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Study the Antimicrobial Activity of Some Medicinal Plant Extract on The Antibiotic Resistance Bacteria Associated with Urinary Tract Infection

A thesis
Submitted to the Council of the College of science for women-University of Babylon in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology

By

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

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Dedication

To the one who delivered the message and fulfilled the trust.. and advised the nation.. to the prophet of mercy and the light of the world's Muhammad bin Abdullah (PBUH).

To whom worried by thinking of my future… to hope candle whom shine my life… to whom bow every letters and pens … my parents (God prolong their ages).

To my brothers.

To my support and strength, my wife.

To those who love them for love, if they passed by a barren land, the springs of love would burst forth from it, my dear children.

To my loyal friends and loved ones.

I present my modest effort, deepest and sincere gratitude for their support.

Ahmed

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List of Abbreviations

Abbreviations	**Complete term**
µl	Micro litter
AMD	antimicrobial resistance
AO	Acridine orange
℃	Degrees Celsius
CLSI	Clinical Laboratory Standards Institute
DNA	Deoxyribo Nucleic Acid
ESKAPE	*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp.*
M	mole
MDR	Multi drug resistant
mg	Milligram
MIC	Minimal inhibitory concentration
min	Minute
ml	Milliliter
mm	Millimeter
nm	nanometer
SDS	Sodium dodecyl sulfate
SIC	Sub lethal concentration
TBE	Tris base-Boric acid-Na_2 EDTA
TE buffer	Tris- EDTA buffer
UTIs	Urinary tract infections
UV	Ultraviolet
WHO	World health organization

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الخلاصة

تعد عدوى المسالك البولية (UTI) واحدة من أكثر المشاكل الصحية شيوعًا في البيئات السريرية. أصبحت مقاومة المضادات الحيوية قضية مقلقة لعدوى المسالك البولية في الوقت الحاضر. تم جمع 130 عينة ادرار من مرضى في المستشفيات العراقية للفترة من بداية تشرين الثاني 2020 وحتى نهاية شباط 2021 لفئات عمرية مختلفة تتراوح بين (20-60) سنة شملت 83 عينة من الاناث و 47 عينة من الذكور. تم زرع العينات على أجار متوسط من الدم ووسط أجار ماكونكي. تم تشخيص المزارع البكتيرية المتنامية بالاختبارات الميكروسكوبية والكيميائية الحيوية حيث كان عدد العزلات التي أعطت نتيجة سلبية (25) عينة بنسبة (19.2٪). بينما كانت النتيجة الإيجابية للمزرعة البكتيرية 105 عزلة بنسبة (80.8٪) حيث كانت 48 عزلة *E. coli* بنسبة 45.7%، 22 عزلة *K. pneumonia* بنسبة 20.9%،11 عزلة *P. aeruginosa* بنسبة 10.5٪ ,24 عزلة *S. aureus* بنسبة 22.9٪.

في هذه الدراسة ، تم إخضاع 105 عزلة من عينات ادرار لاختبار الحساسية للمضادات الحيوية وفقًا لإرشادات (CLSI 2019) حيث تم استخدام سبعة أقراص مختلفة من المضادات الحيوية. وبلغت نسبة المقاومة 80.95٪ ، وبلغت مقاومة للأدوية المتعددة 34.1٪. كانت معدل المقاومة بما في ذلك Trimethoprim, Tobramycin, Piperacillin, Ceftazidime, Cefeime, Aztreonam Rifampicin و Vancomycin.حيث كانت عزلات *E. coli* مقاومة في 34 عزلة (70.83٪) من 48 عزلة. كما كانت عزلات *K. pneumoniae* مقاومة للمضادات الحيوية في 21 عزلة (95.45٪) من 22 عزلة ، بينما كانت *S. aureus* مقاومة للمضادات الحيوية في 20 عزلة (83.33٪) من 24 عزلة و *P. aeruginosa* 11 عزلة (90.90%) من 10 عزلات.

الكشف عن DNA البلازميد في البكتيريا عن طريق استخلاص الكت ثم الترحيل الكهربائي للهلام. وبعد ذلك تم اختبار المعالجة بالبلازميد بواسطة sodium dodecyl sulfate و Acridine orange بتركيزات (12.5 ، 25 ، 50 ، 100 ، 200 ، 400) ميكروغرام / مل. كان MIC لـSDS (200 *E.coli*، *K. pneumonia* 100، *P. aeruginosa* 50، *S. aureus* 50) و كان MIC لAO (200 *E.coli*، *K. pneumonia* 200، *P. aeruginosa* 200، *S. aureus* 100) .

تم اختيار ثلاث عزلات من كل بكتيريا متعددة المقاومة تحتوي على البلازميد لاختبار تأثير المستخلصات النباتية المستخدمة في الدراسة التي حددت المركبات النشطة بيولوجيًا باستخدام تقنية كروماتوغرافيا الغاز -

كتلة الطيف (GC-MS). لم يكن هناك تأثير للمستخلص المائي الساخن والبارد لنبات *Phyllanthus emblica* و *Syzygium aromaticum* و *Citrullus colocynthis* على البكتيريا ، بينما تأثير المستخلص الكحولي لنبات *Phyllanthus emblica* (إيثانول وميثانول) MIC على بكتيريا *E.coli* 1.5 ميكروغرام. / مل ، بكتريا *K. pneumonia* 6 ميكروجرام / مل ، *P. aeruginosa* 0.75 ميكروغرام / مل ، و *S. aureus* 0.75 ميكروغرام / مل) و (*E.coli* 0.75 ميكروغرام / مل ، *K. pneumonia* 6 ميكروغرام / مل ، *P. aeruginosa* 0.75 ميكروغرام / مل ، و بكتريا *S. aureus* 3ميكروغرام / مل) على التوالي.

بينما لم يظهر اختبار المستخلص الكحولي لنبات *Syzygium aromaticum* أي تأثير على بكتيريا (الإيثانول والميثانول) على بكتريا *S. aureus* و *K. pneumonia*. بينما أظهر ذلك تأثيرا على بكتريا *E. coli* و *P. aeruginosa* بواسطة MIC على بكتيريا (*E.coli* 3 ميكروغرام / مل و *P. aeruginosa* 3 ميكروغرام / مل) و (*E.coli* 3 ميكروغرام / مل و *P. aeruginosa* 3 ميكروغرام / مل) على التوالى. حيث ان لم يكن للمستخلص الكحولي لنبات *Citrullus colocynthis* (الايثانول والميثانول) أي تأثير على البكتيريا ، ولكن تم دمج المستخلص النباتي مع أقل تركيز من المضادات الحيوية ، مما أعطى نتائج مثبطة جيدة نتيجة العلاقة التآزرية التي حدثت بين المستخلصات النباتية والمضادات الحيوية.

Summery

In clinical settings, urinary tract infection (UTI) is one of the most common health disorders. Antibiotic resistance has become an alarming issue for UTI management nowadays. Urine samples (130) were collected from patients in Iraqi hospitals for the period from the beginning of November 2020 to the end of February 2021 for different age groups ranging between (20-60) years for included 83 samples of females and 47 samples of males, urine samples were cultured on blood agar and Maconkey agar medium. The growing bacterial cultures were diagnosed with microscopic and biochemical tests by the number of isolates that gave a negative result was (25) sample with (19.2%). The positive result for bacterial culture was 105 (80.8%) include 48 isolate *E. coli* 45.7%, 22 isolate *K. pneumonia* 20.9%, 11 isolate *P. aeruginosa* 10.5%, and 24 isolate *S. aureus* 22.9%.

In this study, the 105 bacterial isolates were subjected to susceptibility testing antibiotics according to the CLSI (2019) guidelines were using seven different antibiotic disks. The resistance rate was 80.95%, and the MDR resistance rate was 34.1%. The resistance rate to Aztreonam, Cefeime, Ceftazidime, Piperacillin, Tobramycin, Trimethoprim, Vancomycin and Rifampicin. *E. coli* isolates were resistant in 34 (70.83%) of the 48 isolates; also, the *K. pneumoniae* isolates were resistant to antibiotics in 21(95.45%) of the 22 isolates, While *S. aureus* was resistant to antibiotics in 20 (83.33%) of the 24 isolates and the *P. aeruginosa* 10 (90.90%) of 11 isolates.

The detection of plasmid DNA in bacteria by kit extraction and then gel electrophoresis. And then, the plasmid curing was tested by sodium dodecyl sulfate and acridine orange, with concentrations 12.5, 25, 50, 100, 200, 400

µg/mL. The minimum inhibitory concentration of SDS was (*E.coli* 200, *K. pneumonia* 100, *P. aeruginosa* 50, *S. aureus* 50) and AO was (*E.coli* 200, *K. pneumonia* 200, *P. aeruginosa* 200, *S. aureus* 100).

Three isolates of each multi-resistant bacteria containing plasmid were selected to test the effect of the plant extracts used in the study that identified the bioactive compounds by using the Gas Chromatography-Spectrum Mass (GC-MS) technique. There was no effect of hot and cold aqueous extract of *Phyllanthus emblica*, *Syzygium aromaticum* and *Citrullus colocynthis* plant on bacteria, while the effect of alcoholic extract of *P. emblica* (ethanol and methanol) MIC on bacteria (*E.coli* 1.5 µg/ml, *K. pneumonia* 6 µg/ml, *P. aeruginosa* 0.75 µg/ml, *S. aureus* 0.75 µg/ml) and (*E.coli* 0.75 µg/ml, *K. pneumonia* 6 µg/ml, *P. aeruginosa* 0.75 µg/ml, *S. aureus* 3 µg/ml) respectively.

While the *S. aromaticum* alcoholic extract test that showed also no effect on bacteria at (ethanol and methanol) on *S. aureus* and *K. pneumonia*, while that showed effect on *E. coli* and *P. aeruginosa* by MIC on bacteria (*E.coli* 3 µg/ml and *P. aeruginosa* 3 µg/ml) and (*E.coli* 3 µg/ml and *P. aeruginosa* 3 µg/ml) respectively. Also, no effect of alcoholic extract of *C. colocynthis* plant, (ethanol and methanol) on bacteria, but the plant extract was combined with the lowest concentration of antibiotics, gave good inhibitory results as a result of the synergistic relationship that occurred between the extract and the antibiotics.

Chapter One

Introduction and Literatures Review

Chapter Two

Materials and Methods

Chapter Three

Results and Discussion

Conclusions and Recommendations

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Appendix

Introduction and Literature Review

1.1. Introduction.

Urinary tract infections are one of the health problems that most countries suffer from, as it comes second after respiratory tract infections, respiratory Tract Infection and urinary tract infections (UTIs) affect all age groups, males and females (Medina, and Castillo-Pino, 2019). Bacteria cause 95% of UTI (Abou Heidar *et al.,* 2019). Due to its abilities to enter the urethra and travel to the bladder and kidneys (Okonko *et al.,* 2009; Hollyer and Ison, 2018).

Clinically infection usually due to Gram-negative bacteria and Gram-positive bacteria, although fungi, viruses, and parasites can also cause infection (Zorc *et al*., 2005; Dao *et al.,* 2020). The etiology of common pathogenic bacterial UTIs includes Gram-Negative bacteria such as *Escherichia*, *Klebsiella*, *Proteus*, *Enterobacter*, *Pseudomonas*, *Serratia spp*, and Gram positive bacteria as Streptococci, Enterococcus sp., and Staphylococcus (Kumar *et al*., 2015).

The presence of multidrug-resistant (MDR) pathogens becomes a cause for serious concern regarding nosocomial infections, the World Health Organization has recently recognized antimicrobial resistance as one of the three most important human health concerns (Bassetti *et al*., 2011). Multidrug resistance bacteria is defined as non-susceptibility to one or more antimicrobials on three or more antimicrobial classes by developing defensive mechanisms against them (Kallen and Srinivason, 2010; Gomila *et al.,* 2018).

The growing numbers of antimicrobial-resistant pathogens, which are progressively associated with nosocomial infection, place a significant burden on healthcare systems and have essential global economic costs, effects include high mortality and morbidity rates, increased treatment costs, diagnostic suspicions, and lack of trust in orthodox medicine, reports using data from hospital-based surveillance studies as well as from the Infectious Diseases

Society of America have begun to refer to a group of nosocomial pathogens as "ESKAPE pathogens" (Rice, 2008; Bush and Jacoby, 2010).

The responsible genes are commonly situated on transposons inside transportable plasmids, which establish them with the possibility to passage horizontally and may in part describe the now worldwide dissemination of this novel mechanism of resistance (Hussain *et al.,* 2021).

People used plant sources for medicinal purposes many decades ago (Giannenas *et al.,* 2020). Role of plant natural products in anti-infective drug discovery medicinal plants has long played essential roles in the treatment of diseases all over the world (Fallah *et al.,* 2006; Schultz *et al.,* 2020). Where plants were found on the globe before humans existed on them and used as essential sources of nutrition and later used to treat patients. In recent times, the researchers' attention has gradually turned to the use of folk medicine methods, which is represented in obtaining therapeutic materials from their primary sources directly, especially herbs and wild plants from them and cultivated. This trend resulted from multiple factors, including the absence of chemicals and industrial plants that can cause many side effects. It has a significantly negative impact on the patient's health and the diagnosis of the active compounds in medicinal plants as well as the ease of use and circulation in it that the effective ones are considered unfocused and easy for absorption by the cells of the body as well as the body's ability to deal and get rid of the surplus from them, as well as in the containment of many active substances that worsen Together to treat disease (Al-Zubaidi *et al.,* 1996; Crini *et al.,* 2020).

Human beings have depended on nature for their simple requirements as sources for medicines, shelters, foodstuff, fragrances, clothing, flavors, fertilizers, and means of transportation throughout the ages, for the large proportions of the world's population, medicinal plants continue to show a

dominant role in the healthcare system, this is mainly true in developing countries, where herbal medicine has a continuous history of prolonged use, the development and recognition of medicinal and financial aids of these plants are on the rise in both industrialized and developing nations (Dar *et al.*, 2017).

The emergence of microbial resistance to many antibiotics is a real medical problem, the most important part of it is the poor and excessive use of microbial antagonists (Beaulac *et al.*, 1998; Kumar *et al.*, 2019). As a result of the development of microorganism resistance to antibiotics, it has become necessary to find the inexhaustible source of many natural products and traditional medicines that are a new source of antimicrobial agents.

1.2. Aims of the present study.

This study aimed to investigate the effect of different plant extract on MDR bacterial isolates that cause UTI by the following study steps.

1. Isolation and diagnosis of resistant bacteria from resistant urinary tract infections.
2. Studying the effectiveness of antibiotics on pathogenic bacteria and their comparison with the effectiveness of plant extracts.
3. Using of plant extracts to inhibit the growth of resistant pathogenic bacteria.
4. The synergistic use of plant extracts that did not show an effect on bacteria with antibiotics
5. Determining the location of the antibiotic resistance genes through the plasmid.

1.3. Literatures review.

1.3.1.Urinary tract infections (UTIs)

Urinary tract infections (UTIs) are one of the most common human infections, occurring both in hospitals and in communities (Tumturk *et al.,* 2019). These infections can affect any part of the urinary system including the kidneys, ureters, bladder, and urethra, it is estimated that approximately 150 million people are affected by UTIs worldwide each year (Durgadevi *et al.,* 2019; Zubair *et al.,* 2019). UTI is a frequent reason for consultation in primary health care in both adult and pediatric populations. It is generally caused by members of the Enterobacteriaceae family, with *E. coli* prevailing in most patients (Nocua-Báez *et al.*, 2017; Quijada-Martínez *et al.*, 2017).

The urinary system is the organs that collect and store urine and then excrete it outside the body and includes the kidneys, ureters, bladder, and urethra, (UTIs) are a term that applies to a variety of people; it is a clinical condition that ranges from the presence of symptoms due to the presence of bacteria in the urine to infection, severe kidney infection with frequent septicemia. Moreover, urinary tract infection 105 cells/ml, which causes the bacteria to invade the urinary tract, is defined as bacteria in the urine of more than the Urinary tract, which leads to injury (Sawalha, 2009).

Women are significantly more likely to experience UTIs than men. Nearly 1 in 3 women will had at least one episode of UTI requiring antimicrobial therapy by 24 years. Almost half of all women will experience UTI during their lifetime (Foxman, 2003). The higher prevalence in women is thought to be related to urethral length. For those who have had prior infections, the risk of another increases dramatically. One study found a recurrence rate was 44% within one

year among women with a history of UTI (Ikähelmo *et al.,* 1996). Likewise, uncircumcised children have a higher rate of infection than circumcised children (Shimda, 2013). In addition to that, several factors help with the infection, including age, gender, marital status, and parent's education level (Sawalha, 2009).

The infection is usually caused by bacteria and viruses and parasites such as *Schitosoma hematoboum* and yeasts such as *Candida albicans*, including common bacterial pathogens such as Gram-negative bacteria *E.coli*, *Klebsiella Pseudomonas, Serratia*, and Gram-positive bacteria such as *Staphylococcus aureus* (Amin *et al.,* 2009). The most common causative pathogens include gram-negative organisms, particularly *E. coli*, which account for 80% of infections (Ronald, 2002). Less common pathogens include Mycobacterium tuberculosis and a variety of anaerobic bacteria, which are of high risk, especially for immunocompromised people. It increases the risk of severe infections such as inflammation kidney and bladder (Griebling, 2007).

1.3.2.Bacterial isolated

Many types of bacteria that cause urinary tract infections (UTIs) were found, which differ according to the patient's resistance and the type of infection, but Gram-negative bacteria belonging to the intestinal family (Kumar *et al*., 2015) are considered the most common types of bacteria that cause urinary tract infections because they are within the plant The normal flora of the body and the bacteria inside the body that cause urinary infections are known as endogenous infections, as well as the exogenous bacteria acquired from the environment surrounding the infection are known as exogenous infections (Foster, 2008).

1.3.2.1. *Klebsiella pneumoniae* (*K. pneumonia*)

K. pneumoniae is a Gram-negative bacillus, non-fastidious, usually encapsulated, rod-shaped bacterium and member of the family Enterobacteriaceae. Infections caused by *Klebsiella* sp. are the most common bacterial pathogens present in healthcare environments, and infections can be endogenous or acquired by direct contact with an infected host (Queenan and Bush, 2007). Fimbrial adhesins and a thick capsule, which serves as an antiphagocytic factor, make *K. pneumoniae* extremely dangerous and a pathogen (Pendleton *et al.,* 2013).

UTIs, nosocomial pneumonia, and intraabdominal infections are all caused by an increase in multiple antibiotic resistance. Carbapenem-resistant *K. pneumoniae* strains, often linked to severe infections with limited treatment options, have emerged as a severe public health concern (Wei *et al.,* 2016). *K. pneumoniae* is a clinically significant pathogen, causing UTI, septicemia, and pneumonia, among other infections. It is one of the top three pathogens of international concern documented in the 2017 World Health Organization's (Shrivastava *et al*., 2018).

In the past few decades, the emergence of MDR and hypervirulent *K pneumoniae*, which pose a severe threat to public health, has been reported worldwide (Chang *et al.,* 2013). Moreover, the hypervirulent and MDR isolates of *K. pneumoniae* were considered mainly not to overlap before the recent emergence of outbreak strains that are simultaneously hypervirulent and MDR (Cheng *et al*., 2012; Liu *et a*l ., 2014; Su *et al.,* 2008; Zhang *et al*., 2015; Zhang *et al.,* 2016). MDR-hypervirulent *K pneumoniae* is a form of convergent clone that resulted from the acquisition of virulence genes encoded on plasmid (Chen *et al.,* 2004) by an MDR strain, or acquisition of MDR genes by a

hypervirulent strain via uptake or transposition of mobile genetic elements such as plasmids, transposons, and integrons (Shields *et al*., 2017).

Antibiotic-resistant *K. pneumoniae* strains often emerge, such as extended-spectrum beta-lactamase-producing bacteria in UTI. *K. pneumoniae* is the second common bacterial pathogen causing UTI in Indonesia. The emergence rate of antibiotic-resistant strains in Indonesia is higher than in Japan, Europe, and America (Gharrah *et al.,* 2017;Kitagawa *et al.,* 2018). The dissemination of extended-spectrum beta-lactamase- is a severe concern worldwide because extended-spectrum beta-lactamase-producing strains increase patient mortality. They are resistant to all penicillins, cephalosporins, and aztreonam, resulting in a decline in available choices for proper therapy (Schwaber *et al.*, 2006). However, *K. pneumoniae* is one of the most frequent causes of both health care- and community-associated infections, including pyogenic liver abscess, urinary tract infections, bacteremia, and pneumonia. Two changing trends in *K. pneumoniae* infections have increasingly caused severe global public health concerns (Keynan and Rubinstein, 2007). One is the emergence of the hypervirulent variant that causes severe infections, and the other is the increase of antimicrobial resistance in *K. pneumoniae*, especially the emergence of carbapenem-resistant (Keynan and Rubinstein 2007; Lin *et al.*, 2010).

The selective pressure imposed by antimicrobial agents had an essential effect on the acquisition and loss of antimicrobial resistance genes in *K. pneumoniae*, leading to the constant evolution of plasmids in *K. pneumoniae*. Studies reporting carbapenem-resistant and hypervirulent *K. pneumoniae* isolates are the most frequently published. However, understanding epidemic traits and dynamics of plasmid acquisition is essential to help anticipate future impacts on human health (Simner *et al*., 2018).

1.3.2.2. *Staphylococcus aureus* (*S. aureus*)

S. aureus is a facultative anaerobic, Gram-positive, round-shaped (coccus) bacteria commonly found as a part of the human skin microbiota. It is typically not harmful in humans with non-compromise immune systems in these environments. *S. aureus* can cause infections when it enters parts of the body that it does not typically inhabit, such as wounds. *S. aureus* can also cause infections on implanted medical devices and form biofilms that make treatment with antibiotics more difficult (Pendleton *et al.*, 2013). The expression of various virulence factors, such as surface proteins, biofilm, exoenzymes, exotoxins, and exfoliative toxins, is linked to *S. aureus'* ability to cause infections. These factors cause bacteria to bind to tissues, causing pathogenesis and infiltrating the immune system, causing toxicity (Costa *et al.*, 2013). Methicillin-resistant *S. aureus* includes strains distinct from other strains of *S. aureus* in the fact that they have developed resistance to β-lactam antibiotics (Pendleton *et al*., 2013). They confer resistance to β-lactam antibiotics by the expression of *mecA* that encodes a low penicillin-binding affinity protein (Smith and Dou 2001). They are linked to a growing array of healthcare-related infections, including infective endocarditis and infections from prosthetic devices. In addition, strains with specific virulence factors and -lactam antibiotic resistance are among the most common causes of community-acquired skin and soft tissue infections (Tong *et al.*, 2015).

Certain *S. aureus* strains have been identified as resistant to Vancomycin-intermediate owing to the overuse of the drug to treat *S. aureus* (Appelbaum, 2007). Meticillin-resistant *S. aureus* is a leading cause of antibiotic-resistant community-associated and health-care-associated infections worldwide (Stefani *et al.*, 2012).

1.3.2.3. *Pseudomonas aeruginosa* (*P. aeruginosa*)

P. aeruginosa is a Gram-negative, aerobic and nonsporulating bacillus. It very occasionally colonizes healthy people (Khalil and Alawi, 2019). *P. aeruginosa* is a gamma-proteobacterium possessing a lowly permeable outer membrane and multiple transport systems that provide its innate resistance to many antibiotics. It also employs a variety of mechanisms such as alterations in porin channels, efflux pumps, target modifications, and β-lactamases that allow it to develop resistance to antimicrobial agents (Tümmler, 2019).

Point mutations on DNA gyrase/Topoisomerase IV provide immense resistance against Fluoroquinolones to *P. aeruginosa* (Morero *et al.*, 2011). Rates of infection due to resistant *P. aeruginosa* continue to increase in the United States and globally, as does resistance to quinolones and carbapenems. Aminoglycoside resistance is emerging as a significant problem (Lepper *et al.*, 2002; Neuhauser *et al.*, 2003). Mutants of *P. aeurginosa* with upregulated efflux pumps also exist that make finding an effective antibiotic or detergent incredibly difficult (Pendleton *et al.*, 2013). There are also some MDR strains of *P. aeruginosa* that express β-lactamases as well as upregulated efflux pumps which can make treatment particularly difficult (Pendleton *et al.*, 2013). *P. aeruginosa* strains secrete several virulence factors associated with extracellular proteins through various genes. Adhesins, pyocyanin, elastase, proteases, hemolysins, an exotoxin, and exoenzymes are some virulence factors identified in *P. aeruginosa* strains (Macin and Akyon, 2017).

P. aeruginosa is the pathogen most frequently isolated. It is often an opportunistic pathogen, causing hospital-acquired infections. These infections are a significant cause of morbidity and mortality worldwide. Ventilator-associated pneumonia, bacteremia, urinary tract infection, and surgical site infection are the main types of infections caused by this organism. Strains of *P.*

aeruginosa resistant to multiple classes of antibiotics are an increasingly concerning problem. The development of innovative interventions and strategies tailored to the health care settings are urgently needed to counteract this threat (Luis *et al.*, 2020). *P. aeruginosa* is an opportunistic pathogen that causes nosocomial infection UTI, especially in immunocompromised hosts. UTI caused by this organism is difficult to treat since *P. aeruginosa* adapts to the biofilm mode of growth on urinary catheters and produces quorum sensing signals to monitor its density and regulate the production of virulence factors (Van Dalden and Iglewski 1998; Wagner *et al.* 2007).

1.3.2.4. *Escherichia coli* (*E. coli*)

E. coli is a Gram-negative bacterium belonging to Enterobacteriaceae, oxidase negative, catalase-positive, non-spore-forming, and facultatively anaerobic (Gillepsie and Hawkey, 2006). *E. coli* is motile by a set of peritrichous flagella and also have fimbriae (Pilli) or fibrillar proteins, often extending in great numbers from the bacterial surface and far out into the surrounding medium (Brenner *et al*., 2004). *E. coli* is the major UTI pathogen (Foxman 2003). UTIs caused by trimethoprim-resistant *E. coli* has been associated with adverse clinical outcomes and an increased workload in general practice (Butler *et al*., 2006). The ability of this bacteria to cause infections in humans belongs to the presence of many virulence factors which the pathogenic forms possess (Pitout, 2012).

Non-pathogenic *E. coli* can become pathogenic following the acquisition of mobile genetic elements by horizontal transfer and interactions with bacteriophages or transposons between *E. coli* and other bacterial species (Kaper *et al.*, 2004; Williams *et al.*, 2010). Virulence factors are specific properties that enable organisms to overcome host defense and cause disease

(Baby *et al.,* 2016). Moreover, *E. coli* virulence factors play a significant role in infection due to helping this bacteria colonize the mucosal neuroepithelium selectively and starting an inflammatory reaction that helps it proceed from the lower urinary tract to renal tissues. The capacity of *E. coli* to produce many virulence factors contributes to its pathogenicity and increases the ability to cause serious infections (Vaish *et al.,* 2016). Mainil (2013) reported that differentiation of pathogenic strains from normal microbiota is based on the production of virulence factors and the identification of mechanisms by which they cause disease, which allows their classification into pathotypes. Infections with *E. coli* pathotypes can result in three common clinical conditions: diarrhea, urinary tract infection (UTI), and sepsis/meningitis (Kaper *et al*., 2004). The relationship may be symbiotic, in that the bacteria, in addition to benefiting from the host, synthesize cofactors and contribute to colonization resistance against pathogenic organisms (Michael, 2013).

However, most *E. coli* strains are harmless, and others cause disease in humans and animals that have evolved to become essential pathogens in their own right, clinically, two distinct types' pathogenic *E. coli* are recognized, one group commonly called extraintestinal pathogenic *E. coli* includes those *E. coli* associated with newborn meningitis or sepsis and urinary tract infections (Gillepsie and Hawkey, 2006). Pathogenic *E. coli* strains can be categorized based on elements that elicit an immune response in O antigen: part of lipopolysaccharide layer, K antigen: capsule H antigen: flagellin (Brenner et al., 2004). *E. coli* has a high capacity to develop intestinal and extra-intestinal disorders in humans and different animals; for example, it is caused by this bacterium, diarrhea, urinary tract infection UTI, cervix, and vagina infections, sepsis, meningitis, abdominal infection, cellulitis, osteomyelitis, avian colibacillosis, and wound infection (Al-Khaqani *et al*., 2017).

1.3.3. Bacterial resistance

Antibiotic resistance has emerged as a threat to global health, food security, and development today. Antibiotic resistance can occur naturally but mainly due to misuse or overuse of antibiotics, which results in recalcitrant infections and antimicrobial resistance (AMR) among bacterial pathogens (Bhatia *et al.,* 2021). In the last four decades, pharmacological industries have produced many new antibiotics, and resistance by microorganisms to these drugs has been accelerated due to the impetuous use of antibiotics. A report submitted to the United Nations in 2019 expects that infections caused by antibiotic-resistant bacteria would cause 10 million deaths per annum and an economic crisis (Carr *et al.,* 2019). The empirical prescription of antimicrobials for UTI is standard practice; however, bacterial resistance to antimicrobials has been increasing globally, representing a decrease in the effectiveness of empirical treatment (Cabrera *et al.,* 2019).

The critical determinants of the development and maintenance of antibiotic resistance are the volume of antibiotic use against bacteria and the biological fitness cost conferred by most resistance mechanisms (Andersson *et al.,* 2007). For several pathogens, the fitness cost measured in vitro has been shown to inversely correlate with the antibiotic resistance rates in clinical settings (Sander *et al.*, 2002; Nilsson *et al*., 2003), supporting mathematical modeling studies that have suggested that fitness cost is a major determinant of resistance rates (Levin *et al*., 1997; Levin 2001).

The MDR strains (multi-drug resistant) of *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species (Bhatia et al., 2021). Each bacterium has its natural resistance pattern that must be taken into account. The

genes encoding these enzymes can be found on the chromosome or plasmids and produced in a constitutive or inducible manner. The main mechanism of resistance to quinolones is the consequence of mutations in DNA gyrase (Gomig *et al.*, 2015; Sedighi *et al.*, 2015).

In UTI, early diagnosis through clinical and paraclinical criteria and the identification of the etiological agent, and the application of a guided antibiotic therapy based on susceptibility tests are essential to avoid complications and improve the patient's prognosis and bacterial multidrug resistance (Chiu *et al.*, 2017; Nocua-Báez *et al.*, 2017; Quijada-Martínez *et al.*, 2017). Based on a limited number of isolates, a 97% decrease in the consumption of sulfamethoxazole /trimethoprim in the United Kingdom between 1991 and 1999 did not result in a reduction in sulfamethoxazole resistance. Additional data from the same area in 2004 showed that sulfamethoxazole and streptomycin resistance in *E.coli* had remained remarkably stable despite deficient use of these drugs (Bean *et al.*, 2005; Chiew *et al.*, 1998). The lack of impact on resistance was due to the existence of co-selection, in which a specific resistance is sustained by its near linkage to another resistance determinant (Enne *et al.*, 2004; Kahlmeter and Menday 2003).

AMR pathogens that cause healthcare-associated infections pose an ongoing and increasing challenge to hospitals, both in the clinical treatment of patients and in the prevention of the cross-transmission of these problematic pathogens (Esposito and Leone, 2007; Schwaber and Carmeli 2007). AMR can be conferred in bacteria via genetic mutation and Horizontal Gene Transfer through chromosomes, plasmids, transposons, and other mobile genetic elements (Giedraitienė *et al.*, 2011). AMR is a natural prevalence connected to a

rise in mortality, morbidity and economic burden of nations worldwide (Zhen *et al.,* 2019).

The prime class of opportunistic pathogens that are a universal threat to humankind are entitled *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* epp. as they are known to "escape" antibiotics and other traditional treatments (Ma *et al.,* 2020). The World Health Organization (WHO) released the global priority pathogen list in 2016 to guide the researcher in the discovery and development of new antibiotics (Shrivastava *et al.,* 2018). These pathogens include vancomycin-resistant Enterococcus sp., methicillin-resistant *Staphylococcus aureus*, fluoroquinolone, carbapenem-resistant Enterobacteriaceae, *Pseudomonas aeruginosa*, and extended-spectrum β-lactamase–producing *Escherichia coli* and *Klebsiella sp*. (Chambers, 2005; Lockhart *et al.,* 2007).

1.3.4.Mechanisms of Antibiotics Resistance

The repeated and inaccurate use of antibiotics has created an increasingly troubling clinical problem, namely, the emergence of resistant bacterial strains whose growth is not inhibited by the MIC application of that antibiotic, and thus the failure to treat many pathological conditions.

The resistance of bacteria can be classified into:

1.3.4.1. Non-genetic Resistance

This type of resistance occurs in abundance in nature in order to provide temporary protection for the pathogen by several means and methods, including what was mentioned in El-Halfawy and Valvano (2012); Sengupta *et al*., (2013).

A) Stop growing : Bacteria stop dividing for some time but remain alive, to avoid the effect of the antibiotic that inhibits cell wall synthesis, such as ampicillin.

B) Hiding and hibernating: Some bacteria hide so deep in the tissues of the host's body that it is difficult to reach them. For example, *Mycobacterium* bacteria live in the tissues for several years, disguised from the body's defenses because they do not multiply, which leads to resistance to many antibiotics. In addition to other mechanisms, the bacteria secrete an outer layer of protective substances around them.

3) Loss of the cell wall

Some bacteria whose osmotic pressure is protected by the host tissues resort to transforming into cells that lack a cell wall (the filamentous shape Like form), and thus these bacteria can resist antibiotics whose target is the cell wall, but the bacteria can return to their normal shape after several generations and is sensitive to antibiotics again.

1.3.4.2. Genetic Resistance

The resistance to antibiotics is either inherited or acquired, as the first resistance represents an inherited trait of origin and is chromosomally encoded for a specific group of antibiotics in some bacterial genera or species (Minarini and Darini, 2012). As for acquired resistance, it is defined as the acquisition of genes that code for resistance to many antibiotics. The genetic resistance of bacteria is divided into two main parts:

A- Chromosomal Resistance: Bacteria acquire this type of resistance by a spontaneous mutation in the chromosomal gene site that controls the functional

and structural shape of the specific receptors to which the antibiotic binds of the parental bacteria to subsequent generations of the strain as well because of the possibility of accumulation of point mutations on the chromosome and their successive inheritance (Velhner *et al.*, 2014).

B- Plasmid Resistance: The resistance is acquired through the transfer of DNA, as the resistance genes for some antibiotics are carried on a plasmid that can be transferred between strains of the same type or other types of bacteria in several ways, such as conjugation, transformation and transduction (Lesouhaitier *et al*., 2009). Also, some antibiotic-resistant plasmids may be carriers of a transposon (transposon elements are defined as pieces of DNA that can change their position from one place to another within the cell's genetic content). Encoding resistance to many antibiotics is most commonly responsible for the accumulation of determinants in the genome of bacterial cells (Carattoli, 2001).

Most antibiotic resistance genes are spread by R-plasmids, which carry resistance genes, often found in antigens or are carried on transposons and are responsible for spreading resistance against many antibiotics and heavy metals (Jacoby and Munoz-price, 2005). There are four horizontal transfer factors for integrons resistance genes: I, II, and III. The fourth class is called super integrons, depending on the type of integrase (int) fusion gene they possess (Gündoğdu *et al*., 2011). It has been shown that the gene packages in the integron gene carry the genes encoding resistance against antibiotics (such as beta-lactams and aminoglycosides), which are often embedded in the chromosome or plasmids (Fluit and Schmitz, 2004). The cassette contains the gene that codes for many functions and proteins (Fluit and Schmitz, 2004; Domingues *et al*., 2012).

Medicinal plants

Conventional medicinal practices have utilized plants against various infections for thousands of years now (Kumar *et al*., 2006; Bibi *et al*., 2011; Cioch *et al*., 2017). Eighty percent of the population of the developing nations depends on easily accessible traditional medications to fulfill their primary medical needs (Maroyi, 2013; World Health Organization, 2013). For a long time, people of India have been using many plant species as traditional medicines for various ailments, including treatment of infectious diseases (Bahmani *et al*., 2015). Indeed, plants synthesize a wide array of compounds known as secondary metabolites or phytochemicals such as quinones, tannins, terpenoids, alkaloids, flavonoids, and polyphenols, which have disease prevention properties and aid them in their self-defense and communication with other organisms in their environment (Harborne *et al*., 1993). Plant extracts as medicines are inevitable substitutions for antibiotics prescribed by physicians. Plant-derived compounds and extracts are commonly used in self-medication due to their easy availability, competence, and nil side effects (Cowan, 1999). People use herbs as a source of medicines, primarily for primary healthcare. Many of them have been studied for their medicinal properties (Bedi *et al*., 2016).

Furthermore, due to acute toxicity and associated side effects of existing chemical medications, there is an increase in inclination towards the traditional source of medicines. Although synthetic drugs are adequate for preventing, curing, and managing countless diseases, their use can cause surplus health vulnerabilities (Liu *et al*., 2012). Plants are a rich source of biologically active compounds, proven to be effective antimicrobial agents. Traditionally, many

plants are used to treat *Mycobacterium* infections. Phytochemicals present in plants inhibit the multidrug efflux system of microbes (Sharma *et al.*, 2010).

In the last four decades, pharmacological industries have produced many new antibiotics, and resistance by microorganisms to these drugs has been accelerated due to the impetuous use of antibiotics. A report submitted to the United Nations in 2019 expects that infections caused by antibiotic-resistant bacteria would cause 10 million deaths per annum and an economic crisis (Carr *et al*., 2019). The Discovery of novel drugs can be accomplished with the use of plants extracts, which is a reservoir of broad-spectrum secondary metabolites (Aisida *et al*., 2019; Madubuonu *et al*., 2019; Ginting *et al*., 2020; Kandemir *et al*., 2020; Ugwoke *et al*., 2020).

Plants have proven themselves effective in preventing and treating the toxicity induced by other toxins or drugs. The efficacy of plant extracts and their derivatives in their antimicrobial activities have paved the way for exploring new and effective treatments against multi-drug-resistant bacteria (Bhatia *et al.*, 2021).

1.3.4.3. *Phyllanthus emblica*

Kingdom: Plantae

Order: Malpighiales

Family: Phyllanthaceae

Genus: *Phyllanthus*

Species: *emblica*

Phyllanthus emblica, colloquially known as the Indian gooseberry (English), Amalaki (Sanskrit), and amla (Hindi), is an essential deciduous tree. This plant belonged to the Euphorbiaceae family and was originally native to India but is today found growing in Pakistan, Uzbekistan, Srilanka, Southeast Asia, China, and Malaysia. The tree is small to medium in size and grows up to 5.5 m. The fruits are the most commonly used plant part and are of both dietary and medicinal use. The raw fruits are green, while; the ripe fruits are yellowish-green in color. The fruits are globular in shape, fleshy and smooth striated with an obovate-obtusely triangular six-celled nut. The fruits are of culinary use and are widely used to make murabba, juice, pickle, chutneys, and vegetables in various dishes (Mirunalini and Krishnaveni, 2010).

Phytochemical studies have shown that amla contains tannins, alkaloids, and phenolic compounds. It is a rich source of vitamin C, and the levels are more than that in oranges, tangerines, or lemon. Amla also contains gallic acid, ellagic acid, chebulinic acid, chebulagic acid, emblicanin-A, emblicanin-B, punigluconin, pedunculagin, citric acid, and isostrictinin (Baliga *et al.*, 2013).

Amla is an important medicinal plant and has been widely used by Ayurvedic practitioners for more than 3000 years. Amla is also of use in Siddha, Unani, Tibetan, Srilankan, and Chinese systems of medicine (Chaudhry, 2019). In the various folk systems of medicine, this fruit is used as an astringent, expectorant, antiasthmatic, laxative, diuretic, spasmolytic, antacid, antipyretic, anti-inflammatory, antidiarrheal, and antidiabetic. It is commonly used to treat various ailments such as hemorrhoids, nervine debility, anemia, jaundice, liver complaints, leucorrhea, hematuria, and inflammation eyes (Mirunalini and Krishnaveni, 2010).

Preclinical studies have shown that amla possesses antibacterial, antifungal, antiviral, antidiabetic, antiulcerogenic, free radical scavenging, antioxidant, anti-mutagenic, anti-inflammatory, immunomodulatory, antipyretic, analgesic, antitussive, antiatherogenic, adaptogenic, snake venom neutralizing, gastroprotective, anti-anemic, wound healing, antidiarrheal, antiatherosclerotic, and nephroprotective (Megraj *et al.,* 2011). *Phyllanthus emblica* was used as alternative medicine, it's one of the essential plants, even in present-day therapeutics, it's contains Phyllemblin and Flavanoids. Phyllemblin is a benzoic compound with a profound antibacterial activity against *S. pyogenes*, *S. aureus*, and *K. pneumoniae* (Javale and Sabnis 2010). At present, the policies for antibacterial agents are of great concern due to the resistance of some strains of microorganisms, drug residues in the fluids and body tissue by antibacterial therapy for bacterial diseases.

1.3.4.4. *Syzygium aromaticum*

Kingdom: Plantae

Order: Myrtales

Family: Myrtaceae

Genus: *Syzygium*

Species: *aromaticum*

Cloves are the aromatic flower buds of a tree; they are native to the Maluku Islands (or the Moluccas) in Indonesia and are commonly used as a spice. Cloves are available throughout the year owing to different harvest seasons in different countries (Kamatou *et al.,* 2012). Cloves are herbaceous annuals of particular breeding, with multiple branches slightly slanting, flower buds are

pale in color at the beginning of flowering and then gradually grow into green color and then ripen to become radiant red color and then ready for harvest, the length of these buds ranges from 1.5 to 2 cm. It has a long goblet that ends with four leaves as flat sepals and four uninflated petals that in turn form a small ball in the center (Bin, 2008).

Cloves have been an effective antagonist to many microorganisms, such as *S. aureus*, *Salmonella enterides*, and *E. coli* (Al-Khayant and Blank, 1985). Time-kill bacterial susceptibility of *E. coli*, *P. aeruginosa*, and *S. aureus* to aqueous extract of *Syzygium aromaticum* seed (Ajiboye *et al.*, 2016). The only sample that showed complete bactericidal effect against all the food-borne pathogens tested *E. coli*, *S. aureus*, and *Bacillus cereus* was the aqueous extract of clove at 3%. The 1% clove extract also showed good inhibitory action (Dorman and Deans, 2000).

Spices as clove, oregano, mint, thyme, and cinnamon have been employed for centuries as food preservatives and as medicinal plants mainly due to their antioxidant and antimicrobial activities. Nowadays, many reports confirm the antibacterial, antifungal, antiviral and anticarcinogenic properties of spice plants. Clove, in particular, has attracted attention due to the potent antioxidant and antimicrobial activities standing out among the other spices (Shan *et al*., 2005). The phytochemical constituents of this plant include eugenol, trans-caryophyllene, α- humulene, eugenol acetate, syzygin A, syzygin B, caffeic acid, ferulic acid, and ellagic acid (Cortés-Rojas *et al.*, 2014).

Clove represents one of the significant vegetal sources of phenolic compounds as flavonoids, hidroxibenzoic acids, hidroxicinamic acids, and hydroxyphenyl propens (Batiha *et al.*, 2020). Eugenol is the main bioactive compound of clove, found in concentrations ranging from 9381.70 to 14650.00

mg per 100 g of fresh plant material (Neveu *et al.*, 2010). Gallic acid is the compound found in higher concentrations of phenolic acids, other phenolic acids found in the clove are caffeic, ferulic, elagic, and salicylic acids. Flavonoids as kaempferol, quercetin, and its derivates (glycosilated) are also found in cloves in lower concentrations (Shan *et al.*, 2005).

Concentrations of up to 18% of essential oil can be found in the clove flower buds. Roughly 89% of the clove essential oil is eugenol, and 5% to 15% is eugenol acetate and β-cariofileno. Another important compound found in the essential oil of clove in concentrations up to 2.1% is α-humulene(Sofia *et al.*, 2007). The antimicrobial activities of clove have been proved against several bacterial and fungal strains (Dorman and Deans 2000).

1.3.4.5. Citrullus colocynthis

Kingdom: Plantae

Order: Cucurbitales

Family: Cucurbitaceae

Genus: *Citrullus*

Species: *coloycynthis*

Bitter melon is a herbaceous creeping plant, the scientific name is *Citrullus coloycynthis*, and its other common names are wild sprouts, bitter apple, infertility, etc. The fruit is considerable in shape that resembles an orange in size, with a green-striped color of 3-5 cm in diameter, and upon maturity, it turns yellow, seeds are dark in color (John *et al*., 1998; Al-Qubaisi, 2004). In Iraq, the plant grows in the north and south of the Jazira region and the southern

and western desert, as well as in the cities of Mosul and Kirkuk and the plain sedimentary region (Hashem and Jamil, 1988).

Bitter melon has been used for hundreds of years in folk medicine for the treatment of constipation and ascitis as an anti-helmintic repellent, as well as for treating diabetes millets and for treating jaundice and other conditions at deficient concentrations that do not exceed two doses (Ziyyat *et al.*, 1997; Zohara *et al.*, 1999; Wikipedia, 2009), and various contagious diseases, including dermatological problems and gynaecological, urinary and pulmonary infections (Marzouk *et al.*, 2009).Studies have shown that the effect of the alcoholic extract is more than the aqueous extract, and the highest concentration of the extract is the most effective (Majeed and Sabah, 2002). Al-Mousawi, 2006 and Marzouk *et al.*, 2011 confirmed that the *C.colocynthis* fruit extract has a high inhibitory effect on Gram-negative and positive bacteria. (Degola *et al.*, 2019) also pointed out that these substances can be used in pharmaceutical treatments such as antibacterial, fungal, parasitic infections, and anti-inflammatory.

2. Materials and methods.

2.1. Materials.

2.1.1. Laboratory Equipment and Instruments.

All equipment and instruments are listed in Table (2-1).

Table (2-1) Laboratory Instruments and Equipment's

Instruments/ Tools	**Company (Origin)**
Autoclave	Hirayama (Japan)
Benson burner	Gemmy (Taiwan)
Biological cabinet (Hood)	Bio Hazard (Korea)
Bioneer Gel electrophoresis	Bioneer (Korea)
Centrifuge	Gemmy (Taiwan)
Deep freezer	Froilabo (France)
Disposable syringes	Supreme (China)
Distill water	Fine Tech (Korea)
E-graph – UV(Gel documentation)	ATTA (Japan)
Eppendorf Centerifuge	Himedia (Germany)
Gel electrophoresis	Mupid (Japan)
Incubator	Binder (USA)
Light Microscope	Olympus/ Japan
Micro centrifuge tubes 1.5mI	BIO BASIC (Canada)
Micropipette	BIO BASIC (Canada)
Nano drop	Bio drop (USA)
Oven	Memmert (Germany)

Platinum Wire Loop	Himedia (India)
Refrigerator	Visel (Korea)
Sensitive Electronic Balance	Sartorius (Germany)
Sensitive electronic balance	KERN (Germany)
UV transilluminator	Eppendorf (Germany)
Vitic 2 compact autoanalyzer supplemented with a. Denisi chek b. Densitometer	Bio merieux (France)
Vortex Mixer	Gemmy (Taiwan)
Water Bath	G.F.L. (Germany)

Table (2-2) Technical Instrument and Disposable Materials.

Item	**Company (Country)**
Conical flask (different sizes)	MBL (UK)
Cylinder	MBL (UK)
Disposable plastic petri dishes	Blastilab (Lebnon)
Medical cotton	Medicare (India)
Medical gloves	Himedia (India)
Millipore filter	Himedia (India)
Para film	Bemis (India)
Plastic test tube 10 ml	Dolphin (Syria)
Sterile swab for streaking	Sigem (Spain)
Test tube rack	Himedia (India)
Tips	Bio bas (Canada)
Wood sticks	Supreme (China)

2.1.2. Chemical and Biological Materials.

2.1.2.1. Chemical Materials.

The essential chemical materials and stains used in this project are listed in Table 2-3.

Table (2-3) Chemical Materials

Chemical material	**Company (Country)**
10 TBE (Tris-Borat- EDTA buffer	Promega (USA)
Agarose	Promega (USA)
Ethanol (70 %) , Ethanol (99%)	Chromagar (France)
Ethidium bromide	Macrogen (Korea)
Glycerol ($C_3H_8O_3$)	(UK)
Gram stain kit	Kimedia (India)
Indole, Catalase reagent (H_2O_2)	Schuchariot (Germany)
Kovac's reagent	Liofilchem (Italy)
Methanol (99%)	Chromagar (France)
Methyl red	UK
Oxidase	B.D.H (England)

2.1.2.2. Biological materials

The culture media has been used in the study are show in Table 2-4.

Table (2-4) Culture media

Culture media	**Utilization**	**Company (origin)**
Blood agar	classify bacterial types according to hemolytic appearance was also used for bacterial cultivated.	
Brain heart	It was used to culture a variety of	

infusion broth	fastidious organisms.	Himedia Accumax (India)
Eosin methylene blue	Differential medium used to distinguish *K. pneumonae* from *E. coli.*	
MacConkey agar	Most gram-negative bacteria were isolated on agar, which can also be used to distinguish between lactose fermenter and non - fermenter bacteria	
Muller – Hinton Agar	It was used in an antibiotic sensitivity test.	
Nutrient agar	It was used for the general test, primary bacterial isolated, used for non- fastidious bacterial type.	
Nutrient broth	It was used to grow and preserve the bacterial isolates.	
Peptone water	It was used to demonstrate the bacterial ability to decompose the amino acid tryptophan into indole.	
kligler iron agar	I was used to identification bacterial ability to fermentation glucose and lactose and possible production of H_2S.	Difco (Michigan)
Simmons citrate agar	It was used for differentiating gram-negative bacteria on the basis of citrate utilization.	

2.1.2.3. Antibiotics discs

The antibiotic discs utilized in this research are mentioned in Table 2-5.

Table (2-5) Antibiotics discs

Antibiotics		**Code**	**Concentra -tion in mg**	**Origin**
Class	**Name**			
Monobactams	Azteronam	ATM	30	Turkey
Cephalosporins	Cefeime	FEP	30	Italy

Cephalosporins	Ceftazidime	CAZ	30	Turkey
Ureidopenicllins	Piperacillin	PRL	30	Turkey
DNA dependant-RNA polymerase	Rifampicin	RA	5	Turkey
Cotrimaxole	Trimethorim	TMP	10	Turkey
Aminoglycosides	Tobramycin	TOB	10	Turkey
Glycopeptide	Vancomycin	VA	30	Turkey

2.1.2.4. Plant material

Plants samples that were used in the study are mentioned in Table 2-6.

Table (2-6) Collecting plant samples.

Plant	**Family**	**Part used**	**Place of collection**
C.colocynthis	Cucurbitaceae	Fruit	The fruit was collected from Badia of Najaf city.
S.aromaticum	Myrtaceae	Flowerbuds	The flowerbuds were got from Herbalists of Al-Najaf City.
P.emblica	Phyllanthaceae	Fruit	The fruit powder from product Organic Veda company in India.

2.1.3.Commercial kit.

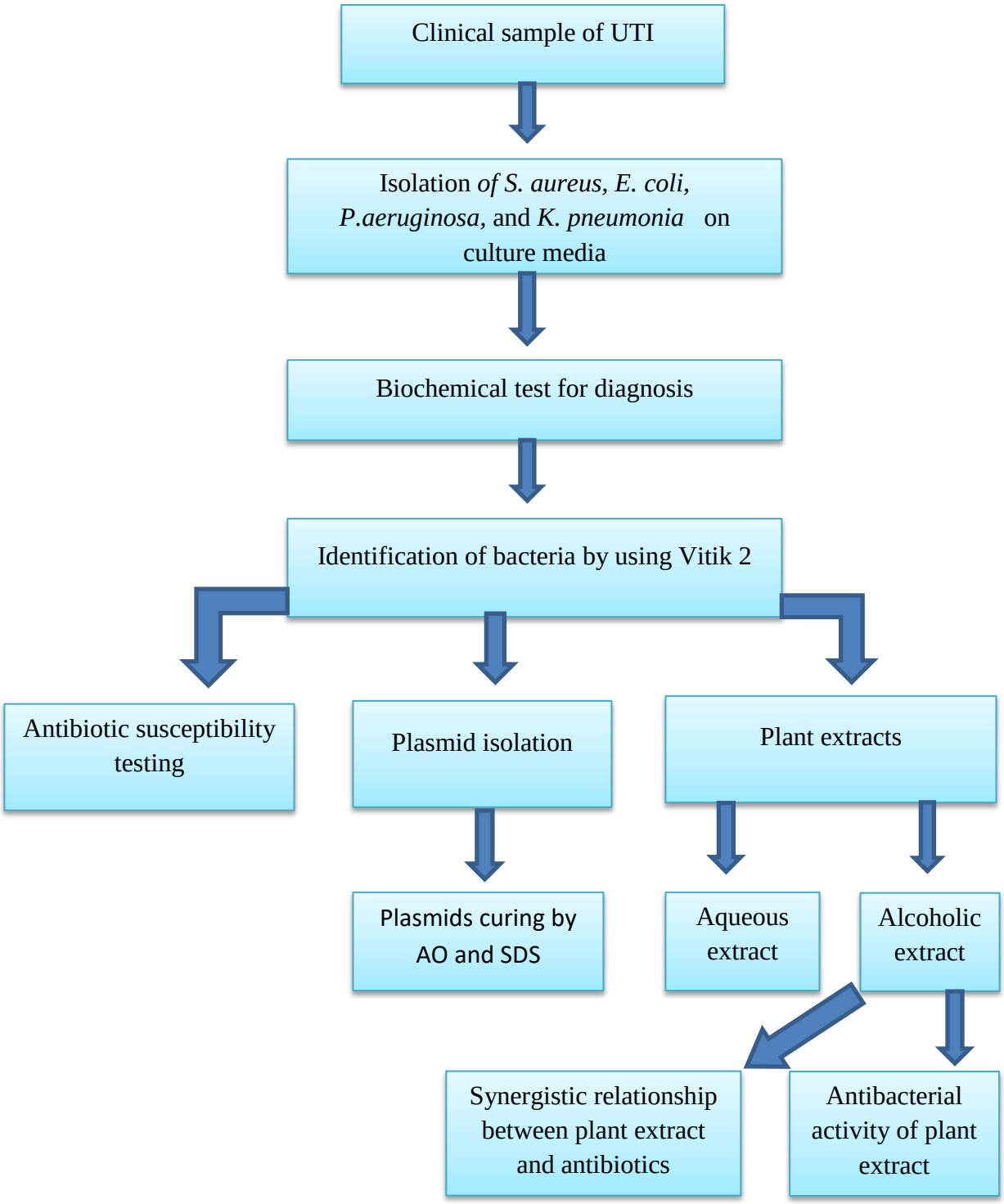
The commercial kit that was used in this study found here Table 2-7.

Table (2-7).Commercial kit in this study.

Type of kite	Company (country)
Plasmid extraction	Favorgen (Taiwan)
Vitik2 system card	Bio merieux (France)

2.2. Methods

2.2.1. Experimental design



2.2.2. Collection of samples

The urine samples (130) were collected from Al-Najaf hospitals patients from the beginning of November 2020 until the end of February 2021. The patient's ages ranged between 20-60 years, 83 samples from females and 47 samples from males, as the information related to the patient's name, age, gender, residence, medical condition, and date of taking the sample. The samples were planted on my media by the planning method at 37 °C for 24 hours. The blood and Maconkey agars were incubated at a temperature, and after that, phenotypic and biochemical tests and diagnostic examinations were performed.

2.2.3. Preparation of reagents

2.2.3.1. Kovacs reagent

This reagent was made by dissolving 5g of P-Dimethyl amino Benzaldehyde (DMAB) in 75mL amyl alcohol, then gradually adding 25 mL concentrated HCL to the mixture. It was used to identify bacterial isolates which can generate indole (MacFaddin, 2000).

2.2.3.2. Methyl red reagent.

Methyl red reagent was used to detect complete glucose hydrolysis. It was prepared by dissolving 0.1g of Methyl red in 300ml of 99% Ethanol. The volume was then completed to 500ml by adding distilled water (MacFaddin, 2000).

2.2.3.3. Voges-Proskauer reagent.

It was used to detect bacterial types that can produce acetyl-methel carbinol. It was prepared according to the method reported by (MacFaddin, 2000).

2.2.3.4. Oxidase reagent

This reagent was prepared according to the method reported by Forbes *et al.,* (2007), by dissolving 0.1g Tetramethyl Para Phenylene diaminedihydro chloride in 10 ml distilled water then stored in a dark container, it was used to detect the ability of bacteria to produce an oxidase enzyme.

2.2.3.5. Catalase reagent

It was prepared by mixing 1 ml of concentrated hydrogen peroxide 30% with 9 ml of distilled water to obtain a concentration of 3% hydrogen peroxide, and kept in the refrigerator in a dark package, used to detect the ability of the bacterial isolates under study to produce catalase enzyme (Forbes *et al.*, 2007).

2.2.3.6. Gram's stain solution

According to the required microbiological methods, the solutions were prepared according to the method reported in *Collee et al.* (1996). It was used for diagnosis gram-positive and gram-negative bacteria.

2.2.3.7. Tris Borate EDTA buffer solution (TBE buffer)

This solution was prepared by dissolving 0.08 mole of Tris-OH and 0.05M boric acid and 0.02M of EDTA in 500 ml of D.W., and then was pH adjusted to 8, after this, an autoclave was used for sterilization and stored at 4°C until use (Sambrook and Rusel, 2001).

2.2.3.8. Ethidium Bromide Solution

This solution can be prepared by dissolving 0.05g of ethidium bromide in 10 ml of D.W. and then stored in a dark container. Ethidium bromide solution is used for staining agarose gel (Sambrook and Rusell, 2001).

2.2.4. Preparation of culture media

All standard culture media that mentioned in table 2-4 were prepared according to the recommendation of their manufacture.

2.2.4.1.Semi-solid medium

This medium was prepared by dissolving 0.5g of agar- agar to 100ml of nutrient broth and then sterilized by autoclave this medium was used for detection of motile and non-motile bacteria (MaclFaddin, 2000).

2.2.5. Biochemical test

Various biochemical tests were done to diagnose and identification of bacterial isolations.

2.2.5.1. Catalase test

It was used to detect the bacterial ability to produce catalase enzyme. This test was done by streaking and selecting bacteria on nutrient agar and incubating for 24h at 37°C, then transferring a small colony by wooden stick to transfer the growth and put it on the surface of a clean slide. When adding one drop of 3% H_2O_2 on bacterial growth, the bubble appeared to be a bubble, indicating a positive result (Forbes *et al.,* 2007).

2.2.5.2. Oxidase test

This test was used to detect bacterial ability to produce oxidase enzymes. One drop of Tetramethyl dye was added to bacterial growth present on filter paper when appearing the purple color within 10 seconds; this indicator to a positive result (Forbes *et al.,* 2007).

2.2.5.3. Vogues- Proskauer test

This test is used to detect bacterial isolate that has the ability to partial hydrolysis of glucose. The test can be occurred by inoculating selected bacterial colonies in the tube containing MR-VP broth and incubating them at 37°C for 24 h. The results were then read by adding 0.6 ml of alpha naphthol (reagent A) and 0.2 ml of 40% of KOH solution (reagent B) when appearing red through 15 minutes this indicate a positive result (Forbes *et al.,* 2007).

2.2.5.4. Indole test

This test was used to indicate the bacterial ability to hydrolysis glucose ultimately. By inoculating selected bacterial colonies in the tube containing MR-VP broth and incubating for 24-48h at 37C°, then added five drops of methyl red reagent when appearance red color indicator positive color (MacFaddin., 2000).

2.2.5.5. Motility test

This test was used to distinguish between motile and non-motile bacterial isolate by stabbing selected bacteria in the tube containing semisolid media and incubating at 37°C for 24-48 h when growth appeared out of stab line meaning positive result (MacFaddin., 2000).

2.2.5.6. Citrate (Simmon's) utilization test

Simmon's citrate considered one of the necessary biochemical tests used to indicate bacterial ability to utilize the citrate as a carbon source, by inoculation selected bacterial colonies in Simmon's citrate slant and incubation for 24 h at 37°C when the color change from green to blue this indicator positive result (MacFaddin., 2000).

2.2.5.7. Methyl red test

Methyl red Vogues-Proskauer broth was inoculated with a young bacterial culture and incubated at 37°C for 24 h. Five drops of methyl red solution were added, mixed, and the result was read immediately. A positive test was the appearance of bright red color (MacFaddin, 2000).

2.2.6. Diagnostic by Vitek 2

The Vitek-2 System was used to identify bacterial isolates according to the manufacturer's instructions. This device was used in the diagnosis of bacteria (Appendix 1).

2.2.7. Samples Culture

The samples were sown directly on the medium of the blood agar in the middle of the Maconkey agar, and the isolates were purified on the medium of the nutrient agar by the planning method, and all dishes were incubated aerobically for 24 hours, and then the phenotypic and biochemical diagnostic tests were performed for the isolates under study.

2.2.8. Protocol for Freezing Bacteria Using Glycerol

Aliquot 500 µl of 30% sterile glycerin into sterile 2 ml tubes containing 4 mm glass pellets and 500 ml of microbial culture was added to the tube and mixed with glycerol using a vortex mixer. The tubes labelled with the organism name, isolate number, date, and tubes were placed in the freezer (Burnett and Crocker, 2005).

2.2.9. Antibiotic susceptibility test

The library-Baure susceptibility test was examined by using a pure culture of selected identified bacterial isolate. Five antibiotic discs were added to each plate, and incubation for 18-24 h, by measurement the inhibition zone can classify bacterial sensitivity (Morello *et al.,* 2006).

2.2.10. Genetic experiment

2.2.10.1. Plasmid DNA Extraction

Plasmid DNA was extracted from clinical isolates. One colony of each isolate cultured on solid medium was inoculated into 5 ml of Brain Heart Infusion broth and grown overnight at 37ºC. DNA was purified from bacterial cells using a Plasmid Extraction Mini kit supplemented by the manufacturing company from these isolate cultures.

The following steps were carried out to plasmid DNA extraction :

1. 1-3 miles of a well-grown bacterial culture was transferred to a centrifuge tube.
2. Centrifuge tube at 11,000 x g for 1 min to pellet the cells and discard the supernatant completely.

3. 200 µl of RNase A was added to the cell pellet and the cells were completely resuspended by immersion.
4. 200 µl of FAPD2 buffer is added and the tube is gently inverted 5-10 times. Incubate the sample mixture at room temperature for 2-5 minutes to lyse the cells.
5. 300 µl of FAPD3 buffer was added and the tube was inverted 5-10 times immediately to neutralize the lysate.
6. Centrifuge at full speed (-18000 x g) for 5 minutes to clarify the lysate. During centrifugation, place the FAPD column into the collection tube.
7. Carefully transfer the suspernatant to the FAPD column and centrifuge at 11,000 × g for 30 sec. I got rid of the flux and put the shaft back into the collecting tube.
8. 400 µl of WI Buffer was added to a FAPD column and centrifuged at 11,000 x g for 30 seconds. I got rid of the flux and put the shaft back into the collecting tube.
9. 700 µl of wash buffer was added to the FAPD column and centrifuged at 11,000 x g for 30 sec. I got rid of the flux and put the shaft back into the collecting tube.
10. Centrifuge at full speed (- 18,000 x g) for 3 min to dry the FAPD column.
11. The FAPD column was placed in a new 1.5 ml centrifuge tube.
12. 50 ul - 100 ul of Elutlon Buffer was added to the center of the membrane of the FAPD column. Stand in the column for 1 minute.
13. Centrifuge at full speed (- 18,000 x g) for 1 minute to extract plasmid DNA and store DNA at -20°C.

2.2.10.2. Preparing the solutions used for agarose gel electrophoresis

Solution Tris-borate-EDTA (TBE) buffer was used in this study at a concentration of (10X (1:9) dilution of the concentration stock). The stock solution was diluted with D.W. and stored at room temperature.

2.2.10.3. Preparation of Agarose Gel

This gel was prepared by dissolving agarose powder in 1X TBE buffer by boiling; then, it was left to cool to 50ºC. The dissolved amount of agarose powder depends upon the aim for which agarose is used. For DNA profile, 1% agarose is used. Ethidium bromide stock solution with a concentration of 10mg/ml was used. Only 5µl of this stock solution was supplemented with 100ml of melted agarose gel to get a final concentration of 0.5µg/ml (Sambrook and Russel, 2001). Then after the addition of ethidium bromide, mixed well and dispensed to the tray of gel electrophoresis.

2.2.10.4. Plasmid curing

The isolates were showing resistance character (100%) subjected to plasmid curing. Acridine orange (AO) and Sodium dodecyl sulfate (SDS) was serially diluted in Nutrient broth. The curing agent was tested at 12.5, 25, 50, 100, 200,400µg/mL concentrations. An overnight growth culture of four isolates subjected to plasmid curing and each of them inoculated into the tube containing 1 mL Muller Hinton broth and incubated at 44°C for 24 h. The minimal inhibitory concentration (MIC) of AO and SDS was determined then the highest concentration permitting growth sub lethal concentration (SIC) was considered as plasmid curing. The overnight culture was inoculated on Nutrient agar with curing agents and incubated at 37°C for two days. The single colonies were picked up by sterile toothpicks and inoculated on a nutrient agar plate (30 colonies/plate). The plates were incubated at 37°C for 24 h and used as master

plates. These colonies were inoculated on Muller - Hinton agar with Rifampicin (5 µg) and on Muller - Hinton agar with Ceftazidime (5 µg) and on Muller - Hinton agar with Cefeime (30 µg) and then incubated at 37°C for 48 h. The colonies that did not grow on the selective medium were known as cured colonies. At the same time, a non-cured culture on a selective medium as a control for curing each marker was performed (Kazeroon, 2015).

2.2.11. Plant material

2.2.11.1. Preparation of plant extracts

The plant samples were cleaned well by washing them with water to get rid of dust and left at room temperature to dry from the water completely. And after making sure that it was dry and not infected with fungi, it was ground using an electric grinder to form a powder and stored in sterile containers until use.

2.2.11.2. Cold aqueous extract preparation

The powdered plant material (500 g) was taken in a round bottom flask and was extracted with water for 24-48 h at room temperature. The solution was filtered, the outcome of filtration was concentrated, and the last trace was removed in a vacuum (Harborn and Svensson 1984).

2.2.11.3. Hot aqueous extract preparation.

The aqueous extract is prepared by dissolving 100 g of dry plant powder in an erlenmeyer flask and adding 500 ml of boiling distilled water. Then the mixture was shaken for two hours using an electric shaker before leaving it at room temperature for 24 h. The mixture was filtered with four layers of gauze and placed in a tube, then the solution was placed in a centrifuge for 10 min at 2000 rpm. The supernatant was filtered by Whatman No. 4. To achieve a dry

raw extract, the filtrate mixture was concentrated by the oven for 72 h to obtain crud extract. This extract was kept at 4 °C in a clean, dark container until used (Zheng Mu *et al*., 1990).

2.2.11.4. Alcoholic extract preparation

The powder was extracted by the maceration method at room temperature using methanol for 48 h to obtain the methanol extract or ethanol for 48 h to obtain the ethanol extract . The solvent extract was filtered using a millipore filter (0.4) to remove particulate matter. The filtrate obtained was concentrated in a rotary evaporator at 37 C°. The extract was conserved at 4 C° in the dark (EI Azhary *et al.*,2017).

2.2.11.5. Preparation of plant extract concentrations.

Five grams of dry residue was taken for each plant extract separately and dissolved in 12.5 ml of distilled water, and the stock solution concentration became 40%, equivalent to 400 mg/ml. From this solution, the concentrations were prepared (3.125, 6.25, 12.5, 25, 50, 100, 200) µg/ml, and thus seven concentrations were obtained for each plant extract according to the formula N1V1 = N2V2. And each concentration was placed in a plain tube capacity of 10 ml and kept in the refrigerator until use (Al-Nakeeb, 2004).

2.2.11.6. Antibacterial activity of plant extracts

The plant extracts were screened for their antibacterial activity by using agar-well diffusion methods described by (CLSI, 2019).

2.2.11.7. Agar-well diffusion method

In this method, Mueller Hinton Agar (MHA) plates were prepared and inoculated as in the well diffusion method. 4 holes of 6 mm in diameter were

made on the inoculated MHA plates using a sterile corn borer, and agar discs were removed. Holes were aseptically filled with 20 µl of plant extract using an automatic microliter pipette and allowed to diffuse at room temperature for 2 h. and after that incubated at 37 C° for rang time 24-48 h (Harborn and Svensson 1984).

2.2.11.8. Synergism between natural plant extracts and antibiotics against bacterial isolates

Natural extracts that did not produce results acted synergistically with synthetic drugs against microbial species. The lowest concentration was 0.75 µg/ml for the alcoholic extract (methanol and ethanol), and the antibiotics used in the experiment gave great results. Where the antibiotics discs is saturated with plant extract and then placed on the culture medium with bacteria.

2.2.11.9. Conditions for analysis of plant extract sample with Gas Chromatography – Mass Spectrometry (GC– MS).

The chemical analysis of plant extracts was performed at the Ibn Al-Bitar Research Center / Iraqi Ministry of Industry, and the device used for the analysis was Agilent Technologies GC System (Appendix 2).

2.2.9.10. Statistical analysis:

Three replicates were used to determine all treatment data. An analysis of variance was done on the data using the SPSS 16.0 program, with a completely randomized project and the least significant difference (L.S.D) set at $P \leq 0.05$ (George *et al*., 2011).

3. Results and Discussion

3.1. Isolation and Diagnosis of Bacterial isolates

3.1.1. Isolation:

The number of isolates that gave a positive result for bacterial culture was 105 (80.8%), where 68 samples from female and 37 samples from males (Table 3-1). While the number of isolates that gave a negative result was 25 (19.2%). Many studies referred to varied percentage of positive urine culture AL-Kubaisy (2013) showed the 32.6% of samples gave the culture while Al-Nuaimi (2014) founded 38.3% of samples gave the result. A high percentage of the results mentioned in the study of Al-Mousawi (2006) who founded 78.6% of the urine samples while Al-Ajili (2007) founded 78.2%. The reason may be due to differences in the size, environment, and nature of the sample because the patient had taken antibiotics before taking the samples, which may lead to the absence of bacterial growth in the sample (Al-Abdali, 2010), or it may be caused by an infection other than bacterial, such as viruses, parasites, fungi, or anaerobic bacterium (Brooks *et al.,* 2007).

The high rate of infection in females may be attributed to the fact that the rate of urinary tract infections in females is due to hormonal and anatomical differences such as the shortness of the urethra (Raka *et al.,* 2004). As for age, the incidence of urinary tract infections in males is high at young ages due to congenital malformations of the urinary tract. After the age of fifty, the infection increases in males due to infection with the prostate, which no longer secretes substances that inhibit the growth of bacteria compared to people who do not have prostatitis (Kelley *et al.,* 2009).

Table (3-1). Number and percentages of urinary tract infections by pathogen bacteria on males and females.

Sex	No. (%) of samples		No. (%) of Positive culture	
	number	%	number	%
Females	83	63.8	68	64.8
Males	47	36.2	37	35.2
Total	130	100	105	80.8

3.1.2. Diagnosis of bacterial isolates:

In this study, four isolates of bacteria were isolated that cause urinary tract infection are positive and negative of Gram stain (Table 3-2). Primarily identified on the Blood agar and MaConaky agar depending on each type of bacteria's diagnostic and differential characteristics. *E. coli, P. aeruginosa, K. pneumonia,* and *S. aureus* were all identified after isolation and purification from urine samples based on the microscopic traits of the bacterial cells and the morphological and cultural characteristics, including the size of the colonies, their color, the edges of the purpose, and the tests used. Diagnosis of all bacteria and compliance with approved diagnostic systems (Cowan and Steels, 2004). In addition to using the familiarizing VITIK2 system (Appendix 3).

The different types of bacteria that cause urinary infection were diagnosed, colonies of bacteria *E. coli* showed on the center colony or medium MaConaky agar color pink as a result of fermented sugar lactose, and solid, medium size, convex, dry, and regular and negative test Oxidase , a positive test for Catalase, a negative Urease test and positive examination indole and instance red and is unable On the consumption of citrate, the microscopic examination showed that after a Gram stain, a smear taken from the pure colonies on the

medium of nourishing clematis showed that their cells were negative for Gram color and had a short bacillary shape and did not form spores (Brooks *al et*., 2007).

As for *K. pneumonia*, it was diagnosed on the center of MaConkey agar aggregate as large circular colonies, with regular edges, pink in color and with a mucous consistency due to having the capsular, and being irregular, as for their colonies on the hemocytes were transparent and shiny, incapable of dissolving blood (Levinson, 2004). It also gave a negative result in the indole test and is positive for the methyl red test and consumption of citrate and urase test; as for the movement test, it was negative for the movement test, as indicated by the results of the microscopic examination of the prepared slides for those colonies and by using the staining in a Gram method (Brooks *al et*., 2007).

Colonies of *P. aeruginosa* were round, smooth, and their colonies were hemolytic-type (hemolytic-Beta) decomposition on the center of the hemolytic cells. Fermented grapes, as the bacteria are negative for a bacillary-shaped grape dye, positive in the oxidase and catalase test, positive for the motility test, negative for the indole and red methyl test, and positive for the cysteine test with a variant (or positive) test result (Brooks *al et*., 2007).

The *S. aureus* was diagnosed with the appearance of Blood agar, inhibit on MaConkey agar appeared in the form of light-colored, creamy colonies, and medium to large-sized colonies appeared, ranging in diameter from 1 to 3 mm, with regular edges, smooth, convex, shiny, and surrounded by a transparent halo. The production of the hemolysin enzyme characterizes it. It gives a complete-type degradation around the bacterial colony known as hemolysis-, as a transparent halo is observed around the bacterial colony due to the blood dissolution added to the medium.

Table (3-2). Microscopic and biochemical diagnosis of bacteria that isolate from UTIs.

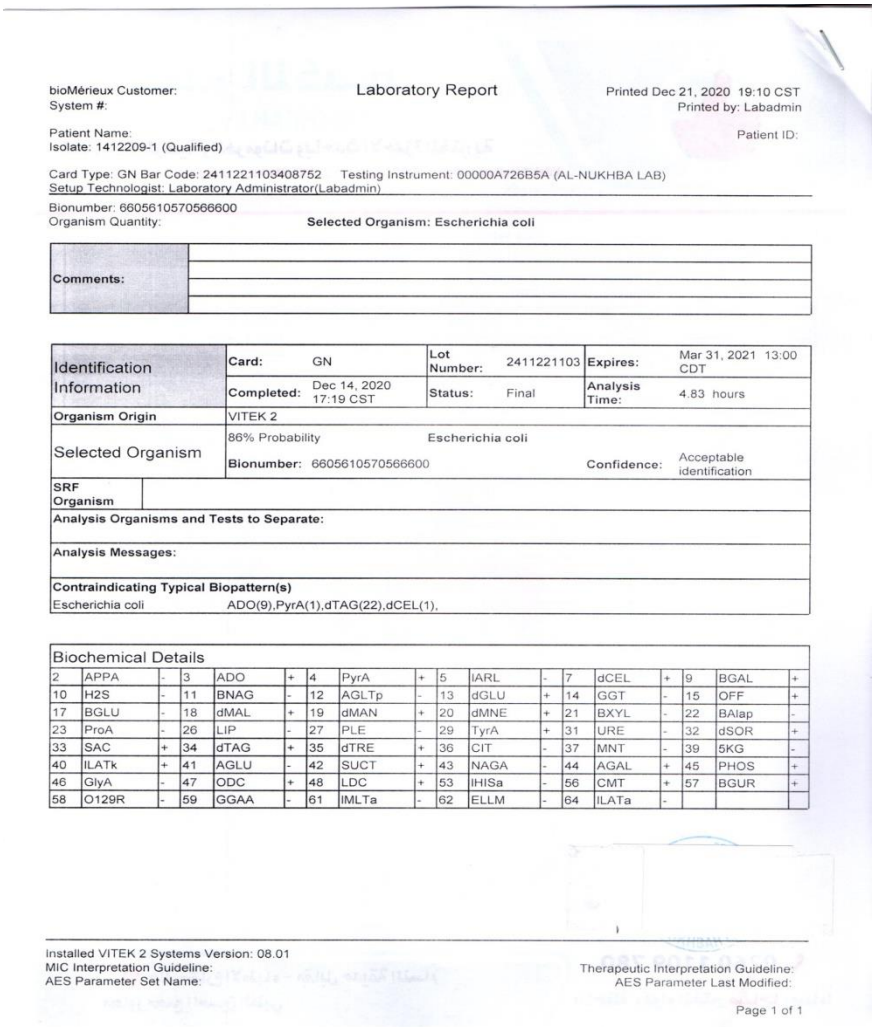
	E. coli	***P. aeruginosa***	***K. Pneumoniae***	***S. aureus***
Gram stain	-	-	-	+
Catalase test	+	+	+	-
Oxidase test	-	+	-	-
Indole test	+	-	-	-
Methyl-Red test	+	-	+	+
Citrate utilization test	-	+	+	-
Motility test	+	+	-	-
Urease test	-	+/-	+	

(+) positive test (-) Negative test (+/-) Heterogeneous result

Bacterial Identification by VITEK2 System, in order to validate the genus and species of some isolates that tested via biochemical test, automated bacterial identification was performed using the VITEK2 System. This method was performed using GP cards (for Gram positive bacteria) and GN cards (for Gram negative bacteria) for rapid and accurate Gram-negative and Gram-positive bacteria diagnosis. The result of VITEK2 system (Figure 1) were conformed our previous result that obtained from standard biochemical method.

The VITEK 2 has proved to be a highly effective and repeatable integrated microbial recognition system in multiple independent studies. With colorimetric reagent cards and associated hardware and software developments, the VITEK 2 offers a cutting-edge technical interface for phenotypic recognition methods. Previous research has found a similar findings among the two methods (GP and GN cards) and the standard biochemical outcomes.

Chen *et al* (2008) reported about 89.7% similarity in diagnosis the Gram-negative rods in standard and VITEK2 methods, Ling *et al* (2003) reported a 95% correlation rate, and Bruins *et al* (2004) reported a 93.0% correlation rate. Ligozzi *et al* (2002) observed that the VITEK 2 ID device could correctly classify over 90% of Gram-positive within 3 hours, with a 99 % characterized as *S. aureus* and this match their standard biochemical approach.



bioMérieux Customer: Laboratory Report Printed Dec 21, 2020 19:10 CST
System #: Printed by: Labadmin

Patient Name: Patient ID:
Isolate: 1412209-1 (Qualified)

Card Type: GN Bar Code: 2411221103408752 Testing Instrument: 00000A726B5A (AL-NUKHBA LAB)
Setup Technologist: Laboratory Administrator(Labadmin)

Bionumber: 6605610570566600
Organism Quantity: **Selected Organism: Escherichia coli**

Comments:	

Identification Information	**Card:** GN	**Lot Number:** 2411221103	**Expires:**	Mar 31, 2021 13:00 CDT	
	Completed: Dec 14, 2020 17:19 CST	**Status:** Final	**Analysis Time:**	4.83 hours	
Organism Origin	VITEK 2				
Selected Organism	86% Probability Escherichia coli				
	Bionumber: 6605610570566600		**Confidence:**	Acceptable identification	
SRF Organism					
Analysis Organisms and Tests to Separate:					
Analysis Messages:					
Contraindicating Typical Biopattern(s)					
Escherichia coli	ADO(9),PyrA(1),dTAG(22),dCEL(1),				

Biochemical Details

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

Installed VITEK 2 Systems Version: 08.01
MIC Interpretation Guideline: Therapeutic Interpretation Guideline:
AES Parameter Set Name: AES Parameter Last Modified:

Page 1 of 1

Figure (1): VITEK2 compact system for identification of Gram- positive and negative bacteria. The result showed an example of tested isolate that characterized as *E coli*.

The results showed the presence of the bacterium *E. coli*, was a common type of bacteria isolated from UTIs (45.7%). Many studies referred to varied percentage of *E. coli* isolates Nabbugodi (2013) showed the 40% of samples gave the UTIs while Al-Kubaisy (2013) founded 70.49% of samples gave the result. The high rate of isolation of the bacterium *E. coli* that causes UTI compared to the rest of the intestinal family bacteria may be due to the presence of this bacteria in considerable numbers in the human gastrointestinal tract as normal flora. In addition, the possession of these bacteria has multiple virulent factors, such as the production of the hemolytic enzyme and their ability to form the biofilm. This helps them in the events of infection and its continuation (Enayat *et al.*, 2011).

While for *K. pneumoniae*, its percentage was 20.9%, Many studies referred to varied percentage of *K. pneumoniae* isolates Al-Zubaidi (1996) showed the 21.8% of samples gave the UTIs while Al-Nuimi (2014) founded 13.63% of samples gave the result. This is because *K. pneumoniae* strains depend on their ability to adhere to the mucous surfaces and are the first step for infection. Without adhesion factors, pathogens in the intestine and urinary system wash away before they cause disease. This virulence factor helps adhesion is the fimbria through which it adheres to the epithelial cells of the respiratory tract and the epithelial cells For the Urinary Channel (Huang *et al.*, 2009).

As for *P. aeruginosa*, its percentage was 10.5%, the study Ahmed (2016) as the rate was 8.7%, while Al-Saadi (2011) founded 41.3% of samples gave the result. These differences may be due to the number of samples that were taken during the study period and circumstances. Patients' health or the development

of resistance by strains to antibiotics, as it is an opportunistic pathogen bacteria that cause UTIs.

While the *S. aureus*, the percentage was 22.9%. Many studies referred to varied percentage of *S. aureus* isolates Al-Qazzaz *et al*., (2009) showed the 19.35% of samples gave the UTIs while Kazem (2005) founded 0.8% of samples gave the result. The isolated ratios of each *S. aureus* in this study may be due to its being among the skin pollutants and thus the ease with which the urinary tract is contaminated, especially in women compared to males due to anatomical differences between the sexes (Wistrom *et al.*, 2014).

Table (3-3). Number of bacterial isolates from UTIs.

Bacterial type	Number of isolates	Percentage (%)
E. coli	48	45.7
K. Pneumoniae	22	20.9
S. aureus	24	22.9
P. aeruginosa	11	10.5
Total	105	100

3.2. Antibiotic activity using the disc method and Multiple Antibiotic Resistance

The sensitivity of the isolates under study was tested for seven antibiotics, including the beta-lactam group (Aztreonam, Cefeime, Ceftazidime, Piperacillin), the aminoclycoside group (Tobramycin), among the group of anti-folic acid inhibitors are Co-Trimoxazol, which is a mixture of (Trimethoprim and Salphamethoxazole) referred to in some sources as the Trimethoprim and group Glycopeptide the Vancomycin. All of them have the same inhibitory

diameters as stated in (CLSI 2019). Rifampicin is an antimicrobial agent that inhibits transcription via binding to the β-subunit of the bacterial DNA-dependent RNA polymerase (Campbell *et al.*, 2001).

The results showed a difference in the response of the isolates understudy to the used antibiotics (Table 4-4). The *E. coli* isolates were resistant in 34 (70.83%) of 48 isolates. Also, the *K. Pneumoniae* isolates were resistant to antibiotics in 21(95.45%) of 22 isolates, While *S. aureus* was resistant to antibiotics in 20 (83.33%) of 24 isolates and the *P. aeruginosa* 10 (90.90%) of 11 isolates.

Table (3-4). Percentages of resistant isolates to the antibiotics used.

Antibiotic	**No. (%) of antibiotic resistant isolates of:**			
	K. Pneumoniae	*P. aeruginosa*	*S. aureus*	*E. coli*
Aztreonam	15	8	13	12
Cefeime	16	7	14	12
Ceftazidime	18	8	14	14
Piperacillin	15	8	13	14
Tobramycin	12	6	9	11
Trimethoprim	14	8	10	8
Rifampicin	11	7	11	10
Vancomycin	10	6	9	11
Total	21	10	20	34
Percentage	95.45%	90.90%	83.33%	70.83%

The Multiple Antibiotic Resistance results showed that 36 isolates were multi-resistant to five different groups of antibiotics from the total of the different antibiotics used in the study, including seven antibiotics as beta-lactam group, aminoclycoside group, Co-Trimoxazol, Glycopeptide and DNA-dependent RNA polymerase group in (Table 3-5).

Table (3-5). Multiple antibiotic resistance of the isolates under study.

Antibiotic	No. (%) of MDR isolates of:			
	K. pneumonia	*P. aeruginosa*	*S. aureus*	*E. coli*
Aztreonam, Cefeime, Ceftazidime, Piperacillin,Tobramycin,Trimethoprim, Rifampicin, Vancomycin	**6**	**3**	**4**	**3**
Aztreonam, Cefeime, Ceftazidime, Piperacillin, Tobramycin, Rifampicin,	**5**	**3**	**2**	**2**
Cefeime, Ceftazidime, Tobramycin, Trimethoprim, Vancomycin	**2**	**1**	**3**	**2**
Total	**13**	**7**	**9**	**7**

The results exposed a variance in the response of the isolates understudy to the antibiotics used. All *E.coli* isolates are resistant to the beta-lactam group (ATM, PRL, CAZ, and FEP) in the percentage of 100%, many studies referred to varied percentage Surah Miri (2014) the resistance to Azteronam 93% and the Mahmoud (2014) for ceftazidime the percentage 100% while the Al-Khalidi (2016) at Ceftazidime in percentage 83% and Azteronam in 54.2%. The 80% of *E.coli* isolates were resistance to aminoclycoside group, Tobramycin's, and this studies Amin (2009) as the percentage of resistance to antibiotics 89.6%. The group of folic acid-inhibiting antibiotics Trimethorim in 95%, many studies

referred the Surah Miri (2014) showed the 89%, while the Al-Khalidi (2016) is 79.1%. The result of resistance Rifampicin antibiotic 80% for *E.coli*.

The sensitivity of bacteria *K. Pneumoniae*, the resistance to antibiotics was 100% for (ATM, PRL, CAZ, and FEP). Many studies referred to varied percentage Sikarwar and Batra (2011) the resistance to cefotaxime 76%, while Sarogamma and Ramakrishna (2011) founded resistance 70% for Azteronam. The rates of *K. pneumoniae* resistance to TOB are 90%. The RA resistance 95%, so the polymyxin-resistant strain *K. pneumoniae* was sensitized by a factor of 24 to Rifampicin in some isolates (Vaara *et al.*, 2010).

The percent of *P. aeruginosa* resistance to antibiotics as (ATM, PRL, CAZ, and FEP) 100%, many studies referred to percentage Falih (2005) the resistance to Azteronam 100%. The TOB resistance was 80%; the studies referred to varied percentage Al-Saadi (2012) resistance percentage of 66%. Concerning TMP the resistance was 100%, the studies referred to percentage Al-Saffar (2005) and Abdullah *et al.*, 2010 showed the 100%. The Rifampicin resistance was 90%, this studies referred to percentage 90% by Hall *et al.*, 2011 so they found that the average fitness of resistant strains was less than that of the wild type.

The *Staphylococcus aureus* resistance to antibiotics (ATM, PRL, CAZ, and FEP) 100%, Rifampicin resistance was found in 59 percent of *S. aureus* clinical isolates evaluated between 1999 and 2008 (Villar *et al.*, 2011). Rifampicin-resistant strains were discovered on MSSA and MRSA biofilms from 24 h onward. Greater rifampicin concentrations could induce rifampicin-resistant mutants faster than lower rifampicin concentrations (Gidari *et al.*, 2020).

3.3. Plasmids isolation

Plasmids DNA was isolated from *S. aureus, E.ccoli, K. pneumonia, P. aeruginosa* using Plasmid Extraction Mini kit, and the separation resulted in a single plasmid band with bands of plasmid on the right (Figure 2). The size of the plasmid of (*S. aureus*, *E. coli*, *K. pneumonia*, *P. aeruginosa*) Where the size of the plasmid of the bacteria was greater than the size of the ladder used. The number of positive isolates for plasmid isolation was *S. aureus* 4 isolates, *E. coli* 3 isolates, *K. pneumonia* 5 isolates, *P. aeruginosa* 3 isolates.

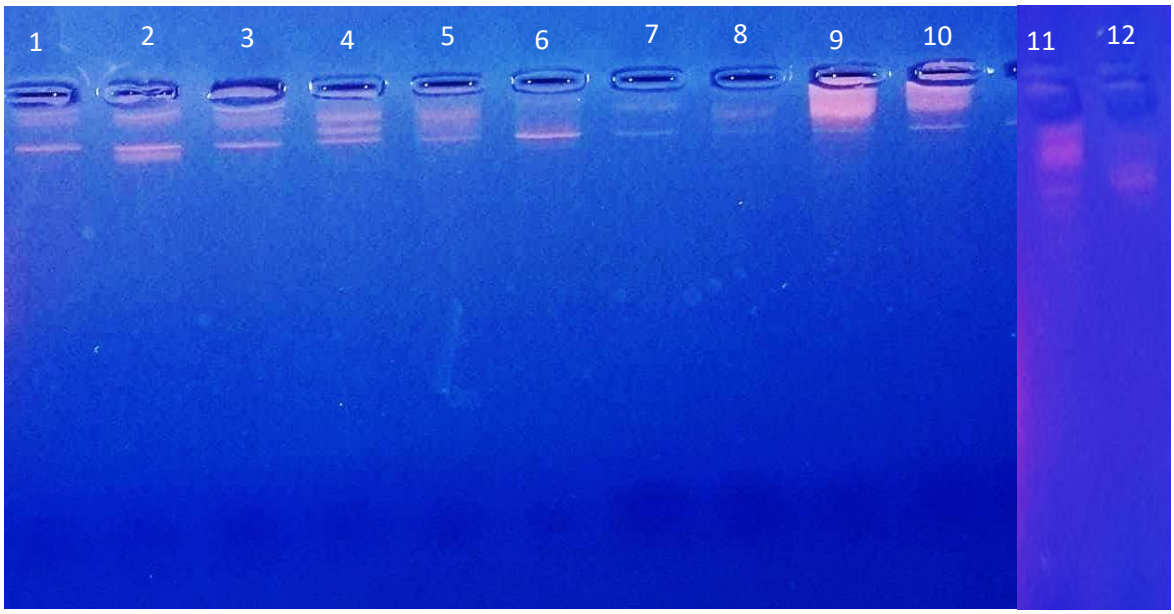


Figure (2): Ehidium bromide stained agarose gel electrophoresis (1% agarose, 70 volt for 50 min products) of plasmid extracted from different bacterial isolate.

1- ***E.coli*** **(E.8)**
2- ***E.coli*** **(E.9)**
3- ***P. aeruginosa*** **(P.1)**
4- ***P. aeruginosa*** **(P.5)**
5- ***S. aureus*** **(S.2)**
6- ***S. aureus*** **(S.3)**
6- ***S. aureus*** **(S.11)**
7- ***K. pneumonia*** **(K.21)**
8- ***K. pneumonia*** **(K.20)**
9- ***K. pneumonia*** **(K.16)**
10- ***E.coli*** **(E.19)**
11- ***P. aeruginosa*** **(P.8)**

Three isolates were selected from each type of bacteria study, as the isolates are multi-resistant (8 antibiotics) and possess the plasmid after their detection, *K. pneumonia* (K.16), (K.20) and (K.21), *P. aeruginosa* (P.1), (P.5) and (p.8), *S. aureus* (S.2), (S.3) and (S.11) , *E. coli* (E.8), (E.9) and (E.19).

3.4. Plasmids curing

Plasmid curing was performed by using Acridine orange (AO) and sodium dodecyl sulfate (SDS). It was observed that growth of *S. aureus*, *E. coli*, *K. pneumonia*, *P. aeruginosa* was MIC inhibited by SDS at concentration 50, 200, 100, and 50 µg/ml, respectively, whereas AO at concentration 200, 200, 200, and 100 µg/ml respectively. (Table 3-6)

Table (3-6). Elimination of antibiotic resistance plasmid from *S. aureus*, *E. coli*, *K. pneumonia*, *P. aeruginosa* by different concentration of AO and SDS.

Curing agent	Bacterial isolates	Concentration tested (µg/mL)					
		12.5	25	50	100	200	400
SDS	*S. aureus*	+	SIC	MIC	---	---	---
	E. coli	+	+	+	SIC	MIC	---
	K. pneumonia	+	+	SIC	MIC	---	---
	P. aeruginosa	+	SIC	MIC	---	---	---
AO	*S. aureus*	+	+	+	SIC	MIC	---
	E. coli	+	+	+	SIC	MIC	---
	K. pneumonia	+	+	+	SIC	MIC	---
	P. aeruginosa	+	+	SIC	MIC	---	---

MIC: minimum inhibitory concentration ; SIC: Sub lethal concentration.

Plasmid curing means the elimination of plasmid from its host cell by using a curing agent and screening the cured bacterial cells to demonstrate the presence of plasmid DNA and specific character gene on its plasmid.

The established sensitivity of a clinical isolate of *S. aureus, E. coli, K. pneumonia,* and *P. aeruginosa* to two anionic detergents, AO, SIC (100, 100, 100,50) µg/mL respectively and SDS, SIC (25, 100, 50, 25) µg/mL respectively, and its resistance to Azteronam, Cefeime, Ceftazidime, Piperacillin, Tobramycin, Rifampicin, Trimethorim (Figure 3,4,and5). Cured cells were achieved with SDS and AO with antibiotics (Table 3-7). The cured colonies lost the Rifampicin, Ceftazidime, and Cefeime resistance On the other hand, no cured cells for *K. pneumonia* were obtained from AO and SDS to Ceftazidime, and Cefeime, and no cured cells for *E. coli* were obtained from AO to Cefeime. When working with some plasmid-containing bacteria, it is often desirable to obtain a plasmid-cured derivative. This allows a direct comparison to be made between the plasmid-containing and plasmid-cured cells. Some plasmids undergo spontaneous segregation and deletion. However, the majority are extremely stable, and require the use of curing agents or other procedures (elevated growth temperature, thymine starvation), to increase the frequency of spontaneous segregation (Buckner et al., 2018).

Table (3-7). Antimicrobials with plasmid curing properties.

Curing Agent	**SDS**			**AO**		
antibiotics / Species	Rifampicin	Ceftazidime	Cefeime	Rifampicin	Ceftazidime	Cefeime
S. aureus	---	---	---	---	---	---
E. coli	---	---	---	---	---	+
K. pneumonia	---	+	+	---	+	+
Ps. aeruginosa	---	---	---	---	---	---

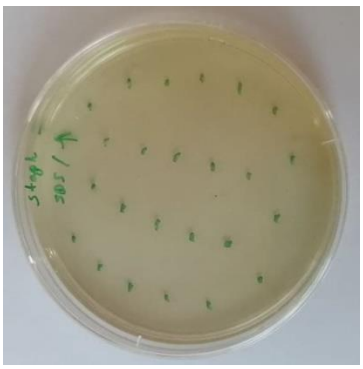
Killer (---), non-lethal (+)

Specific examples of plasmid curing agents in bacteria and the corresponding references are provided in Table (3-8). Although all of the plasmid curing agents discussed in this manuscript have been employed to enhance the frequency of

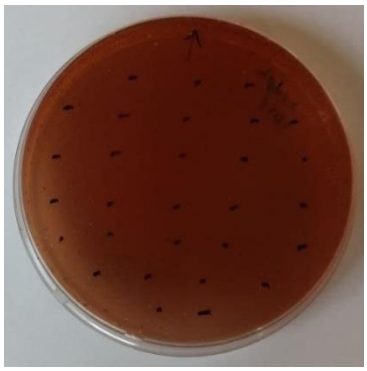
plasmid lost, they are only useful against some plasmids. Therefore, when initially working with new plasmids and bacterial isolates, a wide variety of curing methods may have to be tried prior to obtaining a satisfactory method.

Table (3-8). Some specific examples of plasmid curing of bacteria by AO and SDS.

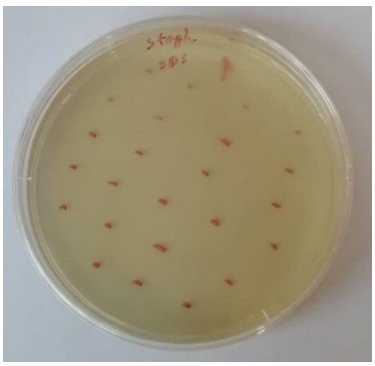
Curing Agent	Species	Plasmid Cured	Main results	Reference
AO	*E. coli*	pBR322	100 µg/mL: 35% CF for plasmids	Keyhani *et al.* (2006)
		Small plasmids (UTI isolates)	75 µg/mL: 11.76% CF for plasmids ≤2.7 mDa	Zaman *et al.*, (2010)
	S. aureus	pED503	15 µg/mL: 3.4% CF for plasmids	Buckner *et al.*, 2018
	K. pneumoniae	M5AI and K12 strain	100 ug/ml	Qureshi & Malik (1990)
	P. aeruginosa	Antibiotic resistance (RP1)	37 to 86% of cells were cured	Abdel-Salam *et al.*, (2007)
SDS	*E. coli*	UTI plasmids	10% w/v: 7.4% CF for plasmids	Zaman *et al.*, (2010)
	S. aureus	Staphyloccocin producing plasmid	30 µg/mL: 100% CF for plasmids	Buckner *et al.*, 2018
	K. pneumoniae	Large indigenous plasmid (96 kb)	4% resulted in 1/8 colonies successfully cured	El-Mansi *et al.* (2000)
	P. aeruginosa	pBC15	10% was effective	Raja and Selvam (2009)



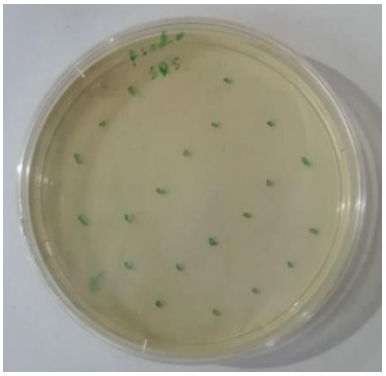
S. aureus/ SDS/ Ceftazidime



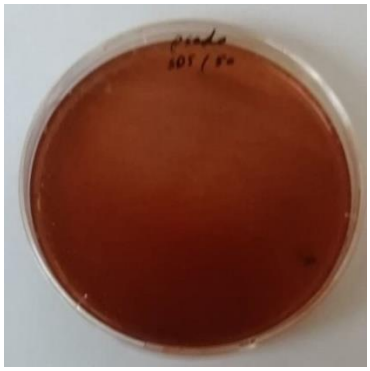
S. aureus/ SDS/ Rifampicin



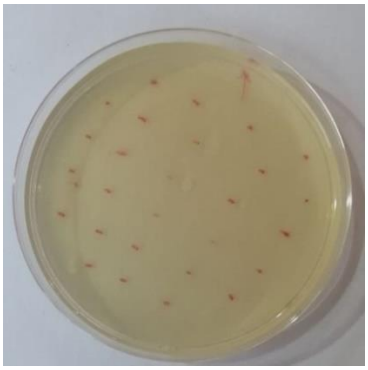
S. aureus/ SDS/ Cefeime



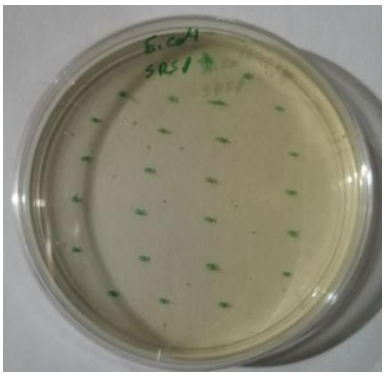
P. aeruginosa/ SDS/ Ceftazidime



P. aeruginosa/ SDS/ Rifampicin

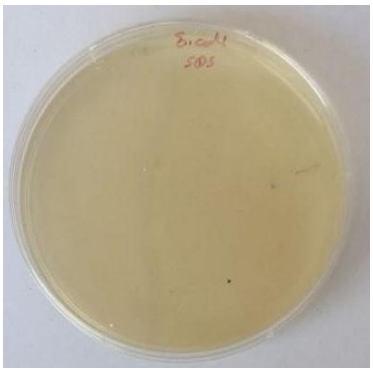


P. aeruginosa/ SDS/ Cefeime



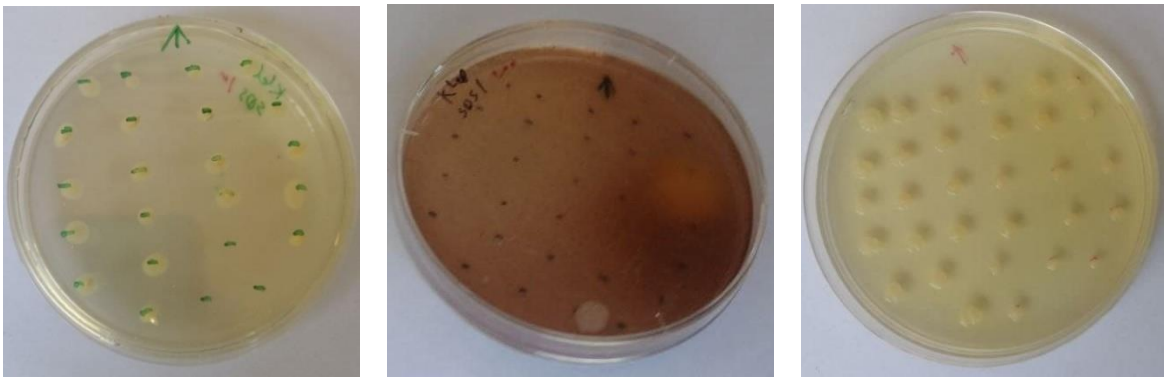
E. coli/ SDS/ Ceftazidime

E. coli/ SDS/ Rifampicin

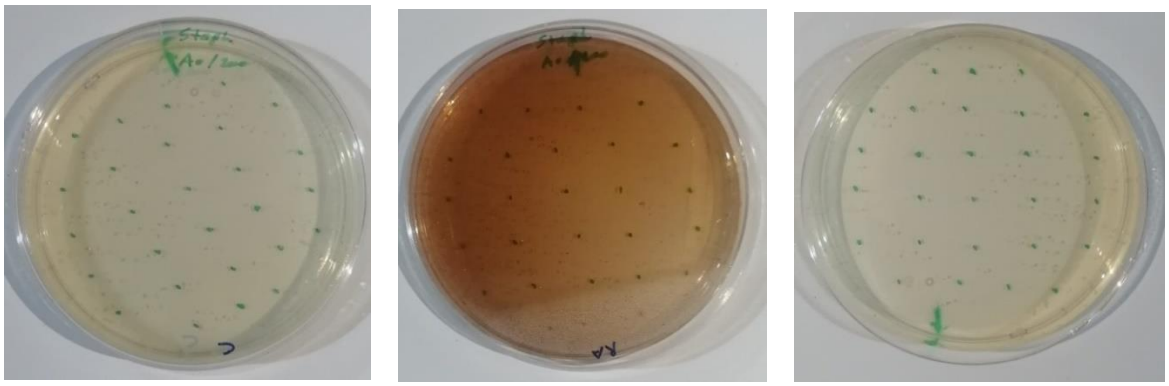


E. coli/ SDS/ Cefeime

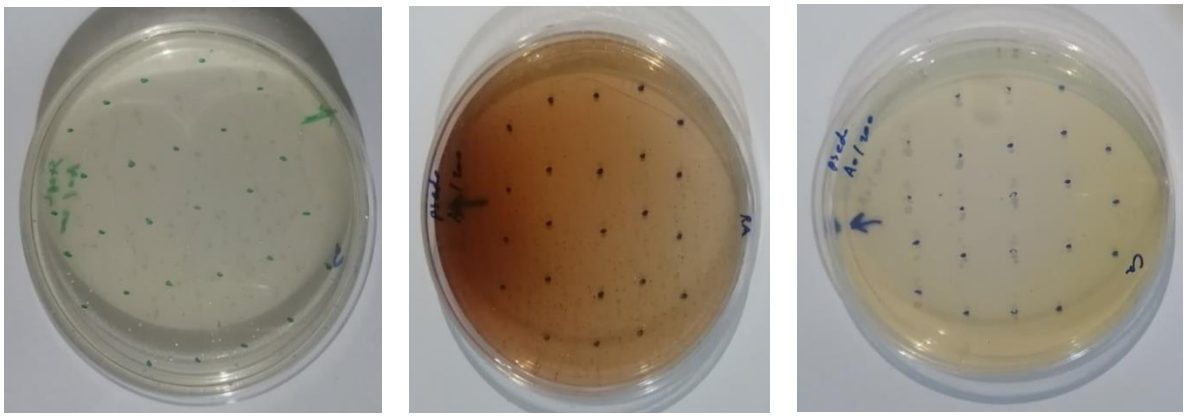
Figure (3). Pick and patch method for selection of cured *S. aureus, P. aeruginosa* and *E. coli.*



K. pneumonia/ SDS/ Ceftazidime *K. pneumonia*/ SDS/ Rifampicin *K. pneumonia*/ SDS/ Cefeime

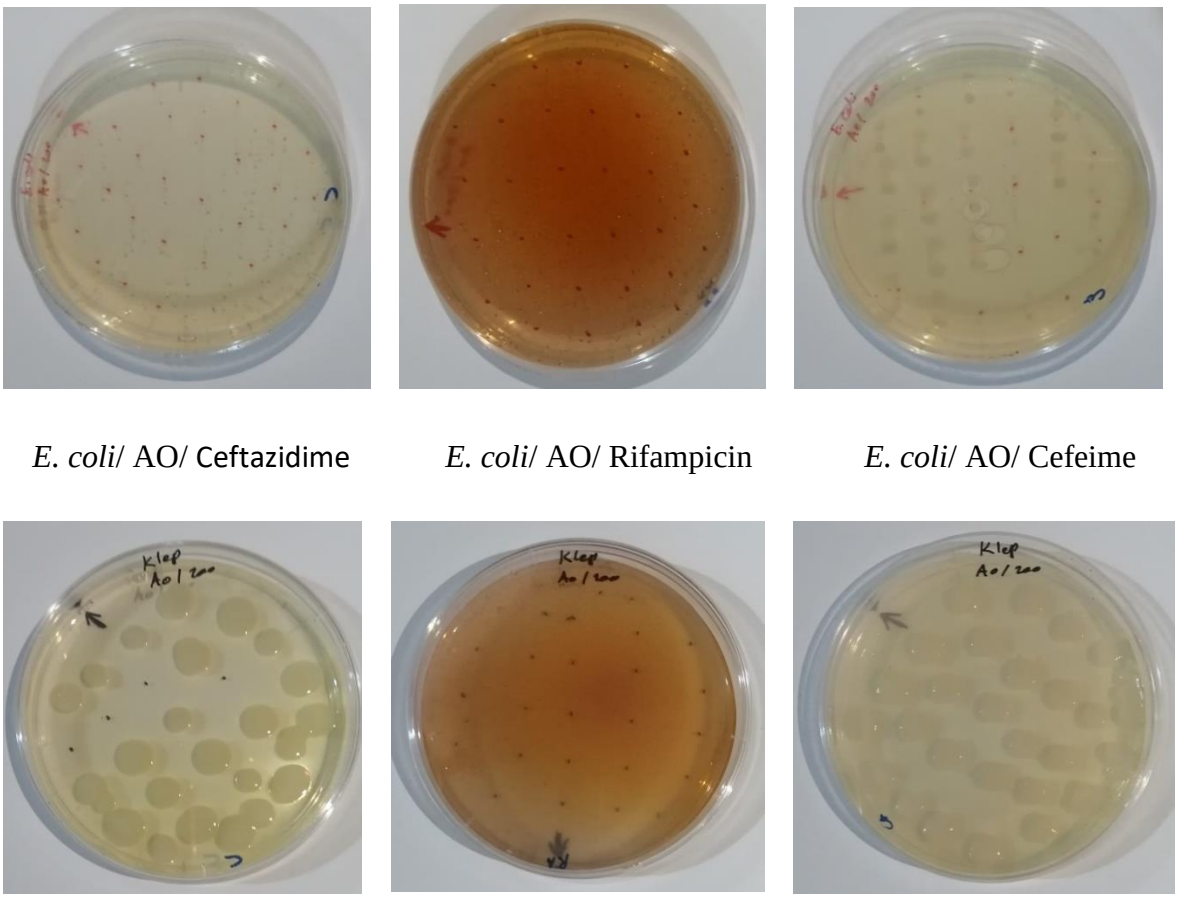


S. aureus/ AO/ Ceftazidime *S. aureus*/ AO/ Rifampicin *S. aureus*/ AO/ Cefeime



P. aeruginosa/ AO/ Ceftazidime *P. aeruginosa*/ AO/ Rifampicin *P. aeruginosa*/ AO/ Cefeime

Figure (4). Pick and patch method for selection of cured *K. pneumonia, S. aureus,* and *P. aeruginosa.*



E. coli/ AO/ Ceftazidime *E. coli*/ AO/ Rifampicin *E. coli*/ AO/ Cefeime

K. pneumonia/ AO/ Ceftazidime *K. pneumonia*/ AO/ Rifampicin *K. pneumonia*/ AO/ Cefeime

Figure (5). Pick and patch method for selection of cured *E. coli* and *K. pneumonia.*

3.6. Inhibitory activity of plant extracts

The effect of the plant extracts used understudy on the bacteria *E. coli*, *K. Pneumoniae*, *S. aureus,* and *P. aeruginosa* using the well diffusion method.

3.6.1. *Phyllanthus emblica* (Amla)

3.6.1.1. Aqueous extract of fruits Amla

The results showed that Amla fruit at hot and cold aqueous extract has an inactive against *E. coli*, *K. Pneumoniae*, *S. aureus*, and *P. aeruginosa*, in

concentration 50, 100, 200, 400 µg/ml was 0% as these bacteria were antibiotic resistance 100% used Azteronam, Cefeime, Ceftazidime, Piperacillin, Rifampicin, Trimethorim, Vancomycin, and Tobramycin.

3.6.1.2. Alcoholic extract of fruits Amla

The antibacterial activity of fruits Amla, using various solvents such as ethanol and methanol, is shown in Table (3-12 and 3-13). The plant's activity was examined using agar well diffusion methods. The results revealed that the extracts of ethanolic and methanol of fruits Amla showed a significant reduction at $p \leq 0.05$ in the growth of *E. coli, K. Pneumoniae, S. aureus*, and *P. aeruginosa* over than the antibiotics resistance 100% used Azteronam, Cefeime, Ceftazidime, Piperacillin, Rifampicin, Trimethorim, Vancomycin, and Tobramycin.

3.6.1.2.1. Ethanolic extract of Amla

Antibacterial activity was applied at 50, 100, 200, and 400 µg/ml and then compared with distilled water (D.W.) as a negative control. The inhibitory zone diameter was increased when increasing the concentration. The results also revealed that the extract by ethanol solvent at 400 µg/ml with average (24.4 mm) showed significant superiority over than the antibiotic resistant in *E. coli*. Whereas the MIC was 1.5 µg/ml.

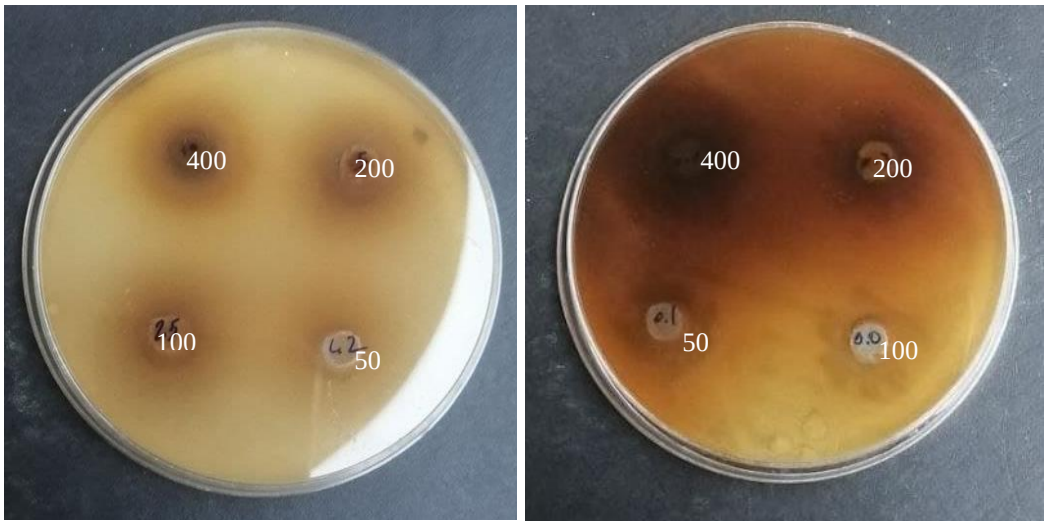
The results of *S. aureus* also revealed that the extract by ethanol solvent at 400 µg/ml with average (26.3 mm) showed significant superiority over than the antibiotic resistant. Whereas the MIC was 0.75 µg/ml. The results of *K. Pneumoniae* also revealed that the extract by ethanol solvent at 400 µg/ml with average (14.3 mm) showed significant superiority over than the antibiotic resistant, the MIC was 6 µg/ml.

On the other hand, the extract by ethanol solvent at 400 mg/ml with average (20.3 mm) showed a different effect between medicinal plant and the antibiotic when applied to *P. aeruginosa*. Whereas the MIC was 0.75 µg/ml, Table (3-9). Figure (6).

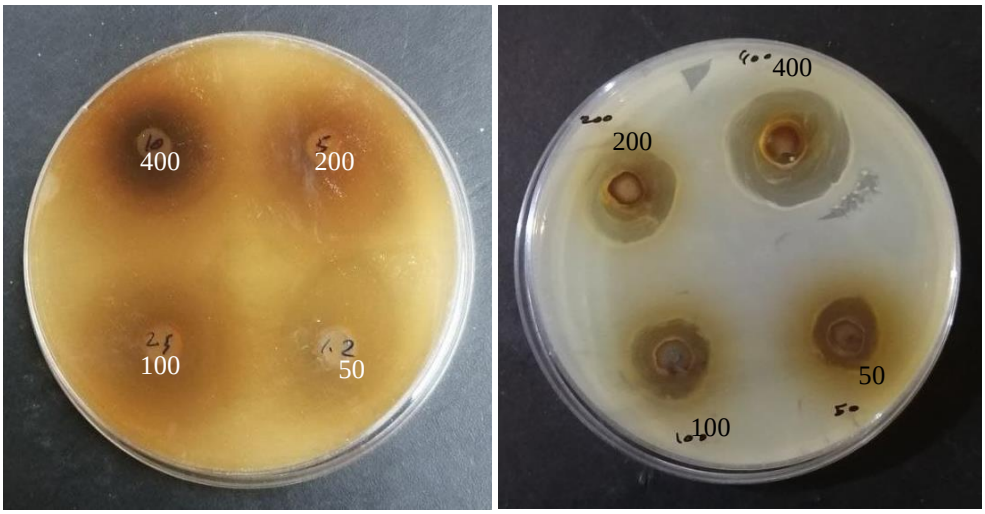
Table (3-9) Antibacterial activity of the crude Ethanolic extract of Amla.

concentration	Inhibition zone/mm											
	E. coli			*P. aeruginosa*			*S. aureus*			*K. Pneumoniae*		
	E.8	E.19	E.9	P.1	P.5	P.8	S.2	S.3	S.11	K.21	K.20	K.16
Control negative (D.W.)	0	0	0	0	0	0	0	0	0	0	0	0
50 µg/ml	17	16	16	16	15	15	15	16	15	---	---	---
100 µg/ml	20	19	19	18	16	17	16	18	17	9	10	9
200 µg/ml	21	20	21	19	18	19	21	21	22	11	13	12
400 µg/ml	25	24	24	21	20	20	26	27	26	14	15	14

L.S.D. value least significance differences at level 0.05 = 2.415



A **B**



C **D**

Figure (6). The effect of different concentration of ethanolic extract of Amla on: A: *K. pneumonia,* **B**: *S. aureus,* **C**: *P. aeruginosa,* **D**: *E. coli*

3.6.1.2.2. Methanol extract of Amla

Antibacterial activity was applied at 50, 100, 200, and 400 µg/ml and then compared with distilled water (D.W.) as a negative control. The inhibitory zone diameter was increased when increasing the concentration. The results also revealed that the extract by methanol solvent at 400 µg/ml with average (25.7 mm) showed significant superiority over than the antibiotic resistant in *E. coli*. Whereas the MIC was 0.75 µg/ml.

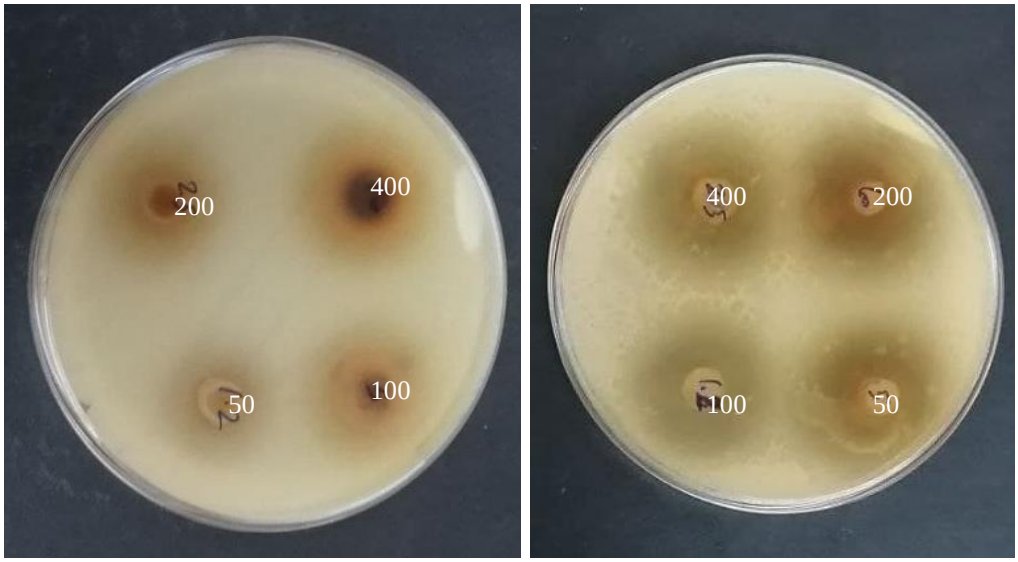
The results of *S. aureus* also revealed that the extract by methanol solvent at 400 µg/ml with average (21 mm) showed significant superiority over than the antibiotic resistant. Whereas the MIC was 1.5 µg/ml. The results of *K. Pneumoniae* also revealed that the extract by methanol solvent at 400 µg/ml with average (15.4 mm) showed significant superiority over than the antibiotic resistant, the MIC was 6 µg/ml.

On the other hand, the extract by methanol solvent at 400 mg/ml with average (24.7 mm) showed a different effect between medicinal plant and the antibiotic when applied to *P. aeruginosa*. Whereas the MIC was 0.75 µg/ml, Table (3-10). Figure (7).

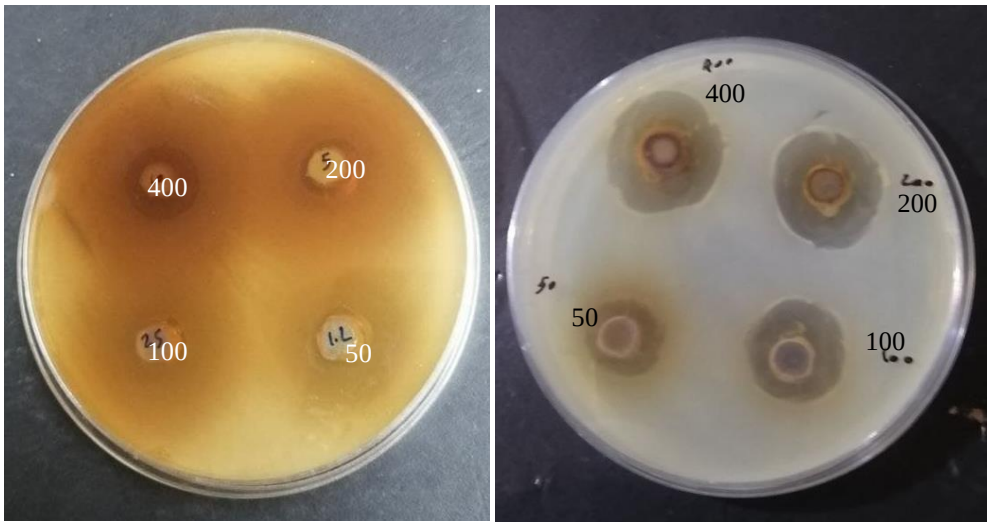
Table (3-10) Antibacterial activity of the crude methanol extract of Amla.

concentration	**Inhibition zone/mm**											
	E. coli			***P. aeruginosa***			***S. aureus***			***K. Pneumoniae***		
	E.8	E.19	E.9	P.1	P.5	P.8	S.2	S.3	S.11	K.21	K.20	K.16
Control negative (D.W.)	0	0	0	0	0	0	0	0	0	0	0	0
50 µg/ml	19	19	18	18	17	18	16	16	15	---	---	---
100 µg/ml	21	20	20	20	19	19	18	17	17	12	11	11
200 µg/ml	23	21	22	22	20	21	20	19	19	14	15	14
400 µg/ml	26	25	26	25	24	25	22	21	20	15	16	15

L.S.D. value least significance differences at level 0.05 = 1.838



A **B**



C **D**

Figure (7). The effect of different concentration of methanolic extract of Amla on: A: *K. pneumonia*, **B**: *S. aureus*, **C**: *P. aeruginosa*, **D**: *E. coli*

The active substances present in the Amla fruit extract included concentrated fatty acid than other active compounds of GC-MS analysis, Figure (8), (Table 3-11). Plants mainly have fatty acids in their bound state, esterified to glycerol, like fats or lipids. Higher plants' leaves contain up to 7% dry weight of lipids, which are vital membrane constituents in cell membranes, chloroplasts, and mitochondria. These fatty acid-derived compounds play a role in interspecies communication and plant defense by acting as intracellular mediators of extracellular signals (Weber 2002). Many plants' seeds and fruits contain significant levels of lipids, which serve as a type of energy storage during germination (Harborne and Baxter 1993). The effect of wood, bark, and leaves of *Brachychiton diversifolius* against the growth of *S.aurese* at a concentration of 2000 mg/ml, and the fatty acids had shown a tremendous antibacterial effect against the growth of *E.coli* (Salem *et al.*, 2014).

Fatty acids are well known for their bactericidal and antifungal effects (Kabara *et al.* 1972). This fatty acid derivative showed an enjoyable antibacterial activity against *E. coli*, *P. aeruginosa*, and *S. aureus* with the MIC value of 9µg/ml against S. aureus (Cerdeiras *et al*., 2000). Described the antibacterial activity of linoleic and oleic acids isolated from the leaves of *Helichrysum pedunculated*. Linoleic and oleic acids inhibited the growth of Gram-positive *B. subtilis*, Micrococcus kristinae, and *S. aureus*, and linoleic acid also showed activity against *Bacillus cereus* and *Bacillus pumilis*. Both acids displayed no activity against Gram-negative Enterobacter cloacae, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and Serratia marcescens (Dilika *et al*., 2000).

Plants' fatty acids are either saturated or simple unsaturated molecules with chain lengths of C16 or C18. Palmitic acid (C16) is the most abundant saturated acid in leaf lipids, as well as several seed oils, but stearic acid (C18) is a major saturated acid in seed fats in some plant groups. Linolenic acid is a common tri-unsaturated fatty acid, as are linoleic and oleic acids (Harborne and Baxter 1993).

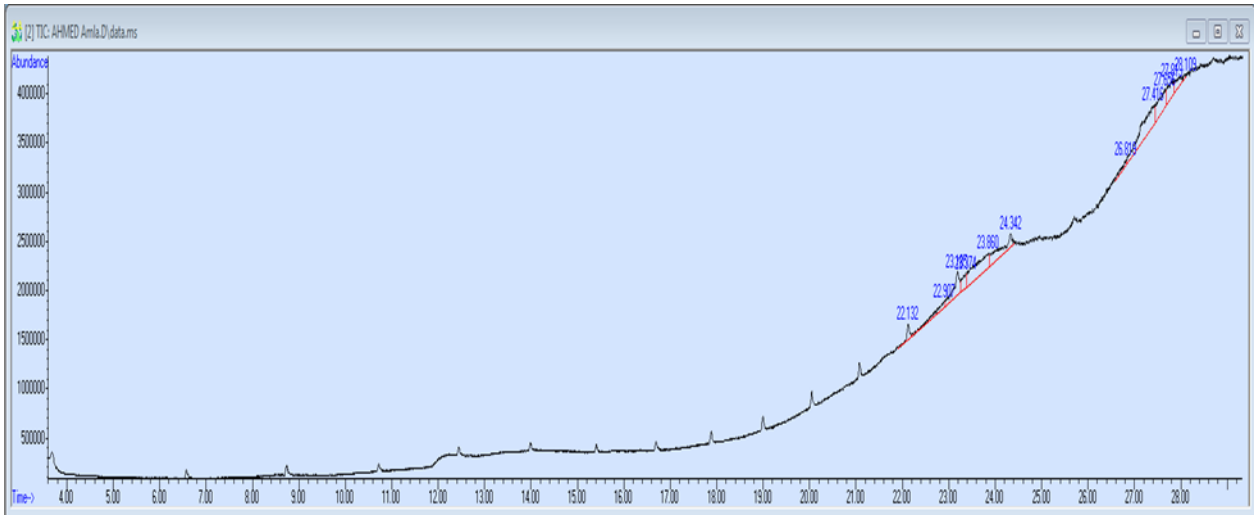


Figure (8). **Gas chromatography–mass spectrometry** (GC-MS) for Amla fruit.

Table (3-11). The active substances in the Amla plant by (GC-MS).

No.	Compound name	R. T. min	Corr. area	cas	corr. Max %	of total%	formula
1	6-Octadecenoic acid	22.132	6418804	000593-39-6	13.83	2.937	$C_{18}H_{34}O_2$
2	Oleic Acid	22.907	10133550	000112-80-1	21.83	4.637	$C_{18}H_{34}O_2$
3	13-Tetradecen-1-ol acetate	23.185	21245238	056221-91-1	45.76	9.722	$C_{16}H_{30}O_2$
4	cis-13-Octadecenoic acid	23.374	10465562	013126-39-1	22.54	4.789	$C_{18}H_{34}O_2$
5	Oleic Acid	23.860	40913346	000112-80-1	88.12	18.723	$C_{18}H_{34}O_2$
6	1-Eicosene	24.342	26172132	000112-80-1	56.37	11.977	$C_{20}H_{40}$
7	Undecanoic acid	26.819	5222445	1000156-09-6	11.25	2.390	$C_{11}H_{22}O_2$
8	Cyclopentane, 1,1,3-trimethyl	27.416	46427818	004516-69-2	100.0	21.247	C_8H_{16}
9	11-bromo-, undecy l ester	27.656	24432616	1000156-09-6	52.62	11.181	$C_{12}H_{23}BrO_2$
10	1,16-Dichlorohexadecane	27.819	16031309	007735-39-9	34.53	7.336	$C_{16}H_{32}C_{l2}$
11	1-Nonadecene	28.106	11054103	018435-45-5	23.81	5.059	$C_{19}H_{38}$

R. T. : Retention time,

3.6.2. *Syzygium aromaticum* (Cloves)

3.6.2.1. Aqueous extract of fruits Cloves

The results showed that Cloves fruit's hot and cold aqueous extract has an inactive against *E. coli, K. Pneumoniae, S. aureus*, and *P. aeruginosa* isolated, in concentration (50, 100, 200, 400) µg/ml was 0% as in the antibiotics resistance 100% used Azteronam, Cefeime, Ceftazidime, Piperacillin, Rifampicin, Trimethorim, Vancomycin, and Tobramycin.

3.6.2.2. Alcoholic extract of fruits Cloves

The antibacterial activity of Cloves flower buds, using various solvents such as ethanol and methanol, is shown in Table (3-12 and 3-13). The plant's activity was examined using agar well diffusion methods. The results revealed that the extracts of ethanolic and methanol of Cloves flower buds showed a significant reduction at $p \leq 0.05$ in the growth of *E. coli, K. Pneumoniae, S. aureus*, and *P. aeruginosa* over than the antibiotics resistance 100% used Azteronam, Cefeime, Ceftazidime, Piperacillin, Rifampicin, Trimethorim, Vancomycin, and Tobramycin.

3.6.2.2.1. Ethanolic extract of Cloves

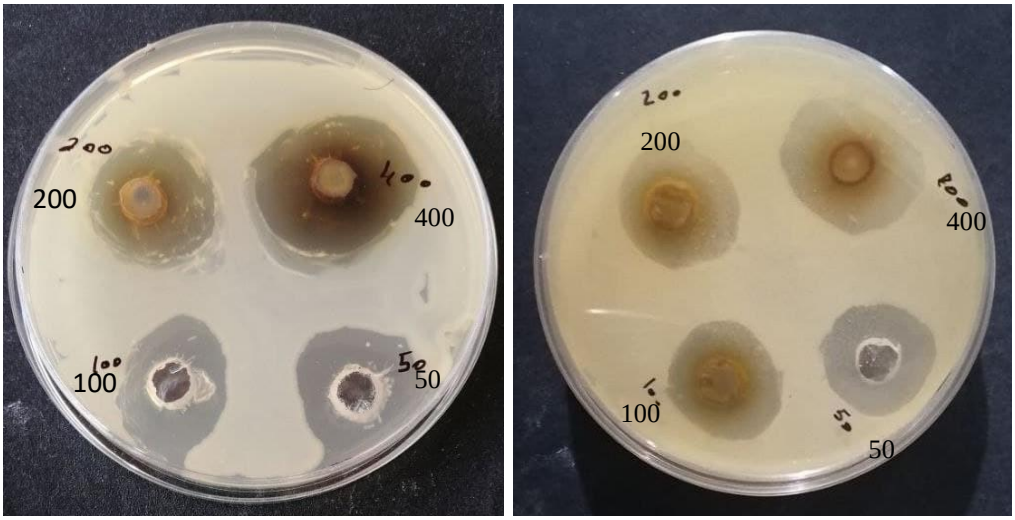
Antibacterial activity was applied at 50, 100, 200, and 400 µg/ml and then compared with distilled water (D.W.) as a negative control. The inhibitory zone diameter was increased when increasing the concentration. The results also revealed that the extract by ethanol solvent at 400 µg/ml with average (22 mm) showed significant superiority over than the antibiotic resistant in *E. coli*, the MIC was 3 µg/ml.

On the other hand, the extract by ethanol solvent at 400 mg/ml with average (26 mm) showed a different effect between medicinal plant and the antibiotic when applied to *P. aeruginosa*. Whereas the MIC was 3 µg/ml, Table (3-12). Figure (9). In contrast, there is no effect the extract by ethanol solvent at 400 µg/ml, to *S. aureus* and *K. pneumonia*.

Table (3-12) Antibacterial activity of the crude ethanolic extract of Cloves against pathogenic bacteria.

concentration	Inhibition zone/mm											
	E. coli			*P. aeruginosa*			*S. aureus*			*K. Pneumoniae*		
	E.8	E.19	E.9	P.1	P.5	P.8	S.2	S.3	S.11	K.21	K.20	K.16
Control negative (D.W.)	0	0	0	0	0	0	0	0	0	0	0	0
50 µg/ml	17	16	16	19	18	20	---	---	---	---	---	---
100 µg/ml	20	18	19	21	20	22	---	---	---	---	---	---
200 µg/ml	21	20	20	24	23	25	---	---	---	---	---	---
400 µg/ml	23	22	21	26	25	26	---	---	---	---	---	---

L.S.D. value least significance differences at level 0.05 = 1.419



A **B**

Figure (9). The effect of different concentration of ethanolic extract of Amla on: **A**: *P. aeruginosa* **B**: *E. coli*

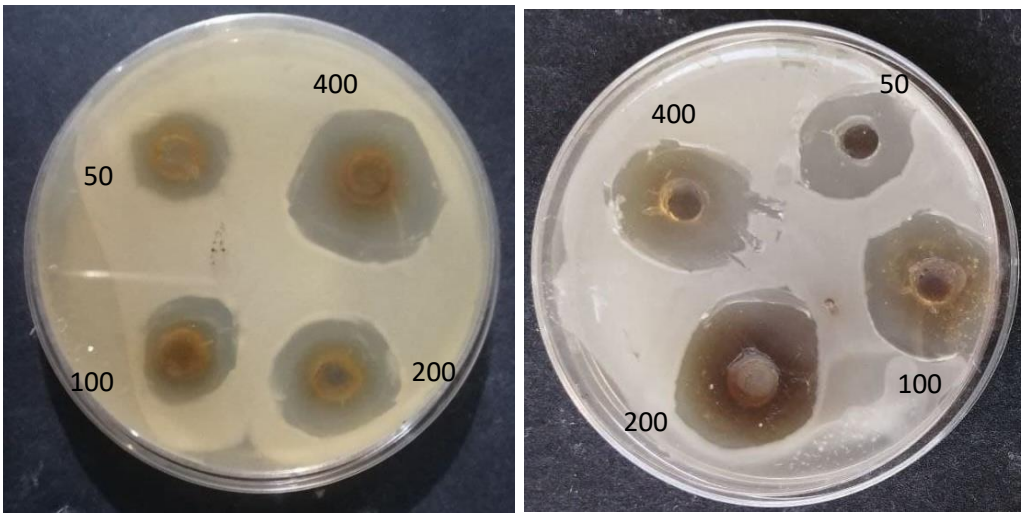
Compared to the negative control D.W., the present study found that increasing the concentration of methanol extract solvent resulted in a considerable reduction in pathogenic bacteria growth (Table 3-13). The results also revealed that the extract by methanol solvent at 400 µg/ml showed clear superiority over the antibiotic as the inhibition in the plant extract compared with (25.7 mm) in the antibiotic when applied to *E. coli*, the MIC was 3 µg/ml.

The extract by methanol solvent at 400 µg/ml showed clear superiority over the antibiotic as the inhibition in the plant extract compared with (27.3 mm) in the antibiotic when applied to *P.aeruginosa*, the MIC was 3 µg/ml. But there is no difference between the extract by methanol solvent at 400 mg/ml and the antibiotics used against *S. aureus* and *K. pneumonia*. Figure (10).

Table (3-13) Antibacterial activity of the crude methanol extract of Cloves against pathogenic bacteria.

concentration	**Inhibition zone/mm**											
	E. coli			*P. aeruginosa*			*S. aureus*			*K. Pneumoniae*		
	E.8	E.19	E.9	P.1	P.5	P.8	S.2	S.3	S.11	K.21	K.20	K.16
Control negative (D.W.)	0	0	0	0	0	0	0	0	0	0	0	0
50 µg/ml	17	16	16	18	17	20	---	---	---	---	---	---
100 µg/ml	20	19	19	21	20	22	---	---	---	---	---	---
200 µg/ml	23	22	21	25	25	26	---	---	---	---	---	---
400 µg/ml	26	26	25	27	28	27	---	---	---	---	---	---

L.S.D. value least significance differences at level 0.05 = 0.958



A **B**

Figure (10). The effect of different concentration of methanolic extract of Amla on: A: *E. coli*, **B**: *P. aeruginosa*,

The active substances present in the Clove extract included concentrated eugenol than other active compounds of GC-MS analysis, Figure (11) (Table 3-14). Eugenol, also called Clove oil, is an aromatic oil extracted from Cloves usedwidely as a flavoring for food. Moreover, eugenol demonstrated the disintegrating capability in the membrane and further increased its permeability, which subsequently causes the death of the organism (Gill and Holly, 2006). Besides its antibacterial activity, it has been reported that eugenol also collapses fungal cell membrane (Atsumi *et al.,* 2001). Eugenol has a protective role in normal cells, promoting cytotoxicity in cancerous cells (Wie *et al.,* 1997; Yoo *et al.,* 2005). The chemo-attractant property of eugenol combined with the observed high antibacterial activity at alkaline pH favors the fact that the compound can work more efficiently when given in *vivo*. Eugenol increased the membrane's permeability, as evidenced by crystal violet assay (Devi *et al.,* 2010).

The antibacterial activities of eugenol against *E. coli* were found in groups treated with 1600 mg/L of eugenol. Samples treated with 1600 mg/L or more of eugenol significantly reduced the number of *E. coli* ($P \leq 0.05$) compared with the control tube (Pei *et al.,* 2009). The addition of 10 mm eugenol to *E. coli* cells resulted in significantly lower ATP levels than both dextrose and buffer-treated cells by 10 min. At the same time, Treatment with 10 mm eugenol increased extracellular ATP significantly. The results depended on the primary mechanism of action of eugenol is disruption of the cytoplasmic membrane, which increases its non-specific permeability. Other secondary effects at sublethal concentrations cannot be discounted and can be expected due to membrane interactions (Gill & Holley, 2006).

Effects of antimicrobials on motility, treatment of *E. coli* with 10 mm eugenol resulted in an immediate cessation of motile behaviour. (Gill & Holley, 2006). While growth of *E. coli* was not observed following treatment with eugenol at a concentration of 1000 ppm at both temperatures. Overall, the lactobacilli appeared to be more resistant to the action of eugenol than the other organisms. Eugenol alone proved highly inhibitory to *E. coli* (Blaszyk & Holley, 1998). With the increasing concentration of eugenol, the growth of bacteria declined. However, the lowest growth rate of *S. aureus* was 11%, achieved at 2000 µg/mL. Also, growth rates of other bacteria, including *K. pneumoniae*, *P aeruginosa*, and *E. coli* at that point, were 12, 30, and 33%, respectively (Moemenbellah-Fard *et al.,* 2020).

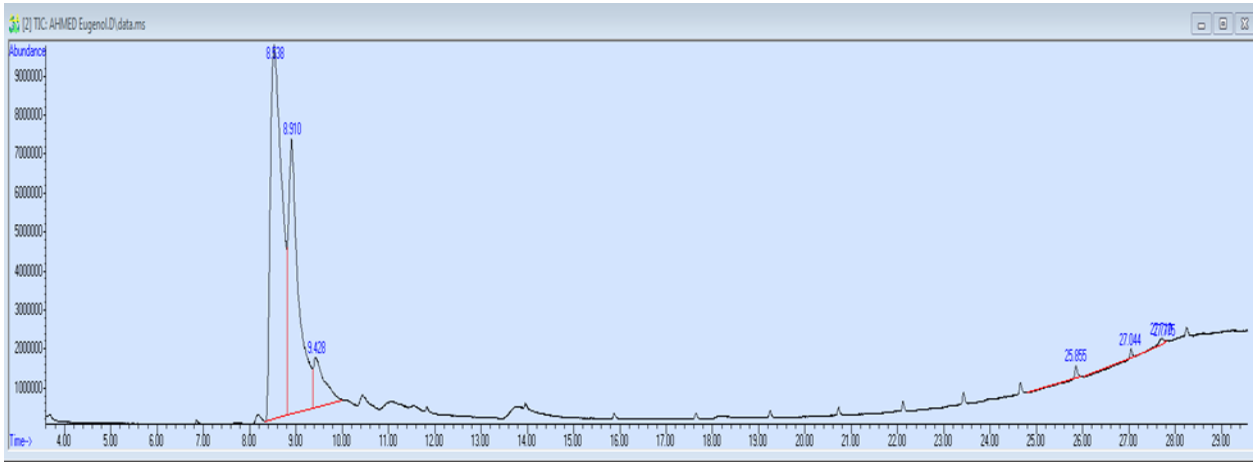


Figure (11). GC-MS for, Cloves flower buds.

Table (3-14). The active substances in the Cloves plant by GC-MS.

No.	Compound name	R. T. min	Corr. area	cas	corr. Max%	of total%	formula
1	Eugenol	8.538	1661060927	0000097-53-0	100.0	55.868	$C_{10}H_{12}O_2$
2	Eugenol	8.910	1084067417	000097-53-0	65.26	36.462	$C_{10}H_{12}O_2$
3	Phenol, 2-methoxy-3-(2-propenyl)	9.428	209258447	001941-12-4	12.60	7.038	$C_{10}H_{12}O_2$
4	Hexasiloxane, tetradecamethyl	25.855	7660842	000107-52-8	0.46	0.258	$C_{14}H_{42}O_5Si_6$
5	Hexasiloxane, tetradecamethyl	27.043	424194	019095-23-9			$C_{14}H_{42}O_5Si_6$
6	Oleic Acid	27.710	10339729	000112-80-1	0.62	0.348	$C_{18}H_{34}O_2$
7	Oleic Acid	27.775	1209045	000112-80-1			$C_{18}H_{34}O_2$

3.6.3. *Citrullus colocynthis*

The study results revealed that *C. colocynthis* extract was not affected by the growth of *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus*. The extract solvent water (cold and hot) and alcohol (methanol and ethanol) were applied in different concentrations. Moreover, the resistance of bacteria to different antibiotics (Azteronam, Cefeime, Ceftazidime, Piperacillin, Rifampicin, Trimethorim, Vancomycin, and Tobramycin). When the plant extract was combined with the lowest concentration with the resistant antibiotics used in the study, it gave good inhibitory results due to the synergistic relationship between the extract and the antibiotics.

The natural extracts acted in synergy with synthetic drugs towards microbial species. The lowest concentration was 0.75 µg/ml of the alcoholic extract (methanol and ethanol), and the antibiotics used in the experiment gave outstanding results.

3.6.3.1. Methanol extract of *C. colocynthis* and antibiotics

The methanol alcoholic extract of *C. colocynthis* and antibiotics combined affected *E. coli*, isolated. Table (3-15) and Figure (12) show the effects of *C. colocynthis* extract and antibiotics on the growth of isolates. The inhibition zone of *E.coli* at concentrations 0.75 µg/ml and antibiotics (FEP, RA, TOB, PRL, TMP, CAZ, and ATM) were 24, 11, 15, 14, 12, 17, and 27 mm, respectively.

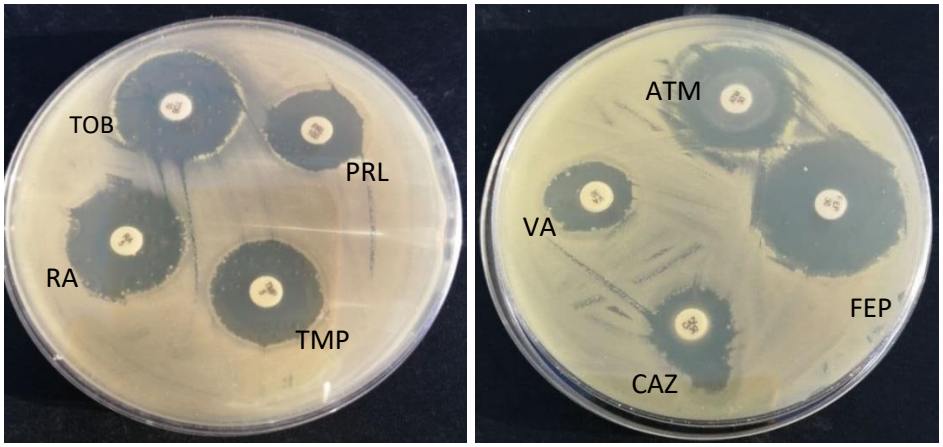
While the inhibition zone of *P. aeruginosa* at concentrations 0.75 µg/ml and antibiotics (FEP, RA, TOB, PRL, TMP, CAZ, and ATM) were 20, 19, 23, 18, 16, 16, and 24 mm, respectively.

As well as the inhibition zone of *S. aureus* at concentrations 0.75 µg/ml and antibiotics (FEP, RA, TOB, PRL, TMP, CAZ, and ATM) were 25, 20, 24, 21, 17, 16, and 20 mm, respectively. Moreover, the inhibition zone of *K. pneumonia* at concentrations 0.75 µg/ml and antibiotics FEP were 8 mm.

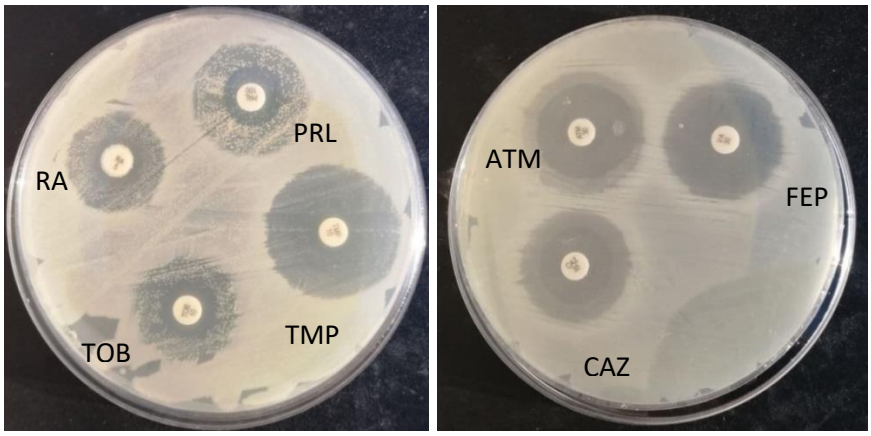
Table (3-15). The methanol *C. colocynthis* extract (0.75 µg/ml) and antibiotics that synergistic action on against pathogenic bacteria.

Bacteria isolate	Inhibition zone/mm							
	FEP	RA	TOB	PRL	TMP	VA	CAZ	ATM
P. aeruginosa	20	19	23	18	16	12	16	24
E. coli	24	11	15	14	12	24	17	15
S. aureus	18	20	---	---	21	18	21	27
K. pneumoniae	8	---	---	---	---	---	---	---

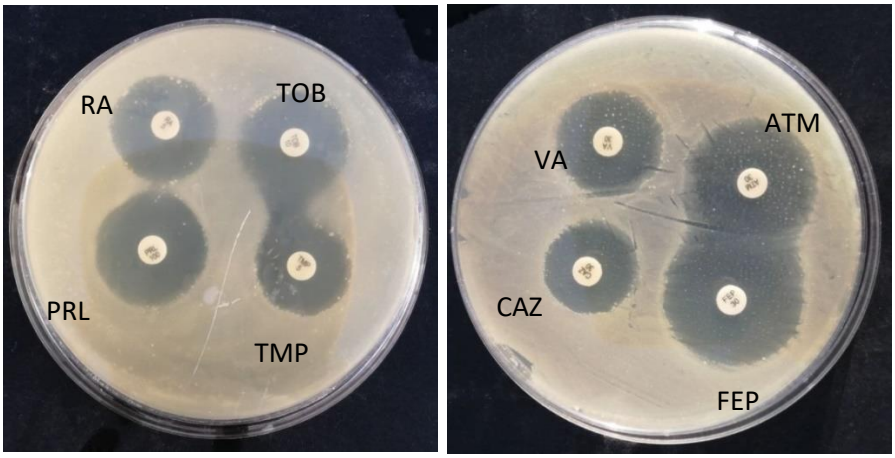
No inhibition (---), FEP: Cefeime, RA: Rifampicin, TOB: Tobramycin, PRL: Piperacillin, TMP: Trimethorim, VA: Vancomycin, CAZ: Ceftazidime, ATM: Azteronam.



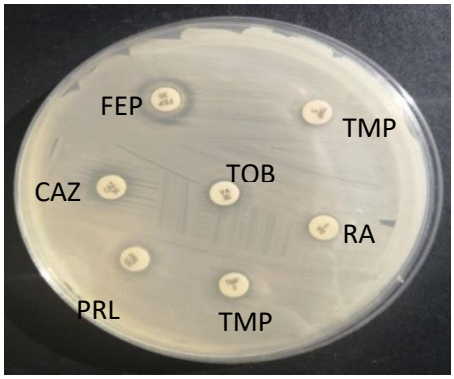
P. aeruginosa/ methanol / *C. colocynthis*



E. coli / methanol / *C. colocynthis*



S. aureus / methanol / *C. colocynthis*



K. pneumonia/ methanol / *C. colocynthis*

Figure (12). The effect of 0.75 µg/ml concentration of methanolic extract of *C. colocynthis* with antibiotics on: A: *K. pneumonia*, **B**: *S. aureus*, **C**: *P. aeruginosa*, **D**: *E. coli*

3.6.3.2. Ethanol extract of *C. colocynthis* and antibiotics

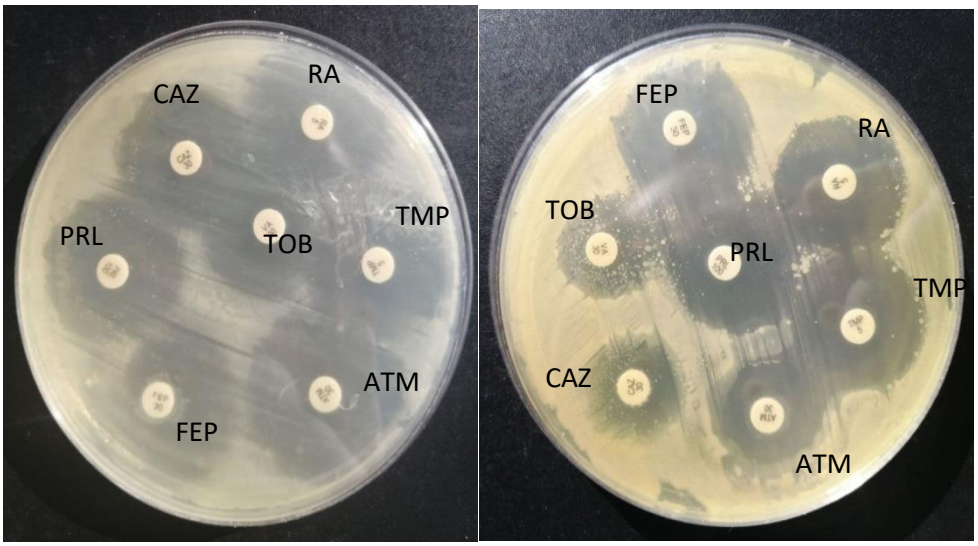
The ethanol alcoholic extract of *C. colocynthis* and antibiotics combined affected *E. coli*, isolated. Table (3-16) and Figure (13) show the effects of *C. colocynthis* extract and antibiotics on the growth of isolates. The inhibition zone of *E.coli* at concentrations 0.75 µg/ml and antibiotics (FEP, RA, TOB, PRL, TMP, CAZ, and ATM) were 25, 20, 16, 15, 24, 24, and 17 mm, respectively.

While the inhibition zone of *P. aeruginosa* at concentrations 0.75 µg/ml and antibiotics (FEP, RA, TOB, PRL, TMP, CAZ, and ATM) were 25, 20, 24, 21, 17, 16, and 20 mm, respectively, showed significant superiority at ($P \leq 0.05$) over the antibiotic. As well as the inhibition zone of *S. aureus* at concentrations 0.75 µg/ml and antibiotics (FEP, RA, TOB, PRL, TMP, CAZ, and ATM) were 25, 20, 24, 21, 17, 16, and 20 mm, respectively. Moreover, the inhibition zone of *K. pneumonia* at concentrations 0.75 µg/ml and antibiotics ATM were 10 mm.

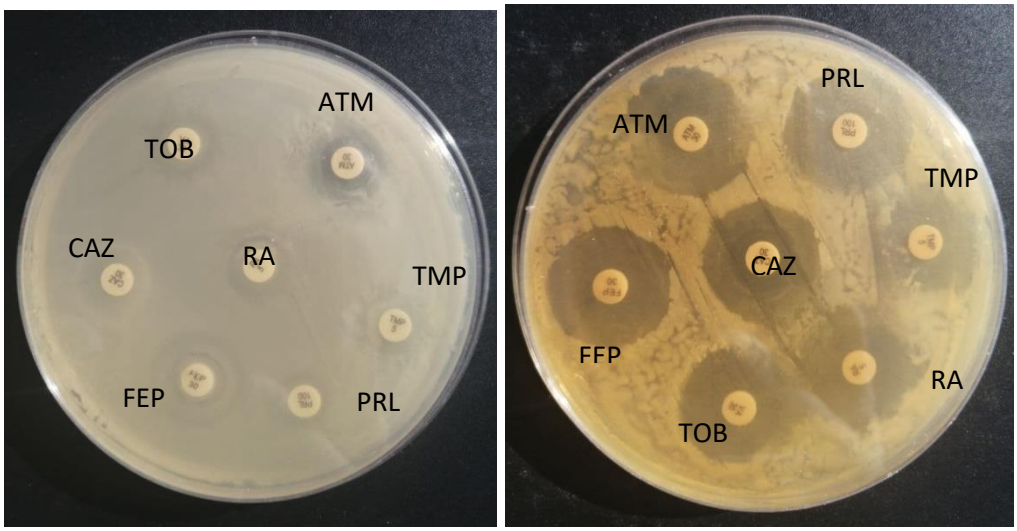
Table (3-16). The ethanol *C. colocynthis* extract (0.75 µg/ml) and antibiotics that synergistic action on against pathogenic bacteria.

Bacteria isolate	Inhibition zone/mm							
	FEP	RA	TOB	PRL	TMP	VA	CAZ	ATM
P. aeruginosa	25	20	24	21	17	12	16	20
E. coli	25	20	16	15	24	25	24	17
S. aureus	25	16	20	25	26	20	25	25
K. pneumoniae	---	---	---	---	---	---	---	10

No inhibition (---), FEP: Cefeime, RA: Rifampicin, TOB: Tobramycin, PRL: Piperacillin, TMP: Trimethorim, VA: Vancomycin, CAZ: Ceftazidime, ATM: Azteronam.



E. coli / ethanol / *C. colocynthis* *P. aeruginosa* / ethanol / *C. colocynthis*



K. pneumonia/ ethanol / *C. colocynthis* *S. aureus* / ethanol / *C. colocynthis*

Figure (13). The effect of 0.75 µg/ml concentration of ethanolic extract of *C. colocynthis* with antibiotics on: A: *K. pneumonia,* **B**: *S. aureus,* **C**: *P. aeruginosa,* **D**: *E. coli*

The active substances present in the *C. colocynthis* extract included concentrated Linoleoyl chloride (9,12-Octadecadienoyl chloride) than other active compounds of GC-MS analysis, Figure (14) (Table 3-17). The *C.*

colocynthis essential can inhibit *S. aureus*, *E. Coli*, *P. aeruginosa*, and *K. pneumonia*. However, its ethanol extract could only inhibit *S. aureus*, *E. Coli*, and *P. aeruginosa* (Doss *et al.*, 2011). Combinational therapy is based on the use of two or more medicines in the treatment of infections that have a beneficial, synergistic effect. Synergy occurs when the antibacterial activity of a mixture of two antimicrobial agents is greater than the sum of the separate components. The efflux pump inhibitors derived from plants will be used in combination therapy (Burt, 2004).

The ethanolic plant extracts of *R. coriaria*, *S. spinosum*, and *R. damascena* were combined with various antimicrobial agents (penicillin G, cephalexin, sulfadimethoxine as sodium, and enrofloxacin) against three test strains of P. aeruginosa using the microdilution method; the MIC was reduced. The means that the plant extracts boosted the drugs' antibacterial effectiveness against the P. aeruginosa test strains, indicating a synergistic interaction (Adwan *et al*., 2010). Souto de Oliveira *et al.,* (2011) tested the synergistic effect of norfloxacin, tetracycline, and erythromycin against S. aureus strains using an ethanol extract of Mangifera indica L. peel. Individual extracts did not have substantial antibacterial activity. However, they did regulate antibiotic activity, resulting in a four-fold reduction in the MIC values for tetracycline and erythromycin when used in combination with antibiotics. According to the findings, mango peel could be used as an antibiotic adjuvant, increasing the value of this mango by-product (Oliveira *et al*., 2011).

Toroglu(2011) investigated in-vitro synergistic effects of different spices and herbs (*Rosmarinus officinalis, Coriandrum sativum*, *Micromeria fruticosa L*., *Mentha piperita*) with gentamicin, cephalothin, ceftriaxone, and nystatin against 13 microbial species. The combination of plant extracts with antibiotics

further reduced drug resistance due to synergistic effects that led to new treatments for infectious diseases (Toroglu, 2011). Exploring phytochemicals that can interfere with bacterial efflux pumps is one exciting strategy. However, the evidence presented in this review suggests that plant extracts and essential oils with antibiotic resistance-modifying action could be employed as adjuvants to antibiotic therapy in the treatment of multidrug-resistant staphylococci (Mikuláová *et al*., 2016). Because the administration of single agents is substantially connected with the establishment of resistance, medication combinations rather than single agents produce better therapeutic outcomes. Due to the complexity of essential oils, no specific bacterial resistance or adaptation has been identified, and secondary effects have yet to be established. A number of studies have recently demonstrated synergistic or additive interactions between plant extract and antibiotics with various resistance mechanisms (reviewed by Kon and Rai 2012; Langeveld et al. 2013).

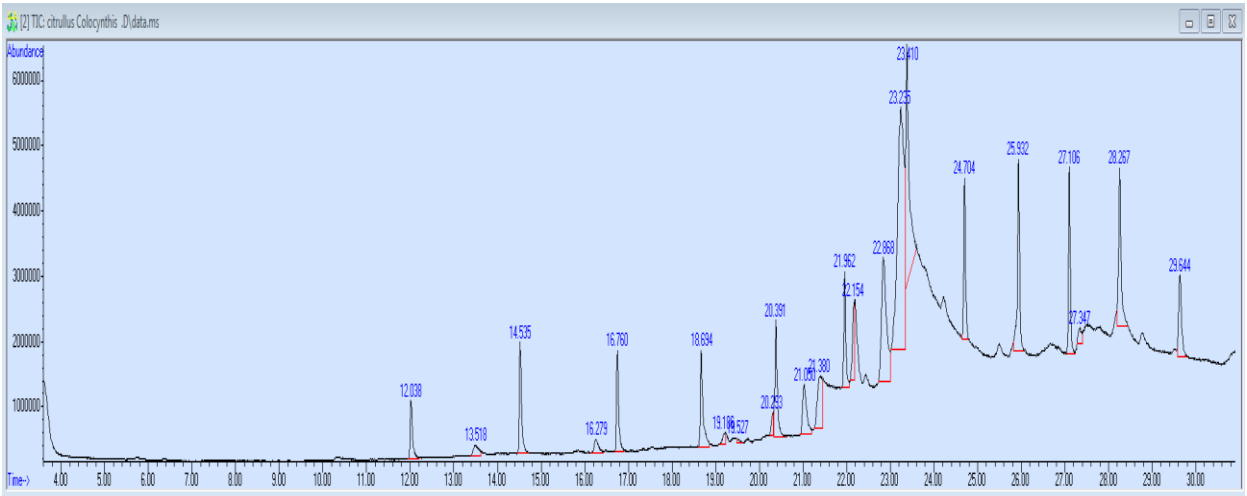


Figure (14). GC-MS analysis *Citrullus colocynthis* flower

Table (3-17).The active substances in the *Citrullus colocynthis* plant by GC-MS.

No.	Compound name	R. T. min	Corr. area	cas	corr. Max%	of total%	
1	Cyclohexasiloxane, dodecamethyl-	12.038	3413955	000540-97-6	8.73	2.002	$C_{12}H_{36}O_6Si_6$
2	Tridecane	13.518	1403596	000629-50-5	3.59	0.823	$C_{13}H_{28}$
3	Cycloheptasiloxane, tetradecamethy	14.535	6218925	000107-50-6	15.91	3.647	$C_{14}H_{42}O_7Si_7$
4	Tetratetracontane			007098-22-8			$C_{44}H_{90}$
5	Cyclooctasiloxane, hexadecamethyl	16.760	5421154	000556-68-3	13.87	3.179	$C_{16}H_{48}O_8Si_8$
6	Cyclononasiloxane, octadecamethyl	18.694	6121685	000556-71-8	15.66	3.590	$C_{18}H_{54}O_9Si_9$
4	Cyclodecasiloxane, eicosamethyl	20.391	6628857	018772-36-6	16.96	3.888	$C_{20}H_{60}O_{10}Si_{10}$
5	Hexadecanoic acid, ethyl ester	21.050	5029449	000628-97-7	12.87	2.950	$C_{18}H_{36}O_2$
6	n-Hexadecanoic acid	21.380	6088166	000057-10-3	15.57	3.571	$C_{16}H_{32}O_2$
7	Cyclooctasiloxane, hexadecamethyl	21.962	5388996	000556-68-3	13.79	3.161	$C_{16}H_{48}O_8Si_8$
8	9,12-Octadecadienoic acid, methyl ester	22.154	4204109	002462-85-3	10.75	2.466	$C_{19}H_{34}O_2$
9	13-Octadecenal, (Z)-	22.868	15640641	058594-45-9	40.01	9.173	$C_{18}H_{34}O$
10	9,12-	23.235	3909175	000060-	100.0	22.93	$C_{18}H_{32}O_2$

	Octadecadienoic acid (Z,Z)-		6	33-3			
11	1,1,1,5,7,7,7 Heptamethyl-3,3-bis(trimethylsiloxy) tetrasiloxane	23.410	19412733	038147-00-1	49.66	11.39	$C_{13}H_{39}O_5Si_6$
12	Cyclononasiloxane, octadecamethyl-	24.704	7266236	000556-71-8	18.59	4.262	$C_{18}H_{54}O_9Si_9$
13	Cyclononasiloxane, octadecamethyl-	25.932	10431260	000556-71-8	26.68	6.118	$C_{18}H_{54}O_9Si_9$
14	Cyclononasiloxane, octadecamethyl-	27.106	5910308	000556-71-8	20.89	4.788	$C_{18}H_{54}O_9Si_9$
15	1,1,1,5,7,7,7-Heptamethyl-3,3-bis trimethylsiloxy)tetra siloxane	28.267	10013374	038147-00-1	25.62	5.873	$C_{13}H_{39}O_5Si_6$
16	Hexasiloxane, tetradecamethyl	29.644	5910308	000107-52-8	15.12	3.466	$C_{14}H_{42}O_5Si_6$

Appendix (1) Diagnostic by Vitek 2

The following steps were carried out to identify bacteria isolation :

1. All the following steps were prepared according to the manufacturer's instructions. Three ml of normal saline are placed in the plane test tube and inoculated with a loop full of the isolated colony.

2. The colony must be aged 24 h, the test tube inserted into a dens check machine for standardization of colony to McFarland's standard solution (1.5x10*cell/ml).

3. The standardized inoculums were placed into the cassette and a sample identification number entered into the computer software via barcode. The VITEK 2 card thus connected to the sample ID number.

4. The cassette is placed in the filler module, when the cards are filled, transferred the cassette to the reader incubator module.

5. All following steps handled by the instrument, the instrument controls the incubation temperature, the optical reading of the cards and continually monitors and transfers test data to the computer for analysis.

Appendix (2) Gas Chromatography – Mass Spectrometry (GC– MS).

The operating conditions of the device were as follows:

1. Separation column type HP-5ms ultra Inert By dimensions (30m× 250µm×0.25µm) which compound of which works in Electron Effect Mode 70-EV (Electron fixed Detector).
2. At a continuous flow rate of 1.2ml/min, helium gas (99.9%) was used as a carrier gas.
3. The volume of injected fluid is 1µ with a split ratio of 1.22 min.
4. Injector temperature is 250 Cº.
5. The temperature of the oven when starting is 80C°, then it increases by 8 degrees per minute to reach 180 C° during a period of 12.5 min, then it continues to increase to 280 C° at a rate of 8 degrees per minute for 25 min, then the temperature increases by 3 degrees per minute to reach a temperature of 310 C°.
6. The device pressure is 11.93 psi with an average of 1.2 ml/min.
7. The total time taken for sample analysis was 31 min.

Appendix (3) Diagnostic of bacterial isolate by Vitek 2

bioMérieux Customer:
System #:

Laboratory Report

Printed Dec 21, 2020 19:10 CST
Printed by: Labadmin

Patient Name:
Isolate: 1412209-1 (Qualified)

Patient ID:

Card Type: GN Bar Code: 2411221103408752 Testing Instrument: 00000A726B5A (AL-NUKHBA LAB)
Setup Technologist: Laboratory Administrator(Labadmin)

Bionumber: 6605610570566600
Organism Quantity:

Selected Organism: Escherichia coli

Comments:	

Identification Information	**Card:**	GN	**Lot Number:**	2411221103	**Expires:**	Mar 31, 2021 13:00 CDT
	Completed:	Dec 14, 2020 17:19 CST	**Status:**	Final	**Analysis Time:**	4.83 hours
Organism Origin	VITEK 2					
Selected Organism	86% Probability		Escherichia coli			
	Bionumber:	6605610570566600			**Confidence:**	Acceptable identification
SRF Organism						
Analysis Organisms and Tests to Separate:						
Analysis Messages:						
Contraindicating Typical Biopattern(s)						
Escherichia coli	ADO(9),PyrA(1),dTAG(22),dCEL(1),					

Biochemical Details

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

Installed VITEK 2 Systems Version: 08.01
MIC Interpretation Guideline:
AES Parameter Set Name:

Therapeutic Interpretation Guideline:
AES Parameter Last Modified:

bioMérieux Customer:
System #:

Laboratory Report

Printed Dec 21, 2020 18:52 CST
Printed by: Labadmin

Patient Name:
Isolate: 1412209-1 (Qualified Duplicate)

Patient ID:

Card Type: GN Bar Code: 2411151203611141 Testing Instrument: 00000A726B5A (AL-NUKHBA LAB)
Card Type: AST-N222 Bar Code: 6221345203511701 Testing Instrument: 00000A726B5A (AL-NUKHBA LAB)
Setup Technologist: Laboratory Administrator(Labadmin)

Bionumber: 0405411550506611
Organism Quantity:

Selected Organism: Escherichia coli

Susceptibility Information	Card: AST-N222		Lot Number: 6221345203	Expires: Aug 2, 2021 13:00 CDT	
	Completed: Dec 13, 2020 20:59 CST		Status: Final	Analysis Time: 11.55 hours	
Antimicrobial	**MIC**	**Interpretation**	**Antimicrobial**	**MIC**	**Interpretation**
Ticarcillin	>= 128	R	Amikacin	>= 64	R
Ticarcillin/Clavulanic Acid	>= 128	R	Gentamicin	>= 16	R
Piperacillin	>= 128	R	Tobramycin	>= 16	R
Piperacillin/Tazobactam	>= 128	R	Ciprofloxacin	>= 4	R
Ceftazidime	>= 64	R	Pefloxacin		
Cefepime	>= 64	R	Minocycline	2	S
Aztreonam	>= 64	R	Colistin		
Imipenem	TRM		Rifampicin		
Meropenem	<= 0.25	S	Trimethoprim/Sulfamethoxazole	>= 320	R

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

AES Findings:	Last Modified: Nov 12, 2019 15:13 CST	Parameter Set: Global CLSI-based+Natural Resistance
Confidence Level:	Consistent	

Installed VITEK 2 Systems Version: 08.01
MIC Interpretation Guideline: Global CLSI-based
Therapeutic Interpretation Guideline: NATURAL RESISTANCE
AES Parameter Set Name: Global CLSI-based+Natural Resistance
AES Parameter Last Modified: Nov 12, 2019 15:13 CST

bioMérieux Customer:
System #:

Laboratory Report

Printed Nov 15, 2020 18:49 CST
Printed by: Labadmin

Patient Name:
Isolate: 4112020-1 (Qualified)

Patient ID:

Card Type: GN Bar Code: 2411143203512955 Testing Instrument: 00000A726B5A (AL-NUKHBA LAB)
Setup Technologist: Laboratory Administrator(Labadmin)

Bionumber: 6607734773564010
Organism Quantity: Selected Organism: **Klebsiella pneumoniae ssp pneumoniae**

Comments:	

Identification Information	Card: GN	Lot Number: 2411143203	Expires: Jan 12, 2021 12:00 CST
	Completed: Nov 4, 2020 14:30 CST	Status: Final	Analysis Time: 4.00 hours
Organism Origin	VITEK 2		
Selected Organism	99% Probability **Klebsiella pneumoniae ssp pneumoniae**		
	Bionumber: 6607734773564010		Confidence: Excellent identification
SRF Organism			
Analysis Organisms and Tests to Separate:			
Analysis Messages:			
Contraindicating Typical Biopattern(s)			

Biochemical Details

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

Installed VITEK 2 Systems Vers
MIC Interpretation Guideline:
AES Parameter Set Name:

Therapeutic Interpretation Guideline:
AES Parameter Last Modified:

bioMérieux Customer:
System #:

Laboratory Report

Printed Nov 7, 2020 19:28 CS
Printed by: Labadmin

Patient Name:
Patient ID:
Isolate: 5112020-1 (Qualified)

Card Type: AST-N222 Bar Code: 6221345203507976 Testing Instrument: 00000A726B5A (AL-NUKHBA LAB)
Setup Technologist: Laboratory Administrator(Labadmin)

Organism Quantity: **Selected Organism: Klebsiella pneumoniae**

Comments:	

Identification Information			
Organism Origin	Technologist		
Selected Organism	Klebsiella pneumoniae		
	Entered: Nov 6, 2020 13:03 CST	**By:**	Labadmin

Analysis Messages:
The following antibiotic(s) are not claimed:
Rifampicin,

Susceptibility Information	**Card:** AST-N222		**Lot Number:** 6221345203	**Expires:** Aug 2, 2021 13:00 CDT	
	Completed: Nov 6, 2020 01:50 CST		**Status:** Final	**Analysis Time:** 8.53 hours	
Antimicrobial	**MIC**	**Interpretation**	**Antimicrobial**	**MIC**	**Interpretation**
Ticarcillin	>= 128	R	Amikacin	>= 64	R
Ticarcillin/Clavulanic Acid	>= 128	R	Gentamicin	>= 16	R
Piperacillin	>= 128	R	Tobramycin	>= 16	R
Piperacillin/Tazobactam	>= 128	R	Ciprofloxacin	>= 4	R
Ceftazidime	>= 64	R	Pefloxacin		
Cefepime	>= 64	R	Minocycline	>= 16	R
Aztreonam	>= 64	R	Colistin		
Imipenem	>= 16	R	Rifampicin		
Meropenem	>= 16	R	Trimethoprim/Sulfamethoxazole	>= 320	R

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

AES Findings:	**Last Modified:** Nov 12, 2019 15:13 CST	**Parameter Set:** Global CLSI-based+Natural Resistance
Confidence Level:	Consistent	

Installed VITEK 2 Systems Version: 08.01
MIC Interpretation Guideline: Global CLSI-based
Therapeutic Interpretation Guideline: NATURAL RESISTANCE
AES Parameter Set Name: Global CLSI-based+Natural Resistance
AES Parameter Last Modified: Nov 12, 2019 15:13 CST

bioMérieux Customer:

Microbiology Chart Report

Printed Feb 22, 2020 09:34 CST

Patient Name:
Location:
Lab ID: 21221-4

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus aureus

Source:

Collected:

Comments:	

Identification Information	**Analysis Time:** 4.85 hours	**Status:** Final
Selected Organism	**Staphylococcus aureus**	
ID Analysis Messages		

Susceptibility Information	**Analysis Time:** 10.45 hours			**Status:** Final	
Antimicrobial	**MIC**	**Interpretation**	**Antimicrobial**	**MIC**	**Interpretation**
Cefoxitin Screen	POS	+	Teicoplanin	>= 32	R
Benzylpenicillin	>= 0.5	R	Vancomycin	>= 32	R
Oxacillin	>= 4	R	Tetracycline	>= 16	R
Gentamicin	>= 16	R	Tigecycline	0.5	S
Tobramycin	8	I	Fosfomycin		
Levofloxacin	>= 8	R	Nitrofurantoin	>= 512*	R
Moxifloxacin	>= 8	R	Fusidic Acid	>= 32	R
Inducible Clindamycin Resistance	NEG	-	Mupirocin		
Erythromycin	>= 8	R	Rifampicin	>= 32	R
Clindamycin	>= 8	R	Trimethoprim/Sulfamethoxazole	20	S

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

AES Findings		
Confidence:	Consistent with correction	
Phenotypes flagged for review:	GLYCOPEPTIDES	VRSA
	BETA-LACTAMS	MODIFICATION OF PBP (mecA)
	OXAZOLIDINONE	RESISTANT
	MACROLIDES/LINCOSAMIDES/STREPTOGRAMINS	MLSB+SA CONSTITUTIVE

bioMérieux Customer:

Microbiology Chart Report

Printed Feb 22, 2020 09:02 CST

Patient Name:
Location:
Lab ID: 21221-1

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Pseudomonas aeruginosa

Source:

Collected:

Comments:	

Identification Information	**Analysis Time:**	7.82 hours	**Status:**	Final
Selected Organism	95% Probability	**Pseudomonas aeruginosa**		
	Bionumber:	0003043103500042		
ID Analysis Messages				

Susceptibility Information	**Analysis Time:** 14.90 hours			**Status:** Final	
Antimicrobial	**MIC**	**Interpretation**	**Antimicrobial**	**MIC**	**Interpretation**
ESBL			Imipenem	>= 16	R
Ampicillin			Amikacin	>= 64	R
Cefazolin	>= 64	R	Gentamicin	>= 16	R
Cefoxitin			Ciprofloxacin	>= 4	R
Ceftazidime	8	S	Levofloxacin	>= 8	R
Ceftriaxone			Tigecycline	>= 8	R
Cefepime	>= 64	R	Nitrofurantoin		
Ertapenem			Trimethoprim/Sulfamethoxazole		

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

AES Findings		
Confidence:	Consistent	
Phenotypes flagged for review:	BETA-LACTAMS	CARBAPENEMASE

Conclusions

1. Widely distribution of UTI among female than male.
2. *E.coli* represented the main causes of UTI.
3. Widely distribution of MDR among bacterial isolates.
4. The irrational and indiscriminate use of these drugs has undoubtedly led to the appearance of multi-resistant isolates. This phenomenon has multiple implications, the most important of which is failure to treat the disease by exhausting the therapeutic options.
5. Describe the patterns by plasmid curing in AO and SDS on bacteria isolated from UTI samples for their appropriate management.
6. Study of medicinal plants alcoholic extracts that selected with GC-MS showed to the plant study *P. emblica*, *S. aromaticum*, *C. colocynthis*.
7. The studded plant aqueous extract has inactive against bacteria.
8. The synergistic relationship between alcoholic *C. colocynthis* extract at 0.75 µg/ml and antibiotics FEP, RA, TOB, PRL, TMP, CAZ, VA, and ATM.

Recommendation

1. Study the possibility of using *P. emblica* as a natural anti-bacterial agent.
2. Study synergistic relationship between the plant extract and antibiotics.
3. The plant extracts should be subjected to a series of pharmacological tests to ascertain there in vivo efficacy, cytotoxicity, interactions and any harmful side effects.
4. Conducting use of these extracts of plants against the other resistance pathogenic bacteria.

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Buckner, M. M., Ciusa, M. L., & Piddock, L. J. (2018). Strategies to combat antimicrobial resistance: anti-plasmid and plasmid curing. *FEMS microbiology reviews*, *42*(6), 781-804.

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