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CULTURE SENSITIVITY TESTS AND VIRULENCE FACTORS UTI CAUSATIVE BACTERIA AMONG WOMEN IN KIRKUK CITY

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ABDULLAH AHMED MOHAMMED MOHAMMED

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By Abdullah Ahmed Mohammed MOHAMMED

May 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

Advisor **:** Assoc. Prof. Dr. Şinasi AŞKAR
Co-Advisor **:** Asst. Prof. Dr. Muna Jalal Ali HUSSEIN

Examining Committee Members:

Chairman **:** Assoc. Prof. Dr. Şinasi AŞKAR
Biology Department
Çankırı Karatekin University

Member **:** Prof. Dr. Nilgün Ünal
Pharmaceutical Microbiology Department
Sağlık Bilimleri University

Member **:** Assoc. Prof. Dr. Mehmet SEZGİN
Biology Department
Çankırı Karatekin University

Approved for the Graduate School of Natural and Applied Sciences

Prof. Dr. Hamit ALYAR
Director of Graduate School

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Abdullah Ahmed Mohammed MOHAMMED

ABSTRACT

CULTURE SENSITIVITY TESTS AND SOME VIRULENCE FACTORS UTI CAUSATIVE BACTERIA AMONG WOMEN IN KIRKUK CITY

Abdullah Ahmed Mohammed MOHAMMED

Master of Science in Biology

Advisor: Assoc. Prof. Dr. Şinasi AŞKAR

Co-Advisor: Asst. Prof. Dr. Muna Jalal Ali HUSSEIN

May 2023

The aim of current study was detection some phenotypic virulence factors of bacteria that isolated from patients with urinary tract infection. Between March 2022 and June 2022, a total of (225) patients at Kirkuk's Azadi Hospital who were both children and adults and suffering from UTI participated in the study. The results showed that 136 samples, or 60.44 % of the total, showed positive results for bacterial growth when they were cultured on the best culture media, including blood agar, mannitol agar and MacConkey agar. The current study shows that the highest percentage of infection was in (26-35 year) group, which were 65(38.2%). Where, the lowest percentage of infection was in (above 45) group, which was 17 (10%) *E. coli*, *Proteus* spp., *Klebsiella* spp. showed total resistance (100%) to amoxicillin/clavulanic acid and ampicillin. *Staphylococcus* spp. and *Enterococcus* spp. showed total resistance to penicillin, oxacillin and cefoxitin. The findings showed different types of virulence factors of studied bacteria. Whereas, percentage of hemolysis production for isolates of *E. coli*, *Proteus* spp., *Klebsiella* spp., *Staphylococcus* spp. and *Enterococcus* spp. was 88.2%, 72.2%, 71.4%, 65.5% and 50%, orderly. Percentage of urease production for isolates of *E. coli*, *Proteus* spp., *Klebsiella* spp., *Staphylococcus* spp. and *Enterococcus* spp. was 90.1 %, 61.1%, 50%, 79.3% and 33.3%, orderly. Percentage of protease production for isolates of *E. coli*, *Proteus* spp., *Klebsiella* spp., *Staphylococcus* spp. and *Enterococcus* spp. was 74.5%, 94.4%, 21.4%, 72.4% and 4.2%, orderly.

2023, 53 pages

Keywords: Antibiotic resistance, Pathogen bacteria, Urinary tract infection, Virulence factors.

ÖZET

KERKÜK'TE KADINLARDA İYE'YE NEDEN OLAN BAKTERİLERİN KÜLTÜR DUYARLILIK TESTLERİ VE BAZI VİRÜLANS FAKTÖRLERİ

Abdullah Ahmed Mohammed MOHAMMED

Biyoloji, Yüksek Lisans

Tez Danışmanı: Doç. Dr. Şinasi AŞKAR

Eş Danışman: Dr. Öğr. Üyesi Muna Jalal Ali HUSSEIN

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Bu çalışmanın amacı, üriner sistem enfeksiyonu olan hastalardan izole edilen bakterilerin bazı fenotipik virülans faktörlerini tespit etmektir. Mart 2022 ile Haziran 2022 arasında, Kerkük'teki Azadi Hastanesi'nde hem çocuk hem de yetişkin İYE'den muzdarip toplam iki yüz yirmi beş (225) hasta çalışmaya katılmıştır. Yüz otuz altı (136) numuneden % 60.44 oranında kanlı agar, mannitol agar ve MacConkey agar dahil olmak üzere kültürlendiğinde bakteriyel üreme için pozitif sonuçlar vermiştir. Mevcut çalışma, en yüksek enfeksiyon yüzdesinin (% 38,2) 26-35 yaş grubunda olduğunu göstermektedir. Burada, en düşük enfeksiyon yüzdesi (% 10) 45 yaş üstü grupta berlirlenmiştir. *E. coli, Klebsiella* spp. ve *Proteus* spp. etkenleri amoksisilin/klavulanik asit ve ampisilin'e karşı tamamı (%100) dirençli bulunmuştur. *Staphylococcus* spp ve *Enterococcus* spp. suşları penisilin, oksasilin ve sefoxitin'e karşı direnç göstermiştir. Bulgular, incelenen bakterilerin farklı virülans faktörlerinine sahip olduğunu göstermiştir. Oysa *E. coli, Proteus* spp., *Klebsiella* spp., *Staphylococcus* spp. ve *Enterococcus* spp. izolatları için hemoliz üretim yüzdesi sırasıyla %88,2, %72,2, %71,4, % 65,5 ve %50, üreaz üretim yüzdesi sırasıyla %90,1, %61,1, %50, %79,3 ve %33,3 proteaz üretim yüzdesi sırasıyla %74,5, %94,4, %21,4, %72,4 ve %4,2 olarak saptanmıştır.

2023, 53 sayfa

Anahtar Kelimeler: Antibiyotik direnci, İdrar yolu enfeksiyonu, Patojen bakteri, Virülans faktörleri.

PREFACE AND ACKNOWLEDGEMENTS

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Abdullah Ahmed Mohammed MOHAMMED

Çankırı-2023

CONTENTS

LIST OF SYMBOLS

/	Divide
g	Gram
>	Greater than
<	Less than
L	Liter
µg	Micro gram
µL	Microliters
Mg	Milli gram
mL	Milliliter
mm	Milli meters
min	Minute
-	Minus
%	Percent
+	Plus
rpm	Revolutions per minute

LIST OF ABBREVIATIONS

ß-lactamases	Beta lactamases
CDC	Centers for disease control
CFU	Colony forming unit
EMP	Eosin methylene-blue
HMO	Health maintentenance orgaization
MRHA	Mannase resistant hemagglutination
SS agar	*Salmonella- shigella* agar
THP	Tamm-Horsfall protein
UTI	Urinary tract infection
VF	Ventricular fibrillation
XLD agar	Xylose lysine deoxycholate agar

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1. INTRODUCTION

The kidneys, ureters, and urethra make up the urinary system, which is one of the crucial organs in the body because of the waste products it eliminates from the body's bloodstream. In addition to the kidney's role in maintaining the body's natural fluid balance, the composition and concentration of urine provide insight into a person's overall health and well-being (Mohammed 2021). Bacteria can cause UTIs by colonizing and multiplying within the urinary system, which includes everything from the urethra to the kidneys (Wagenlehner *et al.* 2002). In addition to filtering blood, the kidney also prevents infection by limiting urine's ability to flow back and forth between the bladder and kidney (Wagenlehner *et al.* 2008).

Without this protective mechanism, the bladder is more vulnerable to UTIs. While simple UTIs are the norm, more severe forms exist, such as those that are symptomatic, acute, or recurrent. Laboratory findings and the type of microbial invasion serve as the basis for categorizing these conditions (Hsieh *et al.* 2017). When a person is healthy, their urine does not include any harmful bacteria, viruses, or fungi because infections happen naturally when bacteria from the intestines travel through the anus and into the urinary system (Johansen *et al.* 2016).

Many cases of UTI are caused by bacteria, the most significant of which is *E. coli*, a gram-negative enteric bacteria that is isolated from the urine of infected individuals (Vernon *et al.* 1997). UTIs are a symptom of the larger problem that widespread, inappropriate antibiotic use has caused (Mohammed 2021). Humans are vulnerable to the infection's potentially devastating repercussions, such as kidney damage, if it is ignored, misdiagnosed, or treated too slowly. However, it is imperative to have an accurate understanding of UTIS and the risk factors connected with it in order to implement swift measures to bring the condition under control (Obi *et al.* 1996).

1.1 Objective of the Thesis

1. Isolation and identification of bacteria causing urinary tract infections in women.
2. Investigation of antimicrobial susceptibility of isolated bacteria to different antibiotics.
3. Investigation of some phenotypic virulence factors of isolated bacteria.

2. LITERATURE REVIEW

2.1 Epidemiology

Urinary tract infections are the cause of almost 10 million annual outpatient healthcare visits (Schappert and Rechtsteiner 2007). After birth, boys are just as likely to get urinary tract infections (UTIs) as girls, although circumcision greatly reduces this risk. Throughout infancy and puberty, girls are at a higher injury than boys, and this risk steadily rises at around 1% every decade, reaching 10% in women by the time they reach age 65. About 50% of women will have a UTI at some point in their life. Urinary tract infections are more common among the elderly, especially among women. Complex UTIs are more common because of the rising incidence of urinary tract abnormalities like urethral catheter insertion, neurogenic bladder, and prostatic hypertrophy. In the senior population, bacteremia most often results from a UTI (Raz and Westham 1993).

Most infections in the senior population in nursing homes are caused by urinary tract infections (Wang *et al*. 2012). UTIs were reported to be 34.2% of all infections and to have an attack rate of 16% in a modern study of 1754 patients in 13 nursing homes in California. Increases in the prevalence of UTIs are anticipated as the population ages; currently, 25% of adults aged 85 and above are residents of nursing homes (Foxman 2002). One of the many known risk factors for getting a UTI is becoming older (Nicolle 2009). In a probable investigation of sexually active young women at a university health center and at a health maintenance organization, the use of a diaphragm containing spermicide, recent sexual intercourse, and a history of recurrent UTI were all associated with an increased risk of UTI (Hooton *et al.* 1996). Recent research found that the annual infection rate was 0.7 times higher among college students (mean age: 23) than it was among HMO members (mean age: 29) (Hooton *et al*. 1996). Many studies have shown that using a spermicide raises the user's risk of developing a urinary tract infection. It is believed that this association arises from a change in vaginal flora that facilitates colonization of the vagina and urethra by uropathogens (Fihn *et al*. 1998). It's common to have another UTI after successfully treating the first one. Of the 179 women

who participated in a prospective study of cystitis associated with coliform bacteria, 44% reported a recurrence of the infection at least once within one year (Ikähelmo *et al*. 1996). UTI recurrences through the one year follow up were caused by the same serotype of *E. coli* as the initial UTI 33% of the time (Hidad *et al*. 2022). Even while reinfection with a different strain is the most common reason for UTI recurrences, some patients can experience recurrences despite obtaining adequate treatment (Harding *et al.* 1991).

2.2 Microbiology

Uropathogenic *E. coli* strains are the leading cause of simple UTIs; they may be responsible for as much as 90% of all UTIS. However, *Klebsiella*, *Enterobacter*, and *Proteus* are prevalent uropathogens, as are *Pseudomonas* and other *Enterobacteriaceae*. Infection can be caused by several Gram-positive bacteria, including *S. saprophyticus* and *Enterococcus* (Kunin 1994). In young women, *S. saprophyticus* accounts for around 10% of UTIs, most often occurring in the warmer months of the year (summer and fall) (Latham and Running 1983). Complications from *Enterococcus* are common in patients with urethral catheters, those on broad-spectrum antibiotics for another infection, and those with weakened immune systems due to a previous infection (Jacobsen *et al*.2008). The presence of *Enterococcus* in the urine is often indicative of the presence of other infectious agents or urinary tract illness. In contrast to primary UTI, secondary UTI is most commonly caused by the bacterium *S. aureus* which travels via the bloodstream to infect the urinary system (Kau *et al*. 2005). A complex UTI often results from exposure to either rare or drug-resistant bacteria. The urinary tract is home to a wide variety of microbes that can cause illness such as *Oligella urethralis* (previously *Moraxella urethralis*), *Nocardia asteroides* (Kunin 1994).

Recently, it has been described in individuals with structural abnormalities of the tract that other enteric bacteria, such as nontyphoidal *Salmonella*, can cause UTI, typically in the context of immunosuppression or concealed structural abnormalitie (Beck-Sague *et al.* 1994). A case of vancomycin-resistant *Enterococcus faecalis* urinary tract infection is presented (Hooton *et al.* 1996). The problems of in- uro-catheter caused persistent

(a severe UTI) and the spread of drug-resistant bacteria (Jacobsen *et al.* 2008). With its source in the urinary system, the microbiologic spectrum of bacteraemia is similar to that of the most common bacteria responsible for UTIs. Among 180 people, research showed that gram-negative organisms, especially *E. coli*, were the leading cause of UTIs in a community hospital (101 of whom were older than 65 years) (Ackermann and Monroe 1996). In patients with long-term indwelling urethral catheters, the frequency of gram-positive (32%) and gram-negative (35%) infections was higher than in patients without catheters (14%) and those with solely *E. coli* (14%) catheters. These results are consistent with those of other studies showing that older age is not a separate risk factor for mortality (Jacobsen *et al*.2008). *Candida fungemia* can also occur, with the infection starting in the urinary tract. *Torulopsis glabrata* is a *Candida* species that is resistant to fluconazole, and it was found in 19% of patients in a Mayo Clinic group (is a nonprofit American academic medical center focused on integrated health care, education, and research) of patients with *Candida fungemia* from an urine source in 1993 (Hooton *et al.* 1996). *Candida* spp. resistant to fluconazole are expected to rise to prominence as fluconazole use increases (Hooton *et al.* 1991).

2.3 Pathophysiology

Urinary tract infections (UTIs) have complex causes due to the interplay between microbial virulence factors and host defensive mechanisms. Although virulence factors have been better established in *E. coli*, many of the same principles may apply to *Klebsiella* and other gram-negative bacteria. This finding was reached by a team of researchers (Ikähelmo *et al.* 1996). Uropathogenic *E. coli* isolates are typically found in a limited subset of the O: K: H serotype and include adhesion organelles termed fimbriae. This is what a team of researchers lead by (Sobel 1995) came to find. Since P fimbriae aid in the attachment of *E. coli* to uroepithelial cells, they are found at a higher frequency in cases of acute pyelonephritis (90%) than in cases of asymptomatic bacteriuria (30%). In addition, a urinary tract infection is more common in children who harbor *E. coli* with P fimbriae in their intestines. Both type 1 fimbriae and receptor 20 have recently been linked to roles in urinary tract infections (UTIs) (Connell *et al.* 1996). Type 1 fimbriae improve *E. coli* pathogenicity by promoting bacterial

persistence in the bladder and upper tract and by enhancing the human inflammatory response (Connell *et al.* 1996). Type 1 fimbriae may work in tandem with P fimbriae to cause disease. Aerobactin mediated iron uptake is less strongly linked to clinical UTI than fimbriae-mediated iron uptake (Subashchandrabose and Mobley 2015). As a cytotoxic agent, hemolysin may cause host cell damage, especially in men with acute pyelonephritis. Despite the fact that nothing is known about its mechanism or its connection to disease in humans. Virulence factors like P fimbriae and hemolysin are clustered together in pathogenicity islands, which can be anywhere from 35 kb to 190 kb in size. Several factors in the host environment can increase susceptibility to urinary tract infections (Wang *et al*. 2020). A colonization of the urethra by uropathogens and subsequent infection is conceivable in otherwise healthy young women due to factors such as sexual activity, spermicide use, and voiding practices. The severity of a woman's menstrual cycle has been linked to the frequency with which she experiences urinary tract infections (Hooton 2001).

The ability of the uroepithelial cells in these women to bind germs may be related to this finding. UTIs are associated with a unique set of host characteristics in the elderly, as compared to young women (Rowe and Juthani-Mehta 2013). It has been hypothesized that urethral catheterization increases the incidence of urinary tract infections because it weakens the body's defenses. A catheter can induce mechanical harm to the surrounding epithelium and aid in the colonization of uropathogenic organisms (Jacobsen *et al*. 2008). At home urethral catheterization increases the risk of bacteriuria by 3-10% daily. Males who use an external catheter, also known as a con dom catheter, are more likely to develop a urinary tract infection (UTI) (Garibaldi *et al*. 1982). Several factors, such as urethral catheters, prostatic hypertrophy, coexisting medical conditions, neurogenic bladder, and nosocomial. acquisition of infection, make it harder to treat urinary tract infections (UTIs) in the elderly (Rowe and Juthani-Mehta 2013).

2.4 Clinical Presentations

Urinary distress and an increase in urine frequency are symptoms of an acute urinary tract infection. There have been reports of urine being cloudy or even bloody. Costovertebral discomfort and fever are symptoms of acute pyelonephritis but not of simple acute cystitis. Cystitis has been associated with suprapubic pain, but a physical examination will normally reveal no abnormalities. A swelling flank lump is a common symptom of perinephric ab scess (Connell *et al.* 1996). However, contrary to common thought, many elderly patients with UTIs have no symptoms at all. It is more common for patients of this age range to encounter sickness. Older patients with pyelonephritis or urosepsis rarely develop a fever. Retention of urine or a drop in output could be a sign of bacteremia or a clogged indwelling urinary catheter. Traditional quantitative measures of infection severity have relied on bacterial counts more than 105 colony-forming units per milliliter. Clinical signs for severe bacteriuria were considered colony counts above 105 CFU/mL, however colony counts below 10^5 CFU/mL were attributed to pollution from neighboring structures. Even though this cutoff has been used in clinical trials and other sorts of research, its usefulness in actual practice has been called into question as of late. Women who experienced urine symptoms had a higher prevalence of " low - count " bacteriuria, defined as 10 colony forming units / mL to 10 colony forming units / mL, than asymptomatic women (Kunin 1994).

Patients experiencing symptoms of UTI who have pyuria and low bacterial colony counts may benefit from treatment, as this suggests that the infection has not progressed to the bladder. Patients who have dysuria and have colony-forming unit counts in the urine of less than 105 CFU/mL have been diagnosed with urethral syndrome. Acute urethral syndrome may be caused by urethritis due to *Chlamydia trachomatis* or *Neisseria gonorrhoeae* infection, genital herpes in infection, vaginitis, or even noninfectious factors including psychological or allergic reactions, in addition to a urinary tract infection. In general, therapy is not essential unless symptoms emerge, however asymptomatic bacteriuria in the elderly is an exception to this norm. In a nursing home population, around 25% of women and 20% of men have significant bacteriuria at any given time, while in the general community of elderly people who do

not live in nursing homes, roughly 20% of women and 5% of men have bacteriuria (Baldassarre and Kaye (1991). Antibiotic therapy has no effect on morbidity or mortality in these patients (Abrutyn *et al.* 1994). In addition, the future risk of antibiotic-resistant illnesses and antibiotic-related adverse effects is raised when persons who don't have symptoms are treated (Gottlieb and Nimmo 2011). Evidence of *Proteus* or other urea-splitting bacteria in the urine is an anomaly. Patients with urinary stones should be treated because of the organisms' ability to cause them (Schwaderer and Wolfe 2017).

2.5 Investigation of Urovirulence Factors

Identifying possible urovirulence factors (uro - VFs) begins with a comparison between the prevalence of a certain bacterial characteristic in urinary isolates and that in faecal strains from healthy control subjects. Urinary isolates can be further categorized into three groups: those with severe pyelonephritis (the sharp very; associated with chills, fever, and the renal), those with cystitis (average intensity; with burning and pain when urinating in addition to suprapubic pain), and those with asymptomatic bacteriuria (the least severe, characterized by the complete lack of symptoms) (Tănase *et al.* 2009). The incidence of the feature of interest can be used to make comparisons among the various UTI symptoms. Notably, although renal scarring has been used as an outcome in epidemiological research, as will be detailed below, very little is known about the specific VFs that contribute to renal scarring. It is possible to determine if in lower (and urethra) or a superior (kidney and ureter) urinary tract infection (UTI) isolate was the source of symptoms for a patient by employing localization procedures (the antibody-coated-test, ureteral catheterization, or Bladder lavage) (Johnson *et al.* 1989).

Accurate anatomical definitions of the bladder's role as VFs. Furthermore, it is useful to compare the host-parasite interactions associated with a specific VF in other residents (patients with normal versus aberrant or instrumented urinary tracts, pregnant versus nonpregnant women,boys versus girls, etc.). Data from multiple studies (gathered on behalf of the review this) may more precisely reflect the prevalence of a VF among uroisolates linked with a specific UTI disease or patient category than data from a single

study. However, care should be taken when interpreting these pooled results, as they might hide important but subtle differences between individual research (due to different techniques and definitions, different study designs, and different populations investigated). Human epidemiological research can reveal links between specific bacterial features and UTI, while animal models allow for through evaluation of the contribution to virulence of this suitable associations. In light of this, it is crucial to select the most applicable animal model and bacterial strains. Animal models of human UTIs cannot be successfully created through the use of intravenous bacterial injection or other nonphysiological treatments in the UT (direct intrarenal injection, ureteral ligation, renal trauma, etc) (Kaijser and Larson 1982).

Most UTI in humans begin in the urethra and progress to the bladder, kidney, and occasionally the bloodstream, even in people with otherwise normal urological anatomy and function (Flores-Mireles *et al*. 2015). The VFs being studied rely heavily on characteristics of the urinary tract, such as cell surface receptors for adhesins, hence the animal species used in the studies must also possess these characteristics in common with humans (Sarowska *et al*. 2019). Comparing to virulence of several wild type progeny that differ in a certain trait is the simplest technique to study the contribution of that property to virulence. However, similar to human epidemiological studies, this method does not clarify whether differences in virulence are caused by the character itself or factors linked with leaves. It is easier to link differences to the factor at hand when comparing progeny that are otherwise genetically similar except for the factor in question (Niu *et al*. 2013). If isogenic tensions are produced utilizing stringent protocols, it is more likely to the factor of interest is the only variable affecting virulence (such as site-directed mutagenesis). In conclusion, possible VFs discovered through epidemiological or animal studies are often examined at the cellular or subcellular level in vitro. This kind of information tends to lend credence to the theory that a particular trait contributes to virulence and may indicate potential methods for taming it. The laboratory is helpful for elucidating the functional importance of putative VFs of *E. coli*, as some of these VFs (such fim briae and hemolysin) are separate bacterial structures or products (Sarowska *et al*. 2019).

2.6 Adherence

There are many different forms of dangerous microorganisms, and they all have the ability to adhere to solid substrates. Examples of these microbes include yeasts, gram-negative and gram-positive bacteria, viruses, and protozoa (Chow *et al.* 1993). However, despite the fact that host cells harboring bacteria might be lost, leading to the elimination of the organisms despite their attachment, microbial pathogens evade eradication by being carried beside through the steady flow of body fluids (blood, urine, digestive contents). Affection is considered a important preliminary step in the colonization of host mucous membrane surfaces and a precursor to invasive infection (Pluschke *et al.* 1983).

Cell Adhesion and Hem agglutination in the Uroepithelium Human uroepithelial cells and human erythrocytes can be agglutinated and adhered to by *E. coli* strains that cause UTIs, even in the incidence of mannose (mannose resistant hem agglutination [MRHA]). The phenomenon was uncovered in the 1970s. Additionally, mannose has no effect on adhesion to uroepithelial cells, and strains expressing mannose-resistant hem agglutination (MRHA) are more common than those displaying only mannose-sensitive hem agglutination (mannose-resistant adherence). Fimbriae are responsible for adherence to epithelial cells and MRHA in the majority of urine isolates (Orikasa *et al.* 1977), which explains why both characteristics are often seen together.

The fimbriae of uroepithelial cells mediate their adherence to one another and their migration in response to hypoxia (MRHA). These results corroborate those postulated that fimbriae mediate MRHA and epithelial - cell adhesion (Pinzón-Arango *et al*. 2009). Fimbriae are tiny fiberlike appendages seen on clinical isolates of *E. coli* that bind to and cross-link erythrocytes, causing agglutination (Connell *et al*. 1996). Subsequently, (Brinton 1965) gave these structures the name "pili" (from the Latin term for "hairs") and showed that they retained their hem agglutinating capacity even after being cleaned and separated from the bacteria they were originally attached to. In addition to interacting with erythrocytes, bacteria can also bind to other cell types, including platelets, leukocytes, pollen, yeast cells, spermatozoa, spores, and latex beads, a process

known as hemagglutination (Valvano *et al.* 1988). Fimbriae have a unique morphology and serve a different purpose than the thicker, longer, more flexible-looking flagella that are responsible for motility but not attachment and the thicker, more phallic-looking sex pili that function in conjugation but not attachment (Tan *et al.* 2016). Brinton's structural research of type 1 fimbriae, which serve as a model for other fimbrial types, reveals that they have a diameter of 7 nm, a length of 0.5 to 2 m, and a center axial hole of 0.2 to 0.25 nm in diameter. Each round of the helix contains anywhere from three to eight of these repeating subunits (Johansen *et al.* 2016).

2.7 Evaluations of Persistence

When bacteria, as well as cells, as well as cells are incubated together to promote adhesion, bacterial adhesion to epithelium cells and cell monolayers can be examined. Washing or filtering out the bacteria that did not adhere and has counting the number of attached bacteria using microscopy or radiometric techniques. The reliability of such investigations is affected by a number of technical aspects, including the epithelial cell's source, the cell type (squamous vs. transitional), and, in some cases, the cell's survival. Adherence tests are complicated by the presence of Tamm-Horsfall protein (THP) (uromucoid, urine slime), which coats uroepithelial cells (Wagenlehner *et al.* 1994).

3. MATERIALS AND METHODS

3.1 Apparatus

Devices, tools that's used in this study for the measurement of parameters, will be it is explained in the following Table 3.1.

Table 3.1 Total devices used in this study

Device	**Origin**
Semi auto analyzer	Eltra (Germany)
Medical Deep Freezer	SANYO (Japan)
Balance	Kern (Germany)
Centrifuge	GallenKamp (England)
Digital Camera	Sony (Japan)
Distillator	G.F.L (Germany)
Electric balance	India MART (India)
Incubator	BINDER (Germany)
Compound light microscope	Olympus (Japan)
Auto vortex mixer	Stuart (England)
Autoclave	Express (U.K.)
pH-meter	GMBH (Germany)
Refrigerator	Vestel (Turkey)
Oven	Lab Tech (Korea)
Water Bath	Gallenkamp (England)
Haematology analyzer	Beckman Coulter (U.S.A)
Anaerobic Jar	Yamato (Japan)

The chemicals, biological materials, and culture media used will be it is shown in the following Table 3.2 and Table 3.3.

Table 3.2 Biological and chemical materials used in this study

Biological and chemical materials name	**Origin**
Acetone	Chemfe (India)
α-naphthol	SDS (India)
Barium chloride ($BaCL_2$ H2O)	CE (Spain)
Boric acid	SDS (India)
Bromothymol blue	Fluka (Switzerland)

Table 3.2 Continue

Chloroform	SDS (India)
CuSo4, 7H2O	East Sussex (U.K)
Ethanol 96%	BDH (England)
Glucose	BDH (England)
Glycerol	BDH (England)
HCL	SDS (India)
H_2O_2	East Sussex (U.K)
Iodine crystal	CDH (India)
$KH_2 PO_4$, K_2HPO_4 & $KHNaPO_4$	East Sussex (U.K)
KOH	CDH (India)
Mannetol	HiMedia (India)
Methanol	HiMedia (India)
Methyl green	GFS (Canada)
Methyl red	GFS (Canada)
Mineral Oil	SDS (India)
Tetramethyl-para-phenylene-diamine-dihydrochloride	SDS (India)
Na_2HPO_4 &NaH_2PO_4	Bioliff (Italy)
Na Cl	Schuchard (Germany)
Na OH	Schuchard (Germany)
Peptone	Mast (U.K)
Potassium iodide (K1)	East Sussex (U.K)
$H_2 SO_4$	East Sussex (U.K)
Starch	CDH (India)
Trypton	Mast (U.K)
Tween 80	Bio Merieux (France)
Urea	Mast (U.K)
Yeast-extract	East Sussex (U.K)
Xylene	SDS (India)

Table 3.3 The culture media's used in this study

Culture media	**Origin**
Blood agar	Oxoid (England)
Brain-Heart infusion broth	Liofilchem (Italy)
Cary-Blair transport media	SDS (India)
DNase Agar	HiMedia (India)
EMB	Oxoid (England)
Gelaten agar medium	Norevo GMBH (Germany)
Kliglers iron agar	Oxoid (England)
Urea agar base	Mast (UK)
MacConky agar	HiMedia (India)
Deman Regosa Sharpe	Oxoid (England)
Simmon citrate agar	HiMedia (India)
Xylose lysine deoxycholate agar	HiMedia (India)
Sorbitol MacConky agar	HiMedia (India)
Skim milk agar	HiMedia (India)
Trypton soy agar broth	Oxoid (England)
Muller- Hinton agar	Liofilchem (Italy)
TSI	HiMedia (India)
Nutrient agar & broth	Oxoid (England)

Reagents, stains, solutions, and antibiotic disc (NCCLs) guideline that's used in this study for the measurement of parameters, will be explained in Tables (Table 3.4, Table 3.5, Table 3.6).

Table 3.4 Solutions, reagents and stains used in this study

Name (Stains and reagents)	**Origin**
Kovac's indole reagent	Rambach (France)
Gram stain solutions	Liofilchem (Italy)
India ink stain	Liofilchem (Italy)
Combo Card test to toxin A , B	NEB (England)

Table 3.5 The antibiotics used in this study for Gram-negative bacteria

NO	**Antibiotic**	**Abbreviation**	**Disk contain (µg)**	**Zone Inhibition Diameter /mm**			**the creator**
				R	**I**	**S**	
1	**Ciprofloxacin**	C I P	5	≤ 15	16_20	≥ 21	Bioanalyse
2	**Amicacin**	A K	30	≤ 14	15_16	**≥17**	Bioanalyse
3	**Cefazolin**	I N N	30	≤ 14	17_15	≥ 18	Bioanalyse
4	**Cefoxitin**	F O X	30	≤14	17_15	≥ 18	Bioanalyse
5	**Ceftazedime**	C A Z	30	≤ 17	18_20	≥ 21	Bioanalyse
6	**Cfepime**	F E P	5	≤ 15	16_18	≥ 19	Bioanalyse
7	**Imipenem**	I P M	10	≤ 19	20_22	≥ 23	Bioanalyse
8	**Gentamicin**	G M	10	≤ 12	13_14	≥ 12	Bioanalyse
9	**Pipracillin**	P R L	100	≤ 17	18_20	≥ 21	Bioanalyse
10	**Tobramycin**	T O B	10	≤ 12	13_14	≥15	Bioanalyse
11	**Levofloxacin**	L E F	15	≤ 13	17_14	≥ 23	Bioanalyse
12	**Tigecycline**	T I	10	≤ 8	_	≥ 8	Bioanalyse
13	**Trimethoprim**	T M P	5	≤ 10	11_15	≥16	Bioanalyse
14	**Ampicillin**	A M	10	≤ 13	14_16	≥17	Bioanalyse

Table 3.6 The antibiotics used in this study are for Gram-positive bacteria

NO	**Antibiotic**	**Abbreviation**	**Disk contain (µg)**	**Zone Inhibition Diameter / mm**			**the creator**
				R	**I**	**S**	
1	**Metronidazol**	M A	30	≤13	14_16	≥17	Bioanalyse
2	**Gentamicin**	G M	10	≤12	13_14	≥12	Bioanalyse
3	**Amicacin**	A K	30	≤14	15_16	≥17	Bioanalyse
4	**Clindamycin**	D A	10	≤14	_	≥15	Bioanalyse
5	**Levofloxacin**	L E F	15	≤13	17_14	≥23	Bioanalyse
6	**Nalidixic acid**	N A	30	≤ 13	14_18	≥19	Bioanalyse
7	**Trimethprim**	T M P	5	≤10	11_15	≥16	Bioanalyse
8	**Ciprofloxacin**	C I P	5	≤15	16_20	≥21	Bioanalyse
9	**Vancomycin**	V A	30	≤ 12	10_11	≥ 9	Bioanalyse
10	**Azithromycin**	A Z M	15	≤ 18	14_17	≥13	Bioanalyse

Also, there are some materials and equipment used will be explained as in Table 3.7.

Table 3.7 Other equipment and materials used in this study

Name materials and equipments	**Origin**
Inoculation needle and wire loop	Volac (England)
Filter, Millipore	Whatman (England)
Benson Burner	GERMANY
Tube rack, Benson BurnerSlides & Cover-Slide	BDH (England)
Glass vial, graduated cylinder, and conical flask	Volac (England)
Washing bottle, Cotton	(Turkey)
Tips and Micropipette	Volac (England)
Small syringe	Kloehn Co (U S A)
Filter paper	Volac (England)
Magnetic drive	Gallenkamp (England)
Tight cups for water	Volac (England)
Plane tupe & Disposable petri dish	Whatman (England)
Wooden sticks & Sterile swabs	Whatman (England)

3.2 Media Preparation

3.2.1 Commercial media without any additive component

Except for XLD Agar and SS Agar, which are prepared by boiling for 1 minute without autoclaving (Table 3.8), all media prepared in accordance with Manufacturer's instructions Steam sterilize at 121°C, pressure 15° for 15 minutes.

Table 3.8 Purpose of preparation of culture media types

	Media	**Purpose of preparation**
1	Brain Heart Infusion Agar	Cultivation and reactivation of isolates
2	Brain Heart Infusion broth	Cultivation and reactivation of isolates
3	Eosin Methylene Blue Agar (EMB Agar)	Gram-negative intestinal bacteria differentiation Lactose-fermenting bacteria, particularly Escherichia coli, manifest as colonies with a metallic green sheen. Colonies that are colourless represent bacteria that do not ferment lactose.
4	Kligler iron agar (KIA)	Test the ability to ferment some carbohydrates and produce acid with or without gas as well as H2S.
5	MacConkey agar	Isolation from gram_negative bacteria and distinction between gram-negative and gram-positive bacteria
6	Muller-Hinton agar	Susceptibility of isolates to antibiotic

Table 3.8 Continue

7	Nutrient agar and broth	General culture medium
8	SS Agar & XLD Agar	Isolation and differentiation of pathogenic enteric bacilli
9	Simmon citrate agar	Determine the ability of the isolates to use citrate as the only source of carbon and energy.
10	Skim milk agar	Cultivation and differentiation of bacteria based on proteolytic activity.
11	Sorbitol MacConkey agar	Differentiation between sorbitol fermenter and non-fermenter
12	Triple Sugar Iron Agar (TSI)	Test the ability to ferment some carbohydrates and produce acid with or without gas as well as H_2S.
13	Blood agar	For beta hemolysis

3.2.2 Commercial media with additive component

A. Egg yolk agar

85 mL of sterile nutrient agar were combined with approximately 15 mL of egg yolk suspension, and the mixture was then cooled to 55 ℃ and put into plates (Collee *et al.* 1996). The separation of bacteria based on lecithinase and lipase synthesis took place in this medium. Lecithinase-producing bacteria are represented as colonies encircled by an area of insoluble precipitate. Lipase-producing bacteria look as pearly, iridescent colonies.

B. Blood agar

According to what was given by the producing company, the blood agar base was made and sterilized at 120 °C for 15 min, cooled for 50 °C, It is then supplemented with 5% Human blood. These bacteria I slept on this media to see if they might cause hemolysis (Baron *et al.* 1994).

C. Medium preservation (BHI)

After being sterilized, the medium is based on the infusion of the heart into the brain and included 15% glycerol as a supplement, was divided into 5 mL sections in sterile

tubes and kept at 4ºC until it was needed. The bacteria isolates were stored in this medium for a lengthy period of time at -20 ºC.

D. Activity of urease test

The 40% urea solution was sterilized by the use of filtration. Christensen urea agar (21gram / 950ml D.W) it was created without the involvement of urea, with pH adjusted at 6.9-6.8 they were autoclaved. After sterile cooling in 50 °C, 50 mL from the urea solution, then 5 ml were aliquots dispensed and aseptically it is dispensed into the screw cap tubes before being left to set the ramp position (Cruickshank *et al.* 1973).

3.2.3 Synthesized media

Synthesized media that used will be explained as in Table 3.9.

Table 3.9 Synthesized media used in this study

Media	References
Britannia broth –Luria	(Sambrook *et al.* 1989)
Medium transport for Carey and Blair	(Atlas 2006)
Motion average test	(Atlas 2006)
Middle of the test indole	(Atlas 2006)
Mannitol average is semi-solid	(Atlas 2006)
Britanni agar Luria	(Sambrook *et al.* 1989)
Medium Sierra	(Atlas 2006)
MRVP Broth (Red methyl Voges Proskauer's broth)	(Atlas 2006)

3.2.4 Preparation of reagents and solutions

A. Catalase reagent

Hydrogen peroxide (3%) was created from the stock solution and tested to see if the isolates could produce catalase in a dark bottle.

B. Methyl red reagent

It was prepared by dissolving 0.1 g of methyl red in 300 mL of 95% ethanol (butnidol) in order to determine whether all of the sugars had been utilized.

C. Oxidase reagent

Tetra methyl-p-phenyl diamine dihydrochloride, 0.1 gm, and 10 mL D.W. were mixed together freshly in a dark bottle to test the isolates' capacity to create the oxidase enzyme.

D. Voges Proskauer detector

This detector consists of two solutions:

Solution A: 5 grams of Naphthol are soluble in 100 millilitres from pure alcohol to make-naphthol (Ethanol 96%).

Solution B: 40 grams from (KOH) were thawed in 100 milliliters from D.W. for make the preparation. It was used to make mixed fermentation and find sugars that were only used partly. They were used to make a mixed fermentation of acids and to find sugars that were only partially used.

A. Iodide component

90 milliliters of D.W. were used to dissolve 2.03 grams of (Iodin) and 5.23 grams of KI, and was the volume then raised to 100 milliliters in D.W. It is kept at four degrees Celsius.

B. Starch component

After being mixed in 100 mL of D.W. with 1 g of soluble starch, the bottle was placed into the water for 10 minutes before being maintained at 4 °C for a week.

C. Normal saline

100 mL of distilled water and 0.85 gram of sodium chloride were combined to create the solution, which was then autoclaved for 15 minutes at 121 °C.

D. Phosphate buffer saline (PBS)

Dissolve component well and tuning the PH to 7.4 and sterilize it at 120 °C to15 minutes:

1.Sodium chloride (NaCl) 8gm

2.Dipotassium Phosphate (K2HPO4) 1.21 gm

3.Potassium dihydrogen phosphate 0.034gm

E. McFarland Standard Solution

For the antimicrobial susceptibility test, a McFarland standard solution consisting of 0.05 ml of BaCl2, 2H2O concentration (1.175 percent) and 9.95 ml of H2So4 concentration was utilized. The use of tube number (0.5) was 1%. The McFarland standard tube No. (0.5) was used to compare the 1.5x108 cell/ml density of the bacterial cells in suspension (Baron *et al.* 1994).

5. Motility test

The medium need to it is heated to room temperature before being injected. By inserting straight needle approximately 1/4 of the way up the center of the medium, carefully chosen colonies from a pure culture were injected (18-23 hours). With the caps off, the tubes were incubated for 24 hours at 37 °C. After the incubation period, it was discovered that 18 growths that moved away from the stab line or were fuzzy over the media were indicators of a motile creature. (Gwendolyn 1988), the motility of these organisms was also examined using a hanging drop wet mount under a light microscope (Mahon and Manuselis 2000).

3.3 Collection Sample and Study Population

Between March 2022 and June 2022, a total of 225 patients at Kirkuk's Azadi Hospital who were both children and adults (outpatients and inpatients) and suffering from UTI participated in the study. For each patient, interviews were conducted.

About 1-2 cc of urine were taken while wearing disposable, clean gloves, and placed in disposable, clean screw-capped containers specifically made for collecting urine samples. Following their collection, all of the specimens were treated right away or, if they were delayed for 1-2 hours, using Carry Blair transit media (Baron *et al.* 1994).

3.4 Identification of Bacteria and Isolation

The initial isolation of the studied bacteria, and collected strains was grown immediately on blood agar and MacConkey agar (Baron *et al.* 1994). The remaining isolates are then selected for definitive microscopic examination, culture characteristics, and biochemical testing to identify bacteria (gram negative and positive) after being they were incubated aerobically at 37 °C for 24 hours (Brooks *et al.* 2007).

3.4.1 Microscopy of gram stain

Swabs of an secluded colony was prepared, placed in sterile slides, Installed by air drying and then stained with a prepared Gram stain. Using in oil immersed objective lins and a 100x light microscope, gram stain reaction and bacterial cell arrangement were studied (Atlas *et al.* 1995).

3.4.2 Characteristics of the culture

The morphological characteristics of isolated colonies were closely investigated using a 100 lens. The colonoies were generally separated into pure and mixed cultures.

3.4.3 Biochemical reaction

A. Catalase test

Put drop of a 3 present H_2O_2 solution was applied to 24 hour an ancient bacterial colony that had been deposited on clean use glass slide a sterilized wood stick. An immediate release of oxygen bubbles denotes a positive outcome (Baron *et al.*1994).

B. Oxidase test

Into a petri dish with filter paper filled with the oxidase detector, colony of the tested microorganism it was convey for filter paper and rubbed over the solution with a rod stick (1 percent dimethyl-p-phenylene-diamine-dihydrochloride). Within 10 seconds, the colour will change to indicate a positive reaction (Baron *et al.* 1994).

C. Indole test

Peptone water broth tubes received bacterial culture before being incubated around 37 °C to 24-48 h. Add a few drops of Kovac's reagent in every tube. When a pink ring appears, the test was positive (Baron *et al.* 1994).

D. Methyl red test

Fresh culture was added to the MR-VP broth, which was subsequently incubated for 24 hours at 37 °C. Each tube received a few drops of methyl red solution. If the colour changes to red, it becomes positive; if it remains yellow, it becomes negative. (Barron *et al*. 1994).

E. Voges-proskauer test

Glucose-peptone phosphate was used as culture medium supplemented with 0.5 mL of VP1 and VP2, generation of a pink to red colour indicates a positive test. The bacteria examined were injected into a liquid MR-VP culture, and then cultured there for 48 hours at a temperature of 37 (Baron *et al*. 1994).

F. Citrate test

Simmons's citrate slope was incubated at 37 °C for 48–72 hours after a fresh culture was injected into it. The change of green for blue on the medium points out a positive outcome (MacFaddin 2000).

G. Coagulase test

Coagulation testing can be performed on slides with a negative control to exclude existing agglutination Two drops of saline solution are placed on the slide labeled with sample number The two drops containing the salt are emulsified with the test object

using a straight wire or wooden stick. A drop of plasma is placed on a drop of test saline, mixed well, and the slide is gently shaken for about 10 seconds (Kateete *et al*. 2010). This test is used to differentiate between types *Staphylococcus* spp.

H. H_2S Production test

Iron-sugar triple agar was injected with the tested bacteria by stabbing the needle containing the purified culture into the back of the TSI tube, then streaking the medium with a sterile loop and incubating at 37 °C for 24 h. The result was positive for carbohydrate fermentation and negative for hydrogen sulfide production (Ditroi *et al*. 2019).

İ. Urea test

Slots were urea agar coated with bacterial culture before being incubated in 37 °C. During 24 hours, results are then read daily for six days. A favorable outcome is indicated by transforming the medium's hue from yellow to purple pink (MacFaddin 2000).

3.4.4 Hemolysin production

On blood agar media, hemolysin production was observed. The bacterial samples were isolated after culture plates were cuddled for 24 hours at 37 ^{o}C, and presence of any hemolysis around the colonies indicated a successful outcome (Collee *et al*. 1996).

3.4.5 Extracellular protease

Using skim milk agar media, extracellular protease was found. Striking on the agar medium was used to cultivate the plates, which were then incubated for 24-48 hours at 37°C. A favourable outcome is indicated by the presence of a clean zone around

bacterial growth (Janda and Bottone 1981). Chemical test properties of gram positive and negative bacteria are given in Table 3.10 and Table 3.11.

Table 3.10 Biochemical tests for gram negative isolated

Species	V P	Coagulase test	Motility	Methyl Red	Citrate test	Oxidase	H_2S	Indole	Catalase
Escherichia.coli	-	-	+	+	-	-	+	+	+
Proteus spp.	+	-	+	+	+	-	+	-	+
Klebsiella spp .	+	-	+	-	+	-	-	-	+

Table 3.11 Biochemical tests for gram positive isolated

Species	Oxidase	Hemolysis	Gram stain	Urea test	Coagulase test	Methyl Red	Motility	V P	Catalase
Staphylococcus spp	-	β	+	+	+	+	-	+	+
Enterococcus spp.	-	-	+	-	-	-	-	+	-

3.5 Antibiotic Susceptibility Test

Disk diffusion method is one of the oldest and most widely used methods in clinical microbiology laboratories for antimicrobial susceptibility testing. It is suitable for testing most common bacterial pathogens without the need for special equipment. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) disc diffusion method is a standard method, but 16-24 hours are required to obtain an antibiotic susceptibility test (ADT) profile. *S. aureus* ATCC 29213 was used as the control strain recommended by EUCAST (EUCAST 2020). With a sterile loop or cotton swab, several colonies with similar morphology are chosen from growth (16–24 hours of incubation) on a non-selective medium. The colonies are then suspended in sterile saline (0.85% NaCl w/v in water) to the density of a McFarland 0.5 standard, which roughly

equates to 1-2 108 CFU/mL for *E. coli*. Preferably, a photometric instrument that has been calibrated with a McFarland standard in accordance with the manufacturer's instructions is used to measure the density of the suspension. A 0.5 McFarland turbidity standard can also be used to visually compare the density of the suspension. The addition of saline or additional organisms causes the suspension's density to be adjusted to McFarland 0.5 (Matuschek *et al*. 2013).

3.6 Arithmetical Analysis

A fully randomized design was employed to perform the experiment, and the Minitab 17 general linear model for analysis of variance (Statistical Analysis System). When there was significant variation in the various averages on a level (P 0.05).

4. RESULTS AND DISCUSSION

4.1 Samples Distribution

The 225 samples in the current study included 225 (59.6%) women with UTI Table 4.1. The results showed that 136 samples, or 60.44 % of the total, revealed positive results for bacterial growth when they were urbane on the best culture media, including mannitol agar, blood agar, and McConkey agar. Of the total samples, 89 (or 39.56 %) showed no evidence of bacterial growth (Figure 4.1).

Table 4.1 The study samples were distributed according to growth

Groups	NO	The percentage
Negative growth	89	39.56%
Positive growth	136	60.44%
Total (%)	225	100%

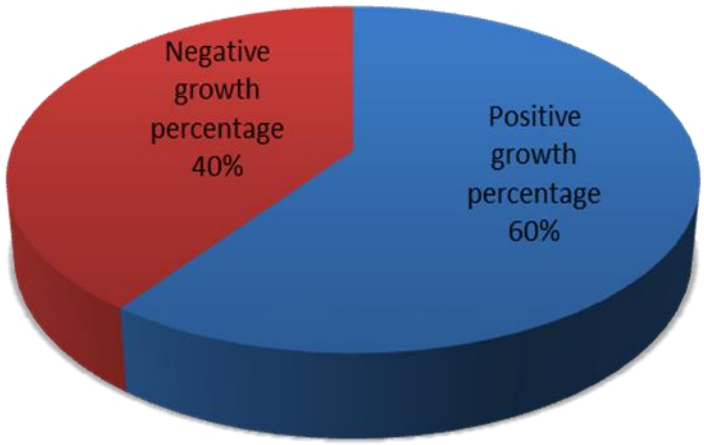

Figure 4.1 Percentage of bacterial isolation rate of patients with UTI

The prevalence of urinary Tract infection in this study samples was 60.44%. These results in this study were closely to the prevalence rate of 71.6% earlier that referred by

(Jellheden *et al.* 1996) in women less than 50 years of age and suffering from acute systems of urinary Tract Infection.

Otherwise, in the current investigation, 89 (39.56%) of all samples, particularly UTIs, had negative findings for bacterial growth, which may have been due to Bacterial growth might be caused by viruses, fungi, or anaerobic bacteria that can't be isolated using the conventional transplant methods used in this study; they might need special cultured and development; they might be caused by antibiotic doses used by infected patient women; they might also be caused by differences in the nature and size of the samples that were taken; and they might be caused by changes in the grading of heat and humidity (Maa'roof and Azeez 2018).

4.2 Identification

We checked morphology and diameter, and forms of bacterial isolates were obtained on blood agar, mannitol agar, and MacConkey agar. Additionally, microscopic and biochemical exam, and that included the specified tests for each of them kind, were used to corroborate the conclusions from the biochemistry diagnosis. Exact copies of the biochemical test results are included in the Tables. (Table 4.2, Table 4.3, Table 4.4).

Table 4.2 Microbial Isolates groups in this study

Isolates	**No**	**Percentage**
Bacterial isolates	42	30.88 %
Bacterial isolates mixed	94	69.22 %
Total	136	100%

Table 4.3 Lineage isolation of Gram negative bacteria

Gram negative bacteria	**No**	**Percentage**
E. coli	51	61.4 %
Proteus mirabilis	18	21.7 %
Klebsiella spp.	14	16.9 %
Total	83	100 %

Table 4.4 Lineage isolation of Gram positive bacteria

Gram positive bacteria	No.	Percentage
Staphylococcus spp.	29	54.7 %
Enterococci spp.	24	45.3 %
Total	53	100%

4.3 Gram-Negative Bacteria Identification

4.3.1 *E. coli*

On MacConkey agar, E. coli forms tiny, light pink colonies (Figure 4.2), while on EMB media; it appears to have a green metallic sheen. It also passed biochemical tests with a negative result of Oxidase test, Voges proskauer test and citrate tests, but it was positive for methyl red, indole test and catalase test.

The current study's findings revealed that both pregnant and non-pregnant women are most frequently affected by bacterial infections of the cervical region and vaginal infections. One of the most persistent pathogens in males of all ages is *E. coli*, which causes UTI infections (Khan *et al.* 2013). According to the most recent statistics, 32.1 percent of women had *E. coli* infections, which was greater than the 14.6 percent isolated proportion reported by Rashid *et al.* (2019). Due to the specific anatomical characteristics of the female urogenital tract, including a vaginal structure and the proximal location of the vagina to the anus, which makes it easy for bacteria to ascend in the reproductive tract, as well as PH, changes in hormonal balance, poor vaginal hygiene, the use of antibiotics and immunosuppressive drugs, the degree of moisture, and concurrent infections, females have a higher incidence of reproductive tract infections (Al-Najjar *et al.* 2020).

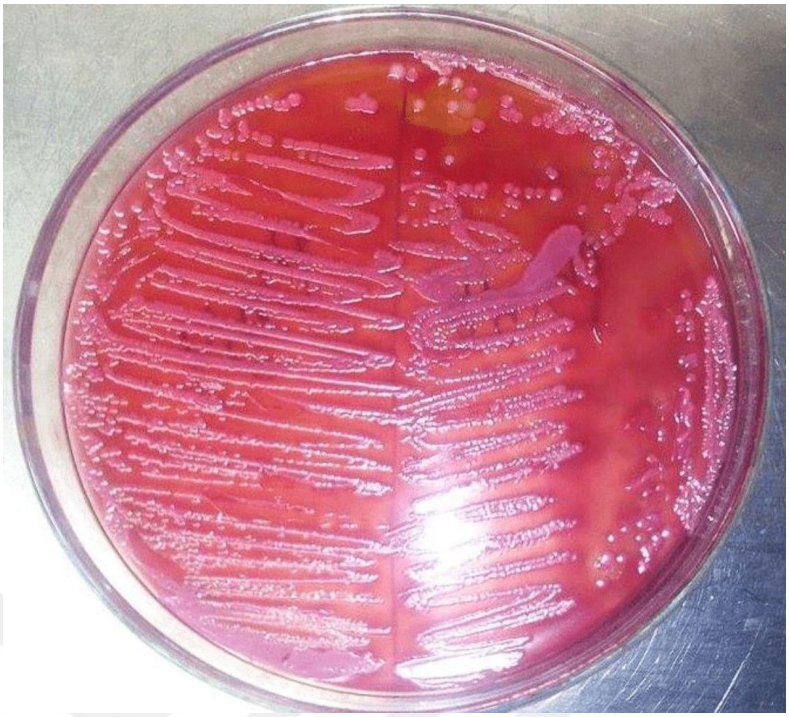

Figure 4.2 *E. coli* colonies on MacConkey agar

4.3.2 *Proteus spp.*

The results of this study showed 18 (21.7 %) isolates of *Proteus* spp. They were isolated from women with UTI. The current findings were in agreement with a study carried out by Abed and Nuha (2016) who referred that the percentages of distribution for gram negative bacteria P. mirabilis were (26.6%).

4.3.3 *Klebsiella spp.*

According to the current study's findings, 14 (16.9%) *Klebsiella* species were identified from UTI women. In research by Jebur and Abbas (2014), vaginitis in women was associated with 19 isolates (14.8 %) of *Klebsiella* spp. (Figure 4.3). This bacterium is rarely found in healthy vagina, but the presence of such bacteria was confirmed in the presence of other pathogenic or non-pathogenic bacteria like Lactobacilli, pointing to its capacity to produce different types of virulence factors in the infected vagina to meet their requirements through their pathogeneses of infection (Lakshmi *et al.* 2012).

Figure 4.3 *Klebsiella* spp. colonies on Blood agar

4.4 Gram- Positive Bacteria Identification

4.4.1 *Staphylococcus spp.*

According to the current study's findings, women with UTIs were the source of 29 (54.7%) *Staphylococcus* spp. isolates. The current findings differ from those of a study conducted by Farhan (2018), who discovered that *S. aureus* made up 11.8% (Figure 4.4) of isolates while *S. saprophyticus* made up 6.97%. The absence of vaginal microorganisms is one of the main causes for the low percentages of *Staphylococcus* spp. Another reason for the low percentages of *Staphylococcus* spp. is that vaginal secretions assist in cleansing the vagina, removing pathogenic bacteria from it, and maintaining vaginal moisture, because vaginal dryness causes killing of beneficial bacteria and causes an imbalance of the microbial balance makes them more susceptible to infection (Yaseen 2010).

Figure 4.4 *S. aureus* colonies on blood agar

4.4.2 *Enterococcus spp.*

According to the findings of the current investigation, 24 (45.3%) isolates of *Enterococcus* spp. were found in UTI women. It was thought that one of the typical pathogens in the urinary system and genital tract was *Enterococcus faecalis* (Baron *et al*. 1994). In a study by Abed and Nuha (2016), it was discovered that more women than in the current study had vaginal infections with *Enterococcus faecalis* (11.6%)

4.5 The Relationship of Age with the Incidence of Bacteria

The age factor and its relationship to the rates of infection of the reproductive system were studied because of its direct relationship and an important role in bacterial infections. The study samples were divided according to age into four age groups as show in Table 4.5. The current study shows that the highest percentage of infection was in (26-35 year) group, which were 65(38.2%). Where, the lowest percentage of infection was in (above 45) group, which was 17(10%).

Table 4.5 The percentage of isolates in all age groups

Age groups	Number of tested samples	Number and percentage of infected samples	Number and percentage of uninfected samples
(15-25) year	72(32%)	41(30.1%)	31(26.1%)
(26-35) year	93(41.3%)	59(43.4%)	34(38.2%)
(36-45) year	42(18.7%)	23(16.9%)	19(21.3%)
Above 45	18(8%)	13(9.6%)	5(5.6%)
Total	225(100)	136(60.44%)	89(39.56%)

In the current study, the results show that the highest percentage of infection was in 26–35 year group. In a study disagreed with the results of the current study, showed that the highest percentage of infection was in 15-25 year group, which were 28 (75.68%), and then infection percentage of 15-25 year was 24 (75%) (Murad *et al.* 2012). Otherwise, the results of current study were agreement with Jabuk (2014) who referred that the positive cultures predominant among women age between 20-40 years and the lowest percentage of infection was in above 40 group, which was 6(12%).

Also, the results of current study were agreement with Naama (2008) who referred that the age group 25-34 have high percent of isolates (29.9%), while the 55-64 represent the low percent of isolates (8.3%), except in candidal infection which was increased in the age group 55-65. The association between age and infection was high significantly ($P<0.05$) The high prevalence rate at 25-34 years of age may be related to the fact that this age group represent the sexually active period, had lower socioeconomic status, had high percentage of contraceptive drugs use more than other ages. This result was corresponding to the result of Barker *et al.* (1991) and Yaseen (2020) who mentioned that, the higher infection was in the age more than 25 years.

4.6 Antibiotic Susceptibility Testing

All kinds of bacteria pose a great danger to humans due to the increase in deaths resulting from diseases caused by bacteria, which leads to the failure of clinical treatment, intravenous treatment, and the accumulation of bacteria in the blood. Most pathogenic bacteria acquire drug resistance over time, which complicates treatment.

This is why it is necessary to monitor antibiotic resistance so that bacterial infectious diseases can be accurately treatment. Antimicrobial susceptibility testing provides a wealth of information about the effectiveness and dosage of antibiotic agents in the treatment of bacterial infections (Behera *et al*. 2019).

4.6.1 *E. coli*

Amoxicillin/clavulanic acid and ampicillin were completely ineffective against *E. coli* (100%) in tests. Nalidixic has a 40% resistance. Other than that, *E. coli* displayed resistance to cefepime, gentamicin, amikacin, imipenem, and azithromycin of 60%, 50%, 10%, 30%, 20%. Antibiotic sensitivity test result for *E. coli* give in Table 4.6.

Table 4.6 This table shows antibiotic sensitivity test for *Escherichia coli*

	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**Percentage**
AMC (10/20mg)	R	R	R	R	R	R	R	R	R	R	100%
AMP (10mg)	R	R	R	R	R	R	R	R	R	R	100%
CN (10mg)	R	S	R	S	R	R	S	S	R	S	50%
CPM (30mg)	R	R	R	R	S	S	S	R	R	S	60%
I M I (10mg)	S	S	S	S	R	R	R	S	S	S	30%
AK (30mg)	S	S	R	S	S	S	S	S	S	S	10%
AZM (15mg)	S	S	S	R	S	S	R	S	S	S	20%
NA (30mg)	S	S	R	S	R	S	R	S	S	R	40%
TMP (5mg)	S	R	S	S	S	S	R	R	S	S	30%
CIP (5mg)	R	R	R	S	S	S	S	S	S	R	40%

S: Antibiotic Sensitive, R: Antibiotic Resistant, AMC: Clavulanic acid and Amoxicillin (Augmentin) , AMP: Ampicillin, TMP: Trimethoprim , IMI: Imipenem, CPM: Cefepime, AZM: Azithromycin , AK: Amikacin, C N: Gentamicin, NA: Nalidixic acid

The current result is consistent with the results of Sakhi (2019) who referred that The usage of these antibiotics often and the transmission of plasmids among bacteria may be to blame for the greatest levels of resistance to ampicillin for pathogenic *E. coli*. Ampicillin resistance in *Enterobacteriaceae* is mostly caused by ß-lactamases (Kliebe *et al.* 1985). Also, the current result is consistent with the results of Al-Helfi (2009) and another study of Prakash (2008) mentioned a high resistance rate in *E. coli* to ampicillin (99%) which is similar to our results (100%). Trimethprim and amikacin showed the lowest resistance among the rest antimicrobials in this study, a study in Kuwait showed

similar result (Noura *et al.* 2005). A study conducted in Cape Town showed that the intestinal family was resistant to ampicillin while being sensitive to amikacin, ciprofloxacin and gentamicin while it was resistant to ceftazidime and ceftriaxone by 53% and 50%, respectively (Mvalo *et al.* 2018).

4.6.2 *Klebsiella spp.*

100% complete resistance to ampicillin and amoxicillin/clavulanic acid was demonstrated by *Klebsiella* spp. nalidixic resistance is 40%. Otherwise, as shown in Table 4.7, *Klebsiella* spp. exhibited 40%, 20%, 10%, 0%, and 10% resistance to azithromycin, cefepime, gentamicin, amikacin, and imipenem, and as described below.

Table 4.7 This table shows antibiotic sensitivity test for *Klebsiella spp.*

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	percentage
AMC (10/20mg)	R	R	R	R	R	R	R	R	R	R	100 %
AMP (10mg)	R	R	R	R	R	R	R	R	R	R	100 %
CN (10/20mg)	S	S	S	S	S	S	S	S	R	S	10%
CPM (30mg)	S	S	S	R	S	S	S	S	R	S	20 %
IMI (10 mg)	S	S	S	S	S	S	R	S	S	S	10%
AK (30 mg)	S	S	S	S	S	S	S	S	S	S	0%
AZM (15 mg)	S	R	S	R	S	S	R	S	R	S	40%
NA (30 mg)	R	S	R	S	S	S	S	S	S	R	30 %
TMP (5mg)	R	R	R	S	R	S	R	R	R	R	80 %
CIP (5 mg)	R	R	R	S	S	S	S	S	S	R	40%
S: Antibiotic Sensitive, R: Antibiotic Resistant, AMC: Clavulanic acid and Amoxicillin (Augmentin) , AMP:Ampicillin, CN:Gentamicin, TMP:Trimethoprim, CPM:Cefepime, AZM:Azithromycin , AK:Amikacin, I M I:Imipenem, NA:Nalidixic acid											

4.6.3 *Proteus spp.*

Amoxicillin/clavulanic acid and ampicillin were completely ineffective against *Proteus spp*. nalidixic has a 50% resistance. As demonstrated in Table 4.10, *Proteus spp*. shown 20%, 10%, 30%, 30%, and 40% resistance to amikacin, imipenem, cefepime, azithromycin, and gentamicin, and as described in Table 4.8.

Table 4.8 This table shows antibiotic sensitivity test for *Proteus spp.*

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	percentage
AMP (10mg)	R	R	R	R	R	R	R	R	R	R	100 %
AMC (10/20 mg)	R	R	R	R	R	R	R	R	R	R	100 %
CN (10 mg)	R	S	S	S	S	S	R	S	R	S	30 %
CPM (30 mg)	R	S	S	R	S	R	S	S	R	S	40 %
IMI (10 mg)	S	S	R	S	S	S	R	S	S	S	20 %
AK (30 mg)	S	S	S	R	S	S	S	S	S	S	10 %
AZM (15 mg)	S	R	S	R	S	S	S	S	R	S	30 %
NA (30mg)	R	S	R	S	R	S	R	S	S	R	50 %
TMP (5 mg)	R	R	R	S	R	S	R	R	R	R	80 %
CIP (5 mg)	S	R	R	S	S	S	S	S	S	R	30 %
S: Antibiotic Sensitive, R: Antibiotic Resistant, AMC: Clavulanic acid and Amoxicillin (Augmentin) , CPM:Cefepime, AZM:Azithromycin, AMP: Ampicillin, CN :Gentamicin, AK:Amikacin, TMP:Trimethoprim, I M I:Imipenem, NA:Nalidixic acid.											

The current findings are consistent with (Kareem and Iman 2011) who referred that the antibiotic resistant of *Proteus* spp. 100% ceftazidime, 66% cefotaxime, 58% nalidixic acid, 50% colistin, cefriaxone chloromphenicol 33% and *Proteus* spp. still sensitive to amikacin. (Al-Jendy and Al-Ofairi 2019) who referred that *Proteus mirabilis* had the highest sensitivity to amikacin, they agrees with present results, and the highest resistance to penicillin, amoxicillin and ampicillin 83.3%, 66.7%, 66.7%.

4.6.4 *Staphylococcus spp.*

Staphylococcus spp. showed total resistance for cefoxitin, penicillin, oxacillin. 40%, 40%, 80%, 10%, 0%, 20% and 20% surrender to azithromycin, ciprofloxacin, trimethoprim, refampicin, nitrofurantoin, erythromycin and gentamicin and as described in Table 4.9.

Table 4.9 This table shows antibiotic sensitivity test for *Staphylococcus spp.*

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	percentage
OX (1mg)	R	R	R	R	R	R	R	R	R	R	100 %
P (10U)	R	R	R	R	R	R	R	R	R	R	100 %
CN (10mg)	S	S	S	S	S	S	R	S	R	S	20 %
F (300mg)	S	S	S	S	S	S	S	S	S	S	0 %
E (15mg)	S	S	R	S	S	S	R	S	S	S	20 %
CX (30mg)	R	R	R	R	R	R	R	R	R	R	100 %
AZM (15mg)	S	S	S	R	S	R	S	S	R	R	40 %

Table 4.9 Continue

RP (5mg)	S	S	S	S	S	S	S	S	R	S	10 %
TMP (5mg)	S	S	R	R	R	R	R	R	S	R	80 %
CIP (5mg)	S	R	S	S	S	S	S	S	S	R	40 %
S: Antibiotic Sensitive, R: Antibiotic Resistant, P: Penicillin, AZM:Azithromycin, CIP:Ciprofloxacin, CN:Gentamicin, O X:Oxacillin, E: Erythromycin, C X:Cefoxitin, I :Intermediate , TMP: Trimethoprim, F: Nitrofurantoin, R P:Refampicin.											

The current findings are consistent with Wenjing *et al.* (2012) who indicated that the (94.3%) of *S. aureus* resistance to rifampin. The causes of rifampin resistant mutations within bacteria might be due to alterations in the gene, which encodes the β-subunit of the RNA polymerase enzyme (Campbell *et al.* 2001).

On the other hand, Adhikari *et al.* (2017) mentioned that clindamycin resistance was found in 10% of *S. aureus* isolates, while (Rağbetli *et al.* 2016) found 13.5% resistance among *S. aureus* isolates. (Prabhu *et al.* 2011) revealed that the percentage of erythromycin resistance among *S. aureus* isolates was 28.42%. While, (Alani 2017) showed that erythromycin resistance among *S. aureus* isolates was (85.7%) and was 78% for azithromycin, this disagrees with the resistance of *S aureus* in current study. While, the current findings are consistent with (Zeidan 2005) who found that tetracycline resistance was 70%, while (Mahmood and Flayyih 2014) who showed that tetracycline resistance was 55%.

4.6.5 *Enterococcus spp.*

Enterococcus spp. showed total resistance for cefoxitin, penicillin and oxacillin. 40%, 0%, 20%, 10%, 0%, 30% and 30% resistance to ciprofloxacin, refampicin, erythromycin, trimethoprim, nitrofurantoin, azithromycin and gentamicin and as described in Table 4.10.

Table 4.10 This table shows antibiotic sensitivity test for *Enterococcus spp.*

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	percentage
OX (1mg)	R	R	R	R	R	R	R	R	R	R	100 %
P (10U)	R	R	R	R	R	R	R	R	R	R	100 %
CN (10 mg)	S	R	S	R	S	S	R	S	S	S	30 %
RP (5 mg)	S	S	S	S	S	S	S	S	S	S	0 %
E (15 mg)	S	R	S	S	S	S	R	S	S	S	20 %
CX(30 mg)	R	R	R	R	R	R	R	R	R	R	100 %
AZM(15mg)	S	R	S	R	S	S	S	S	S	R	30 %
F (300 mg)	S	S	S	S	S	S	S	S	S	S	0 %
TMP (5mg)	R	S	R	R	R	R	R	R	R	R	10 %
CIP (5 mg)	R	S	S	R	S	S	S	R	S	R	40 %
S: Antibiotic Sensitive, R: Antibiotic Resistant, I: Intermediate, CN:Gentamicin, OX:Oxacillin, P: Penicillin, CIP:CiprofloxacinE, RP:Refampicin, CX:Cefoxitin, AZM:Azithromycin, E:Erythromycin , TMP:Trimethoprim, F:Nitrofurantoin.											

The current findings are agree with study carried out by (Al-Marjani 2014) who referred that the antibiotic sensitive of *E. faecalis*, where it was total sensitive to ampicillin. While, it was total resistant to oxacillin and cefepime. Also, the current findings are agree with study carried out by (Oh *et al.* 2007) who referred that the antibiotic resistance of *E. faecium* was 58% among the isolates isolated from the patients. While, the current findings are disagree with (Hassoun and Mohammed 2022) who found that the 27 isolates of *E. faecalis* isolated from urinary tract infections that these isolates are all 100% sensitive to vancomycin.

4.7 The Virulence Factors of Studied Bacteria

Table 4.11 showed different types of virulence factors of studied bacteria. Whereas, percentage of hemolysis production for isolates of *E. coli*, *Proteus* spp., *Klebsiella* spp., *Staphylococcus* spp. and *Enterococcus* spp. was 88.2%, 72.2%, 71.4%, 65.5% and 50%. Percentage of urease production for isolates of *E. coli*, *Proteus* spp., *Klebsiella* spp., *Staphylococcus* spp. and *Enterococcus* spp. was 90.1 %, 61.1%, 50%, 79.3% and 33.3%.

Percentage of protease production for isolates of *E. coli*, *Proteus* spp., *Klebsiella* spp., *Staphylococcus* spp. and *Enterococcus* spp. was 74.5%, 94.4%, 21.4%, 72.4% and

4.2%. Percentage of capsule production for isolates of *E. coli*, *Proteus* spp., *Klebsiella* spp., *Staphylococcus* spp. and *Enterococcus* spp. was 82.4%, 0.0%, 57.1%, 93.1% and 41.7%.

Table 4.11 The virulence factors of studied bacteria

Bacteria isolates	**Virulence factors**				**Isolate No.**
	Hemolysis	**Urease**	**Protease**	**Capsule**	
E. coli	45(88.2%)	49(90.1%)	38(74.5%)	42(82.4%)	51
Proteus spp.	13(72.2%)	11(61.1%)	17(94.4%)	0(0.0%)	18
Klebsiella spp.	10(71.4%)	7(50%)	3(21.4%)	8(57.1%)	14
Staphylococcus spp.	19(65.5%)	23(79.3%)	21(72.4%)	27(93.1%)	29
Enterococcus spp.	12(50%)	8(33.3%)	1(4.2%)	10(41.7%)	24

When grown on blood agar, several different species of bacteria are able to create hemolysin, which results in hemolysis zones that are barely bigger than the colonies themselves (Sharma *et al.* 2007). The ability to penetrate the host's tissues and improve the pathogenic capacity of an organism both of which are referred to as virulence factors are conferred by hemolysin production. This crucial virulence factor, which causes transmembranous holes to develop in the host cell membrane and is cytotoxic (Cruickshank *et al.* 1973).

Another crucial virulence trait of UTIs is the synthesis of hemolysin. Hemolysins cause scarring by directly having cytotoxic effectiveness on the renal epithelium. According to reports, alpha-hemolysin is an antigenic substance having dermonecrotic effects that can be fatal a variety of host tissues and cells, including RBCs, leucocytes, epithelial, and endothelial cells, are hazardous to hemolysins as well (Griffiths and McClain 1988).

When hemolysin production by *E. coli* was tested, it was discovered that 40.4% of the 47 isolates of *E. coli* were capable of producing it on blood agar as a zone of hemolysis encircling bacterial colonies (Bhakdi *et al.* 1988).

Gram negative and Gram positive bacteria isolated from urinary tract infections were studied for their ability to produce extracellular proteases. It was discovered that after

24 hours of incubation, all isolates of this study (*Proteus mirabilis, E. coli*, *Staphylococcus* spp., and *Klebsiella*) were able to produce proteases in M9 media (supplemented with 20 percent glucose and 1 percent gelatin). After adding 3 mL of 5 percent trichloroacetic acid to the colony, a clear hallo of transparent region was discovered around the colony.

This finding corroborated the findings of Choong *et al.* (2001), who discovered that all isolates of uropathogenic bacteria produced extracellular protease. Additionally, these outcomes matched those of (Döring *et al.*1981), who discovered that gelatin was used as substrates into agar and different culture medium compositions to detect extracellular protease from microorganisms on agar plates. Extracellular protease is crucial for cell viability and intercellular communication (Esposito *et al.*1980). Additionally, during the growth of the organism in the medium containing amino acids, the generation of protease was stimulated (Caballero *et al.* 2001).

The exo-products of this microorganism, such as alkaline protease and elastase, which damage tissues by breaking down elastin, collagen, and proteoglycans, have been proposed as virulence factors in a range of diseases caused by them. These enzymes have also been shown to break down proteins involved in host defense in vivo (Amara *et al.* 2009).

5. CONCLUSIONS AND RECOMMENDATIONS

The results showed that 136 samples (60.44%) of the total, displayed positive results to bacterial growth when they were cultured on the better culture media.

The current study shows that the highest percentage of infection was in 26–35 year group, which were 65 (38.2%). Where, the lowest percentage of infection was in above 45 group, which was 17 (10%).

Proteus spp., E. coli and *Klebsiella* spp. showed total resistance (100%) to ampicillin and amoxicillin/clavulanic acid. *Staphylococcus* spp., *Enterococcus* spp. showed total resistance to cefoxitin, penicillin and oxacillin.

The findings showed different types of virulence factors of studied bacteria. Whereas, percentage of hemolysis production for isolates of *Klebsiella* spp., *Proteus* spp., *E. coli*, *Enterococcus* spp. and *Staphylococcus* spp. was 71.4%, 72.2%, 88.2%, 50% and 65.5%, orderly Percentage of urease production was 50% ,61.1%,90.1 %, 33.3% and 79.3%. Percentage of protease production for isolates of, *Klebsiella* spp., *Proteus* spp., *E. coli*., *Enterococcus* spp. and *Staphylococcus* spp. was 21.4%, 94.4%,74.5%, 4.2% and 72.4%. Percentage of capsule production for isolates of *Klebsiella* spp, *Proteus* spp., *E. coli*, *Enterococcus* spp. and *Staphylococcus* spp. was 57.1%, 0.0%,82.4%, 41.7% and 93.1%.

In line with all this information;

It is recommended that women aged 26-35 should be screened regularly to detect urinary tract infection. Such screenings will help to detect the causative agent early, ensure adequate treatment with appropriate antimicrobial drugs, and determine drug sensitivities to prevent any complications for the mother and fetus.

Antibiotics with active ingredients amikacin and Imipenem can be used in the treatment of UTI caused by *Proteus* spp, *Klebsiella* spp and *E. coli*. Antibiotics with active ingredients nitrofurantoin and rifampicin can be used in the treatment of UTI caused by *Staphylococcus* spp. and *Enterococcus* spp.

REFERENCES

Abed, K. A. and Nuha J. K. 2016. Molecular and Bacteriological Detection of Some Bacterial Vaginosis Associate Bacteria in Women. Iraqi J. Sci., 57(3): 1926-1936.

Abrutyn, E., Mossey, J., Berlin, J. A., Boscia, J., Levison, M., Pitsakis, P. and Kaye, D. 1994. Does asymptomatic bacteriuria predict mortality and does antimicrobial therapy reduce mortality in mobile elderly women? Annals of Internal Medicine, 120 (10): 827-833.

Ackermann, R. J. and Monroe, P. W. 1996. Bacteremic urinary tract infection in older people. Journal of the American Geriatrics Society, 44(8): 927-933.

Adhikari, R., Pant, N. D., Neupane, S., Neupane, M., Bhattarai, R., Bhatta, S. and Lekhak, B. 2017. Detection of methicillin resistant *Staphylococcus aureus* and determination of minimum inhibitory concentration of vancomycin for *Staphylococcus aureus* isolated from pus/wound swab samples of the patients attending a tertiary care hospital in Kathmandu, Nepal. Canadian Journal of Infectious Diseases and Medical Microbiology, 2: 33-38.

Alani, H. A., Hassawi, D. S. and Flayih, M. T. 2017. Patterns of antibiotic resistance in *Staphylococcus aureus* isolates and detection the heteroresistance to vancomycin by population analysis method. JUAPS, 11(3): 26-33.

Al-Helfi, M. A.M. 2009. Environmental epidemical study on ows (chrysomya bezziana) villeneuve in basrah province south of Iraq. Basrah J. Veter. Res., 11(1): 307-317.

Al-Jendy. A. S. and Al-Ofairi B. A. 2019. The bacterial pathogens of patients with urinary tract infections and antibiotics susceptibility in Taiz Governorate–Yemen. Al-Mustansiriyah J. Sci., 30(1): 43-51.

AL-Marjani, M. F. 2014. Van A in vancomycin-resistant *Enterococcus faecalis* isolated in Baghdad. African Journal of Microbiology Research, 7(2): 115-119.

Al-Najjar A., Alban R. and Michael K. 2020. Spatial patterns and links between microbial community composition and function in cyanobacterial mats. Frontiers in Microbiology 5(604): 22-26.

Amara, A. A., Salem, S. R. and Shabeb, M. S. A. 2009. Biodetergent: The possibility to use bacterial protease and lipase as detergent. Microbiol, 20(3): 312-320.

Atlas, R. M. 2006. The handbook of microbiological media for the examination of food. CRC Press, 464 page, USA.

Atlas, R., Brown, A. and Parks, C. 1995. Laboratory manual experimental of microbiology. Mosby-Year Book Inc., 565 page, USA.

Baldassarre, J. S. and Kaye, D. 1991. Special problems of urinary tract infection in the elderly. North American Medical Clinics., 75(2): 375-390.

Banerjee, S., Shaikh, C., Wallace, G. R., Murray, P. I. and Scott, R. A. H. 2003. Frozen cucumber as a mount for processing vitreoretinal specimens. British Journal of Ophthalmology, 87(4): 512-512.

Barker, W.W. and Banfield, J.F. 1991 Biologically versus inorganicallymediated weathering reactions: Relationships between minerals and extracellular microbial polymers in lithobiontic communities. Chemical Geology, 132: 59–69.

Baron, E. J., Peterson, L. R. and Finegold S. M. 1994. Bailey and Scott's Diagnostic microbiology. Mosby Louis Publishe, 958 page, USA.

Beck-Sague, C., Villarino, E., Giuliano, D., Welbel, S., Latts, L., Manangan, L.M. and Jarvis, W.R. 1994. Infectious disease and death among nursing home residents: results of surveillance in 13 nursing homes. Infection Control and Hospital Epidemiology, 15(7): 494-496.

Behera, B., Anil Vishnu, G. K., Chatterjee, S., Sitaramgupta V, V. S. N., Sreekumar, N., Nagabhushan, A., Rajendran, N., Prathik, B. H. and Pandya, H. J. 2019. Emerging technologies for antibiotic susceptibility testing. Biosensors & Bioelectronics, 142: 111552.

Bhakdi, S., Mackman, N., Menestrina, G., Fray, L., Hugo, F., Segar, W. and Hollabd, I.B. 1988. The hemolysin of *E. coli*. European J. Epid., 4(2): 135-143.

Brinton, C. C., Jr. 1965. The structure, function, synthesis and genetic control of bacterial pili and a molecular model for DNA and RNA transport in gram negative bacteria. Transactions of the New York Academy of Sciences, 27(8): 1003–1054.

Brooks, G. F., Carroll, K. C., Butel, J. S., Morse, S. A., Mietzner, T. A. and Jawetz, M. 2007. Adelberg’s medical microbiology. Sultan Qaboos Univ. Med. J., 7: 273.

Caballero, A. R., Moreau, J. M., Engel, L. S., Marquart, M. E., Hill, J. M. and O'Callaghan, R. J. 2001. *Pseudomonas aeruginosa* protease IV enzyme assays and comparison to other Pseudomonas proteases. Analytical Biochemistry, 290(2): 330-337.

Campbell, E. A., Korzheva, N., Mustaev, A., Murakami, K., Nair, S., Goldfarb, A. and Darst, S. A. 2001. Structural mechanism for rifampicin inhibition of bacterial RNA polymerase. Cell, 104(6): 901-912.

Choong, S., Wood, S., Fry, C. and Whitfield, H. 2001. Catheter associated urinary tract infection and encrustation. International Journal of Antimicrobial Agents, 17(4): 305-310.

Chow, J. W., Thal, L. A., Perri, M. B., Vazquez, J. A., Donabedian, S. M., Clewell, D. B. and Zervos, M. J. 1993. Plasmid-associated hemolysin and aggregation substance production contribute to virulence in experimental *enterococcal* endocarditis. Antimicrobial Agents and Chemotherapy, 37(11): 2474-2477.

Collee, J.G., Miles, R.S. and Watt, B. 1996. Tests for the Identification of Bacteria. In: Collee, J.G., Marmion, B.P., Fraser, A.G. and Simmons, A., Eds., Mackie and McCartney Practical Medical Microbiology. Churchill Livingstone, pp. 131-151, New York.

Connell, I., Agace, W., Klemm, P., Schembri, M., Mărild, S. and Svanborg, C. 1996. Type 1 fimbrial expression enhances *Escherichia coli* virulence for the urinary tract. Proceedings of the National Academy of Sciences, 93(18): 9827-9832.

Cornely, O., Arikan-Akdagli, S. E. V. T. A. P., Dannaoui, E., Groll, A. H., Lagrou, K., Chakrabarti, A. and Petrikkos, G. 2014. ESCMID and ECMM joint clinical guidelines for the diagnosis and management of mucormycosis 2013. Clinical Microbiology and Infection, 20: 5-26.

Cruickshank, R., Duguid, J.P., Mormon, B.P., and Swain, R.H.A. 1973. Medical Microbiology: A Guide to the Laboratory Diagnosis and Control of Infection. 12th edition, vol. 1 Microbial Infections. Churchill Livingstone, 12 : 667.

Ditroi, T., Nagy, A., Martinelli, D., Rosta, A., Kožich, V. and Nagy, P. 2019. Comprehensive analysis of how experimental parameters affect H2S measurements by the monobromobimane method. Free Radical Biology and Medicine, 136: 146-158.

Döring, G., Obernesser, H. J. and Botzenhart, K. 1981. Extracellular toxins of Pseudomonas aeruginosa. II. Effect of two proteases on human immunoglobulins IgG, IgA and secretory IgA (author's transl). Zentralblatt fur Bakteriologie. 1. Abt. Originale. A: Medizinische Mikrobiologie, Infektionskrankheiten und Parasitologie, 249(1): 89-98.

Esposito, A. L., Gleckman, R. A., Cram, S., Crowley, M., McCabe, F. and Drapkin, M. S. 1980. Community-acquired bacteremia in the elderly: Analysis of one hundred consecutive episodes. Journal of the American Geriatrics Society, 28(7): 315-319.

EUCAST, 2020. European Committee on Antimicrobial Susceptibility Testing. Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST. Version 10: 5-7.

Farhan, W. M. 2018. Molecular study to some Virulance gene of *Staphylococcus* aureus and *Pseudomonas aeruginosa* bacteria isolated from women suffered from bacterial vaginitis. Journal of University of Anbar for Pure Science, 12(1): 3-6.

Fihn, S. D., Boyko, E. J., Chen, C. L., Normand, E. H., Yarbro, P. and Scholes, D. 1998. Use of spermicide-coated condoms and other risk factors for urinary tract infection caused by Staphylococcus saprophyticus. Archives of Internal Medicine, 158(3): 281-287.

Foxman, B. 2002. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. The American Journal of Medicine, 113(1): 5-13.

Garibaldi, R. A., Mooney, B. R., Epstein, B. J. and Britt, M. R. 1982. An evaluation of daily bacteriologic monitoring to identify preventable episodes of catheter-associated urinary tract infection. Infection Control and Hospital Epidemiology, 3(6): 466-470.

Gottlieb, T. and Nimmo, G. R. 2011. Antibiotic resistance is an emerging threat to public health: an urgent call to action at the Antimicrobial Resistance Summit 2011. Med J Aust, 194(6): 281-3.

Griffiths, B. B. and McClain, O. 1988. The role of iron in the growth and hemolysin (Streptolysin S) production in *Streptococcus pyogenes*. J. Basic. Microbiol. 28(7): 427–36.

Gwendolyn, R. 1988. Microbiology for Health Sciences (3rd ed.). J. B. Lippincott Company, 566 page, Philadelphia.

Harding, G. K., Nicolle, L. E., Ronald, A. R., Preiksaitis, J. K., Forward, K. R., Low, D. E. and Cheang, M. 1991. How long should catheter-acquired urinary tract infection in women be treated? A randomized controlled study. Annals of Internal Medicine, 114(9): 713-719.

Hassoun, B. H. and Mohammed, G. J. 2022. Molecular genotyping of *Enterococcus faecalis* isolated from clinical sources by BOX-PCR and ERIC-PCR. International Journal of Health Sciences, 6(1): 11664-11677.

Hidad, S., van der Putten, B., van Houdt, R., Schneeberger, C. and Kuil, S. D. 2022. Recurrent E. coli Urinary Tract Infections in Nursing Homes: Insight in Sequence Types and Antibiotic Resistance Patterns. Antibiotics, 11(11): 1638.

Hooton, T. M. (2001). Recurrent urinary tract infection in women. International Journal of Antimicrobial Agents, 17(4): 259-268.

Hooton, T. M., Scholes, D., Hughes, J. P., Winter, C., Roberts, P. L., Stapleton, A. E. and Stamm, W. E. 1996. A prospective study of risk factors for symptomatic urinary tract infection in young women. New England Journal of Medicine, 335(7): 468-474.

Hooton, T.M., Hillier, S., Johnson, C., Roberts, P.L. and Stamm, W.E. 1991. *Escherichia coli* and contraceptive method. Jameh, 265(1): 64-69.

Hsieh, M. H., Wood, H. M., Dicianno, B. E., Dosa, N. P., Gomez-Lobo, V., Mattoo, T. K. and Bavendam, T. 2017. Research needs for effective transition in lifelong care for congenital genitourinary conditions: a workshop sponsored by the national institute of diabetes and digestive and kidney diseases. Urology, 103: 261-271.

Ikähelmo, R., Siitonen, A., Heiskanen, T., Kärkkäinen, U., Kuosmanen, P., Lipponen, P. and Mäkelä, P. H. 1996. Recurrence of urinary tract infection in a primary care setting: analysis of an I-year follow-up of 179 women. Clinical Infectious Diseases, 22(1): 91-99.

Jabuk, S. I. A. 2014. Prevalence of aerobic bacterial vaginosis among Intrauterine Contraceptive Device users women in Hilla city. Journal of University of Babylon, 22(9): 2431-2424.

Jacobsen, S. M., Stickler, D. J., Mobley, H. L. and Shirtliff, M. E. 2008. Complicated catheter-associated urinary tract infections due to Escherichia coli and Proteus mirabilis. Clinical Microbiology Reviews, 21(1): 26-59.

Janda, J. M. and Bottone, E. J. 1981. *Pseudomonas aeruginosa* enzyme profiling: predictor of potential invasiveness and use as an epidemiological tool. Journal of Clinical Microbiology, 14(1): 55-60.

Jebur, M. S. and Abbas A. H. 2014. Antibacterial susceptibility patterns of *Klebsiella pneumoniae* isolates from vaginitis cases of pregnant women in Baghdad city. Al-Qadisiyah Med. J., 10(17): 196-203.

Jellheden, B., Norrby, R.S. and Sandberg, T. 1996 Symptomatic urinary tract infection in women in primary health care: bacteriological, clinical and diagnostic aspects in relation to host response to infection. Scandinavian Journal of Primary Health Care, 14: 122-130.

Johansen, T. B., Bonkat, G., Cai, T., Tandogdu, Z., Wagenlehner, F. and Grabe, M. 2016. Gray areas are in the area of urinary tract infections. European Focus in Urology, 2(4): 460-462.

Johnson, J.R. and Stam, W.E. 1989. Urinary tract infections in women: diagnosis and treatment. Annals of Internal Medicine, 111(11): 906-917.

Kaijser, B, and Larson, B. 1982. Experimental acute pyelonephritis caused by intestinal bacteria in animals. Journal of Urology, 127(4): 786-790.

Kareem, I. J and Iman Y R. 2011. Antibiotic susceptibilities of gram negative aerobic bacteria isolated from urinary tract infections in community. Iraqi J. Med. Sci., 9(4): 295-300.

Kateete, D. P., Kimani, C. N., Katabazi, F. A., Okeng, A., Okee, M. S., Nanteza, A. and Najjuka, F. C. 2010. Identification of Staphylococcus aureus: DNase and Mannitol salt agar improve the efficiency of the tube coagulase test. Annals of Clinical Microbiology and Antimicrobials, 9(1): 1-7.

Kau, A. L., Martin, S. M., Lyon, W., Hayes, E., Caparon, M. G. and Hultgren, S. J. 2005. Enterococcus faecalis tropism for the kidneys in the urinary tract of C57BL/6J mice. Infection and Immunity, 73(4): 2461-2468.

Khan, J. A., Rathore, R. S., Khan, S. and Ahmad, I. 2013. In vitro detection of pathogenic Listeria monocytogenes from food sources by conventional,

molecular and cell culture method. Brazilian Journal of Microbiology, 44: 751-758.

Kliebe, C., Nies, B. A., Meyer, J. F., Tolxdorff-Neutzling, R. M. and Wiedemann, B. 1985. Evolution of plasmid-coded resistance to broad-spectrum cephalosporins. Antimicrobial Agents and Chemotherapy, 28(2): 302-307.

Kunin, C. M. 1994. Urinary tract infections in females. Clinical Infectious Diseases, 18(1): 1-10.

Lakshmi, K., Chitralekha, S., Illamani, V. and Menezes, G. A. 2012. Prevalence of bacterial vaginal infections in pre and postmenopausal women. Int. J. Pharm Bio. Sci, 3(4): 949-956.

Latham, R. H., Running, K. and Stamm, W. E. 1983. Urinary tract infections in young adult women caused by Staphylococcus saprophyticus. Jama, 250(22): 3063-3066.

Maa'roof, M. N. and Azeez, I. M. 2018. Isolation and Identification of some Bacterial Species From Cervix Infected Women in Salahuddin Governorate. Tikret Journal of Pharmaceutical Sciences, 13(1): 46-52.

Macfaddin, J. F. 2000. Biochemical tests for identification of medical bacteria. Williams and Willkins Compony, 113 page, USA.

Mahmood, H. A. and Flayyih, M. T. 2014. Detection of van A gene of Vancomycin resistant *Staphylococcus aureus* by PCR technique. Int. J., 2(7): 209-216.

Mahon, C. R. and Manuselis, G. 2000. Textbook of Diagnostic Microbiology. Saunders Company, 1230 page, London.

Mandracchia, V. J., Hayes, D. W., Yoho, R. M. and Hayes, M. F. 2000. Diagnosis, differential and treatment options. Nat Rev Microbiol, 13: 269-84.

Mohammed, M. J. 2021. Bacterial study of bacteria species causing urinary tract infection in Kirkuk city. World Bulletin of Public Health, 5: 11-16.

Murad, A. M., Yahya J. S. and Samira S. H. 2012. Bacterial vaginosis and other infectious agents in preterm labour in Kirkuk province. Kirkuk Uni. J. Sci. Stu. 7(2): 42-57.

Mvalo, T., Eley, B., Bamford, C., Stanley, C., Chagomerana, M., Hendricks, M., Van Eyssen, A. and Davidson, A. 2018. Bloodstream infections in oncology patients

at red cross war memorial children's hospital, Cape Town, from 2012 to 2014. International Journal of Infectious Diseases, 77: 40–47.

Naama, J. K. 2008. Study of causative agents of cervicitis in women attending gynecologic outpatient department in Najaf city. Kufa Med. J., 11(1): 166-174.

Nicolle, L. E. 2009. Urinary tract infections in the elderly. Clinics in Geriatric Medicine, 25(3): 423-436.

Niu, C., Yu, D., Wang, Y., Ren, H., Jin, Y., Zhou, W.and Liang, L. 2013. Common and pathogen-specific virulence factors are different in function and structure. Virulence, 4(6): 473-482.

Noura, A., Mahdi A. and Albert, M. J. 2005. Virulence and phylogenetic groups of *Escherichia coli* cultured from raw sewage in Kuwait. Gut Patho., 14(18): 1-6.

Obi, C.L., Tarupiwa, A. and Simango, C. 1996. Scope of urinary pathogens isolated at the public health bacteriology laboratory, Harare: patterns of antibiotic sensitivity to isolates and incidence of hemolytic bacteria. Central African Journal of Medicine, 42 (8): 244-249.

Oh, E., Kwang-Tae S. and Hong-Sik Y. 2007. Antimicrobial susceptibility pattern of *Enterococcus faecalis* and *E. faecium* from fish farms in the southern coast of Korea. Korean J. Fish. Aqu. Sci., 41(6): 435-439.

Orikasa, S. and Hinman Jr, F. 1977. Reaction of the vesical wall to bacterial penetration: resistance to attachment, desquamation and leukocytic activity. Transactions of the American Association of Genito-Urinary Surgeons, 69: 52-60.

Pinzón-Arango, P. A., Liu, Y. and Camesano, T. A. 2009. Role of cranberry on bacterial adhesion forces and implications for Escherichia coli–uroepithelial cell attachment. Journal of Medicinal Food, 12(2): 259-270.

Pluschke, G., Mayden, J., Achtman, M. and Levine, R. P. 1983. The role of the capsule and O antigen in resistance to O18:K1 Escherichia coli in supplement-mediated killing. Infection and Immunology, 42 (3): 907-913.

Prabhu, K., Rao, S. and Rao, V. 2011. Inducible clindamycin resistance in *Staphylococcus* aureus isolated from clinical samples. Journal of Laboratory Physicians, 3(01): 025-027.

Prakash, K. P. 2008. Epidemiology and antimicrobial resistance of enteric pathogens in Dhahira region, Oman. Iranian J. Publ. Health, 37(3): 60-69.

Rağbetli, C., Parlak, M., Bayram, Y., Guducuoglu, H. and Ceylan, N. 2016. Evaluation of Antimicrobial Resistance in *Staphylococcus aureus* Isolates by Years. Interdisciplinary Perspectives on Infectious Diseases, 2: 2-4.

Rashid, A., Saqib, M., Deeba, F. and Khan, J. A. 2019. Microbial profile of burn wound infections and their antibiotic sensitivity patterns at burn unit of allied hospital Faisalabad. Pakistan Journal of Pharmaceutical Sciences, 36(1): 247-255.

Raz, R. and Westham, W. E. 1993. A controlled trial of intravaginal estriol in postmenopausal women with recurrent urinary tract infections. The New England Journal of Medicine, 329 (11): 753-756.

Rowe, T. A. and Juthani-Mehta, M. 2013. Urinary tract infection in older adults. Aging Health, 9(5): 519-528.

Sakhi, R. J. 2019. Isolation of *Escherichia coli* from Diarrhea and test their pathogensity and susceptibility pattern for antibiotic. J. Edu. Pur. Sci., 9(1): 43-48.

Sambrook, J., Fritsch, E. F. and Maniatis, T. 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, 1546 page, USA.

Sarowska, J., Futoma-Koloch, B., Jama-Kmiecik, A., Frej-Madrzak, M., Ksiazczyk, M., Bugla-Ploskonska, G. and Choroszy-Krol, I. 2019. Virulence factors, prevalence and potential transmission of extraintestinal pathogenic Escherichia coli isolated from different sources: recent reports. Gut Pathogens, 11: 1-16.

Schappert SM, Rechtsteiner EA. Ambulatory medical care utilization estimates for 2007. Vital Health Stat, 169: 1-38.

Schwaderer, A. L. and Wolfe, A. J. 2017. The association between bacteria and urinary stones. Annals of Translational Medicine, 5(2): 3-6.

Sharma, S., Bhat, G. and Shenoy, S. 2007. Virulence factors and drug resistance in *E. coli* isolates from extraintestinal infections. Indian. J. Med. Microbiol, 25(4): 369- 373.

Sheinfeld, J., Schaeffer, A. J., Cordon-Cardo, C., Rogatko, A. and Fair, W. R. 1989. Association of the Lewis blood-group phenotype with recurrent urinary tract infections in women. New England Journal of Medicine, 320(12): 773-777.

Sobel, J. D. 1995. Urinary tract infections. Principles and Practice of Infectious Diseases, 3: 582-611.

Subashchandrabose, S. and Mobley, H. L. 2017. Virulence and fitness determinants of uropathogenic Escherichia coli. Urinary Tract Infections: Molecular Pathogenesis and Clinical Management, 5: 235-261.

Tan, M. S., White, A. P., Rahman, S. and Dykes, G. A. 2016. Role of fimbriae, flagella and cellulose on the attachment of Salmonella Typhimurium ATCC 14028 to plant cell wall models. PLoS One, 11(6): e0158311.

Tănase, A., Popov, M., Cepoida, P. and Maximenco, E. 2009. Pielonefrita acută la adult: protocol clinic naţional PCN-59. Această Publicaţie, 23-34.

Valvano, M. A. and Crosa, J. H. 1988. Molecular cloning, expression, and regulation in *Escherichia coli* K-12 of a chromosome-mediated aerobactin iron transport system from a human invasive isolate of *E. coli* K1. Journal of Bacteriology, 170(12): 5529-5538.

Vernon, S., Foo, CK and Coulthard, M.J. 1997. How general practitioners treat children with urinary tract infections: a review in the former Northern District. British Journal of General Practice, 47 (418): 297-300.

Wagenlehner, F. M. E., Niemetz, A. H., Weidner, W. and Naber, K. G. 2008a. The spectrum and antibiotic resistance of urinary pathogens from hospitalized patients with urinary tract infections: 1994-2005. International Journal of Antimicrobial Agents, 31: 25-34.

Wagenlehner, F. M. E., Niemetz, A., Dalhoff, A. and Naber, K. G. 2002b. Spectrum and antibiotic resistance of uropathogens from hospitalized patients with urinary tract infections: 1994–2000. International Journal of Antimicrobial Agents, 19(6): 557-564.

Wang, C., Li, Q., Lv, J., Sun, X., Cao, Y., Yu, K. and Wang, Q. 2020. Alpha-hemolysin of uropathogenic Escherichia coli induces GM-CSF-mediated acute kidney injury. Mucosal Immunology, 13(1): 22-33.

Wang, L., Lansing, B., Symons, K., Flannery, E. L., Fisch, J., Cherian, K. and Mody, L. 2012. Infection rate and colonization with antibiotic-resistant organisms in skilled nursing facility residents with indwelling devices. European Journal of Clinical Microbiology and Infectious Diseases, 31: 1797-1804.

Wenjing, Z., Wulin, S. and Xiaoling, M. 2012. Molecular characterization of rifampicin-resistant *Staphylococcus aureus* isolates in a Chinese teaching hospital from Anhui, China. BMC Microbiology, 12(1): 240.

Yabuuchi, E. and Ohyama, A. 1972. Characterization of "pyomelanin"-producing strains of Pseudomonas aeruginosa. International Journal of Systematic and Evolutionary Microbiology, 22(2): 53-64.

Yaseen, A. A. 2010. Effect of etracction procedure on the inhibition of santonica plant against spectrum of isolated bacteria. Diyala Agricultural Sciences Journal, 2(1): 7-14.

Yaseen, S. A. S. 2020. Study About the Causative Agents of Cervical Infections and Cytopathological Changes in Iraqi Women. Iraqi Journal of Science, 246-253.

Zeidan, I. A. 2005. Genetic and bacteriologic study to Staphylococcus aureus isolated from clinical specimens and resistant to Vancomycin antibiotic. M.Sc. Thesis, Baghdad University, 86 page, Iraq.

CURRICULUM VITAE

Personal Information

Name and Surname : Abdullah Ahmed Mohammed MOHAMMED

Education

MSc	Çankırı Karatekin University Graduate School of Natural and Applied Sciences Department of Biology	2020-2023
Undergraduate	Tikrit University Sciences of College Department of Biology	2011-2015