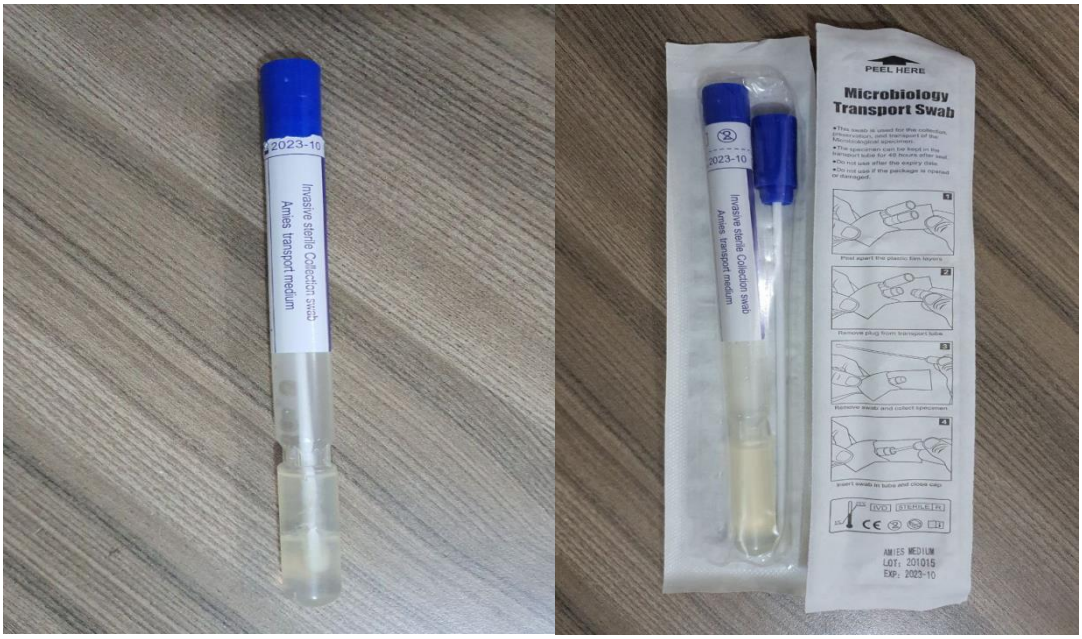


Figure (3-2): The culture of samples on amies transport medium.

Table (3-3): Components of gram stain.

Ingredients	Amount Gm./L
Crystal Violet	250ml
Decolorizer (e.g. ethanol	250ml
Iodine solution	250ml
Safranin	250ml

3.3.2: Urine Specimens Collection:

Urine samples were collected from the same patients from whom the swabs were taken, clean catch midstream urine samples were collected into a sterile screw capped universal container by standard method. Then urine samples are transferred to the parasite laboratory and the necessary steps are taken for microscopic examination.

3.3.3: Preparation of Culture Media

All prepared bacteriology culture media were prepared according to the instructions of the supplying company in the laboratory and the methods described by Atlas (2010) were followed. After the media ingredients were dissolved into distilled water (dH_2O) by boiling at (121) C, then were Autoclaved for (15) minutes [2] and pressure of 15 pounds / inch. The medium was cooled to approximately 50°C under sterile conditions, then poured into sterilized Petri-dishes and left to solidify. The final PH was 7.3 at 25°C, then they were stored at 4°C until used. These media are the following:

Table(3-4) Prepared Media.

NO.	Medium Name	Origin
1-	Blood Agar Base	Himedia (India)
2-	MacConky Agar	Liofilchem (Italy)

3.3.3.1: Blood agar

Blood agar Petri-plates were made by dissolving 40.0 g of blood agar base powder in 1 L of (dH2O) according to the instructions company. Afterwards, the medium autoclaved as mentioned in section 3.3.3, then they were left to cool to approximately 45ºC. 5% human blood was added aseptically and mixed well inside a sterilized hood. Finally, the medium was poured in sterile Petri plates, left to solidify inside the hood, and kept inverted till use in the refrigerator.

3.3.3.2: MacConkey's agar

MacConkey's agar was prepared by weighting 51.5 g in 1 L of (dH2O). The media ingredients were melted, and as mentioned in section (3.3.3., they were autoclaved and poured into sterile Petri plates.

3.4. Examination of the Specimens

3.4.1: Direct Exam of Wet mount

A wet mount technique under microscope examination is the most usual test. Each lady had two vaginal swabs collected, one for direct exam and the other for culture. The first was taken from the lateral wall of the vaginal canal and put in a tube containing 0.5 mL physiological saline solution (0.9% NaCl for wet mount examination. The tube was taken to the laboratory, gently shaken, and a slide was made for instant inspection under a light microscope.

Two slides were prepared: one drop of the mixture was placed on a glass slide and covered with a slide cover, and at least 20 fields were inspected using 10x and 40x objectives to identify the motile *Trichomonas vaginalis*. The activists were known for their jerky movements, which were distinguished by the undulating membrane movement, as in Figure (4-6), as well as *Candida* spp. which were also distinguished by this method using 40x then 100x objective, as in Figure (4-5).

The second slide was stained with Gram stain, which was made using the materials listed in Table (3-3), and a wet mount of vaginal discharge was produced and inspected using a compound microscope at a magnification of 40X (Nwokah *et al*., 2019), observing the four flagella of the parasite stained with Gram stain is diagnosis of the parasite infection in the stained specimen, as in Figure (4-7).

The Gram-stained smear was also examined under oil immersion (100x) for the following morphotypes: large Gram-positive rods (*Lactobacillus* morphotypes), small Gram-variable rods (*Gardnerella vaginalis* morphotypes) round epithelial cells (clue cells), 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.

The second swab cultivation samples with sterile collection swab amies transport medium (Biozek Medical, Netherlands) were incubated for several days at a temperature of 37$_{o}$C.

3.4.2. General Urine Examination (GUE)

Urinary samples were collected from all female patients and control group from whom vaginal swabs were taken. The samples were collected into a sterile screw capped universal container by standard method. After that, the samples were centrifuged at 3000 rpm for 5 minutes to separate the deposit which was placed on a clean slide and covered with cover slip to be examined under a light microscope for the detection of *T. vaginalis* motile with 10X and then 40X magnification, as in figure (4-8).

Automated urine examination are also done for the total samples to detect its components, especially the number of white blood cells, to find out the immune system response to infection with the parasite or pathogenic microorganisms or both.

The magnitude of white blood cell within the urine sample was classified as follows: (i) normal 1 to 3 p/PHF, (ii) moderate: 4 to 30 p/PHF, and (iii) high: > 30 p/PHF.

3.5: Cultivation of Vaginal Discharge:

The second swab cultivation samples with sterile collection swab amies transport medium (Biozek Medical, Netherlands) were incubated for several days at a temperature of 37$_{o}$C. Then, the samples were inoculated into the following media for microorganisms identification: blood agar and MacConkey agar. Finally, vaginal swab culture and identification of microorganisms were performed according to bacteriological diagnosis Vitek2 system.

3.5.1. Culturing Vaginal Specimens for Bacteriological Diagnosis:

The vaginal specimens were cultured for bacteriological diagnosis after the samples were examined in the laboratory to diagnoses the presence of infection with *T.vaginalis*. These samples which were infected with parasites were streaked onto blood agar and MacConkey's agar plates so that non-infected samples were incubated aerobically at 37ºC for 24hr (Markey *et al*., 2014).

After that, the plates were examined for bacterial growth, and the colonies were classified according to their characteristics, hemolysis, and response on blood and MacConkey's agars, respectively.

The Gram's response was also used to identify the isolates. Gram's stain was used on a pure colony from each isolate to identify the morphology, organization, and Gram's response of the bacteria inside the colony.

3.6. Vitek2 Compact Diagnostic System

According to the manufacturer's instructions, the automated Vitek2 compact system (bio-Merieux SA, France) and its supplements were used to identify positive and negative microorganisms. The reagent cards in this system feature 64 wells, each of which may hold an independent test substrate.

The phenotypic identification of bacterial species was based on the findings of 64 colorimetric substrates. First of all, the bacterial isolates were cultured for 18 to 24hr onto MacConkey's agar and blood agar at 37°C. After that, the suspension of bacteria was made by suspending the microorganisms in 3 ml of sterile saline (aqueous 0.45% - 0.50% NaCl, pH 4.5 -7.0) inside a polystyrene tube.

The density of suspension was adjusted to be equivalent to number 0.5-0.63 McFarland standard (1x108 CFU/mL) by using a DensiCheck turbidity meter (bio-Merieux). Afterwards, the tube containing suspensions of microorganism and identification cards ID (GP ID and GN ID) were placed into a special rack (Vitek2 cassette), and the card was auto-inoculated within the Vitek2 instrument via a vacuum-release method (Hombach *et al*., 2015).

The cassette can accommodate 30 tests. During incubation, each well in the card was read and scanned every 15 min, to measure either color or turbidity products of substrate metabolism, with a total incubation time of approximately 3-9hr (Gardner and Altman, 1995).

Table (3-5): Vitek2 diagnosis of the negative bacterial isolates.

Biochemical Details	*E. coli*	*K. Pneumoniae*	*E. cloacae complex*	*Salmonella group*	*Enterobacter aerogenes*
APPA	_	_	-	-	_
ADO	_	+	-	-	+
PyrA	_	+	-	-	+
IARL	_	_	-	-	_
dCEL	_	+	+	-	+
BGAL	+	+	+	-	+
H2S	_	_	-	-	_
BNAG	_	_	+	-	_
AGLTp	_	_	-	-	_
dGLU	+	+	+	+	+
GGT	_	+	+	-	+
OFF	+	+	+	+	+
BGLU	_	+	-	-	+
dMAL	+	+	+	+	+
dMAN	+	+	+	+	+
dMNE	+	+	+	+	+
BXYL	_	+	+	-	+

BAlap	_	_	-	-	_
ProA	_	_	+	+	_
LIP	_	_	-	-	_
PLE	_	+	+	-	+
TyrA	_	+	+	+	_
URE	_	+	-	-	_
dSOR	+	+	+	+	+
SAC	+	+	+	-	+
dTAG	_	+	-	-	_
dTRE	+	+	+	+	+
CIT	_	_	+	-	+
MNT	_	_	+	-	+
5KG	_	_	-	-	_
ILATk	_	+	+	+	+
AGLU	_	_	-	-	_
SUCT	_	_	+	+	+
NAGA	_	_	+	-	_
AGAL	+	+	+	+	+
PHOS	_	+	*(+)*	*(+)*	+
GlyA	_	_	+	-	_
ODC	+	_	+	+	+
LDC	+	+	+	+	+
IHISa	_	_	-	-	_
CMT	+	_	-	+	+
BGUR	+	_	+	+	+
O129R	+	+	+	+	+
GGAA	_	_	-	-	_
IMLTa	_	_	-	*(+)*	_
ELLM	+	_	+	*(+)*	_
ILATa	_	_	-	*(+)*	_

Table (3-6): Vitek2 diagnosis of the positive bacterial isolates.

Biochemical details	***Kocuria Kristine***	***Enterococcus faecalis***	***Staphylococcus hominis***	***Staphylococcs aureus***	***Lactococcus garvieae***	***Staphylococcus haemolyticus***	***Streptococcus Uberis***
AMY	_	+	_	_	_	_	-
PIPLC	_	_	_	_	_	_	-
dXYL	_	_	_	_	_	_	-
ADHI	+	+	_	+	+	+	+
BGAL	_	_	_	_	_	_	+
AGLU	_	_	_	+	+	_	-
APPA	+	_	_	_	+	_	-
CDEX	_	+	_	+	_	_	-
AspA	_	+	_	_	_	_	-
BGAR	_	_	_	_	_	_	-
AMAN	_	_	_	_	_	_	-
PHOS	_	_	_	+	+	_	-
LeuA	+	_	_	_	+	_	+
ProA	+	_	_	_	+	_	-
BGURr	_	_	_	_	_	_	+
AGAL	_	_	_	_	_	_	-
PyrA	+	+	_	+	+	+	-
BGUR	_	_	_	_	_	_	+
AlaA	+	+	_	_	+	_	+
TyrA	_	+	_	_	+	_	-
Dsor	_	+	_	_	_	+	+
URE	_	_	+	_	_	_	-
POLYB	+	+	_	+	+	_	+
dGAL	+	+	_	_	_	_	+
dIRB	_	+	_	+	+	_	+
ILATk	+	_	_	+	_	_	-

LAC	_	+	_	_	_	_	-
NAG	_	+	_	+	+	+	+
dMAL	+	+	_	+	+	+	-
BACI	+	+	_	+	+	+	+
NOVO	+	+	_	_	_	_	+
NC6.5	+	+	+	+	+	+	-
dMAN	_	+	_	+	+	+	+
dMNE	+	+	_	+	_	_	
MBdG	_	+	_	+	+	_	-
PUL	_	_	_	_	_	_	
dRAF	_	_	_	_	_	_	
O129R	_	_	_	+	+	+	
SAL	_	+	_	+	_	_	
SAC	+	+	+	+	+	+	
dTRE	_	+	_	+	+	+	
ADH2s	_	_	_	_	_	_	
OPTO	+	+	+	+	+	+	

3.7. The Statistical Analysis

The statistical analysis was done by using Statistical Package for Social Science (SPSS version 24). Chi-Square test ($\chi 2$), Fisher exact test, were used to determine relationship between the infection rate and variables that used in the current study as well as percentage, with a probability (p) value of 0.05 or less considered as statistically significant (Al-Majidii *et al.*, 2020).

Chapter Four

Results

Results

This chapter presents the results of the data analysis systematically in tables and these corresponded with the objectives of the study as follows:

Table 4.1:Distribution of *Trichomonas vaginalis* Parasite test in patient and control group

Test		Patient		Control		P. Value
		F	%	F	%	
Trichomonas vaginalis in Swab	Negative	212	91.38	25	100.0	0.234 ns
	Positive	20	8.62	0	0	
Total		232	100.0	25	100.0	
Trichomonas vaginalis In Urine (in positive swab)	Negative	4	20.0	25	100.0	0.001 sig
	Positive	16	80.0	0	0.0	
Total		20	100.0	25	100.0	

ns= not significance (Fisher's exact test used in comparison),
sig: significant (chi square used in comparison)
F= frequency, % =percentage

Table 4.1 shows that 20 (8.62%) of patients were positive *Trichomonas vaginalis* in Swab and 80% of them were positive for *Trichomonas vaginalis* in the urine. All controls were negative for *T.vaginalis* in Swab and in urine a significant differences between patient group and control group regarding to *T.vaginalis* in Swab and in urine.

Table 4-2: Percentage of detection of *Trichomonas vaginalis* infection in women (n = 232) by three laboratory methods

Test	Total number of examined women	No. of infected women	% from study group	% from infected cases
Wet smear	232	20	8.62	100
Gram stain	232	20	8.62	100
General urine	232	16	6.89	80

Table 4-3: Demographic features of the study samples.

Characteristics	Ranking And Intervals	Patients (n=232)		Control (n=25)		P. value
		F	%	F	%	
Age /year	14-20	29	12.5	6	24.0	0.624
	21-27	71	30.6	8	32.0	
	28-34	70	30.2	5	20.0	
	35-41	45	19.4	5	20.0	
	42-48	14	6.0	1	4.0	
	49-56	3	1.3	0	0	
Marital status	Married	227	97.8	25	100.0	0.760
	Widow	2	0.9	0	0	
	Divorced	3	1.3	0	0	
Occupation	Employed	29	12.5	1	4.0	0.306

status	Housewife	197	84.9	24	96.0	
	Student	6	2.6	0	0	
Residence	Urban	111	47.8	9	36.0	0.259
	Rural	121	52.2	16	64.0	
Educational status	Illiterate	39	16.8	8	32.0	0.289
	Primary	89	38.4	10	40.0	
	Secondary	41	17.7	4	16.0	
	Intermediate	15	6.5	1	4.0	
	Bachelor and above	48	20.7	2	8.0	
F= frequency, % =percentage						

Table 4.3 shows that less than one third 71 (30.6%) of patients and 8 (32%) of controls age groups within (21-27) years. Most of patient group 227 (97.8%) and all of control group 25 (100%) were married. Regarding to occupation status, the majority of patient group 197 (84.9%) and most of control group 24 (96%) were housewife, on the other hand ; more than half of patient group 121 (52.2%) and vast majority of control group 20 (80%) were living in rural area and finally, more than third of patient group 89 (38.4%) and about two fifth of control group 10 (40%) were having a primary level of education.

Table 4-4: Gynecology history of the study subjects.

Characteristics	Ranking and Intervals	Patients (n=232)		Control (n=25)	
		F	%	F	%
Pregnant	No	171	73.7	20	80.0
	Yes	61	26.3	5	20.0
Vaginal discharge	No	25	10.8	23	92.0
	Yes	207	89.2	2	8.0
Vaginal itching	No	93	40.1	25	100.0
	Yes	139	59.9	0	0
Dysuria	No	102	44.0	25	100.0
	Yes	130	56.0	0	0
Dyspareunia	No	94	40.5	25	100.0
	Yes	138	59.5	0	0
Malodor	No	83	35.8	25	100.0
	Yes	149	64.2	0	0
Contraceptives used	No	163	70.3	15	64.0
	Yes	69	29.7	9	36.0
Type of contraceptive	Intrauterine device	28	12.1	6	24.0
	Pills	34	14.7	4	16.0
	depo-provera	6	2.6	0	0
	Implanon	1	.4	0	0

	None	163	70.3	15	60.0
F: frequency, % :percentage					

Table 4.4 exposed that more than third 171 (37.7%) of patient group and vast majority 20 (80%) of control group were having no pregnancy, while most of patient group 207 (89.2%) having a vaginal discharge and more than half of control group 14 (56%) having no vaginal discharge. Regarding to dysuria, more than half of patient group 130 (56%) were having a dysuria and all of control group 25 (100%) were having no dysuria, on the other hand ; more than half of patient group 139 (59.9%) were having vaginal itching and all of control group 25 (100%) were having no vaginal itching. Moreover, more than half of patient group 138 (59.5%) were having dyspareunia and all of control group 25 (100%) were having no dyspareunia, while more than two third of patient group 149 (64.2%) were having malodor and all of control group 25 (100%) were having no malodor, ,and finally, the higher percentage of patient group 34 (14.7%) were using a pills as a contraceptive and less than one quarter of control group 6 (24%) were using an intrauterine device as a contraceptive.

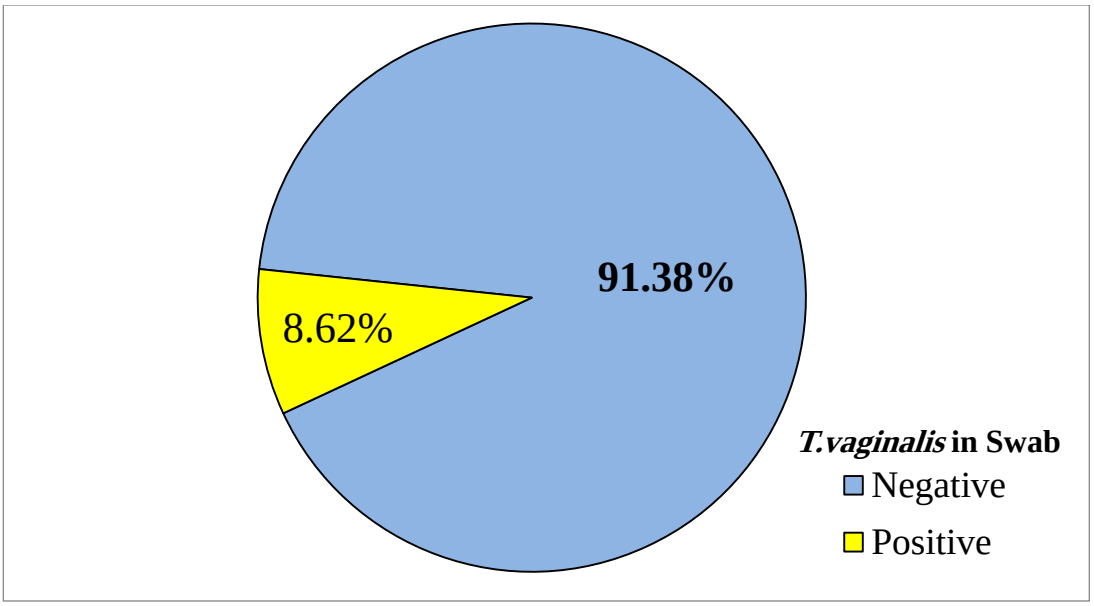


Figure 4.1: Percentage of *Trichomonas vaginalis* in Swab (N=232).

This figure shows that most of patient group (91.4) having a negative swab for *T.vaginalis*.

As shown in Figure 4.1, only (8.62%) of the studied group had positive swab for *T.vaginalis*.

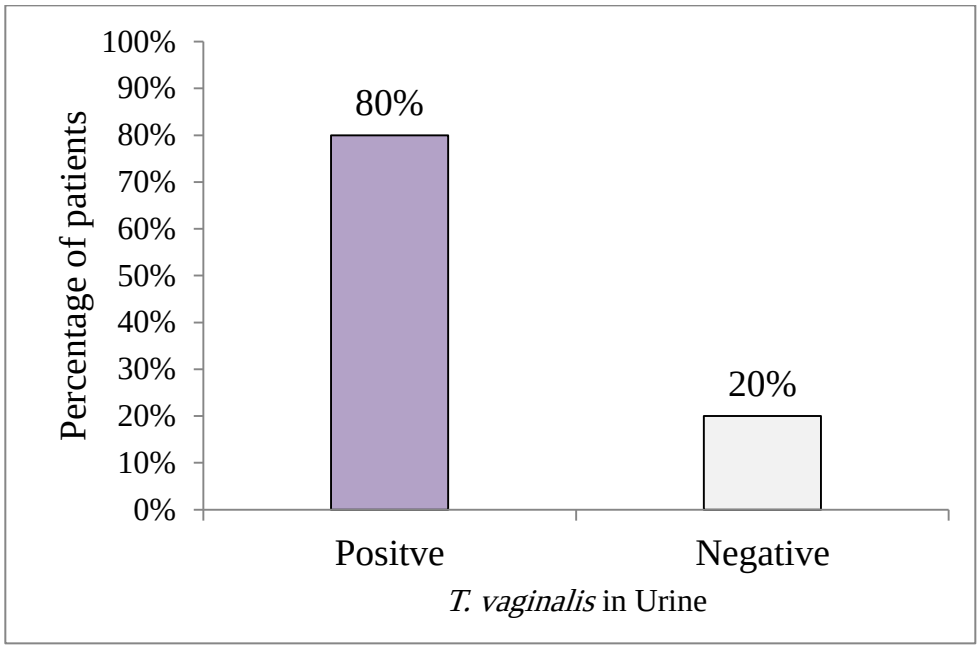


Figure 4.2: Percentage of *Trichomonas vaginalis* in Urine (in positive swab patients) (N=20).

This figure shows that 80% of the 20 *T.vaginalis* swab positive patients were positive for *T.vaginalis* in urine.

Table (4-5): Frequency distribution of Microbial Species detected in the Vaginal Swabs.

Microbial	NO.	Percentage
Kocuria kristinae	**67**	**28.88%**
Enterobacter cloacae complex	39	16.81%
Streptococcus uberis	27	11.64%
Trichomonas vaginalis	**20**	**8.62%**
Salmonella group	16	6.90%
***Candida* spp**	15	6.47%
Klebsiella pneumonia* ssp *pneumonia	**5**	**2.16%**
Escherichia coli	4	1.72%
Enterococcus faecalis	3	1.29%
Staphylococcus hominis* ssp *hominis	1	0.43%
Enterobacter aerogenes	1	0.43%
Staphylococcus aureus	1	0.43%
Lactococcus garvieae	1	0.43%
Staphylococcus haemolyticus	1	0.43%

Table (4-5) shows several pathogenic and non-pathogenic microbial species detected in vaginal swabs of the studied group where *Kocuria kristinae was* the more frequent detect in 28.87% followed by *Enterobacter cloacae complex* in 16.81%, *Streptococcus uberis* in 11.63% , *T. vaginalis in 8.62%*, *Salmonella group* in 6.9%, *Candida* spp in 6.47% ,*Klebsiella pneumonia* ssp *pneumonia* 2.16%, *E.coli* 1.72%, *Enterococcus faecalis in* 1.29% and each of *Staphylococcus hominis* ssp *hominis*, *Enterobacter*

aerogenes, , *Staphylococcus aureus*, *Lactococcus garvieae* and *Staphylococcus haemolyticus*, where the least frequent, each detected in only *0.43%* of the studied group

Table 4.6: Distribution of Pathogenic Microbes Associated with Trichomoniasis in positive swab patient and control group

Test		Patient (N=20)		Control (N=25)		P-value
		F	%	F	%	
Candida spp	Negative	19	95.0	25	100.0	0.444 ns
	Positive	1	5.0	0	0.0	
E. coli	Negative	16	80.0	20	80.0	1.00 ns
	Positive	4	20.0	5	20.0	
Klebsiella pneumonia ssp pneumoniae	Negative	15	75.0	25	100.0	0.013 sig
	Positive	5	25.0	0	0.0	
Staphylococcus hominis ssp hominis	Negative	19	95.0	25	100.0	0.444 ns
	Positive	1	5.0	0	0.0	
Enterobacter aerogenes	Negative	19	95.0	25	100.0	0.444 ns
	Positive	1	5.0	0	0.0	
Enterococcus faecalis	Negative	17	85.0	25	100.0	0.08
	Positive	3	15.0	0	0.0	
Staphylococcus aureus	Negative	19	95.0	25	100.0	0.444 ns
	Positive	1	5.0	0	0.0	
Staphylococcus haemolyticus	Negative	19	95.0	25	100.0	0.444 ns
	Positive	1	5.0	0	0.0	

sig: significant, ns: not significance, F: frequency, % :percentage
* Fisher's exact test used in all comparison (Chi-square was inapplicable)

Table 4.6 shows that no significant differences between patient group and control group in all pathogenic bacteria associated with trichomoniasis except for

Klebsiella pneumonia ssp pneumoniae (P. value <0.05) where it was more frequent in patients than controls; 25% vs. 0.0%, respectively.

Table 4.7: Relationship between demographical data and *Trichomonas vaginalis* in Swab (n=232).

Variable		*Trichomonas vaginalis* in Swab				Total (n=232)	P. value
		Negative (n = 212)		Positive (n=20)			
		No.	%	No.	%		
Age (year)	14-20	27	93.1	2	6.9	29	0.201 ns
	21-27	63	88.7	8	11.3	71	
	28-34	64	91.4	6	8.6	70	
	35-41	44	97.8	1	2.2	45	
	42-48	12	85.7	2	14.3	14	
	49-56	2	66.7	1	33.3	3	
Marital status	Single	0	0.0	0	0.0	0	1.00 ns
	Married	207	91.2	20	8.8	227	
	Widow	2	100.0	0	0.0	2	
	Divorced	3	100.0	0	0.0	3	
Occupation	Employed	28	96.6	1	3.4	29	0.701 ns
	Housewife	178	90.4	19	9.6	197	
	Student	6	100.0	0	0.0	6	
Residence	Urban	100	90.1	11	9.9	111	0.503 ns
	Rural	112	92.6	9	7.4	121	
Educational level	Illiterate	37	94.9	2	5.1	39	0.665 ns
	Primary	82	92.1	7	7.9	89	
	Secondary	35	85.4	6	14.6	41	
	Intermediate	14	93.3	1	6.7	15	
	Bachelor or higher	44	91.7	4	8.3	48	

ns: not significant, Fisher's exact test used in all comparisons except for residence (chi-square used)

Table 4.7 shows that there are a no significant relationship between *T.vaginalis* in Swab and any of demographic data at **$p \leq 0.05$**.

Table 4.8: Relationship between gynecology history and *Trichomonas vaginalis* in Swab (n=232).

Variable		Trichomonas vaginalis in Swab				Total	P. value
		Negative (n = 212)		Positive (n=20)			
		No.	%	No.	%		
Pregnant	No	158	92.4	13	7.6	171	0.355 ns
	Yes	54	88.5	7	11.5	61	
Vaginal discharge	No	23	92.0	2	8.0	25	0.632 ns
	Yes	189	91.3	18	8.7	207	
Vaginal itching	No	86	92.5	7	7.5	93	0.627 ns
	Yes	126	90.6	13	9.4	139	
Dysuria	No	96	94.1	6	5.9	102	0.188 ns
	Yes	116	89.2	14	10.8	129	
Dyspareunia	No	85	90.4	9	9.6	94	0.669 ns
	Yes	127	92.0	11	8.0	138	
Malodor	No	76	91.6	7	8.4	83	0.940 ns
	Yes	136	91.3	13	8.7	149	
Contraceptive use	No	148	90.8	15	9.2	163	0.627 ns
	Yes	64	92.8	5	7.2	69	

ns: not significant, chi-square used in all comparisons except for vaginal discharge (Fisher's exact test used)

Table 4.8 shows that no significant relationship was found between *T.vaginalis* in Swab and any of gynecological history , in all comparisons, P. value > 0.05.

Table 4.9: Relationship between demographical data and *Trichomonas vaginalis* in urine (n=20).

Variable		Trichomonas vaginalis in Urine				Total	P. value
		Negative (n=4)		Positive (n=16)			
		No.	%	No.	%		
Age (year)	14-20	1	50.0	1	50.0	2	0.913 ns
	21-27	2	25.0	6	75.0	8	
	28-34	1	16.7	5	83.3	6	
	35-41	0	0.0	1	100.0	1	
	42-48	0	0.0	2	100.0	2	
	49-56	0	0.0	1	100.0	1	
Marital status	Married	4	20.0	16	80.0	20	NA
	Widow	0	0.0	0	0.0	0	
	Divorced	0	0.0	0	0.0	0	
Occupation	Employed	0	0.0	1	100.0	1	0.800 ns
	Housewife	4	21.1	15	78.9	19	
	Student	0	0.0	0	0.0	0	
Residence	Urban	3	27.3	8	72.7	11	0.591 ns
	Rural	1	11.1	8	88.9	9	
Educational level	Illiterate	0	0.0	2	100.0	2	0.280 ns
	Primary	1	14.3	6	85.7	7	
	Secondary	2	33.3	4	66.7	6	
	Intermediate	1	100.0	0	0.0	1	
	Bachelor or higher	0	0.0	4	100.0	4	

ns: not significant, Fisher's exact test used in all comparisons,
NA: not available (cannot be calculated)

Table 4.9 shows that there were no significant relationships between *T. vaginalis* in urine and any of demographic data at $p \leq 0.05$.

Table 4.10: Relationship between Gynecology History and *Trichomonas vaginalis* in Urine (n=20).

Variable		Trichomonas vaginalis in Urine				Total	P. value
		Negative (n = 4)		Positive (n=16)			
		No.	%	No.	%		
Pregnant	No	4	30.8	9	69.2	13	0.249 ns
	Yes	0	0.0	7	100.0	7	
Vaginal discharge	No	1	50.0	1	50.0	2	0.368 ns
	Yes	3	16.7	15	83.3	18	
Vaginal itching	No	2	28.6	5	71.4	7	0.587 ns
	Yes	2	15.4	11	84.6	13	
Dysuria	No	2	33.3	4	66.7	6	0.549 ns
	Yes	2	14.3	12	85.7	14	
Dyspareunia	No	2	22.2	7	77.8	9	0.622 ns
	Yes	2	18.2	9	81.8	11	
Malodor	No	1	14.3	6	85.7	7	0.561 ns
	Yes	3	23.1	10	76.9	13	
Contraceptive use	No	4	26.7	11	73.3	15	0.530 ns
	Yes	0	0.0	5	100.0	5	
ns: not significant, Fisher's exact test used in all comparisons,							

Table 4.10 shows that no significant relationship had been reported between *T.vaginalis* in urine and any of gynecological history at **$p \leq 0.05$**.

Table 4.11: Relationship between *Trichomonas vaginalis* in swab and Pathogenic microbial Associated with Trichomoniasis.

Pathogenic microbe		*Trichomonas vaginalis* in swab				P. value*
		Negative (N=212)		Positive (N=20)		
		F	%	F	%	
Candida spp	Negative	197	92.9	19	95.0	0.598 Ns
	Positive	15	7.1	1	5.0	
E. coli	Negative	212	100.0	16	80.0	0.001 Sig
	Positive	0	0.0	4	20.0	
Klebsiella pneumonia ssp *pneumoniae*	Negative	212	100.0	15	75.0	0.001 Sig
	Positive	0	0.0	5	25.0	
Staphylococcus hominis ssp *hominis*	Negative	212	100.0	19	95.0	0.086 Ns
	Positive	0	0.0	1	5.0	
Enterobacter aerogenes	Negative	212	100.0	19	95.0	0.086 ns
	Positive	0	0.0	1	5.0	
Enterococcus faecalis	Negative	212	100.0	17	85.0	0.001 Sig
	Positive	0	0.0	3	15.0	
Staphylococcus aureus	Negative	212	100.0	19	95.0	0.086 ns
	Positive	0	0.0	1	5.0	
Staphylococcus haemolyticus	Negative	212	100.0	19	95.0	0.086 ns
	Positive	0	0.0	1	5.0	

sig: significant, ns: not significance, F: frequency, % :percentage
* Fisher's exact test used in all comparison (Chi-square was inapplicable)

other pathogens including Staphylococcus haemolyticus, Staphylococcus aureus, Enterobacter aerogenes, Staphylococcus hominis ssp *hominis, Candida* spp were not significantly associated with *T. vaginalis* in swab , (P. value > 0.05).

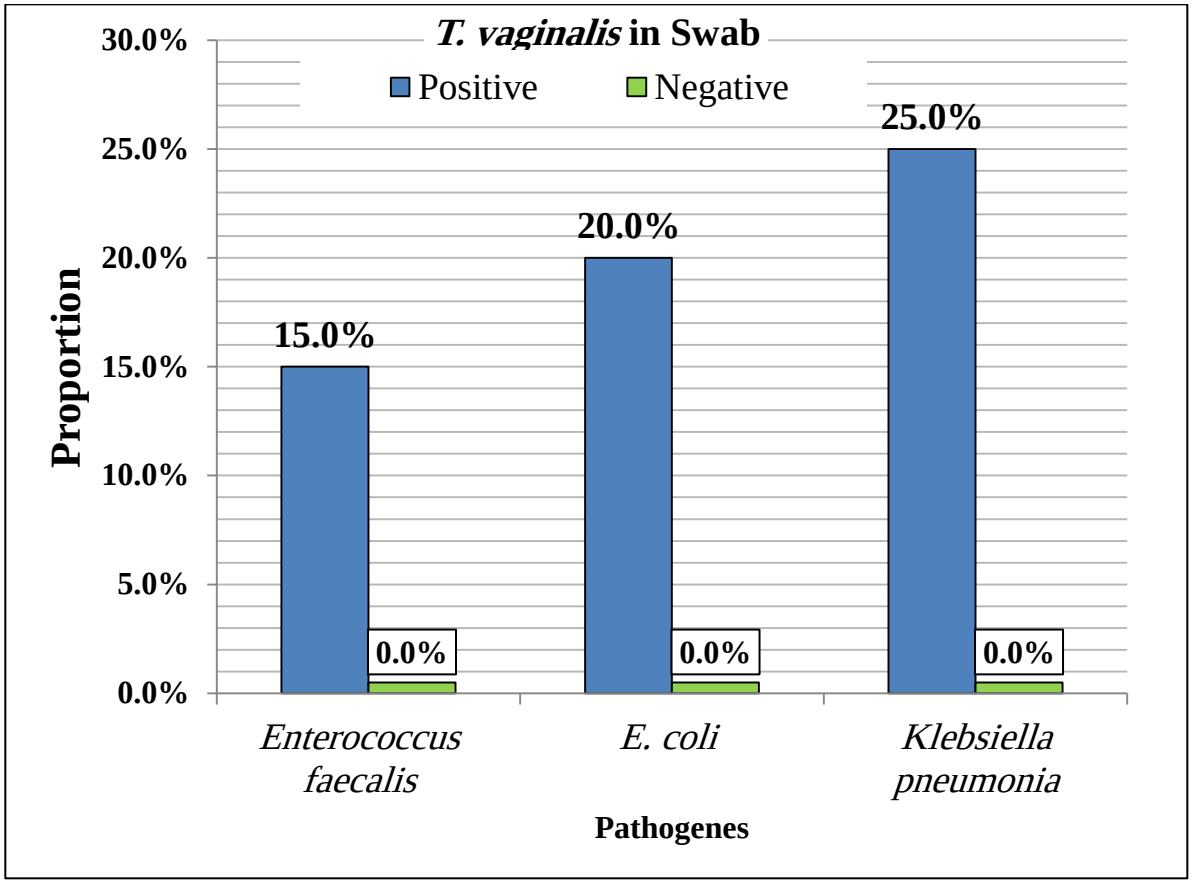


Figure 4.3: Proportional distribution of the pathogenic microbes that significantly associated with positive *T. vaginalis* (N=20)

Figure 4.3, graphically compare the proportions of the pathogenic microbes that significantly associated with positive *T. vaginalis*, where the higher proportion of 25% reported for *Klebsiella pneumonia* ssp *pneumoniae* followed by *E. coli* in a proportion of 20% and *Enterococcus faecalis* that contributed for 15%

Table 4-12:Distribution of WBC in patient and control group in urine.

WBC/HPF	**Patients (n=232)**		**Control (n=25)**		**P. value**
	F	%	F	%	
0-3	119	51.3	20	80.0	0.011 sig
4-30	67	28.9	5	20.0	
>30	46	19.8	0	0	
Total	232	100.0	25	100.0	

Chi square test used in comparison,

Sig: significance,

F= frequency, % =percentage

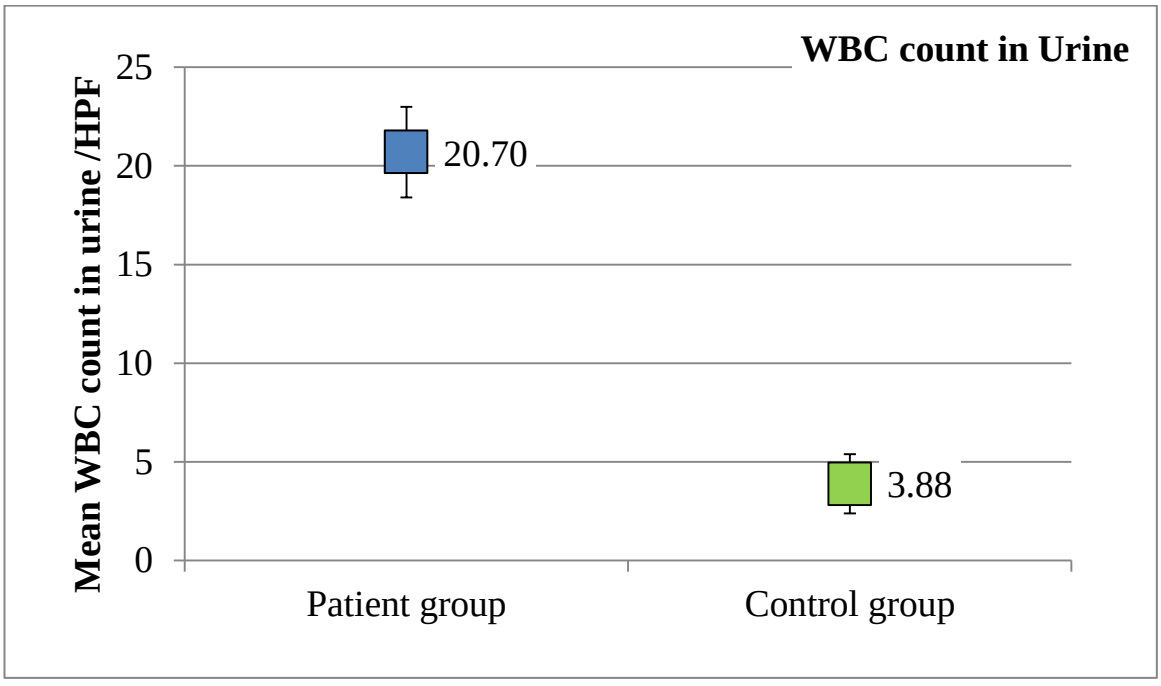


Figure 4.4. Comparison of mean WBC count in urine / HPF of the studied groups

In table (4-12) more than half of the study group, 119/232 (51.3%) and 20/25 (80%) of controls had WBC in the urine that was within the normal range of WBCs in urine, while 67(28.9%) of study group and 5(20%) of control group with mediate increase value of WBCs in urine, and 46(19.8%) of study group with high increase value compare to none of controls. From other point of view, when the mean values of WBC in urine compared among patients with positive *T. vaginalis*, negative *T. vaginalis* and controls, it had been significantly found that both subgroups of positive and negative *T. vaginalis* patients had significantly higher mean number of WBC in urine compared to control. Moreover, patients with positive *T. vaginalis* in swab had much higher number of WBC in urine compared to those with negative *T. vaginalis* in swab, (Figure 4.4.).

Table4-13: Comparison of mean urine WBC count in patients with positive swab *Trichomonas vaginalis*

Pathogens	Test results	No. of patients	WBC in urine/HPF		P. value
			Mean	SD	
Candida spp	Negative	19	95	47	0.177
	Positive	1	26	0	
E. coli	Negative	16	93	54	0.840
	Positive	4	87	22	
Klebsiella pneumoniae. ssp *pneumonia*	Negative	15	96	53	0.515
	Positive	5	79	39	
Staphylococcus hominis. ssp.*hominis*	Negative	19	95	47	0.190
	Positive	1	25	0	
Enterobacter. Aerogenes	Negative	19	93	50	0.580
	Positive	1	64	0	
Enterococcus faecalis	Negative	17	89	52	0.528
	Positive	3	109	27	
Staphylococcus aureus	Negative	19	87	46	0.077

	Positive	1	176	0	
Staphylococcus haemolyticus	Negative	19	95	48	0.177
	Positive	1	26	0	

Furthermore, as shown in table (4-13) the mean urine WBC count in patients with positive swab *Trichomonas vaginalis* was compared according to the results of testing for each pathogen (positive vs. negative), results of these comparisons revealed no significant differences in mean urine WBCs in patients who were positive vs. those who were negative for these pathogens, (P. value > 0.05). Interestingly, despite the statistical insignificance, positive swab *Trichomonas vaginalis* patients who had negative pathogens results appeared to had higher WBCs count compared to those with positive pathogens, this indicates that the higher WBCs count in the urine could be mainly attributed to the presence of *Trichomonas vaginalis* rather than the other microbes like *Candida, E coli*, …,etc.

Table4-14: Comparison of mean urine WBC count in patients with Negative swab *Trichomonas vaginalis*

Pathogens	Test results	No. of patients	WBC in urine/HPF		P. value
			Mean	SD	
Candida spp	Negative	197	15	7	0.230
	Positive	15	7	3	
E. coli	Negative	212	14	6	NA
	Positive	0	-	-	
Klebsiella pneumoniae ssp *pneumonia*	Negative	212	14	6	NA
	Positive	0	-	-	
Staphylococcus hominis. ssp.*hominis*	Negative	212	14	6	NA
	Positive	0	-	-	
Enterobacter aerogenes	Negative	212	14	6	NA
	Positive	0	-	-	

Enterococcus faecalis	Negative	212	14	6	NA
	Positive	0	-	-	
Staphylococcus aureus	Negative	212	14	6	NA
	Positive	0	-	-	
Staphylococcus haemolyticus	Negative	212	14	6	NA
	Positive	0	-	-	
NA: comparison was not available because all patients were negative for this pathogen					

In Table (4-14) the total patient with no *T.vaginalis* was 212, the mean WBCs count in their urine was compared according to the results of laboratory culture of the different pathogens, no significant difference was found in the mean WBCs count between patients with positive or negative *Candida* spp. , the mean WBCs was 15 ± 7 and 7 ± 3 cell/HPF, respectively (P. value >0.05). In the negative swab *T. vaginalis* group, culture for all other pathogens was negative, and the mean WBCs in the urine was 14 ± 6 cell/HPF in all subgroups of patients who were negative for these pathogens.

From other point of view, it is worth mentioned that in patients with negative swab *T. vaginalis* the mean WBCs in the urine was lower than that in the positive swab *T. vaginalis* group, which again reflected the association between positivity of *T. vaginalis* in swab and higher WBCs count in the urine.

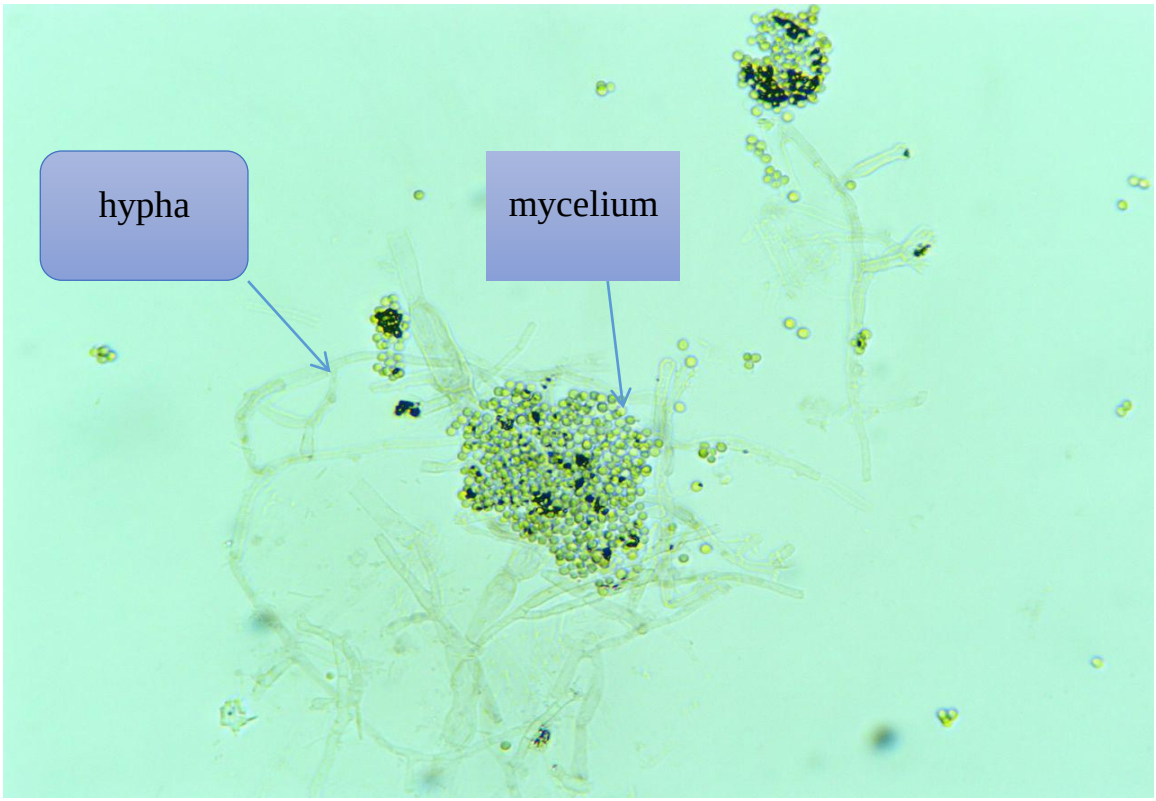


Figure (4-5) ***Candida*** **spp in wet smear with 100X objective by microscope camera system**

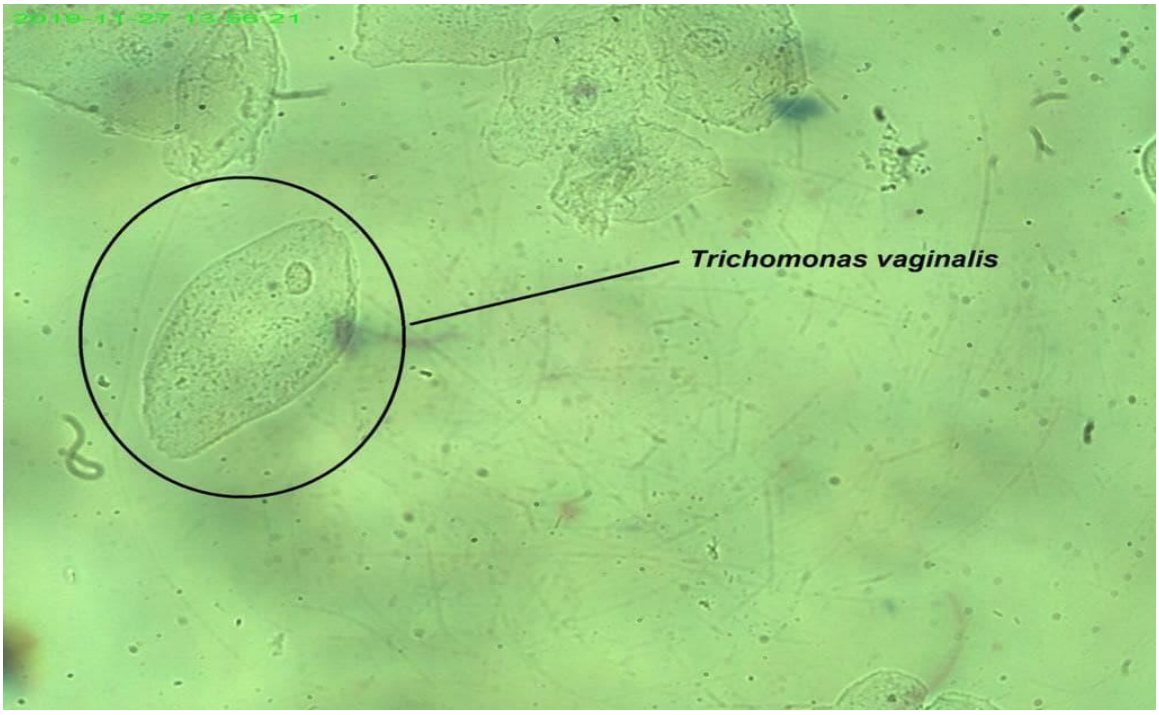


Figure (4-6) ***Trichomonas vaginalis*** **in direct exam with 100X objective by microscope camera system**

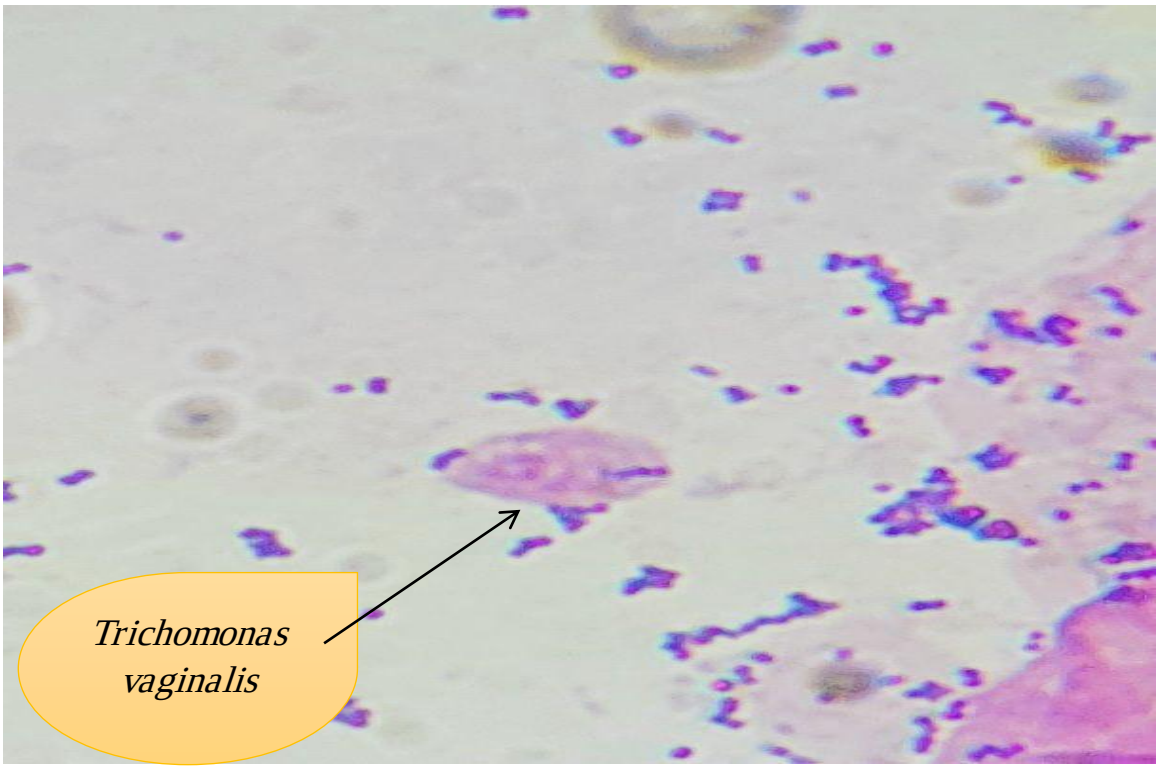


Figure (4-7) ***Trichomonas vaginalis*** **with gram stain with 100X objective by microscope camera system**

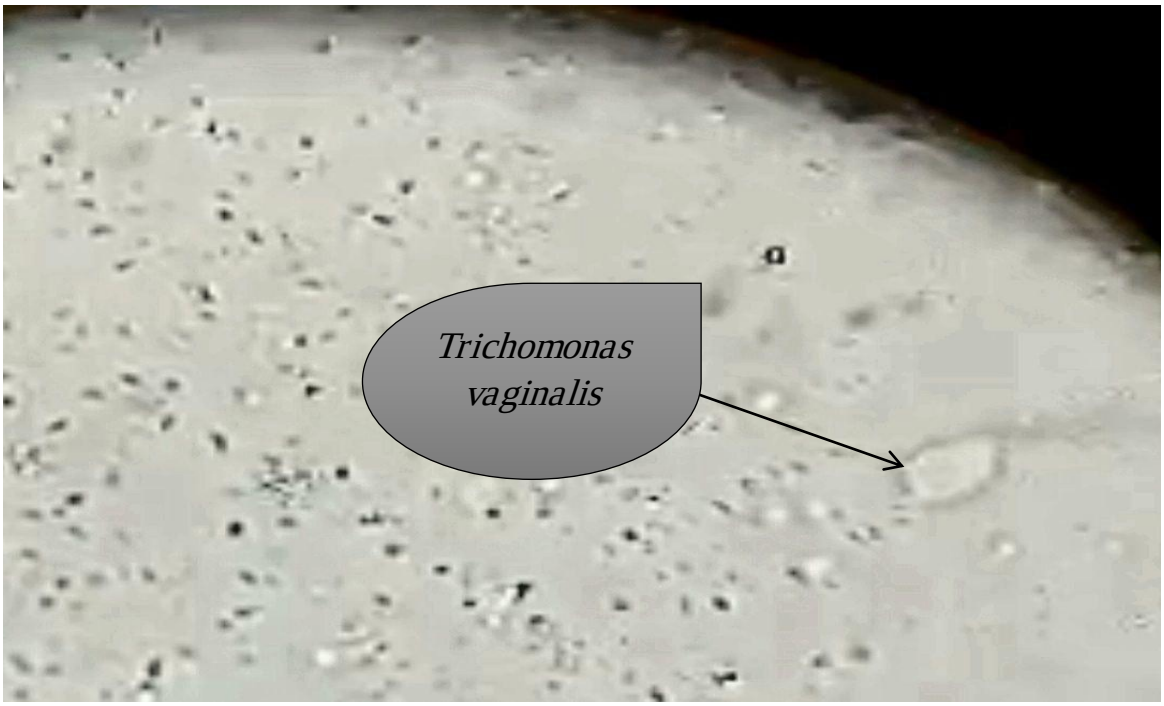
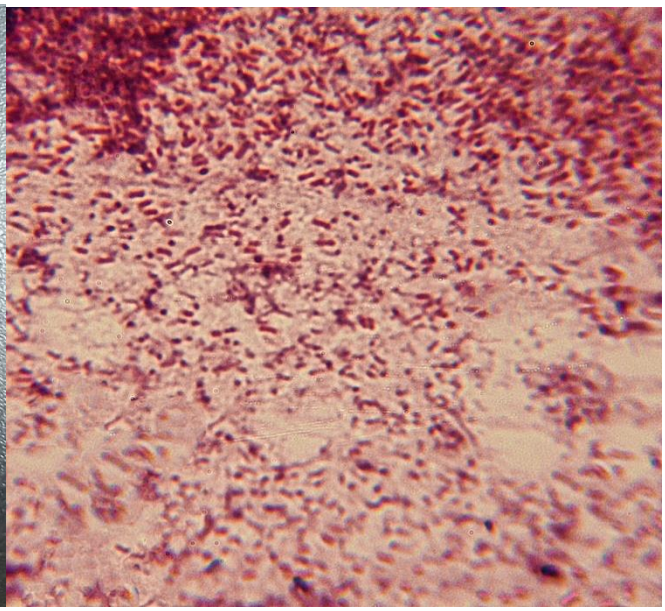
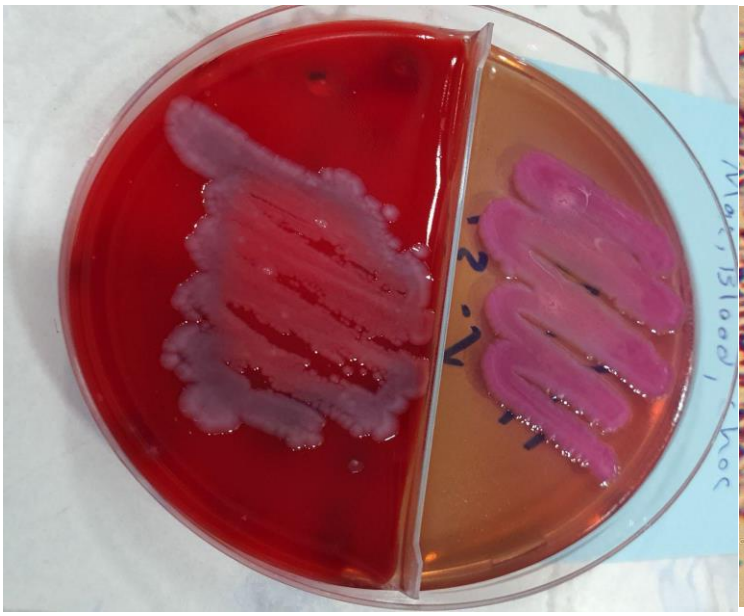


Figure (4-8)*Trichomonas vaginalis* in urine sample with 40X objective by microscope camera system



Figure(4-9) A. *Escherichia coli* culture on MakConky agar

B .colonies of the bacteria with gram stain under microscope 100x.



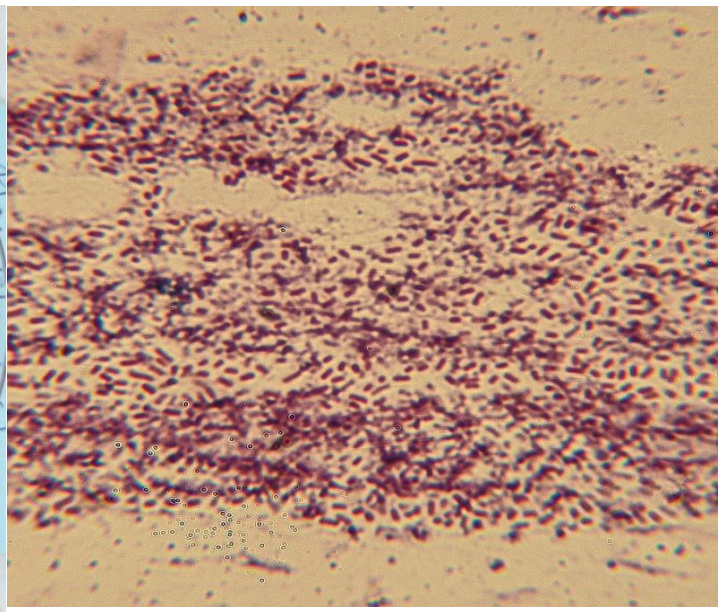


Figure (4-10) A. *K. Pneumoniae* culture on Blood and MakConky agar

B. Colonies of the bacteria with gram stain under microscope 100x.

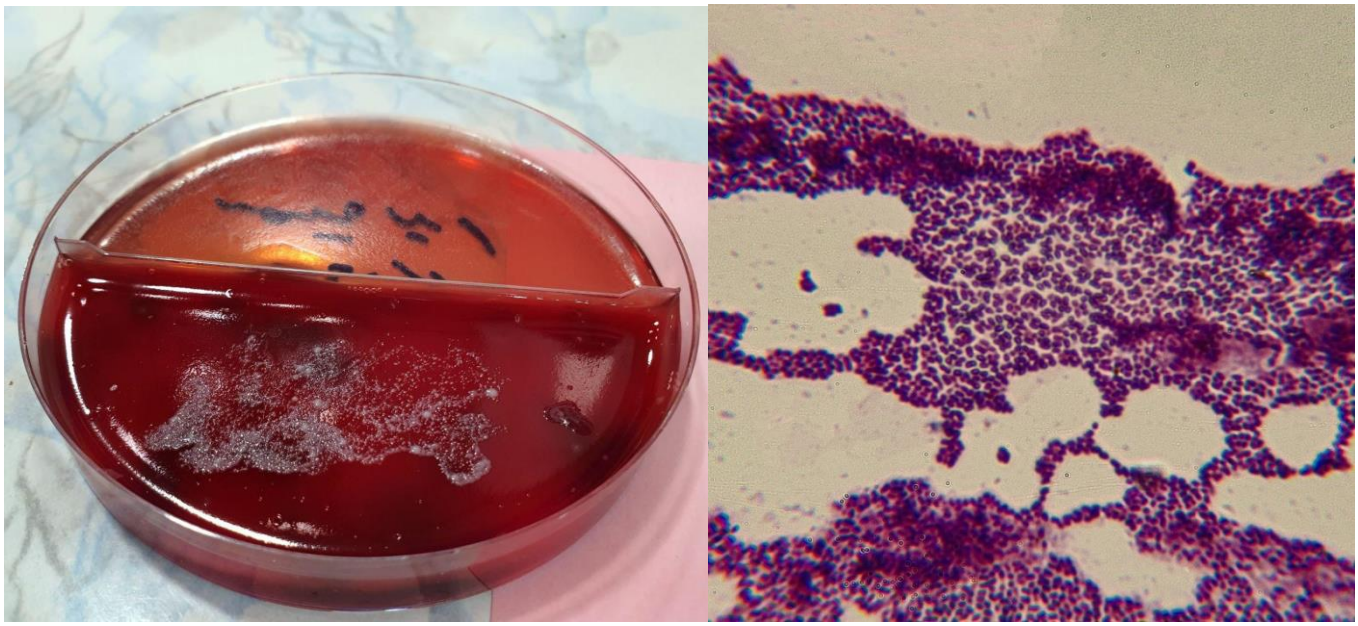


Figure (4-11) A. *Kocuria Kristine* culture on Blood agar

B. Colonies of the bacteria with gram stain under microscope 100x

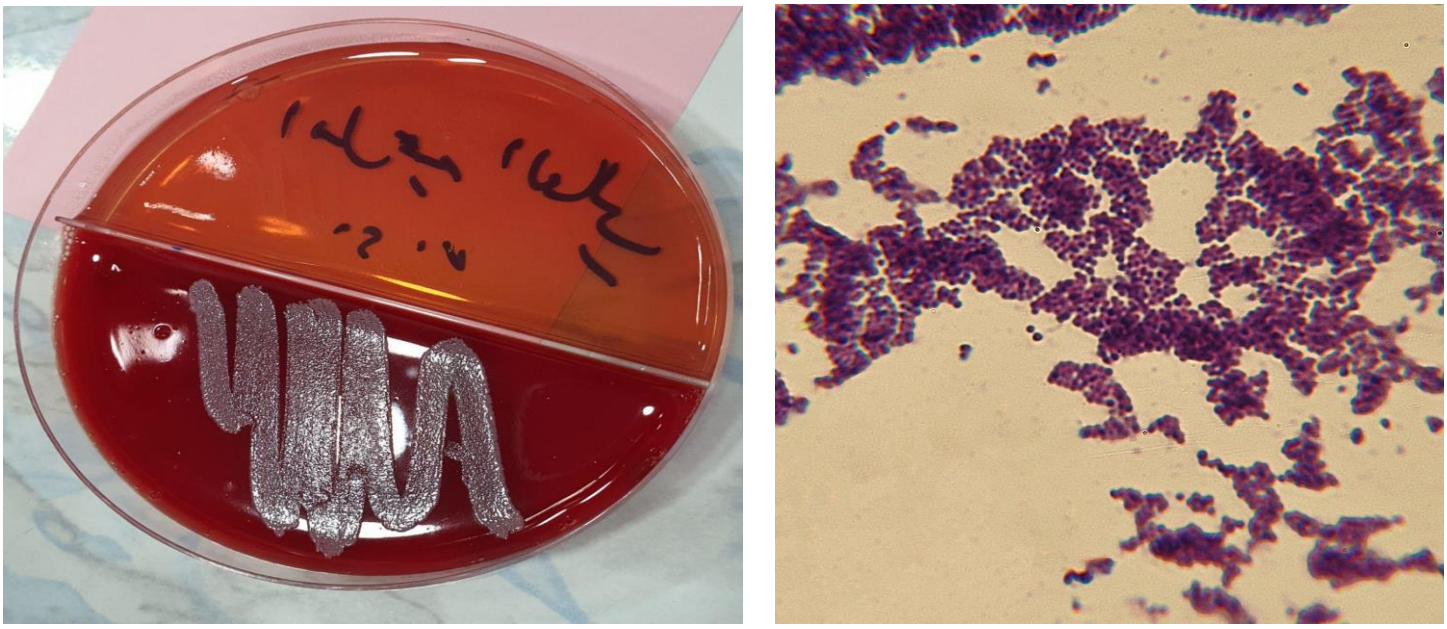
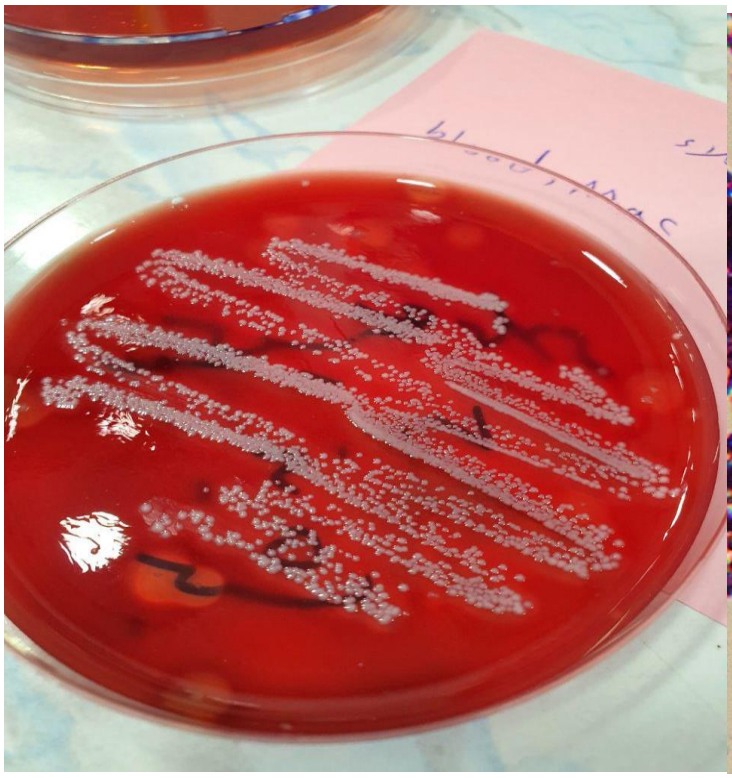
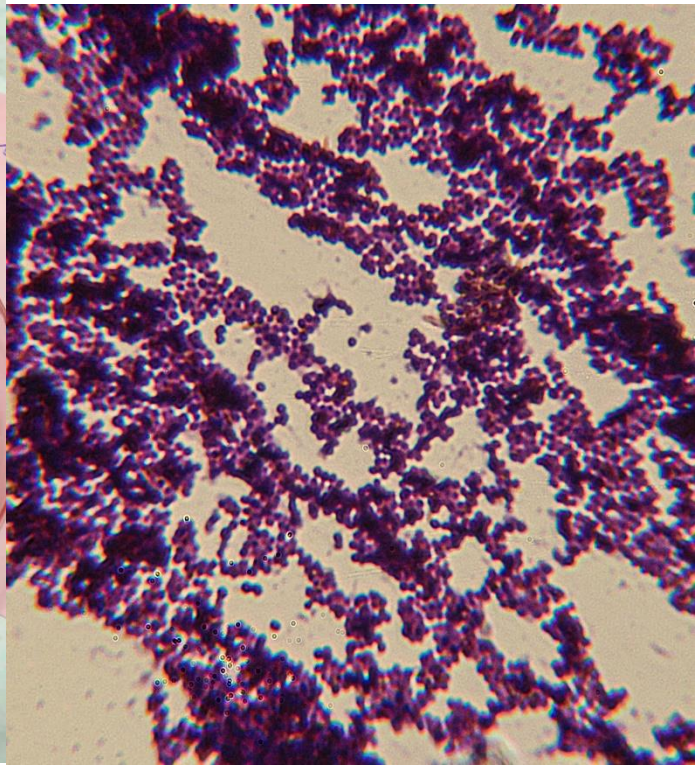


Figure (4-12) A. *S.hominis* on Blood agar

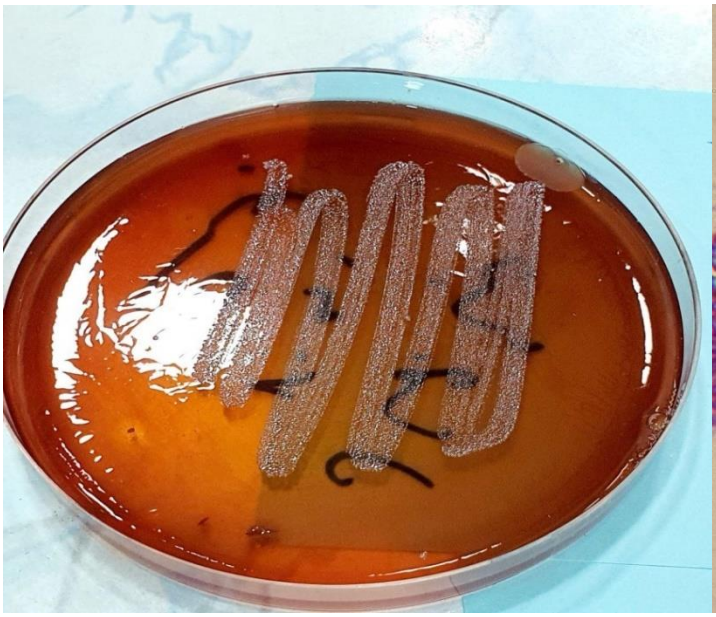
B. Colonies of *S.hominis* with gram stain under microscope 100x.

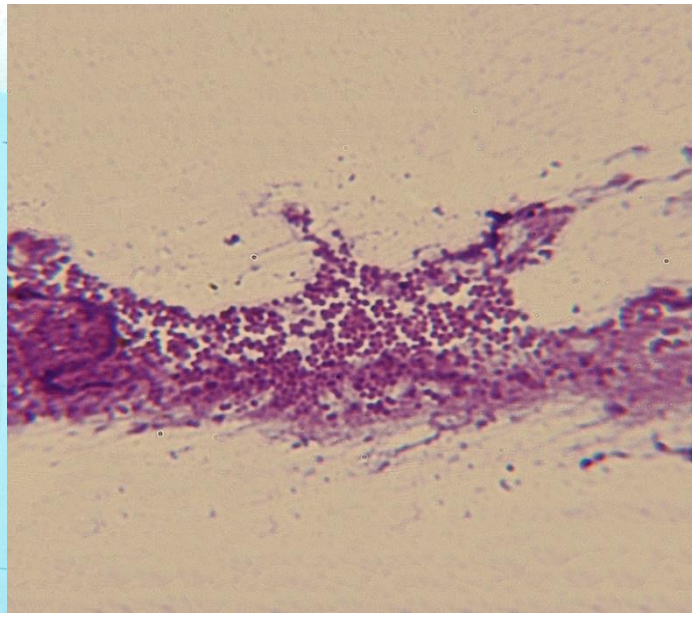




Figure(4-13) A. *Staphylococcus haemolyticus* on Blood agar

B.colonies of *Staphylococcus haemolyticus* with gram stain under microscope





Figure(4-14) A. ***Enterococcus faecalis*** on Blood agar

B.Colonies of ***Staphylococcus haemolyticus*** with gram stain under microscope 100x.

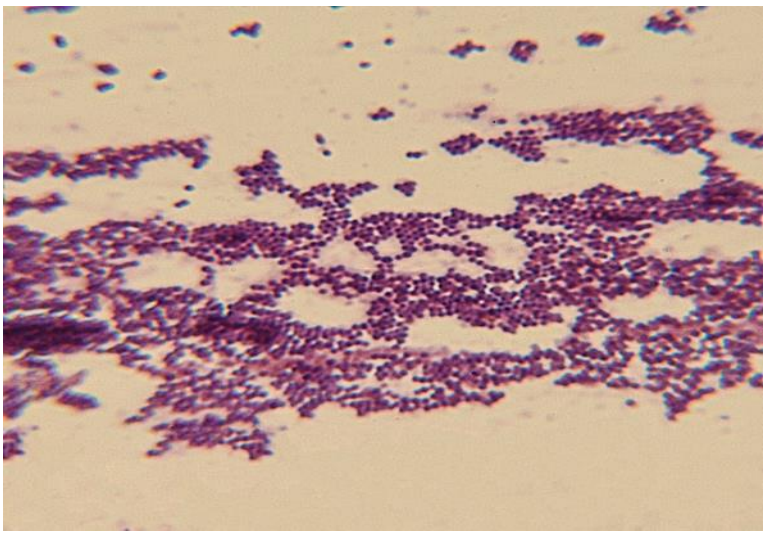
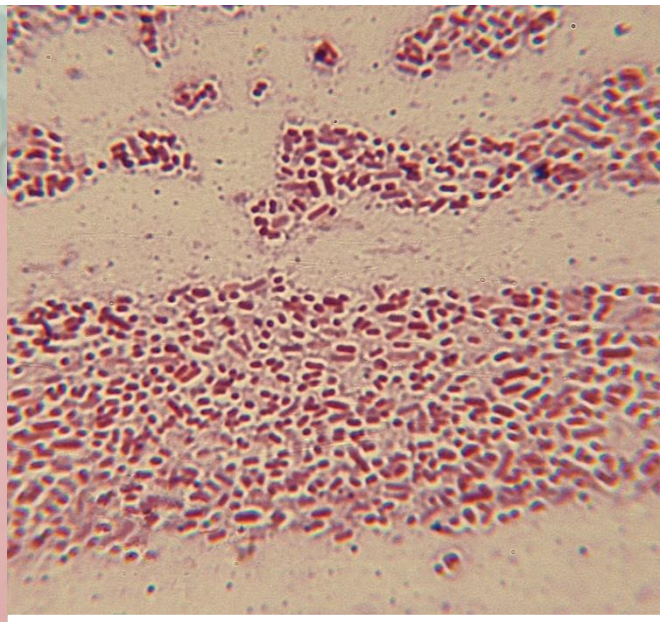


Figure (4-15) Colonies of *Staphylococcs aureus* with gram stain under microscope 100x.

Figure(4-16) A. *Enterobacter aerogenes* on Blood and MacKoncy agar.



B.Colonies of ***Enterobacter aerogenes*** with gram stain under microscope 100x.

Chapter Five
Discussion

Discussion

The results of the current study for microscopic examination of samples obtained from the swabs of 232 women showed that the total infection rate of *T. vaginalis* in the vaginal swab samples with wet mount and gram stain for women was 20 (8.62%). This is consistent with the study conducted in Karbala (Al-Haidari, 2011) where it was found that the total infection rate with the direct exam (wet mount preparation and gram stain) was (9.5%). This result is slightly more than the result of the current study but very close to it, as well as the result by Deivam *et al.* (2014) in India which where the infection rate was 8.1%. The prevalence rate was also similar in other studies, as in Obunge *et al*, 2009 in Nigeria which showed that the highest infection rate with wet mount was 10.5%.

The results of the current study (wet smear) differed from a number of studies, some of them showed results at low rates from the results of the current study, as by Al-Saeed, (2011) in Dohuk which where the infection rate was 2.4%, and by Daoud and others, (2013) in Kirkuk which showed that the highest infection rate with wet mount was 2.7%, while by Matini and other (2012) in Hamedan, western Iran which showed that the highest infection rate with wet mount was 1.7%, and by Kassem & Majoud, (2006) in Turkey which where the infection rate was (3.2%).

The results of other studies differed by showing the results of a higher infection rate with the parasite by direct examination, including the findings by(Dahab *et al*., 2012) in Sudan which where the infection rate was 12%, by Al-Marsomy, (2020) in Baghdad which showed that the highest infection rate was 15.38%, by Lewis *et al.* (2013) in South Africa which where the infection rate was 23.6%, by Al-Hussuny, (2015) in Diyala which where the

infection rate was (24.60%), by Al-Abodi *et al*., (2019) in Muthanna which where the infection rate was (26.00%). While the highest infection rate was recorded by Al- Majidii *et al*, (2020) in Iraq with a recent study in Maysan using direct examination was (75.22%).

The results of examining 232 urine samples of the same women from whom the swab was taken in table (4-1) showed that the total infection rate appeared in 16 (80%) of those infected using direct exam, with a rate of 6.89% of the total number examined. This percentage is consistent with what was found in the study by Al-Quraishi, (2013) in Babylon (7.38%), while it differed from other studies as the rate of infection in urine samples was higher than the results reached by Al-Haidari, (2011), where the infection rate in urine samples was (7.1 %) as well. In Amadi and Nwagbo, (2013) in Nigeria, the incidence rate of both in urine samples was 3.5%.

The recorded percentages were higher by Awadh and Al-Quraishi, (2016) in Babylon Governorate in another study which where the infection rate was (13.48%). The researcher explained that the reason may be due to the fact that the study is a pathological and not an epidemiological one, i.e. samples were collected from patients only and not randomly, while the current study was epidemiological. There are significant differences (p=0.001) among study groups with trichomoniasis in urine.

This discrepancy in percentages is due to the difference in the number of samples and the type of samples, as studies recorded the highest rate of infection when examining vaginal secretions compared to urine examination (Al-Ibrahim, 2008). The parasite multiplies and as a result of crowding and competition for food and available space, it penetrates the urinary canal, so the appearance of the parasite in urine is an accidental or emergency situation

(Al-Ibrahim, 2008), and the nature of social traditions in different regions of the world, especially multiple sex partners and different living conditions, have a clear impact on recording different rates of infection (Ponte & Gross, 2000). Thus, Verteramo *et al*. (2008) indicated that the sexual lifestyle is one of the factors that increase the spread of trichomoniasis.

The discrepancy may be due to the difference in the methods of diagnosis, as there are multiple methods for diagnosing the *Trichomonas vaginalis* parasite that differ in their sensitivity, as well as some patients taking Flagyl before consulting the gynecologist (Al-Badri and Al-Tikriti, 2013).

Diagnostic procedures for large-scale screens must meet extremely high standards in terms of specificity, sensitivity, and test result duration. Although enzyme immunoassays and PCR-based *T. vaginalis* detection match some of these criteria, these tests are only available in specialist laboratories due to their high prices. Wet preparation and culture procedures will continue to be used as the first line of diagnostics. Because most infected patients did not show usual indicators of infection, clinical symptoms are unreliable (Al-Haidari, 2011).

Relationship between Demographical data and *Trichomonas vaginalis* in Swab

The current study showed low infection rate among the study group. This rate is lower than those in most other Iraqi cities, and it shows that the age group (49-56) years constitutes 3 (1.3%) of the total number of examined women with the highest infection rate 1(33.33%) within the total number of age groups, 1(0.43%) within the total number of study group, and then the age group (42-48)years with 2 (14.28%) percentage within the total number

of age group, and 2(0.86%) within the total number of study group. The result of the emergence of an increased incidence of infection within these two categories may be due to the small number of samples examined. Accordingly, the category (21-27) was adopted, the highest percentage of infection within the total number of the category 71 (30.6%) with 8 (11.26%) within the age group and 8 (3.44%) of the total number of infected women20 (%8.62), followed by the group (28-34), which constitutes 70 (30.2%) of the total number of women examined. The infection rate was 6 (8.57%) within the age group (28-34) and 6 (2.58%) among the total number of infected women. The higher infected rate among the age group 21-27 years is attributed to the high sexual activity, the productivity, abortion and malnutrition. The lowest percentage was 1(2.2%) within the age group (35-41). All of these results are very close to Al-Haidari (2011) in Karbala who found that the highest infection rate was in the age group (40 and above) years with 3 (14.2%) within the age group and 3(2.6%) from total infection, and according to infection rate within the total infection women was the highest IR within the age group (21-30), with 5(4.34%) percentage of the total number of the infection 11 (9.5%), so the lower IR was (31-40) with 2 (4.8%) within the age group. This result disagree with Al-Majidii *et al*. (2020) who found that the group (34-40) years was the most affected, which interpreted the result as increasing at these ages because of the high level of estrogen that makes the vaginal environment suitable to grow of the *T. vaginalis* (Nwokah *et al.*, 2019). There is no significant differences among age and trichomoniasis infected (p=0.201).

Table (4-3) shows that the educational level of the total examined women was 39(16.8%) illiterate, 89(38.4%) primary stage, 41(17.7%)

secondary stage, 15(6.5%) intermediate stage and bachelor and above was 48(20.7%).

In Table (4-7) shows that there is no significant relationship between the trichomoniasis IR and education levels of women (p=0. 665), despite that the women with secondary educational level have the highest infection rate (14.6%) and the lowest was (5.1%) at women with illiterate level. These findings agree with Al-Habib and other (2005) and did not agree with Al-Majidii *et al.* (2020) who found that the illiterate was the highest infection rate; this may be due to poor health care and lack of women's awareness programs which put the women at the risk of infection (Yeh *et al.,* 2013).

Table (4-3) shows that the marital status among the total examined women, married women represented the largest group 227 (97.8%), widow 2 (0.9%), divorced 3 (1.3%).

Results showed, including Table (4-7), that all infected with the parasite in relation to marital status appeared in married women with a percentage of 8.8%, This may be due to transmission of infection that occurred through sexual intercourse, while no infection appeared in the categories of divorced and widowed women. The reason may be due to the fact that the percentage of the number of female examinations is small from these two groups due to the lack of health follow-up and the review of health centers and outpatient clinics by these two groups. While unmarried females were not examined Because of their refusal to participate, this is due to the region’s habits in refusing to test for unmarried women, and these results are consistent with what researchers Al-Majidii *et al.* (2020) found that the highest rate of infection was among married women with a rate of (80.92%). Al-Kahfaji (2020) found that the highest infection rate was (81.90%), while it

contradicted the findings of Tine *et al.* (2019) as it was found that the highest infection rate was among the divorced women with a rate of (11.1%). However, without additional information about the participant's sexual behavior, educational level, or knowledge about STDs, the researcher could not clearly explain the relationship between marital status and the prevalence of Trichomoniasis as there is a non-significant differences (p=1.00).

In Table (4-3), the distribution of the total samples on the basis of the place of residence, as they were distributed in a percentage and number urban with 111(47.8%) and rural 121 (52.2%). The current study, as shown in Table (4-7), that the highest infection rate was in the urban with 10%, compared to the rural with a rate of (7.43%), but it does not find a significant difference between the area of residence and the vaginal trichomoniasis (p=0.503), This result is in agreement with the results of the study by Ali *et al.* (2017), as well as with Barbuceanu and Vacarel (2014) in Romania, where the infection rate in the urban area was 66.4% higher than in the rural 33.6%, and disagreeing with the results of Taher and Shaker (2018).

The researcher attributed the reason for this to the fact that women in rural areas are infected but have less opportunity to get a periodic gynecological examination from the women of the urban. Therefore many infected women are not aware that they have been infected with the parasite. Also, the total number of sample does not represent the governorate with all its urban and rural residents, as it may show a difference in the percentages of women infected in rural and urban areas in the case of a larger number to represent the study.

In Table (4-3), the occupation status of the study group was 29 (12.5%) employed, housewife 197 (84.9%) while the student was 6 (2.6%) from total examined women.

Based on the occupation status as in Table (4-7), the results of the case study showed that the highest infection rate appeared in housewives with infection rate (9.6%). The study results agreed with the two studies Al-Majidii *et al.* (2020) and Nas *et al.* (2020) which showed that the highest infection rate among housewives. This may reflect a return to poverty, which resulted in a loss of immunity as a result of malnutrition, a lack of knowledge, and a failure to seek adequate treatment (Al-Majidii *et al*., 2020). It differed from the results by Mahdi *et al.* (2001), and the infection appeared in a low percentage among the female employees, with (3.4%). No infection appeared among the students examined in the current study, with non-significant differences (p=0.701).

Relationship between Gynecology History and *Trichomonas vaginalis* in Swab (n=232).

Table (4-4) showed that the the pregnant women were 61(26.3%) of total examined women 232 (100%), and 171(73.7%) were non-pregnant women. The pregnant women in the control group was 20 (80%) and the non-pregnant women was 5 (20%). The results Table (4-8) showed that the highest infection rate was among pregnant women with a rate of 11.47% compared to the rate of non-pregnant women, which was 7.6%. However, there is no significant difference between pregnancy and *T.vaginalis* infection (p=0.355), which agrees with Kadhum (2012), and disagreed with Abdul-Aziz *et al.* (2019). This may be due to the hormonal changes that occur in the

body during pregnancy, as hormones are essential during the reproductive age directly affecting the ability of *T.vaginalis* (Arbabi *et al.*, 2014).

Table (4-4) showed that from the total examined women, 232(100%), 207 (89.2%) had vaginal discharge, while 25 (10.77%) were without vaginal discharge. The percentage of women with vaginal discharge was 23 (92%) in the control group and without discharge was 2 (8%). As seen in the Table (4-8), 207 (89.22%) of the total number of the study group examined with a wet mount and gram stain suffer from vaginal secretions of different colors, of which 20 (8.62%) were infected with T. vaginalis from total examined women were 18 (7.75%) within the total number of infected suffering from vaginal discharge . In comparison, of the total number of the study group25 (10.77%) did not suffer from vaginal secretions , of which 2 (8%) from total examined women are infected with parasite. Infection rate and number were 2 (0.86%) of the total infected women, and this shows the highest infection rate 18 (90%) of infected women with vaginal secretions.

The reason why most women infected with the parasite suffer from vaginal infections may be due to the effect that the parasite has on the membranes upon adhesion, which leads to irritation of the area and scratching of the adhesion sites, which causes an increase in the secretions associated with infection in several forms.

Other symptoms showed vaginal itching, dysuria, dyspareunia, and malodor, as well as contraceptives used; from total examined women, 139 (59.9 %) were with vaginal itching, while 93 (40.1%) without suffering from itching. All control group did not suffer from itching 25 (100%). 130 (56.0%) women were suffering from dysuria symptom, while 102 (%44.0) were not. Dyspareunia appeared in 138 (%59.5) examined women have dyspareunia

and 94 (40.5%) were without dyspareunia. All control group did not suffer from dyspareunia 25 (100%). In the current study, 149 (64.2%) had malodor, and 83 (35.8%) did have any. All control group did not suffer from malodor 25 (100%). Among total study group, 163 (70.3%) did not use any contraceptive, while 69 (29.7%) women used different types, pills 34 (14.7%), intrauterine device 28 (12.1%), depo-provera 6 (2.6%) and implanon 1 (0.4%). In control group intrauterine device 6 (24%), pills 4 (16%), and 15 (60%) did not use any contraceptive.

Other symptoms are reported, including itching, in 139 (59.9%), 13 (9.35%) are infected with the parasite, and 13 (5.6%) of the total number of the women are infected. In comparison, the number and IR 7(5%) of all women examined did not suffer and 7 (3%) within the total infected women did not suffer from itching. The highest IR with dysuria was (10.76%) of the total number of women examined. Fourteen women out of 130 (56.03%) suffered, and 14 (6%) percentage of the total number of infected women. In comparison, 6 (2.6%) of the infected women did not suffer from dysuria.

The presence of malodor in 149 (64.22%) of the examined women, the highest IR was 13 (8.72%) of the total number of concerned women suffering from malodor, and 13 (5.6%) percentage of the total number of infected women with malodor. It shows that the symptoms of itching, dysuria, and malodor are high among infected women by 65%, 70% and 65%, respectively. The results of this study agree with what was recorded by Al-Ibrahimi,(2008), Dahab *et al*. (2012), Patil *et al*. (2012), Al-Badri and Al-Tikriti (2012), where the highest percentage of clinical symptoms was vaginal secretion with 58.4%, 15.5%, 18% and 100%, respectively. The time when the natural secretions are few and also these secretions are thicker than usual or watery.

The findings of Al-Badri and Al-Tikriti (2013) agreed that the percentage of women infected with Trichomonas, accompanied by symptoms of itching, burning and malodor, was 83.87%, 54.84% and 90.32%, respectively.

Suleiman (2008) pointed out to a clear relationship between *T.vaginalis* parasite and itching in women who suffer from vaginal secretions, which was 4.8%, while the lowest infection rate was when there was no vaginal itching which was 1.4%. The reason for the appearance of symptoms of irritation and itching is attributed to rotational and rapid movement of the parasite and the movement of its flagella and tapered caudal spina, non-significant differences among vaginal discharge, vaginal itching, dysuria and malodor with trichomoniasis, respectively (p=0.632, p=0.627, p=0.188 p=0.940).

The current study showed that the percentage of female patients suffering from dyspareunia was 138 (59.48%). The infected women suffering from dyspareunia was 11 (7.97%) of the total number suffering women and 11 (4.74%) of the all infected women. In contrast, the results showed that the percentage of female patients of the total number of female subjects who did not suffer from dyspareunia, 94 (40.51%) and 9 (3.88%) of all infected women, and this shows a slight increase in the problem of dyspareunia when infected with *T.vaginalis*, to the age groups in the current study in which the highest infection rate was recorded (42-48) and (49-56), as it may be due to the decrease of sexual activity in this group or the hormonal changes occurring in this age stage may affect as a result of the reduction in estrogen and its implications, there are non-significant differences (p=0.669).

In the current study, it was found that the percentage of infected women who do not use any type of contraceptive is 15 (75%) of the total infected with the parasite 20 and 9.2% of the total number of examined women who did not use contraceptives, which is a much higher percentage than the number of women infected with the parasite and who used contraceptives, which was 5 (25%) of the total number of 20 infected women, and 7.4% of the total number of women examined (study group who used contraceptives), and since the highest number of infections with the parasite It appeared in the current study in the two age groups (21-27) and (28-34) with the number 8 and 6, respectively which were 70% of the total number of infected with the parasite within these two groups which is the productive stage, and those who were suffering from delayed pregnancy, this may explain the fact that there is a great link between infection with the parasite and the occurrence of delayed pregnancy.

Relationship between demographical data and *Trichomonas vaginalis* in urine (n=20).

The Table (4-2) showed that from 232, the total number examined women with direct exam and general urine exam for each woman. The result showed the direct exam with true positive 20 women. In comparison, 16 positive cases result with general urine exam and four false negatives from the total infected women with wet mount direct exam test.

In the Table (4-9), according to age group, the age group (14-20) had one case positive, and one false negative, the age group (21-27) had two false-negative and six true positives, the age group (28-34) had one case with false-negative and five actual positive, age group (35-41) (42-48) (49-56) had no false-negative and 1, 2, 1 true positive, respectively. So the maximum IR

was among age group (21-27) with 6 (8.45%), and the minimum IR was among (35-41) with 1 (2.22%) and number, this agreed with Al-Quraishi (2014) found that the maximum infection percentage for female was 22.9% for ages (20-29) year while disagreed with the minimum percentage that he found which was 2.94% for ages (40-50) year.

According to the marital status, all positive cases (20) in the direct exam were within the married group, so the total positive cases were (16) and false-negative within this status. In occupation status, one employed positive cases and no false negative, while in housewife status, there were 15 positive results and four patients with a false negative.

In the same table, according to the residence, in general urine exam in urban areas, there were three false-negative and eight were true positive. In comparison, in rural areas, there was one case false-negative and eight cases true positive. This result disagreed with the finding of Al-Quraishi (2014) who found the maximum infection rate was in female with (12.16%) in rural areas, while (7.38%) in urban areas.

regarding educational status, no false-negative was reported and two true positives in illiterate level, in primary level one case was false-negative and six were true positives, in secondary level the result was two false-negative results and four true positives, no false-negative in both intermediate, bachelor and above levels with one and four true positives results, respectively. No significant relationship between *T. vaginalis* in urine and any demographic data age, occupation status, residence, and educational status with p=0.913, p=0.800, p =0.591, p =0.280, respectively.

Relationship between gynecology history and *Trichomonas vaginalis* in urine (n=20).

Table (4-10) showed the gynecology history of the study group and the relationship with trichomoniasis infection in urine. Among pregnant women, the IR among pregnant women was seven cases with positive results, with no false-negative results, while nine positive cases and four false-negative cases among non-pregnant women were reported. These results disagreed with Al-Haidari (2011) who found that all positive cases in urine were two cases among pregnant women. There is no significant relationship between *T. vaginalis* in urine and pregnant status (p= 0.249).

The Table also showed that among total infected results in urine test (16), fifteen women were positive with vaginal discharge and just one without any, while three false-negative among women with vaginal discharge and one without vaginal discharge (p=0.368) were found.

According to vaginal itching among the study group, there were three false-negative and fifteen positive results with vaginal itching; only one false-negative and the other one positive case without vaginal itching among women. In comparison, the table showed two false-negative cases and four positive cases without dysuria, while two false-negative cases and 12 positive cases with dysuria. Infected women with dyspareunia were two false-negative and nine positive cases, while two false-negative and seven positive cases were without dyspareunia among infected women (P=0.587) (P=0.549) (p=0.622), respectively were found.

Malodor appeared in three false-negative and ten positive cases, while one false-negative and six positive cases without malodor. The result of the current study showed four false-negative and eleven infected women not

using contraceptives, without false-negative results, and five positive results among infected women using contraceptives. No significance different among contraceptives used and malodor (p=0.639) (p=0.197), respectively were found. There is no significant relationship between *T. vaginalis* in urine and any gynecologic history.

The difference in the number of infected women between the two urine samples and wet swab samples may be due to various reasons, including the parasite may not actually be present in the genitourinary tract and this usually occurs in recent infections, or the parasite may be present but it was not detected by direct examination of the urine because the sample was given in a way Randomness by the patient and the failure to apply the correct typical method for taking the sample by him, and also how long is the period between taking the sample and conducting the direct examination, is the sample fresh or not informative, the accuracy of the examiner for diagnosis and other reasons that may give a false negative result.

Microbiological Study

Distribution of Pathogenic Microbial Associated with Trichomoniasis in Women (positive swab) and Control Group

The results of the current study showed that among the total study group of 232 women, the swab sample cultured in appropriate media and then diagnosis with Vitek2 system result in several pathogenic and non-pathogenic microbial, including *Candida* spp, *E.coli*, *Kocuria kristinae*, *Klebsiella pneumonia* ssp *pneumonia*, *Staphylococcus hominis* ssp *hominis*, *Enterobacter aerogenes*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Lactococcus garvieae*, *Staphylococcus haemolyticus*, *Salmonella* group *Enterobacter cloacae complex* and *Streptococcus uberis*.

The percentage of infected with *Candida* spp. was 16 (6.9%), *Kocuria kristinae* 67 (28.87%), *Salmonella* group 16 (6.9%), *Enterobacter cloacae complex* 39 (16.81%), *Streptococcus uberi* 27 (11.63%), *E.coli* 13 (5.6%), *Klebsiella pneumonia* ssp *pneumonia* 5 (2.15%), *Staphylococcus hominis* ssp *hominis* 1(0.43%), *Enterobacter aerogenes* 1 (0.43%), *Enterococcus faecalis* 3(1.3%), *Staphylococcus aureus* 1 (0.43%), *Lactococcus garvieae* 1(0.43%) and *Staphylococcus haemolyticus* 1 (0.43%).

Mixed infection of *T. vaginalis* with other pathogenic microbial, *E.coli* and *T. vaginalis* 4 (20%) (p= 0.001), *Klebsiella pneumonia* ssp *pneumonia* and *T. vaginalis* 5 (25%, 2.15%) (p=0.001), *Staphylococcus hominis* ssp *hominis* 1 (5%, 0.43%) (p=0.086), *Enterobacter aerogenes*1 (5%, 0.43%) (p=0.318), *Enterococcus faecalis* 3 (15%, 1.3%) (p=0.001), *Staphylococcus aureus* 1 (5%, 0.43%) (p=0.086), *Staphylococcus haemolyticus* 1(5%, 0.43%) (p=0.086), *Candida* spp. and *T.vaginalis* 1 (5%, 0.43%) (p= 0.598) percentages within the infected women with the parasite and the total examined women, respectively.

Mixed infection of *T. vaginalis* with other non-pathogenic microbial, with *Kocuria kristinae* 3 (15%) (p=0.083), percentages within the infected women with the parasite and the total examined women, respectively.

It was found that the highest percentage of association of pathogenic microorganisms with *T. vaginalis* was *Klebsiella pneumonia* ssp *pneumonia* by 25%, followed by *E.coli* infection 4 (20%), then *Enterococcus faecalis* 3 (15%), *Staphylococcus hominis* ssp *hominis*, *Enterobacter aerogenes*, *Staphylococcus aureus* and *Candida* spp with lowest percentage 1 (5%) each.

The infection with *Klebsiella pneumonia* ssp *pneumonia* by 25% associated with the parasite, while no bacterial infection with this bacteria

among parasite non-infected women as well as among the control group. This may give a great indication that there is a certain correlation between the presence of these types of pathogenic bacteria and parasites, as one of them may be qualified for the presence of the other. The rate of infection with the fungi accompanying the parasite reached 0.43%, which is lower than the percentage of non-infected with the parasite (5.17%). This is consistent with what was found by study Al-Maqdadi (2010) which found that *T. vaginalis* is associated with *Candida* by 14.8%, which is much less than the non-parasite group (27%). This may be due to the possibility of secretion of inhibitory substances from yeasts that inhibit the growth of the parasite. There is significant relationship between *T. vaginalis* infected with *Klebsiella pneumonia* ssp *pneumonia* (p=0.001), while no significant relationship between *T. vaginalis* infected and *Candida* spp. (p=0.598).

As for the other pathological microorganisms, the percentage of their presence in the group infected with the parasite, despite the few percentages that appeared for each type. These types did not appear in women who were not infected with the parasite as well as, did not appear in control group. Therefore, it was found from these results that *T. vaginalis* parasite is responsible for the change in the vaginal normal flora (Al-Marsomy, 2015), or that the intestinal bacteria appeared to accompany the parasite, as the two infections resulted from a lack of awareness and health care that led to infection with the parasite and the transmission of intestinal bacteria to the vagina as a secondary infection.

Occurrence of WBC in patient and control group in urine.

In Table (4-12) distribution of WBC in patient and control group, which shows the only 46 (19.8%) showed high rate of WBCs in urine, while

no one have high rate within control group. There are a significant differences between the values among study group and control group (p=0.011).

From the Table (4-12) and figure (4-4), it became clear that the mean value of WBCs in urine in the study group is higher than that in the control group, and that the mean value of WBCs in urine is higher than that of non-parasite infected women, despite the presence of bacterial infection alone, and here it may be an indication of a clear and significant increase in the values of WBCs in infected with both parasites and bacteria are mainly due to parasitic infection

In the Table (4-12), it is clear that the women examined were women who were not infected with the parasite, whose percentage was 212 (91.18 %) more than half of the number 119. The percentage of white blood cells they had in the urine test was normal (0-3 p/HPF), and less than a third of the number 64-67 had a percentage of (4-30 p/HPF) white blood cells, and a small percentage of them had a high white blood cell count (>30 p/HPF). The percentages were normal or low for the number of white blood cells that appeared in the control samples. These results appeared in women who were not infected with the parasite and were not infected with bacteria and fungi mostly which were associated with infection with the parasite.

While those who were infected with the parasite in the wet smear examination, whose number was 20 infected, and the parasite infection was accompanied by a bacterial or fungal infection, it was found that they had a high rate of white blood cells or an average increase in percentage. These results are consistent with Lay *et al*. (2018), who found that the percentage of

white blood cells rises in infection with the parasite associated with bacterial vaginosis infection more than the infection alone.

Comparison of mean urine WBC count in patients with positive swab *Trichomonas vaginalis*

From the two Tables (4-13) and (4-14), the results showed that the mean value for those infected with the parasite and not infected with microbial is higher than the mean value for those infected with the parasite and microbial together, despite the fact that the mean values are relatively high for them. They have a high level of more than 95 of the 5 cases that were accompanied by a parasitic and bacterial infection together and whose mean value was 79 and this is what we notice in the table for mostl the mean values for both bacterial and parasitic infections together and the parasitic infection alone. This gave a great indication that the main cause of elevated WBCs is parasitic infection. Although there is no significant differences in mean urine WBCs in patients who were positive vs. those who were negative for these pathogens, (P. value > 0.05).

Also, the negative result of the white blood cells' mean values in urine for all non-infected with the parasite and the pathogenic bacteria that were associated with it 212 reinforces what was shown by the results of the previous tables on the mean effect of the parasitic infection in raising the percentage of blood cells. no significant difference was found in the mean WBCs count between patients with positive or negative *Candida* spp. , (P. value >0.05).

From the results, we notice that most of the cases in which a bacterial infection was accompanied by a parasite infection, the percentage of white blood cells was medium (4-30 p/HPF) or high (>30 p/HPF), while the results

showed that the presence of Bacterial infection alone with these species gave different results, ranging from normal percentages (0-3 p/HPF) in many cases and less with an average percentages of (4-30 p/HPF), and a few cases of infection who had a high rate of white blood cells (>30 p/HPF).

It may be concluded from this that accompanying bacterial infection with parasite infection increases the immune response and thus an increase in the number of white blood cells than if the infection was one of them alone, and this is consistent with what he found Ley *et al*. (2018) that the immune response to *T. vaginalis* has been investigated most extensively in pregnant women when compared to bacterial vaginosis alone, *T. vaginalis* positive women who are pregnant with bacterial vaginosis (Nugent 7–10) exhibited more vaginal IL-1 and neutrophils.

T. vaginalis reduced colonisation by *Lactobacillus* but not by BV species, which were found inside epithelial cells. *T. vaginalis* increased interleukin (IL)-8 and suppressed secretory leucocyte protease inhibitor (SLPI), which is similar to Fichorova *et al*. (2013).

Chapter Six

Conclusion and Recommendations

Conclusions

1- The prevalence of infection with Trichomoniasis was relatively low in Holy Karbala governorate.

2- By linking the marital status, age groups, use of contraceptives and the parasite infection rate within the study group, we conclude the reality of the effect of parasite infection on the occurrence of delayed childbearing.

3- The pathogenic infections associated with trichomoniasis are not present individually in the control group and in the non-infected-parasite women. This may be due to the fact that the parasite creates the appropriate conditions for infection with other pathogens, or vice versa.

4- The infection with *T. vaginalis* resulted in an increase of WBC as a marker of the important defence system.

Recommendation:

1- Farther comprehensive studies, taking in considering more sample size, more area for study, and more longer duration.

2- Studying the effect of pathogenic microbes that appear with parasite infection, separately for each one of them, to find out the relationship leading to their association, whether the effect was parasitic, microbial, or both.

3- Many studies may be carried on the interaction between immunity system responses and infection with *T. vaginalis*.

4- Study some of *T. vaginalis* virulence factors and its affect.

5- Study of the relationship between *Candida* inhibitory factors and *T. vaginals* growth

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Appendices

Appendix A

Date of Visit: _____/_____/_____

Trichomoniasis/ Questionnaire

Demographic data:

Age ☐

Marital status: Single ☐ Married ☐ divorced ☐ Widow ☐

Occupation status: Employed ☐ Housewife ☐

Residence: Urban ☐ ; Rural ☐

Educational status: Illiterate ☐ Primary ☐ Secondary ☐

Intermediate ☐ Bachelor and above ☐

Gestational age: Up to 36 weeks ☐ More than 36 weeks ☐

Treatment: Yes ☐ No ☐

Previous STIs: Yes ☐ No ☐

Antenatal care: Yes ☐ No ☐

Vaginal discharge: Yes ☐ No ☐

Vaginal itching: Yes ☐ No ☐

Dysuria: Yes ☐ No ☐

Dyspareunia: Yes ☐ No ☐

Contraceptives used before pregnancy: Yes ☐ No ☐

Pills ☐ Depo-Provera ☐ Norplant ☐ Loop ☐

Appendix B

موعد الزيارة_____/_____/_____ :

داء المشعرات / استبيان

البيانات الديموغرافية:

العمر ☐

الحالة الاجتماعية: عزباء ☐ متزوجة ☐ أرملة ☐ مطلقة ☐

حالة المهنة: موظف ☐ ربة بيت ☐

الإقامة: حضري ☐ ؛ قروي ☐

الحالة التعليمية: أمي ☐ ابتدائي ☐ ثانوي ☐ متوسط ☐ بكالوريوس فأكثر ☐

فترة الحمل: حتى 36 أسبوعًا ☐ أكثر من 36 أسبوعًا ☐

رعاية ما قبل الولادة: نعم ☐ لا ☐

إفرازات مهبلية: نعم ☐ لا ☐

حكة المهبل: نعم ☐ لا ☐

عسر البول: نعم ☐ لا ☐

عسر الجماع: نعم ☐ لا ☐

موانع الحمل المستخدمة قبل الحمل: نعم ☐ لا ☐

حبوب منع الحمل ☐ لولب ☐ حقن منع الحمل ☐ شريحة ☐

Appendix C

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

Ministry of Higher Education
and Scientific Research
University of Babylon
Faculty of Graduate Studies

جامعة بابل
كلية الدراسات العليا
شؤون الطلبة /العلوم الطبيعية

No:
Date:

العدد : ٢٦٧٩
التاريخ : ٢٠/١٠/١٤

الصادر الى/ دائرة صحة كربلاء

مدينة الامام الحسين الطبية ـ مستشفى النسائية والتوليد ـ مستشفى الهندية العام

م/ تسهيل مهمة

تحية طيبة:-

يرجى تفضلكم بتسهيل مهمة طالبة الدراسات العليا (الماجستير) (زينب عبد الحسن فاضل ابراهيم)في قسم(علوم الحياة) كلية العلوم بجامعتنا والمقبولة للعام الدراسي (٢٠١٩-٢٠٢٠) (على قناة النفقة الخاصة) لغرض اكمال متطلبات بحثـــها .

..مع الاحترام..

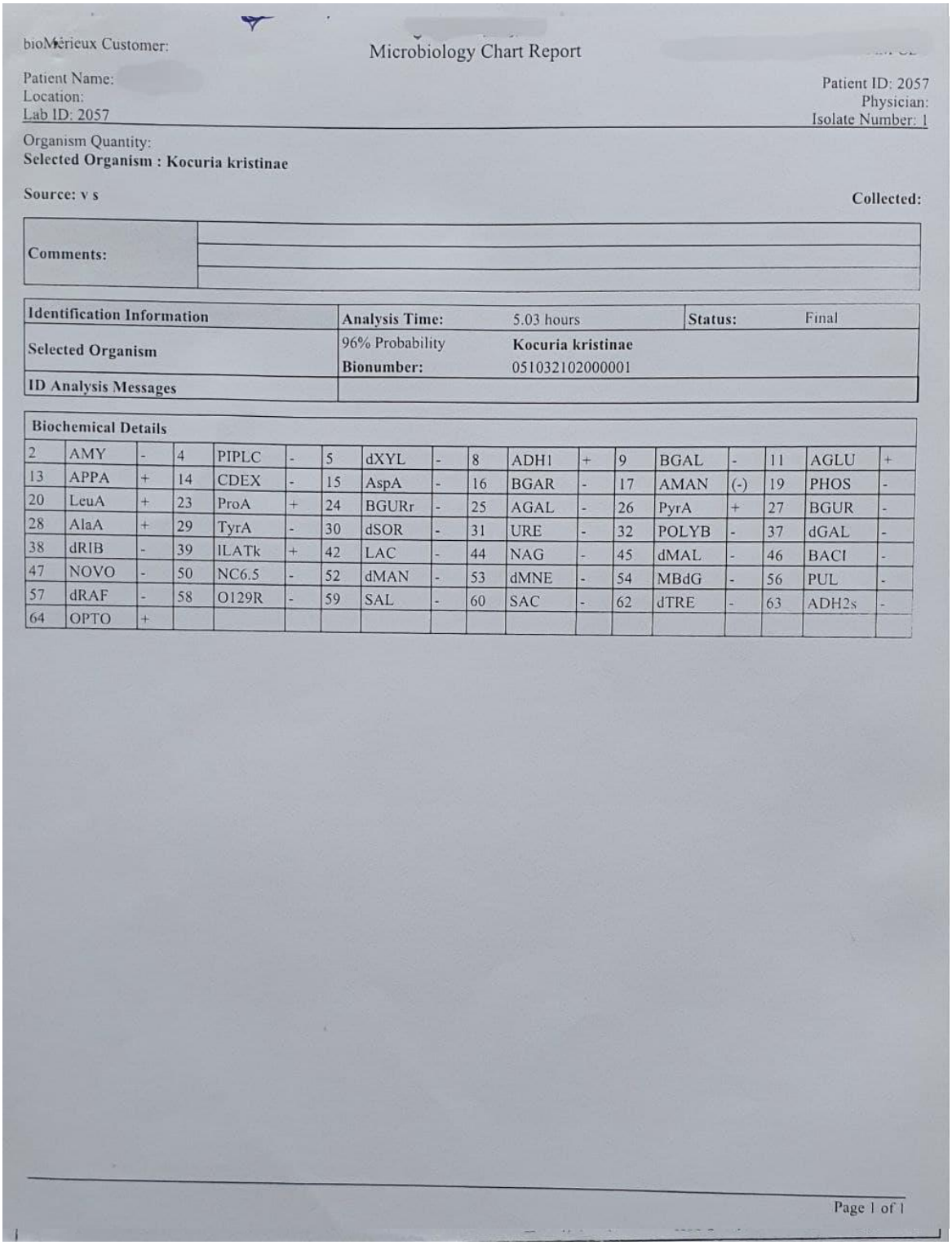
الاستاذ الدكتور
سعد مرزة حسين الاعرجي
معاون عميد كلية الدراسات العليا
٢٠٢٠/١٠/١٤

نسخة منه إلى //
- كلية العلوم ..مع الاحترام
- شعبة شؤون الطلبة / العلوم الطبيعية ..
- الصادرة .

graduatefaculty@uobabylon.edu.iq
graduatefaculty@gmail.com

سعدية

Appendix D



bioMérieux Customer:

Microbiology Chart Report

Patient Name:
Location:
Lab ID: 2057

Patient ID: 2057
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Kocuria kristinae

Source: v s

Collected:

Comments:	

Identification Information	**Analysis Time:** 5.03 hours	**Status:** Final
Selected Organism	96% Probability **Kocuria kristinae**	
	Bionumber: 051032102000001	
ID Analysis Messages		

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	+
13	APPA	+	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	(-)	19	PHOS	-
20	LeuA	+	23	ProA	+	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	+	29	TyrA	-	30	dSOR	-	31	URE	-	32	POLYB	-	37	dGAL	-
38	dRIB	-	39	ILATk	+	42	LAC	-	44	NAG	-	45	dMAL	-	46	BACI	-
47	NOVO	-	50	NC6.5	-	52	dMAN	-	53	dMNE	-	54	MBdG	-	56	PUL	-
57	dRAF	-	58	O129R	-	59	SAL	-	60	SAC	-	62	dTRE	-	63	ADH2s	-
64	OPTO	+															

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Appendix E

bioMérieux Customer:

Microbiology Chart Report

Patient Name:
Location:
Lab ID: 2044

Patient ID: 2044
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Enterobacter cloacae complex

Source: v s

Collected:

Comments:	

Identification Information	**Analysis Time:**	5.97 hours	**Status:** Final
Selected Organism	89% Probability	**Enterobacter cloacae complex**	
	Bionumber:	0627635553577411	
ID Analysis Messages			

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	+	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	+	29	TyrA	+	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-
40	lLATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	+	44	AGAL	+	45	PHOS	(+)
46	GlyA	+	47	ODC	+	48	LDC	+	53	lHISa	-	56	CMT	-	57	BGUR	+
58	O129R	+	59	GGAA	-	61	lMLTa	-	62	ELLM	+	64	lLATa	-			

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Appendix F

bioMérieux Customer:

Microbiology Chart Report

Patient Name:
Location:
Lab ID: 2047

Patient ID: 2047
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Streptococcus uberis

Source: v s

Collected:

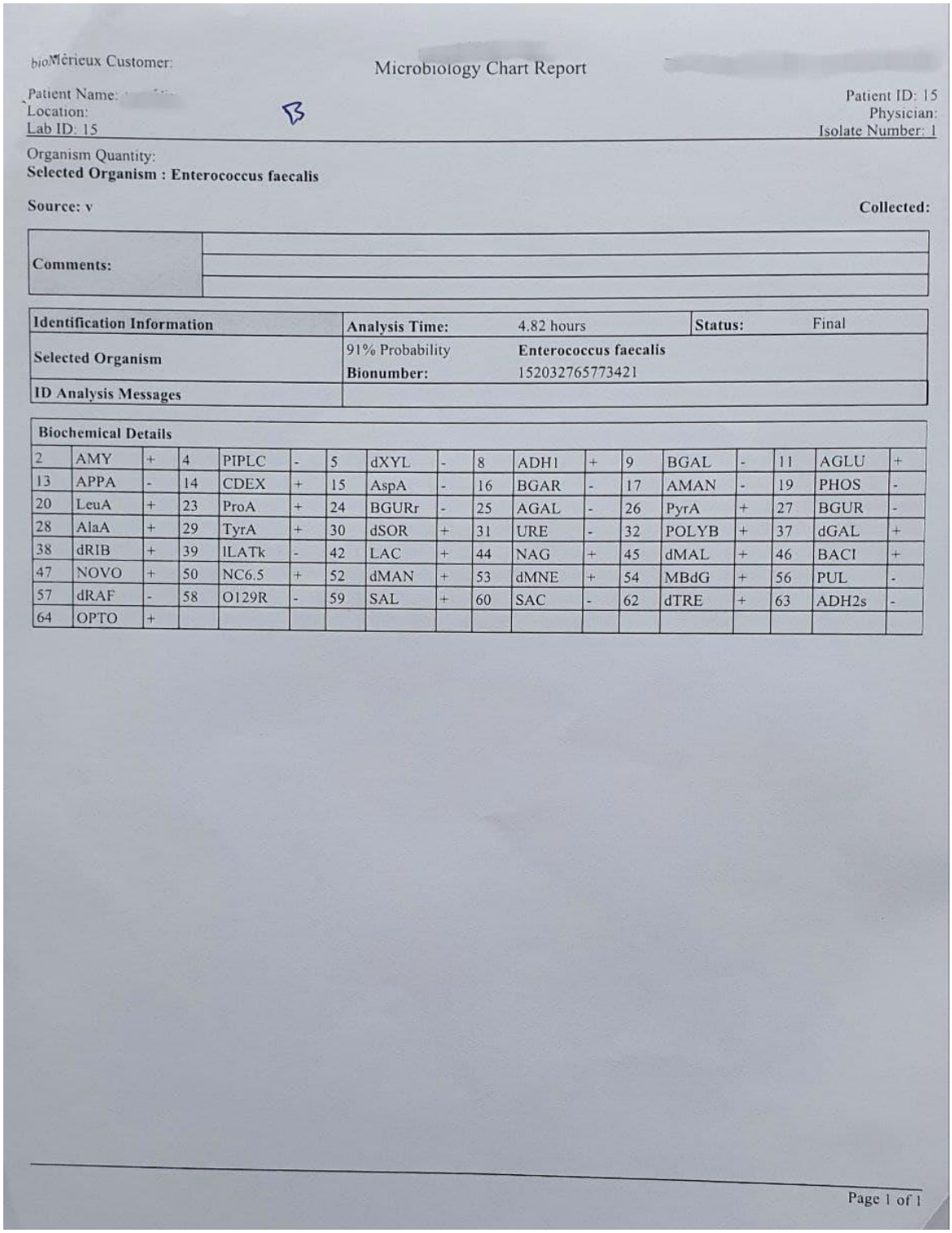
Comments:	

Identification Information	**Analysis Time:**	2.85 hours	**Status:**	Final
Selected Organism	98% Probability	**Streptococcus uberis**		
	Bionumber:	030054545551131		
ID Analysis Messages				

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	+	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	+	23	ProA	-	24	BGURr	+	25	AGAL	-	26	PyrA	-	27	BGUR	+
28	AlaA	+	29	TyrA	-	30	dSOR	+	31	URE	-	32	POLYB	-	37	dGAL	+
38	dRIB	+	39	ILATk	-	42	LAC	+	44	NAG	+	45	dMAL	-	46	BACI	+
47	NOVO	+	50	NC6.5	-	52	dMAN	+	53	dMNE	+	54	MBdG	-	56	PUL	-
57	dRAF	+	58	O129R	-	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Appendix G



bioMérieux Customer:

Microbiology Chart Report

Patient Name:
Location:
Lab ID: 15

Patient ID: 15
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Enterococcus faecalis

Source: v

Collected:

Comments:	

Identification Information	**Analysis Time:**	4.82 hours	**Status:**	Final
Selected Organism	91% Probability	**Enterococcus faecalis**		
	Bionumber:	152032765773421		
ID Analysis Messages				

Biochemical Details

2	AMY	+	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	+
13	APPA	-	14	CDEX	+	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	+	23	ProA	+	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	+	29	TyrA	+	30	dSOR	+	31	URE	-	32	POLYB	+	37	dGAL	+
38	dRIB	+	39	ILATk	-	42	LAC	+	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	+	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	-	59	SAL	+	60	SAC	-	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

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Appendix H

bioMérieux Customer:

Microbiology Chart Report

Patient Name:
Location:
Lab ID: 4

Patient ID: 4
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Lactococcus garvieae

Source: v

Collected:

Comments:	

Identification Information	**Analysis Time:**	5.22 hours	**Status:**	Final
Selected Organism	86% Probability	**Lactococcus garvieae**		
	Bionumber:	051432321762231		
ID Analysis Messages				

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	+
13	APPA	+	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	(+)
20	LeuA	+	23	ProA	+	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	+	29	TyrA	+	30	dSOR	-	31	URE	-	32	POLYB	+	37	dGAL	-
38	dRIB	+	39	ILATk	-	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	-	50	NC6.5	+	52	dMAN	+	53	dMNE	-	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

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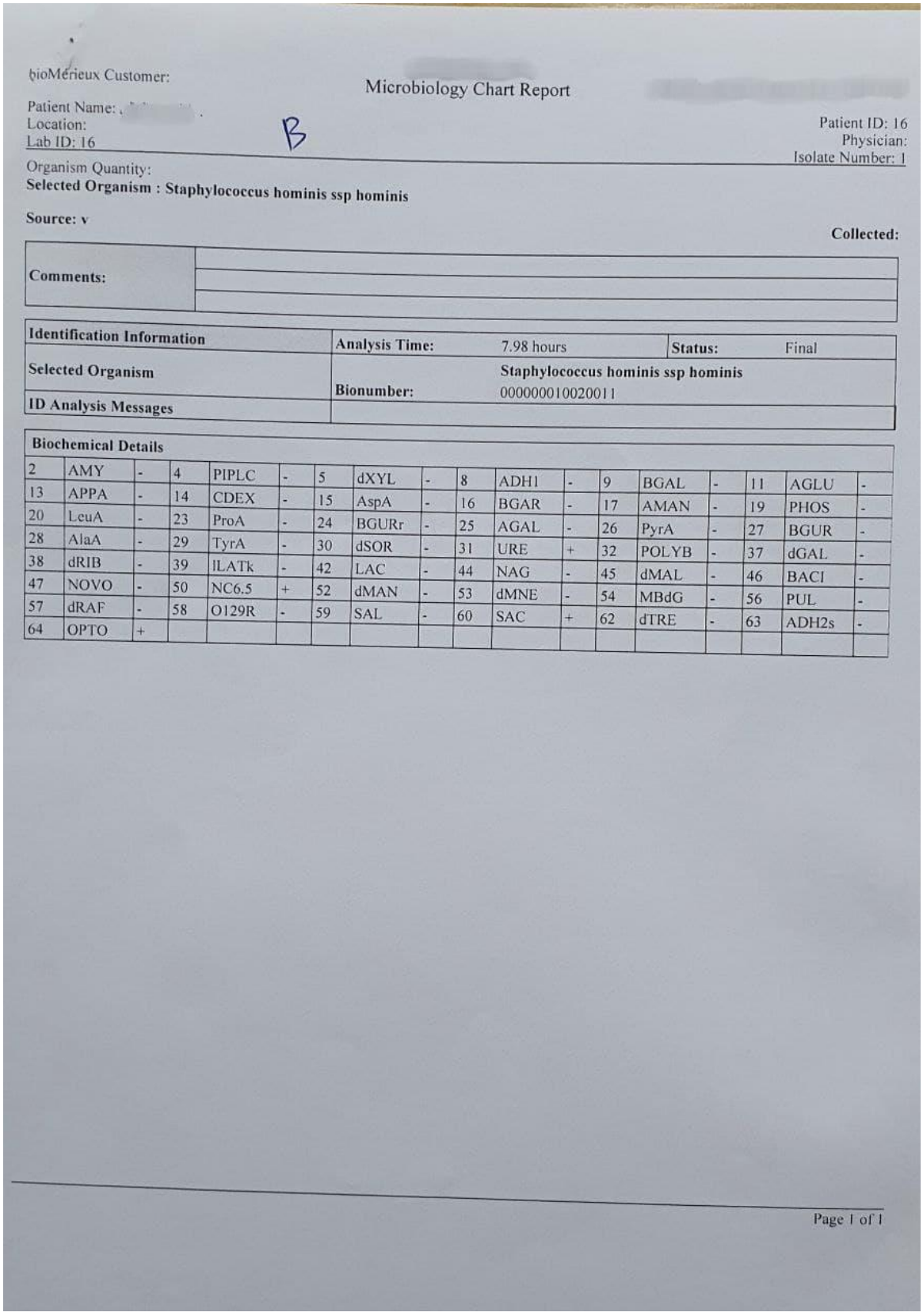
Appendix I



bioMérieux Customer:

Microbiology Chart Report

Patient Name:
Location:
Lab ID: 16

B

Patient ID: 16
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus hominis ssp hominis

Source: v

Collected:

Comments:	

Identification Information	**Analysis Time:**	7.98 hours	**Status:**	Final
Selected Organism	**Bionumber:**	**Staphylococcus hominis ssp hominis** 00000001002001 1		
ID Analysis Messages				

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	-	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	-	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	+	32	POLYB	-	37	dGAL	-
38	dRIB	-	39	ILATk	-	42	LAC	-	44	NAG	-	45	dMAL	-	46	BACI	-
47	NOVO	-	50	NC6.5	+	52	dMAN	-	53	dMNE	-	54	MBdG	-	56	PUL	-
57	dRAF	-	58	O129R	-	59	SAL	-	60	SAC	+	62	dTRE	-	63	ADH2s	-
64	OPTO	+															

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Appendix J

bioMérieux Customer:

Microbiology Chart Report

Patient Name:
Location:
Lab ID: 16

B

Patient ID: 16
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus hominis ssp hominis

Source: v

Collected:

Comments:	

Identification Information	**Analysis Time:**	7.98 hours	**Status:**	Final
Selected Organism	**Bionumber:**	**Staphylococcus hominis ssp hominis** 00000001002001 1		
ID Analysis Messages				

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	-	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	-	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	+	32	POLYB	-	37	dGAL	-
38	dRIB	-	39	ILATk	-	42	LAC	-	44	NAG	-	45	dMAL	-	46	BACI	-
47	NOVO	-	50	NC6.5	+	52	dMAN	-	53	dMNE	-	54	MBdG	-	56	PUL	-
57	dRAF	-	58	O129R	-	59	SAL	-	60	SAC	+	62	dTRE	-	63	ADH2s	-
64	OPTO	+															

Appendix K

bioMérieux Customer:

Microbiology Chart Report

Patient Name: [redacted]
Location:
Lab ID: 13

Patient ID: 13
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus haemolyticus

Source: v

Collected:

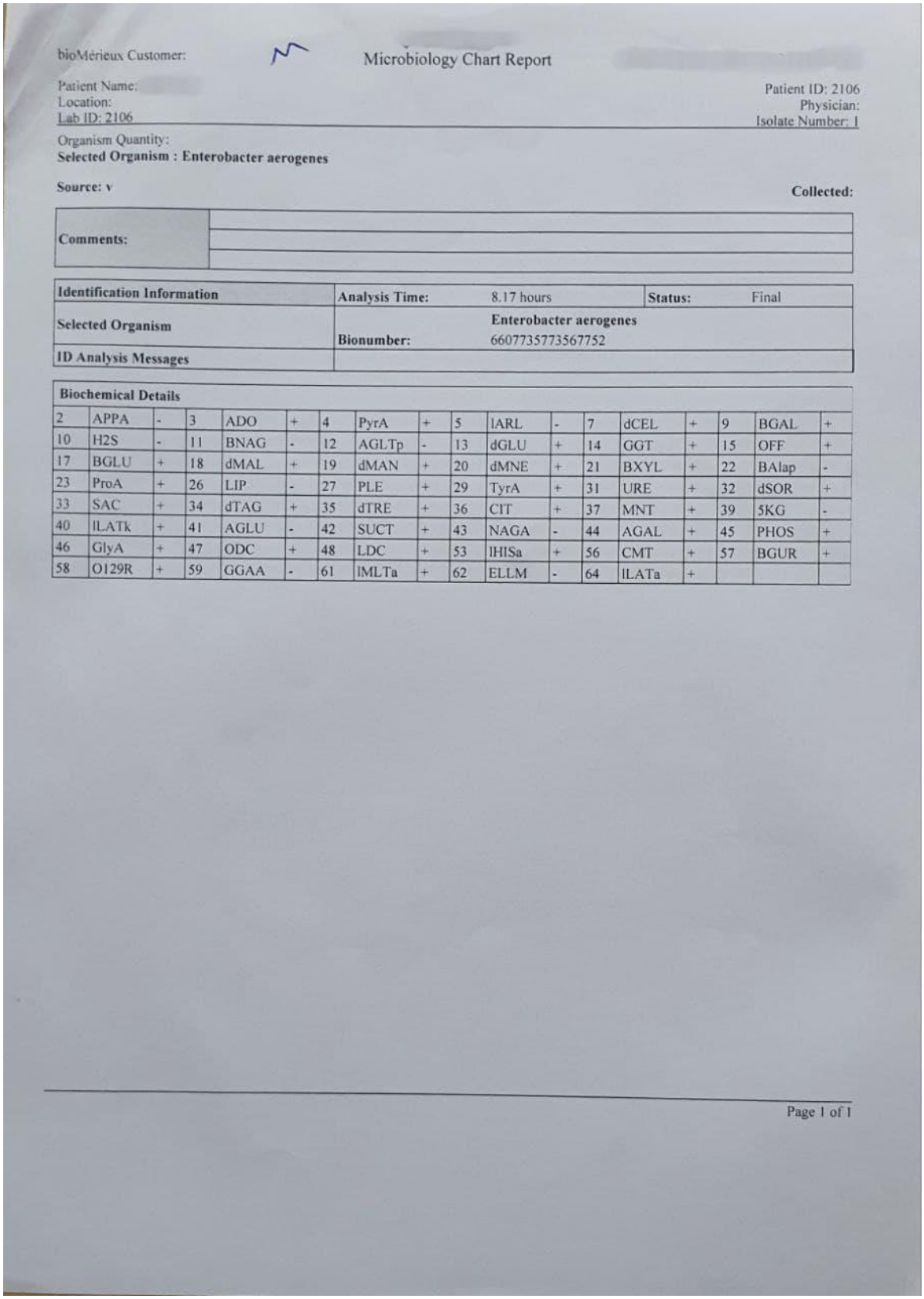
Comments:	

Identification Information	**Analysis Time:**	5.82 hours	**Status:**	Final
Selected Organism	96% Probability	**Staphylococcus haemolyticus**		
	Bionumber:	010002400760231		
ID Analysis Messages				

Biochemical Details

2	AMY	(-)	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	+	31	URE	-	32	POLYB	-	37	dGAL	-
38	dRIB	(-)	39	ILATk	-	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	-	50	NC6.5	+	52	dMAN	+	53	dMNE	-	54	MBdG	-	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Appendix L



bioMérieux Customer:

Microbiology Chart Report

Patient Name:
Location:
Lab ID: 2106

Patient ID: 2106
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Enterobacter aerogenes

Source: v

Collected:

Comments:	

Identification Information	**Analysis Time:**	8.17 hours	**Status:**	Final
Selected Organism	**Bionumber:**	**Enterobacter aerogenes** 6607735773567752		
ID Analysis Messages				

Biochemical Details

2	APPA	-	3	ADO	+	4	PyrA	+	5	lARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	+	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	+	29	TyrA	+	31	URE	+	32	dSOR	+
33	SAC	+	34	dTAG	+	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-
40	lLATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	+
46	GlyA	+	47	ODC	+	48	LDC	+	53	lHISa	+	56	CMT	+	57	BGUR	+
58	O129R	+	59	GGAA	-	61	lMLTa	+	62	ELLM	-	64	lLATa	+			

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Appendix M

bioMérieux Customer:

Microbiology Chart Report

Patient
Location:
Lab ID: 11

Patient ID: 11
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Escherichia coli

Source: v

Collected:

Comments:	

Identification Information	**Analysis Time:**	3.88 hours	**Status:**	Final
Selected Organism	99% Probability	**Escherichia coli**		
	Bionumber:	0405610450046610		
ID Analysis Messages				

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	-	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	-	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	lLATk	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	+
46	GlyA	-	47	ODC	+	48	LDC	+	53	lHISa	-	56	CMT	+	57	BGUR	+
58	O129R	+	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	-			

Appendix N

bioMérieux Customer:

Microbiology Chart Report

Patient Name:
Location:
Lab ID: 11

Patient ID: 11
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Escherichia coli

Source: v

Collected:

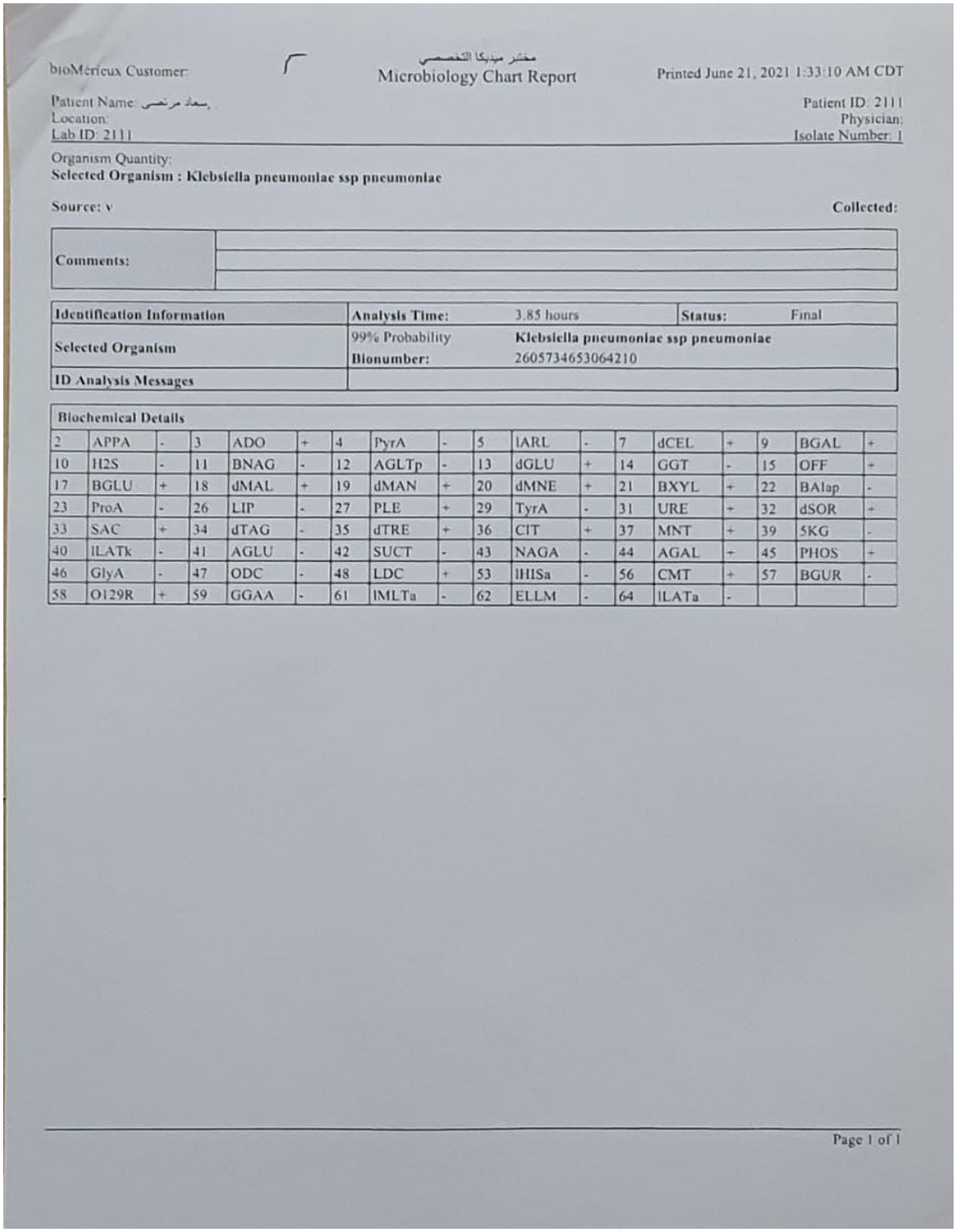
Comments:	

Identification Information	**Analysis Time:**	3.88 hours	**Status:**	Final
Selected Organism	99% Probability	**Escherichia coli**		
	Bionumber:	0405610450046610		
ID Analysis Messages				

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	-	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	-	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	lLATk	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	+
46	GlyA	-	47	ODC	+	48	LDC	+	53	lHISa	-	56	CMT	+	57	BGUR	+
58	O129R	+	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	-			

Appendix O



bioMérieux Customer: مختبر مديكا التخصصي

Microbiology Chart Report

Printed June 21, 2021 1:33:10 AM CDT

Patient Name: سعاد مرتضى
Location:
Lab ID: 2111

Patient ID: 2111
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Klebsiella pneumoniae ssp pneumoniae

Source: v **Collected:**

Comments:	

Identification Information	**Analysis Time:** 3.85 hours	**Status:** Final
Selected Organism	99% Probability **Bionumber:**	**Klebsiella pneumoniae ssp pneumoniae** 2605734653064210
ID Analysis Messages		

Biochemical Details

2	APPA	-	3	ADO	+	4	PyrA	-	5	lARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	+	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	+	29	TyrA	-	31	URE	+	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-
40	lLATk	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	+	45	PHOS	+
46	GlyA	-	47	ODC	-	48	LDC	+	53	lHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	-			

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الخلاصة

تهدف الدراسة الحالية إلى الكشف عن علاقة الإصابة بـالمشعرات المهبلية *T. vaginalis* بأنواع أخرى من مسببات الأمراض البكتيرية والفطرية في الإفرازات المهبلية وعينات الادرار ، وكذلك إيجاد العلاقة بين العوامل الديموغرافية ونتائج البحث. في هذه الدراسة تم جمع مسحة مهبلية رطبة لـ 232 أنثى و عينات ادرار من نفس المرضى بالإضافة إلى 25 عينة من الإناث السليمة (مجموعة السيطرة) بأعمار مختلفة تراوحت بين (14-58) سنة الذين حضروا المستشفيات والعيادات الطبية من الاقضية الاربعة لمحافظة كربلاء من الفترة 1 تشرين الثاني 2020 حتى 5 حزيران 2021.

أظهرت نتائج التحضير الرطب وصبغة الجرام المباشرة و فحص الادرار العام أن أعلى معدل إصابة كان 20 (8.62٪) بالمشعرات المهبلية، أظهرت نتائج التحضير الرطب المباشر ومسحة الجرام المباشرة والفحص العام للادرار أن أعلى معدل إصابة كان 20 (8.62٪) مع *T. vaginalis* في الرطب المباشر وصبغة الجرام نفس العدد والنسبة المئوية للاختبارين بينما في فحص الادرار العام كان العدد والنسبة 16 (6.89٪). أظهرت النتائج وجود فارق معنوي بين مجموعة المرضى ومجموعة السيطرة بخصوص *T. vaginalis* في المسحة والادرار (P <0.001).

بالإضافة إلى وجود بعض الكائنات الحية الدقيقة التي ظهرت في مزرعة المسحة المصاحبة للإصابة بالطفيلي على MacConky وأجار الدم تم التعرف عليها بنظام Vitik 2. ترتبط هذه العدوى بالطفيليات ، حيث كان أعلى معدل للعدوى المشتركة هو الالتهاب *الرئوي Klebsiella* (2.15 5 *pneumonia* ssp 25٪) (٪ (، ثم الإشريكية القولونية*E.coli* 4 (20٪ ، 1.72٪) ثم15) 3 *Enterococcus faecalis* 1.3٪ (، *Staphylococcus hominis* ssp *hominis* ، *Enterobacter aerogenes* ، *Staphylococcus aureus* و *Candida* spp. بأقل نسبة 1 (5٪ ، 0.43٪) لكل منها.

وجد أن أعلى نسبة ارتباط بين الكائنات الحية الدقيقة مع المشعرات المهبلية كانت مع بكتريا الالتهاب الرئوي) 5 *Klebsiella* 2.15% 25٪) وعلى الرغم من قلة عدد النساء المصابات ببكتريا الالتهاب الرئوي *Klebsiella* ، كانت جميع الحالات المصابة بالالتهاب الرئوي .*Klebsiella* مصاحبة للعدوى بالطفيلي فقد أظهرت النتائج وجود علاقة معنوية بين *T. vaginalis* و *Klebsiella pneumonia* و *Escherichia coli* و *Enterococcus faecalis* (P <0.001، كما كانت هناك معدلات إصابة منخفضة للغاية للأنواع البكتيرية الأخرى 1 (5٪ ، 0.43٪) التي

ارتبطت بالعدوى بالطفيلي ولم تتواجد مع الأنواع الأخرى في النساء غير المصابة بالطفيلي كما كانت هناك معدلات إصابة مشتركة منخفضة جدًا لأنواع بكتيرية أخرى.

أظهرت نتائج الدراسة أن أعلى عدد ونسبة إصابة كانت 8 (11.3٪) على التوالي في الفئة العمرية (21-27) وأدناها في الفئة العمرية (35-41) كانت 1 (2.2٪) على التوالي. كانت جميع المصابات متزوجات وبنسبة 20 (8.8٪) عدد ونسبة. أعلى عدد ونسبة إصابة كانت الإناث في المرحلة الثانوية تحصيل دراسي بنسبة 6 (14.6٪) ، بينما أدنى نسبة إصابة 2 (5.1٪) بين الأميات. كان لدى النساء المصابات الحوامل معدل إصابة أعلى من غير الحوامل و بنسبة 7 (11.5٪) عدد ونسبة مئوية على التوالي. كانت النساء المصابات بالحكة المهبلية أعلى من النساء المصابات بدون حكة مهبلية بنسبة 13 (9.4٪) ، والنساء المصابات اللواتي يعانين من عسر الادرار أعلى من النساء المصابات بدون 14 (10.8٪).

وفقًا لعدد WBC في عينة الادرار باستخدام التحليل الآلي ، أظهرت النتائج قيمة كبيرة ($P < 0.011$) من WBC في مجموعة المرضى ومجموعة السيطرة. أظهرت النتائج أن متوسط مجموعة المرضى (20.7) ، والمتوسط للمصابين (212) الذين لديهم اصابة بالميكروبات غير الطفيليّة على حده كان 14 ، في حين كان المتوسط لدى ال20 امرأة المصابة بالعدوى المرافقة للطفيلي 92 ، مما اوضح أن أعلى ارتفاع. عدد WBC بين النساء المصابات بالعدوى المصاحبة.

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انتشار طفيلي المشعرات المهبلية *Trichomonas vaginalis* والإصابات البكتيرية المصاحبة في نساء محافظة كربلاء المقدسة

رسالة مقدمة الى
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