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**قابلية التأثر لبكتريا القولون البرازية المعزوله من المرضى المصابين بألتهابات المجاري البولية للعاثيات البكترية**

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# CHAPTER ONE

# INTRODUCTION AND LITERATURE REVIEW

# CHAPTER TWO

# MATERIALS AND METHODS

# CHAPTER THREE

# RESULTS AND DISCUSSION

# CHAPTER FOUR

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# Committee certification

We examining committee, certify that we have read this thesis and examined the student in its contents and that according to our opinion is accepted as a thesis for the degree of Master of Science in Biotechnology.

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## 4.1 conclusions:

1- Gram negative bacteria (81.11%) are common causative agent of UTI, whereas Gram positive bacteria (18.89%) rank in the second place.

2- *E. coli* was found being the common causative agent of UTI in comparison with other Gram negative bacterium, and their incidence rate was higher in female than male.

3- Bacteriophages specific to *E. coli* were isolated from sewage water and it was possible to group them in respect of their infectivity of *E. coli* isolates.

4- Therapeutic effect of bacteriophage of skin's wound of experimental mice infected with *E. coli* might encourage research to provide an alternative method for treatment of some bacterial infections.

## 4.2 Recommendation:

1- Further studies are needed for isolation and identification of bacteriophages specific to various bacterial species.
2- Phage culture bank is required to maintain the collection of all types of bacteriophages to help the researchers for future studies.
3- Research studies should be directed to ascertain bacteriophage lysing effect on antibiotic resistant bacteria.
4- Studies are required to ascertain the phage therapy of bacterial infections in comparison with antibiotic treatment.

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# List of Abbreviations

| Abbreviations | Words |
|---|---|
| API | Analytical Profile Index |
| CNF | Cytotoxic Necrotizing Factor |
| CLDT | Cytolethal Distending Toxin |
| DNA | Deoxyribonucleic acid |
| EAEC | Enteroaggregative *E. coli* |
| *E. coli* | *Escherichia coli* |
| EHEC | Enterohaemorrhagic *E .coli* |
| EIEC | Enteroivassive *E. coli* |
| EPEC | Enteropathogenic *E. coli* |
| ETEC | Enterotoxigenic *E. coli* |
| GIT | Gastrointesinal Tract |
| gm | Gram |
| LPS | Lipopolysaccharides |

| | |
|---|---|
| LT | Heat labile toxin |
| ml | Millilitter |
| mg | Milligram |
| PFU | Particle Forming Unit |
| RNA | Ribonucleic acid |
| Rn | Phage number |
| RPM | Rotation Per Minutes |
| SIDS | Sudden Infant Death Syndromes |
| ST | Heat stable toxin |
| STx | Shiga Toxin |
| UPEC | Uropathogenic *E. coli* |
| UTI | Urinary Tract Infection |
| U.V | Ultra violet light |

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## 1.1 Introduction:-

Urinary tract infection (UTI) is an infection involving part or all of the urinary tract which remains a common and troublesome health problem in many different countries all rounds the world (Usien *et al*., 2001).

In most hospitals a very large numbers of UTI were recorded daily among different ages and sexes, these infections may involve the urethra , bladder , ureters , the kidney and prostates, the extent of infection depends on the interaction between the bacteria and host's defense mechanism, an infection can occur anywhere along this tract, but the lower parts of the urethra and bladder are most commonly involved, this is called cystitis, if the infection travels up the ureters to the kidney it is called pyelonephritis (Nicolle, 2001; Josip, 2006).

The urinary tract infections remain a major clinical problem and are considered the most common infectious disease affecting the humans throughout their life span, and occur in all population, from neonate to geriatric patients (Tolkoff-Rubin *et al*., 2004).

The urinary tract infections have received a great attention from the researchers and workers in the clinical field, and considered one of the problems which becomes at the second stage after the respiratory tract infections. The main clinical concerns come from the increasing of death rates and from the great damage which cause the renal failure, therefore nowadays scientists are directing their investigations toward identification of agents which cause the infections (Neu, 1992).

The most important causes of UTI are represented by Gram negative bacteria belong to family *Enterobacteriacae* [this family is classified as part of Group5, which includes aerobic and facultative anaerobic Gram negative bacilli] especially *Escherichia coli* (Holt *et al*., 1994).

*Escherichia coli* were discovered in 1885 by Theodor Escherich a German bacteriologist (Mandal *et al*., 2001; Stamm, 2002).

Although *E.coli* is a major component of the normal intestinal flora, but it is recognized as very active opportunistic pathogen associated with UTI (Blanco *et al*., 1996). *E. coli* has several virulence factors which contribute to its pathogenicity like: adherence, O-antigen, capsule-K-antigen, aerobactin, hemolycin, endotoxin lipopolysaccharides and others (Pass *et al*., 2000; Ruiz *et al*., 2002).

Bacterial viruses (bacteriophages), also called "phages", the name came from Greek word 'phagein' meaning 'eat', bacteriophages are viral parasites of bacteria and are considered the simplest self-replicating system that contain an outer shell (Boyd, 2005). Phages can be robust antibacterial agents *in vitro,* however, trials for their possible use as therapeutic agents were carried out during 1920s to the 1950s (Ackermann and Du-Bow, 1987).

In recent years, well-controlled animal models have demonstrated that phages can rescue animals from a variety of fatal infections, while non-controlled clinical reports published in Eastern Europe have shown that phages can be effective in treating drug-resistant infections in humans, these encouraging data, combined with the fact that drug-resistant bacteria have become a global crisis, have created a window of opportunity for possible phage therapy to be ascertained , this time using modern technologies and placebo-controlled designs (Alisky *et al*., 1998).

Prior to the discovery and widespread use of antibiotics, it was suggested that bacterial infections could be prevented and/or treated by the administration of bacteriophages (Slopek *et al*., 1985).

Phages are considered to be very safe for therapeutic use, so far only a very few side-effects have been reported in the patients undergoing phage

therapy; it had been suggested that the observed side effects might be associated directly with the therapeutic process (Hanlon, 2007).

The phage or virion is a complete viral particle which consists of a nucleic acid core that is made up, depending on the phage, of DNA or, less often, of RNA. Surrounding this nucleic-acid genome is a protein called capsid, and constituting the infective form of a virus. The phage life cycle consists of an extra cellular search for attachment to susceptible bacteria, phage-genome uptake into bacteria, production of phage progeny, and subsequent release of their progeny into the extra cellular environment (Adams, 1969; Ackermann, 2003).

It is worth to mention that bacteriophages exhibit considerable diversity in the nature in respect of their nucleic acid contents, and different modes of replication, fundamentally different propagation strategies are exhibited by lytic and temperate phages, lytic phages produce many copies of themselves in a single burst of growth, whereas temperate phages establish themselves as prophages either by becoming part of an established host's replicon or by forming an independent replicon (Jawetz *et al*., 1998).

## Aims of the study:-

1- Isolation and identification of *E.coli* from patients with urinary tract infections.

2- Isolation of phages from natural sources (sewage).

3- Study the bacteriophages Susceptibility of *E. coli*.

4- Study the possible therapeutic effect of bacteriophage in treatment of infected mice skin wound with *E. coli*.

## 1.2 Literature review

### 1.2.1 Discovery of Urinary Tract Infection

The clinical description and treatment of UTIs were noticed and recorded many centuries before the discovery of bacteria and perhaps the earliest description of UTI was presented by physician Abu Bakr Al-Rhazi in ninth century AD (Warren, 1997). In Britain, however, the symptoms of UTI were not defined until some 500 years later; John of Arderne (1412) spoke of symptoms such as pain in the kidneys. Culpepper (1653) wrote about the treatment of UTI and also observed the distinction between upper and lower tract symptoms. Thereafter, Pasteur (1863) observed that human urine was a good culture medium. Roberts (1881) demonstrated the relationship between the finding of bacteria in urine and the development of cystitis, and also described the appearance of rod-shaped bacteria in urine (Van Nostrand *et al*., 2000).

Escherich (1894), who had found the organism *Escherichia coli* in the fecal flora, also identified the same organism in the urine of children with cystitis (Svanborg and Godaly, 1997).

### 1.2.2 Definition of Urinary Tract Infection

Urinary tract infection could be defined as presence of more than $10^5$ cells/ml bacteria as determined in properly collected specimen of urine (Sleigh and Timbury, 1994).

Infections of the urinary tract are the second most common type of infection in the body, after the flu and common cold (Forbes *et al.,* 1991). UTI bacterial infection occur in both male and female however, it is

particularly common in females, (10 – 20%) of women have UTI at some time in their life and significant number have recurrent infection (Mims *et al*., 1987).

Urinary tract infections are the most common medical complaint among women in their reproductive years, women are 30 times more likely to have cystitis than men are; every year, about one in nine American women have at least one UTI, and up to 60% of all women will develop a urinary tract infection at some time in their lives, a third of these women will have a recurrence within a year, furthermore, each year about 250,000 women develop kidney infections (pyelonephritis) and 100,000 are hospitalized for treatment (Stapleton and Stamm, 1997).

Urinary tract infections in men are not as common as in women but can be very serious when they do occur, women tend to have urinary tract infections more often than men because bacteria can reach the bladder more easily in women, the urethra is shorter in women than in men, which is 1.5 inches compared to 8 inches in men, so bacteria have a shorter distance to travel, the urethra is also located near the rectum in women, bacteria from the rectum can easily travel up the urethra and cause infections (Asscher, 1980).

Sexual activity increase the risk of UTI in women because bacteria can be pushed into the urethra, using a diaphragm can lead to infections because diaphragms push against the urethra and make it harder to completely empty the bladder, the urine that stays in the bladder is more likely to grow bacteria and cause infections (Roberts, 1999).

Uncircumcised boys younger than one year old also have a slightly higher risk of developing a UTI, other risk factors that increase a child's chance of developing a UTI include:

An abnormality in the structure or function of the urinary tract, for example, a malformed kidney or blockages somewhere along the tract of normal urine flow.

An abnormal backward flow (reflux) of urine from the bladder up the ureters and toward the kidneys, this condition, known as vesicoureteral reflux (VUR), is present at birth, and about 30% to 50% of children with a UTI are found to have it (Cunha, 1996).

A common source of infection is catheters, or tubes, placed in the urethra and bladder, a person who cannot void or who is unconscious or critically ill often needs a catheter that stays in place for a long time, some people, especially the elderly or those with nervous system disorders who lose bladder control, may need a catheter for life, bacteria on the catheter can infect the bladder (Warren, 1987).

The symptoms of UTI are frequent urination, flank pain, dysuria, burning with urination and some time fever (Walter and Norrby, 2001).

## 1.2.3 Classification of UTI

Different classifications have been devised to help choose the treatments and determine the causes of UTIs.

## A- Primary or Recurrent:

UTIs are classified as primary or recurrent, depending on whether they are the first infection or whether they are repeat events, Community- or Hospital-Acquired. UTIs are also sometimes grouped according to where they are acquired:

1. Community-Acquired Infections. Most UTIs are thought to develop in the community at large. It is unclear how primary community-acquired infections occur or how they are spread.
2. Hospital-Acquired Infections. UTIs are also commonly acquired in the hospital, often due to contaminated urinary catheters (Fisk *et al*., 1999).

## B- Uncomplicated and Complicated:

UTIs are also sometimes further defined as either being uncomplicated or complicated depending on the factors that trigger the infections (Forbes *et al*., 1991).

1. Uncomplicated infections are only associated with bacterial infection, most often (*E. coli*) and these include:

a. Cystitis: is the most common urinary tract infection and is sometimes referred to as acute uncomplicated UTI. It occurs in the lower urinary tract (the bladder and urethra).

b. Pyelonephritis (Kidney Infection): When infection spreads to the upper tract (the ureters and kidneys) it is called pyelonephritis.

c. Urethritis: When infection is limited only to the urethra, the infection is known as urethritis. This is a most common sexually transmitted disease in men (Macleode and Edwards, 1995).

2. Complicated infections, which occur nearly as often in men as in women, are also caused by bacteria but they occur as a result of some anatomical or structural abnormality (Elgavish and Pattanik, 1993; Lennatte *et al*., 1985).

### C- Classifications Based on Symptoms and Levels of Infection:

UTIs can also occur without symptoms and with symptoms but with very low bacterial levels.

1. When bacteria are present and there are no symptoms it is called asymptomatic UTI or also bacteriuria.

2. Some patients can also have symptoms of infection with very low bacterial counts, in such cases, the condition is called acute urethral syndrome (Zhanel *et al.*, 1990).

## 1.2.4 Routes of UTI

Many routes have been described by which the bacteria are causing UTI which could be summarized as follows:

**1- Ascending route:**

It is most important mean by which the urinary tract becomes infected (Santoro and Kaye, 1978).

It includes ascend of infectious agents from the external (genital and perineal) to the urinary tract and cause infection (Orga and Faden, 1985). Ascending infection is most common cause of UTI in women than in men (Tannagh and MacAninh, 1995).

The short urethra of women favors the ascend of bacteria from urethra to the bladder, however the antibacterial properties of prostatic fluid may also account for increase resistance of UTI observed in men (Stamy *et al*., 1971; Glauser, 1986).

**2- Haematogenous route:**

It is common route, and includes the transfer of bacteria from circulating blood which contains bacteria to urine through the kidney (O'Gradyfwl, 1980).

**3- Lymphatic route**:

Infection of urinary tract by means of lymphatic channels probably occurs, but is rare, in this pathway bacterial pathogens may travel through the rectal and colonic lymphatic to the prostate bladder, and through the preuterine lmphatics to the female urogenital tract (Meares, 1984; Sobel *et al*., 1993).

## 1.2.5 Etiologic agents

Bacteria are most common cause of urinary tract infection, organism invading the urinary tract range from Gram positive cocci to Gram negative bacilli (table 1.1) in this respect, Gram negative bacilli are considered the most common bacteria incriminated in UTI (Chkraborty, 1996). And the *Enterobacteriaceae* are considered as the major causing organism of UTI

which are originating in the gut before entering the urethra (Stamy *et al*., 1971).

Tabel (1-1) the common microorganisms which are associated with UTI

| Group | Organism | References |
|---|---|---|
| **Gram negative bacteria** | *E. coli* | Howard, 2004. |
| | *Proteus mirabilis* | Mobley and belas, 1995 |
| | *Klebsiella spp* | Mims *et al.*, 1987 |
| | *Enterobacter spp* | Glauser, 1986 |
| | *Serratia marsecens* | Mims *et al.*, 1987 |
| | *Salmonella spp* | Mims *et al.*, 1987 |
| | *Pseudomonas aureginosa* | Maskell,1988 |
| | *Heamophilus infleunzae* | Navarro *et al*., 1994 |
| | *Brucella spp* | Terai *et al.,* 1994 |
| | *Nisseria gonorrhoea* | Navarro *et al*., 1994 |
| **Gram positive bacteria** | *Staphylococcus* | Mitchell, 1964 |
| | *saprophyticus* | Sobel *et al.*, 1993 |
| | *Staphylococcus epidermidis* | Stamm., 1998 |
| | *Staphylococcus aureus* | Glauser., 1986 |
| | *Staphylococcus hemolyticus* | Jawetz *et al.*, 1998 |
| | *Staphylococcus hyicus* | Maskell., 1988 |
| | *Streptococcus feacalis* | Navarro *et al*., 1994 |
| | *S.milleri* | Navarro *et al*., 1994 |
| | *Enterococci* | Terai *et al*., 1994 |
| | *Coryebacterium spp* | |
| **Others** | *Candida spp* | Navarro *et al.*, 1994 |
| | *Chlamydia trachomatis* | Mobley and Belas, 1995 |
| | *Mycoplasma hominis* | Mims *et al*., 1987 |
| | *Cryptococcus neoformans* | Tolkoff-Rubin *et al*., 2004 |
| | *Herpes simplex virus* | Mims *et al*., 1987 |

### 1.2.٥.1 Gram negative bacteria

It has been reported that there are several species in *Enterobacteriaceae* which are considered an important causes of UTI (Jawetz *et al*., 1987). *Escherichia coli* is the most common cause of urinary tract infection, accounting for about 85% of community acquired and 50% of hospital-acquired infections, it predominates strongly at most ages (Johnson, 1991).

### 1.2.٥.2 Gram positive bacteria

Gram Positive bacteria encountered in UTI are less frequent than Gram negative bacteria, most important species is *Staphylococcus epidermidis* which causes mild infection (Mitchell, 1964). *Staphylococcus aureus* and *Enterococci* are more associated with UTI in hospitalized patients (Glauser, 1986; Stamm, 1998).

### 1.2.٥.3 Others

Yeast especially that belongs to *Candida* could also cause UTI, *Candida albicans* was found in diabetic women and in patient with indwelling catheters but usually this species could represent harmless colonization (Stamm and Turck, 1998).

Viruses also can cause UTI such as *Herpes simplex virus* may produce an active uritheritis (Stamm, 1998).

Several other classes of microorganism such as *Mycoplasma spp* and *Chlamydia trachomatis* can invade the urinary tract, *C.trachomatis* has been clearly shown to be an important cause of acute uretheral syndrome, while *Mycoplasma spp* implicated as causes of chronic pyelonephritis (Tolkoff-Rubin *et al*., 2004).

## 1.2.6 Genus *Escherichia*

The genus *Escherichia* is Gram negative, rod in shape, measuring (2-6 μm) in length and (1.1-1.5μm) in diameter, motile by peritrichous flagella, facultative anaerobic, non spore forming, non capsulated, catalase positive, oxidase and urease negative, give negative reactions in the Voges-Proskauer, phenylalanine deaminase and gelatin hydrolysis tests, lactose, D-mannitol and D-mannose fermented, do not produce $H_2S$ in triple sugar iron agar and most strains form gas from glucose (Collee *et al*., 1996).

There are five species in the *Escherichia* genus, *E. blattae, E. coli, E. fergusonnii, E. hermanni, and E. vulneris. E. coli* is the most important species of the genus (Collee *et al*., 1996).

*E. hermanni* and *E. vulneris* are most frequently obtained from wound infections but also isolated from infections at other body sites (Cruikshank *et al.,* 1975). *E. fergusonnii* has been most frequently obtained from human feaces. *E. blattae*, is not isolated from human specimens (Roberto *et al.,* 2004).

Theodre Escherich first described *E. coli* in 1885, as bacterium coli commune whom he isolated from the feces of newborns, it was later renamed *Escherichia coli* (*E. coli*) and its relative are known to microbiologists as "entericbacteria" because they live in the intestinal tract of human and animals as normal flora (Kaper *et al*., 2004; Howard, 2004).

*E. coli* becomes a very popular laboratory organism because scientists could grow it quickly on both simple and complex media. *E. coli* can grow in presence of air, using oxygen as terminal electron receptor (aerobically) or

without air by what is called fermentative metabolism, the ability to grow both aerobically and an aerobically classifies the *E. coli* bacteria as a facultative anaerobe. *E. coli* colonizes the gastrointestinal tract (GIT) of human and other animals within hours or few days after birth (Prescott *et al*., 1990).

Over 700 antigenic types (serotypes) of *E. coli* are recognized based on the presence or absence of heat stable somatic antigens (O-antigen), somatic antigen composed of polysaccharide chains linked to the core of lipopolysacchride (LPS) complex common to all Gram negative bacteria and more than 170 different O-group/H-antigen combinations, the H antigens are the flagellar antigen there are at least 56 types and the last factor of serotyping classification is K-antigen (capsular antigen) (Rivera and Keen, 2001; Walter and Stamm, 2006).

Pathogenic *E. coli* was divided into five categories that differ in their virulence factors enteropathogenic *E. coli* (EPEC) that have pathogenicity island (locus of enterocyte effacement) encoding proteins involved in the formation of attaching and effecting lesion on host intestinal cells, enterotoxogenic *E. coli* (ETEC) which produce heat labile and/ or heat stable enterotoxin; enterohemorrhagic *E. coli* (EHEC) which represent an important pathogen around the world and is characterized by the production of shiga like toxin and intimin; enteroivasive *E. coli* (EIEC) and enteroaggrigative *E. coli* (EAEC) (Roberto *et al.*, 2004).

## 1.2.7 Pathogenicity of *E. coli*

*E. coli* is the predominant non pathogenic facultative flora of the human intestine, the presence of *E.coli* and other kinds of bacteria within our intestine is necessary for us to maintain health, the bacterium makes the vitamin K and B-complex (Alraek *et al.*, 2002; Naster *et al.*, 2001).

Although *E. coli* is the predominant coliform species in the healthy colon, but it can cause disease when it escapes from its usual gastrointestinal habitats, invasion of the blood stream can cause infection, it is known that *E.coli* is associated with urinary tract infection, neonatal meningitis, intestinal disease and some time food poisoning (Ming *et al.,* 2002; xie *et al*., 2006).

The ability of *E. coli* pathogenicity to cause disease belongs to the presence of one or more virulence genes these genes encoded the virulence traits, these virulence determinants which include (adhesions, motility, chemotaxis, toxins, antiphagocytic properties and defense against immune responses) (Roos and Klemm, 2006; Brzuszkiewicz *et al.,* 2006).

These virulence gene may be located on the transmissible genetic elements such as transposons, plasmids or bacteriophage., in addition, other genes may be part of particular regions on the bacterial chromosomes termed pathogenicity island (Pais), which present in the genome of pathogenic strains of given species but absent in the nonpathogenic variants of species (Guyer *et al.*, 1998; Bingenbidois *et al.,* 2002; Skyberg *et al.,* 2006).

## 1.2.8: Virulence factors of *E. coli*

Uropathogenic *E. coli* are the most important group of microorganism responsible for UTI that differ from non-pathogenic *E. coli* as a normal flora and other *E. coli* pathotype by its production of specific virulence factors (Pass *et al.*, 2000; Gehua *et al.,* 2002).There are different virulence factors which include :-

**1- Adhesion: -** This factor has important role during establishment of UTI, besides their primary function as adhesion molecule several other additional

functions, such as invasions, promote biofilm formation and transmit signals to epithelial cells resulting in the inflammation (Oelschlaeger *et al.,* 2002).

*E.coli* has different type of adhesion like:

a-Fimbrial adhesion which include type I -fimbria (*afa*), P-fimbria (*pap*) and S-fimbria (*sfa*), the genes encoding for fimbrial adhesion (*afa, pap, sfa*) are closely linked on the chromosome of different types of pathogenic *E.coli* (Roberto *et al*., 2004).

b- Non-fimbrial adhesion (Intimin): It is outer membrane protein encoded by *eae* gene, this gene is a part of LEE (Locus of enterocyte effacement) pathogenicity island (Johnson *et al.,* 2001).

Intimin is responsible for attachment and affecting host intestinal or urothelial cells (intimate adherence between and enterocyte membrane), there are three variants of intimin produced by EPEC and EHEC (Monica *et al*., 2000; Peterfeng *et al.,* 2001).

**2- Hemolysin: -** hemolysin is pore forming toxin, it is the best characterized member of the (RTX) toxin family, which lysis the cells by creation of pores in the target cell membrane and affect erythrocyte, leukocytes and renal tubular cells, there are three types of hemolysin produced by *E.coli*: extra cellular free hemolysin called alpha, cell associated hemolysin called beta and gamma hemolysin produced by strain have the resistance for nalidexic acid antibiotic (Russo *et al.,* 2005).

**3- Enterotoxin: -** which is produced by non invasive enterotoxogenic *E.coli* (ETEC) which has the genes carried on the plasmid encoded for these toxins that include two types that labile enterotoxin (LT) and heat stable enterotoxins (ST) (Escobar-paramo *et al.,* 2004).

**4-Verotoxin or shiga like toxins: -** this term is based on the reaction of toxins on vero cells, at least two families of these toxins ST x1 and ST x2, which produced by EHEC strains that have the genes carried on the

chromosome encoded for these toxins, these *E.coli* strains cause spectrum of human illness ranging from uncomplicated diarrhea to hemorrhagic colitis and hemolytic-uremic syndrome (Johnson *et al*., 2000).

**5- Capsules: -** represent extra protective barrier which make the host more susceptible and provide the protection of bacteria against desiccation and act as antiphagocytosis, anticomplement and serum resistance (Mark *et al*., 2004).

**6- Lipopolysacharide: -** which is active as endotoxins (Amor *et al.,* 2000).

**7- Other toxins like cytotoxic necrotizing factor (CNF)** that interfere with phagocytosis, aerobactine act as sidrophore, sudden infant death syndromes (SIDS) and cytolethal distending toxin (CLDT) (Emody *et al*., 2003).

## 1.2.9 Phage

Phage is small virus that specifically infects bacteria and kills them or lysis bacteria but it has no effect on human, animals and plants. Viruses, in contrast to cells that can grow and reproduce by division, are assembled from pre-made components (Romero *et al.,* 1990).

A particular phage may be very specific in that it will infect only a few strains of a certain bacterial species, on the other extreme, there are phages which can infect strains of two or more species of a particular genus, susceptibility to lysis by a particular phage may be the only apparent phenotypic difference between two bacterial strains and may be the only means by which a strain causing an outbreak of disease can be recognized, this observation is the basis for phage-typing, and a procedure was developed for characterizing and detecting bacterial strains by their reaction (susceptibility or resistance) to various known strains of phages (Wagner and Waldor, 2002).

Bacteria if given the proper nutrients, can grow and reproduce on their own, but viruses cannot "live" or reproduce without getting inside some other living cells, whether it is a plant, animal, or bacteria, bacteriophage can be isolated from different sources for example sewage water and they can also be extracted from corpuses (Zierdt *et al.*, 1960).

Viruses are capable of survival, but not growth, in the absence of a cell host, replication of the viral genome depends upon the metabolic energy and that macromolecular synthetic machinery of the host, frequently this form of genetic parasitism result in debilitation or death of the host cell, therefore successful propagation of the virus requires:

(1) A stable form that allows the virus to survive in the absence of its host.

(2) A mechanism for invasion of a host cell.

(3) Genetic information required for replication of the viral components within the cell.

(4) Additional information that may be required for packaging the viral components and liberating the resulting virus from the host cell (Jawetz *et al.*, 1998).

## 1.2.10 Discovery of bacteriophages

The first report about what we now recognize as bacteriophage (bacterial viruses that invade bacterial cell) was published more than a century ago, in 1896, Ernest Hankin a British bacteriologist, reported that something in the waters of the Ganges and Jumna rivers in India had marked antibacterial action and could pass through a very fine porcelain filter (Carlton, 1999). Two years later, the Russian bacteriologist Gamaleya observed a similar phenomenon while working with *Bacillus subtilis*, and the

observations of several other investigators are also thought to have been related to the bacteriophage phenomenon (Van Helvoort, 1992).

After 20 years Hankin's observation, a bacteriologist from England Frederick Twort actually isolated filterable entities capable of destroying bacterial cultures and producing small cleared areas on bacterial lawns (1915), Twort did not further explore his finding. Two years after Twort's discovery, Felix d'Herelle, a French Canadian microbiologist working at the Pasteur Institute in Paris, reported the same phenomenon (clear spots or clear areas caused by invisible microbe a virus parasitic on bacteria) D'Herelle called the virus bacteriophage or bacteria-eater or devour from Greek (Bergh *et al*., 1989).

## 1.2.11 Structure of phage

Viruses are composed of nucleic acid molecules surrounded by protective coating, some phages also contain lipid, but these are exceptional, considerable variability is found in the nucleic acid of phage, the nucleic acid inside the coating, called the **phage genome** in bacteriophage, encodes most of the gene products needed for making more phages (Boyd *et al.*, 2001).

Many phages contain double-stranded DNA, others contain single-stranded RNA, and some contain single-stranded DNA, unusual bases such as hydroxymethylcytosine are sometimes found in the phage nucleic acid (Jawetz *et al.*, 1998).

All phages have a chromosome encased in a capsid that is composed of phage-encoded proteins, for many phage types, the capsid is attached to a tail structure that is also made from phage-encoded proteins (Olson *et al.*, 2001).

## 1.2.12 Lifecycle of bacteriophage

All phages must carry out a specific set of reaction in order to make more of themselves, **first** the phage must be able to recognize a bacterium that it can multiply in by binding to the bacterial cell surface, **next** the phage must inject its genome and the genome must be protected from the bacterial nucleases in the cytoplasm (Levin and Bull, 2004).

The phage genome must be replicated, transcribed, and translated so that large number of genomes, capsid proteins, and tail proteins (if present), are produced at the same or nearly the same time, complete phage particles are then assembled and the phage must get out of the bacterium, phages are very choosy as to what bacteria they infect, this is referred to as the **host range** of the phage, for example, lambda only infects certain *E.coli*, whereas spo1 phage infect only *Bacillus subtilis* (Hendrix, 2002).

The number of phages that can be released from one bacterium after infection and growth by one phage is known as the **burst size** and every phage has a characteristic burst size, different phages also take different amounts of time to go through one growth cycle and the phage has successfully reproduced when it is possible to detect **plaques** or circular areas with little or no bacterial growth on an agar plate covered with a thin layer of bacteria (You and Yin, 1999).

The virus is capable of infecting *E. coli* bacteria and after this infection; virus can follow either one of two different pathways:

(1) The virus integrates its DNA into the host bacterial DNA and duplicates when the bacterium divides this pathway is known as lysogeny,

(2) The virus uses the bacterial molecular machinery to make many viral copies and leave (after killing the host bacterium) to infect other bacteria this called lysis pathway, once the virus is in the lysogenic state, it can shift to the lysis state under certain conditions, e.g., if the bacterial culture is irradiated with ultraviolet (UV) light, the molecular regulatory mechanism responsible for the lysogeny/lysis decision is known as the phage switch (Gottesman, 1999).

The most thoroughly studied lytic phages, T-even (e.g., T2, T4) phages of *E. coli* have, demonstrated the need for precisely timed expression of viral genes in order to coordinate events associated with phage formation, temperate phage are able to enter a nonlytic prophage state in which replication of their nucleic acid is linked to replication of host cell DNA, bacteria carrying prophage are termed lysogenic because a physiologic signal can trigger a lytic cycle resulting in death of the host cell and libration of many copies of the phage, the best characterized temperate phage is the *E. coli* phage lambda, filamentous phages exemplified by the well-studied *E. coli* phage M13, are exceptional in several respects, their filaments contain single- stranded DNA complexes with protein and are extruded from their hosts (Jawetz *et al.*, 1998).

Some temperate bacteriophages exemplified by *E. coli* phage P1, can be established in the prophage state as plasmid, the double-stranded DNA of other temperate bacteriophages is established as prophage by its insertion into the host chromosome, the site of insertion may be quite specific, as exemplified by integration of *E. coli* phage lambda at a single *int* locus on the bacterial chromosome, the specificity of integration is determined by of the shared DNA sequence by the *int* locus and a corresponding region of the

phage genome, other temperate phage, such as *E. coli* phage Mu, integrate in any of a wide range of chromosomal sites and in this respect resemble transposons (Jawetz *et al.*, 1998).

The capsid plays three important roles in the phage life cycle: **(i)** protecting the phage genome during the extracellular search (e.g., from DNA-degrading enzymes); **(ii)** effecting phage adsorption, which is the attachment of the virion particle to a susceptible bacterium; and **(iii)** the subsequent delivery (uptake) of the phage genome into the cytoplasm of the now-infected bacterium, the extra cellular search occurs via phage diffusion through an aqueous milieu. During this period the phage must avoid physical damage while waiting to encounter a susceptible bacterium. The likelihood that an individual phage will find a bacterium to adsorb is a function of time, the phage diffusion rate, and the local density of phage-susceptible bacteria, with more bacteria resulting in faster phage adsorption (Summers and d'Herelle, 1999).

## 1.2.13 Phage therapy

Phage therapy is the therapeutic use of lytic bacteriophages to treat pathogenic bacterial infections. Phage therapy is a potential alternative to antibiotics, it has been extensively used and developed mainly in former Soviet Union countries for about 90 years (Mc Grath and Van Sinderen, 2007).

Phage therapy was used to treat infection throughout countries of the former Soviet Union, and was part of the general standard of care there, used especially in pediatric, burn and surgical hospital settings. Phage preparation was carried out on an industrial scale. Tons of tablets, liquid preparations and

spray containers of carefully-selected mixtures of phages for therapy and prophylaxis were shipped throughout the former Soviet Union each day (Parfitt, 2005).

The largest use was in hospitals, to treat both primary and nosocomial infections, alone or in conjunction with chemical antibiotics, they played a particularly important role when antibiotic-resistant organisms were found, the military is still one of the strongest supporters of phage therapy research and development, because phages have proven so useful for wound and burn infections as well as for preventing debilitating gastrointestinal epidemics among the troops (Brüssow, 2007).

There are reports on using phage therapy in other countries. In the period after his discovery, D'Herelle promoted the use of phages as therapeutic agents for the treatment of infectious diseases; the first reported application of phages to treat infectious diseases of humans came from Bruynoghe and Maisin in France in 1921, which used bacteriophages to treat staphylococcal skin disease. Phages have been used, since that time, for prophylaxis and therapy in the United States (early 1930s) and, for the last five decades, in Eastern Europe (Duckworth and Gulig, 2002).

The international literature contains several hundred reports on phage therapy, with the majority of the publications coming from researchers in the former Soviet Union and eastern European countries. Phages have been reported to be effective in treating skin infections caused by *Pseudomonas, Staphylococcus, Klebsiella, Proteus, and E. coli, staphylococcal* lung and pleural infections, *P. aeruginosa* infections in cystic fibrosis patients, neonatal sepsis, and surgical wound infections (Christin and Thomas, 2003).

The emergence of pathogenic bacteria resistant to most, if not all, currently available antimicrobial agents has become a critical problem in modern medicine, particularly because of the concomitant increase in immunosuppressed patients. The concern that humankind is re-entering the "pre-antibiotics" era has become very real and the development of alternative anti-infection modalities has become one of the highest priorities of modern medicine and biotechnology (Kapil, 2005).

## 1.2.14 Bacteriophage Typing as an Epidemiological Tool for Urinary *Escherichia coli*

Phage typing was used to identify strains of *E. coli* isolated from urinary and non urinary sources, identification of strains of *E. coli* in epidemiological studies has been primarily by serological typing, these studies indicated that a small number of strains are responsible for the majority of infections of the urinary tract and these strains are different from those responsible for infantile diarrhea (Joseph *et al*., 1969).A method for studying bacteriuric strains by phage typing has been developed, the study reported the use of phage typing for the identification of bacteriuric strains of *E. coli* and discusses the feasibility of phage typing in epidemiological studies of UTI (Brown and Parisi, 1966).

Bacteriophage that infect *E. coli* sometimes are referred to **coliphage**, generally, bacteriophage are referred to simply as **phage** as this is true for all bacterial viruses, phage can replicate only within host cells, in other words, coliphage can replicate only within *E. coli*, phage must attach to a receptor on the surface of a bacterial cell in order to initiate an infection, this interaction between the phage and receptor is very specific - a given phage type only will

bind to a **specific receptor molecule**, thus, all phage are not alike (Brüssow, 2006).

The specific identification of microorganisms with viruses has been known for some time, it is known that most viruses will only attach to and infect a specific host; the principle of phage-typing is to exploit this relationship and use the viruses as "tags" to identify microorganisms in the environment at the sub-species level (Bradley, 1963; Pajunen *et al.*, 2002).

## 1.2.15 General comparison between phages and antibiotics therapies

Over the past several years researchers have been looking at the potential of bacteriophage as an alternative to antibiotics to prevent and treat some diseases. Phage therapy is the nature's own way of controlling bacteria and they are safe because they have no known effect on animal or plant cells (Stroj *et al*., 1999).

Some investigators have listed the advantages and disadvantages of phages and antibiotics therapies:

**A) Advantages:**

**Bacteriophage: -** Pirisi, (2000) listed the following advantages for phage therapy:

1- Very specific, affects the targeted bacterium only; therefore, "dysbiosis" (and chances of developing secondary infections) is avoided.

2- No side effects have been described so far - Is able to self-reproduce as long as corresponding host-bacteria are present in the environment, therefore the need to repeatedly administer the phage is greatly reduced (except when the bacteria have enterotoxin).
3- Since phage is targeted to receptors on bacterial membrane or capsule, which are important virulence determinants, development of phage-resistance usually means changes in those structures and may, therefore, lead to attenuation of the strains in virulence.
4- Selecting a new phage (e.g., against phage-resistant bacteria) is a rapid process and frequently can be accomplished in days.
5- Production is simple and relatively inexpensive.

**Antibiotics: -** Yacoby *et al*.,( 2007) emphasized on one of the important characteristics of antibiotic therapy , that is it can be used without knowing exact characteristics of the disease-causing bacteria.

**B) Disadvantages:**

**Bacteriophages: -** Yacoby *et al*. (2006) suggested that because of the high specificity of phages, the disease-causing bacterium has to be identified before the phage therapy can be started.

**Antibiotics: -** Verbeken *et al.* (2007) listed the following disadvantages of antibiotics therapy:

1) Non-specific action that targets not only the pathogenic microorganisms but also a normal micro flora, this can affect the

microbial balance in the gut, which, in turn, often leads to serious secondary infections.

2) Multiple side effects, including yeast infections, intestinal disorders, and allergies.
3) Repeated administrations are often needed
4) Developing a new antibiotic (e.g., against drug-resistant bacteria) is a time consuming process and may take several years
5) Production of antibiotics is expensive.

# 2. Materials and methods.

## 2.1 Materials

### 2.1.1 Equipments

| Equipments | Company (Country) |
|---|---|
| Autoclave | Karlkob (Germany) |
| Balance | Ohaus (France) |
| Compound light microscope | Olympus (Japan) |
| Deep freezer | Sanyo (Japan) |
| Distillator | Gallenkamp (England) |
| Hot plate with magnetic stirrer | Gallenkamp (England) |
| Incubator | Gallenkamp (England) |
| Micropipette | Witeg (Germany) |
| Millipore filter | Millipore and Whatman (England) |
| Oven | Gallenkamp (Germany) |
| pH-Meter | Gallenkamp (Germany) |
| Power supply | LKP (Sweden) |
| Portable centrifuge | Gallenkamp (Germany) |
| Sensitive balance | Mettler (Switzerland) |
| Water bath | Gallenkamp (Germany) |

## 2.1.2 Chemicals

| Chemicals | Company (Country) |
|---|---|
| Crystal violet | BDH (England) |
| Ethanol | Riedel-De Haen (Germany) |
| Gelatin | Difco(U.S.A) |
| Glycerol | BDH(England) |
| Iodine | BDH (England) |
| Methyl red | BDH (England) |
| Peptone | Himedia (India) |
| Safranin | BDH (England) |
| Sodium chloride (NaCl) | Fluka (Switzerland) |
| Xylol | BDH(England) |

## 2.1.3 Media

| Medium | Company (Country) |
|---|---|
| Agar Agar | Himedia (India) |
| Blood agar base | Mast-diagnostic (England) |
| Brain heart infusion broth | Oxoid (England) |
| Eosin methylene blue | Himedia (India) |
| MacConkey agar (Mac agar) | Himedia (India) |
| MR-VP media | Himedia (India) |
| Nutrient agar (N.A) | Himedia (India) |
| Nutrient broth (N.B) | Himedia (India) |
| Simon citrate media | Difco (U.S.A) |
| Triple sugar iron agar (TSI) | Difco (U.S.A) |
| Urea agar base | Difco (U.S.A) |

## 2.1.4 Analytical profile index 20E Kit (API Bio merieux, Lyon, France)

API 20 E Kit consists of:

**A) The galleries.**

The gallery is a plastic strip with 20 micro-tubes containing dehydrated reactive gradients.

**B) API 20 E Reagents.**

1. Oxidase reagent (1% tetra –methyl P-phenylene diamine).

2. Kovac's reagent (P-dimethyl amino benzaldehyde at 4% in HCL isoamyl alcohol).

3. Voges –Prskauer reagent:
   - **a.** VP1 (40% potassium hydroxide).
   - **b.** VP2 (6% alpha-nephthol).

4. Ferric chloride (3 - 4%).

## 2.1.5 Reagents preparation

### 1- Oxidase test reagent (Baron *et al*., 1994)

A solution of 1% N,N,N,N tetramethyl-p-phenylene diamine dihydrochroide was prepared in sterile distilled water (D.W) when needed.

### 2- Kovac's reagent (Collee *et al*., 1996)

It was prepared by dissolving one g of para-dimethyl aminobnzaldehyde in 15 ml of isoamyl alcohol, and then five ml of concentrated HCL added carefully and gradually. The prepared reagents was kept in refrigerator, this reagent was used in indole test.

## 2.1.6 Experimental animal

Three groups of local mice were used for experimental study. These mice were obtained from the animal house of Biotechnology Research Center of Al-Nahrain University.

## 2.2 Methods

## 2.2.1 Media preparation

### 2.2.1.1- Nutrient agar, Eosin methylene blue agar and MacConkey agar.

These media were prepared in Petri-dishes as recommended by manufacturing companies, autoclaved at 121°**C** for 15 minutes.

### 2.2.1.2- Blood agar (Collee *et al.*, 1996).

It was prepared by autoclaving blood agar base at 121°**C** for 15 minutes, cooled to 50°**C**; the blood was added to give final concentration of 5%, mixed well and poured in Petri-dishes.

### 2.2.1.3- Urea agar base medium (Collee *et al.*, 1996).

Prepared by adding 24 gm of urea base to 950 ml of distilled water, pH was adjusted to (6.5-7.0) and sterilized by autoclaving for 15 minutes, then was let to cool to 50°**C**. Then 50 ml of 20% urea solution was added and sterilized by using Millipore filter unit and 0.45 micron membrane filter, after that it was placed in sterile tubes to solidify as slants.

### 2.2.1.4- Peptone water for Indol test (Collee *et al.*, 1996).

20 gm of peptone and five gm of NaCl were dissolved in 1000 ml of distilled water and sterilized by autoclave at 121°**C** for 15 minutes.

### 2.2.1.5- Triple sugar iron agar (TSI) and Simone citrate media.

These media were prepared as recommended by manufacturing company, then autoclaved at 121°**C** for 15 minutes, and the media were dispensed as slants.

### 2.2.1.6- Gelatin medium (Baron and Finegold, 1994).

Brain heart infusion broth was used after adding 12% of dissolved gelatin to it; pH was adjusted to six and then sterilized by autoclave.

### 2.2.1.7- Semi solid agar (Collee *et al.*, 1996).

It was prepared by dissolving 0.5% of agar in nutrient broth and sterilized by autoclaving. Then 10 ml were dispensed in test tubes and left to solidify in the vertical position, it was used for motility test.

### 2.2.1.8- Potassium cyanide medium (Collee *et al.*, 1996).

It was prepared by dissolving three gm of peptone, five gm of NaCl, 0.23 gm of $KH_2PO_4$ and 5.24 gm of $Na_2HPO_4$ in 950 ml of distilled water, pH was adjusted to 7.6, and then distributed into (Bijou bottles) five ml for each one and sterilized by autoclaving at 121 °**C** for 15 minutes. 0.5 gm of potassium cyanide was dissolved in 100 ml of sterile distilled water, and 0.015 ml was added to each Bijou bottles.

## 2.2.2 Sterilization methods.

### 2.2.2.1 Moist heat sterilization.

Autoclave was used to sterilize media and solutions at 121 °**C** for 15 minutes.

### 2.2.2.2 Dry heat sterilization.

Electric oven was used to sterilize glass wares and others by heating at 180 °C for 2 hours.

### 2.2.2.3- Filtration.

Millipore filtration unit with 0.45 μm pore diameter membrane filter was used to sterilize, urea, and crystal violet solutions.

## 2.2.3 Collection of urine samples.

Midstream urine samples were collected in sterile cups from patients attending AL-Kadhmya hospital, private urology clinic and central public health laboratory during the period 1/12/2006 to 15/3/2007. A total of 277 samples were collected and transported to the laboratory during one hour by using cool box.

## 2.2.4 Bacterial isolation (*E. coli*)

Loopful of undiluted urine samples were spread on blood agar, Eosin methylene blue agar and MacConkey agar plates. Plates were incubated overnight at 37°**C**. Then single pink colonies which were lactose fermenters on MacConky plates and those which gave metallic green color on EMB agar were picked off and were transferred to new MacConkey agar plate for further purification by dilution streaking to obtain single isolated colonies, then used for further diagnosis.

## 2.2.5 Maintenance of bacterial isolates

Maintenance of bacterial isolates was performed according to (Sambrook *et al*., 1989) as following**:**

**A - Short term storage.**

Bacterial isolates were maintained for short periods of (2 – 3 weeks) on MacConkey agar plate; the plates were tightly wrapped in parafilm and stored at 4°**C**.

**B - Medium term storage.**

Bacterial isolates were maintained in stab culture for period of few months; such cultures were prepared in small screw-cup bottles containing (5) ml of nutrient agar media as slants and stored at 4°**C**.

## C - Long term storage.

Bacterial isolates were stored for longer periods in medium containing 20% glycerol at low temperature without loss of significant viability; this was done by adding (one ml) of glycerol to (four ml) of nutrient broth bacteria culture in small screw-cup bottles and stored at -20°**C**.

## 2.2.6 Identification of bacteria:

## 2.2.6.1- Cultural identification

Identification of bacterial isolates was carried out by observing main features of colony morphology (shape, color, size, edges) on surface of MacConkey plates.

## 2.2.6.2 - Microscopic identification

A loop full of suspected *E. coli* isolate was fixed on microscopic slide then stained by Gram stain to examine (cell shape, grouping, size, Gram reaction and spores forming) (Atlas, 1995).

## 2.2.6.3- Biochemical identification

Biochemical tests which are more specific in identification of bacteria were carried out as following:

## 1-Oxidase test (Atlas, 1995)

The test was done by using a moisten filter paper with a few drops of a freshly prepared solution of N, N, N, N, Tetra- methyl-p-phenylene diamine dihydro-chloride (2.1.5.1), then aseptically a clump of cells were picked up from slant growth with a sterile wooden stick and smeared on the moistened paper, the development of violet or purple color within 10 seconds indicated a positive test.

## 2-Ureas test (Atlas, 1995)

Urease activity was detected by inoculating the surface of Christensen urease agar slants (2.2.1.3) with bacterial growth and incubated at 37°**C** for 24 hrs, the appearance of red violet color indicates a positive test; whereas yellow-orange color indicates negative test.

## 3- Triple sugar iron agar (TSI) test (Atlas, 1995)

Bacteria are cultured on TSI agar slant by stabbing and streaking on TSI slant (2.2.1.5) surface then incubated for (24 – 48 hrs) at 37 °**C**. If the color of medium is changed from red to yellow this indicated acid formation;

and appearance of black precipitate indicated ferric sulfate formation; whereas pushing and cracking the agar to the top indicated $CO_2$ formation.

## 4- Simon citrate test (Collee *et al*., 1996)

Simon citrate agar (2.2.1.5) was inoculated by streaking a loopful of bacterial growth on the surface of the medium, incubated at 37°**C** for 24- 48 hrs, when the color of media is changed from green to blue this indicated positive result.

## 5-Indol test (Collee *et al*., 1996)

This test demonstrates the ability of bacteria to decompose the amino acid tryptophan to indol which accumulates in the medium, five ml of peptone water inoculated by bacterial colony, incubated at 37°**C** for 48 hrs, then 0.05 ml of Kovac's reagent (2.1.5.2) was added with gentle mixing of the contents of the tube, appearance of red ring on the surface indicated positive result.

## 6- Methyl red test (Collee *et al.*, 1996).

This test is employed to detect the production of sufficient acid during the fermentation of glucose. The MR-VP media was prepared and three ml were dispensed in five ml test tubes and sterilized by autoclave, the isolate was inoculated, then incubated at 37°**C** for 48 hrs, and five drops of Methyl red reagent were added and mixed gently, appearance of red color represent positive result, while appearance of yellow color represent negative result.

## 7- Catalase test

This test demonstrates the presence of catalase, an enzyme that catalyze the release of oxygen from hydrogen peroxide, this was performed

by smearing a colony on glass slide, then a drop of 3% $H_2O_2$ was added, appearance of bubbles indicated a positive result.

## 8- Gelatinase test (Atlas *et al.*, 1995)

Gelatin agar was used to detect gelatin liquefaction; this was done by inoculating tubes containing the medium which was prepared according to (2.2.1.6) and incubating at 37°**C** for 48 hrs, after that the cultures were kept in the refrigerator at 4°**C** for 30 min. positive result was recorded by observing gelatin liquefaction.

## 9- Potassium cyanide test (Collee *et al.*, 1996)

This test was done for detection the ability of bacteria to grow in the presence of cyanide; the medium prepared according to (2.2.1.8) was inoculated with one loop-full from 24 hrs nutrient broth culture and incubated at 37°**C** for 24 hrs, the cover of tube was tightly screwed down to prevent air exchange. The appearances of the turbidity indicated the positive result.

## 10- Motility test (Cruickshank *et al.*, 1975)

In this test semi solid agar medium was used, motile bacteria (swarm) will show spreading growth (diffuse) that is easily recognized by the naked eye. The medium prepared according to (2.2.1.7). The bacteria were inoculated by stapping in straight line, the culture was incubated at 37°**C** for 24 hrs, and motile bacteria would grow and diffuse around the line.

## 2.2.7 Identification of isolates by API E20 Kit

Identification of bacterial isolates was carried out by transferring single well isolated colonies from MacConkey agar plates into API 20 E microtubes system. This system is designed for the performance of more than 20 standard biochemical tests from a single colony on plating medium. Each test is performed within sterile plastic micro tube which contains the appropriate substrates and is affixed to an impermeable plastic strip (gallery). Each gallery contains 20 micro tubes (each of which consists of a tube and a cupules section).

## 2.2.8 Isolation of phage (Wietzorrek *et al.*, 2006).

### A) Isolation of phages

Phages were isolated from local samples of water likely to contain high quantities of bacteria and bacteriophages from sewage water. The procedure of isolation includes the following steps:

1- 1000 ml of raw sewage samples were collected from different regions in Baghdad.

2-The samples of raw sewage water were centrifuged at speed of 8000 rpm for 10 minutes.

3-The supernatant of each sample was poured off into new sterile centrifuge tube to separate it from the pellet.

4- The supernatant was filtrated through 0.45μm filter membrane (Millipore corp.).

5- The filtrate ($10^{13}$ PFU/ml) was stored in a sterile tube and preserved at -18°**C** until used.

**B) The detection of Bacteriophage**

1- Nutrient agar plates were swabbed with purified *E. coli* isolated from UTIs patients.

2- 0.1ml of prepared sewage filtrates (2.2.8/A) were placed by pasteure pipette on above mentioned *E. coli* plates then left the plates in room temperature for 15 to 20 minutes.

3- The plates were incubated at 37°**C** overnight.

4- Presence of bacteriophage in the filtrate was demonstrated by formation of plaque (zone lysis) on the site of filtrate spots.

**C) Purification and propagation of bacteriophage**

1- By aid of Pasteur pipette, samples from the clear lysis zones were taken and cultured on the same bacteria in another nutrient agar plate.

2- After the drops had been absorbed thoroughly (left for approximately 15 to 20 minutes at room temperature), the plates were incubated overnight at 37°**C**.

3- The plates were examined for visible plaque region of no bacterial growth, the selected plaque will represent one million particles of a purified phage and specific for tested *E. coli* isolate.

4- Screw-cap bottles each contains 10 ml of nutrient broth were incubated separately with *E. coli* bacteria.

5- The isolated bacteriophage specific for each bacteria was added (0.2ml) to each of screw cap bacterial culture bottle, then the tubes incubated at 37°**C** for 24 hrs.

6- After incubation the content of each tube was centrifuged at speed 8000 rpm for 10 minutes. And the supernatant was filtered through 0.45 μm membrane filter.

7- The specific bacteriophage for each bacterium were poured into sterile tubes and stored at -18°**C** for future studies.

## 2.2.9 The effect of phage on bacterial infection of mice skin (Harely and Prescott, 1996).

The *in vivo* study of possible therapeutic effect of isolated bacteriophage on bacterial infections was performed on mice. The bacteriophage which was specific in term of lysing the *E. coli* isolate was prepared and applied locally on the skin of experimentally infected mice.

In this study, three groups of mice were used, each group includes three mice, and the experiment was done according to the following steps:

1- The hair of the skins of the back of mice was removed, and then the shaved regions were cleaned and disinfected with cotton swab saturated with 70% alcohol.
2- Direct scraping of the skin of each mouse (in the three groups) was done by sterile pathological scalpel was performed to produce abrasion on one half of the mouse's back skin, while the other half of mouse's back skin was left non-scraped. The fourth group (other half none treated) of mice was left as a control.
3- After half an hour the mice skins of each of the following groups were subjected to following treatments :

**First group:** - represented by three mice, each one was smeared by suspension of *E. coli* isolate number (E14) and phage (R6). Cotton swab was moistened by a suspension of *E. coli* and phages isolated from it, at the same time.

**Second group:** - each of three mice was smeared by suspension of *E. coli* (E14) and after 24 to 72hrs of treatment the inflamed mice skin was treated with 0.1 ml of bacteriophages (R6) specific in term of lysing the *E. coli* isolate.

**Third group:** - Each of three mice represented in this group were infected with *E. coli* isolates alone.

The fourth group (half of mice in each group) was left as a control.

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## 3.1 Isolation and identification of *E. coli* bacteria

### 3.1.1 Identification of Gram's positive and negative bacteria

Two hundred and seventy seven midstream urine samples were collected from patients suffering from symptoms referred as urinary tract infections. Samples were collected from different hospitals and private urology clinics in Baghdad during period from 1/12/2006 to 15/3/2007.

Out of 277 urine samples, 180 (64.98%) samples gave bacterial growth on MacConkey agar and blood agar, 109 (60.56%) cultures referred to female patients while 71 (39.44%) cultures referred to male patients. These results are comparable to the results reported by Ibraheem (2006), Sebahi (2003) and Mohammed (1989), who found that the percentages of positive cultures of urine samples on MacConkey and blood agar were 61.5%, 66% and 64% respectively , while AL-Shukr (2005), AL-Bayati (1999), Barkat (1997) and Younis (1986), reported higher percentages (93.7%, 83%, 89% and 84% respectively) than the results reported in this study. On the other hand, AL-Shaikhli (2004) found that the positive cultures of urine samples on the above mentioned media were 49%.

The reason behind this variation in the observed percentages may be due to difference in sample's size and number, site of collection, season, number of hospitals surveyed and medication especially exposure to antibiotics.

Similar studies done in other countries gave different results. A study carried out by Obi and his colleagues (1996) showed that the percentage of positive cultures of urine samples was 27%.

In this study, Gram's stain tests and microscopical examination of the isolates showed that 34 (18.89%) isolates out of 180 (64.98%) midstream urine samples were classified as Gram positive bacteria and 146 (81.11%) isolates as Gram negative bacteria as shown in figure (3 – 1).

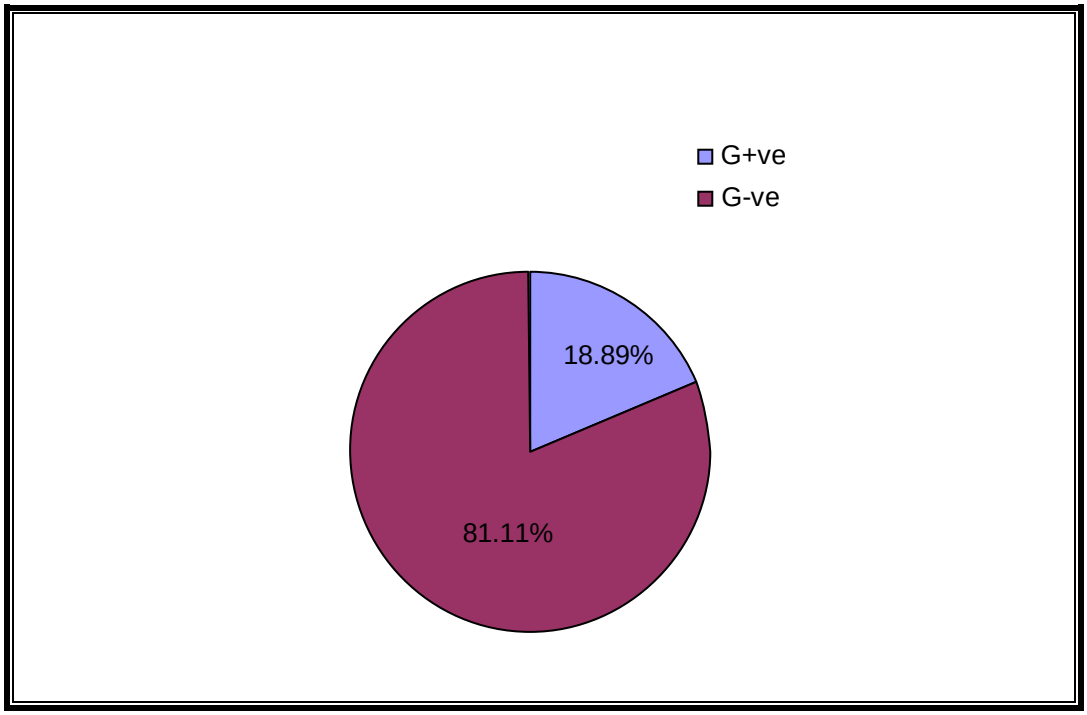


Figure (3 – 1) percentage of G+ve and G–ve bacteria isolated from UTIs patients.

A similar result was reported by AL-Jaboury (2001) who found that G–ve bacteria were responsible for 86% of UTIs cases and G+ve bacteria were responsible for about 14%. Other study carried out by AL-Hmedouy (2005), showed that G–ve bacteria were the dominant and G+ve represented the second infectious agent that cause UTI.

## 3.1.2 Identification of bacterial isolates.

Identification of bacterial isolates as *E. coli* was carried out according to cultural, morphological, and biochemical tests. The identification of presumed *E .coli* isolates was done in accordance to their pink, non viscous colonies appearance on MacConkey agar, lactose fermentation, and grayish white,

moderately opaque with or without zone of hemolysis on blood agar, and motile bacteria appearance on semi solid agar medium; while the microscopically features were Gram negative bacilli, non spores former. The results of biochemical tests indicated positive for indole, Methyl red and Catalase tests, gas production, A/A reaction, no $H_2S$ production in TSI agar, while Oxidase, urease, Vogas-Proskuar, citrate, Gelatin and Potassium cyanide (KCN) were negative.

The isolates were identified as *Escherichia spp* depending on identification scheme suggested by (Forbes *et al*., 1991). However a confirmation of *E. coli* identification was done using API 20E tests.

The obtained results indicated that out of 180 UTI bacterial cultures, only 98 cultures were identified as *E.coli*, and so the isolation percentage of *E.coli* in UTI cultures was 67.12%. This result was comparable with the results reported by Younis (1986) and Read *et al.,* (1989) who found that the isolation percentages of *E.coli* from UTIs cases were 79.5% and 67% respectively. However, different results were obtained by Ibraheem (2006) and Mohammed (1989) who found that the isolation percentages of *E.coli* from urine samples of UTI patients was 33.3% and 39.9% respectively.

It is worth to mention that the results obtained in this study had shown that biochemical and Api 20 E tests (which were performed on G–ve bacterial isolates which were isolated from midstream urine samples) had revealed that 5 (3.4%) isolates were identified as *Enterobacter sp*. , 11 (7.53%) isolates *Klebsiella sp.* , 15 (10.27%) isolates *Pseudomonas sp*. , 17 (11.64%) isolates *Proteus sp*. and 98 (67.12%) isolates were identified as *E. coli* as shown in figure ( 3 – 2).

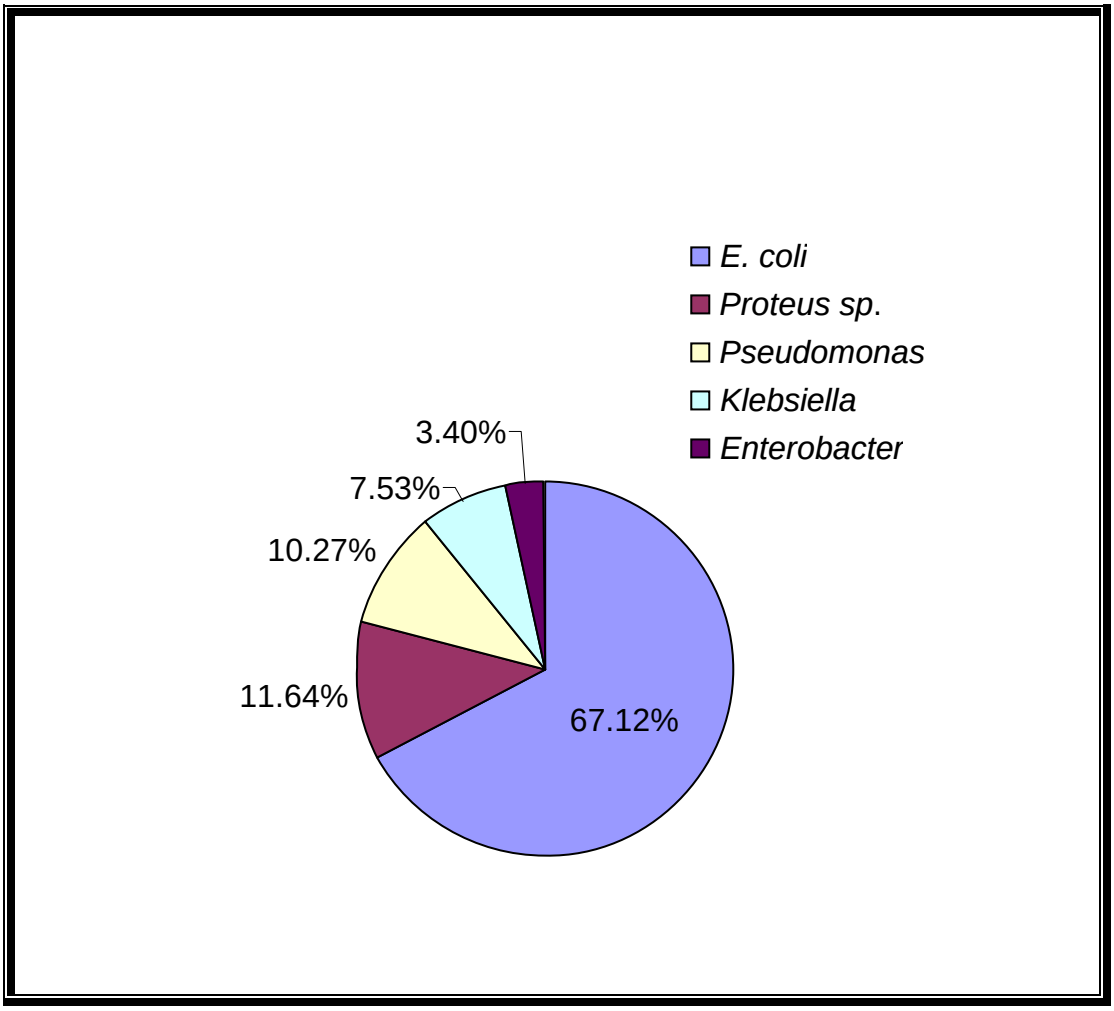


Figure (3 – 2) percentages of *E. coli* to other G–ve bacteria isolated from urine of patients suffer from UTIs.

Our results were comparable to the results reported by Hryniewicz *et al.*, (2001) who found that the percentage of *E. coli* as causative agent of UTIs was (73%), for *Klebsiella sp*. (9.5%), for *Proteus sp*. (8.5%), and other members of *Enterobacteriaceae* was (2.2%).

Members of *Enterobacteriaceae* family considered the main causative agents of UTIs and especially *E.coli* which is responsible for about (75 – 85%) of UTIs cases. *E. coli* is found to cause most of the UTI because of its expression of a number of virulence factors that are responsible for its

pathogencity and thus it is a common causative agent of UTI (Kunin and Buesching, 2000; Jasmina *et al.*, 2001).

Present results were also closely related the results of AL- Shukr (2005), in which that *Klebsiella sp.* and *Proteus sp*. were considered the second causative agent of UTIs after *E.coli* with frequency of infection 11% and 8%, respectively.

Other species of bacteria may contribute and led to infection but with lower frequency like *Enterobacter sp.*, *Staphylococcus sp*. (Mims *et al.*, 1987 ; Anderson *et al.*, 2004).

## 3.2 UTIs among gender

The results showed the prevalence of *E.coli* in both sexes, 71 isolates out of 180 were identified in male samples (39.44%), while in female samples 109 isolates were identified as *E.coli* (60.56%), with female /male ratio 1.54/1 as shown in figure (3 – 3).

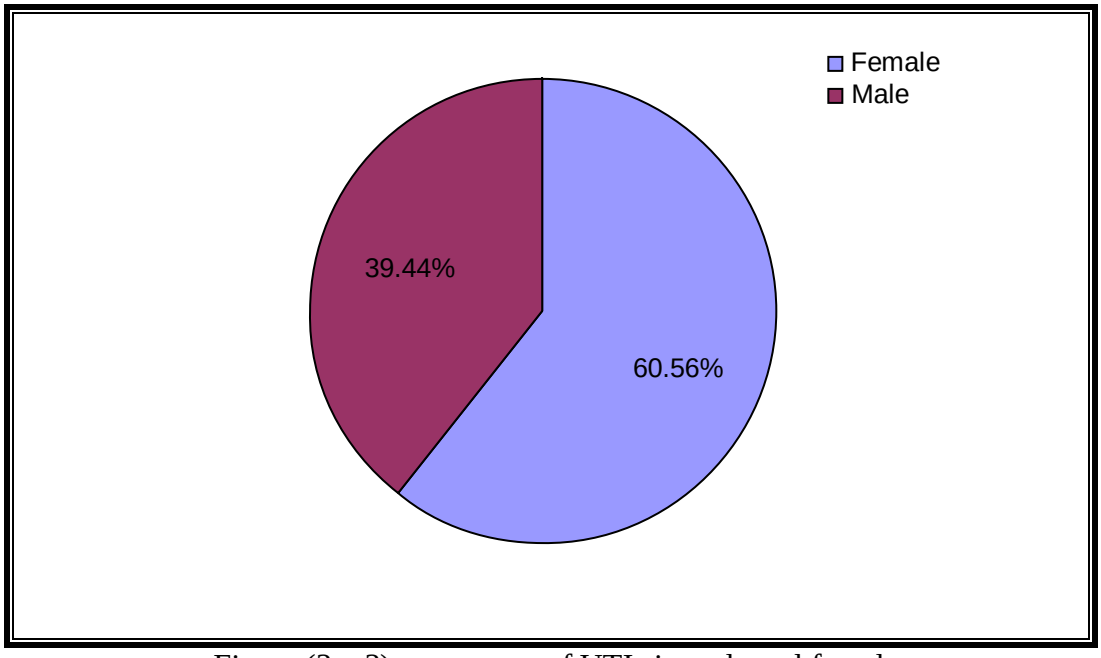


Figure (3 – 3) percentage of UTIs in male and female

It is known that the incidence of UTIs is generally higher in females than in males world wide for several reasons that are the shorter female urethra so it is less effective deterrent to infection than the male urethra, sexual intercourse facilitates the movement of microorganisms up the urethra particularly in female, so that the incidence of UTIs is higher among sexually active than celibate women. However the antibacterial properties of prostatic fluid may also account for increased resistance to UTI observed in men. In male infants UTIs are more common in the uncircumcised and this is associated with colonization of the inside of prepuce and urethra with fecal organism (Mims *et al.*, 1987).

In this respect there was a comparable agreement between the results reported in this study and the results recorded by AL- Jaboury (2005), which found that the percentage of UTIs in males and females were (37.5%) for males and (62.5%) for females; as will as with the results of AL- Hmedouy (2005), who recorded that the percentage of UTIs among gender was (38.1%) for males and (61.9%) for females.

## 3.3 Isolation and propagation of phages

In the following experiments attempts were made for isolation of bacteriophages specific for *E. coli* isolates obtained from different UTI patients. 7 isolates of bacteriophages were recovered from sewage water of different sites in Baghdad city. The samples of raw sewage were centrifuged at speed 8000 rpm for 10 minutes to remove bacteria and other cells derbies in the precipitated pellets at the bottom of the centrifuge tubes, and after filtration of the supernatant through 0.45µm membrane filter it was possible to obtain filtrate free of bacteria and suitable for isolation of bacteriophages.

Drops of 0.1ml of the filtrate presumed to contain bacteriophages were placed on nutrient agar Plates swabbed with pathogenic *E. coli* and incubated at 37°**C** for 24 hrs, plates which showed lytic effect (plaques) on *E. coli* isolates were separated and stored at 4° C for further tests. Seven plaques (bacteriophages) were identified from different sewage waters. During further purification, phages showed stability and it was possible to maintain the cultures. The seven isolated bacteriophages were propagated and further purified, as described in materials and method (2.2.8.C).

In this study we selected randomly 20 *E. coli* from our collection (98 *E. coli* isolates) for isolation of phages. The results are shown in table (3 – 1) and figure (3 – 4).

Table (3 – 1) Isolation of bacteriophages specific to *E. coli* from sewage samples.

| *E. coli* isolates | Bacteriophage isolates | | | | | | |
|---|---|---|---|---|---|---|---|
| | R1 | R2 | R3 | R4 | R5 | R6 | R7 |
| E1 | + | + | + | + | + | + | + |
| E2 | – | – | – | – | – | – | – |
| E3 | + | + | + | + | + | + | + |
| E4 | + | + | + | + | + | + | + |
| E5 | + | + | + | + | + | + | + |
| E6 | + | + | + | + | + | + | + |
| E7 | + | + | + | + | + | + | + |
| E8 | + | + | + | + | + | + | + |
| E9 | + | + | + | + | + | + | + |
| E10 | + | + | + | + | + | + | + |
| E11 | – | – | – | – | – | – | – |
| E12 | + | + | + | + | + | + | + |
| E13 | + | + | + | + | + | + | + |
| E14 | + | + | + | + | + | + | + |
| E15 | + | + | + | + | + | + | + |
| E16 | + | + | + | + | + | + | + |
| E17 | + | + | + | + | + | + | + |
| E18 | + | + | + | + | + | + | + |
| E19 | + | + | + | + | + | + | + |
| E20 | + | + | + | + | + | + | + |

E: *Escherichia coli.* R: Phage isolates.

+: Lytic zone (Plaque appearance on *E. coli* plates).

_: Non lytic zone.

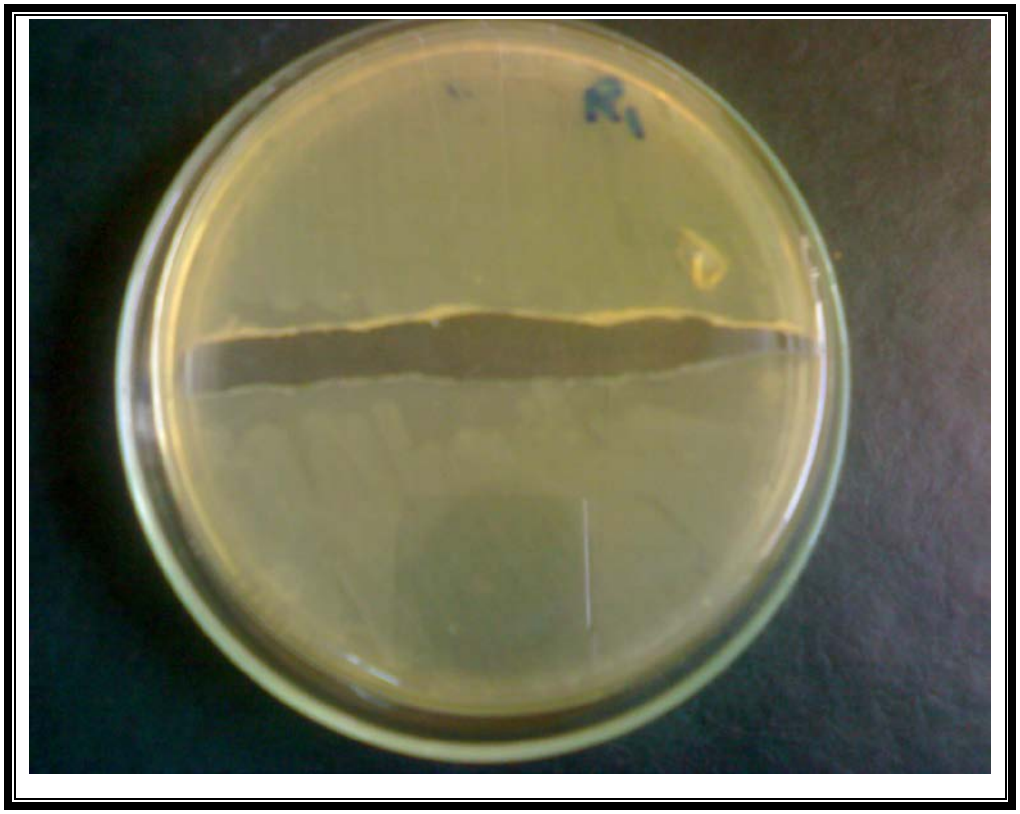

Figure (3 – 4) upper part with *E. coli* culture was not treated with phage, the lower part treated with specific phage.

## 3.4 Phage susceptibility of *E. coli*.

The infectivity of obtained phages was ascertained on different *E. coli* isolates. Thus, the seven phages obtained after further propagation in specific *E. coli* isolates were investigated for their effect on the 20 randomly selected *E. coli* isolates (out of 98 *E. coli* isolated from UTIs by this experiment). The lytic activities of the phage differed on different *E. coli* isolate as shown in table (3 – 2). Moreover, the results demonstrated that the *E. coli* isolates (E2 & E11) were resistant to all seven bacteriophages.

The obtained results showed that phage typing classified the 20 *E. coli* isolates into 18 *E. coli* isolates. This finding is not too surprising since there are more than 700 serotype of *E. coli*.

Table (3 – 2) Lytic effect of bacteriophages (R series) on 20 different isolates of *E. coli*.

| *E.coli* isolates | R1 | R2 | R3 | R4 | R5 | R6 | R7 |
|---|---|---|---|---|---|---|---|
| E1 | + | + | +++ | _ | ++ | _ | + |
| E2 | _ | _ | _ | _ | _ | _ | _ |
| E3 | +++ | + | + | + | ++ | +++ | + |
| E4 | + | _ | _ | _ | ++ | _ | _ |
| E5 | _ | + | ++ | + | +++ | +++ | + |
| E6 | + | + | + | ++ | ++ | ++ | ++ |
| E7 | ++ | ++ | ++ | +++ | _ | _ | _ |
| E8 | + | + | +++ | ++ | ++ | _ | _ |
| E9 | + | + | ++ | ++ | + | + | + |
| E10 | + | + | _ | _ | _ | _ | _ |
| E11 | _ | _ | _ | _ | _ | _ | _ |
| E12 | _ | _ | _ | _ | _ | _ | + |
| E13 | + | + | ++ | + | + | + | + |
| E14 | + | +++ | + | +++ | + | +++ | + |
| E15 | + | _ | ++ | + | _ | _ | _ |
| E16 | ++ | + | + | ++ | +++ | ++ | + |
| E17 | ++ | ++ | ++ | + | + | + | _ |
| E18 | +++ | ++ | + | + | + | ++ | +++ |
| E19 | _ | _ | + | + | + | _ | + |
| E20 | _ | _ | _ | _ | +++ | + | +++ |

+++: Large plaque (2.5mm). ++: Medium plaque (2mm).

+: Small plaque (1mm). _: no plaque.

In this study three types of phage's plaques were observed, according to the diameter of the plaque. First group represented by three pluses (large plaque with a diameter equivalent to 2.5 mm), two pluses (medium plaque, 2 mm) and one plus (small plaque, 1 mm). The results are shown in table (3 – 2), and figures (3 – 5), (3 – 6), (3 – 7).

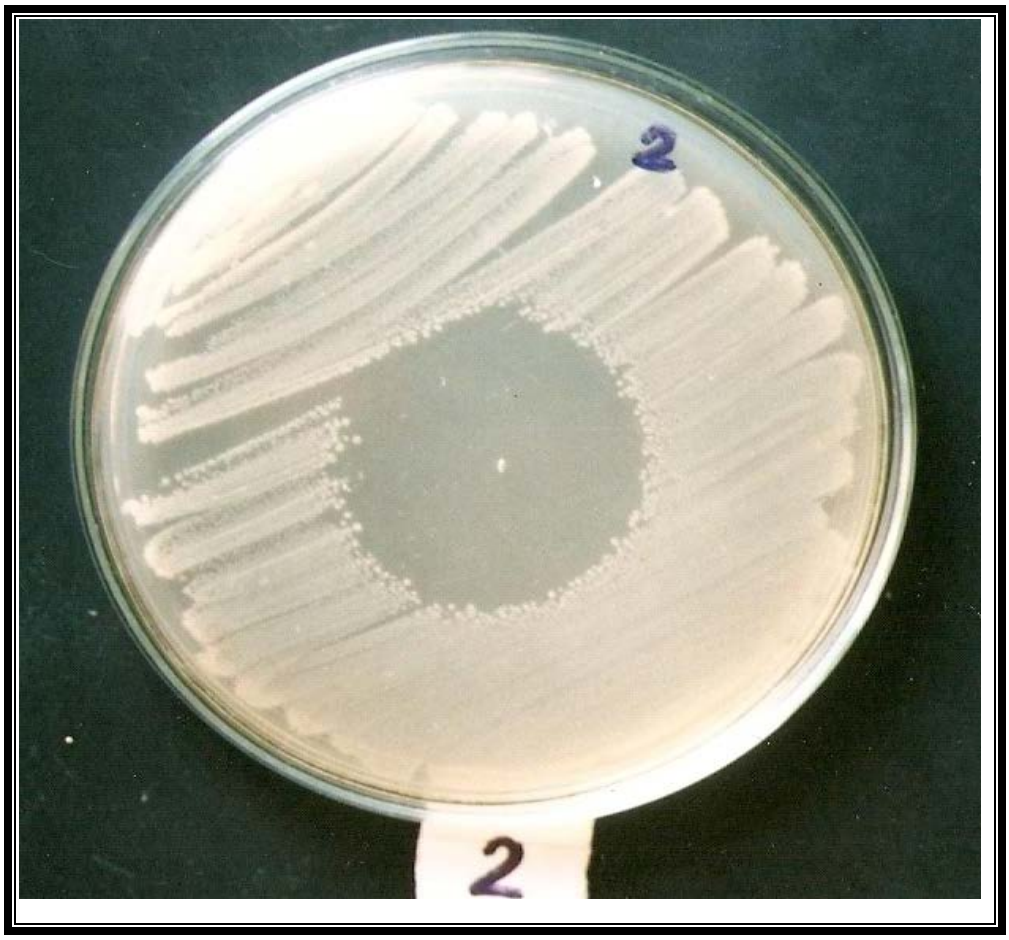


Figure (3 – 5) formation of large plaque by phage R6 (2.5 mm) on *E. coli* 5 culture.

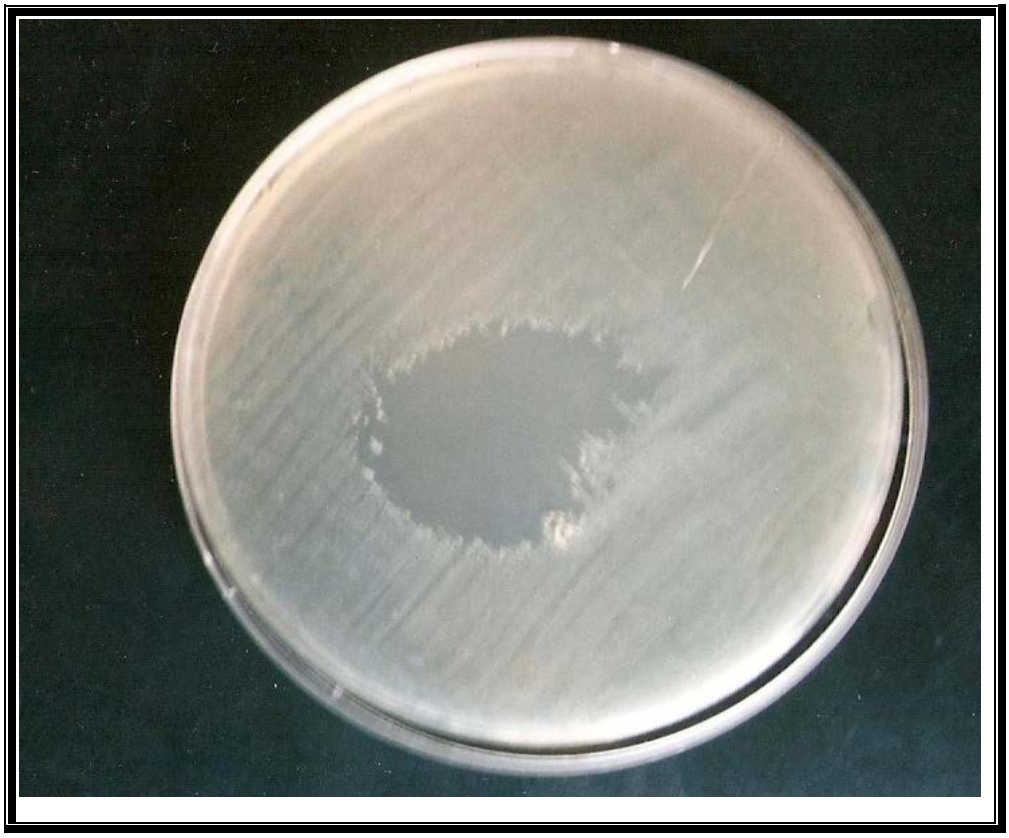

Figure (3 – 6) formation of medium plaque of specific phage R3 (2 mm) on *E. coli* 5 culture.

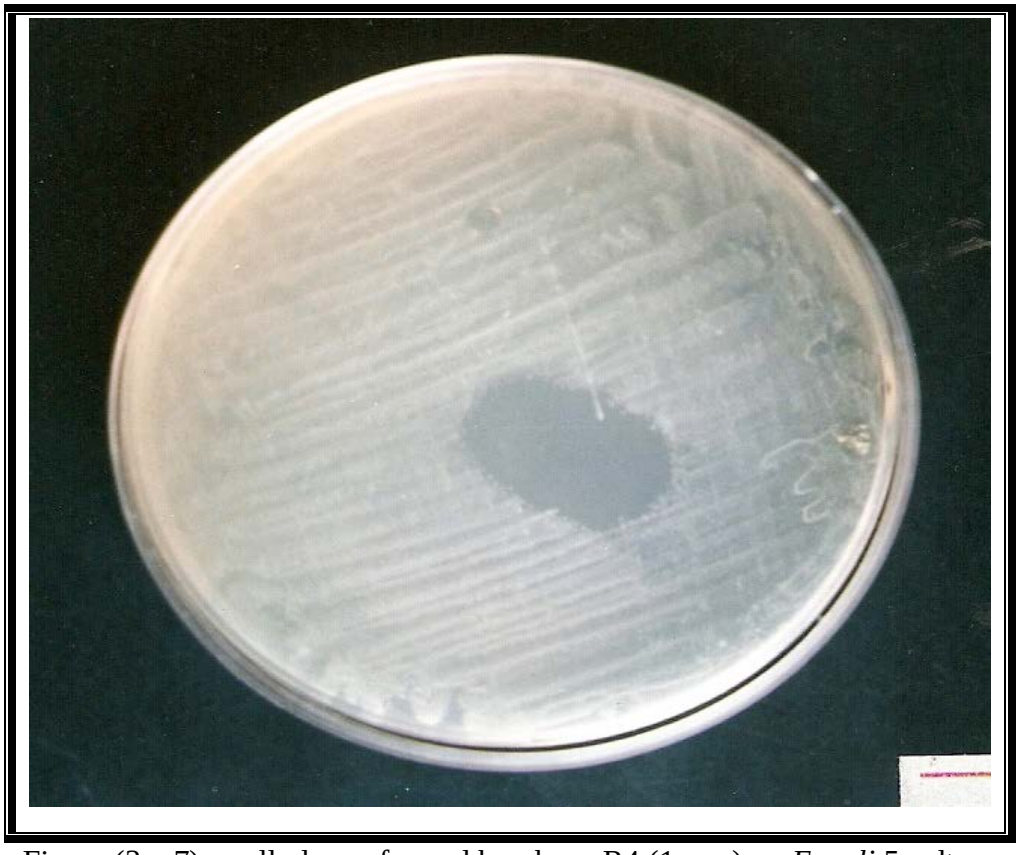

Figure (3 – 7) small plaque formed by phage R4 (1 mm) on *E. coli* 5 cultures.

It as been reported that there are more than 1500 phages expected to infect 700 *E. coli* serotypes and there are different phage families that infect *E. coli* , examples of coliphages are the following: T1, T2, T3, T4, T5, T6, T7, N4, Lambda, M12, M13, Ms2, F2 and fr (Joklik *et al*., 1984).

It is worth to mention that not all *E. coli* isolates are alike, and probably possess some differences in their surface molecules and, as a result, one would expect there are differences in the phage receptors sites present on their surfaces molecules and serve some important functions for the bacteria; they do not exist on the cell surface merely as portals of entry for phage (Tanji *et al.*, 2005).

The results which were shown in figure (3 – 8), demonstrated the specificity of two phages isolated in this study against two *E. coli* isolates. Indicating the differences in phage receptor sites presents on surface of various isolates of *E. coli*.

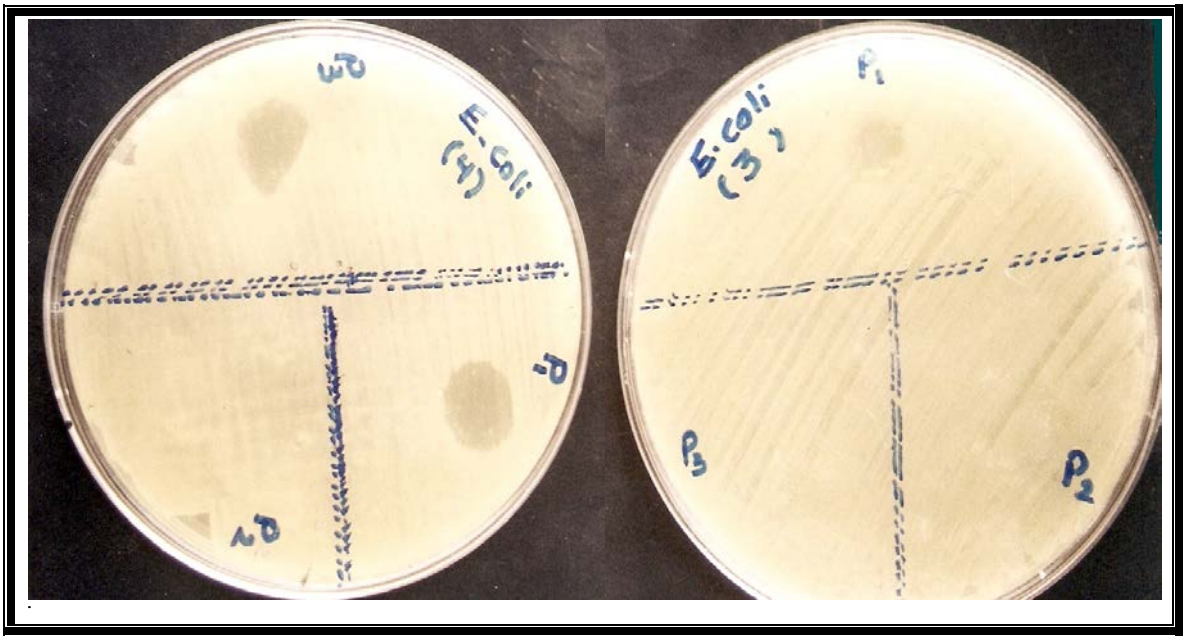


Figure (3 – 8) specificity of three phages against two *E. coli* isolates.

The sensitivity of more uropathogenic *E. coli* isolated in this study toward various phages isolated from sewage were shown in figures (3 – 9), (3 – 10), (3 – 11) and (3 – 12).

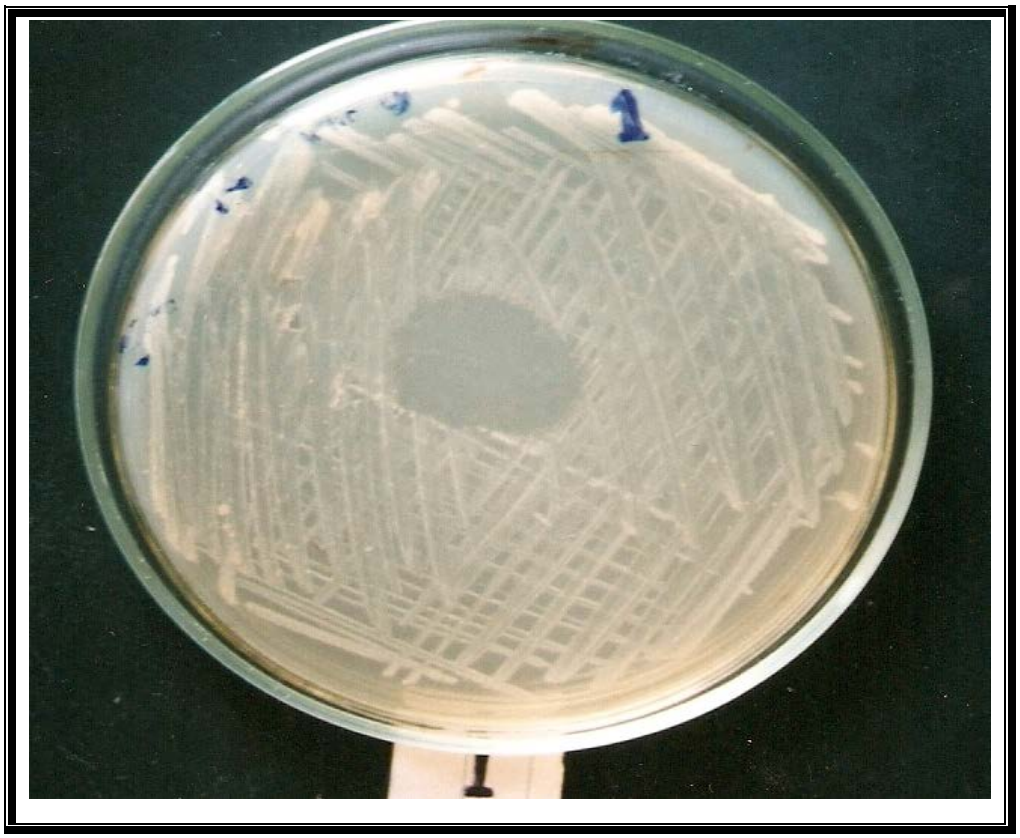


Figure (3 – 9) the specific bacteriophage R1 for *E. coli* 1

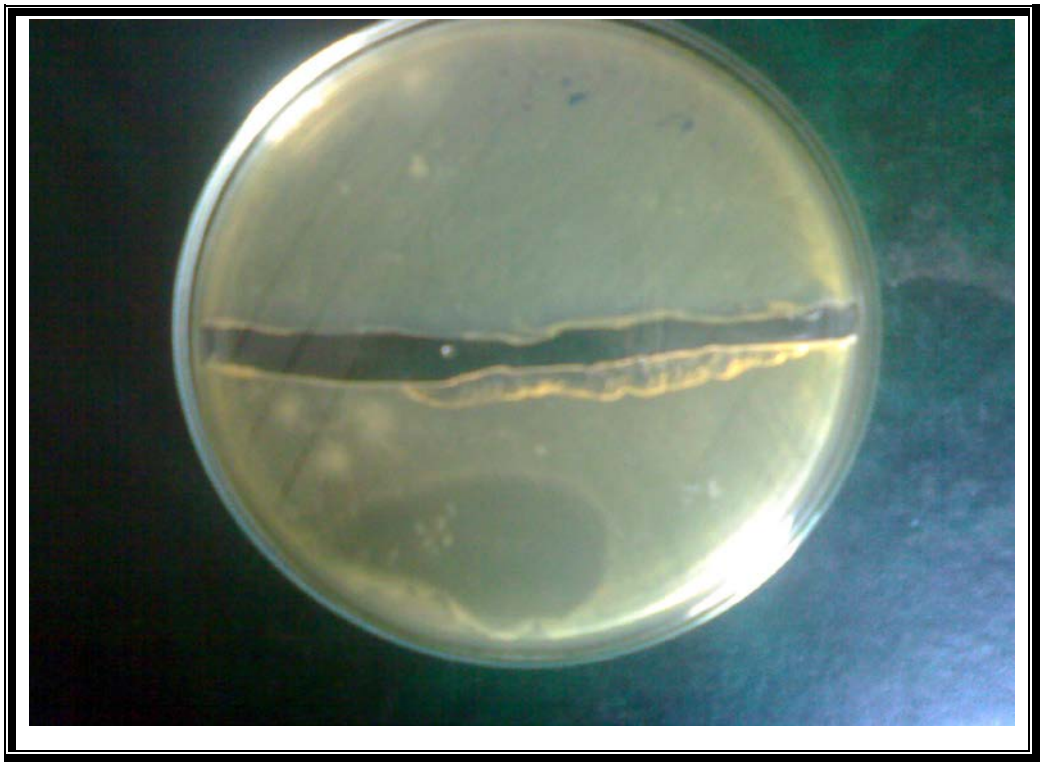

Figure (3 – 10) in upper part of plate *E. coli* 1 treated with R4. In lower part *E. coli* 1 treated with R3.

Figure (3 – 11) in upper part *E. coli* 2 treated with R2. In lower part *E. coli* 8 treated with R4

Figure (3 – 12) in upper part *E. coli* 11 treated with R6. In lower part *E. coli* 3 treated with R6.

The ability of phage to distinguish varieties among identical serovar (serotype) led to development and acceptance of phage typing as significant epidemiological procedure (Weber-Dabrowska *et al*., 2000).

Phage typing can expand potential of these procedures by characterizing isolates on a higher order of delineation and conceivably may substitute even for the more expensive immunological methods in use, aside from relating an isolate to an out break; phage typing can be used for assessing strain distribution (Merril *et al.*, 1996).

Phages typing of cultures from patients with chronic bacteriuria, to determine whether strains with the same phage type were responsible for recurrences of bacteriuria, patients with chronic bacteriuria were chosen from

the out patients. At each clinic visit, the urine was cultured and any organism identified as *E.coli* was phage – typed (Winterbauer *et al.,* 1967).

Relation of phage type to antibiotic resistance, number of phage types of strains resistant to the antibiotics tested. Although a number of phage types were associated with antibiotic resistance, no relation was found between a specific phage type and a particular pattern of antibiotic resistance (Yacoby *et al.,* 2007).

It is worth to mention the results reported by Gershman (1977) who indicated phage typing can contribute significantly to control of mastitis by helping the practitioner and health – related personal to identify disease _ associated strains and to monitor the response of these isolates to treatment.

The results reported in this work which indicated the ability of bacterial viruses (bacteriophages) to lyse and kill bacterial cells led to the hope that they might be therapeutically effective in the treatment of infections related to *E. coli.*

## 3.5 Phage treatment of *E. coli* infected animal's wound

Experiments were performed in this study to ascertain the possible therapeutic effect of bacteriophages obtained in this work on mice wound which were infected with *E. coli* isolates recovered from UTI patients. The results of this investigation were shown in figures (3 –13), (3 – 14), (3 – 15) and (3 – 16), table (3 – 3).

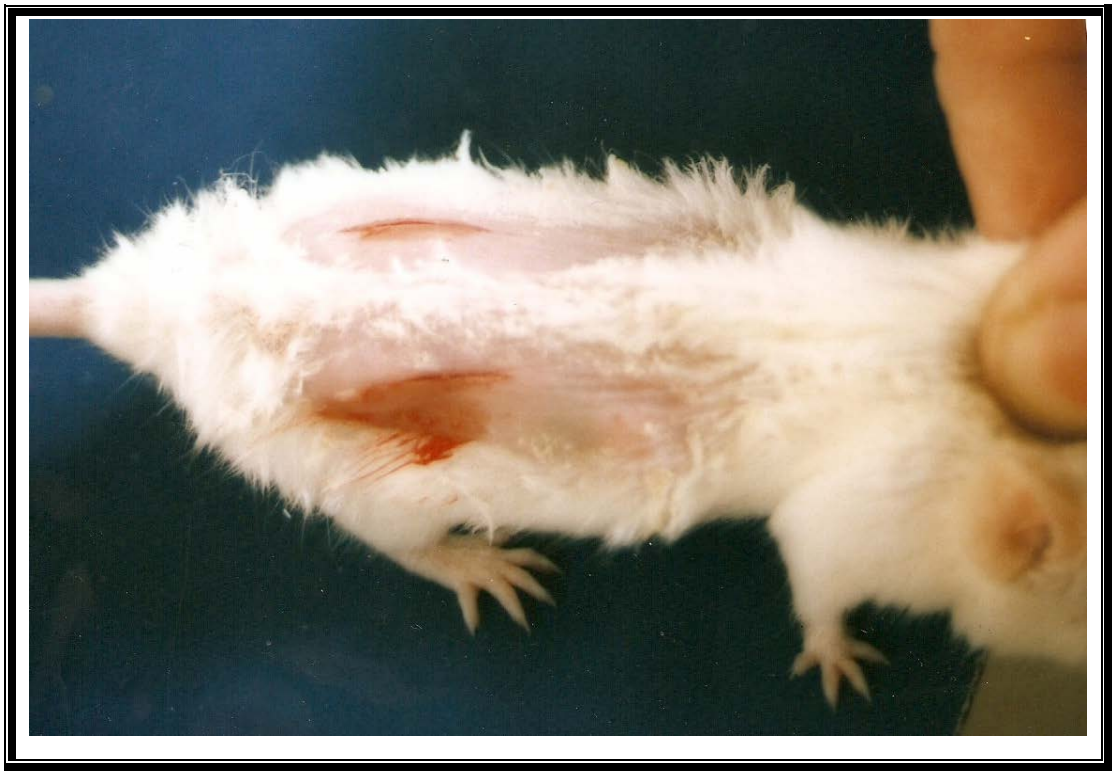

Figure (3 – 13) mice before Infected with *E. coli* 14.

Figure (3 – 14) treatment of mice infected with *E. coli* 14 with specific bacteriophage R6.

Figure (3 – 15) treated mice with specific bacteriophage R6

Figure (3 – 16) finally treated mice.

Table (3 – 3) Effect of bacteriophage (R6) on *E. coli* (14) infected mice wound

| Groups of experimental mice | Type of treatment with *E. coli* (E14) or phage (R6) | Results |
|---|---|---|
| First group | Infected with *E. coli* and phage at the same time. | Non–inflammatory appearance occurred on the skin. |
| Second group | Infected with *E. coli* and treated with phage after the appearance of infection. | Complete recovery of skin after 3 days. |
| Positive control (Third group) | Infected with *E. coli* only. | Complete recovery of skin after 2 weeks. |
| Negative control | None treated and none infected. | Normal healthy mice |

The results revealed the therapeutic effect of bacteriophage isolate number (R6) against infection mice wound with *E. coli* isolate number (E14). And we select the bacteriophage isolate number R6 because it's more sensitive than other isolates of phage.

Animal infected with *E.coli* after appearance of inflammation treated with bacteriophage have shown certain regenerative changes.

Our results were in agreement with the reported results by Bull and colleagues (2002), who indicated decline in the microbiological efficacy of phage with delay in treatment. These results showed that bacteria became

refractory to treatment over the term of infection; this may be attributed to a decline in the rate of replication of bacteria once inside the experimental mouse.

Smith and his colleagues (1987) at the institute for Animal Disease Research in Houghton, Great Britain. Reported the successful use of phages for treatment of experimental *E. coli* infections in mice, and found that a single dose of specific *E. coli* phage reduced, by many orders of magnitude, the number of target bacteria in the alimentary tract of calves, lambs and piglets infected with diarrhea – causing *E. coli* strain. The phage treatment also stopped the associated fluid loss and all animals treated with phages survived the bacterial infection. Sulkvelidze and his colleagues (2001) reported the effectiveness of phages against infection caused by several bacterial pathogens, including multidrug – resistant mutants (*Staphylococcus, Pseudomonas, E.coli, Klebsiella*) , in their experiments , phages were administrated locally by applying a few drops of phage suspension to the eye, middle ear, or nasal and orally. The rates of success (marked to complete recovery in skin with negative cultures) ranged from 75% to 100% and were even higher (99%) with patients for whom antibiotic therapy was infective.

Soothil (1994) reported the utility of phages in preventing and treating experimental disease in mice and guinea pigs infected with *Pseudomonas aeruginosa* and *Acinetobacter*, and they suggested that phages might be efficacious in preventing infections of skin grafts used to treat burn patients.

The applications of phages in practice of orally, topically on infected wounds or spread on to surfaces, or used during surgical procedures, Injection is rarely used, avoiding any risks of trace chemical contaminants that may be present from the bacteria amplification stage, and recognizing that the

immune system naturally fights against viruses introduced into the blood stream or lymphatic system (Summers, 2001).

Using the injection as another type of application. Bacteriophage were used as therapy for the treatment of variety of bacterial infections including laryngitis, skin infections, dysentery, conjunctivitis, periodontitis, gingivitis, sinusitis, urinary tract infections, and intestinal infections, burns, boils, and other disease, also polymicrobial biofilms on chronic wounds, ulcer and infected surgical (Perepanova *et al.*, 1995).

We must avoid using bacteriophages against bacteria having enterotoxin which is dangers, because may the phages help bacteria to release enterotoxin of it (Levin and Bull, 2004).

In other publications from Poland, phages were reported to be effective in treating cerebrospinal meningitis in a newborn (Stroj *et al*., 1999).

Cislo and his colleagues (1987) reported phage therapy of skin infections caused by *Pseudomonas, Staphylococcus, Klebsiella, Proteus*, and *E. coli*.recurrent subphrenic and subhepatic abscesses, and various chronic bacterial diseases (Kaczkowski *et al.*, 1990). In addition phage therapy was found being effective in the treatment of long-term suppurative infections (Weber-Dabrowska *et al*., 2000).

# Summary

In this study 227 urine samples were collected from patients suffering from symptoms referred as urinary tract infection. The urine samples were obtained from different hospitals in Baghdad during the period from 1 / 12 / 2006 to 15 / 3 / 2007.

180 (64.98%) urine samples gave positive bacterial growth on MacConkey agar and / or blood agar. The obtained positive cultures showed that 34 (18.89%) were Gram positive bacteria and 146 (81.11%) samples were Gram negative. The Gram negative samples revealed that 98 (67.12%) bacterial isolates were identified as *Escherichia coli* and 48 bacterial isolates were identified as other species of Gram negative bacteria, 5 (3.4%) cultures were identified as *Enterobacter sp*., 11 (7.53%) isolates as *Klebsiella sp.*, 15 (10.27%) isolates as *Pseudomonas sp*., and 17 (11.64%) isolates as *Proteus sp*.

The percentage of *E. coli* isolates in samples taken from females was 60.56%, while the percentage of *E. coli* isolates which were identified in male samples was 39.44%.

In this study we isolate seven bacteriophages from sewage water at different sites in Baghdad city. The isolates of phage were further purified, propagated and maintained for further tests to ascertain their ability to lyse various *E. coli* isolates that were obtained from UTI patients.

In this study the lytic effect of seven bacteriophages was ascertained on 20 *E. coli* isolates. The results revealed 18 different isolates, and variable lytic activities were observed in different *E. coli* isolates.

Therapeutic effect of bacteriophage was studied in treatment of experimentally infected mice skins with *E. coli* isolate obtained in this study. The results revealed the therapeutic effect of bacteriophage isolate number (R6) against infection of mice wound with *E. coli* isolate number (E14).

# Supervisors certifications

We, certify that this thesis was achieved under my supervision in the College of Science, Al-Nahrain University as a partial requirement for the degree of Master of Science in Biotechnology.

Signature: Signature:

Supervisor: Supervisor:

Scientific degree: Scientific degree:

Date: Date:

In review of the available recommendations, I forward this thesis for debate by the examining committee.

Signature:

Name: **Dr.Kadhim Mohamed Ibrahim.**

Scientific degree:

Chairman of Biotechnology department.

Date:

**Republic of Iraq**
**Ministry of Higher Education**
**And Scientific Research**
**Al-Nahrain University**
**College of Science**
**Biotechnology Department**


# Bacteriophage's Susceptibility of *Escherichia coli* Isolated from Patients with Urinary Tract Infections

**A Thesis**

**Submitted to the College of Science / Al-Nahrain University**

**In Partial Fulfillment of the Requirements for the Degree of**

**Master of Science in Biotechnology**

**By**

*Rejwan Khaleel Ibrahim Al-Mzoory*

**B. Sc. In Biotechnology (2005)**

**Al – Nahrain University**

**July** **Rajab**

**٢٠٠٨** **1429**

# الاهداء

الى الروح الخالده... الى من لم تتركني وتبتعد عن ذهني حتى وان غابت.....

جدتي الغاليه

(رحمها الله)

الى نبراسي الدائم ومصدر قوتي واصراري وعزيمتي.....

والدي العزيز

الى منبع الحنان الدافئ وعين الطيبة الى ملكة حياتي ونور العينين وشمس يومي الجديد

والدتي الحبيبة

الى ياسمين حياتي...الى من كانت ضيائي في دربي الطويله...الى من وافتني دائما وابدا

خالتي الغاليه (ام ازاد)

الى عصفورة الدار..... الى من تحمل رقة الورد وبراءة الاطفل وثالث العينين.

اختي الغاليه (جيان)

الى من كنزته ذخرا لحياتي ومستقبلي وسندي في الدنيا.....

اخي الغالي (اركان)

الى كل من قدم لي يد المساعده من عائلتي والاصدقاء لاتمام دراستي.... الى كل احبتي

اهدي ثمرة جهدي المتواضع.

ابنتكم رجوان

بِسْمِ ٱللَّهِ ٱلرحمَن ٱلرَّحِيمِ

نَرفَعُ دَرَجَاتٍ مَن نَشَاءُ

وَفَوقَ كُلِّ ذِي

عِلمٍ عَلِيمٌ

صَدَقَ ٱللَّهُ ٱلعَظِيمِ

سورة يوسف

# الخلاصة


في هذه الدراسه تم جمع ٢٢٧ عينة ادرار من مرضى يعانون من التهاب المجاري البولية. تم الحصول على عينات الادرار من عدة مستشفيات مختلفه في مدينة بغداد خلال الفترة من ١/ ١٢/ ٢٠٠٦ الى ١٥/ ٣ / ٢٠٠٧ .

وجد بان 180 (64.98%) عينة ادرار اعطت نتيجة ايجابية لنمو البكتريا على اوساط الزرعية الماكونكي ووسط اكار الدم. اظهرت النتائج التي حصل عليها بان 34 (18.89%) هي بكتريا قابله للصبغه الموجبة و 146 (81.11%) عينة هي كانت بكتريا قابلة للصبغه السالبة. وقد تم التوصل بان ٩٨ (67.12%) عزلة بكتيريا شخصت على انها بكتريا القولون *E. coli* و ٤٨ عزلة بكتيريا شخصت لانواع اخرى مختلفة من بكتريا القابلة للصبغه السالبة والانواع كانت كالاتي :

5 (3.4%) عزله شخصت لبكتريا *Enterobacter sp.*

11 (7.53%) عزلة بكتريا شخصت لبكتريا *Klebsiella sp*

15 (10.27%) عزلة بكتريا شخصت لبكتريا *Pseudomonas sp.*

و17 (11.64%) عزلة بكتيريا شخصت لبكتريا *Proteus sp.*

نسبة بكتريا القولون او *E. coli* من العينات المعزولة للنساء كانت 60.56% بينما كانت النسبه تختلف في الرجال فكانت نسبة بكتريا القولون *E. coli* المعزوله من الرجال هي .39.44% .

في دراستنا هذه تم عزل ٧ عزلات لل bacteriophages من مياه الفضلات في عدة مناطق مختلفه في عاصمة بغداد.

تم اكثار وتنقية عزلات phage لاجراء الفحوص الاضافية لتحقيق قدرته لتحليل مختلف عزلات بكتريا القولون *E. coli* المعزوله من مرضى التهاب المجاري البولية.

في هذه الدراسة تم اختبار التاثير التحللي للانواع السبعه من ال bacteriophages في ٢٠ عزلة من بكتريا ال *E. coli*. بينت النتائج المتوصل اليها على ١٨ عزلة مختلفه لبكتريا ال *E. coli*، ولوحظ بان فعاليات التحلل متغيرة في مختلف عزلات ال *E. coli* .

تم دراسة التاثير العلاجي لل bacteriophages في معالجة جلد الفئران المصابه تجريبيا ببكتريا *E. coli* التي تم الحصول عليها خلال هذه الدراسة ، اظهرت النتائج بأن التاثير العلاجي لرقم العزلة R6 ل bacteriophage ضد عزلة E14 لبكتريا *E. coli* والتي اصيبت بها الفئران .

جمهورية العراق
وزارة التعليم العالي و البحث العلمي
جامعة النهرين
كلية العلوم
قسم التقانة الأحيائية


# قابلية التأثر لبكتريا القولون البرازية المعزوله من المرضى المصابين بألتهابات المجاري البولية للعاثيات البكترية

رسالة
مقدمة الى كلية العلوم / جامعة النهرين
وهي جزء من متطلبات نيل درجة ماجستير
علوم في التقانة الاحيائية

من قبل
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بكلوريوس تقانة احيائية / كلية العلوم
جامعة النهرين (٢٠٠٥)

رجب تموز

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