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INVESTIGATION OF CYTOKINES CHANGES ASSOCIATED WITH GENOTYPE OF *ESCHERICHIA COLI* STRAINS AND *CYTOMEGALOVIRUS* ISOLATED FROM IN WOMEN WITH VAGINITIS IN MISAN PROVINCE, IRAQ

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August 2023

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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ABSTRACT

INVESTIGATION OF CYTOKINES CHANGES ASSOCIATED WITH GENOTYPE OF *ESCHERICHIA COLI* STRAINS AND *CYTOMEGALOVIRUS* ISOLATED FROM IN WOMEN WITH VAGINITIS IN MISAN PROVINCE, IRAQ

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Master of Science in Biology

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August 2023

The investigation of cytokine changes associated with the genotype of *Escherichia coli* (*E. coli*) strains and *Cytomegalovirus* (CMV) isolated from women with vaginitis in Misan Province, Iraq, represents a critical exploration into the intricate interplay of microorganisms and the immune response in the context of vaginal infections. Vaginitis, a common gynecological condition, can be caused by a variety of pathogens, including *E. coli* and CMV. Understanding how specific genotypes of *E. coli* and strains of CMV impact the cytokine profiles of affected individuals is pivotal in advancing our knowledge of the immunological dynamics in vaginal infections. The study aims to detect *E. coli* using biochemical tests and molecular techniques. Identification of virulence genes of *E. coli*. Immunological detection of *Cytomegalovirus* IgM and IgG. Cytokine changes which associated with the virulence genes of *E.coli* in vaginitis women. The study was carried out in the obstetrics and gynecology department of AL-Sadder Teaching Hospital in Maysan governorate and under the supervision of competent staff during the period March 2022–August 2022. the number of samples were collected 200 samples divided into high vaginal swabs (125) and serum (75%). All study samples were taken from women with vaginitis in 15–69 years. In the study, pathogenic microorganisms in Vaginitis Women were found to had a high rate of mixed infection (72%). CMV was the second most common outcome, occurring in 53 cases (26.5%). *E. coli* was prevalent in the age groups 20–29 years (30%) and 30-39 years (30%), while *Cytomegalovirus* had a high rate in the 20-29 years age group (32.1%). Among women aged 20-29 years, mixed infection had a high rate (43.1%), while *E. coli*

was more prevalent in the age group 20-29 years to 30-39 years (30%). In cases where abortion did not occur, mixed infection had a high rate (29.2%) compared to *Cytomegalovirus* (32.1%). However, in cases of abortion, mixed infection had a high rate (70.8%), while *E. coli* had a lower rate (64%). Abortion with *Cytomegalovirus* was recorded in 36 cases (67.9%). Regarding virulence genes in *E. coli* in Vaginitis women, PCR technology revealed that DNA was found in all *E. coli* strains, with a high percentage rate (100%). Among the four genes of *E. coli* (*16srRNA, Tra t, Vat, Fim H*), gene *16srRNA* had a high rate (100%), while gene *Vat* had a lower percentage (18%). In terms of antibiotic resistance, *E. coli* strains showed the highest resistance to Ampicillin (80%) and the lowest resistance to Meropenem, Piperacillin, Ceftazidime, Cefepime, Ertapenem, Imipenem, Meropenem, and Amikacin. Intermediate antibiotic resistance was highest for Fosfomycin (4%). Moreover, all *E. coli* strains were most sensitive to Meropenem (100%), while Ampicillin had the lowest sensitivity (18%). Regarding immunoglobulins of *Cytomegalovirus* in Vaginitis women, those who tested positive for IgM had previously contracted the virus or had it reacti*Vat*ed, while those who tested positive for IgG had recently contracted CMV. In positive cases, IgG had a high rate (100%), while IgM had a low rate (0%). In negative cases, IgM had a high rate (100%), and IgG had a low percentage (0%). The study also recorded interleukin levels for *Cytomegalovirus*, with IL-6 at 0.79730±0.245659, IL-8 at 0.69881±0.281192, IL-10 at 0.81726±0.307895, and TNF-α at 0.54758±0.284395.

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Keywords: Vaginitis, Bacterial vaginosis, *Escherichia coli*, Virulence factors of *E.coli*, *Cytomegalovirus*, Interleukins (IL-6, IL-8, TNF- α, IL-10)

ÖZET

IRAK'IN, MİSAN İLİNDEKİ VAJİNİTLİ KADINLARDAN İZOLE EDİLEN *ESHERİCHİA COLİ* SUŞLARI VE *SİTOMEGALOVİRUS* GENOTİPİ İLE İLİŞİLİ SİTOKİN DEĞİŞİKLİKLERİNİN ARAŞTIRILMASI

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Misan İlinden İkincil İnfeksiyonları Bulunan Kadınlardan İzole Edilen *Escherichia coli* (*E. coli*) Suşlarının ve *Sitomegalovirüsün* (CMV) Genotipi ile İlişkilendirilen Sitokin Değişikliklerinin Araştırılması, vajinal enfeksiyonlar bağlamında mikroorganizmaların ve bağışıklık sisteminin karmaşık etkileşimini inceleyen kritik bir araştırmayı temsil etmektedir. Vajinit, yaygın bir jinekolojik durumdur ve *E. coli* ve CMV dahil olmak üzere çeşitli patojenler tarafından tetiklenebilir. Belirli *E. coli* genotipleri ve CMV suşlarının bireylerin sitokin profillerini nasıl etkilediğini anlamak, vajinal enfeksiyonlardaki bağışıklık dinamiklerine dair bilgimizi ilerletmede önemlidir. Çalışma, *E. coli*'nin biyokimyasal testler ve moleküler teknikler kullanılarak tespit edilmesini amaçlamaktadır. *E. coli*'nin virülans genlerinin tanımlanması. Sitomegalovirüs IgM ve IgG'nin immunolojik tespiti. *E.coli*'nin vajinitisli kadınlardaki virülans genleri ile ilişkilendirilen sitokin değişiklikleri. Çalışma, Maysan Valiliği'nde bulunan AL-Sadder Öğretim Hastanesi'nin kadın hastalıkları ve doğum bölümünde ve yetkili personelin gözetiminde gerçekleştirildi ve Mart 2022-Ağustos 2022 dönemini kapsamaktadır. Toplam 200 örnek toplandı ve bunlar yüksek vajinal sürüntüler (125) ve serum (75%) olarak bölümlere ayrıldı. Tüm çalışma örnekleri, 15-69 yaş arasındaki vajinitli kadınlardan alınmıştır. Çalışmada, Vajinitis hastalarındaki patojen mikroorganizmaların yüksek oranda karışık enfeksiyon (%72) gösterdiği bulunmuştur. CMV ikinci en yaygın sonuç olarak, 53 vakada (%26.5) görülmüştür. *E. coli*, yaş grupları 20-29 yaş (%30) ve 30-39 yaş (%30) arasında yaygındı, ancak

sitomegalovirusun en yüksek orana sahip olduğu yaş grubu 20-29 yaş (%32.1) idi. 20-29 yaşındaki kadınlarda karışık enfeksiyon yüksek bir orana sahipti (%43.1), *E. coli* ise 20-29 yaş ile 30-39 yaş arası yaş gruplarında daha yaygındı (%30). Düşük yapmayan vakalarda karışık enfeksiyonun yüksek bir oranı vardı (%29.2) ve sitomegalovirusa göre (%32.1) daha yüksekti. Bununla birlikte, düşük yapma durumlarında karışık enfeksiyonun yüksek bir oranı vardı (%70.8), *E. coli* ise daha düşük bir orana sahipti (%64). Sitomegaloviruslu düşük 36 vakada (%67.9) kaydedilmiştir. Vajinitisli kadınlarda *E. coli*'deki virülens genlerine gelince, PCR teknolojisi DNA'nın tüm *E. coli* suşlarında bulunduğunu ortaya koymuştur ve yüksek bir yüzde oranına sahiptir (%100). *E. coli*'un dört geni (*16srRNA, Tra t, Vat, Fim H*) arasında, *16srRNA* geni yüksek bir orana sahipti (%100), *Vat* geni ise daha düşük bir yüzdeye sahipti (%18). Antibiyotik direnci açısından, *E. coli* suşları Ampisilin'e en yüksek dirence (%80) sahipti ve en düşük dirence ise Meropenem, Piperacillin, Ceftazidime, Cefepime, Ertapenem, Imipenem, Meropenem ve Amikasin'e sahipti. Orta düzeyde antibiyotik direnci en yüksek Fosfomisin için (%4) kaydedilmiştir. Ayrıca, tüm *E. coli* suşları en duyarlı olduğu antibiyotik olarak Meropenem'e (%100) sahipti, Ampisilin ise en düşük duyarlılığa (%18) sahipti.Vajinitisli kadınların *Cytomegalovirus*'un immünoglobülinleri açısından, IgM pozitif olanlar virüsü daha önce kapmış veya yeniden aktive etmiş olabilirken, IgG pozitif olanlar son zamanlarda CMV ile enfekte olmuş olabilirler. Pozitif vakalarda IgG yüksek bir orana sahipti (%100), IgM ise düşük bir orana sahipti (%0). Negatif vakalarda ise IgM yüksek bir orana sahipti (%100), ve IgG düşük bir yüzdeye sahipti (%0). Ayrıca, çalışma *Cytomegalovirus* için interleukin seviyelerini kaydetmiştir; IL-6 için 0.79730±0.245659, IL-8 için 0.69881±0.281192, IL-10 için 0.81726±0.307895 ve TNF-α için 0.54758±0.284395 olarak saptanmıştır.

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Anahtar Kelimeler: Vajinit, Bakteriyel vajinoz, *Escherichia coli*, *E coli*'nin virülans faktörleri, *Sitomegalovirüs*, İnterlökinler (IL-6, IL-8, TNF-α, IL-10)

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CONTENTS

LIST OF SYMBOLS

µL	Microliter
Min	Minutes
mL	Milliliter
%	Percentage
rpm	Revolutions per minute

LIST OF ABBREVIATIONS

AES	Advanced expert system
Afa	Afimbrial adhesins
AI	Avidity index
AST	Antibiotic susceptibility testing
AST-GN	Gram negative susceptibility
AV	Aerobic vaginitis
BV	Bacterial vaginosis
CDC	Centers for Disease Control and Prevention
CLSI	Central Laboratory Standards Institute
CMV	*Cytomegalovirus*
CMVM	VIDAS CMV IgM
CVEC	Cervico-vaginal *E.coli*
ddH_2O	Double distilled water (ddH_2O)
ELFA	Enzyme-linked fluorescence immunoassay
ELISA	Enzyme-linked immunosorbent assay
ESBLs	Extended Spectrum Beta-Lactamase
EtBr	Ethidium bromide
FOM	Fosfomycin
GCV	Ganciclovir
GN	Gram-Negative
HCMV	Human *Cytomegalovirus*
HIV	Human immunodeficiency virus
HSV	Herpes simplex virus
HSV-1	Herpes simplex virus-1
HVS	High Vaginal Swabs
ID-GN	Gram negative bacillus identification
IE	Immediate early
IL	Interleukin
KOH	Postassium Hydroxide
L	Late
MIC	Minimum inhibitory concentration
MTCT	Mother-to-child transmission
NK cells	Natural killer cells
OD0	Optical density
OMPs	Outer membrane proteins
PCR	Polymerase chain reaction
PID	Pelvic inflammatory disease
rRNA	Ribosomal RNA
SPATEs	Serine protease autotransporter protein

SPR®	Solid Phase Receptacle
STDs	Sexually transmitted diseases
STIs	Sexually transmitted infections
TNF-	α-Tumor necrosis factor - α
TV	Trichomonal vaginitis
UP1a	Uroplakin 1A receptor
UTIs	Urinary tract infections
Vat	Vacuolating autotransporter toxin
VEC	Vaginal *Escherichia coli*
VFs	Virulence factors

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

Vaginal inflammation, known as vaginitis, is infection by a variety from viral, bacteria and parasite and non-infectious reasons. There are three most frequent causes of infectious vaginitis are *Trichomonal vaginitis* (TV), *vulvovaginal candidiasis*, and bacterial vaginosis (BV) (Donders 2007, Workowski and Bolan 2015). There have been cases of vaginal infections that were caused by bacteria belonging to the family *Enterobacteriaceae*. And included in this type of disease. There are a wide variety of distinct strains of Gram-negative bacteria that fall under the umbrella of the Enterobacteriaceae family.*E.coli*, *Salmonella*, and other bacteria are included in this group (Ongradi 2002, Puerta and Mateos 2010). *Escherichia coli* is normal bodily, vaginal, intestinal, and other flora. Comparatively, vaginal *E.coli* is little defined. It is capable of causing a wide range of diseases, some of which manifest symptoms while others do not. Several studies have demonstrated that there are correlations between normal bacteria such as *E.coli* and certain disorders in the reproductive system and vaginal infection (Sáez-López *et al.* 2016).

Women who have BV have been shown to have significant inflammatory changes in their vaginal tracts, BV therapy reduces cervical inflammatory cytokines. CMV in the lower vaginal tract makes BV infection easier (Yudin *et al.* 2003, Mao *et al.* 2003). *Cytomegalovirus* is a complicated virus with a wide range of immunomodulating properties which cause local inflammation and vaginitis (Handsfield *et al.* 1985). Reinfection with a fresh CMV strain can infect the fetus and spread intrauterine. CMV infection is widespread in seropositive women in areas with high rates of STDs and BV. (Boppana *et al.* 2001).

1.1 Aims of the Study

Detection of *E.coli* by biochemical test and molecular technique. Identification of virulence genes of *E.coli*. Immunological detection of *Cytomegalovirus* IgM and IgG. Cytokine change which associated with virulence genes of *E.coli* in vaginitis women.

2. LITERATURE REVIEW

2.1 Vaginitis

A variety of microorganisms live in the vagina, which is a complex ecology for growth it. Is also considered a microorganisms sanctuary with a lot of nutrients (Larsen and Monif 2001, Danielsson *et al.* 2011). Host factors include age, as well as changing hormone levels (due to pregnancy, menstruation, or because of using hormone contraceptives, and during menopause), as well as sexual and hygiene habits, and other genital infections, which affect vaginal microbiome (Ma *et al.* 2012). Although some of these commensal microbiotas are opportunistic pathogens that can lead to premature birth, chronic infections, or fatal conditions for both the mother and the fetus, The vast majority of them do, in fact, maintain symbiotic connections with the people that host them (Ravel *et al.* 2011).

Lactobacillus dominates vaginal microbiota. Bacterial population fluctuations affect vaginal fluid, secretions, and odor. The proliferation of bacteria, which are already normally present in the vagina, causes vaginitis and increases excretions (Machado *et al.* 2017, Aduloju *et al.* 2019).

Vaginitis is a common vaginal infection or inflammation in clinical medicine (Yenidunya *et al.* 2012). Vaginal infections are usually responsible for abnormal secretions, in addition to some non-infectious causes, when compared to physiological normal discharge, abnormal vaginal discharge is different in consistency and color (thick, thin, frothy, yellow, gray, green, or white), and is sometimes accompanied by other symptoms including an unpleasant odor and itchiness (Donders *et al.* 2002, Syed and Braverman 2004).

If left untreated, vaginal infections can raise the risk of morbidity and can even develop to chronic inflammatory disease (PID). PID can lead to tubal infertility, pregnancy complications, reproductive problems, preterm labor, and a low birth weight if the

condition is left untreated. Infections in the vaginal tract can be treated with antibiotics. Vaginal canal infection can induce cervical dysplasia, HIV, HSV-1, and other problems (Yenidunya *et al.* 2012). Fungus, bacteria, parasites, and viruses can cause vaginal infections (Giraldo *et al.* 2012).

2.2 Bacterial Vaginosis

The most common kind of vaginal infection is called disease, which is often referred to as BV. This disorder is characterized by an excess of a range of microorganisms that generally exist within the Lactobacillus of normal vaginal flora. Examples of these microorganisms include yeast, bacteria, and fungi. (Anderson *et al.* 2004). BV is typically asymptomatic, but it can be identified by the discharge of foul secretions that are homogenous and grayish-white in color; an ele*Vat*ion in the vaginal pH of more than 4.5; and a fishy odor after menstruation or after sexual activity (Paladine and Desai 2018).

Gardner and Dukes described bacterial vaginosis in human for the first time in 1955 (Scott *et al.* 1987). There is a correlation between bacterial vaginosis, an infection of the reproductive system that can affect women of childbearing age, and adverse pregnancy outcomes. These effects include premature brith, preterm labor, post-abortion infection, early abortion, and recurrent miscarriage. Infections and other problems can cause preterm births., which can have devastating and even deadly consequences for the infants born prematurely (Africa *et al.* 2014).

The most significant and prevalent risk factor in today's society for the development of BV is the practice of having a significant number of sexual encounters with people who have the same sexual orientation. It has been postulated that this technique promotes BV by inducing a shift inside the native microbial composition as a consequence of exposure to alkali sperm. This change in native vaginal flora is thought to contribute to the development of BV, using tampons, and sharing clothes, flowers, and herbs (Vodstrcil *et al.* 2015). In contrast to the (34%) of women in monogamous relationships, and found that BV was more common in (63%) of women who had several sexual

partners. Many women experience symptoms for the first time soon after beginning sexual activity (Kent 1991). BV promotes HIV and HSV-2 vaginal shedding and viral multiplication (Castellano Filho *et al.* 2010). According to the findings of one study, there is a significant link among BV and HIV infection. If the women are HIV positive, BV will increase by 60% and HIV will increase by 40% if the women are also BV positive (Cohen *et al.* 2012).

2.3 Diagnosis of Bacterial Vaginosis

Amsel's clinical criterion and Nugent Gram smearing testing in the lab are the two gold standard criteria that have been used to identify bacterial vaginosis. Use of Amsel's Criteria in Diagnosis:

Amsel provided an explanation of the first method, which is one of two that are applied in the BV diagnostic process (Amsel *et al.* 1983). The Amsel criteria, which have been reliable for diagnosing bacterial vaginosis, are the industry standard (Egan and Lipsky 2000). A thin, uniform vaginal discharge, an pH more than 4.5, and a positive smell or taste test with 10% KOH after being added to a vaginal fluid are all indicators of chlamydia. An examination using a microscope of moist preparations of saline showed the presence of tip cells (Nugent *et al.* 1991).

Three of the four criteria indicate bacterial vaginosis, which accounts for 90% of cases. Vaginal discharge with short gram-negative or gram-variable rods Gram stain and shorter *lactobacilli* indicate bacterial vaginosis. Longer *lactobacilli* indicate bacterial vaginosis (Sobel 2000). Nugent recommends vaginal smear Gram stain scores (Nugent *et al.* 1991). Bacterial vaginosis causes 10–30% of vaginitis infections, making clue cell identification one of the most effective approaches. Bacterial vaginosis patients have vaginal discharge with clue cells (Sobel *et al.* 2011). On wet mount, more than 20% of clue cells were sensitive and specific for bacterial vaginosis (Chandeying *et al.* 1998). Clue cells in vaginal discharge predicted post-operative infection (Larsson and Platz-Christensen 1991).

2.4 Epidemiology of Bacterial Vaginosis

The causes of vaginal bacteria are unknown, which accounts for the difference in prevalence around the globe (Kenyon and Colebunders 2013). The clinical environment, sociodemographic characteristics, medical standards, gestational time, and other factors, among others, all have an impact on the proliferation of diseases in women (Trabert and Misra 2007).

Southern and eastern portions of Africa had the highest incidence of bacterial vaginosis, with a rate of (58%), according to research that aimed to assess the global epidemiology of bacterial vaginosis using data for Nugent scoring criteria to identify bacterial vaginosis patient. With a frequency of (37%), Gambia has the second-highest rate of bacterial vaginosis after Uganda (Kenyon and Colebunders 2013). The proliferators rate among South African pregnant women was reported to be (17.6%). According to research done in Iraq, BV rates ranged from (28 - 37.5 %) in both pregnant and non-pregnant women (Naji 2005).

2.5 Pathogenicity of Bacterial Vaginosis

When it comes to female patients seeking medical attention from gynecologists, infections of the vaginal tract are one of the most common reasons why they do so. It is possible for neonates to catch these diseases before or while they are being born. Despite the fact that there is a significant amount of microbial colonization of the cervicovaginal epithelium. The existence of bacteria in particular areas (endometritis or pelvic inflammatory disease), for instance, has been connected to the onset of a great deal of different illnesses (Teisala 1987). Placenta, fetal membranes, and cervical mucus all work together to create a protection for the growing baby from harmful microorganisms while the mother is pregnant. This barrier is formed during pregnancy. The development of the fetus was able to proceed normally during this time period. Introducing germs into the uterus cavity can cause miscarriage and other obstetric problems (McDonald and Chambers 2000).

Women who have BV get an increased chance of having pelvic inflammation disease, infections as just a consequence of regular gynecological treatments, or infections after surgery. This higher risk is due to the fact that BV increases the likelihood of infection, sexually transmitted diseases such as HIV, as well as significant complications during pregnancy, such as intrauterine infections and preterm deliveries (Marrazzo *et al.* 2010).

BV seems to be polymicrobial in nature, in contrast to the majority of common infectious illnesses. The intricacy and heterogeneity of BV, whose bacterial composition can vary from day to day and from one person to another, have been recently highlighted by genomic research (Gajer *et al.* 2012).

2.6 Clinical Manifestations

There is widespread consensus that pregnancy is one of the most important risk factors for BV. (Brocklehurst *et al.* 2013). In the United States, bacterial vaginosis affects one out of every three women, making it the most common sexually transmitted disease (BV) (Allsworth and Peipert 2007).

The majority of cases of irregular vaginal discharge in women of reproductive age are due to bacterial vaginosis (BV). BV affects approximately thirty percent of women of reproductive age (Yenidunya *et al.* 2012). Because of a microbial imbalance in the vaginal flora, which identified by an overgrowth of complex communities that are characterized by *Actinobacteria* , Gram-negative bacteria as well as a paucity of lactobacilli, this condition can cause vaginal infections (Srinivasan *et al.* 2010).

Bacterial vaginosis is a significant inflammatory disorder that should be treated as soon as possible because of the serious complications that can result from it, including immaturity, early birth, rupture of both the uterine membrane, and spontaneous miscarriage, in addition to the risk of obtaining sexually transmitted illnesses (Joyisa *et al.* 2019).

2.7 Characteristic of *Escherichia coli*

Within the population that was the focus of this research, an exceptionally high frequency of isolates from the family *Enterobacteriaceae* was discovered. Even in situations in which this bacterial family's genus is a natural resident of the intestinal system, the infection can still occur, it is nevertheless possible for them to be implicated in infections of the intestines, genitourinary systems, and blood, among other organs and systems (Kaushik *et al.* 2017).

The normal vaginal microflora is made up of anywhere from five to fifteen different species of bacteria, including anaerobes and aerobes, In addition to this, it has the potential to bring on a wide variety of clinical syndromes and diseases, including such vaginosis (e.g. pelvic inflammatory disease). facultative gram-negative anaerobic One of the organisms that is frequently discovered in the microbiome of females is called *Escherichia coli*., regardless of whether or not they are pregnant. There have been reports that *E.coli* has been discovered in anywhere from 9 to 28% of women who are not pregnant and anywhere from 24 to 31% of women who are expecting a child (Chow *et al.* 1986, Hillier *et al.* 1993).

Escherichia coli are Gram-negative enteric bacilli that are usually found in women's vaginal tracts. They can cause vaginal infections. These microorganisms have been linked to a number of infections that can occur in pregnant women. Some of these infections include vaginal and endocervical colonization, intra-amniotic, puerperal, and neonatal infections, as well as early and late neonatal sepsis, which can occasionally include meningitis or urinary tract infections. It is possible for maternal *E. coli* to colonize a baby and cause an early neonatal illness if the *E. coli* infects the amniotic fluid, the membranes break, or the newborn passes through the vaginal canal after brith (Soto *et al.* 2008).

2.7.1 Classification of *Escherichia coli*

Escherichia coli was been classified as the flowing (Cole *et al.* 2009):

- Kingdom: Bacteria
- Phylum: Proteobacteria
- Class: Gamma Proteobacteria
- Order: Enterobacteriales
- Family: Enterobacteriaceae
- Genus: Escherichia
- Species: *Escherichia coli* (*E.coli*)

2.7.2 Pathogenicity of *Escherichia coli*

Even while *Lactobacillus and Streptococcus* species form the majority of the vaginal flora, other bacteria such as *E.coli* is quite significant. This is despite the fact that these bacteria do not usually indicate an infection. There is a connection between *vaginal E. coli* and newborn sepsis, and vaginal *E.coli* has the capacity to trigger signs of infections (Percival-Smith *et al.* 1983). It is possible to explain why As a result of *E.coli's* opportunist capacity to cause a variety of infections of women, particularly UTI or vaginitis, it is prevalent in both healthy vaginal and those of patients who do have vaginitis. *E.coli* is found both in healthy vagina and those of people who have vaginitis. Because of this ability, *E.coli* can be the root cause of a wide variety of infections in women.. This bacteria's a contaminant in the vagina (Ahmed 2015).

Aerobic bacterial vaginitis, which has been reported to be present in up to (23%) of symptomatic women, has been connected to *E.coli*, which was found to be the causative agent. An *E.coli* infection in the vaginal canal is associated with mucosal colonization and results in a robust inflammatory response (Villaseca *et al.* 2015).

E.coli was the bacterial pathogens that had been isolated the most, and its various strains were genotyped (divided into commensals and virulent groups). In symptomatic individuals, the study discovered a greater incidence of vaginal infection due to *E.coli* (35.11%), which is higher than the rate recorded by Donders *et al.* (2017).

2.7.3 Virulence factors of *Escherichia coli*

The presence of a variety of virulence factors in these strains makes it possible for them to colonize the vaginal and endocervical tissues. It investigated the virulence characteristics of the *E.coli* that was discovered as well as the prevalence of *E.coli* in vaginal and endocervical samples taken from pregnant and nonpregnant women (Cook *et al.* 2001).

Immunosuppression is the main cause of vaginal infections (Kalia *et al.* 2019). *Escherichia coli* can develop many vaginal virulence factors due to its genetic potential (Torcia 2019). *E.coli* produces iron receptors, siderophores, toxins, fimbriae, flagella, aphymic adhesins, other immunological evasion mechanisms (Figure 2.1) (Lüthje and Brauner 2014, Klemm *et al.* 2010).

There is a correlation between the presence of a large number of virulence genes in *E. coli* isolates and their capacity to colonize and cause illnesses (Guiral *et al.* 2011). These colonizing isolates have the potential to occasionally cause complications during pregnancy or to be passed on to the infant, when they can cause neonatal infection (Watt *et al.* 2003). There is evidence to suggest that vaginal *E.coli* colonization raises the risk of premature birth as well as other complications during pregnancy (Krohn *et al.* 1997).

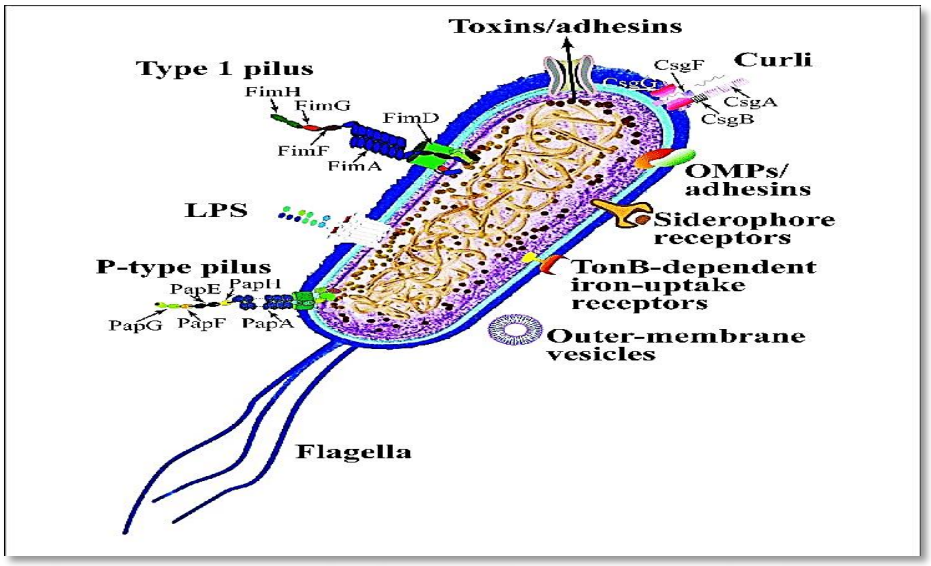

Figure 2.1 Virulence factors of *Escherichia coli* (Klemm *et al.* 2010)

2.7.4 Genetic identification diagnosis of *E.coli*

2.7.4.1 16 srRNA and virulence factors gene of *E.coli*

Clinical microbiology laboratory identified and discovered novel bacteria using 16S rRNA gene sequencing (Fattahi *et al.* 2013). Gram staining, culture, and biochemical assays have been used to identify hazardous bacteria (O'hara 2005). This is the gold standard for bacterial identification.

PCR amplification of 16S ribosomal RNA (rRNA) genes is used to detect microorganisms (Sárvári *et al.* 2018). 16S rRNA, found in all bacteria, can be used for taxonomic investigations and species identification. This gene encodes bacterial ribosome 30S subunit RNA (Metso *et al.* 2014).

The 16S ribosomal RNA (rRNA) gene, which could become the new gold standard for bacterial categorization, is highly conserved within and between species (Woo *et al.* 2000). 16S rRNA gene sequences are useful for bacterial taxonomy and phylogeny. Despite being present in almost all bacterial species and usually part of a multigene family or operon, the 16S rRNA gene has not changed over time. This suggests that

random sequence changes are a more precise measure of time, and the 1500-base-pair 16S rRNA gene is sufficient for informatics (Patel 2001).

Developments such as 16S rRNA gene sequencing, which is used for identification of the gut microbiome and the vaginal microbiome, which are extremely important for women's health, have only recently made it possible to accurately identify an individual's microbiome. This is due to the fact that the ability to correctly identify an individual's microbiome only became possible relatively recently (Gupta *et al.* 2019).

Phylogenetic study with the 16S rRNA gene sequence began in 1985. (Lane *et al.* 1985). The 16S rRNA gene sequence is the most extensively used marker gene for profiling bacterial populations because it has highly conserved regions for primer design and hyper-variable areas for phylogenetic analysis (Tringe and Hugenholtz 2008).

The information that is encoded on mRNA is converted into proteins by the bacterial ribosome, which is composed of three rRNA molecules and fifty-four proteins. The complex structural composition of the ribosome is responsible for the ribosome's ability to do this task (Schuwirth *et al.* 2005). Ribosome components have coevolved to sustain function (Jain *et al.* 1999). Since the 16S and 23S rRNAs form the structural core of the ribosome (Schuwirth *et al.* 2005).

Escherichia coli's ribosome can absorb foreign 16S rRNAs, even if they appear to be species-specific (Kitahara *et al.* 2012). Ribosome function may allow hundreds of simultaneous nucleotide changes. The lowest identity of a functioning 16S rRNA gene to *E.coli* was 80%, making this possible (Brodersen *et al.* 2002).

2.7.4.2 Trat gene in *E.coli*

One of the protectins that contribute to the formation of the virulence factors (VFs) in *E. coli* is known as the serum resistance protein (traT) (Montenegro *et al.*1985). TraT is an *E.coli* conjugative plasmid (F plasmid) genetic component that is involved in the

process of transferring the R factor. It is one of the genes that consists the tra operon (Elwell and Shipley 1980). 20,000–30,000 copies of the traT gene product are present in each cell. This protein has an outer membrane molecular weight of 23 kda and is noncovalently bound to peptidoglycan. It performs a role that is analogous to that of a number of other lipoproteins associated with peptidoglycan. It is found in the membrane in the form of multimeric aggregates, a sizeable portion of which can be observed on the exterior of the membrane that surrounds the cell (Achtman *et al.* 1977).

TraT's role in serum resistance consists in preventing the complement membrane assault complex from correctly assembling itself or inserting itself into the membrane. This is the primary function of TraT (Timmis *et al.* 1985).

The surface exclusion of *E.coli* bacteria carrying the F plasmid reduces their ability to act as recipients in conjugation with cells carrying identical or nearly related plasmids. This is one of the characteristics of the F plasmid. Because of this, when compared to a F negative cell, cells harboring the F plasmid often have a capability that is anywhere from 100 to 300 times lower to behave as recipients (Achtman *et al.* 1977). The TraT protein inhibits conjugation in a stage that comes before the establishment of stable mating aggregation (Manning *et al.* 1980).

2.7.4.3 *Vat* virulence gene

The *Vat* toxin, also known as the vacuolating autotransporter toxin, is a serine protease autotransporter protein that belongs to the family *Enterobacteriaceae* (SPATEs). The *Vat* toxin is a class II cytotoxic SPATE that is a 110 kDa secreted protein that is exported by the Type Va secretion system. It is an SPATE (Nichols *et al.*2016). It is interesting to note that both enteroaggregative and enteropathogenic *Escherichia coli* can cause harm to epithelial cells. This damage is caused by virulence factors such SPATEs (Sanchez-Villamil *et al.* 2019).

Members of the autotransporter group are found in *E.coli* and other gram-negative bacteria. These claimed virulence factors include enterotoxins, cytotoxins, mucinases, immunoglobin proteases, heme-binding proteins, and adhesions (Henderson *et al.* 2004). According to a number of articles, a single strain of *E.coli* was discovered to contain multiple autotransporters (Parham *et al.* 2004).

2.7.4.4 *Fim H* virulence gene

Approximately one 100% of *E.coli* have the fimH gene, which is a virulence gene (Obata-Yasuoka *et al.* 2002). According to Rashki (2014), fimH was found in 71.21% of the Cervico-vaginal *E.coli* samples (CVEC). Because FimH plays a critical role in interbacterial adhesion, urogenitoepithelial adhesion, and biofilm formation, one can conclude that it is significant in bacterial vaginosis. This is because the presence of FimH in an isolate makes the isolate more virulent (Melican *et al.* 2011).

Fimbriae, Afa, and membrane proteins are the three main forms of adhesins (OMPs). Most are fimbriae (Lindberg *et al.* 2008). The chaperon-usher pathway assembles and secretes Gram-negative bacteria's sticky and virulence-associated surface components. A specialized chaperone in the bacterial periplasm binds nascent pilus subunits and an integral outer membrane channel protein called the usher polymerizes them into the pilus fiber at the outer membrane (Werneburg and Thanassi 2018).

The production of surface adhesins increases the virulence of pathogenic *E.coli* by making it possible for the bacteria to come into close proximity with the cell wall of the host. There have been several modifications made to bacterial adhesins so that they can colonize different habitats (Hagan and Mobley 2007). Three more genes, namely fimF, fimG, and fimH, are responsible for regulating the sticky property as well as the longitudinal modulation. In order to enable invasion and the production of intracellular structures that are similar to biofilms, the FimH adhesin connects to the uroplakin 1A receptor (UP1a) on the surface of a fibrillum at the end of a pili rod made of FimA proteins (Rangel *et al.* 2013).

E.coli strains and other Gram-negative bacteria are able to stick to the surfaces of their host cells thanks to the unique interactions that take place between the adhesins found on bacterial fimbriae and the cell receptors found on eukaryotic cells. *E.coli* can create a wide variety of different forms of fimbriae, however type 1 fimbriae are most commonly observed on the surface of *E.coli.* Type 1 fimbriae are encoded by a fim gene cluster, which consists of nine different genes that are required for their production. The presence of the fimH gene, which directs the production of the specific adhesin found in these fimbriae, is essential (Pusz *et al.* 2014).

The following virulence-related genes were investigated by PCR in the vaginal isolates: fimH (Schubert *et al.* 1998). According to Lüthje and Brauner (2014), the genomic capabilities of *E.coli* makes it possible for the organism to create a wide array of virulence factors that cause infection. The fimH gene product plays an essential part in the early stages of an infection because it has the ability to locate receptors on the surface of the host cell that are responsible for promoting bacterial colonization (Brannon *et al.* 2020).

Despite the fact that they are classified as commensal strains, this reveals that the isolated strains possess the virulence factors that are necessary to initiate an infectious process. The pathogenic potential of the *E.coli* bacteria will also be determined by the number and virulence of the genes that are present in combination, as well as the amount and appropriate virulence of those genes. In addition, the pathogenic potential will depend on the combination of those genes (Bien *et al.* 2012).

2.7.5 Treatment of *Escherichia coli*

Since maternal *E.coli* colonization increases the risk of neonatal infection, it has been suggested that the amniotic fluid and vagina in pregnant women operate as barriers that select for *E.coli* strains that are high-risk for neonates (Watt *et al.* 2003). These colonizing isolates become resistant and serve as a reservoir for infections in the mother and newborn, decreasing their therapeutic options (Al-Mayahie 2013a).

Since this type of infection can cause chronic infection, pelvic inflammatory sickness, infertility, ectopic pregnancy, reproductive dysfunction, surgical infection, HIV acquisition, and premature birth, it is important to identify the causative agents and antibiotic resistance profile (Mulu *et al.* 2015). In underdeveloped nations with high incidence of infectious illnesses, malnutrition, and poverty, antibiotic resistance is a major public health issue (Moges *et al.* 2014). It is one of the main reasons infectious diseases like *E.coli* infections fail to be treated, which increases morbidity and death and medical expenditures (Bouza and Cercenado 2002).

The problem of *E.coli* that is resistant to many drugs is becoming more widespread over the world (EL Kholy *et al.* 2003). It is clear that there are regional differences in the prevalence and susceptibility patterns of clinical *E.coli* isolates, as well as that there are significant differences between populations, clinical samples, and environments (Erb *et al.* 2007).

2.7.5.1 Antimicrobial susceptibility

VITEK® 2 system cards (AST-P580, AST-N222, and AST-P580) and (AST-GN76). Ampicillin, Cefazolin, and Trimethoprim/Sulfamethoxazole fight *E.coli* better than other antibiotics. Gentamycin, Ciprofloxacin, Levofloxacin, Nitrofurantoin, Imipenem, Meropenem, and Ertapenem are *E.coli*-effective antibiotics (Michie *et al.* 2003).

According to the results of antimicrobial susceptibility tests carried out with the "VITEK 2" system, all of the *E.coli* isolates were found to be susceptible to the antibiotics "meropenem, cefuroxime, cefotaxime, cefepime, amoxicillin / clavulanate, piperacillin / tazobactam, amikacin, gentamicin, ciprofloxacin, and nitrofurantoin." In the meantime, 38.1% of *E.coli* isolates were resistant to the antibiotic combination trimethoprim-sulfamethoxazole, and 42.9% of *E.coli* isolates were resistant to ampicillin and piperacillin. Some researchers came to the same conclusion as Akerele *et al.* (2002), who found that every single *E. coli* isolate they examined showed a favorable reaction to ciprofloxacin (Barcaite *et al.* 2012).

Researchers in Lithuania found that cefuroxime, cefotaxime, amikacin, and ciprofloxacin worked against all isolates of *E.coli* (100%) in every exam they did. Taking these results into account (Al-Mayahie 2013a).

In Iraq, it was found that all *E.coli* isolates were resistant to meropenem (100 percent), whereas only 31.5% of them were resistant to trimethoprim/sulfamethoxazole. Meropenem was shown to be effective against 31.5% of the *E.coli* isolates. In contrast to this, Al-Mayahie says (Al-Mayahie 2013a). In Lithuania, it was discovered that cefuroxime, cefotaxime, amikacin, and ciprofloxacin were all 100% effective against all *E.coli* isolates. On the other hand, ampicillin, piperacillin, ampicillin/sulbactam, gentamicin, and piperacillin/tazobactam were all discovered to be 25%, 16%, 7.8%, and 1.0% resistant, respectively. The present findings were found to be within the same range as that which Zai *et al.* (1996). They found that 14.8% of the isolates in Pakistan were able to be treated with ampicillin, and that 77.7% of the isolates were able to be treated with gentamicin. Ciprofloxacin use is discouraged during pregnancy by certain doctors and other health care providers, particularly in the first trimester of pregnancy (Meyer and Rodvold 1995).

2.8 Characteristics of *Cytomegalovirus*

The primary differentiating factors between the three *herpesviral* subfamilies—alpha, beta, and gamma—are the biological properties of the viruses, as well as the organization of their genomes and the sequencing of their genetic information. *Herpesviruses* all have the ability to live throughout the entirety of their host's life, despite the fact that members of the same subfamily may exhibit fairly distinct biological characteristics.

Certain human disorders require this for long-term in vivo persistence. *Betaherpesvirinae's* prototype is HCMV. It is highly species-specific, has a long replication cycle, can cause cell enlargement (*cytomegaly*), and persists in myeloid lineage cells (Sinclair 2010). HCMV-infected cells have swollen, granular cell bodies, which Ribbert first characterized in 1881 (Ribbert 1904). Goodpasture and Talbert

believed viruses induced cellular changes until 1921. This theory was never confirmed (Goodpasture and Talbot 1921). Three different groups isolated human *Cytomegalovirus* (HCMV) from cell culture (Smith 1956). Included in the family of *herpesviruses* is the giant enveloped human *Cytomegalovirus*, which is extremely widespread among human populations. The double-stranded DNA of this organism contains around 160 open reading frames. A sizeable portion of the human CMV genome contains the genetic coding for gene products that either inhibit or subvert the activity of the host immune system or interact with inflammatory substances in order to facilitate the virus's ability to remain dormant and to proliferate throughout the host (Mocarski *et al.* 2007).

Cytomegalovirus, sometimes known as CMV, is one of the most prevalent causes of congenital infections and a prominent opportunistic pathogen in immunocompromised individuals. The spread of CMV is significantly influenced by the fact that it can be passed on through sexual contact (Handsfield *et al.* 1985). In addition, seropositive women from populations with a higher prevalence of bacterial vaginosis and sexually transmitted diseases have an increased risk of contracting CMV reinfections. These reinfections with novel CMV strains can lead to intrauterine transmission of the virus as well as harmful infections in the fetus (Boppana *et al.* 2001).

2.8.1 Classification of *Cytomegalovirus*

CMV was been classified as the flowing (Zimmer 2013):

- Kingdom: Heunggongvirae
- Phylum: Peploviricota
- Class: Herviviricetes
- Order: Herpesvirales
- Family: Herpesviridae
- Subfamily: Betaherpesvirina
- Genus: *Cytomegalovirus*

2.8.2 Structure of *Cytomegalovirus*

CMV is an extremely sophisticated virus, and different strains of it have a large amount of genetic variation from one another. It has been shown that the highly polymorphic amino-terminal portion of the enzyme glycoprotein gpUL73 (gN) can be broken down into four distinct genotypes (Pignatelli *et al.* 2001).

The diameter of the icosahedral, spherical to pleomorphic, and round geometries of the *Cytomegalovirus* ranged from one hundred fifty to two hundred nanometers. The size of its genome, which is not fragmented and is linear, is around 200 kb (Sinclair 2010). In addition, HSV-5 is the name given to this virus by Arvin *et al.* (2007), and it can be seen in Figure 2.2 (Ma *et al.* 2012).

Due to the fact that their genomes are so enormous, *herpesviruses* have the longest genomics and one of the longest genomes of any human virus in general. This is because *herpesviruses* encode hundreds of different proteins (Sijmons *et al.* 2014) Because CMV is a complicated virus with a wide variety of immunomodulatory roles, several of its gene products have the potential to trigger inflammation in specific areas (Mocarski 2002).

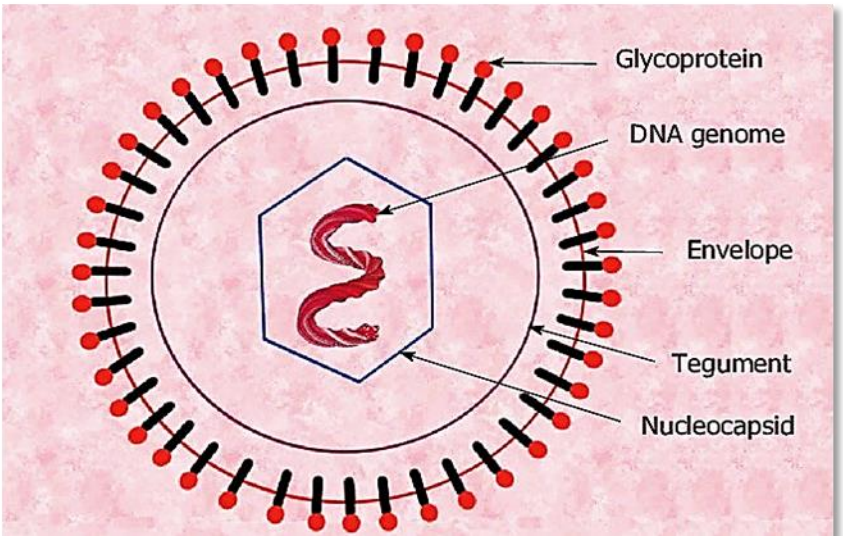

Figure 2.2 Structure of *Cytomegalovirus* (Ma *et al.* 2012)

2.8.3 Life cycle of *Cytomegalovirus*

CMV can be passed from person to person through intimate contact with infected bodily fluids. Viral replication can be observed in many compartments (blood, saliva, milk, cervical secretions, and urine) following primary infection, reacti*Vat*ion of an endogenous latent virus, or reinfection with a different strain of CMV (Cannon *et al.* 2011). CMV shedding from mucosal surfaces is the source of the virus that causes horizontal transmission among populations. This virus is responsible for the spread of the disease. The one and only exception is breast milk (Hamprecht and Goelz 2017).

It has been discovered that all significant leukocyte populations, in addition to fibroblasts, smooth muscle cells, epithelial cells, and endothelial cells, all contain HCMV nucleic acids (Sinzger *et al.* 1995). In contrast to fibroblasts, infections caused by HCMV in monocytes and macrophages are contagious from cell to cell and do not result in cytolysis (Fish *et al.* 1995).

In all *herpesviruses*, lytic infection triggers a carefully orchestrated chain reaction of viral gene expression. This happens as a direct result of extensive viral genome transcription, which in turn encourages viral DNA replication and the production of infectious virions. HCMV lytic infection is accompanied by a transcription program that begins with the expression of immediate early (IE) genes, then moves on to the expression of early (E) genes, and eventually ends with the expression of late (L) genes. After the initial infection, HCMV will continue to be present in the host for the remainder of that host's life. This persistence most likely involves both dormant infection sites and active infection sites in the host that are continuously subjected to low levels of productive infection.

During the period of latency, the lytic transcription program is suppressed, and the generation of viral transcription is restricted to the creation of a small number of genes related with latency. As a consequence of this, the genome of the virus is maintained and can be passed on even when there is no infectious virus present. It is important to

note that this dormant virus might routinely reawaken in vivo (Sinclair 2010, seen in Figure 2.3), as the passage indicates (White and Spector 2007).

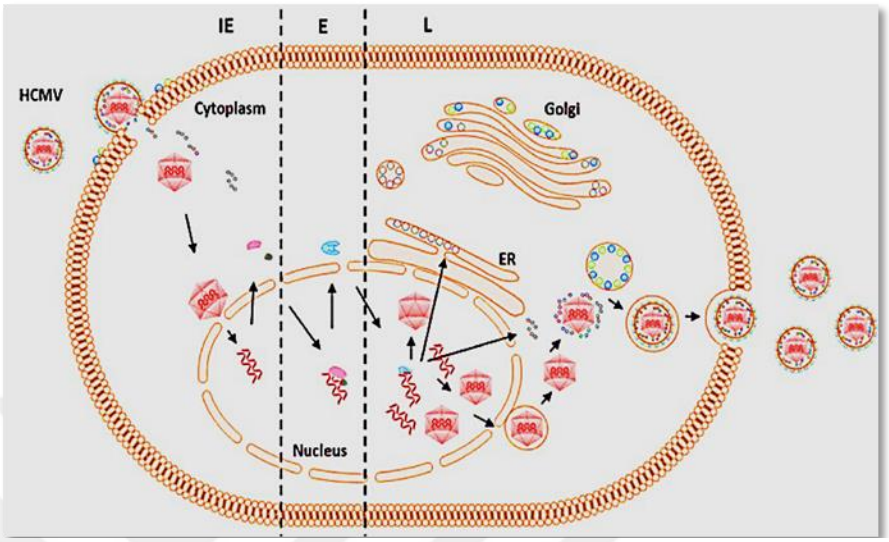

Figure 2.3 Life cycle of *Cytomegalovirus* (White and Spector 2007)

2.8.4 Pathogenesis and transmission of *Cytomegalovirus*

The transfer of *Cytomegalovirus* (CMV) from mother to child transmission (MTCT) is an important contributor to the virus's continued dominance in the human population. The vast majority of CMV's genome is devoted to gene products that can interact with, avoid, or benefit from the human innate and adaptive immune systems as well as inflammatory reactions. This is because CMV's genome is so enormous. This makes it easier for CMV to survive in its human hosts (Schleiss 2011). In a previous study conducted at our organization, trichomoniasis, gonorrhea, and BV were all found to be independently associated to the intrauterine transmission of CMV (Fowler and Pass 1991).

When they come into touch with the skin, biological fluids including as saliva, urine, blood, sperm, and cervical secretions are among those that have the potential to carry the virus. There are three potential routes that an infection can take to travel vertically: through the placenta while the mother is pregnant; through contact with blood and cervical secretions when giving birth; and through breast-feeding after the kid is born

(Jorgensen and Pfaller 2015). CMV is capable of being passed on through sexual contact, and a number of studies have linked the virus to less than optimal reproductive outcomes (Batwa *et al.* 2016).

2.8.5 Clinical features of *Cytomegalovirus* infection

Infection in the womb, hearing loss, and neurological dysfunction in offspring are among symptoms that can be caused by the *Cytomegalovirus* (CMV) (Manicklal *et al.* 2013). And the most prevalent source of congenital infection, which affects between 0.3 and 0.7 % of live newborns, is bacteria that mothers carry in their wombs (Kenneson and Cannon 2007).

Sensorineural hearing loss, organomegaly, microcephaly, cerebral calcification, and chorioretinitis are the most common symptoms in 10–15% of congenitally infected neonates. The virus directly cytopathically affects the fetus, causing these symptoms (Munro *et al.* 2005). CMV infects the fetus through placental infection. The virus infects syncytiotrophoblasts, cytotrophoblasts, and endothelial cells to cross the placenta. Infection induces inflammation, leukocyte infiltration, and cytokine production, which damage the placenta and fetus (Pereira and Maidji 2008).

A mild mononucleosis or flu-like disease with a protracted fever and tiredness can sometimes present as a primary infection during pregnancy. However, around 75–95% of moms who have a primary infection during pregnancy do not exhibit any symptoms (Nigro 2009). Every year, between 30,000 and 40,000 infants in the United States are born with a congenital infection caused by the *Cytomegalovirus* (CMV), which results in at least 150 infant deaths and more than 5,500 additional infants being born with disabilities such as impaired vision or hearing, as well as cognitive difficulties such as mental retardation (Dollard *et al.* 2007).

2.8.6 Diagnosis of *Cytomegalovirus*

The use of serologic testing is necessary in order to diagnose a primary maternal CMV infection. Normal practice dictates that testing should follow after receiving certain ultrasound results, such as those indicating fetal echogenic bowel, intrauterine growth retardation, or brain calcifications (Hughes *et al.* 2016).

A patient's blood anti-CMV IgM and IgG levels can indicate CMV infection. CMV infections usually start with IgM production, followed by IgG (Verma *et al.* 2015). In the early weeks after a primary infection, IgG avidity, a marker of antibody maturity, is low (antibodies bind to the antigen weakly) and gradually increases (i.e., antibodies bind tightly to the antigen). A positive IgM and low avidity IgG result can detect initial infections (Macé *et al.* 2004). CMV-specific IgG seroconversion in matched acute and convalescent samples three to four weeks apart can also detect initial infections. IgM can survive months after a first infection and be found following reacti*Vat*ion or reinfection (Hagay *et al.* 1996).

According to (Bodéus *et al.* 1999), approximately one quarter of all fetuses that are born to expectant moms who have primary CMV infection during the first trimester will have the virus. Therefore, a congenital CMV infection is highly likely to develop in infants whose mothers had a primary CMV infection while they were pregnant. This is the case for children born to mothers who had the virus during pregnancy. It is common practice to use maternal serum CMV immunoglobulin (IgM) antibody testing in order to locate the point of first infection. On the other hand, only 20–25% of pregnant women who have positive findings for CMV IgM testing are determined to actually have primary CMV infection (Revello *et al.* 2011).

This is because there is a possibility of CMV IgM persistence six to nine months after the initial infection, or it could be identified when latent reacti*Vat*ion takes place (Lazzarotto *et al.* 2011, Revello *et al.* 2011). Confirmatory testing using the serum CMV IgG avidity index (AI) is utilized for the purpose of identifying the main infection that occurs during pregnancy (Lagrou *et al.* 2009). Additionally, fetal CMV infection

can be caused by a reaction of a dormant virus or reinfection with a new strain of CMV, and this occurs in 1% to 2% of pregnant women (Kenneson and Cannon 2007).

2.8.7 Elisa test

ELISA is widely acknowledged as the method that is utilized most frequently in clinical settings such as hospitals and medical clinics all around the world. It has also been put to extensive use in the biotechnology industry for the accurate detection and quantification of biological molecules (mostly proteins and polypeptides), and it is currently playing an increasingly important part in the fields of environmental, therapeutic, and food safety applications (Lai *et al.* 2004). It is generally accepted that ELISA has been utilized in some form or another in each and every laboratory that has ever been in operation. Because it produces data that are both highly reproducible and quantifiable, ELISA is an invaluable piece of biotechnology for use in scientific research and clinical diagnostics (Hosseini *et al.* 2018).

2.8.8 Interleukins associated in vaginitis women

2.8.8.1 Interleukin-6 (IL-6)

Interleukin-6 (IL-6) is considered to be one of the most important proinflammatory cytokines that are present during the beginning of an infection (Carlquist *et al.* 1999). Additionally, interleukin-6 (IL-6) is considered to be an essential component in trophoblast activity because it plays a significant role in the proliferation, invasion, and differentiation of trophoblast cells. This is one of the reasons why IL-6 is regarded as an essential component in trophoblast activity (Das *et al.* 2002).

The interleukin (IL-6) is an important factor in the regulation of a number of different cellular and biological processes (Guzeloglu-Kayisli *et al.* 2009). It has been proven that IL-6, a well-known potent regulator of the host's innate and humoral immune

responses, is up-regulated during a variety of viral infections, one of which is CMV. This is the case for a number of other viral infections as well (Rossini *et al.* 2012).

Human endometrial and tubal cells have been recognized as significant regulators of endometrial receptivity and blastocyst implantation. In addition to secreting IL-6 and the unique chemicals that it produces, tubal cells and endometrial cells both produce IL-6 (Cuman *et al.* 2013). In addition, abnormal expression of IL-6 in the endometrium has been associated to poor reproductive outcomes, including abortion and the inability to successfully implant embryos (Dimitriadis *et al.* 2007).

The human *Cytomegalovirus* is capable of infecting a diverse selection of human cell types; however, in immunocompetent hosts, the mucosal epithelium is often the site where primary infection and viral replication commence (La Rosa and Diamond 2012). Alterations in the expression of immune signaling molecules and interferon response genes are two of the ways the virus can circumvent the defenses of the host immune system (Gealy *et al.* 2005).

IL-6 is a potent acute phase response inducer and it works best in situations when there is infection or inflammation. By regulating the generation of other cytokines and antibodies by B lymphocytes, it is also an important component in the acquired immunity that protects against a wide variety of infectious agents. Additionally, its levels rise when there is chronic inflammation in the body (Mihara *et al.* 2012). Furthermore, it has been proven that human CMV infection boosts the expression of IL-6 mRNA even when viral genes are not expressed from the beginning of the process (Gealy *et al.* 2005). It is noteworthy to note that recent in vitro study suggests that high IL-6 levels may have a role in the reacti*Vat*ion of human CMV. This is something that needs further investigation (Rossini *et al.* 2012).

2.8.8.2 Interleukin-8 (IL-8)

Overexpression of IL-8 has been linked to having the *Cytomegalovirus* (CMV) infection; hence, IL-8 is of particular significance (Redman *et al.* 2002). It's possible that IL-8 is able to cause HCMV replication and the commencement of early HCMV replication events because it stimulates the transcription of HCMV late genes with cytokines before DNA replication takes place (Lee *et al.* 1999, Capobianchi *et al.* 1997). In addition to increasing HCMV replication, IL-8 attracts and acti*Vat*es neutrophils at infection sites, which may contribute to HCMV-associated inflammation. IL-8 involved in HCMV-associated inflammation (Baggiolini *et al.* 1994). Infected neutrophils may spread the virus to other organs. It has been shown that interleukin-8 can make a major contribution to the pathophysiology of gastrointestinal inflammation brought on by HCMV. (Oppenheim *et al.* 1991).

2.8.8.3 TNF- α

One of the most important inflammatory cytokines that monocytes make is called tumor necrosis factor alpha, or TNF-α) for short. This cytokine exists in two distinct forms: membrane-bound and soluble (Dinarello 1997). The immune cell from which cytokines arise and the immunological effects that they exert are the two primary factors that have traditionally been used to classify cytokines into families (Basu *et al.* 2015). The pro-abortogenic effects of TNF-α have been attributed to a number of different pathways. These pathways include trophoblast invasion, placentation, and the stimulation of pro-apoptotic gene expression in human fetal membranes. This, in turn, speeds up the deterioration of the membranes and raises the risk of premature rupture. It has also been claimed that TNF-α indirectly causes miscarriage by acti*Vat*ing macrophages or NK cells. This theory is supported by the findings of the aforementioned study (Li *et al.* 2017).

The early and late phases of gestation are the primary times when TNF-α is expressed (rather than the middle). According to, it stimulates uterine activity during childbirth and acts to promote blastocyst implantation throughout pregnancy. It also regulates

trophoblast proliferation, migration, and differentiation, maintains keeps a healthy balance between trophoblast turnover and renewal. It plays multiple roles in the septic response, including causing apoptotic cell death and recruiting immune effector cells during inflammation. It also plays a role in the septic reaction. Despite the fact that TNF-α plays an important part in the regulation of placental growth and function, aberrant production of the protein has been related to adverse pregnancy outcomes, which lead to the injury or death of the fetus (Haider and Knöfler 2009).

2.8.8.4 Interleukin-10 (IL-10)

Interleukin (IL-10), an important anti-inflammatory cytokine that is produced by monocytes, has the capacity to inhibit the proliferative response of T cells, reduce the production of proinflammatory cytokines, and suppress the presentation of antigen by monocytes. In spite of the fact that IL-10 has a wide range of immune-regulatory effects, its precise role in CMV infection remains unknown. It is well established that IL-10 has the ability to inhibit a variety of monocyte and macrophage functions, one of which is the production of TNF-α. In addition, IL-10 may indirectly compromise the antiviral defensive mechanisms of T cells and NK cells by influencing the synthesis of IL-12, for instance. This may be one of the ways that IL-10 does this (Mosman 1994). Additionally, IL-10 can inhibit the growth of T cells in two different ways: directly and indirectly. Infection of monocytes by CMV, as a last point (Perrin *et al.* 1999, Taylor-Wiedeman *et al.* 1991). IL-10 promotes monocyte-to-macrophage differentiation but only replicates in macrophages (Allavena *et al.* 1998). Thus, an increase in IL-10 levels, such as we detected in both symptomatic and asymptomatic CMV infection, weakens the antiviral defense and may contribute to CMV-induced immunosuppression, as shown by superinfections with other microorganisms. CMV infection caused superinfections with other pathogens. Patients suffering from CMV infection, on the other hand, have a chance of experiencing considerable beneficial outcomes when they take IL-10. The physiologic effects of IL-10 are determined by how it interacts with other cytokines, notably those that promote inflammation such as TNF-α (Weissman et al. 1994).

3. MATERIAL AND METHODS

3.1 Materials

3.1.1 Subjects

About 200 samples high vaginal swabs and serum were taken from women with vaginitis in Al-Sadr Teaching Hospital under the direct supervision of a gynecologist physician. Each patient had a unique serial number, and the study was carried out with the approval of the Maysan Health Department. The symptoms were characterized by purulent vaginal discharge, a foul odor, itching, fever, and preterm birth.

3.1.2 Apparatus and equipments

The tools and gadgets employed in this investigation are listed in Table 3.1 below.

Table 3.1 The apparatus and equipments that used with their producing companies and the origin

Apparatus and Equipments	Origin / Company
AURA TM PCR Cabiant	EuroClone/Italy
Autoclave	Hirayama/Japan
Balance	Kernpfb/Germany
Beakers	Iso Lab/Germany
Bio TDB-100, Dry thermostatbuilt	Bio San/Germany
Bio Variable Volume (2-20µl,0.5-10µl,20-200µl,10-100µl,100-1000µl)	Labnet/ Germany
Biosafety Cabinet	Lab Tech/France
Burner	Indiamart/India
Combi-spin	Biosan/ Lativa
Cooling centerfuge	Eppendroff/Germany
Cover slides	Superestar/India
Cylinder	Iso Lab/Germany
Disposable Petri dishes	Al-malak company/Iraq
distilled water	Gfr/Germany
Document system	Labent/USA
Electrophoreses	CBS, Scientific/USA
ELISA	HumanReader /Germany
Flask (250-500)	Iso Lab/Germany
Funnel glass	Iso Lab/Germany
Gloves	Broche/Malaysia
Incubator	Human Lab/Korea

Table 3.1 The apparatus and equipments that used with their producing companies and the origin (Continuous)

Light Microscope	Nikon/Japan
Loop	John Blotn/England
Micropipettes	DragonMED/china
Microspin	Biosan/ Lativa
Microspine 12, High-speed Mini-centrifuge	Bio San/Germany
Microwave	Gosonic/China
Miniature Power Supply with Voltages of 300V and 2200V	Supplier/Chain
MultiGeneOptiM ax Gradient Thermal Cycler	Labent/USA
Oven	Memmert/Germany
Slides	Superestar/India
Standard Wire Loop	John Bolten/England
Swaps	AFCO/Jordan
Syringes	Sterileo/China
UV.transmission	Vilber lourmat/Farance
V-1 Plus, Personal Vortex for tubes	Digsystem
Vitek-2-Compact System	Biomerieux/France
Wood stick	Supteme/china

3.1.3 Chemistry and biology materials

All Chemistry and biology materials are used in this study, as in the Table 3.2.

Table 3.2 Chemistry and biology materials used

NO	Materials	Corporation
1	Absolute Ethanol	Schariauiau/Spain
2	Agarose	Conda / USA
3	Blood	Blood bank/Mysan city
4	i-genomic BYF DNA Extraction Mini Kit	Intron biotechnology/Korea
5	Ladder 100 bp	Intron / Korea
6	Normal saline	Iraq
7	Oil Immersion	Jourilabs
8	Pre mix pcr	Intron / Korea
9	Primer	Alph DNA/USA/Canada
10	Red safe staining souluion	Intron / Korea
11	TBE buffer 10 X	Conda / USA
12	6X Loading dye	Intron / Korea

3.1.4 Culture media

Media are used in this study, as in the Table 3.3.

Table 3.3 Culture Media

NO	Media	Company
1	Amies Transport medium	Sterile transport medium swab/jordan
2	Blood agar	Neogen/USA
3	MacConkey agar	Neogen/USA
4	Nutrient agar	Neogen/USA

3.1.5 Diagnostic kit

Equipment that was utilized in this investigation, as shown in Table 3.4.

Table 3.4 Kit use in this search

NO	Kit type	Purpose	Company
1	Gram stain	Differentiation between microorganism	Himedia/India
2	Human IL-10 ELISA Kit	To quantitative detection of IL-10 in human serum.	MyBioSource/USA
4	Human IL-6 ELISA Kit	To determine the level of IL6 in Human serum	MyBioSource/USA
3	Human IL-8 ELISA Kit	To quantitative detection of IL-8 in human serum.	MyBioSource/USA
5	Human TNF-α (Tumor Necrosis Factor Alpha)	For quantitative determination of Human TNF-α concentrations in serum.	MyBioSource/USA
6	MiniVidas CMV G	To detect IgG specific for *Cytomegalovirus*	Biomerienx/ France
7	MiniVidas CMV M	To detect IgM specific for *Cytomegalovirus*	Biomerienx/ France
8	Vitek2 AST-N204	Determining the appropriate treatment for *Escherichia coli*	Biomerienx/ France
9	Vitek2 GN Kit	Identification of Gram-Negative bacteria	Biomerienx /France

3.2 Methods

3.2.1 Sample collection

The total of samples (200), including high vaginal swabs (125) and (75) serum. All study samples were taken from women with vaginitis in age (15-69) years. The samples were collected in the obstetrics and gynecology department in AL-Sadder Teaching Hospital in Maysan governorate and under the supervision of the competent staff during

the period March 2022 to August 2022, and were used common biochemical tests to identified (Forbes *et al.* 2002).

3.2.2 Questionnaire sheet

Data were taken direct from patient and clinically history from each case, the information were arranged in detail formulation paper as shown in.

3.2.3 Collection of vaginal swabs

3.2.3.1 High vaginal swabs

1- The vaginal walls has been separated used a speculum.
2- Used a cotton swab to remove any surplus cervical mucus.
3- Insert it into the vagina with care approximately two inches (five centimeters) past the introitus, and then make sure the swab hits the vaginal walls by gently rotating it for ten to thirty seconds.

3.2.3.2 Specimen transport and storage

1- Vaginal swab samples must be transported to the lab in the specified tube and medium for swab specimen transportation.
2- Vaginal swabs samples must be transported to the lab between (2 °C) and (30 °C) degrees Celsius and tested within 60 days of collection.
3- Freeze at (-20 - -70) °C for up to 12 months after collection if prolonged storage is needed.

All samples in this study were transferred and culti*Vat*ed immediately after collection on culture media.

3.2.4 Preparation of culture media

3.2.4.1 Preparation of macconcy agar media

1- This media was used as differentiation media of gram positive and gram negative.
2- Put and culture media in autoclave for sterilization at (121°C) for 15 minutes and followed the manufacturer's recommendations when use this media.

3.2.4.2 Preparation of blood agar media

1- By followed the manufacturer's recommendations when using this media.
2- Sanitizes in an autoclave at (121°C) for 15 minutes, then is allowed to cool.
3- About (5%) of human blood was added to it and Mix well**.**

3.2.4.3 Preparing of nutrient agar

1- According to the manufacturer's recommendations when using this media.
2- 28 grams of nutrient agar should be dissolved in a liter of dripped water.
3- Sterilized for 15 minutes at a temperature of (121°C) in the autoclave.

3.2.5 Diagnosis bacteria isolation

3.2.5.1 Identification of bacterial vaginosis

According to color, size, ele*Vat*ion, and margin of the colonies on enrichment, selective and differential media (Blood agar and MacConcy agar, Nutrient agar) are among the morphological characteristics of the bacterial vaginosis developing colonies (Collins *et al.* 2004).

3.2.5.2 Staining

3.2.5.2.1 Gram stain

1. After preparing the smear of suspension on the slide with the help of a loop and allowing it to dry with air, the smear can be fixed with heat.
2. After pouring Crystal Violet into the container, the mixture was left undisturbed for around 30 seconds to 1 minute before being rinsed with water.
3. After flooding for one minute with gram's iodine, you should then rinse with water.
4. Rinse with water after washing for approximately ten to twenty seconds with acetone or alcohol that contains 95%.
5. After approximately a minute, add some safranin, and then wash it off with water.
6. Dry the sample by blotting it, then allow it to air dry before examining it under a microscope.

3.2.5.2.2 Wet amount preparation

1. Add one or two drops of normal saline solution on a slide with a coverslip.
2. Examined under microscopic (Collins *et al.* 2004).

3.2.5.3 Biochemical examination

3.2.5.3.1 Oxidase test

1. Tetramethyl phenylenediamine dihydrochloride (oxidase reagent) (1 percent) was placed two to three drops on a piece of filter paper.
2. With the edge of a slide, a piece of the culture was scraped off and spread across the surface of the impregnated paper.
3. A dark purple color was produced within ten seconds, signaling a positive response (Cowan and Steel 1965).

3.2.5.3.2 Catalase test

1. Use a bacteriological loopful or an antiseptic wood rod to transfer a small amount of colonization the growth from the surface of a neatly cleanly glass slip.
2. A drop of hydrogen peroxide (3%) should be applied to the inoculum (Crucitti *et al.* 2011).

3.2.5.4 VITEK 2 diagnostic system

Principle: The Vitek 2 Compact (30 card capacity) device uses a 64-well barcoded card for organism identification and susceptibility testing. The AST-GN and ID-GN test kits identify gram-negative bacilli. The Vitek 2 ID-GN card can identify 154 species of *Enterobacteriaceae* and a few glucose non-fermenting gram negative bacteria in 10 hours. The results of the Vitek-2 Antimicrobial Susceptibility Tests (AST) for *Staphylococcus spp., Enterococcus spp., and Streptococcus agalactiae* as well as the majority of clinically important aerobic gram-negative bacilli are available in less than 18 hours.

Procedure**:** The Vitek-2 device was used to diagnose bacteria isolates from culture media. Sterile swabs or wooden utilize were used to transport one to two colonies of pure culture. Bacteria were then suspended in (0.3) ml of pasteurized brine in clear Polystyrene test tubes. Turbidity was adjusted in accordance with McFarland, ranging gram negative from (0.5-0.63). Then, the cassette bear with 10 Card, suspense, tube, and bar code to input data, followed by loading it to the automated transport system (Pincus 2006).

3.2.6 Microscopically examination of *E.coli*

The wet quantity method under microscope examination is the most common type of test. This method requires taking a swab from the vaginal cervix and mixing it with three milliliters of normal saline. After that, one drop of the mixture is placed on a glass

slide, and the slide is covered with the slide. This is followed by an examination of the sample using a light microscope with a magnification of 40X.

3.2.6.1 Identification of *E.coli* isolates

The samples that were transported were plated onto MacConkey agar and incubated aerobically for 24 hours at (37°C). Based on gram stain results, colony morphology, and common biochemical assays, the isolates were identified (Collee *et al.* 1996). Using the Vitek-2 technology (bioMerienx, France), in accordance with the manufacturer's instructions, the identified *E. coli* was then confirmed. Accordance to the current Clinical Laboratory Standards Institute standards, antimicrobial susceptibility testing (AST) (CLSI) (Zlatian *et al.* 2018).

Gram-negative bacteria can be identified with the help of the AST-N204 cards. Some examples of these bacteria are Escherichia coli and other species of Enterobacter. The antibiotic microdilution method is the cornerstone of the automated bacterial antibiotic susceptibility testing that is now available:

1. In each of the cells from the card that have different antibiotic concentrations, the bacterial strain is culti*Vat*ed.
2. Each cell is read three times once in every 15 minutes, and for each reading, 16 points are used to calculate the absorbance of the cell, which is proportional to the amount of bacteria present.

3.2.6.2 Molecular technique

3.2.6.2.1 Protocol *E.coli* sample

1. Gram-negative bacterium sample.
2. Transfer 1–2 ml of grown bacteria to a 2 ml tube.

3. Discard all but 50 µl of the supernatant after centerifugate bacteria for 1 minute at 13,000 rpm.
4. Tap or vigorously votex to completely resuspension the bacterial pellet into supernatant.
5. Vortex 300 µl Buffer MG, 20 µl Proteinase K, and 5 µl RNase A Solution in the sample tube.
6. Incubation of the lysate for 15 minutes at 65 degrees Celsius.
7. After complete lysis, add 250 µl Buffer MB and gently invert five to six times or pipet. Never vortex. Spin down to remove lid droplets after mixing.
8. Invert or pipet 250 µl 80% ethanol into the lysate. Never vortex. Spin down to remove lid droplets after mixing.
9. Pipette 750 µl of step 8 mixture into the spin column in a 2.0 ml collecting tube. Discard the flow-through and collecting tube after one minute of 13,000 rpm (RT) centrifugation.
10. Put the spin column into a new 2.0 ml collection tube, add 700 µl Buffer MW, and continue to spin for another minute (13,000 rpm). To dry the membrane, centrifuge it for one more minute before reusing the collection tube. Discard the flow-through.
11. Place the spin column in a new 1.5 ml tube (one that was not provided) and directly apply between 50 and 100 l of Buffer ME to the membrane of the spin column. After centrifuging for one minute at room temperature, elute the sample by subjecting it to 13,000 revolutions per minute.

3.2.6.3 Agarose gel electrophoresis of DNA

After the DNA has been extracted, electrophoresis can be utilized to determine the sizes of individual DNA fragments. Additionally, electrophoresis can be utilized to determine the outcome of PCR when standard DNA is present. developed with the use of agarose gel (Sambrook et al. 1989).

Currently, 3 l of the processor loading buffer (Intron/Korea) and 5 l of the DNA that is going to be electrophoresed are being loaded into the pores of the gel. It took anything from one to two hours, using an electric current of seventy volts per square centimeter,

but eventually the tincture made its way to the other side of the gel. An ultraviolet lamp with a wavelength of 336 nanometers was used to analyze the gel after it had been stained with a red safe nucleic acid staining solution and immersed in a pool made up of 500 milliliters of distilled water and 30 microliters of the solution.

3.2.6.4 Red safe nucleic acid staining solution

For use with agarose gels, the Red Safe Nucleic Acid Staining Solution (20,000x) is a risk-free and one-of-a-kind alternative to the ethidium bromide (EtBr) stain. When it is attached to DNA or RNA, it emits a green glow. This peculiar stain has two fluorescence excitation maxima when it is linked with nucleic acid; the first one is at (309 nm), and the second one is at (419 nm).

At a wavelength of 514 nanometers, there is an apparent excitation. The fluorescence emission core of DNA that is linked to Red Safe is 537 nanometers. The Red Safe Nucleic Acid Staining Solution is Highly Sensitive (20,000x), Comparable to EtBr.

The staining done with EtBr is analogous to the staining done with Red Safe Nucleic Acid Staining Solution (20,000x). In the Ames test, the potent mutagen EtBr induced a greater number of mutations than the 20,000x concentration of Red Safe Nucleic Acid Staining Solution.

Both the mouse marrow chromophilous erythrocyte micronucleus test and the mouse spermary spermatocyte chromosomal aberration test were unsuccessful when performed with a 20,000x concentration of Res Safe Nucleic Acid Staining Solution. Instead of using EtBr (Catalog Number 21141), it is recommended to use RedSafe Nucleic Acid Staining Solution (20,000x) for the purpose of recognizing nucleic acid in agarose gels.

3.2.6.5 primers used in the interaction

The primers were lyophilized, and then they were dissolved in free double distilled water (ddH_2O) to give a final concentration of 100 pmol/µL as stock solution. A stock was kept at-20℃, and 10 pmol/ul concentration of work primer was prepared by suspending 10µL of the stock solution in 90µL of free ddH_2O water to reach a final volume of 100 µL. This experiment was conducted by Alpha DNA (Table 3.5)

Table 3.5 The specific primers (*16srRNA, Tra t, Vat*, FimH) of genes

NO	Target gene	Primer	Sequence	Tm (°C)	GC (%)	Product size	R
1	*16srRNA*	Forward Reverse	5′-AGAGTTTGATCCTGGCTCAG-3′ 3′- GGTTACCTTGTTACGACTT-5′	54.3 49.4	50.0 42.1	1250 base pair	(Srinivasan *et al.* 2015)
2	*Tra t*	Forward Reverse	5'-GGTGTGGTGCGATGAGCACAG - 3' 5'-CACGGTTCAGCCATCCCTGAG - 3'	74 74	68 68	290 base pair	(Cruz-Cruz *et al.* 2021)
3	*Vat*	Forward Reverse	5'-TCCTGGGACATAATGGTCAG - 3' 5'- GTGTCAGAACGGAATTGT - 3'	68 62	60 52	981 base pair	(Cruz-Cruz *et al.* 2021)
4	FimH	Forward Reverse	5'-TCGAGAACGGATAAGCCGTGG - 3' 5'-GCAGTCACCTGCCCTCCGGTA - 3'	72 76	66 70	508 base pair	(Cruz-Cruz *et al.* 2021)

3.2.6.6 Maxime pcr premix kit (i-Taq) 20µlrxn (CAT. No. 25025)

Intron's Maxime PCR PreMix Kit includes a 2X Master mix solution and numerous premix kits depending on experience and use. The i-Taq DNA Polymerase, deoxynucleotide triphosphate (dNTP) mixture, reaction buffer, or other PCR components are all contained in a single tube of the Max PCR Pre Mix Kit (i-Taq). This product provides the most ease and best results.

A first advantage is the fact that it possesses all of the elements that are necessary for PCR; hence, in order for us to carry out PCR, everything that is left for us to do is add a DNA Template, primer set, and D.W. The availability of a gel loading buffer for electrophoresis is the second factor. This buffer enables us to do gel loading without first undergoing any kind of pretreatment. Because of how quick and simple the usage procedure is, it is suitable for a wide range of different types of experiences (Table 3.6).

Table 3.6 The Components of the Maxime PCR PreMix kit (i-Taq)

NO	Material	Volume
1	i-Taq DNA Polymerase	5U/µL
2	DNTPs	2.5mM
3	Reaction buffer (10X)	1X
4	Gel loading buffer	1X

3.2.6.7 Molecular diagnosis of virulence genes in *E.coli*

Table 3.7 Combination of the particular gene interactions necessary for diagnosis

NO	Components	Concentration
1	Taq PCR PreMix	5µL
2	Forward primer	10 picomols/µL (1 µL)
3	Reverse primer	10 picomols/µL (1 µL)
4	DNA	1µL
5	Distill water	17 µL
6	Final volume	25 µL

After performing numerous experiments, the best conditions for (initial denaturation) and annealing were found. Using gradient PCR, the temperature was altered for each of the samples, and the concentration of the DNA template was varied to be between 1.5 and 2 µL. This was done so that the optimal conditions could be determined. These two considerations are crucial for primer annealing with the complement (Table 3.7, Table 3.8, Table 3.9, Table 3.10).

Table 3.8 The optimum condition of detection (*16srRNA*)

NO	Phase	Tm (°C)	Time	No. of cycle
1	Initial Denaturation	94°C	3 min	1 cycle
2	Denaturation-2	94°C	45sec.	35 cycle
3	Annealing	56°C	1 min	35 cycle
4	Extension-1	72°C	1 min	35 cycle
5	Extension-2	72°C	7 min	1 cycle

Table 3.9 The optimum condition of detection (*Tra t*)

NO	Phase	Tm (°C)	Time	No. of cycle
1	Initial Denaturation	94°C	5 min	1 cycle
2	Denaturation-2	94°C	30sec.	35 cycle
3	Annealing	57°C	30sec.	35 cycle
4	Extension-1	72°C	2 min	35 cycle
5	Extension-2	72°C	7 min	1 cycle

Table 3.10 The optimum condition of detection (*Vat*, FimH)

NO	Phase	Tm (°C)	Time	No. of cycle
1	Initial Denaturation	94°C	5 min	1 cycle
2	Denaturation-2	94°C	30sec.	35 cycle
3	Annealing	55°C	30sec.	35 cycle
4	Extension-1	72°C	2 min	35 cycle,
5	Extension-2	72°C	7 min	1 cycle

3.2.7 *CYTOMEGALOVIRUS* (CMV) samples

3.2.7.1 Principle of minividas

The interaction of two components is the foundation of the VIDAS® principle: the coated SPR® receptacle, which contains antigens or antibodies, and the Strip, which is composed of a succession of wells carrying the correct quantity of reagent required for the test. Within the SPR, all reactions take place in two key phases: immunological reaction.

Principle of the procedure (IgM)

After a first stage that involves the absorption of IgG and rheumatoid factor, the specimen is put through a number of cycles in which it is placed both inside and outside of the SPR for a certain amount of time throughout each cycle. Due to the fact that, anti-CMV IgM antibodies are able to bind to the CMV antigen that was coated on the interior of the SPRs that were present in the specimen. A sample that has not been bonded can have its components washed away. Mouse monoclonal anti-human IgM antibodies conjugated with alkaline phosphatase that cycle in and out of the SPR will bind to human anti-CMV IgM that has been attached to the wall of the SPR. During the final wash phase, any unbound conjugate is eliminated. In order to see the SPR, a fluorescent substrate known as 4-methylumbelliferyl phosphate is added. The enzyme that is still present on the wall of the SPR is responsible for catalyzing the reaction that transforms the fluorescent substrate into the fluorescent product, 4-methylumbelliferone. The intensity of the fluorescence is determined by the optical scanner of the equipment. After the VIDAS CMV IgM (CMVM) assay has been completed, the instrument will immediately do an automatic analysis of the results, provide a test value, and print a report for each sample.

Assay procedure

1. Take out of the kit only what is necessary, and put the rest back into storage at a temperature between 2 and 8 ° C. Check to see that the temperature of the components has reached room temperature (approximately 30 minutes).
2. It is necessary to make use of one CMVM strip and one CMVM SPR for each sample, control, or standard that is being investigated. After the appropriate SPRs have been removed, check to see that the storage pouch has been carefully resealed before proceeding. The test can be detected by looking at the instrument and seeing the code "CMVM." by "S1", The calibrator needs to be identified and put through two separate tests. If the positive control is going to be examined, then C1 needs to be used to determine what the positive control is. In the event that the negative control is to be evaluated, the identification of the negative control by C2 is

required. In the event that it is required, affix the correct sample identification number to each of the "CMVM" Reagent Label strips.

3. Mix the standard, the controls, and the samples together using a mixer of the vortex type (for serum that has been separated from the pellet). In this particular test, the sections for the calibrator, the control, and the sample test all amount to (100 uL).
4. Put the "CMVM" Reagent Strips and SPRs in their respective locations on the instrument. Verify that the color labels that are printed on the SPRs and the Reagent Strips that hold the assay code are same. Begin the processing of the assay in accordance with the instructions in the User Manual. Each step of the test is carried out mechanically by the apparatus. Following the completion of the pipetting procedure, the vials should be resealed and placed in a refrigerator between 2 and 8 ° C.
5. It should only take about an hour and a half to complete the test. After the assay has been completed, the SPRs and the strips should be removed from the instrument. Used SPRs and strips should be disposed of by placing them in the appropriate receptacle.

Principle of the procedure (IgG)

After an initial stage of sample dilution, the sample is sent through a series of cycles in which it is both inside and outside of the SPR for a fixed amount of time. Anti-CMV IgG antibodies present in the specimen will attach to the purified CMV antigen coating the interior of the SPR The unbound components of the sample are removed by washing them. A monoclonal anti-human IgG antibody that has been conjugated with alkaline phosphatase is cycled in and out of the SPR. This antibody will attach itself to any human IgG that is bound to the wall of the SPR. The last wash phase eliminates any unbound conjugate that may have been present.Add 4-methylumbelliferyl phosphate to see the SPR.

Enzyme on the SPR wall will transform the substrate into 4-methylumbelliferone, a luminous product (450 nm) Its optical scanner measures fluorescence intensity. CMV IgG levels determine this intensity.After the VIDAS CMV IgG (CMVG) Assay has

been completed, the data are sent to a computer where they are analyzed in an automated fashion. When calculating the amount of anti-CMV IgG that is present in the sample, the device makes use of a calibration curve that is kept within the device itself. For each sample, a report is printed.

Assay procedure

1. Take out of the kit only what is necessary, and put the rest back into storage at a temperature between 2 and 8 ° C. Check to see that the temperature of the components has reached room temperature (approximately 30 minutes).
2. It is necessary to make use of one CMVG strip and one CMVG SPR for each sample, control, or standard that is being investigated. After the appropriate SPRs have been removed, check to see that the storage pouch has been carefully resealed before proceeding. The test can be recognized on the instrument by the code "CMVG," which identifies it (to do so, refer to the Instrument User Manual). by "S1", The calibrator needs to be identified and put through two separate tests. If the positive control is going to be examined, then C1 needs to be used to determine what the positive control is. In the event that the negative control is to be evaluated, the identification of the negative control by C2 is required. In the event that it is required, affix the correct sample identification number to each of the "CMVM" Reagent Label strips.
3. When you are mixing the calibrator, the controls, and the sera, you should use a mixer that has a vortex (for mixing serum that has been separated from the pellet). In this particular test, the sections for the calibrator, the control, and the sample test all amount to (100 uL).
4. Put the "CMVG" Reagent Strips and SPRs in their respective locations on the instrument. Verify that the color labels that are printed on the SPRs and the Reagent Strips that hold the assay code are same. Begin the processing of the assay in accordance with the instructions in the User Manual. Each step of the test is carried out mechanically by the apparatus. Following the completion of the pipetting procedure, the vials should be resealed and placed in a refrigerator between 2 and 8 ° C.

5. It should only take about an hour and a half to complete the test. After the assay has been completed, the SPRs and the strips should be removed from the instrument. Used SPRs and strips should be disposed of by placing them in the appropriate receptacle.

The results that expacted in this stude were shoed in Table 3.11 and Table 3.12

Table 3.11 Cutoff MiniVidas CMV (IgM)

NO	Single/cutoff Ratio	Interpretation
1	<0.7	Negative
2	≥0.7 to <0.9	Equivocal
3	≥0.90	Positive

Table 3.12 Cutoffs of the MiniVidas CMV (IgG)

NO	Single/cutoff Ratio	Interpretation
1	<4.0	Negative
2	4.0≤ to <6.0	Equivocal
3	≥6.0	Positive

3.2.8 Human Interleukin 6 (IL-6)

3.2.8.1 Materials supplied

All the material that used in this study was shoed in Table 3.13

Table 3.13 The Materials

Items	Materials	Covers' various hues	96 well kit
1	Microelisa Stripplate	---	96 well plate
2	Standards	S1(Red), S2(Pink), S3(Blue), S4(Green), S5(Yellow), S6(White)	0.5mL×6 vials
3	Sample Diluent	Blue	6.0mL×1 bottle
4	HRP-Conjugate Reagent	Red	10.0mL×1 bottle
5	20×Wash Solution	White	25 mL×1 bottle
6	Stop Solution	Yellow	6.0 mL×1 bottle
7	Chromogen Solution A	Purple	6.0 mL×1 bottle
8	Chromogen Solution B	Black or Brown	6.0 mL×1 bottle
9	Closure Plate Membrane	---	2×pieces
10	Manual	---	1×paper

3.2.8.2 Samples collection and storage according to the manufacturer's recommendations (mybiosource cat.no: mbs0 2 1993)

Serum should be collected and centrifuged at 1000 ×g (or 3000 rpm) for around (20) minutes. Carefully collect the supernatant. Assay right away or keep samples in storage at (-20°C or -80°C). Steer clear of frequent freeze-thaw cycles.

3.2.8.3 Reagent preparation and storage

Store the kits (the Plate and all reagents) between 2 and 8 degrees Celsius. Dilute one volume of Wash Solution (twenty volumes) with nineteen volumes of deionized or distilled water to make Wash Solution (1 volume). At 2–8°C, the diluted wash solution lasts one month. Other reagents and the undiluted Wash Solution can be kept at 2–8 °C for 6 months.

3.2.8.4 Assay procedures according to the manufacturer's recommendations (mybiosource cat. no: mbs021993)

1. When commencing the test methods, make sure that the Plate, all of the reagents, and the samples have all reached room temperature, which is defined as a temperature that is between 18 and 25 degrees Celsius.
2. Prepare the wells to contain the blanks, standards, and samples respectively. There should be nothing added to any of the wells that are designated as Blank, and a volume of 50 µL should be added to each of the wells that are designated as Standard. Each sample well ought to have a material quantity of 50 µL. Add one hundred microliters of HRP-conjugate Reagent to each well in order to create blank wells. Incubate the plate at 37°C for an hour while it is covered with a membrane from a closure plate.
3. Wash each well, including the ones that are blank, a total of four times. The manual washing method is one of the available options: Dump the mixtures that were used to incubate the wells into a sink or another suitable waste receptacle. Utilizing either

a pipette or a squirt bottle, completely saturate each well with the Wash Solution (1%). After approximately a minute, flip the plate over and pound it on some absorbent sheets or paper towels until there is no trace of moisture left on the surface. Proceed in this manner for a total of four times.

4. Add 50 µL of Chromogen Solution A to each well, followed by 50 µL of Chromogen Solution B. (Keep Chromogen Solution B away from all sources of light.) After giving the plate a light stir and incubating it for 15 minutes at 37˚c, you should repeat the process. (The Plate should be shielded from light.) It is recommended that the substrates progress from colorless to a gradation of blue.
5. Add fifty microliters (50 µl) of the Stop Solution to each well. The color that has developed in the wells before the addition of the Stop solution will shift from blue to yellow once it has been introduced. After adding the Stop Solution, wait no more than 15 minutes before reading the optical density (O.D.) at 450 nm with an ELISA reader (around 5 minutes is usually the best time).

3.2.8.5 Calculation of Results

1. In order to subtract the average optical density of the Blank (OD0), it is necessary to do an arithmetic average of the duplicate readings for each standard and sample.
2. Utilizing professional curve fitting software allows for the creation of the standard curve (which is often a linear, quadratic, or cubic curve) as well as the determination of the analyte level.

3.2.9 Human Interleukin 8 (IL-8) (mybiosource cat.no: mbs 355339)

Kit components

1- One plate with 96 wells that has been coated in an anti-human IL-8 antibody in advance.
2- Lyophilized Human IL-8 standards consist of 2 tubes, each containing 10 ng.

3- 30 µl is the amount of sample and standard diluent buffer. 4. Biotin-conjugated anti-human IL-8 antibody (in a concentrated form): 130µl. Dilution:1:100
4- Antibody diluent buffer: (12mL)
5- The concentrated form of the Avidin-Biotin-Peroxidase Complex (ABC) is 130µl. Dilution: 1:100
6- ABC diluent buffer: (12mL)
7- TMB substrate: (10mL)
8- Avoid the solution: (10mL)
9- Wash buffer (25X): …..(30mL).

Samples collection and storage

1-Serum: It is recommended that the serum be allowed to clot at ambient temperature for approximately (4 hours). Perform a centrifuge for fifteen minutes at about (1000 g). Either do an analysis on the serum right away, or aliquot it and store it at -20 ° C.

2-Wash buffer: Wash buffer concentrated solution should be diluted with 25 times the volume of distilled water (1:25). (That is, put 30 mL of concentrated wash buffer into 720mL of distilled water).

Assay procedure

Equilibrate the ABC working solution and TMB substrate (Kit Component 8) for at least 30 minutes at room temperature (37°C) before adding to the wells. Plotting a standard curve for each test is advised.

1. Place an aliquot of standard solutions (0.1 mL) into each standard well. The concentrations of the standard solutions should be as follows: 1000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.2 pg/mL, and 15.6 pg/mL. Put (0.1 mL) of the Sample/Standard diluent buffer in the well designated as the control (which is zero) (Kit Component 3). The test sample wells need to be filled with 0.1

mL of a sample that has been adequately diluted (human serum), and the plate needs to be incubated at 37 degrees Celsius for 90 minutes while it is covered.

2. After removing the cover and emptying the plate's contents, place the plate upside down on absorbent filter papers or another material that may soak up liquid. To begin, add 0.1 mL of the biotin-conjugated anti-human IL-8 antibody work solution to each of the wells described above (standard, test sample and zero wells). It is recommended that the solution be added to the bottom of each well, away from the sides of the well. After you have covered the plate, place it in an incubator set to 37 degrees Celsius for an hour.
3. After removing the cover, perform three washes of the plate utilizing the Wash Buffer (Kit Component 10) manual washing is one of the methods that can be used: Do not let the solution in the plate come into contact with the side walls when you discard it. Clap the plate on top of some absorbent substance, like filter papers, for example. After completely filling each well with Wash buffer (Component 10 of the Kit), place the plate on an ELISA shaker and give it a gentle vortex for two minutes. After that, aspirate the contents of the wells, and then clap the plate on absorbent filter sheets or another absorbent material. The total of three washes comes from carrying out this process two more times.
4. The ABC working solution should be added to each well at a concentration of 0.1 mL. Cover the dish, and incubate it at 37 degrees Celsius for half an hour. After removing the cover, wash the plate five times using the wash buffer (Component 10 of the Kit), allowing the wash buffer to sit in each well for one to two minutes each time.
5. Put 0.1 mL of TMB substrate, which is component 8 of the kit, into each well, cover the plate, and incubate it at 37 Celsius in the dark for half an hour. The first three to four wells, which contain the most concentrated Human IL-8 standard solutions, are the only ones that display shades of blue. The other wells do not display any color that is easily distinguishable. Add 0.1 mL of the Stop solution (Kit Component 9) to each well, and then combine the contents of the wells completely. The hue shifts abruptly from blue to yellow.

6. Check the optical density absorbance at 450 nm in a microplate reader as soon as possible after applying the stop solution. The time limit for this check is 30 minutes. For the purpose of calculation, "the relative O.D.450" equals "the O.D.450 of each well" minus "the relative O.D.450" (the O.D.450 of Zero well). It is possible to plot the standard curve by comparing the relative optical density at 450 nm (Y) of each standard solution to the individual concentrations of each standard solution (X). It is possible to extrapolate, based on the standard curve, the human IL-8 concentration of the samples.

3.2.10 Human Interleukin 10 (IL-10) (mybiosource cat.no: mbs355300)

Kit components

1. One plate with 96 wells that has already been coated with an antibody against human IL-10.
2. Lyophilized Human IL-10standards call for two tubes of ten nanograms each.
3. 30 milliliters is the volume of the sample and standard buffers.
4. 130 ul of a biotin-conjugated anti-human IL-10 antibody that has been concentrated. Dilution: 1:100.
5. Antibody diluent buffer: (12mL)
6. The concentrated form of the Avidin-Biotin-Peroxidase Complex (ABC) is 130 ul. Dilution: 1:100
7. ABC diluent buffer: (12mL)
8. TMB substrate: (10mL)
9. Avoid the solution: (10mL)
10. Cleanse the buffer (25X): (30mL).

Preparation of sample and reagents

1-Serum: The serum can be allowed to coagulate either at (4°C) for the entire overnight period or at room temperature for approximately 4 hours. Centrifuge at about for ten minutes (1000 g). Either do an analysis on the serum right away, or aliquot it and store it at -20 degrees Celsius.

2-Wash buffer: 25 times as much distilled water should be added to the concentrated Wash buffer (1:25). (that is, include 30 milliliters of highly concentrated wash buffer into 720 milliliters of distilled water).

Assay procedure

Before adding to wells, equilibrate the ABC working solution and TMB substrate (Kit Component 8) at 37°C for 30 minutes. Each test should have a standard curve.

1. The standard wells should each have 0.1 mL aliquots of standard solutions with the following concentrations: 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.3 pg/mL, 15.6 pg/mL, and 7.8 pg/mL. 3-Pour 0.1 milliliters of the Sample/Standard diluent buffer into the control well, which is labeled "zero" (Kit Component 3). Fill the test sample wells with 0.1 milliliters of a sufficiently diluted sample (human serum), and then incubate the plate at 37 degrees Celsius for 90 minutes while covering it with a lid. This step is essential.
2. After removing the cover and emptying the plate's contents, place the plate upside down on absorbent filter papers or another material that may soak up liquid. To begin, add 0.1 milliliters of the biotin-conjugated anti-human IL-10 antibody work solution to each of the wells described above (standard, test sample and zero wells). It is strongly suggested that the solution be poured to the base of each well, keeping it away from the well's edges. After you have finished covering the plate, put it for an hour in an incubator with the correct temperature to 37 degrees Celsius.
3. Remove the plate's cover, and using one of the methods, which is manual cleaning, wash the plate three times with the Wash Buffer (Kit Component 10). Do not let the

solution in the plate come into contact with the side walls when you discard it. Place the plate upside down on top of some absorbent material, such filter papers. After completely filling each well with Wash buffer (Component 10 of the Kit), place the plate on an ELISA shaker and give it a gentle vortex for two minutes. After that, aspirate the contents of the wells, and then clap the plate on absorbent filter sheets or another absorbent material. The total of three washes comes from carrying out this process two more times.

4. The ABC working solution should be added to each well at a concentration of 0.1 milliliter. Cover the dish, and incubate it at 37 degrees Celsius for half an hour. After removing the cover, wash the plate five times using the wash buffer (Component 10 of the Kit), allowing the wash buffer to sit in each well for one to two minutes each time.

5. Put 0.1 milliliters of TMB substrate, which is component 8 of the kit, into each well, cover the plate, and incubate it at 37 degrees Celsius in the dark for half an hour. The first three to four wells, which contain the most concentrated Human IL-10 standard solutions, are the only ones that display shades of blue. The other wells do not display any color that is easily distinguishable. Add (0.1 mL) of the Stop solution (Kit Component 9) to each well, and then thoroughly mix the contents of the wells. The hue shifts abruptly from blue to yellow.

6. Check the optical density absorbance at 450 nm in a microplate reader as soon as possible after applying the stop solution. The time limit for this check is 30 minutes. For the purpose of calculation, "the relative O.D.450" equals "the O.D.450 of each well" minus "the relative O.D.450" (the O.D.450 of Zero well). It is possible to plot the standard curve by comparing the relative optical density at 450 nm (Y) of each standard solution to the individual concentrations of each standard solution (X). Interpolation from the standard curve allows one to determine the Human IL-10 concentration of the samples.

3.2.11 Human Interleukin TNF-α (Tumor Necrosis Factor Alpha) (mybiosource cat.no: mbs2502004)

Kit components and storage

Kits can be kept at 4°C for a month without being opened. Once the kit is received, store the components individually in accordance with the guidelines below if they won't be used within a month (Table 3.14).

Table 3.14 Kit components and Storage

NO	Item	Specification	Storage
1	Micro ELISA Plate (Dismountable)	96T: 8 Wells × 12 strips	-20°C, 6 months
2	Reference Standard	96T: 2 vials	-20°C, 6 months
3	Concentrated Biotinylated Detection Ab (100×)	96T: 1 vial, 120 µL	-20°C, 6 months
4	Concentrated HRP Conjugate (100×)	96T: 1 vial, 120 µL	-20°C (Protect from light)
5	Reference Standard and Sample Diluent	1 vial, 20 mL	4°C, 6 months
6	Biotinylated detection Ab Diluent	1 vial, 14 mL	4°C, 6 months
7	HRP Conjugate Diluent	1 vial, 14 mL	4°C, 6 months
8	Concentrated Wash Buffer (25)	1 vial, 30 mL	4°C, 6 months
9	Substrate Reagent	1 vial, 10 mL	4°C (Protect from light)
10	Stop Solution	1 vial, 10 mL	4°C
11	Plate Sealer	5 Pieces	4°C
12	Product Description	1 copy	4°C
13	Certificate of Analysis	1 copy	4°C

Sample collection and reagents

1-Serum: Prior to centrifuging for 15 minutes at (1000 g) at (4°C), allow samples to clot for 2 hours at room temperature or overnight at (4°C). To conduct the assay, collect the supernatant.

2-Wash Buffer: To make (750 mL) of Wash Buffer, Dilute (30 mL) of Concentrated Wash Buffer with (720 mL) of deionized or distilled water.

Assay procedure

1. Put 100 microliters of either the standard or the sample into each well. Incubate for an hour and a half at 37 °C. Take care not to spill it. Add one hundred microliters of the working solution for the biotinylated detection antibody. To cover, make use of the Plate sealer. Combine in a mild manner. Incubate for one hour at a temperature of 37 Celsius.
2. After aspirating or decanting the solution from each well, add 300ul of wash buffer to each well. Aspirate or decant the solution from each well after it has been allowed to soak for half a minute, and then wipe each well dry with some new absorbent paper. Perform the step of washing your hands three times. Add one hundred microliters (100 uL) of the HRP Conjugate working solution to each well. To cover, make use of the Plate sealer. Incubate for half an hour at 37 degrees Celsius.
3. The solution should be removed from each well by aspiration or decantation, and the washing procedure described in step 3 should be carried out five times.
4. Add 90uL of the substrate reagent to each well. When covering the plate, apply a new plate sealer. Incubate for approximately 15 minutes at 37 ° C. obstruct the light from reaching the plate. Add 50uL) of the Stop Solution to each well.
5. Make use of a microplate reader set to 450 nm and perform simultaneous calculations to determine the optical density (OD value) of each well.

Calculation of results

After taking the average of the duplicate readings for each standard and sample, deduct the average value of the zero standard's optical density. Draw a four-parameter logistic curve on a piece of log-log graph paper, positioning the optical density values along the y-axis and the standard concentration along the x-axis.

3.2.12 Statistical analysis

In this investigation, the statistical software program known as spss version (26) was utilized to determine the chi-square, frequency, and percentage, in addition to locating the significant differences that existed between the variables and having a p-value < 0.05.

4. RESULTS AND DISCUSSION

4.1 Pathogenic Microorgansims in Vaginitis Women

In Table 4.1 and Figure 4.1 explain the different pathogenic in vaginitis women, most them were mix infection between bacterial vaginosis, *Cytomegalovirus* and *Escherichia coli*, after screening 200 samples of vagianl swabs isolated from vaginitis women, the high rate was mix infection 72 (36.0%) and low rate *Cytomegalovirus* 53 (26.5%), *Escherichia coli* 50 (25.0%), no infection 25 (12.5%).

Table 4.1 Pathogenic microorgansims in vaginitis women

Infection	Frequency	%	Mean ±SD
No infection	25	12.5	2.22±1.467
E.coli	50	25.0	
CMV	53	26.5	
Mix infection	72	36.0	
Total	200	100%	

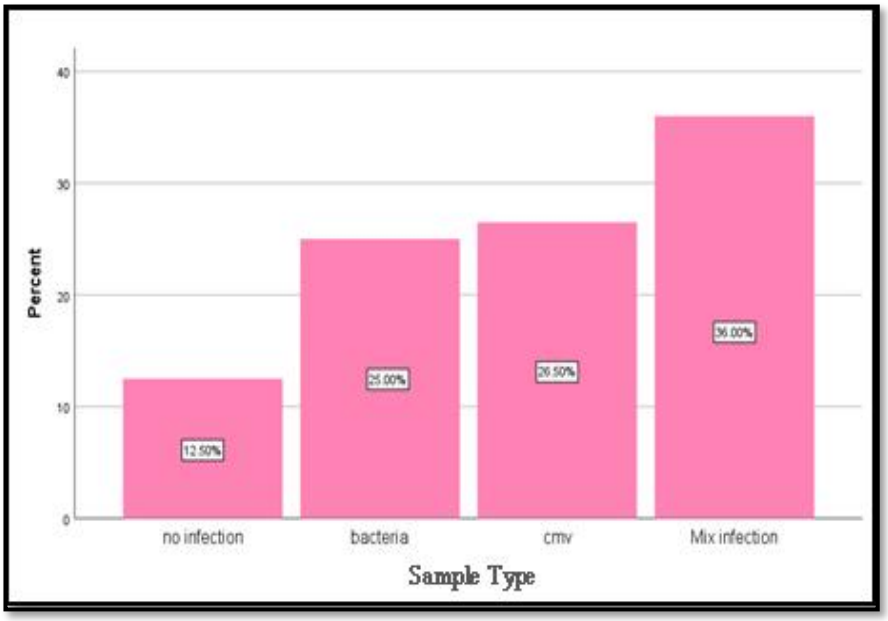

Figure 4.1 Pathogenic Microorgansims in Vaginitis Women

According to pathogenic microorgansims in Vaginitis Women was recorded a high rate with mix infection as 72(36.0%) the current study was lower than the reported result (Shrestha *et al.* 2011) who was recorded a high rate with mix infection in Nepal (39.0%) in among women seeking medical care due to complicated interactions between opportunistic microorganisms that live in both aerobic and anaerobic environments (Han *et al.* 2019). (Maladkar *et al.* 2015) who was reported the presence of mixed infections is one of the most important variables that makes diagnosing and treating vaginitis more difficult, so it has been significance of culture-based diagnosis is highlighted by the possibility of infection recurrence in cases of inaccurate diagnosis or failure to recognize mixed illnesses (Fan *et al.* 2013). (Kazi *et al.* 2012) this agrees with the present study which recorded *E.coli* as 50(25.0%), since it was determined that the total prevalence of *E.coli* in the high vaginal swab samples tested was 25%. According to the findings of a number of studies, *E.coli* is a common pathogen that can be found in a diverse range of pathogenic microorganisms. Infection with *E.coli* can result in a number of negative outcomes for both the mother and the fetus, including spontaneous abortion, neonatal hemolytic uremic syndrome, intrauterine growth restriction, and even the death of the fetus (Tempera *et al.* 2006, Sacerdoti *et al.* 2018). The current investigation found that CMV 53 (26.5% of cases) is the second most common outcome. This study agrees with (Begnel *et al.* 2021). (Yamada *et al.* 2018) who showed infection with HCMV has been connected to pregnancy outcomes such as low birth weight, premature birth, antepartum hemorrhage, prenatal hypertension, megaloblastic anemia, and deformity, according to earlier investigations.

4.2 Age Groups with Vaginitis Women

The age categories at which vaginitis women occur are described in Table 4.2. In this study, *Escherichia coli* a high percent was recorded in age (20-29) years 15(30%) and (30-39) years 15(30%), the low rate was in age (60-69) years 1(2%). *Cytomegalovirus* is the high rate was recorded in age (20-29) years 17(32.1%) and the low rate was in age (60-69) years 1(1.9%). Mix infection was a high percentage and recorded in age group (20-29) years 31(43.1%) and the low rate was in age (60-69) years 0(0%).

Table 4.2 Age groups with vaginitis women

Variables	No infection	*E.coli*	CMV	Mix infection	Total	Chi2 with p- value
Age	%	%	%	%	%	
15-19	0	2(4)	3(5.7)	4(5.6)	9(4.5)	12.07
20-29	5(20)	15(30)	17(32.1)	31(43.1)	68(34)	
30-39	6(24)	15(30)	13(24.5)	18(25)	52(26)	
40-49	8(32)	11(22)	14(26.4)	12(16.7)	45(22.5)	0.33
50-59	5(20)	6(12)	5(9.4)	7(9.7)	23(11.5)	No significant
60-69	1(4)	1(2)%	1(1.9)	0	3(1.5)	
Total	25(100)%	50(100)%	53(100)	72(100)%	200(100)%	

In the present study, different age group range from (15-69) years were reported in Table 4.2. women in the age group (20-29) years was reported a high rate of the Mix infection 31(43.1%) and in the age group (20-29) (30-39) years was reported a high rate of the *Escherichia coli* 15(30%), this due the higher frequency of unprotected sexual contact between newlywed teenage and young women contribute to the incidence of BV in younger women by altering the vaginal environment and raising the likelihood of BV (Vallor *et al.* 2001). There are additional factors, such as pH changes during the menstrual cycle, which alter the natural flora and encourage the growth of bacteria in the vagina (Winberg *et al.* 1974). *Cytomegalovirus* in the age group (20-29) years was reported a high rate as 17(32.1%) due to the two primary times of increased CMV infection over the course of a lifetime are the perinatal period and the years of sexual maturation. A significant method of transmission is through sexual activity, particularly in nations with a high percentage of seronegative people (Jindal and Aggarwal 2005). (Hizel *et al.* 1999) who found CMV reacti*Vat*e due to altered physiological circumstances and weakened immunity during pregnancy, As a result, seropositive mothers can still deliver CMV-infected children through reinfection during pregnancy or the reacti*Vat*ion of the latent virus.

4.3 Education Levels with Vaginitis Women

A high percentage of vaginitis women with primary education had been documented a high percentage rate of infection, In primary education the high rate was recorded in Mix infection 45(62.5%) and the low percent in *Cytomegalovirus* 28(52.8%), In

secondary education the high rate was in Mix infection 9(12.5%) while, the low rate in *Cytomegalovirus* 6(11.3%), In higher education high rate in *Cytomegalovirus* 19(35.8%) and the low rate in *Escherichia coli* 13(26%) (Table 4.3).

Table 4.3 Education levels with vaginitis women

Variables	No infection	*E.coli*	CMV	Mix infection	Total	Chi2 with p- value
Education	%	%	%	%	%	
Primary	14(56)	30(60)	28(52.8)	45(62.5)	117(58.5)	2.17 0.45 No Significant
Secondary	3(12)	7(14)	6(11.3)	9(12.5)	25(12.5)	
Higher	8(32)	13(26)	19(35.8)	18(25)	58(29)	
Total	25(100)%	50(100)%	53(100)	72(100)	200(100)%	

This data is consistent with those of (Zhang *et al.* 2010, Vink *et al.* 2012) that show that higher levels of education and employment are associated with lower rates of vaginitis in pregnant women, perhaps because more educated women are more likely to seek medical assistance when they become ill (Culhane *et al.* 2001). Women with lower socioeconomic status, lower levels of education, the lack of a female consultant at the health service center, a reluctance to seek medical assistance, and societal and cultural norms all contribute to the disproportionately high rate of vaginitis among women with lower levels of education, as found by (Fiscella and Klebanoff 2004). Regarding to education level of CMV in vaginitis women. It was seen that women with primary education was recorded a high level was 28(52.8%) that due to lack of education results in a lack of health awareness programs and is one of the leading causes of CMV infection in women with primary education and poor hygiene (AlShammmari and Hummoud 2021).

4.4 Residence Associated with Vaginitis Women

According to the residence associated with vaginitis women as in Table 4.4, was seen that the women live in rural recorded high percentage in Mix infection 41(56.9%) and low rate in *Cytomegalovirus* 31(58.5%), in city the high rate was in Mix infection 31(43.1%) and the low rate was in *Escherichia coli* 15(30%).

Table 4.4 Residence associated with vaginitis women

Variables	**No infection**	***E.coli***	**CMV**	**Mix infection**	**Total**	**Chi2 with p-value**
Residences	%	%	%	%	%	
Rural	14(56)	35(70)	31(58.5)	41(56.9)	121(60.5)	2.57 0.23
City	11(44)	15(30)	22(41.5)	31(43.1)	79(39.5)	
Total	25(100)	50(100)	53(100)	72(100)	200(100)%	

In the current study, in rural with Mix infection was recorded high rate 41(56.9%) and *Cytomegalovirus* was recorded low rate in 31(58.5%) and in the city women with Mix infection was recorded high rate 31(43.1%) and *Escherichia coli* was recorded low rate 15(30%) as in Table 4.4. The current study agree with (Shrestha *et al.* 2011) who found that women coming from rural areas made up the majority of the cases of BV and higher proportion the reason due to poor hygienic habits, a lack of time to maintain their health, a low level of living, ignorance, and difficulty getting to instant medical services (Kadir *et al.* 2014).

(Enright and Prober 2004) who reported residents of underdeveloped nations had greater rates of CMV seropositivity than citizens of developed countries, according to an analysis of CMV seroprevalence research carried out around the world. In poor nations and the lower socioeconomic echelons of affluent nations, CMV is typically acquired earlier in life this agree with this study (Seale *et al.* 2006) since the *Cytomegalovirus* is unstable in the environment, it must be transmitted through close or pri*Vat*e contact so one of the main factors of transmission is crowding (Hizel *et al.* 1999).

4.5 Abortion Associated with Vaginitis Women

In Table 4.5 show abortion associated with vaginitis women, In no abortion occurred was recorded high rate in Mix infection 21(29.2%) wich was not abortion occurred and low rate in *Cytomegalovirus* 17(32.1%), In yes abortion occurred the high rate was in Mix infection 51(70.8%) and the low rate was in *Escherichia coli* 32(64%).

Table 4.5 Abortion associated with vaginitis women

Variables	No infection	*E.coli*	CMV	Mix infection	Total	Chi2 with p-value
Abortion	%	%	%	%	%	0.788 0.42 No Significant
No	9(36)	18(36)	17(32.1)	21(29.2)	65(32.5)	
Yes	16(64)	32(64)	36(67.9)	51(70.8)	135(67.5)	
Total	25(100)	50(100)	53(100)	72(100)	200(100)	

Early in pregnancy, spontaneous abortion is a common issue (Wang *et al.* 2004). In present study, abortion with vaginitis women the high rate was in Mix infection 51(70.8%) and the low rate was in *Escherichia coli* 32(64%) in table 4.6. The current study was high rate then the reported result in (Chard 1991) who found even more severe, the loss of a subclinical pregnancy is estimated to be 60% based on the measurement of human chorionic gonadotrophin levels. According to recent studies, women with BV during pregnancy had a two- to three-fold higher risk of spontaneous abortion than those without BV (Ugwumadu *et al.* 2003).

In addition, it was proven by Meningistie *et al.* (2014) that BV was present in expecting moms who had a history of spontaneous abortion (Mengistie *et al.* 2014). Some authors suggested that BV cause miscarriages in the first trimester, while others claimed that BV infection in the early stages of pregnancy induces miscarriages in the second trimester and preterm labor. On the other hand, some authors claimed that BV infection in the early stages of pregnancy induces miscarriages and preterm labor (Ugwumadu *et al.* 2003). Abortion with *Cytomegalovirus* was 36(67.9%).

This current data agree with (Meng *et al.* 2011) who found that unusual uterine cavities, parental karyotypes, endocrine variables, infections, and autoimmune diseases can all result in abortions and this is related to how the *Cytomegalovirus* affects the development of the fetus in the uterus and the causative moral virus inflectional, or weak immunity, have contraception, change the natural flora in the uterus and vagina, and promote viral to the rising (Teissier *et al*. 2014).

4.6 Virulence Genes and *16srRNA* in *Escherichia coli* in Vaginitis Women

In Table 4.6 and Table 4.7, show virulence genes in *Escherichia coli* in vaginitis women by used PCR technology. In this study, DNA was found in all *Escherichia coli* strains had a high percentage rate was recorded in positive 50(100%). while, the four genes of *Escherichia coli* (*16srRNA*, *Tra t*, *Vat*, *Fim H*), in positive, the high rate was recorded in gene *16srRNA* 50(100%) and the low percent in gene *Vat* 9(18%). In negative the high rate was in gene *Vat* 41(82%) and the low rate in gene *16srRNA* 0(0%) (Figure 4.2, Figure 4.3, Figure 4.4, Figure 4.5, Figure 4.6).

Table 4.6 Virulence genes and *16srRNA* in *Escherichia coli* in vaginitis women

Name of Genes	Frequency		Total%
	Positive %	Negative %	
DNA results	50(100)	0	50(100)
16sRNA	50(100)	0	50(100)
Tra t	32(64)	18(36)	50(100)
Vat	9(18)	41(82)	50(100)
Fim H	47(94)	3(6)	50(100)

According to virulence genes in *Escherichia coli* the high rate was in gene *16srRNA* 50(100%) the current data agree with (Budding *et al.* 2010) due to the currently the gold standard for microbiome profiling is 16S rRNA gene sequencing it has been widely utilized for a variety of purposes to investigate bacterial taxonomy using the 16S rRNA gene sequence. These include first, practically all bacteria contain it, frequently in the form of multigene families or operons. Secondly, the fact that the 16S rRNA gene's function has remained constant over time, indicating that random sequence changes are a more accurate gauge of time (evolution) (Bosshard *et al.* 2003).

Gene *Vat* rate was 9 (18%), This outcome was consistent with earlier research where *Vat* gen accounted (18.1%) (Nichols *et al.* 2016) that cause a number of cytopathic effects, such as swelling and vacuolation, can be induced in target host cells by the vacuolating autotransporter toxin (*Vat*) (Ewers *et al.* 2007).

Table 4.7 PCR Result Gene of *Escherichia coli* in vaginitis women

Sample	DNA Result	PCR Result Gene traT 290 bp	PCR Result Gene *Vat* 981 bp	PCR Result Gene FimH 508 bp	PCR Result Gene *16srRNA* 1250 bp
1	+	+	-	+	+
2	+	+	-	+	+
3	+	+	-	-	+
4	+	+	-	+	+
5	+	+	+	+	+
6	+	+	+	+	+
7	+	+	+	+	+
8	+	+	+	+	+
9	+	-	-	+	+
10	+	-	-	+	+
11	+	-	-	+	+
12	+	-	+	+	+
13	+	+	+	+	+
14	+	+	-	+	+
15	+	+	-	+	+
16	+	-	+	+	+
17	+	+	-	+	+
18	+	+	-	+	+
19	+	+	-	+	+
20	+	-	-	+	+
21	+	+	+	+	+
22	+	+	-	+	+
23	+	-	-	+	+
24	+	+	-	+	+
25	+	+	-	+	+
26	+	+	-	+	+
27	+	+	-	+	+
28	+	-	+	+	+
29	+	-	-	+	+
30	+	+	-	+	+
31	+	+	-	+	+
32	+	-	-	-	+
33	+	+	-	+	+
34	+	-	-	+	+
35	+	-	-	+	+
36	+	+	-	+	+
37	+	-	-	+	+
38	+	-	-	+	+
39	+	-	-	+	+
40	+	+	-	+	+
41	+	+	-	+	+
42	+	+	-	+	+
43	+	+	-	+	+
44	+	+	-	+	+
45	+	-	-	-	+
46	+	+	-	+	+
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50	+	-	-	+	+

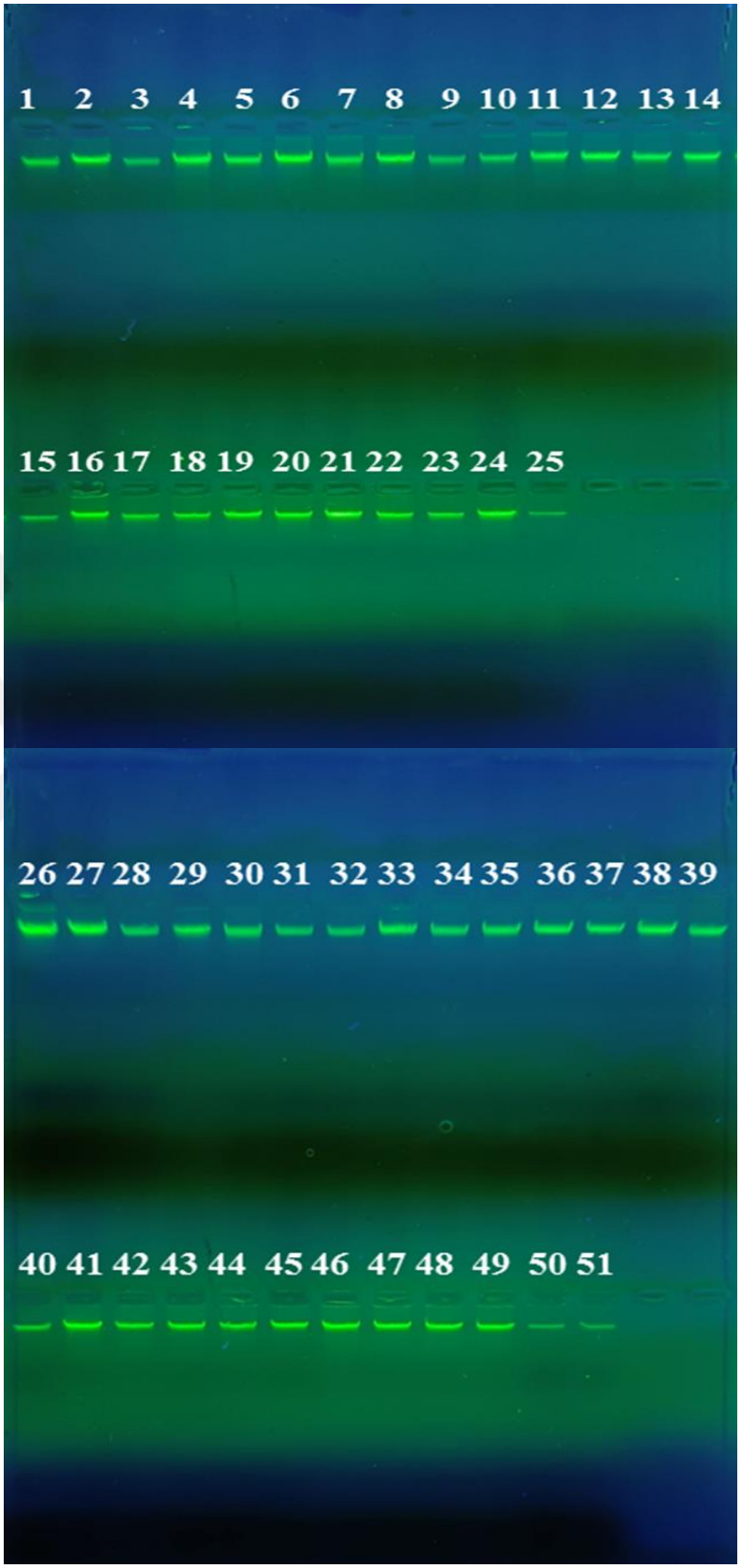

Figure 4.2 Gel electrophoresis of genomic DNA extraction from Bacteria, 1% agarose gel at 5 vol /cm for 30 min

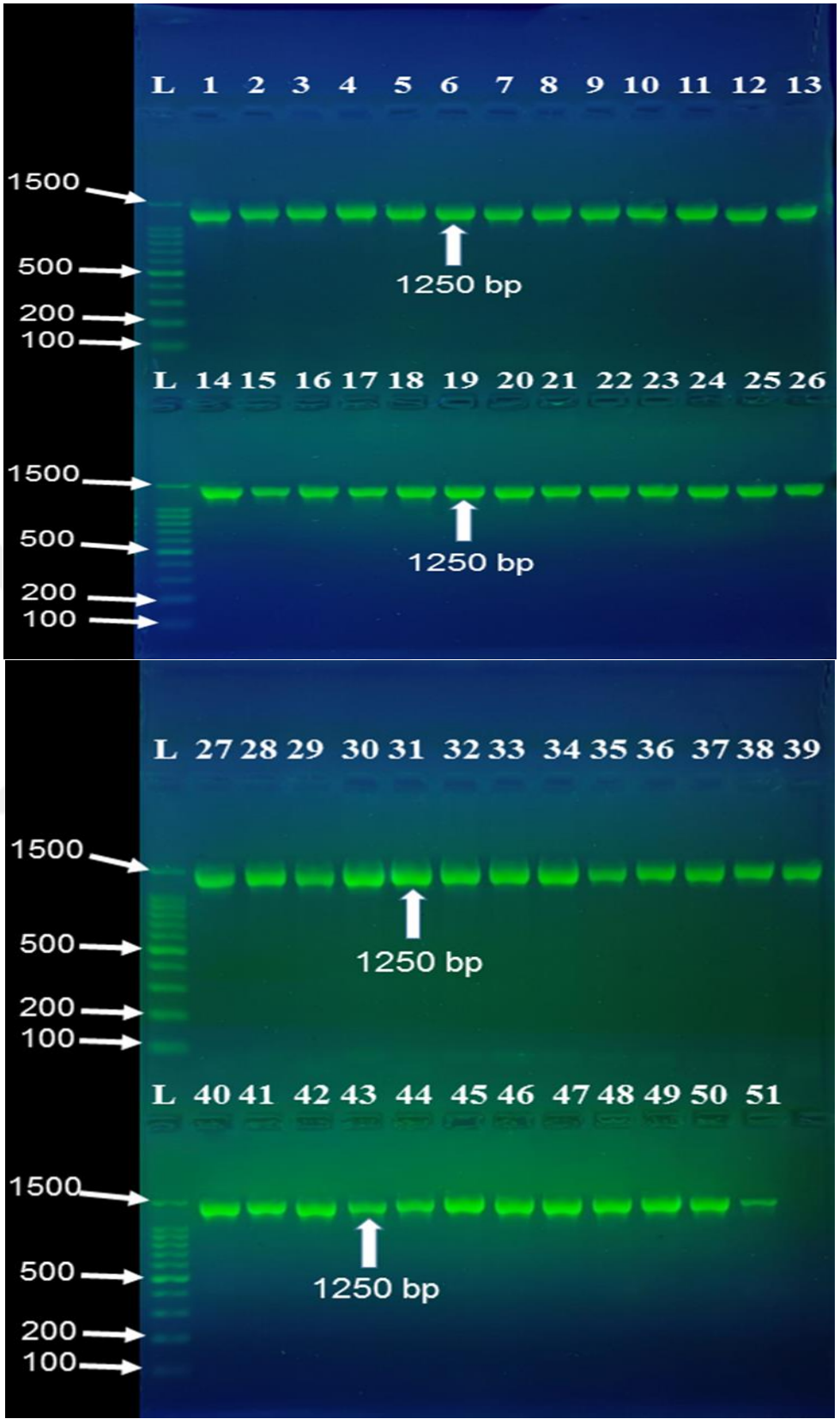

Figure 4.3 PCR band size 16sRNA 1250bp. At 5 volt/cm2, 1.5% agarose electrophoresed the product. 1x 1:30 TBE buffer. DNA ladder (100)

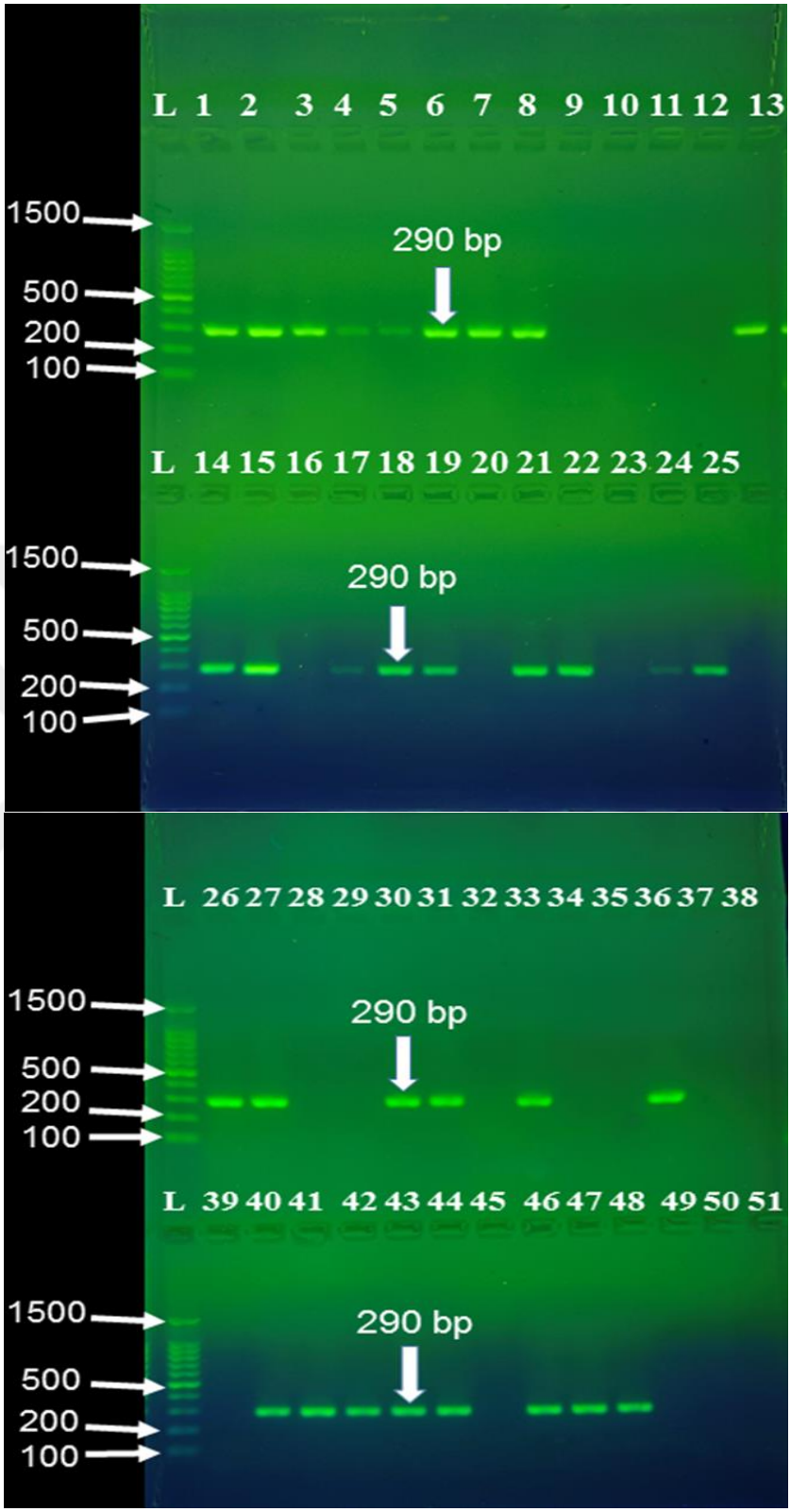

Figure 4.4 Tra-t 290 bp PCR product. 1.5% agarose was electrophoresed at 70 volt/cm2. 1x 1:30 TBE buffer. DNA ladder (100)

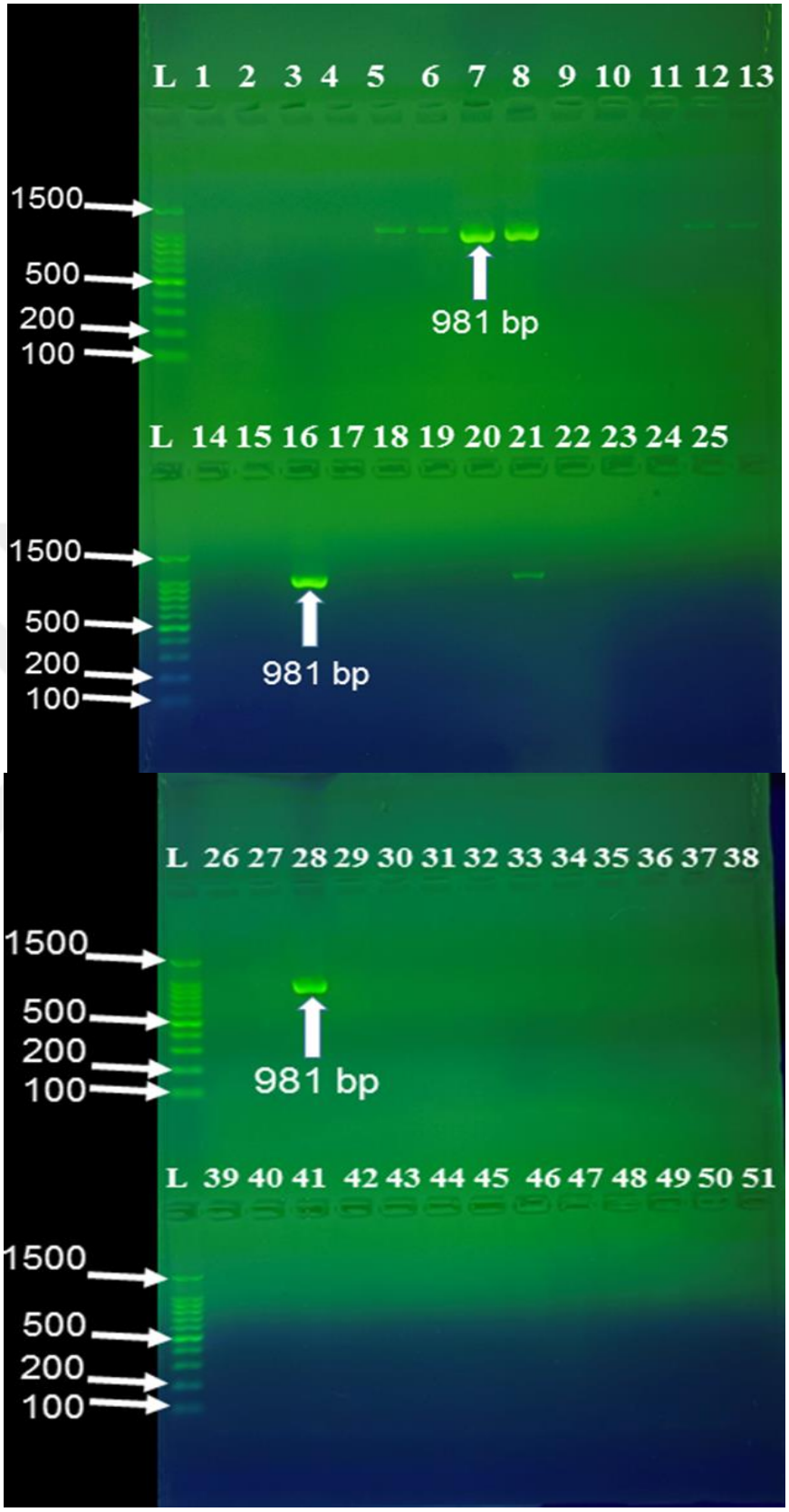

Figure 4.5 *Vat* 981 bp PCR product. 1.5% agarose was electrophoresed at 70 volt/cm2. 1x 1:30 TBE buffer. DNA ladder (100)

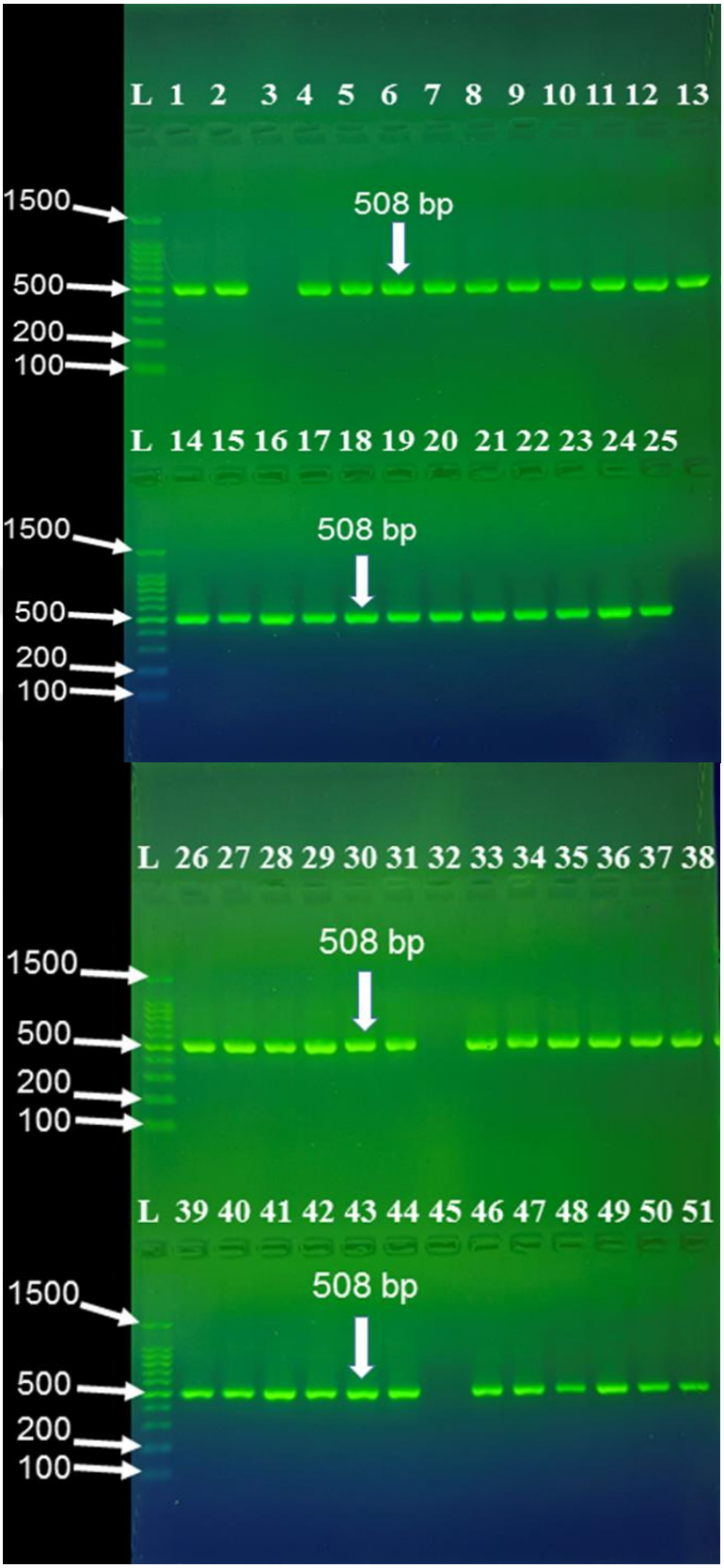

Figure 4.6 508 bp PCR product. 1.5% agarose was electrophoresed at 70 volt/cm2. 1x 1:30 TBE buffer. DNA ladder (100)

4.7 Antibiotic Resistances And Sensitive Of *Escherichia coli* In Vaginitis Women

In Table 4.8, show the susceptibility of every isolate of *E.coli* in vaginitis women to antimicrobial drugs was testested. *E.coli* strains was recorded the highest rate of resistances Ampicillin 40 (80%) and the lowest rate Meropenem 0(0) %, intermedium antibiotics was the high rate Fosfomycin 2(4%) and the low rate was Piperacillin 0(0) % Ceftazidime 0(0) %, Cefepime 0(0)%, Ertapenem 0(0)%, Imipenem 0(0)%, Meropenem 0(0)%, Amikacin 0(0)%. In addition, all *E.coli* strain was the highest rate sensitive Meropenem 50(100%) and the lowest rate was Ampicillin 9(18%).

Table 4.8 Antibiotic resistances and sensitive of *Escherichia coli* in vaginitis women

Name of antibiotics	Resistances R	Intermedium I	Sensitive S
Ampicillin	40 (80)%	1(2)%	9(18)%
Amoxicillin	15 (30)%	1(2)%	34(68)%
Piperacillin	33(66)%	0	17(34)%
Ceftazidime	30(60)%	0	20(40)%
Cefepime	30(60)%	0	20(40)%
Ertapenem	2(4)%	0	48(96)%
Imipenem	1(2)%	0	49(98)%
Meropenem	0	0	50(100)%
Amikacin	1(2)%	0	49(98)%
Gentamicin	3(6)%	1(2)%	46(92)%
Ciprofloxacin	8(16)%	1(2)%	41(82)%
Norofloxacin	8(16)%	1(2)%	41(82)%
Fosfomycin	5(10)%	2(4)%	43(86)%
Nitrofurantoin	3(6)%	1(2)%	46(92)%
Trimethoprim	29(58)%	1(2)%	20(40)%

Vaginal *E.coli* colonization a risk factor for pregnancy problems, particularly if these strains are ampicillin-resistant and exhibit numerous virulence characteristics that lead to infection or treatment failure and these strains have a negative clinical effect and have been linked to obstetric and neonatal infections (Watt *et al.* 2003, Saez-Lopez *et al.* 2013).

Al-Mayahie (2013b) who found these colonizing isolates' development of resistance serve as a reservoir for infections in both the mother and the newborn, affecting their ability to receive therapy. In the present study, antibiotic of *Escherichia coli* strains in

vaginitis women was high rate of resistances Ampicillin as 40 (80%). This study agree with (Hilbert *et al.* 2009) who reported that (80.8%) of *E.coli* strains were thought to be ampicillin-resistant. That due to antibiotic use, overuse, and misuse are creating selective pressures that are causing antibiotic resistance to gradually rise and the formation of multidrug-resistant bacterial strains. Previously treatable bacterial diseases are now frequently incurable or necessitate the use of antibiotics as a last resort (Sherchan *et al.* 2015).

Escherichia coli was highest rate sensitive Meropenem 50(100%). The current data agree with (Narayana-Swamy *et al.* 2015) who reported that *Escherichia coli* was most sensitive to meropenem (100%) due that meropenem more recent antibiotic, is both pricey and highly effective. Proven to be efficient in delivering a clinical and microbiological cure through clinical trials (Patel *et al.* 2008, Fuchs *et al.* 2007).

Intermedium antibiotics of *Escherichia coli* was Fosfomycin the high rate as 2(4%) the current study agree with (Tseng *et al.* 2015) who reported according to a study, Fosfomycin (FOM) intermediate *E.coli* strains are more frequently antimicrobial drug resistant than Fosfomycin susceptible ones. It has been established that Fosfomycin resistance occurs infrequently. And because Fosfomycin has a distinct method of action, it has been predicted that the frequency of cross-resistance between Fosfomycin and other antibiotics is quite low.

4.8 Immunoglobulin (Igm And Igg) of *Cytomegalovirus* in Vaginitis Women

Regarding to immunoglobulin of *Cytomegalovirus* in vaginitis women in Table 4.9. Women who tested positive for IgM had already contracted the virus or had it reacti*Vat*e, but those who tested positive for IgG were found to have recently contracted CMV. In positive, the high rate was recorded in IgG 53(100%) and the low rate in IgM 0(0%). In negative the high rate was in IgM 53(100%) and the low percent in IgG 0(0%).

Table 4.9 Immunoglobulin (IgM and IgG) of *Cytomegalovirus* in vaginitis women

Type of Antibodies	Negative <0.7	Equivocal 0.7-0.8	Positive ≥0.9	Mean ±SD
IgM	53(100) %	0	0	0.025±0.078
	Negative <4	Equivocal 4-5	Positive ≥6	Mean ±SD
IgG	0	0	53(100)%	34.32±10.827

In the present study, immunoglobuline of CMV in vaginitis women was IgM 0(0%) this result was similar to study in Iran, the prevalence of positive IgM was 0.06% (Sharghi *et al.* 2019) who reported that IgM is an antibody whose titer rises with the beginning of sickness before IgG does. Additionally, during the stages of active infection and reinfection, this antibody is generated. Among women of childbearing age, the prevalence of CMV antibodies varies substantially depending on the demographic. Immunoglobulin of *Cytomegalovirus* was IgG 53(100%). This current data agree with (Brooks *et al.* 2001) who reported the incidence of CMV IgG antibodies is higher in developing nations (90–100%), according to reports. Because, it was discovered that women from rural backgrounds had higher IgG antibody levels than women who lived in metropolitan areas (De Jong *et al.* 1998).

4.9 Interleukins of *Cytomegalovirus* in Vaginitis Women

In Table 4.10 show Interleukins of *Cytomegalovirus* in vaginitis women by used ELISA technology. In this study, Mean ±Std Deviation of Interleukin was recorded in IL-6 (0.79730±0.245659), IL-8 (0.69881±0.281192), IL-10 (0.81726±0.307895) and TNF-α (0.54758±0.284395) (Figure 4.7, Figure 4.8, Figure 4.9, Figure 4.10).

Table 4.10 Interleukins of *Cytomegalovirus* in vaginitis women

Type of Interleukins	**Mean ±Std Deviation**
IL-6	0.79730±0.245659
IL-8	0.69881±0.281192
IL-10	0.81726±0.307895
TNF-α	0.54758±0.284395

In present study, Interleukins of *Cytomegalovirus* in vaginitis women was recorded in IL-6 (0.79730±0.245659), IL-8 (0.69881±0.281192), TNF-α (0.54758±0.284395) in table (4-10) these was the most important proinflammatory cytokines that are present during the beginning of an infection. These results was low from the reported results with (Geist and Dai 1996) who reported that IL-6 (281.2 ± 85.5), IL-8 (167.2 ± 38.0), TNF-α (10.4 ± 1.2) due that a direct correlation between increased serum cytokine levels and the emergence of both active CMV illness and asymptomatic CMV infection.

Although IL-8 and TNF-a were demonstrable, serum IL-6 levels show the clearest correlation. The observed cytokine increases could be a result of particular immune system interactions with CMV or they could be a result of a general acute-phase response. (Lee *et al.* 1999, Capobianchi *et al.* 1997) who found that cytokine-stimulated transcription of HCMV late genes prior to DNA replication IL-8 is able to cause HCMV replication and initiation of early HCMV replication events. It has also been demonstrated that IL-6 levels rise while human CMV replication (Gealy *et al.* 2005).

Interleukin (IL-10), an important anti-inflammatory cytokine whose levels increase during acute and chronic infection, such as the ones it secretion and discovered in both symptomatic and asymptomatic CMV infection associated to a weakening of the antiviral defense, and it play a substantial role in the immunosuppression experienced during CMV infection, was found. This result was lower than the finding in (Nason *et al.* 2016), who reported interleukin IL-10 was (1.14). The biological effects of IL-10 are determined by the ways in which it interacts with other cytokines, notably cytokines that promote inflammation such as TNF- α (Mosman 1994).

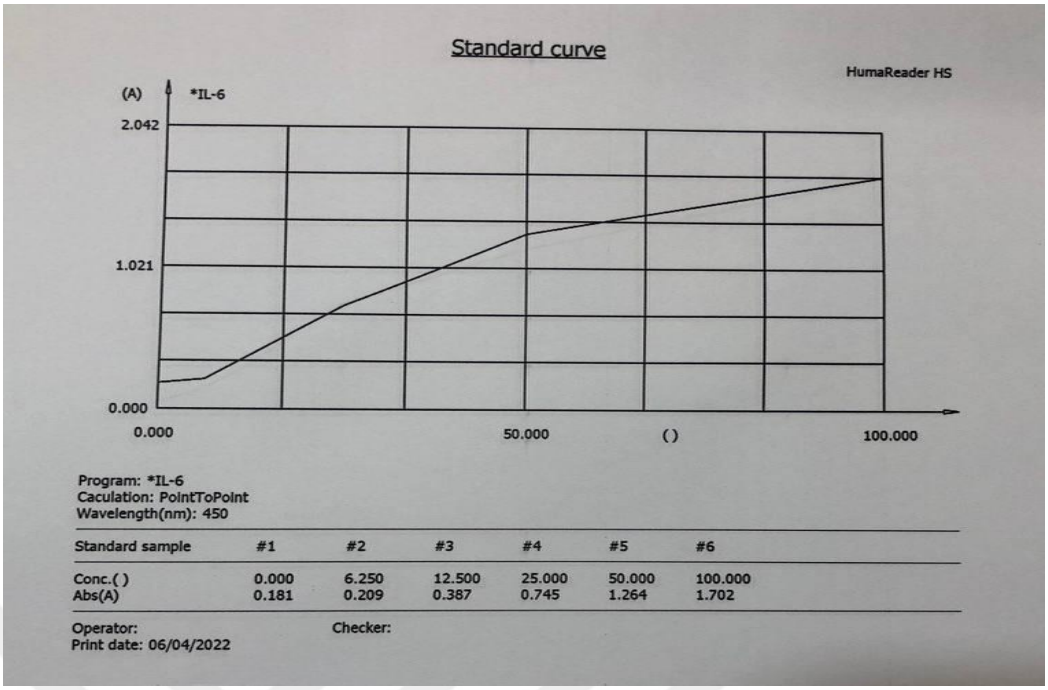

Figure 4.7 Standard curve for the determination of IL-6

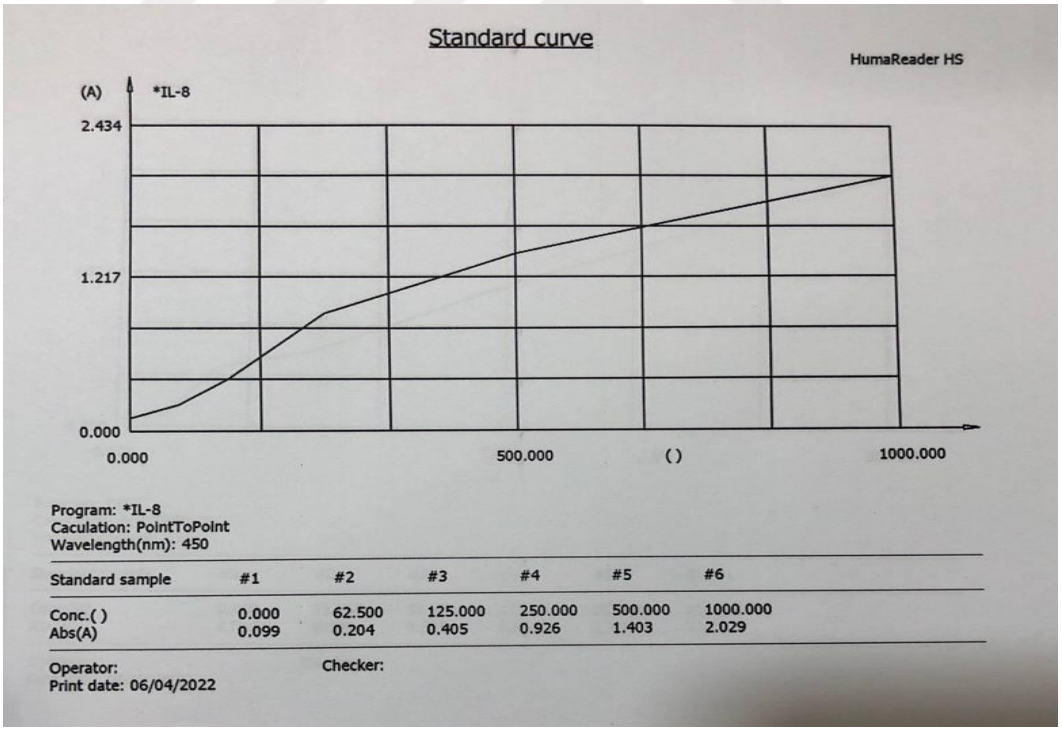

Figure 4.8 Standard curve for the determination of IL-8

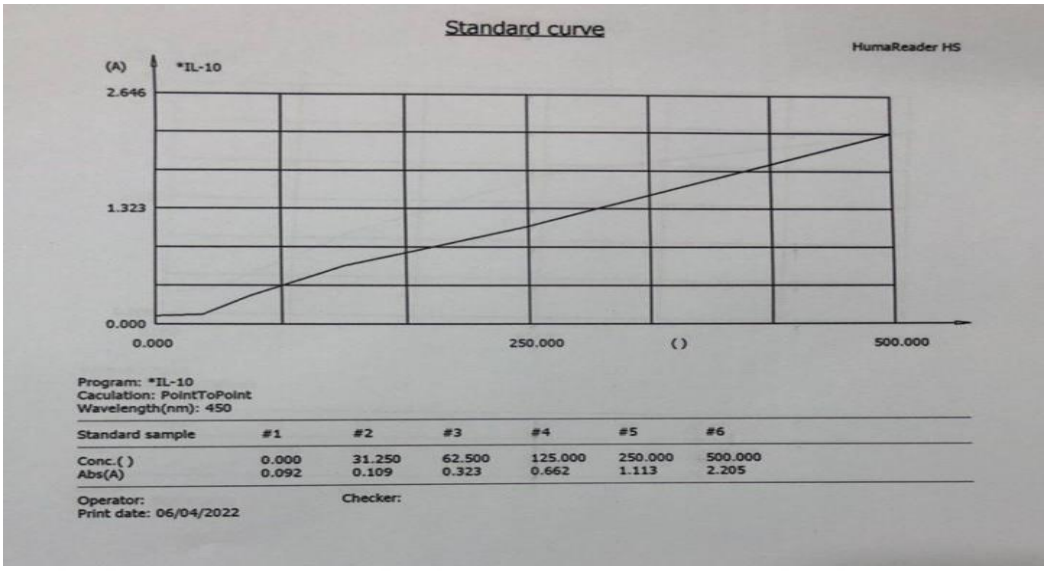

Figure 4.9 Standard curve for the determination of IL-10

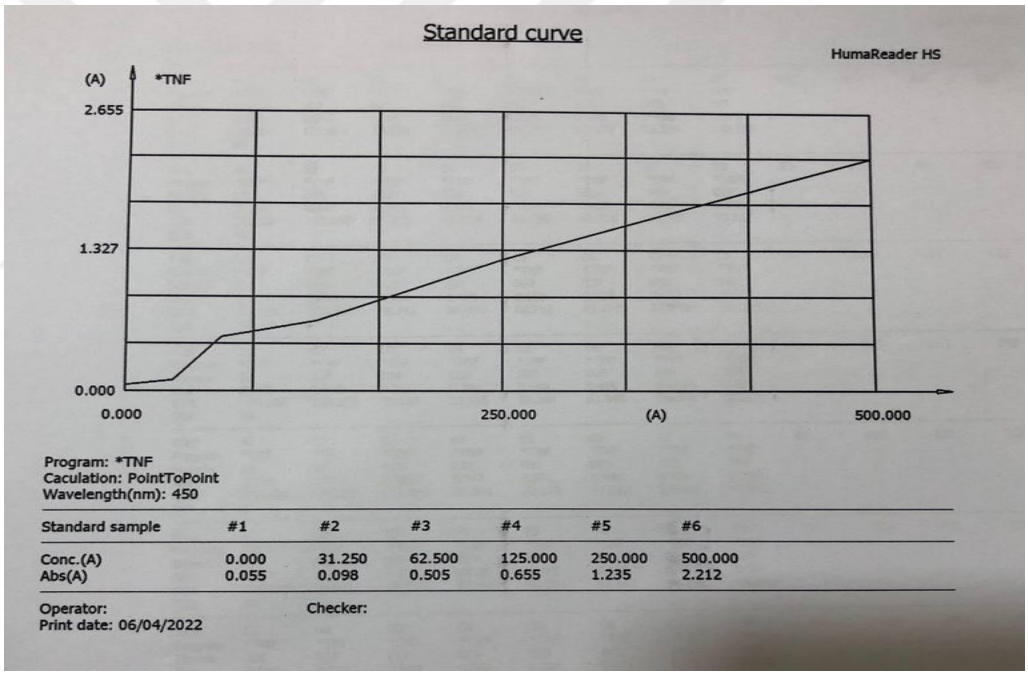

Figure 4.10 Standard curve for the determination of TNF-α

5. CONCLUSION AND RECOMMENDATION

1. Pathogenic microorgansims in Vaginitis Women was recorded a high rate in mix infection 72(36.0%).
2. The most infected age group with vaginitis women in age (20-29) years due that to sexual contact and reproductive age was recorded a high rate in mix infection as 31(43.1%).
3. *16srRNA* gene of *Escherichia coli* in positive recorded as 50(100%) was the gold standard for microbiome profiling.
4. Meropenem was highly effective and is antibiotic for *Escherichia coli* was recorded in sensitive as 50(100%).
5. Immunoglobulin of *Cytomegalovirus* IgG was been reported a high rate in vaginitis women 53(100%).
6. Interleukin 10 is a more common cytokine that is more ele*Vat*ed than cytokines in all vaginitis women patients was recorded a high rate (0.81726±0.307895).

Recommendation

1. Detection other virulence gene factors for *E.coli* associated with *Trichomonas* vaginalis.
2. Use real time PCR technology for detection gene expression of virulence gene in *E.coli*.
3. Detection antibiotics resistance for *E.coli* in vaginitis women.
4. Detection ele*Vat*ed other interleukins associated with *Cytomegalovirus* in vaginitis women.
5. Correlation between the role of H2o2 peroxide hydrogen in *Lactobacillus* and *E.coli* in vaginitis women.

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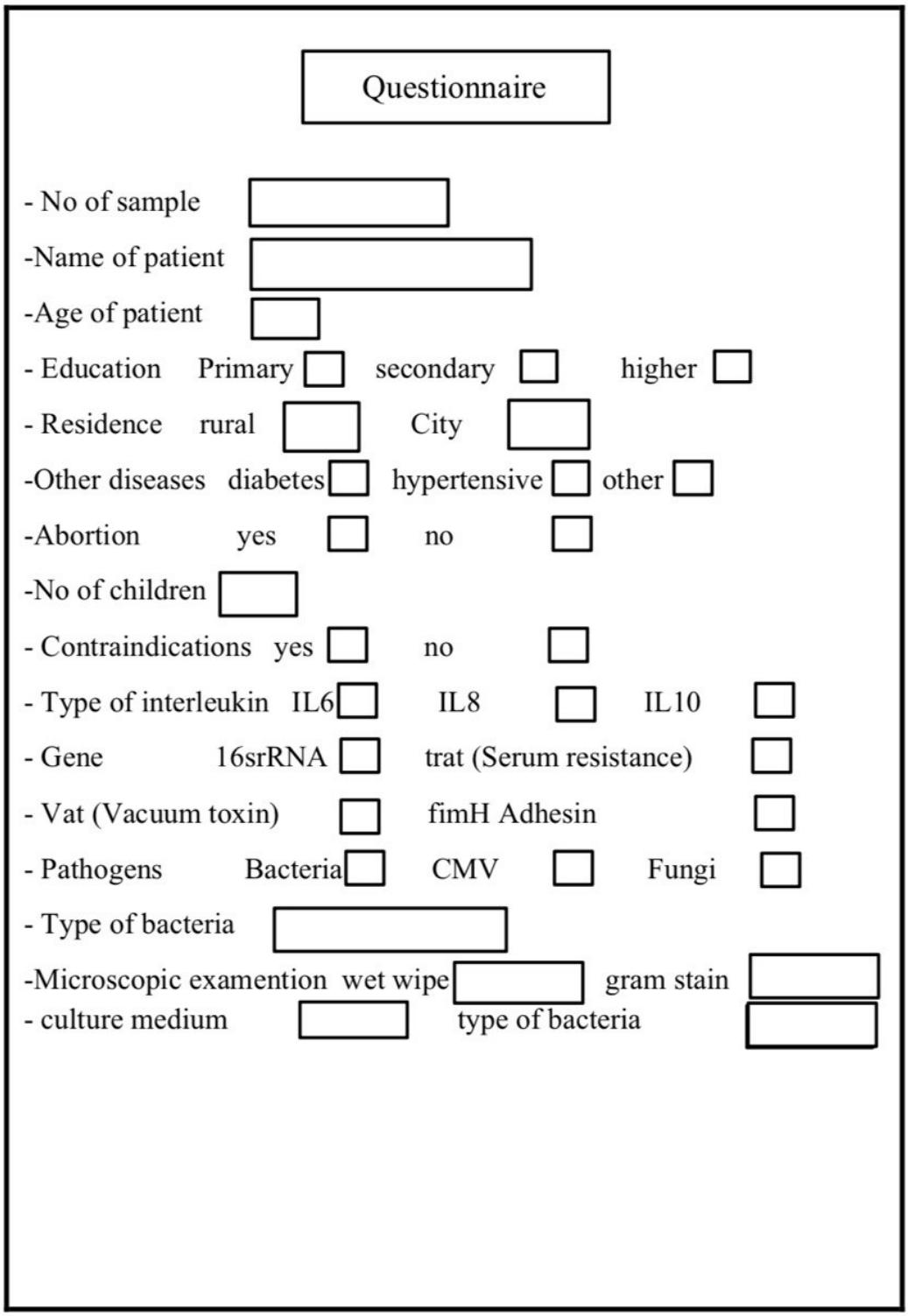
APPENDIX

APPENDIX 1. Questionnaire paper

APPENDIX 2. Laboratory report Antibiotic of *Escherichia coli*

APPENDIX 3. A formal report to facilitate the task

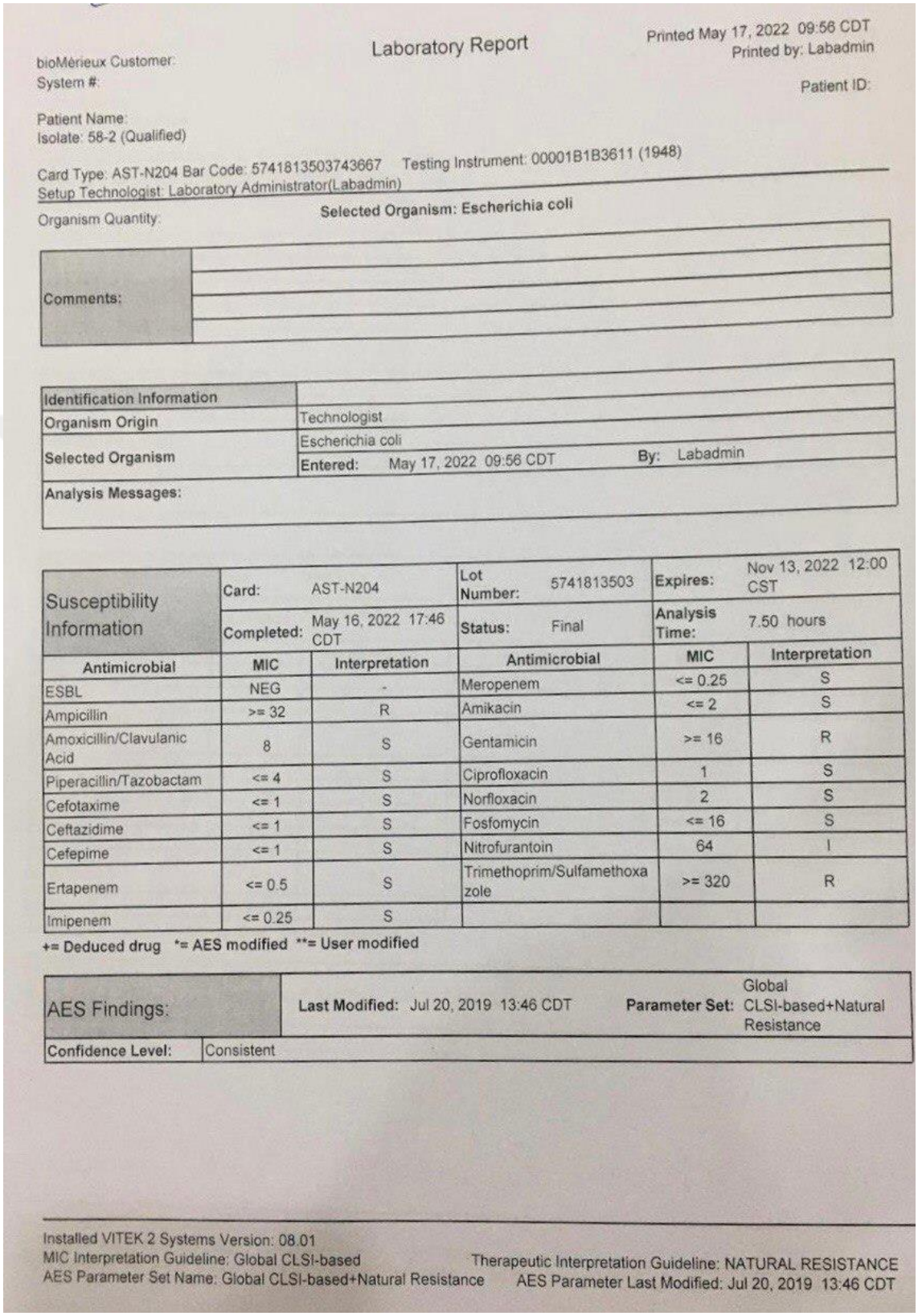
APPENDIX 1. Questionnaire paper



Questionnaire

- No of sample
-Name of patient
-Age of patient
- Education Primary secondary higher
- Residence rural City
-Other diseases diabetes hypertensive other
-Abortion yes no
-No of children
- Contraindications yes no
- Type of interleukin IL6 IL8 IL10
- Gene 16srRNA trat (Serum resistance)
- Vat (Vacuum toxin) fimH Adhesin
- Pathogens Bacteria CMV Fungi
- Type of bacteria
-Microscopic examention wet wipe gram stain
- culture medium type of bacteria

APPENDIX 2. Laboratory report Antibiotic of *Escherichia coli*



Laboratory Report

Printed May 17, 2022 09:56 CDT
Printed by: Labadmin

bioMérieux Customer:
System #:

Patient ID:

Patient Name:
Isolate: 58-2 (Qualified)

Card Type: AST-N204 Bar Code: 5741813503743667 Testing Instrument: 00001B1B3611 (1948)
Setup Technologist: Laboratory Administrator(Labadmin)

Organism Quantity: **Selected Organism: Escherichia coli**

Comments:	

Identification Information	
Organism Origin	Technologist
Selected Organism	Escherichia coli
	Entered: May 17, 2022 09:56 CDT By: Labadmin
Analysis Messages:	

Susceptibility Information	Card: AST-N204		Lot Number: 5741813503	Expires: Nov 13, 2022 12:00 CST	
	Completed: May 16, 2022 17:46 CDT		Status: Final	Analysis Time: 7.50 hours	
Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
ESBL	NEG	-	Meropenem	<= 0.25	S
Ampicillin	>= 32	R	Amikacin	<= 2	S
Amoxicillin/Clavulanic Acid	8	S	Gentamicin	>= 16	R
Piperacillin/Tazobactam	<= 4	S	Ciprofloxacin	1	S
Cefotaxime	<= 1	S	Norfloxacin	2	S
Ceftazidime	<= 1	S	Fosfomycin	<= 16	S
Cefepime	<= 1	S	Nitrofurantoin	64	I
Ertapenem	<= 0.5	S	Trimethoprim/Sulfamethoxazole	>= 320	R
Imipenem	<= 0.25	S			

+= Deduced drug *= AES modified **= User modified

AES Findings:	Last Modified: Jul 20, 2019 13:46 CDT	Parameter Set: Global CLSI-based+Natural Resistance
Confidence Level:	Consistent	

Installed VITEK 2 Systems Version: 08.01
MIC Interpretation Guideline: Global CLSI-based Therapeutic Interpretation Guideline: NATURAL RESISTANCE
AES Parameter Set Name: Global CLSI-based+Natural Resistance AES Parameter Last Modified: Jul 20, 2019 13:46 CDT

APPENDIX 3. A formal report to facilitate the task

CURRICULUM VITAE

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