A Study on the Possible Involvement of Antibiotic Resistant Plasmids in Adherence of Uropathogenic *E.coli* to Uroepithelial Cells

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1.1 Introduction

The family *Enterobateriaceae* is the widest and most heterogeneous group of medically important Gram negative bacteria. About a third of the 30 genera known in this family are human pathogens, causing a variety of diseases ranging from intestinal infections to urinary tract infections, nosocomial respiratory tract infections, wound infections, and septic shock. Urinary tract infections (UTIs) remain a major clinical problem and is 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). In this regard *Escherichia coli* is a common infectant of the urinary tract and bladder in man, and was isolated from 30 – 90% of all uncomplicated urinary tract infections(Rakasha, 2003; Ziegler *et al*., 2004).

Uropathogenic *E.coli* (UPEC) possess a variety of virulence factors which enhance the ability of bacteria to overcome host defences, colonization of urinary tract, and subsequently cause UTI. These virulence factors include hemolysin, adherence, O-antigen, capsule-K-antigen, and others (Changyun Ye and Jianguovu, 2001). Many of these virulence factors encoded by gene cluster located on either the *E.coli* chromosome or plasmids (Rakasha *et al*., 2003). Attachment of UPEC to uroepithelial cells of urinary tract is of great importance in the pathogenesis of UTI. In the process of bacterial cytoadherence, infectious agent interfere with specific molecules on epithelial cells(Svenberg-Eden, 1993). In this respect bacteria

possess small surface-project called pili (fimbriae) that enable the organisms to attach to specific receptors on Uroepithelial cells. UPEC expresses an array of adhesins including ; P, S, Dr, and type-1 pili, which encoded by chromosomal gene clusters (Zieglar *et al*., 2004).

The major antibiotics classes currently in use for *E.coli* infections are the β-lactams, quinolones, aminoglcosides, teracyclines, and sulfonamides. Resistance in *E.coli* can result from gene mutations or transfer of resistance determinants (R- determinants) between stains or species. Clinically, gene transfer is the most common mechanism of transferring resistance. R-determinants are typically on plasmids, but they also may be part of mobile genetic elements(transposnes), which could move between plasmids or chromosomes in the same organisms or to new organisms.

Plasmid-associated resistance genes of *E.coli* have been discovered for the majority of known antimicrobials, including the quinolones, tetracyclines, chloramphenicol or trimethoprime, thus, *E.coli* is considered as candidate species in which new multi-resistance plasmids may be evolved (Sherley *et al*., 2004).

It is worth to mention that to date we know of no study that has attempted to infer possible correlation between plasmid antibiotics resistance and adherence of UPEC. However, there are studies which correlate between plasmids and adherence capability of diarrohgenic *E.coli* (Knutton *et al*., 1987; Di Martino *et al*., 1997); whereas, chromosomal determinant have shown being responsible for UPEC adherence(Zhang *et al*., 1997).

1.2 The aims of this study

1- Isolation and identification of *E.coli* from UTI patients .

2- Investigation of the antibiotic resistance of *E.coli* isolates and determination the antibiotic resistance patterns.

3- An attempt was made to infer the process of multiple resistance.

4- Role of plasmids in antibiotics resistance of UPEC.

5- The possible role of R plasmids in adherence ability of UPEC.

1.3 Discovery of urinary tract infection

Urinary tract infections (UTI) are among the most common bacterial infection that lead patients to seek medical care. Approximately 10% of human will have a UTI at some time during their lives (Forbes *et al*., 1991). 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. 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. Escherich (1894), who had previously found the organism *Escherichia coli* in the fecal flora, identified the same organism in the urine of children with cystitis. These findings were followed by the argument about the route of infection and whether it is haematogenous or ascending routes. Later on, the rapid development of antimicrobial agents was considered as a great success in the management of all types of infections including UTI (Asscher , 1980).

Thereafter, understanding the ultra structure of the causative organisms and the host –parasite interaction in the urinary tract, both have provided insights into the molecular basis of the disease process such as pathogen adhesion. Therefore, new approaches to prophylaxis and treatment of UTI have been explored (Eden *et al*, 1989).

1.4 Definition and symptoms

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).

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

1.5 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).

Table (1-1) The common microorganisms which are associated with UTI

Group	Organism	References
Gram negative bacteria	*E.coli*	Jawetz *et al*., 1998
	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 saprophyticus*	Glauser., 1986
	Staphylococcus epidermidis	Sobel. *et al*., 1993
	Staphylococcu aureus	Stamm., 1998
	Staphylococcus hemolyticus	Glauser., 1986
	Staphylococcus hyicus	Jawetz *et al*., 1998
	Streptococcus feacalis	Maskell., 1988
	S.milleri	Navarro *et al*., 1994
	Enterococci	Navarro *et al*., 1994
	Coryebacterium spp	Terai *et al*., 1994
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
	HIV	Glauser, 1986
	Herpes simplex virus	Mims *et al*., 1987

1.5.1 Gram negative bacteria

UTI studies indicated that there are several species in *Enterobacteriaceae* are found to be important causes of this disease. *Escherichia coli* is by far 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 (Jawetz *et al*., 1987; Johnson, 1991).

1.5.2 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). *S.aureus* and *Enterococci* are more associated with UTI in hospitalized patients (Glauser, 1986; Stamm, 1998).

1.5.3 Others

Yeast especially that belongs to *Candida* could also cause UTI. On other hand, *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. 6 Classification of UTI

There are several systems for classification of UTI.

A/ According to the site of infection, UTI can be classified into three major groups (Forbes *et al*., 1991).

- Uretheritis: infection of urethera.
- Cystitis: infection of bladder.
- Pyelonephritis: inflammation of the kidney parenchyma calices and pelvis.

B / Some investigators classified UTI according to type of pathogenesis into two groups

1- Uncomplicated UTI: occur in functionally and anatomically normal urinary tract and this in turn either symptomatic or asymptomatic. (Macleode & Edwards, 1995).

2-Complicated UTI: in this case there is functionally and anatomically abnormalities such as bladder out flow obstruction or prostate hyperplasia (Elgavish and Pattanik, 1993; Lennatte *et al*., 1985).

1. 7 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 become infected (Santoro and Kaye, 1978) , it includes the 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 (Stamey *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*., 1992).

1. 8 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 gelatine hydrolysis tests, lactose, D-mannitol and D-mannose fermenter, 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, *Escherichia blattae, E.coli, E.fergusonnii, E.hermanni, and E.vulneris. The* type species of the genus is *E.coli* (Collee *et al*., 1996).

E.coli bacteria were discovered in the human colon in 1885 by German bacteriologist Theodor Escherich. Soon after its discovery, *E.coli* become a very popular laboratory organism because scientists could grow it quickly on both simple and complex media. *E.coli* can grow in air, using oxygen as terminal electron receptor (aerobically) or without air by what is called fermentative metabolism. The ability to grow both aerobically and anaerobically 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).

E.coli is responsible for three types of infections in human:

1-Urinary tract infection (UTI).

2- Neonatal meningitis.

3-Gastroenteritis. By diarrohgenic group of *E.coli*, Enteropathogenic *E.coli* (EPEC), Enterotoxigenic *E.coli* (ETEC), Enterohaemorrhagic *E.coli* (EHEC), Enteroivassive *E.coli* (EIEC), Enteroaggregative *E.coli* (EAEC) (Forbes e*t al*., 1991).

E.hermanni and *E.vulneris* are most frequently obtained from wound infections but also isolated from infections at other body site (Betteileim, 1992). *E.fergusonnii* has been most frequently obtained from human feaces. *E.blattae*, is not isolated from human specimens (Cherly *et al*., 1999).

1 .9 Virulence factors of Uropathogenic *E.coli* (UPEC)

Uropathogenic strains of *E.coli* are characterized by expression of distinctive bacterial properties, products or structures referred to as virulence factors because they help the organism to overcome host defenses and colonize or invade the urinary tract (Johanson, 1991; Blanco, 1997).

1.9.1 Fimbriae and adherence ability

The ability to adhere to uroepithelial cell is considered as important virulence factor in UPEC, however presence of fimbriae on cell surface help the bacterium to attach at specific site on uroepithelial cells, which allow the bacteria to colonize host mucosal surface and invading host tissues, foil host defense mechanisms, and incite an injurious host inflammatory response (Zhang *et al*., 1997).

1.9.2 O -antigen (somatic or cell wall antigen)

Which is a lipopolysccharide in the outer membrane of *E.coli* consist of two parts, first the lipid part that is necessary for toxic activity; while, the second is a polysaccharide which consist of small constant region (core) and an outer part (O- polysaccharide), which is an important virulence factor of *E.coli*, and gives rise to different serotypes .

The most frequent ones found in UTI are type: O_1 ,O_2 ,O_4 ,O_6 ,O_7 ,O_8 ,O_9 ,O_{11} O_{16} ,O_{18} ,O_{22} ,O_{25} ,O_{39} ,O_{50} ,O_{62} , O_{75} and O_{78}, but almost any type may produce UTI although it is extremely rare (O'Hanely *et al*., 1985).

1.9. 3 Capsule - K - antigen

Capsule is slimy layer that is made of polysaccharide and is

considered as an important virulence factor of *E.coli* which causes attachment of bacteria to the epithelial cells or layers prior to the urinary tract invasion.Capsule-K- antigen pathogenicity is due to the prevention of complement dependent bactericidal action of the normal serum and inhibit phagocytosis such as K1 antigen (Gemski, 1980; Welch *et al*., 1978).

More urinary than fecal *E.coli* isolates are encapsulated and produce greater amounts of capsule, certain K types are more common among cystitis and pyelonephritis than fecal strains. K1 antigen is most common in neonatal meningitis and cross- react with a *Neisseria meningitides* group B capsule (Gemski *et al*., 1980).

1.9. 4 Hemolysin production

Which is an exotoxin, plays a role in permitting *E.coli* to initiate or sustain extra-intestinal infection, it is a virulence marker to symptomatic and asymptomatic UTI, this character is more common in strains causing UTI than those of normal flora of the intestine (Green and Thomas, 1981; Hughes *et al*., 1983). There are two types of heamolysin, first the filterable (α - hemolysin) which is cell free supernate and it's effect is directly on the bladder mucosa in case of UTI, it is also contributes to the damage of mucosal epithelial cells, especially in the bladder, furthermore, such toxin may impair the function of lymphocytes and other cell involved in the defense mechanism of the body (Mobley and Belas, 1995). The second type is the non-filterable (β-hemolysin) which associated with bacterial cell (Younis, 1986; Gupte, 1988).

1.9.5 Other virulence factors

Uropathogenic *E.coli* has other virulence factors that enhance colonization and facilitate invasion of the urinary tract causing UTI, these factors include:

1- Colicin-v (bacteriocins). These are antibiotic-like bactericidal substances produced by certain strains of bacteria and are active against some other strains of the same or closely related species, bacteriocin-producing strains are resistant to their own bacteriocin, thus it can be used for typing of organisms (Qauckenbush and Falkow, 1979). Colicin factor-1 is antimicrobial agent produced by Uropathogenic *E.coli* harboring non-conjugative plasmid (Sorsa *et al.*, 2003).

2- Siderophores. Uropathogenic strains of *E.coli* usually produce siderophores that play an important role in iron acquisition for the bacteria during or after colonization(Tolkoff-Rubin *et al.*, 2004). Uropathogenic *E.coli* have multiple iron acquisition mechanism including siderophore-mediated iron uptake system, which controlled chromosomally, but also can be transferred horizontally (i.e. can be controlled by genes on the plasmid) (Sorsa *et al.*, 2003).

3- Sensitivity to the bactericidal effect of normal human serum.

It was found that *E.coli* strains isolated from UTI patients are more resistant to the effect of human serum than strains of faecal origin (Svanborg-Eden *et al.*, 1978).

1.10 Bacterial adhesion

Bacterial adherence to host cells is a crucial step in the initiation of various infections, thus attachment of UPEC to specific sites on uroepithelial cell is of great importance in pathogenesis of UPEC.

Uropathogenic *E.coli* strains possess an array of adhesins including: type 1, P, S, and G fimbriae (some times called pili) as well as adhesion of Dr family and non fimbriae adhesins (NFAs).Adherence factors facilitate the colonization and persistence in the colon or vagina which may serve as reservoir for ascending infection in the urinary tract (Zhang *et al.*, 1997).

Matsumoto in 1998 found that the existence of adherence factor with bacterial cell surface thus enabling the bacteria to adhere to the tissue and so to develop infection. This suggests that the adhesion factor is an important virulence factor which assists the bacteria to colonize and make infection. The adherence of bacteria to epithelial cells is the net result of a complex process which often involve (lock and key type) interaction between lectin–like bacterial attachment protein called adhesin and specific complex carbohydrate structures of the host cell membrane (receptors) (Jackson *et al*., 1977; Fowler and Stammy, 1978).

However, it is found later that some bacteria may exhibit adhesive properties without expressing pili, these bacteria express soluble adhesin that surrounded the bacterial cells like a capsule (K- antigen) and was called non fimbriae adhesin (NFA) (Rosentein *et al*., 1984).

Studies carried out by several investigators show that fimbrial

adhesions have an independent genetic determination than those of NFA (Goldhar *et al*., 1987).

The adherence of bacteria to uroepithelial cells (UECs) assists bacteria to resist the mechanical wash by the flow of urine, which enables them to persist, colonize and invade the tissue (Hagberg *et al*., 1981).

The ability of bacteria to attach to UECs might be a virulence factor for *E.coli* strains which cause symptomatic UTI (Svanborg-Eden, 1979).

Eden in 1980 found that most of the *E.coli* strains tested attached to both sequamous and transitional epithelial cells.

1.10.1 Molecular basis of Bacterial adhesion

An array of adhesins in addition to type -1 pili (common pili), P, S, G, and X fimbriae as well as adhesins of Dr family and non fimbrial adhesins (NFAs) have been identified in UPEC (Zhang *et al*., 1997). These above mentioned adhesins are encoded by clustering of virulence genes on the chromosome termed pathogenicity–associated islands (PAIs) and these stretches of DNA are not found in non UPEC (Tolkoff-Rubin *et al*., 2004). P.fimbriae production is regulated by chromosomal *pap* operon contaning 11 genes (Maning *et al*., 2001).

However other pathogenic *E.coli* such as: ETEC possess specialized pili called Colonization factor antigens(CFAs), which is responsible for attachment of ETEC to intestinal epithelial cells and are encoded by plasmid(Hardy, 1986).

1. 11 Fimbriae (pili)

Fimbriae are proteinaceous rigid hair-like extensions projected from bacterial cells which recognize specific receptor molecules on the surface of host cell membrane usually carbohydrate, glycolipids or glycoproteins (Kallenius *et al*., 1986). The diameter of fimbriae range between 7 – 10 nm, therefore they can be demonstrated by electron microscope. They develop in freshly isolated strains and in liquid culture and tend to disappear following subculture on solid media (Gaastra and Dewraaf, 1987).

Fimbriae are important for bacterial attachment to mucosal surface, which is the initial step in the pathogenesis of UTI (Mandell *et al*., 2000).

According to their morphology, several types of pili have been identified in *E.coli* strains causing UTI (table 1 - 2) which include:

1- Mannose – sensitive (type 1) pili. Also called common pili of *E.coli*, these are very stable protein firmly attached to the bacterium, they are more difficult to remove than flagella or sex pili (Novotny *et al*., 1969).

Type -1 fimbriae is known to bind to D-mannose residues on epithelial cells causing agglutination of guinea pig erythrocytes, the agglutination is inhibited by presence of mannose and is called mannose sensitive (MS) (kallenius *et al*., 1981). MS fimbriae are widely found on most *E.coli* isolates, and various studies suggest that virtually all Uropathogenic *E.coli* strains have at least the capacity to express these fimbriae *in vitro* (Eden *et al*., 1989).

Table (1 – 2) Uropathogenic *E.coli* adhesins and corresponding epithelial receptors (Sobel *et al*., 1993).

Adhesin	Receptor
Type 1 fimbriae (MS)	D- mannose on epithelial cells
P-fimbriae (MR)	Gal α 1-4 Gal (P-blood group antigen)
S-fimbriae (MR)	α Sialyl 2-3 galactoside
Type 1C (MR)	Undetermined
G-fimbriae (MR)	Terminal N-acetyl-D-glucosamine
M-fimbriae (MR)	Galactose-n-acetyl-glucosamine
Non –fimbrial adhesins	Undetermined
Dr hemagglutinin	Dr blood group antigen

MS = Mannose sensitive; MR = Mannose resistant.

2- Mannose resistant pili.

They are thin and show no inhibition in the presence of mannose so they are called mannose resistant (MR) pili (Leffler and Svanborg, 1981).

These adhesins have been given a variety of names such as, (P fimbriae, P pili, pap pili (pyelonephritis associated pili)) reflecting their association with pyelonephritis and particular receptor(Tolkoff-Rubin *et al*., 2004). P pili bind to α-D-Gal (1-4) B-D-Gal belonging to the globoseries of glycolipids which are bound on P-blood group antigens and on uroepithelial cell (Dobrindt *et al*., 2001). Both MS and MR pili may coexist on the same bacterial strains (Sobel *et al*., 1993).

3- X- fimbrial type, has been described by Schoolnick *et al*., 1993; this type resemble P-fimbriae (in term of haemagglutination i.e. mannose resistant) but appear to attach to a totally different human epithelial cell receptors (Sobel *et al*., 1993).

4- Several other fimbrial adhesins have been identified in a smaller number of Uropathogenic *E.coli* species, these include: S, type-1C, G-fimbriae, M- and F- adhesin (Sobel *et al*., 1993).

There are other types of pili exist on the surface of UPEC which include:

1- Fertility pili (F) or sex pili. They are longer than the common and colicin pili, they are about 20 nm in length, but in fewer numbers than common pili on the surface of the cells, they are involved in fertilization through DNA passage from one bacterium to another by conjugation process (Crawford and Gesteland, 1964; Novotny *et al*., 1969).

2- Colicin pili: (col-1). They are about 2 nm in length and are associated with colicin factor which is antimicrobial agent produced by *E.coli* harboring small non conjugative plasmid (Jawetz *et al*., 1998).

1.12 Genetic of Uropathogenic *E.coli* virulence

Virulence factors in bacteria may be encoded on chromosomal DNA, bacteriophage DNA, plasmids, or transposons in either plasmid or the bacterial chromosome, thus when bacterial cells harbor genes which are responsible for coding certain virulence factors, bacterial cell will be transferred from avirulent cell to virulent one (Tolkoff-Rubin *et al*., 2004).

The virulent *E.coli* strains that cause UTI are distinct in their virulence factors(table 1 – 3) from most intestinal commensal *E.coli* types(Chang Yun and Juguo xu ,2001). In this respect diarrheagenic *E.coli* and UPEC strains possess different types of adhesins (table 1 – 4)(Tolkoff-Rubin *et al.,* 2004).

Table (1 – 3) Virulence factors of UPEC and their encoded gene location(Tolkoff-Rubin *et al.,* 2004).

Virulence factors	Chromosomal encoded	Plasmid encoded
1- Adhesion factors type-1, P, S, G, and X	+	-
2- O -somatic antigen	+	-
3- Capsule K - antigen	+	-
4- Heamolysin production	+	-
5- Bacteriocin production (colicin – V)	-	+
6- Siderophore production	+	+

Table (1 – 4) Different types of adhesins and their genes location in different bacterial isolates.

Bacteria	**Adhesins**	**Gene location**	**Reference**
1- ETEC	CFAs	chromosomal +Plasmids	Qadri *et al.*, 2000
2- EPEC	EAF	Plasmids	Knutton *et al.*, 1986
3- EHEC	BFP	Plasmids	Knutton *et al.*, 1986
4- UPEC	P- fimbriae	Chromosomal	Maning *et al.*, 2001
5- *Proteus spp*	MR / P fimbriae	Chromosomal	Bahrani and Molby, 1994
6- *K.pneumoniae*	MR/P fimbriae	Chromosomal	Old and Adegbold, 1985

1.13 Antibiotics treatment of UTI

Many criteria considered for choice of drug for treatment of UTI, these include: the drug is active against the infecting organisms, non toxic, the dose obtained, the effect of pH and possess no or little effect on normal flora of intestine and other regions (Glauser, 1986).

1.13.1 β-lactam antibiotics

β-lactam antibiotics irreversibly inhibit enzymes involved in the final steps of cell wall synthesis, the enzymes inhibited by β-lactam drugs mediate the formation of peptide bridges between adjacent strands of peptidoglycan which called penicillin–binding–proteins (PBPs) (Nester *et al*., 2001).All members of this group have a shared chemical structure called β-lactam ring (Chambers *et al*., 2001).

β-lactam include two groups: Penicillin and Cephalosporines .The penicillin group is the most widely used group of antibiotics due not only to their action but for their stability to gastric acid, thus, they are suitable for oral administration, and also for their possessing less toxicity than the most of the other antibiotics (Jawetz *et al*., 1998).

Ampicillin and amoxicillin are given orally to treat UTI, otitis, and lower respiratory infection, they are active against Gram negative such as : *E.coli* and *P.mirabilis,* but they are inactivated by β-lactamases enzyme (Cercerado *et al*., 1990), they have the same spectrum and activity but amoxicillin is better absorbed from the gut, less making diarrhea and also give high blood level (chambers *et al*., 2001).

A combination of amoxicillin with β-lactamase inhibitor clavulanic acid, that interfere with the activity of some types of β-lactamases and thus amoxicillin is protected against enzymatic destruction(Nester *et al*., 2001; Chambers *et al*., 2001).

Cephalosporines are derived from an antibiotic produced by the fungus called *Cephalosporium acremonium*, they are β-lactam antibiotics with a nucleus of 7-aminocephalosporic acid, natural cephalosporines have low antibacterial activity, but the attachment of various R1 and R2 groups result in proliferation of enormous array of active drugs with varying pharmacologic properties and antimicrobial activity (Jawetz *et al*., 1998).

Cephalosporines were originally introduced for use in patients with allergy with penicillins (Davis *et al*., 1990).

Cephalosporines are divided into 4 major generations depending mainly on the spectrum of antimicrobial activity (Chambers *et al*., 2001).

First generation cephalosporines include: cephalexin, cephalothin, cefadroxil, cefazolin, and cephradine, are active against Gram positive bacteria and are moderately active against some Gram negative rods such as *E.coli*, *Proteus*, and *Klebseilla* (Mitsuhashi, 1980).

Cephalexin, cephradine, and cefadroxil are absorbed from the gut to variable extent, and urine concentration is usually very high, so oral drugs may be used for the treatment of UTI (Chambers *et al*., 2001).

Second generation cephalosporines include: cefaclor, cefuroxime, cefprozil, loracarbef, and cefonicid. All these antibiotics are less active against Gram positive bacteria than first generation (Chambers *et al*., 2001),

but are more active than the first generation by their activity against Gram negative rods, such as *E.coli* , and *Proteus* (Jawetz *et al*., 1998). Cefuroxime is resistant to β-lactamase, so it could be used against the *Enterobacteria* which produce β-lactamase (Neu, 1991).

The third generation of cephalosporines have major advantages as their expanded Gram negative coverage and the ability of some to cross the blood brain barrier(Chambers *et al*., 2001). Cefotaxime is one of the antibiotics which belong to the third generation cephalosporines and it had a strong activity against *E.coli*, *Proteus*, *Klebsiella*, and *Serratia* (Mitsuhashi, 1980).

Cefepime is an example of fourth generation cephalosporines, it is more resistant to hydrolysis by chromosomal β-lactamases, and some extended–spectrum β-lactamases that inactivate many of the third generation cephalosporines (Chambers *et al*., 2001).

1.13.1.1 Resistance to β-lactam

The resistance of bacteria to β-lactam is due to degradation of drug by β-lactamase which is produced by bacteria, lack or poor permeability to the drug by bacterial cell and lack or altered PBP which is drug receptors on the cell wall and some of these are enzymes involved in transpeptidation reaction (Nester *et al*., 1998).

The major basis for bacterial resistance to penicillines is the inactivation of drug by β-lactamase, which inhibit the activity of β-lactam antibiotics by breaking the β-lactam ring of antibiotic (Arakwa *et al*.,1989).

The inheritance of β-lactamase is either encoded by chromosome or plasmid (Wiedemann, 1990).

β-lactamase encoded by chromosome was observed in most Gram negative bacteria, while β-lactamase such as, penicillinase is encoded mostly by plasmid in *S.auerus* bacteria (95%of *S.aureus* producing plasmid encoded β-lactamase), however some strains produce both types of β-lactamase (i.e. chromosomal or plasmid mediated) (Hardy, 1986).

Most of the enzymes produced by Gram negative bacteria belong to the TEM-1 or TEM -2 types (Davis *et al*., 1990). Recently , it was found that members of *Enterobacteriaceae* such as, *E.coli*, *Klebsiella,* and *Proteus mirabilis* have the ability to produce plasmid encoded enzymes called Extended spectrum β-lactamase (ESBLs) which enable the bacteria to resist most active β-lactams which are third generation cephalosporines, in addition at least six chromosomally mediated β-lactamases have been distinguished in ampicillin resistant *E.coli* by isoelectric focusing (Di Martino *et al*., 1997; Luzzaro *et al*., 2001).

Resistance of *E.coli* against β-lactam and β-lactam inhibitor combination arisen from the resistant *E.coli* strains which produce high level of β-lactamase than the amount of inhibitor permeating into the cell would be insufficient to inactivate all the β-lactamase (Chambers *et al*., 2001).

Resistant to β-lactam spreads among member of *Enterobacteriaceae* by plasmids. Morever, Rassol *et al.,* in 2003 successeded in transfer the resistant to β-lactam from *Kebsiella pneumoniae* to *E.coli* by transconjugation.

1.13.2 Aminoglycosides

Aminoglycosides are group of bactericidal antibiotics originally obtained from various *Streptomyces* species, have a hexose ring, to which various amino sugars are attached by glycosidic linkages (Chambers *et al*., 2001). All aminoglycosides inhibit protein synthesis by attaching to and inhibiting the function of 30S subunit of bacterial ribosome (Davis *et al*., 1990). Streptomycin is considered as oldest aminoglycoside drug which also still drug of choice for treatment of bacteria which resist to other type of drugs, however common Gram negative bacteria develop resistant to this drug (Mingeot-lecterco *et al*., 1999).

Aminoglycosides especially gentamicin used widely in treatment of UTI, so it is considered as drug of choice in this field (Merin *et al*., 1988).

Amikacin is one of the important drugs which belongs to aminoglycoside group, many Gram negative enteric bacteria including *E.coli*, *Proteus*, *Serratia*, and *Pseudomonas* are inhibited by amikacin, which is resistant to many enzymes that inactivate gentamicin and tobramicin (Chambers *et al*.,2001). Several studies on uropathogenic *E.coli* strains show that the amikacin is more active than streptomycin and gentamicin (Sotto *et al*., 2001).

1.13.2.1 Resistance to aminoglycosides

The resistance to aminoglycoside was due to enzymatic destruction (inactivation) of drug (plasmid mediated transmissible resistant), alteration or deletion of ribosomal receptor (chromosomal mutant), and impaired

entry of aminoglycosides into cell (Chambers *et al*., 2001).

In clinical isolates of Gram negative organism resistance to aminoglycosides is due to the production of enzyme that specifically modify the antibiotic, so that it can no longer gain entry into the bacterial cell (Joklik *et al*., 1984). The enzymes inactivate the aminoglycosides by: acetylation of amino groups (acetyltransfrase), phosphorylation of hydroxyl group (phosphortransferas), or adenylylaion of hydroxyl group (adenyltransferase) (Forbes *et al*., 1998).

1.13.3 Quinolones

The mode of action of all quinolones is blocking bacterial DNA synthesis by inhibiting bacterial DNA gyrase, which prevents the relaxation of positively supercoiled DNA that is required for normal transcription and replication (Chambers *et al*., 2001).

Quinolones include many antibiotics such as: Naldixic acid, ciprofloxacin, norfloxacin, and ofloxacin (Pimental *et al*., 1998).

Quinolones are potent bactericidal agents, they have a broad spectrum of activity that includes Gram negative and gram positive bacteria (Forbes *et al*., 2001).

Ciprofloxacin and Naldixic acid are effective antibiotics in UTI even when caused by Multidrug resistant bacteria (Chambers *et al*., 2001).

Ciprofloxacin has become one of the most widely prescribed antimicrobial agents and has low incidence of side effect (Mulhall and Bergann, 1995).

1.13.3.1 Resistance to Quinolones

Chromosomal resistance to Quinolones is developed by mutations which either cause an alteration in the A subunit of the target enzyme (DNA gyrase) or change in outer membrane permeability, which on other hand result in decrease of drug accumulation (Forbes *et al*., 2001).

It was found that resistance to naldixic acid and ciprofloxacin, could be encoded by plasmid which could be transferred between *Enterobacteriaceae* and *P. aeroginosa* (Martinez – Martinez *et al*., 1998).

1.13.4 Macrolides

The macrolides are group of closely related compounds characterized by a macro-cyclic lactone ring, the erythromycin (representative of this group) was obtained in 1952 from *Streptomyces erythreus* (Chambers *et al*., 2001). Macrolides have been widely used to treat various infections, they bind to 25S rRNA on the 50S ribosomal subunit resulting in blockage of transpeptidation and or translocation resulting inhibition of protein synthesis (Kawamura-sato *et al*., 2000). The antimicrobial activity of macrolides is broad spectrum; thus macrolides antibiotics are used against Gram positive and some Gram negative bacteria (Jawetz *et al*., 1998).

1.13.4.1 Resistance to macrolides

Resistance to erythromycin result from an altered rRNA receptor, this is caused by chromosomal mutation, or under control of transmissible plasmids, commonly found in *S.auerus* and *Streptococcus* species (Chambers *et al*., 2001).

1.13.5 Tetracyclines

The tetracyclines are large group of drugs with a common basic structure and activity, tetracyclines obtained by catalytic dehalogenation of chlortetracycline, which (chlortetracycline) is isolated from *Strptomyces aureofaciens* (Chambers *et al*., 2001).

Tetracycline is concentrated intracellularly by sensitive bacteria and as a result protein synthesis is stopped by inhibit binding of aminoacyl-tRNA to the 30S subunit of bacterial ribosome (Joklik *et al*., 1984).

Tetracyclines are broad spectrum antibiotics and bacteriostatic antibiotics for many Gram positive bacteria and several intracellular bacterial pathogens such as *Chlamydia*, *Rikettsia* (Forbes *et al*., 2001).

1.13.5.1 Resistance to tetracyclines

The most important mechanisms of resistance to tetracycline is by decreasing intracellular accumulation due to either impaired influx, or increased efflux, by an active transport protein pump, this pump protein encoded by transmissible plasmid (Chambers *et al*., 2001; Nester *et al*., 2001).

Tn 10 is one of the transposone found in *Enterobacteriaceae* which causes for tetracycline resistance, the transposone encodes a protein pump in the envelop of *E.coli* (Hardy, 1986). Other mechanisms have been included in tetracycline resistance such as: enzymatic inactivation of tetracycline, and ribosomal protection by interfering with tetracycline binding to the ribosome (Chambers *et al*., 2001). Chopra in 1985 found that the wide use of tetracycline result in spread bacterial resistance in most *Enterobacteriaceae*.

1.13.6 Chloramphenicol

Chloramphenicol was first isolated in 1947 from cultures of *Streptomyces venezuelae*, it was synthesized in 1949, becoming the first completely synthetic antibiotic of importance to be produced commercially (Chambers *et al*., 2001).

Chloramphenicol: is a potent inhibitor of microbial protein synthesis, it binds to the 50S subunit of bacterial ribosome, inhibiting the peptidyltransferase step of protein synthesis (Nester *et al*., 2001).

Chloramphenicol is bacteriostatic and broad spectrum, it is active against both aerobic and anaerobic Gram positive and Gram negative organisms (Chambers *et al*., 2001).

1.13.6.1 Resistance to chloramphenicol

Clinically significant resistance to chloramphenicol is due to production of chloramphenicol acetyltransferase, a plasmid-encoded enzyme that inactivates the antibiotic (Jawetz *et al*., 1998).

The enzyme is found intracellulary and is synthesized constitutively in Gram negative bacteria; while in *S.aureus* it is induced by the presence of the antibiotic(Joklik *et al*., 1984).

1.13.7 Trimethoprim-sulfamethaxazol (SXT)

Frequently trimethoprim is combined with a sulfonamide (usually sulfamethaxazol) to produce bactericidal agent that can simultaneously attach two targets (competition with enzymes) on the same folic acid metabolic pathway (Forbes *et al*., 1998). SXT has been the most widely used

antimicrobial for the treatment of acute UTI, it is effective against most common uropathogens including *E.coli, Klebsiella*, and *Proteus spp* (Schaeffer, 1998).

1.13.7.1 Resistance to SXT

Most common cause for resistance is due to production of resistance enzymes to both antibiotic, which are often under plasmid control(Chambers *et al*., 2001). SXT resistance among urinary tract isolates has been reported with an increase frequency in Canada and united states (Zhanel *et al*., 2000).

1.13.8 Nitrofurantion

It is one of urinary antiseptics which exert antibacterial activity in the urine, but have little or no systemic antibacterial effect, its usefulness is limited to lower UTI (Chambers *et al*., 2001). It is bacteriostatic and bactericidal for many Gram positive and Gram negative bacteria, the mechanism of action is not clearly defined and clinical drug resistant emerges slowly (Forbes *et al*., 1998).

1.14 Bacterial plasmids

1.14.1 Definition

Plasmids are extra chromosomal molecule of DNA that multiply independently of the chromosome, they have own replication origins which can stably inherited through generations (Boyd, 1988; Nester *et al*., 2000). Plasmids are found in most bacterial species, they are also present in some fungi, protozoa, and present in some species of eukaryotes(Freifelder, 1987)

1.14.2 Structure and physical properties

Bacterial plasmids are molecules of double–stranded DNA their molecular weights range from about 1×10^6 to 200×10^6 (less than 1 K Dalton – 200 K Dalton), therefore range in size from about 0.04% to 8% of *E.coli* chromosome (Hardy, 1986; Boyd, 1988). Plasmid can be found in different forms; covalently closed circular (CCC), open circular, and linear (Boyd, 1988).

Most of plasmid DNA inside bacteria is in the form of CCC, meaning that there are no breaks in either of two polynucleotide strands which comprise the double helix (Hardy, 1986), most of the CCC plasmid molecules isolated from bacteria are twisted to form supercoiled molecules which have superhelical twists (Hardy,1986; Davis *et al*., 1990).

If one of the two polynucleotide strands in a closed-circular plasmid is broken or nicked, an open circle is formed, if the superhelical twists of supercoiled plasmid are lost, the molecule unwinds and becomes relaxed, when both polynucleotides are broken a linear molecule is formed (Hardy, 1986).

1.14.3 Plasmids replication

Many plasmids are maintained in bacterial cultures, they are heavily dependent on the metabolic functions of the host cell, for their reproduction, they can only replicate within host cell, they replicate in semi conservative manner and maintain circularity throughout replication cycle (Hardy, 1986; Freifelder,1987).

All plasmids have a replicon function, meaning that they contain an origin of replication and a mechanism to control the frequency of initiation of replication(Prescott, 1990).

All types of plasmids replicate by one of two general mechanisms, unidirectional, and bidirectional replication (forming θ replication (butterfly mode)), however some plasmids in which both modes are present (Freifelder, 1987). Plasmids replication is conveniently divided into 3 major stages: initiation, elongation of polynucleotide chains by semi-conservative synthesis, and termination (Hardy, 1986).

1.14.4 Plasmids function

Plasmids have relatively few genes, generally less than 30, their genetic information is not essential but it is useful to the host, and bacteria that lack them usually function normal (Prescott, 1990).Plasmids carry optional genes that confer additional properties to bacterial cell which include:

1- Resistance to various antibiotics, metal ions, ultraviolet irradiation and serum bactericidal activity (Davis *et al*., 1990).

2- Production of bacteriocins, protease, heamolysin, surface antigens (adhesins), and antibiotics (Boyd, 1988).

3- Fertility, which responsible for sex pili production (Prescott, 1990).

4- Degradation of certain compounds such as: toluene, oil, salicylate, and lactose (Prescott *et al*., 1990).

1.14.4.1 Resistant plasmids (R plasmids)

R plasmids (R-factor) are the most important group of plasmids that carry genes for resistance to one and often several antimicrobial drugs (Davis *et al*., 1990).

Many R plasmids composed of two parts; resistance R genes, which code for the resistance traits, these genes are mostly located in transposons (small elements of DNA that are capable of movement from one position to another in the DNA), and resistance transfer factor RTF, which code for transfer of plasmid to other bacteria by conjugation (Davis *et al*., 1990; Nester *et al*., 2001).

R plasmids were discovered in 1959 in Japan as the cause of a rapid increase in multiple drug resistance to sulfonamides, streptomycin, chloramphenicol, and tetracycline in *Shigella dysenteriae*, and they were also found to move freely between this pathogen and ordinary *E.coli* strains (Joklik *et al*., 1984; Hardy, 1986; Davis *et al*., 1990).

The R factors are large, self transmissible plasmids, widely distributed among E*nterobacteria* and also other Gram negative organisms(Davis *et al*., 1990). R plasmids have wide host ranges and can multiply in a wide variety of different Gram negative genera (Nester *et al*.,2001).

Some of R plasmids are non conjugative plasmids, they are smaller in mass (about 5×10^6 Daltons), than conjugative plasmids (40×10^6 to 200×10^6 Daltons) and rarely encoding for more than two antibiotic resistance genes

(Hardy, 1986; Freifelder, 1987), their transfer is mediated by co-resident conjugative plasmids by the process of mobilization (Joklik *et al.*,1984).

1.14.4.2 Virulence plasmids

Virulence plasmids make their host more pathogenic because the bacterium is better able to resist host defense or to produce toxins, for example, colonization factor antigens CFAs (adhesins) which are produced by Enterotoxigenic *E.coli* strains enabling the bacteria to adhere intestinal cells are plasmid-determined(Hardy, 1986). Two types of toxins; heat labile toxin (LT) and heat stale toxin (ST), are also produced by *E.coli* responsible for traveler's diarrhea, both toxin genes are plasmid borne(Prescott *et al.*, 1990).

1.14.5 Plasmid curing

1.14.5.1 Definition

Plasmid curing defined as the process by which the whole plasmid (or part of it) can be eliminated or removed from the host cell by using either chemical or physical agents. However curing may also occur spontaneously (Bguanchan *et al.*, 1969; Hahan and Ciak, 1976; Prescott *et al.*,1990), spontaneous segregation occurs to some plasmids during cell division, then lost from bacterial cell (Hardy, 1986).

The location of antibiotics resistance determinants either on plasmid or chromosomal could be determined by carrying out plasmid curing experiments (Rassol *et al.*, 2003).

1.14.5.2 Plasmid curing by chemical agents

Chemical agents which are used in plasmid curing are intercalating with DNA replication, such as, acridine orange and ethidium bromide, these two agents have been used in plasmid curing in most *Enterobacteriaceae* and *S.aureus* (Rubin and Rosenbium, 1971).

Other compounds are found to have plasmid curing ability, SDS has the ability to cure some plasmids, this chemical was found to be effective in eliminating plasmids from bacterial cells, and was found more effective as compared with ethidium bromide or physical treatment in Gram positive bacteria (Sonstein and Baldwin, 1972).

Trevors in 1986 found that acridine orange, ethidium bromide, mitomycin C, and acriflavin have curing ability of plasmids in different degrees.

Some antibiotics such as, rifampicin and novobiocin have been found the ability to cure plasmid DNA (Johnston and Richmond, 1970).

Chemical curing agents if administered to bacterial population in sub lethal doses, can lead to inhibit plasmid replication without harming bacterial chromosomal replication, and thus maintaining the ability of bacteria to reproduce and generate offspring (Hahan and ciak, 1975).

2.1 Materials

2.1.1 Equipments

Equipments	Company (Country)
Autoclave	Gallenkamp(England)
Balance	Ohans (France)
Compound light microscope	Olympus (Japan)
Distillator	Gallenkamp (England)
Electrophoresis machine	Gallenkamp (England)
Hot plate with magnetic stirrer	Gallenkamp(England)
Incubator	Gallenkamp(England)
Micropipette	Witeg(Germany)
Millipore filter	Millipore and Whatman(England)
Oven	Memert (Germeny)
pH- Meter	Meter-GmpH Tdedo(U.K)
Power supply	Aurora instruments Ltd. (England)
Portable centrifuge	Hermle labortechnik(Germany)
Refrigerator centrifuge	Harrier(U.K)
Shaker incubator	GFL(Germany)
Spectrophotometer	Aurora instruments Ltd. (England)
U.V transilluminator	Gallenkamp (England)
Water bath	GFL(England)

2.1.2 Media

Medium	Company (Country)
Blood base agar	Mast-diagnostic (England)
MacConkey agar (Mac agar)	Oxiod (England)
Muller hinton agar	Biokit S.A (spain)
Nutrient agar (N.A)	Oxiod (England)
Nutrient broth (N.B)	Oxiod (England)
Simon citrate media	Difco (U.S.A)
Triple sugar iron agar (TSI)	Difco (U.S.A)
Trypton	Difco (U.S.A)
Urea agar base	Oxiod (England)
Yeast extract	Pharmacos LTD

2.1.3 Chemicals

Chemicals	Company (Country)
Acridine orange	BDH (England)
Agarose	BDH (England)
Bromophenol blue	BDH (England)
Crystal violet	BDH (England)
Disodium hydrogen phosphate	BDH (England)
EDTA	Fluka (Switzerland)
Ethanol	Riedel-De Haen (Germany)
Glacial acetic acid	Certified analytical reagents (England)

Glucose	BDH (England)
Hydrochrolic acid	BDH (England)
Methanol	BDH (England)
Potassium chloride	BDH (England)
Potassium dihydrogen phosphate	BDH (England)
Sodium dihydrogen phosphate	BDH (England)
Sodium hydroxide	Fluka (Switzerland)
Sodium chloride	Riedel-De Haeny (Germany)
Sodium dodecyle sulphate (SDS)	Fluka (Switzerland)
Tris –base	SIGMA (Germany)
Urea	BDH (England)

2.1.4 API 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 Antibiotic disks

Antibiotic agents	Code	Concentration (µg)	Source (Country)
Amikacin	AK	30	Bioanalyse (Turkey)
Ampicillin	AM	10	Bioanalyse (Turkey)
Amoxicillin + clavulanic acid	AMC	20/10	Bioanalyse (Turkey)
Cefotaxime	CTX	30	Bioanalyse (Turkey)
Cephalexin	CL	30	Bioanalyse (Turkey)
Chloramphenicol	C	30	Bioanalyse (Turkey)
Ciprofloxacin	CIP	5	Bioanalyse (Turkey)
Erythromcin	E	15	Bioanalyse (Turkey)
Gentamicin	CN	10	Bioanalyse (Turkey)
Naldixic acid	NA	30	Bioanalyse (Turkey)
Nitrofuratoin	F	300	Bioanalyse (Turkey)
Streptomycin	S	10	Bioanalyse (Turkey)
Tetracycline	TE	30	Bioanalyse (Turkey)
Trimethoprim + Sulphamethoxazole	SXT	1.25 /23.75	Bioanalyse (Turkey)

2.2 Plasmid

Plasmid	Source (Country)	Concentration	Molecular weight
pBR 322	Invitrogen ,life technologies (U.S.A)	0.25 µg/µl	2.8×10^6 Daltons (4363 base pairs)

2.3 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 D.W when needed.

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

It was prepared by dissolving 1 g of para-dimethyl aminobnzaldehyde in 15 ml of isoamyl alcohol, then 5 ml of concentrated HCL added carefully and gradually and kept in refrigerator, this reagent was used in indole test.

2.4 Buffers used in adhesion test

2.4.1 Phosphate Buffer Saline (PBS) pH 7.0 (Cruikshank *et al.*, 1975)

NaCl	8 g
KCl	0.2 g
Na_2HPO_4	1.15 g
Distilled water	1000 ml

Sterilized by autoclave at 121°C for 15 minutes.

2.5 Solutions and buffers used for plasmid DNA isolation (Sambrook *et al.*, 1989)

2.5.1 TE buffer

10 mM Tris. Cl (pH 8.0)

1 mM EDTA (pH8.0)

It was prepared by dissolving 0.12 g of Tris, 0.037g of EDTA with 60ml D.W, the pH was adjusted with concentrated HCL to the desired (pH 8.0), cooled to room temperature, the volume was then completed to 100 ml with D.W, sterilized by autoclave at 121°C for 5 minutes, and stored in refrigerator.

2.5.2 Solution I

It was prepared by dissolving the following ingredients in 60 ml D.W

Glucose	0.9 g
Tris base	0.3 g
EDTA	0.37 g

The pH was adjusted by concentrated HCL to the desired (pH 8.0), the volume was then completed to 100 ml with D.W, sterilized by filtration with Millipore filter paper, and stored in refrigerator.

2.5.3 10 N NaOH (stock solution).

It was prepared by dissolving 4g of NaOH to 10 ml of D.W, and stored in refrigerator until needed.

2.5.4 10% SDS (stock solution)

It was prepared by dissolving 10 g of SDS in 90 ml D.W, heated to 68°**C** to assist dissolution, the pH was adjusted to (pH 7.2) by adding few drops of concentrated HCL, and the volume was completed to 100 ml with D.W.

2.5.5 Solution II (freshly prepared)

It was prepared by adding 0.1 ml of 10 N NaOH (2.5.3) to 0.5 ml of 10% SDS (2.5.4), the volume was completed to 5 ml with D.W, and used directly.

2.5.6 5 M potassium acetate

It was prepared by dissolving 4.91g of Potassium acetate in 6 ml of D.W, the pH was adjusted to (7.5), and the volume was completed to 10 ml.

2.5.7 Solution III

To 6 ml of 5 M K-acetate (2.5.6), 1.15 ml of glacial acetic acid and 2.85 ml of D.W were added, and stored in refrigerator.

2.5.8 70 % ethanol

It was prepared by mixing 70 ml of absolute ethanol with 30 ml D.W stored in room temperature.

2.6 Solutions and buffers used in electrophoresis (Sambrook *et al.*, 1989)

2.6.1 TAE buffer (50 X)

Tris –base	242 g
Glacial acetic acid	57.1 ml
0.5 M EDTA	100 ml

pH adjusted at 8.0, and stored at room temperature.

2.6.2 TAE buffer (1X)

This buffer was prepared by dilution 1:49 V:V of 50X TAE (2.6.1.) with D.W.

2.6.3 Ethidium bromide (10 mg / ml) (Ausubel *et al.*, 1987)

This solution was prepared by adding 50 g Ethidium bromide in 5 ml D.W. and kept in brown container.

2.6.4 Loading buffer

loading buffer was obtained as prepared buffer from Analytica company with following composition:

Sucrose	50 %
EDTA	0.1 M (pH 8.0)
Bromophenol blue	0.2 %

2.7 Solution used in curing experiment

2.7.1 Acridine orange stock solution (1 mg / ml) (Miller, 1972)

It was prepared immediately before use by dissolving 10 mg of acridine orange in 10 ml sterile D.W. and diluted as required.

2.8 Methods

2.8.1 Media preparation

1- Nutrient agar, nutrient broth, MacConkey agar, and Muller Hinton agar.

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

2- Blood agar.

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-dishs.

3- Urea agar.

An amount of 950 ml of urea agar base (Christnsens media) was prepared as recommended by manufacturing company, autoclaved at 121°C for 15 minutes, cooled to 50°C, then 50 ml of 40% urea was added and sterilized by filtration. The medium was dispensed as slant.

4- 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.

5-Luria Bertani broth medium (L.B broth medium) (Sambrook *et al.*, 1989).

It was prepared by dissolving the following ingredients in 90 ml of D.W:

Trypton	1 g
Yeast extract	0.5 g
NaCl	1 g

pH was adjusted to 7.0, and then the volume was completed to 100 ml with D.W, sterilized by autoclaving at 121°**C** for 15 minutes.

2.8.2 Collection of urine samples

Midstream urine samples were collected in sterile cups from outpatient people of private urology clinic and central public health laboratory during the period 1/12/2005 to 21/3/2006.

2.8.3 Bacterial isolation

Loopful of undiluted urine samples were spread on blood agar and MacConkey agar plates. Plates were incubated overnight at 37°**C**, then single colonies were observed for their lactose fermenting ability.

Then the colonies which showed positive reaction were transferred to new MacConkey agar plate for further purification by dilution streaking to obtain single isolated colonies, then used for further diagnosis.

2.8.4 Maintenance of bacterial isolates

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

1- 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.

2- 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 2-3 ml of nutrient agar media and stored at 4°C .

2.8.5 Biochemical tests for characterization of bacterial isolates.

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 (2.3.1), 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 indicate a positive test.

2- Urease test (Atlas, 1995).

Urease activity was detected by inoculating the surface of Christensen urease agar slants(2.8.1.3) with bacterial growth and inoculated 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 (2.8.1.4) surface then incubated for (24 – 48 hrs) at 37 °C. If

the color of medium is changed from red to yellow this indicates acid formation; and appearance of black precipitate indicates ferric sulfate formation; whereas pushing and cracking the agar to the top indicate CO_2 formation.

4- Simon citrate test (Collee *etal.*, 1990)

Simon citrate agar(2.8.1.4) 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 indicates positive result.

5- Indole test (Collee *etal.*, 1996)

5 ml of peptone water inoculated by bacterial colony, incubated at 37°C for 48 hrs, then 0.05 ml of Kovac's reagent (2.3.2) was added with mixing the contents of tube gently, appearance of red ring on the surface indicates positive result.

2.8.6 Identification of isolates

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).

The biochemical tests included in this system are the following:

1- Beta-galactoside test ONPG.

2- Arginine dihydrolase test ADH.

3- Lysine decarboxylase test LDC.

4- Ornithine dearboxylase test ODC.

5- Citrate utilization test CIT.

6- Hydrogen sulphide test H_2S.

7- Urease test URE.

8- Tryptophane deaminase test TDA.

9- Indole test IND.

10- Voges proskauer test VP.

11- Gelatin liquefaction test GEL.

12- Glucose fermentation test GLU.

13- Mannitol fermentation test MAN.

14- Inositol fermentation test INO.

15- Sorbitol fermentation test SOR.

16- Rhamnose fermentation test RHA.

17- Sucrose fermentation test SAC.

18- Melibiose fermentation test MEL.

19- Amygdalin fermentation test AMY.

20- Arabinose fermentation test ARA.

The above 20 tests were performed according to the manufactures instruction as follows

1. Preparation of the galleries.

Five ml of tap water dispensed into incubation tray to provide a humid atmosphere during incubation.

2. Preparation of bacterial suspension.

A well-isolated colony was picked up by loop from MacConkey agar plates and was suspended in 5 ml sterile distilled water by rubbing against the side of the tube and mixed thoroughly with the water.

3. Inoculation of the galleries.

The twenty micro tubes were inoculated by a sterile pasture pipette, according to the manufactures instruction both the tube and the tube section of CIT, VP, and GEL microtubes were filled.

After inoculation couple section of the ADH, LDC, ODC, H_2S, and URE microtubes were completely filled with sterile mineral oil.

4. Incubation of the galleries.

After inoculation, the plastic lid was placed on the tray and the galleries were incubated for 18 to 24 hours at 37°**C** .

5. Reading of the galleries.

All the reactions not requiring reagent were recorded first, then the following reagents were added to the corresponding microtubes,

1- One drop of 3.4% ferric chloride to the TDA microtube.

2 –One drop of Kovac's reagent to the IND microtube.

3- One drop of Voges-proskuer reagent to VP microtube.

4- One drop of the oxidase reagent to ONPG microtube.

The biochemical reactions performed by the API 20E and their interpretation are listed in table (2 – 1).

Table (2-1) Interpretation of reactions performed by API 20E.

Microtube	Positive	Negative
ONPG	Yellow	Colorless
ADH	Red / orange	Yellow
LDC	Orange	Tallow
ODC	Red/orange	Yellow
CIT	Blue-green /green	Pale green/yellow
H_2S	Black deposit	Colorless /grayish
URE	Red/orange	Yellow
TDA	Dark brown	Yellow
IND	Red ring	Yellow ring
VP	Pink/red	Colorless
GEL	Diffusion of black pigment	No diffusion
GLU	Yellow	Blue/blue-green
MAN	Yellow	Blue/blue-green
INO	Yellow	Blue/blue-green
SOR	Yellow	Blue/blue-green
RHA	Yellow	Blue/blue-green
SAC	Yellow	Blue/blue-green
MEL	Yellow	Blue/blue-green
AMY	Yellow	Blue/blue-green
ARA	Yellow	Blue/blue-green

6- Identification of the isolate using the analytical profile index (numerical coding) for rapid identification at species and biotype level (supplied by the manufacturer).

For using the index, the biochemical profiles obtained have to be transformed into numerical profiles and to compare it with those listed in the index by transforming all 20 biochemical results into seven-figure numerical profile (i.e., seven digit number) by placing them into group of three and consigning a specific value for each of the positive as follows:

Group 1			**Group 2**			**Group 3**			**Group 4**		
ONPG	ADH	LOC	ODC	CIT	H2S	URE	TDA	IND	VP	GEL	GLU
1	2	4	1	2	4	1	2	4	1	2	4

Group 5			**Group6**			**Group7**	
MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA
1	2	4	1	2	4	1	2

Each positive reaction is given a value equal to 1, 2, or 4 according to the position of the test in its group. The sum of these values given the corresponding figure. Thus, the figure can have a value from 0 to 7 (zero for negative reaction). The seven digit numerical profile is then looked up in the index and the identification is determined.

2.8.7 Sensitivity test to antibiotics

10 ml of nutrient broth was inoculated with the *E.coli* colony, and incubated at 37°C for 18-24 hrs ($O.D_{625}$ about 0.1) giving 1×10^8 CFU /ml of broth which also equal to the turbidity of 0.5 STD McFarland.

0.1 ml of bacterial culture was transferred to Muller Hinton agar plate, a sterile cotton swab was used to spread the inoculum on the plate surface in three different planes (by rotating the plate approximately 60° each time) to obtain an even distribution of inoculum through out the plate. The inoculated plates were then placed at room temperature for 30 minutes to allow absorption of excess moisture.Then with sterile forceps, the selected antibiotic disks were placed on the inoculated plates and incubated at 37°C for 18 hrs in an inverted position.

After incubation, the diameter of inhibition zones were noted and measured by ruler, results were determined according to the national committee for laboratory standard (NCCL, 1991).

2.8.8 Bacterial adhesion test (Iwahi *et al.*, 1982)

2.8.8.1 Selection of isolates.

Six isolates of *E.coli* were selected on the bases of their multiple antibiotics resistance, each isolate was cultured in 10 ml nutrient broth, incubated overnight at 37°C and prepared for adhesion test.

2.8.8.2 Preparation of bacterial suspension.

10 ml of nutrient broth medium was inoculated with 24 hrs old bacterial colony, the culture was incubated at 37°C overnight to ($O.D_{625}$ about 0.1) giving 1×10^8 CFU / ml. Cultures were washed twice in PBS and bacterial cells were collected by centrifugation at 1000 rpm for 20min and resuspended in PBS.

2.8.8.3 Preparation of the epithelial cell.

Uroepithelial cells were isolated from the urine of three healthy females by centrifugation of urine at 1000 rpm for 10 minutes. The cells

were resuspended in PBS to a concentration of 10^5 cells /ml. (OD_{625} about 0.25).

2.8.8.4 Adhesion test.

1. A mixture of 0.2 ml of bacterial suspension, 0.2 ml of the epithelial cell suspension and 0.1 ml of BPS (2.4.1) were mixed and incubated with shaking at 37°**C** for one hour.
2. Unfixed bacteria were removed by centrifugation in PBS at 1000 rpm for 10 minutes.
3. The final pellet was resuspended in 50 µl PBS, then transferred onto a sterile glass microscope slide, air dried, fixed with methanol : acetic acid (3:1) and stained with crystal violet.
4. The number of adherent bacteria on epithelial cells were counted under light microscope(1000 x).
5. Control experiment was carried out in which only epithelial cells were included in absence of bacteria.

2.8.9 Plasmid curing experiment

Acridine orange (AO) was used as curing agent for plasmid DNA, the experiment was according to procedure described by (Miler, 1972).

2.8.9.1 Preparation of bacterial suspension.

10 ml of LB broth media was inoculated with single colony of antibiotic resistant bacteria, the culture was incubated for 18 hrs at 37°**C** to ($O.D_{625}$ about 0.1) giving 1×10^8 cell / ml

2.8.9.2 Curing experiment.

1- Serial dilutions of AO(stock solution 1 mg/ml) were made to give(5, 10, 20, 40, 80, and 100 µg/ml) in LB broth media tubes.

2- 0.1 ml of bacterial suspension prepared in (2.3.9.1) was inoculated into each of above mentioned tubes to determine the inhibitory concentration and sub inhibitory concentration of AO.

3- The sub inhibitory concentration was selected as curing concentration and used in further curing experiments. and the bacterial cells were allowed to grow in LB broth supplemented with sub inhibitory concentration of AO at 37°**C** for 24 hrs.

2.8.9.3 Selection of the cured bacteria.

1-After growing bacterial culture exposed to sub inhibitory concentration of AO, 0.1 ml samples of suitable dilutions (10^{-1} – 10^{-3}) were plated on nutrient agar plate.

2- Ten growing colonies were picked up and sub cultured on nutrient agar plate overnight at 37°**C**.

3- Antibiotic sensitivity test was done for each growing colony on Muller Hinton agar plate as described in (2.8.7).

5- After incubation at 37°**C** for 24 hrs the observed inhibition zone was measured for control (pre AO treatment) and AO treated colonies (post AO treatment).

Note: AO untreated bacterial cells were used as control.

2.8.10 Plasmid DNA Isolation by alkali lysis (Sambrook *et al.*, 1989)

2.8.10.1 Preparation of bacterial isolates.

1- A single bacterial colony was transferred into 2 ml of L.B broth media (2.8.1.5) in a loosely capped 15-ml tube, the culture was incubated overnight at 37°**C** with vigorous shaking.

2- 1.5 ml of the culture was transferred into a microfuge tube and centrifuged at 12000 rpm for 30 seconds at 4°**C** in a microfuge.

3- The medium was removed, leaving the bacterial pellet as dry as possible.

2.8.10.2 lysis by alkali

1- The bacterial pellet which was prepared in (2.8.10.1), was suspended in 100 µl of ice-cold solution I (2.5.2) by vigorous vortexing.

2- 200 µl of freshly prepared solution II (2.5.5) were added, then the tube was closed tightly and the contents were mixed by inverting the tube rapidly five times.

3- 150 µl of ice-cold solution III (2.5.7) were added; the tube was closed, mixed by vortexing gently for 10 seconds, and stored on ice for 3-5 minutes.

4- The tube was centrifuged at 12000 rpm for 5 minutes, the supernatant was transferred to a fresh tube.

5- DNA was precipitated with 2 volumes of absolute cold ethanol, mixed by vortexing, and the mixture was allowed to stand for 2 minutes.

6- The mixture was centrifuged at 12000 rpm for 5 minutes at 4°**C** in a microfuge.

7- The supernatant was removed by micropipette, the tube was stood in an inverted position on a paper towel to allow all of the fluid to drain away, any fluid drops adhering to the walls of the tube were removed.

8- The pellet of DNA was rinsed with 1 ml of 70 % ethanol at 4°**C**, centrifuged again, the supernatant was removed as described in step 8, and the pellet of DNA was allowed to dry in the air for 10 minutes.

9- The Nucleic acids were dissolved in 50 µl of TE (pH 8.0), mixed by vortexing gently and the DNA were stored at -20°**C**.

2.8.11 Gel electrophoresis (Sambrook *et al.*, 1989)

1- Preparation of agarose gel.

The gel (0.7 %) was prepared by dissolving of 0.28 g of agar into 40 ml 1X TEA Buffer (2.6.2), boiled until all agarose was dissolved and left to cool at 50°**C,** 2 µl of ethedium bromide (2.6.3) was added, the gel poured in tray with well former (comb), after the gel was solidified the comb removed gently, then the gel put in the tank which filled with TAE buffer until the surface of gel was covered.

2- Sample loading.

The DNA Samples were loaded by mixing 10 µl of DNA obtained in (2.8.10.2) with 2 µl of loading buffer (2.6.4) using micropipette, then the wells filled carefully with mixture, pBR 322 plasmid was loaded as control, the electrodes were connected.

3- Running of electrophoresis.

The electrophoresis apparatus was joined to power supply and turned on, the voltage was adjusted at 5-7 v/cm of gel, for 2.5 hrs.

After the electrophoresis is completed the current turned off and the gel was visualized by using UV- transilluminator, at 320 nm.

2.8.12 Determination of DNA concentration (Sambrook *et al.*, 1989)

The DNA which obtained in (2.8.10.2) was diluted with TE buffer (2.5.1) 1:100, then the absobance of U.V. light was read at 260 nm against TE

buffer blank to determination the concentration of DNA which was calculated accordingly from the flowing equation:

Concentration Of DNA = $O.D_{260}$ × dilution factor × 50 µg /ml = no. µg /ml.

3.1 Isolation and identification of *E.coli*

3.1.1 Isolation of bacterial UTI

Two hundred midstream urine samples were collected from patients suffering from symptoms referred as urinary tract infection. Samples were collected from patients of Central Public Health Laboratory and private urology clinic, during period from 1/12/2005 to 21/3/2006.

Out of two hundred urine samples, 123 (61.5%) samples gave bacterial growth on MacConkey agar and blood agar, 74(61%) cultures referred to female patients while 49(39%) cultures referred to male patients.

These results are similar to results reported by Sebahi (2003) and Mohammad (1989), who found that the percentages of positive cultures of urine samples on MacConkey and Blood agar were 66% and 64.6% respectively, while higher percentages were reported by Al-Shukr (2005), Barakat (1997), and Younis (1986) who reported percentages of positive cultures of urine samples on these two media equivalent to 93.7%, 89%, 84% respectively. On the other hand lower percentages were reported by Al-Shaikhli (2004), Obi and his colleagues (1996) who found that the positive cultures of urine samples on the above mentioned media were 49.4%, 27% respectively.

The reason behind differences in the observed percentages may be due to difference in size, number, site of collection, season, and medication especially exposure to antibiotics.

3.1.2 Identification of isolates

Identification of isolates was carried out according to cultural, morphological, and biochemical tests. In accordance to their pink colony appearance on MacConkey agar as lactose fermenters, and grayish white moderately opaque with or without zone of hemolysis on blood agar, as well as the results of biochemical tests which shown in table (3–1); positive indole test, gas production, A/A reaction, no H_2S production in TSI agar, and urease and citrate negative, the isolates were identified as *Escherichia spp* depending on identification scheme suggested by (Forbes *et al*., 1998), however a confirmation of results was done using API 20E tests. Results shown in figure (3-1) indicate that out of 123 UTI bacterial cultures, only 41 cultures were identified as *E.coli*, and so the isolation percentage of *E.coli* in UTI cultures was 33.3%. This result is Similar to result reported by Mohammed (1989) who found that the isolation percentage of *E.coli* from urine samples of UTI patients was 39.9%. However, different results were obtained by Younis (1986) and Read *et al*., (1989) who found that the isolation percentages of *E.coli* from UTI cases were 79.5% and 67% respectively.

Table (3 – 1) Some biochemical tests for characterization of *E.coli.*

Test / Isolate	TSI			Urease	Citrate	Indole
	Gas	H_2S	Reaction			
E.coli	**+**	**-**	**A/A**	**-**	**-**	**+**

A/A = acid /acid

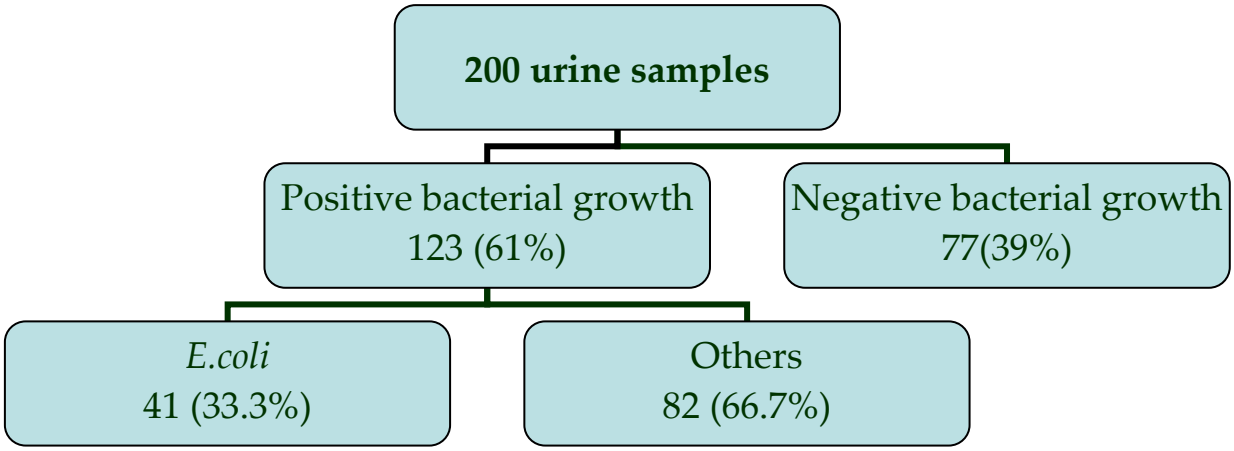


Figure (3 – 1) Schematic diagram summarizes the isolation procedures of uropathogenic *E.coli*.

Results in (table 3-2) show prevalence of *E.coli* in both sexes, 14 isolates out of 41 were identified in male samples (34%), while in female samples 27 isolates were identified as *E.coli* (66%), with female/male ratio 1.93/1 .

Similar result was obtained by Al-Fahdawi (2001) who found that the isolation percentage of *E.coli* from UTI cases was 38 %, and the female/Male ratio was 1.72/1 of UPEC isolates,

Table (3-2) Infectivity of *E.coli* in both sexes

Sex	No. of *E.coli* isolates	Percentage
Male	14	34%
Female	27	66%
Total	41	100%

3.2 Antibiotic sensitivity

41 *E.coli* isolates were screened for their resistance profiles to fourteen antibiotics representing different groups, depending upon their mode of action. Results shown in table (3–3) indicate that variable resistance profiles among isolates against antibiotic were noticed. The recorded percentage of resistance of *E.coli* isolates to tested antibiotics (table 3-4), indicate that all *E.coli* isolates were 100% resistant to Erythromycin, ampicillin, and cephalexin, while (88%) were resistant to (Amoxicillin + clavulanic acid combination). (83%) to cefotaxime and streptomycin. On the other hand (5%) were resistant to amikacin and ciprofloxacin , (9.8%) to Naldixic acid, and (17%) to chloramphenicol, while others distributed in between. Similar results were reported by Al-Fahdawi (2001), and Al-Alosi (2004), who reported that no *E.coli* isolates from UTI patients were sensitive to ampicillin, Abd-alsattar (2004) reported resistance percentage (95%) of *E.coli* isolates to ampicillin.

In this study high resistance percentage (88%) was reported to amoxicillin/clavulanic acid (AMC) combination, Sharma and Grover(2004) reported that (60%) of *E.coli* isolates were resistance to AMC in India; while Sotto *et al.,* (2000) and Johnson *et al.,* (1995)reported that resistance percentages of *E.coli* isolates to AMC were (20%) and (11%) respectively.

Table (3 – 4) Percentages of antibiotic resistance in 41 isolates of *E.coli* isolated from UTI samples.

Antibiotics	Number of resistant isolates	Resistance Percentage %
β-lactam penicillin		
Ampicillin(AM)	**41**	**100**
β-lactam penicillin + β-lactamase inhibitor		
Amoxicillin + clavulanic acid(AMC)	**36**	**88**
Cephalosporine		
Cephalexin(CL)	**41**	**100**
Cefotaxime(CTX)	**34**	**83**
Aminoglycoside		
Streptomycin(S)	**34**	**83**
Gentamycin(CN)	**21**	**51**
Amikacin(AK)	**2**	**5**
Macrolides		
Erythromycin(E)	**41**	**100**
Tetracyclines		
Tetracycline(TE)	**15**	**36.6**
Quinolones		
Naldixic acid(NA)	**4**	**9.8**
Ciprofloxacin(CIP)	**2**	**5**
Others		
Chloramphenicol(C)	**7**	**17**
Nitrofuration(F)	**24**	**58.5**
Trimethoprim + slfamethoaxazole(SXT)	**26**	**63**

Resistance to (β-lactam / β-lactamase inhibitor combination) may be due to over production of β-lactamase by resistant isolates which overcome the β-lactam inhibitor action(Chambers *et al*., 2001).

This study also showed high resistance to cephalosporines; all *E.coli* isolates (100%) were resistant to cephalexin (belong to 1st generation cephalosporines). Lower resistance percentages were reported by Al-Shaikhli (2004) and Al-Alosi (2004), they found that *E.coli* isolates resistant to cephalexin were in the percentage range between 43% and 60%.

The reported result also showed that the resistance percentage to cefotaxime was (83%) which is considered being high. Similar result reported by Al-Alosi (2004) who found that 80% of *E.coli* isolates were resistant to Cefotaxime. While Rodrigues *et al.,* (2004) found that resistance percentage of *E.coli* against Cefotaxime was (60%).

The reason of high resistant percentages of *E.coli* isolates to β-lactam antibiotics may due to wide and wiseless use of antibiotics which is leading to development of resistance by the action of β-lactamase enzymes which may be either chromosomally or plasmid mediated (Rodrigues *et al*., 2000). Resistance to 3rd generation cephalosporines (Cefotaxime) was mainly due to ESBL enzyme that can hydrolyze 3rd generation cephalosporines and aztreonam antibiotics (Babypadmini and Appalaraji, 2003).

The reported resistance to aminoglycosides in this study were found variable. Amikacin was the most effective one against *E.coli* isolates; it showed a resistant percentage of (5%).

Babypadmini and Appalaraji (2003) found in their study which was carried out in India that the resistance percentage of *E.coli* isolates to amikacin was 14%. Valdivieso *et al.,* (1999) had studied urinary isolates which were obtained from 11 Chilean hospitals, they found that 1.3% of *E.coli* isolates were resistant to amikacin.

In this study we recorded highest resistance percentage to streptomycin among tested aminoglycosides which was 83%. However, Johnson *et al*., (1995) recorded (26%) of resistance to streptomycin.

Our study showed that 51.2% of *E.coli* isolates were resistant to gentamycin, similar result recorded by Abd-alsattar (2004) who found that 55% of *E.coli* isolates were resistant to gentamycin, however Al-Shaikhli (2004), Al-Alosi (2004), and Al-Fahdawi (2001) found that the resistance to gentamycin were 24%, 30%, and 74% respectively, while Valdivieso *et al.,* (1999) found 4.2% of *E.coli* isolates were resistant to gentamycin.

E.coli could resist aminoglycosides by producing modifying enzymes which modify the aminoglycoside antibiotic and prevent it from binding to ribosome (Jawetz *et al*., 1998).

Our study also showed (36.6%) of isolates were resistant to tetracycline, higher resistance percentage reported by Al-Shaikhli (2004) and Al-Fahdawi (2001) they reported (69%) and (88%) respectively.
Meulir and his colleagues (1988) found that *Enterobacteriaceae* were resistant to tetracycline and their resistant was due to resistant gene which carried by plasmid and these genes could be transformed among *Enterobacteriaceae*.

Quinolones on the other hand show a good effect on *E.coli* isolates, as long as most isolates found sensitive to them for example, ciprofloxacin showed a resistance of (4.9%) of isolates, similar results reported by Valdivieso *et al*., (1999), and Abd-Alsattar (2004); they found that the resistance percentages were 5% and 5.6 % respectively, higher percentage of resistance was obtained by Al-Shaikhli (2004) who found that the ciprofloxacin resistance percentage of *E.coli* isolates was 19.4%. Although the ciprofloxacin is newly used in treatment in comparision with other antibiotics, but resistant developed as a result of overuse and misuse of this antibiotic, moreover Rice and his colleagues (1992) show that increasing uses of antibiotic was associated with development of resistance against it.

Our results showed that 9.8% of *E.coli* isolates were resistant to Naldixic acid, which belong to quinolones groups. Al-Alosi (2004) found that the resistance percentage of *E.coli* to naldixic acid was 20%. While Sharma and Grover (2004), found the resistance percentage of *E.coli* to Naldixic acid was (86%) which considered to be high, also Al-Fahdawi and Al-Shaikhli reported resistance percentage of (35%) and (48%) respectively.

Resistance of *E.coli* to ciprofloxacin and Naldixic acid may be due to the development of resistant gene carried by conjugative plasmid (Martinez-martinez *et al.,* 1998).

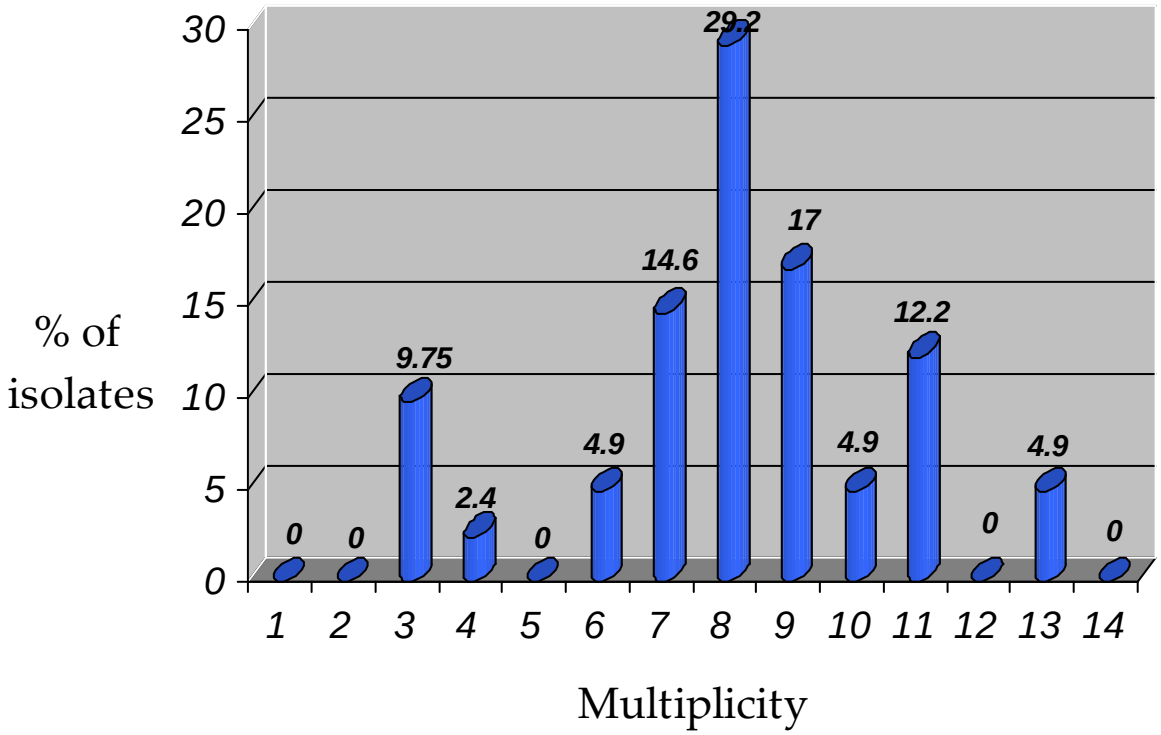
Our result indicates that chloramphenicol is still good antimicrobial agent against *E.coli* as long as a percent of 17% resistance was recorded. Al-Shaikhli (2004) reported that only (39 %) of *E.coli*

isolates were resistant to chloramphenicol. As much as (58.5%) of *E.coli* were found resistant to nitrofurantion, however Valdivieso *et al.*, (1999) reported that 4.3% of isolates were resistant to nitrofurantion. Only (63 %) *E.coli* isolates were found resistant to cotrimoxazole (SXT). Similar result was recorded by Al-Shaikhli (2004) who found that 66% of *E.coli* were resistant to SXT, lower percent was reported by Valdivieso *et al.*, (1999) they found that the resistance percentage was 43%, while higher resistance percentage recorded by Sharma and Grover (2004), they found 81% of *E.coli* isolates from UTI patients were resistant to SXT.

3.3 Multiple antibiotic resistance

Grouping of isolates in order to obtain a pattern of resistance is important for clearing the view of their infectivity behavior. Accordingly, the results shown in figure (3 - 2) indicate that the tested *E.coli* isolates (41 isolates) showed multiple resistance to various types of antibiotics which were used in this study.

The obtained results showed that two isolates namely no. 25, 35 have the highest multi-resistance to antibiotics which represent 4.9 % of the isolates; these two isolates resist 13 antibiotics, while isolates no. 7, 9, 17, 39 have the lowest multi-resistance which represent 9.75%; they resist only 3 antibiotics, moreover the highest percentage was observed in the group which resist 8 antibiotics; in this group the isolates are no. 2, 21, 26, 27, 36, 37, 34, 5, 20, 29, 12, and 32, which represent 29.2% of isolates.



Figure(3-2) Percentage of multiple antibiotics resistance

Multiple antibiotic resistance of bacteria could be due to transferable plasmid carrying resistant genes which transferred among pathogenic bacteria such as pBS13, pBS12, pB2, and pMS4 plasmids (Tenova, 1996; Mandal *et al*., 2004). In addition to that, certain mutation could occur as a result of random use and over use of antibiotics (Chambers *et al*., 2004).

Figure (3-3) showed the evolution of multiplicity patterns in the *E.coli* isolates. It is interesting to note that the 21 isolates shown in the figure start the resistance pattern with three antibiotics(AM, E, CL). These three antibiotics are found to give resistance in isolates no. 7, 9, 17, 39. However resistance in rest of 17 isolates increases gradually to reach a level which gives resistance to 11 antibiotics. These results might suggest evolutionary pattern of resistance which builds up gradually in *E.coli*.

3.4 Adhesion of Uropathogenic *E.coli*

The ability of *E.coli* to adhere to uroepithelial cells is of great importance in pathogenesis of UTI (Ziegler *et al*., 2004). Thus in this study the adherence capacity of *E.coli* isolates no. 5, 6, 30, 31, 35, and 41 was examined, the isolates were selected according to their antibiotic resistance patterns.

Adherence ability of *E.coli* to uroepithelium was determined by the number of bacteria on uroepithelial cells (table 3 – 5). The mean numbers of bacteria adhering to 20 uroepithelial cells were counted under light microscope (Iwahi *et al*., 1982).

The results which are shown in table (3 – 5) do not suggest the possible correlation between degree of multiplicity of antibiotics resistance and adherence ability. Such correlation could not be observed in isolates no. 35, 41, as long as isolate no. 35 (resistant to 13 antibiotics) has adherence ability equivalent to 29.9 bacteria /cell, while isolate no. 41 (resistant to 7 antibiotics) has adherence ability 29 bacteria /cell.

The obtained results showed that *E.coli* no. 5 (resistant to 8 antibiotics) gave the highest mean no. of adhering bacteria to uroepithelium cell (34 bacteria per uroepithelium cell) (figure 3 – 4).

Whereas *E.coli* no. 31 (resistant to 11 antibiotics) showed the lowest mean no. of adherent bacterial cell to uroepithelium cell(16.4 bacteria/cell), other tested *E.coli* isolates were in the range between (18 – 33 bacteria /cell). Vidya *et al.,* (2005) found that highest mean no. of adherence of *E.coli* to uroepithelium was 24 bacteria / cell.

Table (3 – 5) The adherence of *E.coli* to uroepithelial cells.

Isolate No. of *E.coli*	**Multiplicity of antibiotic resistance**	**In phosphate buffer saline (PBS)**				
		NO. of adherent *E.coli* to no .of uroepithelial cells				**Mean no. of adherent *E.coli* /cell**
		0	**1-5**	**6-2**	**>20**	
5	**8**	0	0	3	17	34
16	**7**	4	3	10	3	18
30	**9**	0	1	1	18	33
31	**11**	5	2	11	2	16.4
35	**13**	2	2	4	12	29.9
41	**7**	0	0	3	17	29
Final mean number of adherent *E.coli* /uroepithelial cell for all *E.coli* isolates						26.6

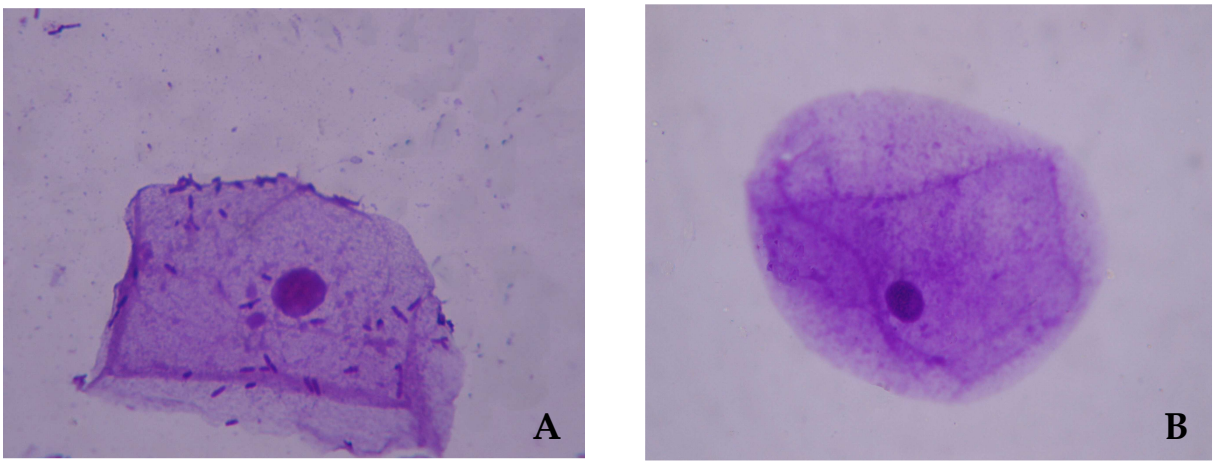


Figure (3 – 4) Adherence of bacterial cells of isolate no. 5 to uroepithelial cell(A) in comparison with untreated uroepithelial cell (B) (1000x).

3.5 Plasmid profile

In this study plasmid profiles of six isolates (no. 5, 16, 30, 31, 35, 41) were studied, in which certain antibiotic resistant patterns were observed. The obtained results showed that most *E.coli* (no.16, 30, 31, 35) isolates had one large plasmid (mega plasmid) with slight variations in size among isolates. However the reported results indicate no detectable band for isolates no. 5 and 41, which could be assumed that these cells are plasmid free. Whereas, isolates no. 16, 30, 31, and 35 have several bands of small plasmids(1-3 bands) in addition to large one as observed on in agarose gel (figure 3–5). The reported results indicate the dissemination of plasmids among *E.coli* isolates which may be carrying resistant genes against wide spectrum of clinically used antibiotics, which may explain the reason of evolution antibiotic resistant patterns in studied bacterial cultures, similar results were obtained by Rasmussent *et al*., (1999) and Barros *et al*., (1999).

Various investigators reported that most *E.coli* isolates contain at least one mega plasmid in addition to other smaller plasmids, which encode for several virulence factors such as heamolysin, toxins, siderophores production, in addition to antibiotic resistant markers (Tosini *et al*., 1998; Al-Alosi, 2004; Al-Moosawi, 2005).

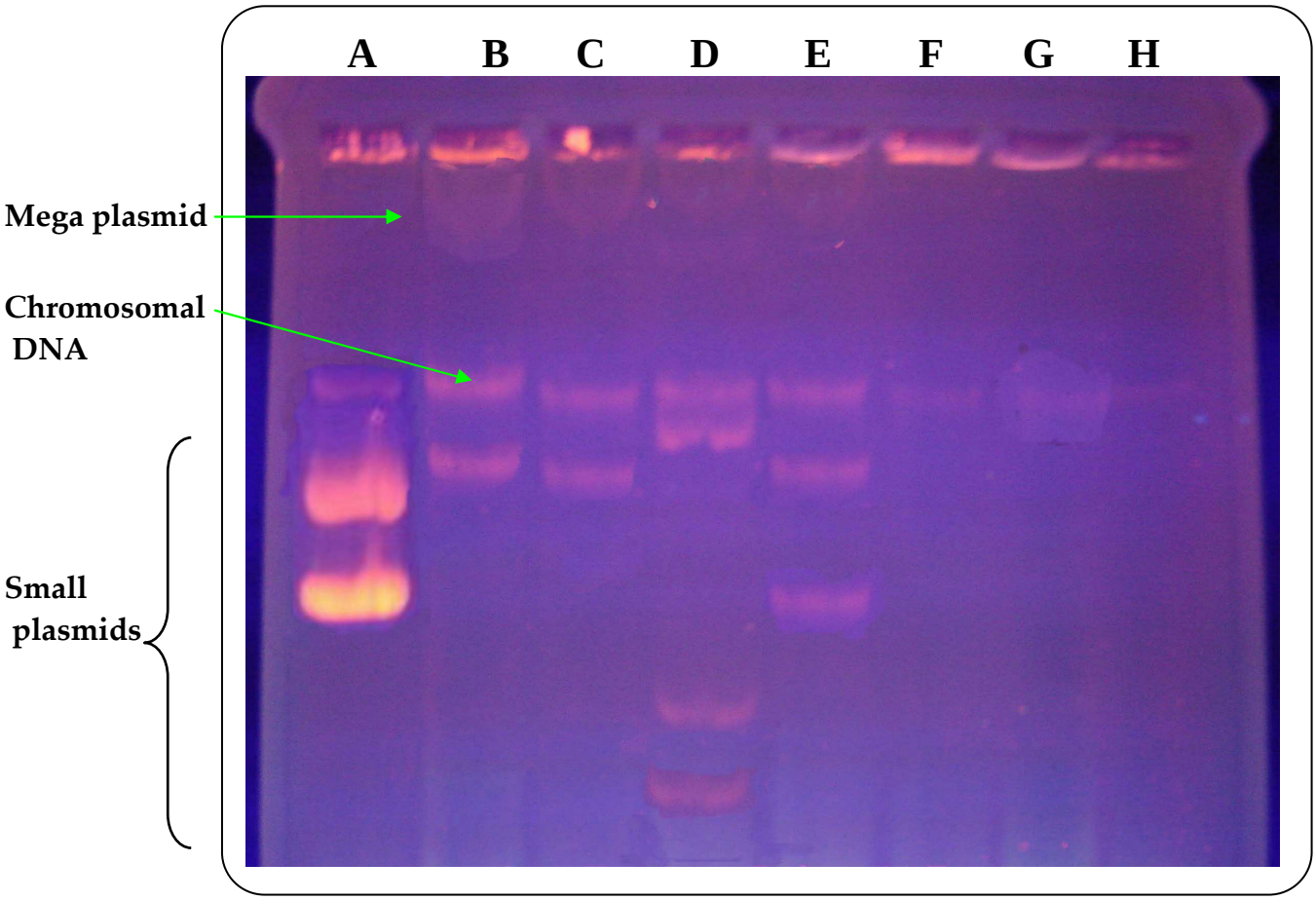


Figure (3 - 5) Gel electrophoresis of plasmids of selected *E.coli* isolates.

A : pBR 322

B: Plasmid content of isolate no. 16

C: Plasmid content of isolate no. 30

D: Plasmid content of isolate no. 35

E: Plasmid content of isolate no. 31

F: Plasmid content of isolate no. 5

H: Plasmid content of isolate no. 4

3.6 The relationship between plasmid content of *E.coli* isolates and antibiotic resistance

The location of antibiotic resistance determinants on chromosome or plasmid could be ascertained by plasmid curing experiments. In this study acridine orange (intercalative agent) was used as curing agent of plasmids from the selected isolates no. 16, 30, 31, and 35, which have indicate the highest multiplicity of antibiotic resistance and contain several plasmid bands.

The obtained results showed that sub- inhibitory concentration of acridine orange for tested isolates were in the range of 80 -100 μg / ml, and at this range the plasmids of isolates no. 16, 30, and 35, were eliminated from bacterial cultures without harming the bacterial growth.

Acridine orange if administered to bacterial populations in sub-lethal doses, can lead to the elimination of plasmid DNA (inhibit plasmid replication) without harming the bacterial chromosome and thus maintaining the ability to reproduce and generate offspring (Singleton and Sainsbury, 2001). Jacob *et al.,* (1963) noted the sensitivity of the replication of plasmid to Acridines to be greater than the sensitivity of *E.coli* chromosome to these compounds, and they proposed that this may be due to the plasmid DNAs are circular supercoiled and DNAs of this conformation have greater affinity for intercalative compounds.

Thus in this work the antibiotic resistant patterns and plasmid profiles of *E.coli* isolates no. 16, 30, 31, 35 were studied and compared before and after AO treatment.

The results in (table 3 – 6) demonstrated that S, SXT resistance determinants were lost in isolate no. 16, whereas CN, TE, AK resistance determinants were lost in isolate no. 31, on the other hand AMC, S, CN, TE, SXT, N.A, CIP resistance determinants were lost in isolates no. 35 after treatment of the isolates by AO. Loss of resistance was accompanied by disappearance of the plasmid bands in the three isolates (no. 16, 30, and 35) (figure 3 - 6). This might indicate that the resistance determinants of above antibioti0cs may be located on one or more plasmids. Similar results have been observed by Al-Alosi (2004) who observed the loss of CN, TE, N.A, S, and SXT resistant determinant after curing of the plasmids of resistant cells.

Our result indicated that AMP, CL, E, and CTX resistant determinants in all isolates were still expressed and have not been affected by AO treatment. This may be explained by that the resistant property is found on the chromosome (Aryes – sia *et al*., 1996).However our results also show that the plasmids of isolate no. 31 was not eliminated from bacterial culture and the antibiotic resistance property not affected , this may be explained that their plasmids were uncurable plasmids by AO.

Table (3 – 6) Antibiotic resistance of *E.coli* isolates before and after AO treatment

Isolate NO. of *E.coli*	**Pre-curing**		**Post-curing**	
	Resistance patterns	**Plasmid profiles**	**Resistance patterns**	**Plasmid profiles**
16	AMP, CL, E, CTX, AMC, S, SXT	one large band, one small band	AMP, CL, E, CTX, AMC	No plasmid bands
30	AMP, CL, E, CTX, AMC, S, CN, TE, AK	one large band, one small band	AMP, CL, E, CTX, AMC, S	No plasmid Bands
31	AMP, CL, E, CTX, AMC, S, CN, TE, SXT, C	one large band, two small bands	AMP, CL, E, CTX, AMC, S, CN, TE, SXT, C	One large band, two small band
35	AMP, CL, E, CTX, AMC, S, CN, TE, SXT, N.A, CIP	one large band, three small bands	AMP, CL, E, CTX	No plasmid bands

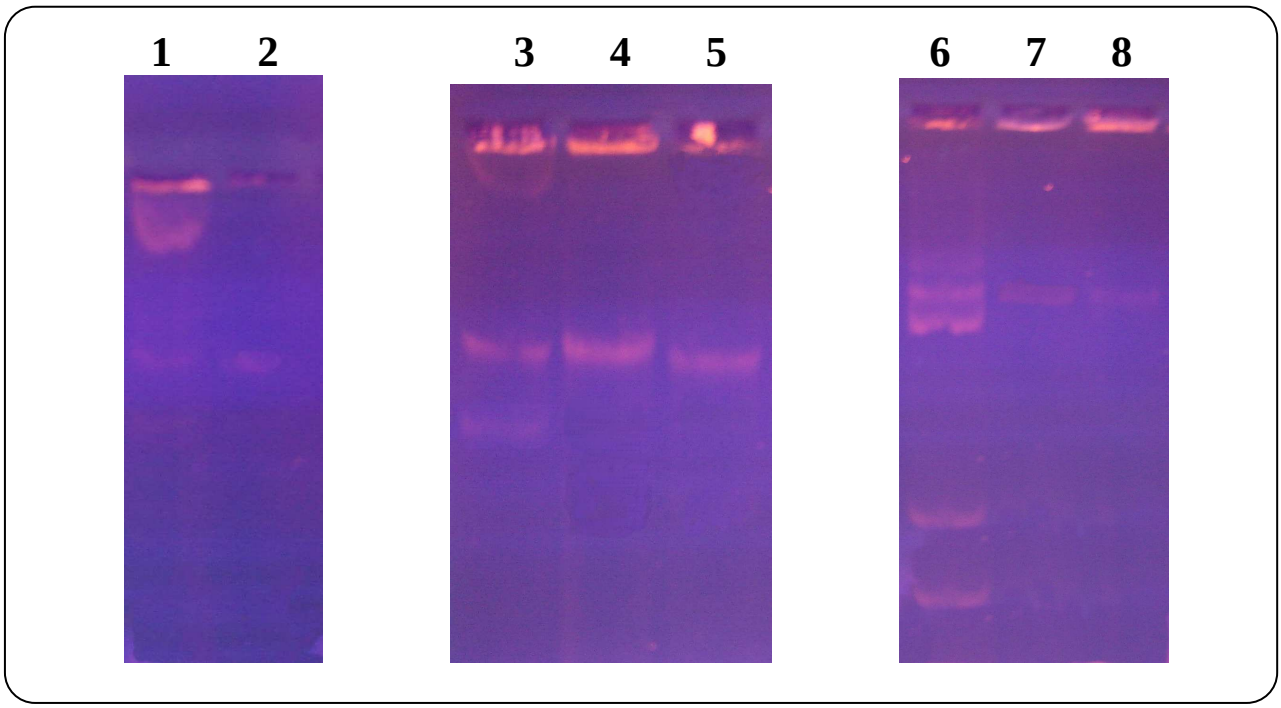


Figure (3 - 6) Gel electrophoresis of plasmids of isolates no. 16, 30, 35 before and after AO treatment.

1: Plasmid content of isolate no. 16 before AO treatment.

2: Plasmid content of isolate no. 16 after AO treatment.

3: Plasmid content of isolate no. 30 before AO treatment.

4: Plasmid content of isolate no. 30 after AO treatment.

5: Plasmid content of isolate no. 30 after AO treatment.

6: Plasmid content of isolate no. 35 before AO treatment.

7: Plasmid content of isolate no. 35 after AO treatment.

8: Plasmid content of isolate no. 35 after AO treatment.

3.7 Adhesion ability of cured isolates

The ability of adhesion for isolates no. 16, 30, 35 (pre and post curing experiments) were examined in order to determine the role of plasmids in adhesion capability. Table (3 – 7) shows that the ability of adherence of 16, 30, and 35 isolates after curing of plasmids were not affected, indicating that adherence ability of *E.coli* isolates to uroepithelial cells were not affected by R plasmids contents,(Figure 3 – 7) shows the adherence of isolate no. 35 to uroepithelial cell after AO treatment.

These results indicate that the adhesion determinants of tested *E.coli* isolates do not located on plasmid and the adhesins antigen such as (P, S, Dr, and others) which are responsible for uropathogenic *E.coli* adherence to uroepithelial cells are more likely chromosomally encoded (Tolkoff-Rubin *et al*., 2004).

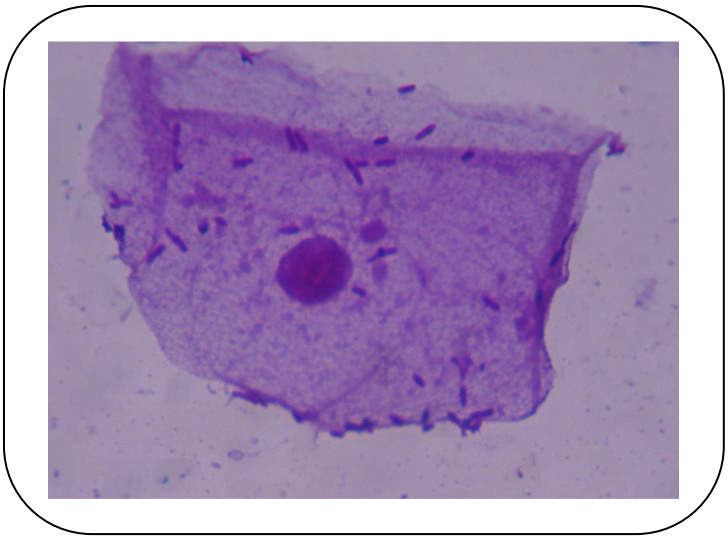


Figure (3 – 7) Microscopic examination of adherence capability of isolate No. 35, after AO treatment (1000x).

Table (3 – 7) Adherence of *E.coli* to uroepithelial cells before and after AO treatment.

Isolate no. of *E.coli*	**In phosphate buffer saline (PBS)**	
	Mean no. of adherent *E.coli* / cell before AO treatment	**Mean no. of adherent *E.coli* /cell after AO treatment**
16	19	18
30	32	32
35	29	29.2

4.1 Conclusions

1- *E.coli* was the common causative agent of Bacterial UTI, and the incidence rate was higher in females than males.

2- High percentage of *E.coli* isolates were resistant to Ampicillin, Erythromcin, Cephalexin, Amoxicillin/clavulanic acid combination, while the most effective antibiotics against UPEC were Amikacin and ciprofloxacin.

3- Acridine orange showed a powerful activity as curing agent in elimination of plasmid(s) responsible for antibiotics resistance in *E.coli*.

4- There is no correlation between adhesion ability of UPEC and multiplicity of antibiotics resistance.

5- Plasmid curing experiment suggested that

- The resistance determinants for SXT, G, TET, CIP, N.A, and AK antibiotics were under plasmids control.
- The adhesion ability of UPEC is not determined by plasmid.

4.2 Recommendation

1- Further research is required on the multiplicity of antibiotics resistance, to investigate the evolution of multiple antibiotics resistance of uropathogenic *E.coli*.

2- Further studies to investigate the possible involvement of plasmids of UPEC in adhesion.

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Figure (3 – 4) Evolutionary multiplicity pattern of antibiotic resistance of *E.coli* isolates

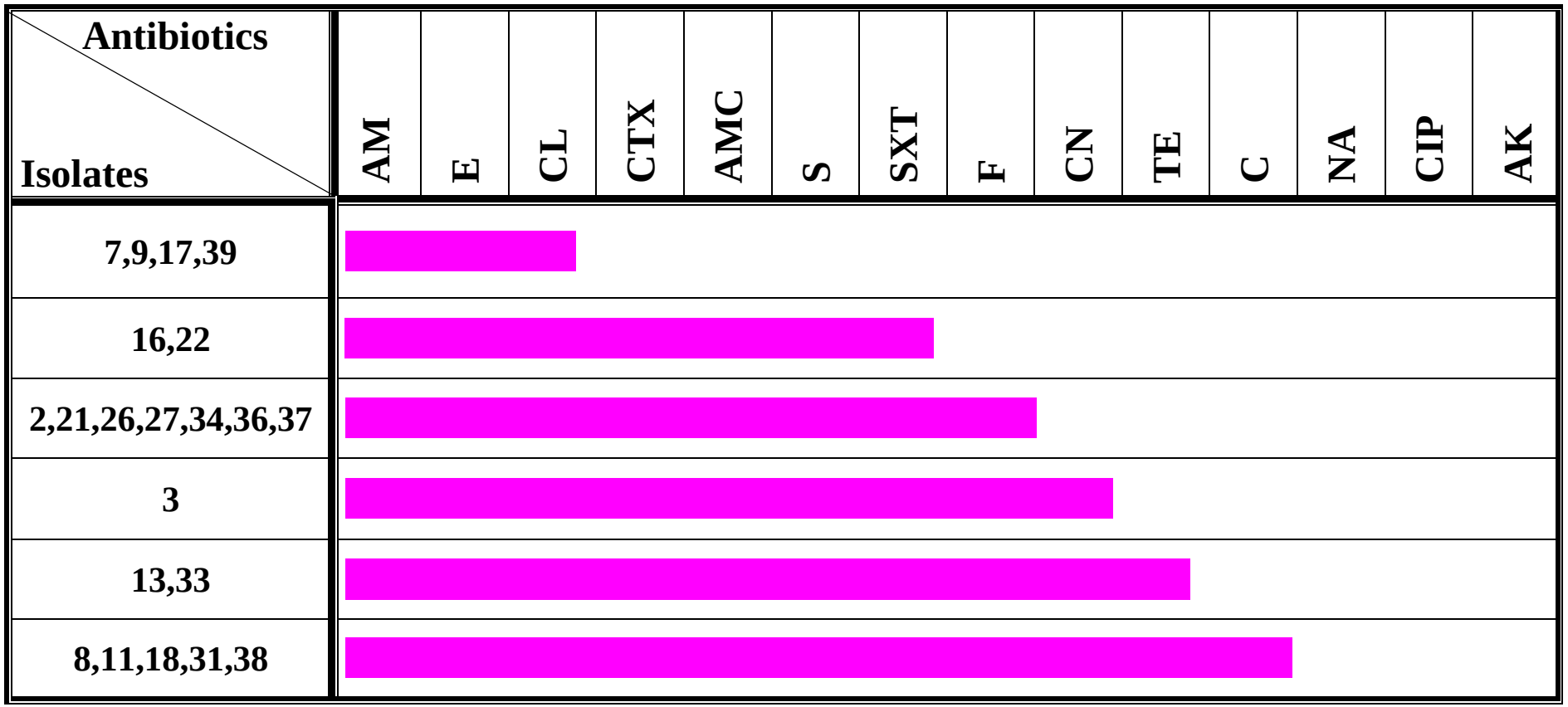

Figure (3 – 4) : Antibiotic resistance patterns of *E.coli* isolates

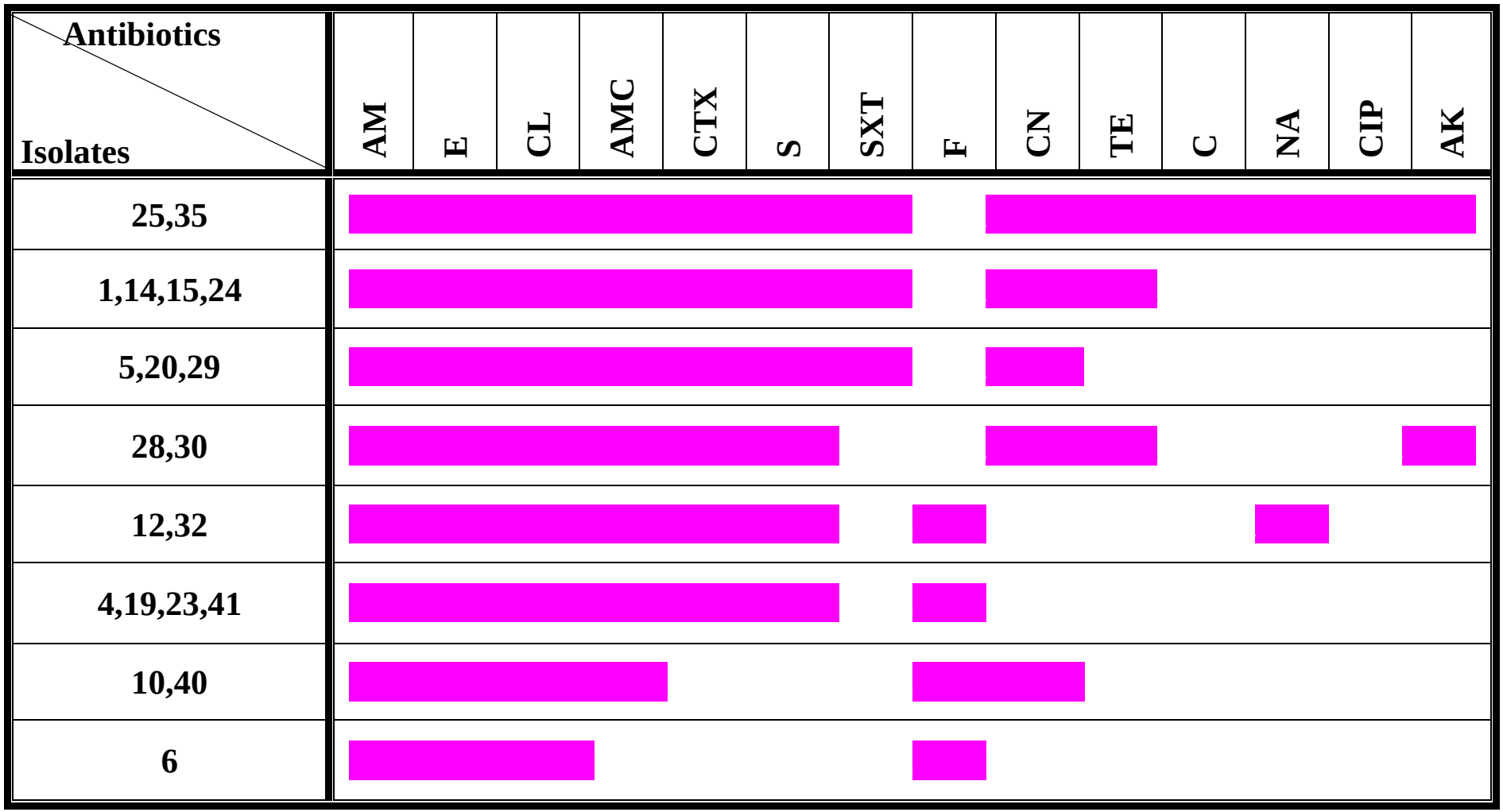

Table (3 – 3) Antibiotic resistance of 41 UTI *E.coli* isolates

Antibiotic / Isolate	AM	E	CL	CTX	AMC	S	SXT	F	CN	TE	C	NA	CIP	AK
1	R	R	R	R	R	R	R	S	R	R	S	S	S	S
2	R	R	R	R	R	R	R	R	S	S	S	S	S	S
3	R	R	R	R	R	R	R	R	R	S	S	S	S	S
4	R	R	S	R	R	R	S	R	S	S	S	S	S	S
5	R	R	R	R	R	R	R	S	R	S	S	S	S	S
6	R	R	S	R	R	S	S	R	S	S	S	S	S	S
7	R	R	S	R	S	S	S	S	S	S	S	S	S	S
8	R	R	R	R	R	R	R	R	R	R	R	S	S	S
9	R	R	S	R	S	S	S	S	S	S	S	S	S	S
10	R	R	S	R	R	S	S	R	R	S	S	S	S	S
11	R	R	R	R	R	R	R	R	R	R	R	S	S	S
12	R	R	R	R	R	R	S	R	S	S	S	R	S	S
13	R	R	R	R	R	R	R	R	R	R	S	S	S	S
14	R	R	R	R	R	R	R	S	R	R	S	S	S	S
15	R	R	R	R	R	R	R	S	R	R	S	S	S	S
16	R	R	R	R	R	R	R	S	S	S	S	S	S	S
17	R	R	S	R	S	S	S	S	S	S	S	S	S	S
18	R	R	R	R	R	R	R	R	R	R	R	S	S	S
19	R	R	R	R	R	R	S	R	S	S	S	S	S	S
20	R	R	R	R	R	R	R	S	R	S	S	S	S	S
21	R	R	R	R	R	R	R	R	S	S	S	S	S	S
22	R	R	R	R	R	R	R	S	S	S	S	S	S	S
23	R	R	R	R	R	R	S	R	S	S	S	S	S	S
24	R	R	R	R	R	R	R	S	R	R	S	S	S	S
25	R	R	R	R	R	R	R	S	R	R	R	R	R	S

Table (3 – 3) Antibiotic resistance of 41 UTI *E.coli* isolates (continue)

Antibiotic / Isolate	AM	E	CL	CTX	AMC	S	SXT	F	CN	TE	C	NA	CIP	AK
26	R	R	R	R	R	R	R	R	S	S	S	S	S	S
27	R	R	R	R	R	R	R	R	S	S	S	S	S	S
28	R	R	R	R	R	R	S	S	R	R	S	S	S	R
29	R	R	R	R	R	R	R	S	R	S	S	S	S	S
30	R	R	R	R	R	R	S	S	R	R	S	S	S	R
31	R	R	R	R	R	R	R	R	R	R	R	S	S	S
32	R	R	R	R	R	R	S	R	S	S	S	R	S	S
33	R	R	R	R		R	R	R	R	R	S	S	S	S
34	R	R	R	R	R	R	R	R	S	S	S	S	S	S
35	R	R	R	R	R	R	R	S	R	R	R	R	R	S
36	R	R	R	R	R	R	R	R	S	S	S	S	S	S
37	R	R	R	R	R	R	R	R	S	S	S	S	S	S
38	R	R	R	R	R	R	R	R	R	R	R	S	S	S
39	R	R	S	R	S	S	S	S	S	S	S	S	S	S
40	R	R	R	R	S	S	S	R	R	S	S	S	S	S
41	R	R	R	R	R	R	S	R	S	S	S	S	S	S

S: Sensitive **AM:** Ampicillin **E:** Erythromycin **CL:** Cephalexin

R: Resistant **CTX:** Cefotaxime **S:** Streptomycin **F:** Nitrofuration

CN: Gentamycin **TE:** Tetracycline **C:** Chloramphenicol **NA:** Naldixic acid

CIP: Ciprofloxacin **AK:** Amikacin **AMC:** Amoxicillin+ clavulanic acid

SXT: Trimethoprim + sulfamethaxzole

Table (3 – 3) Antibiotic resistance of 41 UTI *E.coli* isolates

Antibiotic / Isolate	AM	E	CL	CTX	AMC	S	SXT	F	CN	TE	C	NA	CIP	AK
1	R	R	R	R	R	R	R	S	R	R	S	S	S	S
2	R	R	R	R	R	R	R	R	S	S	S	S	S	S
3	R	R	R	R	R	R	R	R	R	S	S	S	S	S
4	R	R	S	R	R	R	S	R	S	S	S	S	S	S
5	R	R	R	R	R	R	R	S	R	S	S	S	S	S
6	R	R	S	R	R	S	S	R	S	S	S	S	S	S
7	R	R	S	R	S	S	S	S	S	S	S	S	S	S
8	R	R	R	R	R	R	R	R	R	R	R	S	S	S
9	R	R	S	R	S	S	S	S	S	S	S	S	S	S
10	R	R	S	R	R	S	S	R	R	S	S	S	S	S
11	R	R	R	R	R	R	R	R	R	R	R	S	S	S
12	R	R	R	R	R	R	S	R	S	S	S	R	S	S
13	R	R	R	R	R	R	R	R	R	R	S	S	S	S
14	R	R	R	R	R	R	R	S	R	R	S	S	S	S
15	R	R	R	R	R	R	R	S	R	R	S	S	S	S
16	R	R	R	R	R	R	R	S	S	S	S	S	S	S
17	R	R	S	R	S	S	S	S	S	S	S	S	S	S
18	R	R	R	R	R	R	R	R	R	R	R	S	S	S
19	R	R	R	R	R	R	S	R	S	S	S	S	S	S
20	R	R	R	R	R	R	R	S	R	S	S	S	S	S
21	R	R	R	R	R	R	R	R	S	S	S	S	S	S
22	R	R	R	R	R	R	R	S	S	S	S	S	S	S
23	R	R	R	R	R	R	S	R	S	S	S	S	S	S
24	R	R	R	R	R	R	R	S	R	R	S	S	S	S
25	R	R	R	R	R	R	R	S	R	R	R	R	R	S

Table (3 – 3) Antibiotic resistance of 41 UTI *E.coli* isolates (continue)

Antibiotic / Isolate	AM	E	CL	CTX	AMC	S	SXT	F	CN	TE	C	NA	CIP	AK
26	R	R	R	R	R	R	R	R	S	S	S	S	S	S
27	R	R	R	R	R	R	R	R	S	S	S	S	S	S
28	R	R	R	R	R	R	S	S	R	R	S	S	S	R
29	R	R	R	R	R	R	R	S	R	S	S	S	S	S
30	R	R	R	R	R	R	S	S	R	R	S	S	S	R
31	R	R	R	R	R	R	R	R	R	R	R	S	S	S
32	R	R	R	R	R	R	S	R	S	S	S	R	S	S
33	R	R	R	R		R	R	R	R	R	S	S	S	S
34	R	R	R	R	R	R	R	R	S	S	S	S	S	S
35	R	R	R	R	R	R	R	S	R	R	R	R	R	S
36	R	R	R	R	R	R	R	R	S	S	S	S	S	S
37	R	R	R	R	R	R	R	R	S	S	S	S	S	S
38	R	R	R	R	R	R	R	R	R	R	R	S	S	S
39	R	R	S	R	S	S	S	S	S	S	S	S	S	S
40	R	R	R	R	S	S	S	R	R	S	S	S	S	S
41	R	R	R	R	R	R	S	R	S	S	S	S	S	S

S: Sensitive **AM:** Ampicillin **E:** Erythromycin **CL:** Cephalexin

R: Resistant **CTX:** Cefotaxime **S:** Streptomycin **F:** Nitrofuration

CN: Gentamycin **TE:** Tetracycline **C:** Chloramphenicol **NA:** Naldixic acid

CIP: Ciprofloxacin **AK:** Amikacin **AMC:** Amoxicillin+ clavulanic acid

SXT: Trimethoprim + sulfamethaxzole

Chapter One

Introduction
And
literature review

Chapter Two

Materials And Methods

Chapter Three

Results And Discussions

Chapter Four

Conclusions And Recommendations

Committee Certification

We, the 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 thesis for the degree of Master of Science in Biotechnology.

Signature:
Name:
Scientific Degree:
Date:
(Chairman)

Signature:
Name:
Scientific Degree:
Date:
(Member)

Signature:
Name:
Scientific Degree:
Date:
(Member)

Signature:
Name:
Scientific Degree:
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(Member)

I hereby certify upon the decision of the examining committee

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Scientific Degree: Assistant Professor
Title: Dean of College of Science
Date:

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List of Abbreviations

Abbreviation	Key
BFP	Bundle-forming pilus
CCC	Covalently closed circular
CFU	Colony forming unit
CFAs	Colonization factor antigens
DNA	Deoxyribonucleic acid
EAEC	Enteropathogenic adherence factor
EAF	Enteroaggregative *E.coli*
EHEC	Enterohaemorrhagic *E.coli*
EIEC	Enteroivassive *E.coli*
EPEC	Enteropathogenic *E.coli*
ETEC	Enterotoxigenic *E.coli*
LT	Heat labile toxin
MS	Mannose – sensitive
MR	Mannose –resistant
ml	Millilitter

mg	Milligram
O.D.	Optical density
NFAs	Non fimbriae adhesins
PBPs	Penicillin – binding – proteins
PAIs	Pathogenicity associated islands
pap	Pyelonephritis associated pili
RTF	Resistance transfer factor
ST	Heat stale toxin
SDS	Sodium dodecyl sulfate
UECs	Uroepithelial cells
UPEC	Uropathogenic E.coli
U.V	Ultra violet light
UTI	Urinary tract infection

Summary

200 urine samples were collected from patients expected to have urinary tract infection, 123 samples gave positive bacterial growth in culture. From positive cultures, (41) bacterial samples were identified as *E.coli* (33.3%). Percentage of *E.coli* in samples taken from females was 66% while 34% of *E.coli* isolates were identified in male samples.

Sensitivity tests toward 14 antibiotics were carried out , results showed that ciprofloxacin, amikacin, and Naldixic acid were the most effective antibiotics and their resistance percentage were 5%, 5%, and 9.8% respectively. While ampicillin, cephalexin, and erythromycin were not effective and their resistance percentage were 100%.

Six isolates namely, no.5, 16, 30, 31, 35, and 41 were selected depending on the result of antibiotic sensitivity , and tested for adherence ability to uroepithelial cells from normal females. All tested isolates were found to adhere to uroepithelial cells . however , isolate no 5 showed the highest ability of adherence with the mean no. of adhering bacteria equivalent to 34 bacteria per uroepithelial cell .

Plasmid profiles of the six selected isolates were investigated by gel electrophoresis. The isolates no. 16, 30, 31, 35 showed several small bands(1-3 bands) in addition to large one (probably mega plasmid), however isolates no. 5, 41 have no plasmids.

Curing experiments were carried out by treating the isolates no. 16, 30, 31, 35 with Acridine Orange to study the possible role of plasmids of *E.coli* in adherence ability.

The obtained results showed that isolates no. 16, 30, and 35 lost their antibiotic resistance determinants of Cotrimoxazole, Gentamicin, Naldixic acid, Tetracycline, and Ciprofloxacin, indicating that resistance to these antibiotics is under plasmid control, whereas Ampicillin, Cephalexin, Erythromycin, and Cefotaxime resistant determinants were not affected .

Adherence experiment showed that the ability of plasmid cured isolates for adherence to uroepithelial cell were not affected.

Supervisor Certification

I 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 :
Supervisor :Dr.**Mohammed Abdul-kadir Ibrahim**
Scientific Degree: Professor

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

Signature:
Name: **Dr. Nabeel Al-Ani**
Scientific degree: Assistant professor.
Title: Head of Biotechnology Department.
Date:

إلى فلذة كبدي ....

ابنتي الغالية روان

أحمد

الخلاصة

تم جمع ٢٠٠ عينة إدرار من مرضى يعانون من التهاب المجاري البولية، وجد أن ١٢٣ عينة أعطت نتيجة ايجابية لنمو البكتيريا على الأوساط الزراعية(ماكونكي و وسط أكار الدم) منها ٤١ عزلة تعود إلى بكتيريا *E.coli* و بنسبة عزل ٣٣,٣% ، و كما أعطت النتائج أن ٢٧ عزلة من عزلات بكتريا الـ *E.coli* عزلت من النساء (٦٦%) في حين ١٤ عزلة (٣٤%) عزلت من الرجال.

تم فحص حساسية جميع عزلات الـ *E.coli* لأربعة عشرة مضاد من مختلف مجاميع مضادات الحياة ، و أظهرت النتائج أن مضادات الحياة Ciprofloxacin و Amikacinو Naldixic acid لها أفضل فعالية ضد بكتريا الـ *E.coli* من بين مضادات الحياة المستخدمة، وكانت النسبة المئوية لمقاومة العزلات لها هي : ٥% و ٥% و ٩,٨ % على التوالي ، في حين المضادات Ampicillin و Cephalexin وErythromicn لم تظهر أي فعالية تذكر حيث كانت النسبة المئوية لمقاومة العزلات لها ١٠٠% .

تم اختيار ستة عزلات (ذات الأرقام ٥ و ١٦ و ٣٠ و ٣١ و ٣٥ و ٤١) وفق نتيجة اختبار حساسية مضادات الحياة لها، حيث كانت متعددة المقاومة(٧ – ١٣ مضاد حيوي) ، و التي اختبرت فيما بعد لمعرفة مدى قابليتها للالتصاق بالخلايا الطلائية للمجاري البولية و المأخوذة من نساء غير مصابات ، لقد وجد انه كل العزلات المختارة لها القابلية على الالتصاق بالخلايا الطلائية و كانت العزلة ٥ الأكثر قابلية على الالتصاق إذ كان معدل الخلايا الملتصقة ٣٤ خلية بكتيرية / خلية طلائية.

تم التحري عن المحتوى البلازميدي للعزلات المنتقاة من حيث مقاومتها لمضادات الحياة بواسطة تقنية الترحيل الكهربائي ، و لقد أظهرت النتائج أن العزلات ١٦ و ٣٠ و ٣١ و ٣٥ تحتوي على حزم بلازميدية صغيرة يتراوح عددها (١ – ٣) بالإضافة إلى حزمة كبيرة لكل عزلة، بينما لم تظهر العزلاتان ٥ و ٤١ أية حزمة بلازميدية.

و في محاولة لدراسة دور بلازميدات المقاومة لمضادات الحياة لبكتريا *E.coli* في قابلية هذه البكتيريا على الالتصاق بالخلايا الطلائية للمجاري البولية تم معاملة العزلات ١٦ و ٣٠ و ٣١ و ٣٥ بصبغة الاكريدين البرتقالية .أظهرت النتائج أن العزلات ١٦ و ٣٠ و ٣٥ قد فقدت صفة المقاومة لمضادات الحياة: Cotrimoxazole و Gentamicin و acid Naldixic و Tetracycline و Ciprofloxacin ، بينما بقت صفة المقاومة لمضادات الحياة : Ampicillin و Cephalexin و Erthromycin و Cefotaxime .

وأظهرت نتائج فحص التصاق البكتيريا بالخلايا الطلائية بان العزلات المحيدة-البلازميد ، لم تتأثر قابليتها للالتصاق بالخلايا الطلائية مقارنة بالخلايا الأم .

Republic of Iraq
Ministry of Higher Education
and Scientific Research
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A Study on the Possible Involvement of Antibiotic Resistant Plasmids in Adherence of Uropathogenic *E.coli* to Uroepithelial Cells

A Thesis

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بسم الله الرحمن الرحيم

فَبَدَأَ بِأَوْعِيَتِهِمْ قَبْلَ وِعَاءِ أَخِيهِ ثُمَّ اسْتَخْرَجَهَا مِن وِعَاءِ أَخِيهِ كَذَلِكَ كِدْنَا لِيُوسُفَ مَا كَانَ لِيَأْخُذَ أَخَاهُ فِي دِينِ الْمَلِكِ إِلا أَنْ يَشَاءَ اللهُ نَرْفَعُ دَرَجَاتٍ مَنْ نَشَاءُ وَ فَوْقَ كُلِ ذِي عِلْمٍ عَلِيمٌ

صدق الله العظيم

سورة يوسف الآية ٧٦

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

دراسة دور بلازميدات المقاومة للمضادات الحيوية في التصاق بكتريا القولون *E.coli* للخلايا الطلائية للمجاري البولية

رسالة

مقدمة إلى كلية العلوم / جامعة النهرين

وهي جزء من متطلبات نيل درجة ماجستير علوم في التقانة الاحيائية

من قبل

احمد خليل إبراهيم حميد

بكالوريوس مختبرات طبية جامعة صنعاء ٢٠٠١

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