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Introduction

Urinary tract infections remains a common nosocomial problem. Women having Symptoms of lower urinary tract infections account for more than 5 million office visits per year in the U.S.A (Stamm et al., 1980).

The prevalence of bacteriuria increases with advancing age and with increasing functional disability. (Nicolle, et al., 1983).

There is a high prevalence of bacteriuria in geriatric population. The majority of elderly subjects with bacteriuria do not have symptoms of urinary tract infections i.e they have asymptomatic bacteriuria (Boscia et al., 1986).

Bacteriuria in adult and elderly population has been associated with increased mortality rate. This association may result from either a direct effect of the bacteriuria itself or from factors that increase both bacteriuria and mortality. (Nordenstam, et al., 1986)

The Aim of This Work

This work was planned to assess the extent of significant bacteriuria in elderly population and determine the correlation between bacteriuria and sex. Also to determine the association between bacteriuria and symptoms of urinary tract infections as dysuria, urgency, incontinence, anorexia, insomnia, fatigue and malaise etc. Also to determine the presence of the plasmid, responsible for bacterial resistance.

REVIEW OF LITERATURE

Normal Flora of The Urethra

The urethra in male and female is normally sterile or contains, in the neighbourhood of the meatus few organisms. The organisms found in normal urine are contaminant from the lower part of the urethra, vagina and perineum, as bladder urine is normally sterile. The organisms encountered in normal urine may be Staphylococci (Coagulase negative), Diphtheroid bacilli, Coliform bacilli, Enterococci, Proteus Species, Lactobacilli, Alpha hemolytic Streptococci, saprophytic yeasts and Bacillus Species. (Baily and Scotte 1974).

The anterior urethra of both sexes contains small numbers of the same types of these organisms regulary appear in normal voided urine in numbers of 10^{2} - 10^{4} /ml. (Jawetz, et al., 1983).

Normal Flora of The Vagina

Soon after birth, aerobic lactobacilli (Dorderlein's bacilli) appear in the vagina and persist as long as the pH remains acid for several weeks, when the pH becomes neutral, a mixed flora of the cocci and bacilli is present. At puberty, lactobacilli appear in large numbers and contribute to the maintenance of acid pH through the production of acid from carbohydrates, particulary glycogen. This appears to be an important mechanism in preventing the

establishment of other, possibly harmfull microorganisms in the vagina. If lactobacilli are suppressed by the adminstration of antimicrobial drugs, yeasts and some other bacteria increase in numbers and cause irritation and inflammation. After the menopause, lactobacilli again diminish in numbers and a mixed flora returns. The normal vaginal flora often includes also group B hemolytic Streptococci, anaerobic Streptococci (Peptostreptococci), Bacteroids species Clostridia, Gardenerella (Haemophillus) vaginalis, ureaplasma urealyticum and sometimes Listeria. The Cervical mucus has antibacterial activity and contains lysozyme. In some women the vaginal introitus contains a heavy flora resembling that of the perineum and perianal area. This may be a predisposing factor in recurrent urinary tract infections. (Jawetz, et al., 1983).

Urinary Tract Infections

Urinary tract infections was defined by John, (1986) as microbial invasion of any part of the genito urinary tract extending from the renal cortex to the urethral meatus. These infections may be considered as either upper urinary tract infections (renal, parenchymal and ureters) or lower urinary tract infections (bladder, urethral, and in males the prostate gland). Urinary tract infection was classified as uncomplicated acute infection when there is no underlying structural or neurological abnormality, or complicated infection when there is one or more of such lesions.

John, (1986) defined the clinical syndromes of urinary tract infections as :

A) Acute bacterial cystitis : manifested by dysuria, frequency of urination and nocturia associated with the bladder bacteriuria, pyuria, usually and hematuria occasionally.

B) Acute bacterial pyelonephritis : manifested by pain, Tenderness, flanks, fever, bacteriuria, pyuria and occasionally hematuria.

C) Acute urethral syndrome : the urethral syndrome is the presence of urinary symptoms such as frequency and dysuria without bacteriuria. (Braude, 1981).

Bacteriuria was defined by Kass, (1957) as the presence of more than (10^5) organisms per milliliter in freshly voided urine. He believed that any bacterial count less than (10^5) organisms /ml indicated simple contamination and is not indicative of true infection.

Stamey, et al., (1981) found that one third of urinary tract infections were associated with count below 100,000 bacteria per milliliter. At the level of more than 10^5 organisms /ml not only colony count should be taken into account but also the number and type of the species found.

If bacteriuria is associated with few or no symptoms, it is known as asymptomatic bacteriuria (Kunin, 1974).

Maskeli, (1974), suggested that the urethral syndrome (dysuria, frequency, without significant bacteriuria may be due to fastidious anaerobic bacteria. Several reports have shown that the recovery of such organisms is the same in women with urethral syndrame as in healthy women.

Anaerobic urinary tract infections was high in renal dialysis patients and in patient with renal transplantation. [Ribot et al., 1981]

Bacteriuria and Urinary Tract Infections in Elderly People.

Dontas, et al., (1966) found that glomerular filtration rate and renal plasma flow are progressively reduced with advancing age and these decreases are closely associated so that the filtration fraction shows only a slight increase with age. The tubular maximum excretory and concentrating ability also become reduced with age. Elderly people with bacteriuria display a more significant functional impairement tubular capacity being more restricted than the other measures of renal function than do these without bacteriuria.

Bacteriuria and overt infection are rarely seen in males before the age of 50 years in the absence of urinary tract instrumentation. After this age obstruction or any other diseases, leading to instrumentation of the tract are the major causes of infection. Infection tends to persist in male when a residual volume of urine remains in the bladder and if the prostate becomes involved, it may continue to seed the urinary stream. Bacteriuria has been reported to be present in 3.5% of men over 70 years of age and may be as frequent as 15% in hospitalized elderly men. (Calvin, 1979).

The prevalence of bacteriuria in the female rises with age and sexual activity and may be as 10% in elderly women.

It is often higher in hospitalized patients. [Calvin, 1979].

The prevalence of bacteriuria increases with the advancing age and in the elderly the prevalence also increases with the functional disability. In the elderly nursing home population 20 to 50 percent of male and female residents will have bacteriuria. Despite the high frequency with which it occurs, the bacteriuria in this group remains poorly characterized. The site of infection within the urinary tract is not well defined and the naturally history of bacteriuria and its contribution are unknown. Some authors have reported decreased renal function in association with bacteriuria but other studies don't support this findings. (Nicolle, et al., 1983).

The authors added that why urinary tract infections increase with advancing age. Many of the phenomena that accompany aging may be contributed, including changes in bladder function, pelvic musculature, prostate size and immunologic as well as concomitant illness. For the present, however, it is not possible to give particular weight to any of these factors. [Nicolle et al 1983].

Studies by Stamey have suggested that urinary tract infections in women is preceeded by introital colonization by Enterobacteriaceae. Data demonstrated an increasing frequency of pharyngeal carriage of gram negative bacilli

with advancing age raise the question whether similar changes in periurethral bacterial colonization are important in the pathogenesis of urinary tract infections in the elderly. (Stamey, et al. 1985).

Urinary tract infections are more frequently encountered in elderly than in young. Although several mechanical factors such as obstruction, stasis and neuro logic bladder may contribute to the increased frequency of bacteriuria, other factors as yet undetermined may play a role. (Sobel and Kaye, 1985).

One hundred and sixty-one non catheterized consecutive acute geriatric admission were screened for bacteriuria on the day following admission, both in the morning and in the afternoon. The prevalence of bacteriuria was 29% on admission. A correlation between bacteriuria and leucocyturia was shown. The diagnosis of urinary tract infections made prior to admission. There was a significant relationship between incontinence and bacteriuria on admission. 12% of abacteriuric patients become bacteriuric between day 1 and day 7 following admission. Escherichia coli accounted for 51% of the isolates on admission to hospital. (Choudhurg, et al., 1990).

Asymptomatic Bacteriuria

The majority of elderly subjects with bacteriuria do not have symptoms of urinary tract infections i.e they have asymptomatic bacteriuria. (Bascio, et al. 1986).

Bacteriuria is common in elderly people and is usually asymptomatic. In the absence of symptoms, pyuria is the only readily available way to differentiate urinary infections with inflammation from infections without inflammation. (Boscia, et al. 1989).

Quantitative culture of mid stream urine fails to yield a significant microorganism in many patients with acute urinary symptoms. Bladder urine obtained by aspiration from symptomatic adults with equivocal findings on standard testing of mid stream urine for low numbers of conventional uropathogens and fastidious bacteria (561) 31% of (1817) women and 36 (12%) of 300 men to be culture positive. Five hundred-eighty-one (70%) of 830 isolates were fastidious bacteria. 191 (34%) of 561 culture positive women and of 36 culture-positive men had polymicrobic bacteriuria. Bacterial count, were $<10^5$ colony forming units/ml in 67% of samples; 204 of 406 patients with single species infections had increased leucolyte counts in urine. Patients with symptoms of urinary tract infection who are culture negative on standard testing may harbour convetional uropathogens in the bladder. In these patients, culture of bladder aspiration

urine for low counts and fastidious species is necessary to diagnose bacteriuria. (Fairley and Birch, 1989).

Three groups of elderly patients who have bacteriuria can he identified :

- those with classical symptoms of urinary tract infections.
- those who have no symptoms. (the majority),
- and those who present atypically with falls, immobility, confusion or poor general health. (Evans, et al., 1989).

In the elderly, asymptomatic bacteriuria is prevalent and in the absence of obstruction, uropathy or infection stones generally pursues a benign course and requires minimal or no treatment. (Schaeffer, 1991)

Abrutyn et al., (1991), studied asymptomatic bacteriuria in elderly ambulatory women residents without indwelling catheters, in self - contained apartment houses at Philadelphia Geriatric centre in the nursing home. The reversion rate from a positive to a negative culture was high (about 34%). Persistent infection with the same organism was uncommon. Infection risk was associated with residence which was partially explained by a factor evaluating mobility but was unrelated to age or scores evaluating activties of daily living or mental status. Besides mobility, other more complex factors play a role in acquisition of infection.

Recurrent Urinary Tract Infections in Men

Smith et al.,(1979) found that all men with recurrent urinary tract infections had a positive antibody coated bacteria test and 52% had evidence for prostate infection. Escherichia coli infection was present in 74% and urinary tract symptoms in 57% of those randomized. Recurrences were usually with the same organism and most occured within 4 weeks of discontinuing therapy.

Nicolle et al., (1983) found that long term treatment of recurrences was no more successful than single dose therapy thus the effort to sterilize the urine for prolonged periods with discrete courses of antibiotics has resulted in failure. (Nicolle et al 1983).

Giamarellou et al., (1991) found that in prospective randomized study, ofloxacin (400 mg orally once daily) versus co-trimoxazole (320/mg, 600 mg orally once daily) were given for 3 weeks in 30 and 22 elderly semi-mobile patients respectively, suffering from asymptomatic bacteriuria, from the obtained results it was evident that : (a) ofloxacin was superior to Co-trimoxazole regarding eradication of bacteriuria particulary in patients with a positive antibody coated bacteria test (b) a high rate of super-infections and reinfections with strains resistant to Co-trimoxazole was observed in both groups indicative of hidden

underlying conditions predisposing to urinary tract infections and (c) ofloxacin did not accumulate in serum during prolonged therapy but the half life ranged between 8.3 and 10.2 h.

Bacteriuria and Mortality in Elderly Population

Dontas et al., (1981) found that bacteriuria in old age was associated with reduction in survival of 30 to 50 per cent.

Evans, et al (1982) compared the risk of death among bacteriuric women and non bacteriuric women and had found that there is a positive aggociation between bacteriuria and mortality in the general population of women. In a prospective study, 131 of 1458 patients acquired urinary tract infections during indwelling bladder catheterization. Seventy-six patients (25 infected and 51 non infected died during hospitalization, death rates were 19 per cent in infected patients and 4 per cent in non infected patients. Multiple logistic regression analysis demonstrated that seven of 21 prespectively monitored variables were associated with mortality among the catheterized patients.

The acquisition of infection was not associated with the severity of underlying disease. Among patients who died infections occured in 38 percent of those classified as having non fatal underlying disease (15 of 39) and in 27 percent of those classified as having fatal disease (10 of 37). Twelve deaths may have been caused byacquired urinary tract infections. Two patients had urinary tract pathogens in premortem blood cultures. Another 10 died with clinical

picture compatible with serious infection, but no diagnostic cultures were performed.

Platt et al., (1982), concluded that the acquistion of urinary tract infection during indwelling bladder cathetrization is associated with nearly a threefold increase in mortality among hospitalized patients, but the reason for this associaton is not yet clear.

Nordenstam, et al., 1986, found that bacteriuria in adult and elderly population has been associated with an increased mortality rate. This association may result from either a direct effect of the bacteriuria itself or from factors that increase both bacteriuria and mortality.

schaeffer, (1991) concluded that despite improved treatment, mortality associated with urinary tract infections in the elderly remains high particulary in hospitalized institutionalized and , or catheterized patients.

Predisposing Factors for Urinary Tract Infections in the Elderly

1- Anatomical Abnormalities

Certain anatomic abnormalities as stenosis of the external urethral meatus usually in male, the presence of the urethral valve, periurethral diverticula and abnormality of the collecting system in kidney, all these might lead to obstruction of the urine flow which predisposes to infection. (Youmans, 1975).

2- Tumours

Benign and malignant tumours may produce partial or complete obstruction of urine flow, either due to compression of urinary tract by expanding tumour or by direct invasion of the bladder. (Ries and Kaye, 1976). The common clinical associatation between U.T.I and cancer is largely due to the tendency for neoplasma of the prostate, bladder or retro peritoneal space to obstruct the flow of urine. Infection of the tract from a variety of causes such as instrumentation to relieve obstruction, may then lead to further complication and sepsis. Another common association is that pus or tumour cells in the urine may be mistaken for infection, even though there is no growth on culture. Ordinarily, the association would not be further extended. There is however some evidence that urinary tract infections produced by Proteus species may form nitrosamines

by a reaction between secondary amines and nitrite produced by the organism. Most Enterobacteriaceae are capable of reducing dietary nitrate excreted into the urine to nitrite. Indeed, this is the basis for a rapid chemical test used to detect infections with these organisms. (Calvin 1979)

3- Mechanical Factors :

Indwelling urinary catheters are associated with high incidence of urinary tract infection (U.T.I). The condom catheter collecting system has generally replaced indwelling catheters in the management of urinary incontinence in an effort to avoid infection. (Hirsh et al. , 1979).

The most common factor predisposing to the development of urinary tract infection in hospital is catheterization. (Kunins, 1980).

At any given time at least 85% of patients with long term indwelling bladder catheters will have significant bacteriuria. (Grahn, et al. 1985).

Catheterization, cytoscopy and other forms of urethral instrumentation, especially the use of indwelling catheter are procedures associated with a high risk of indwelling urinary tract infections. Prolonged catheter drainage is associated with urethral diverticula, chronic bacterial prostatitis, chronic fibrotic cystitis, vesico-urethral reflux and pyelonephritis. A single transient

catheterization of the bladder induces bacteriuria in approximately 1% of ambulatory and 10% of bed ridden patients. (Sherris, et al. 1986).

For each day that an indwelling urinary catheter is kept in a patient, the rate of bacteriuria increases by 5 to 10 percent, virtually all patients in whom such instrumentation is maintained will eventually become infected. In 10 to 15 percent of cases bacteriuria results from other forms of urologic instrumentation e.g cystoscopy, transurethral surgery or urethral dilatation. Urethral procedures and catheterization have become common and 5 to 16 percent of patients community hospitals have Foley catheters. Although many cases of catheter associated or instrument associated bacteriuria are asymptomatic, at least 1 to 3 percent are associated with secondary bacteriuria and more than 2 percent are associated with wound infection. (Childs, 1986).

Bacterial adherence plays an important part in the pathogenesis of community acquired urinary tract infections in long term catheterization. (Daifuku, and Stamm, 1986)

4- Calculi

Calculi in the urinary tract are prone to cause obstruction of urine flow and increase susceptibility of infection. (Rocha 1969).

On the other hand Kamel (1980) has reported that infection with urea splitting bacteria e.g proteus are likely to cause stone formation.

5- Neurogenic dysfunction

Neurogenic dysfunction predisposing to urinary tract infection because of the inability to initiate or control bladder emptying. (Youmans 1975).

6- Diabetes mellitus

Diabetic patients who require hospitalization have an increased incidence of urinary tract infection due to catheterization. However it was reported that glucose makes urine a better culture medium. (Forland & Thomas, 1977).

7. Schistosomiasis

According to Al. Ghorabe (1981); secondary infection of urinary tract is a common complication and is difficult to overcome.

Urinary Trcat Obstruction and Infection

Obstruction any where in the urinary tract increases susceptibility of the kidney to infection. Increase intrarenal pressure, with its resulting decrease renal flow, appears to be responsible. The exact role of urinary stasis in obstruction related infection, however, has not been defined clearly. (Richard, and Gadon 1983).

Routes of Urinary Tract Infection

1) Ascending Infection :

Work done by Stamey (1981) has shown that, ascending route of infection (i.e urethral organisms spreading to or invading the bladder) is the most important means by which the urinary tract becomes infected.

a) Organisms causing initial and recurrent infections in females are Coliform bacteria that colonize the vaginal introitus and short urethera and, subsequently the bladder.

b) In males, the prostate may play a role in recurrent urinary tract infections although such a role is less clearly defined. This type of infection is often associated with obstruction of the urinary tract.

2) Hematogenous Infection

It is much less common, but urinary tract infections secondary to bacteremia may occur.

a) Renal abscess : The kidney may be the site of Staphylococcal abscess in patients with bacteriuria or endocarditis due to Staphylococcus aureus.

b) Acute pyelonephritis : This can occur by the hematogenous route as a result of gram negative bacteremia of any cause, but, again this occurence is relatively uncommon. (Stamey, 1981).

3- Lymphatic Infection

Experimental evidence suggests that lymphatic connection between upper & lower tracts may play a role in the urinary tract infection in animals. The role of lymphatics in human urinary tract infection, however is unknown. (Stamey 1981).

4- Infection Through Trauma

If traumatic opening is made in the urinary tract this may cause infection if bacteria are introduced; the infection will be more serious if urinary tract is obstructed below the injury or if urine escapes through the traumatic opening and infiltrates the surrounding tissue. (Kamel, 1980).

5- Infection by direct extension from other organs

Direct extension of infection may occur by penetration of bacteria from a nearly infected area through the intact wall of the urinary tract, by rupture of an abscess or by involvement of the wall of the urinary tract in necrosis due to invasive neoplasm. (Kamel, 1980).

Clinical Manifestations of Urinary Tract Infections

Localization studies have been performed, it is clear that clinically it is very difficult, if not impossible to distinguish upper from lower urinary tract infections. Urinary frequency, dysuria and lower abdominal suprapubic discomfort are non specific, and seen both in upper and lower urinary tract infection. (Turch, 1980).

a) Upper Tract Infection

Some patients with acute pyelonephritis have characteristic findings of higher fever 39.5 ^{o}C - 40.5 ^{o}C, shaking chills implies that urinary tract infection may be complicated by bacteremia. These findings may develop rapidly over a period of few hours or 1-2 days. Nausea, vomiting and diarrhea or constipation also may be seen. The examination often reveals pain on palpation of the costovertebral angle and often, of the flank areas but this may be a non specific finding. The urine analysis usually reveals significant pyuria and bacteria, but pyelonephritis can occur with a rather benign urine analysis. (Fairley, 1967).

b) Lower Tract Infection

Clinically one can not distinguish cystitis from the acute urethral syndrome. Acute cystitis is presented with dysuria or burning after urination, as well

as urgency and frequency of urination. (Seinford, 1975).

Suprapubic pain together with fullness or pressure also may be present alone, the patient may complaint of flank discomfort making these findings, unreliable in distinguishing upper from lower urinary tract infection. Physical examination in females is often unremarkable . The patient may complaint of pain on suprapubic palpation. The urethral orifice may be erythematous. Ordinarily no systemic symptoms are present in uncomplicated lower urinary tract infection. Fever is generally absent, although low grade temperature are not uncommon. (Seinford, 1975).

Stamm (1980), stated that acute urethral syndrome (A.U.S) is a very common problem in female, studies have shown that as many as thirty five up to fifty percent of women presenting to general practitioners with symptoms of lower urinary tract infection appear to have this syndrome. These patients exhibit symptoms of frequency and dysuria and therefore, the clinical presentation may be identical to that in cystitis. In general, patients with acute urethral syndrome are febrile. The urine analysis may or may not show pyuria. To distinguish acute urethral syndrome from cystitis, it is important to obtain a urine culture. In patients with acute urethral syndrome, the urine culture is sterile.

Micro - Organism Encountered in U T I

Since the normal flora of the gastrointestinal tract serves as the endogenous source of the great bulk of urinary tract infections, and since urine is a good culture medium for gram-negative enteric bacilli, the predominant organisms include those of the genera Escherichia, Proteus, Klebsiella, Enterobacter (Aerobacter), Serratia and Pseudomonas (Bartell et al., 1974).

Escherichia coli accounts for more than 90% of acute infections in patients with structurally normal urinary tracts. The relative resistance of E. coli to the inhibitory effects of vaginal fluid, its possession of pili that aid its attachment to the epithelial cells of the urinary tract, and its mobility appear to contribute to its effectiveness as a uropathogen. It has been shown that the relationship of E.coli to acute symptomatic U T I is limited to relatively few of the more than 150 O sertypes of this organism found in stool. These serotypes, O1, O2, O4, O6, O7 and O75 are more resistant to the bactericidal effects of serum suggesting that seroresistance also contributes to virulence. Strains of E. coli isolated from patients with upper urinary tract disease possess K surface antigens which are absent from organisms isolated from the lower urinary tract (Sherris et al., 1986).

Proteus species are a common cause of U T I usually in patients having chronic bacteriuria with obstructive uropathy and history of repeated instrumentation (Coetzee, 1972). Proteus mirabilis accounted for 4.9% of the total urinary pathogens in hospitals (Gruneberg, 1980). Proteus vulgaris and Proteus morganii each accounted for about 1% of urinary pathogens isolated from urines derived from general practice and hospital patients (Gruneberg, 1976). Experimental evidence suggests that Proteus mirabilis possesses pili that facilitates its adherence to the mucosa of the renal pelvis. In addition, urease production by all species of Proteus leads to hydrolysis of urea, formation of ammonium hydroxide and alkalinization of the urine. The elevated pH of the urine is directly toxic to renal cells and stimulates the formation of magnesium and ammonium phosphate struvite urinary calculi, which can contribute to the chronicity of infection by producing ureteral obstruction and sheltering the bacteria from the patient's defensive mechanism and the physician's antimicrobial agents (Sherris et al., 1986).

Klebsiella species is the cause of some urinary tract infections and may lead to the formation of urinary calculi by elaborating polysaccharides, mucin and slimes that are suitable lignads (adhesion) for divalent cations such as Ca^{++} (Dewardner, 1967). Klebsiella has three major O

antigens and 72 K antigens. Capsular type 2 and untypable strains have been recovered most frequently from urine (Bartell et al., 1974). Kledsiella and Enterobacter species accounted for 13.9% to 21.6% of organisms causing U T I in hospital while these organisms accounted only for 2.3% to 4.6% of organisms causing U T I in general practice (Gruneberg, 1980).

Other gram-negative bacilli (Providentia Serratia, Hafnia and Citrobacter freudii) accounted for 1.9% to 4% of organisms causing U T I in general practice and from 4.5% to 7% of organisms causing U T I in hospital patients (Gruneberg, 1980).

Pseudomonas aeruginosa : U T I is rare in general practice; it occurs usually in patients who have been instrumented or who have underlying pathology (Coetzee, 1972). Gruneberg (1980), reported Pseudomonas aeruginosa as a cause of U T I only in hospitalized patients (2.7% to 5.2%) during the period 1971 - 1978.

Staphylococci are the causative organisms from 2.7% to 6.9% of urinary tract infections in hospital and from 5.2% to 9.8% in general practice. Staph. epidermidis, a coagulase - negative, novobicin sensetive organism was the causative organism of urinary tract infections in 0.27% as reported by Williams et al., (1976). Staph. saprophyticus

resemble Staph. epidermidis closely but differs in its resistance to novobiocin. It is now recognized as the cause as much as 20% of symptomatic urinary tract infections in young, sexually active women (Sherris et al., 1986). Less than 1% of U T I are caused by Staph aureus (Gruneberg, 1980).

Enterococci have been recognized as the infecting organisms in 1-4% of urinary tract diseases in general practice and 3.4-5.6% of urinary tract diseases in hospital patients. (Gruneberg, 1976 and Gruneberg, 1980). Infection with B-Streptococci have reported by Moulin et al., (1974).

Typhoid and paratyphoid Bacilli, which not primarily pathogens of the urinary tract, may be found in the urine (Turk et al., 1984).

Mycobacterium tuberculosis : occurs as a secondary phenomenon to infections elswhere in the body (Mallinson et al., 1981).

Yeasts, particularly species of candida, may be isolated from catheterized patients receiving antibacterial therapy and from diabetics, but they seldom produce symptomatic diseases, Chlamydia Trachomatis, in contrast, can produce acute urethral syndrome (sherris et al., 1986).

The formation of spheroplast, protoplasts and L-forms in 20% of patients is of problems associated with chronic

bacteriuria and chronic pyelonephritis in adults. During therapy, specially with pencillin, tetracyclines and chloramphenicol, the causative bacteria may manage to survive under conditions of increased tonicity. After discontinuation of antimicrobial therapy, the spheroplasts, protoplasts, or L-forms may revert to the parent bacteria, grow extensively and cause a relapse (Bartell et al., 1974).

Antibiotic Resistance

There are two distinctly different ways by which bacteria may develop mechanisms of resistance to a given antimicrobial agent. These are mutation or inheritance. (Sykes, 1982)

Mutation

Mutation are changes in Nucleotide sequence in the DNA of bacterial chromosome that lead to the synthesis of an altered, but often still functional, protein or other macromolecule. This altered macromolecule is inert or can interfere with the activity of antibiotics. Such mutational lesions are the result of substitution of DNA bases, or insertion, deletion or from shifting in part of the coding sequence. (Drake, 1970)

The latter changes usually cause the production of non functional proteins leading to antibiotic resistance phenotypes only if the protein normally produced was non essential under some conditions, or if the protein's function could be substituted in some other way. For an antimicrobial agent to exert its inhibitory effect, it must enter the cell, bind to its target site and block a step (or steps) in cell metabolism. (Davis and Kagan, 1977).

Mutation that lead to resistance may alter a component of the cell wall or the cell membrane so that, the drug can

no longer be transported into the cell. A number of antimicrobial agents are known to require active transport (whether these transport systems are specific for the antibiotic or whether the antimicrobial agent parasitizes the transport system for a normal and useful metabolite is not known). In teleological terms, it seems unlikely that bacteria would have specific transport systems for chemicals that would kill them, however, they possess specific surface receptor sites for a variety of toxic agents such as bacteriophages, these toxic agents lead to reduction of uptake of the antimicrobial agent so that the cell is resistant. (Barker, 1972).

mutation can alter the target site or binding site for the antimicrobial agent within the cell. It is easy to imagine how change in a single amino acid in a protein could affect the binding of antimicrobial agent to that protein or protein complex without affection normal function. In many such cases, drug binding and biological function don't necessarily require the same functional domains within a protein sequence. Altered ribosomes, DNA or RNA polymerases, and enzymes have been characterized from bacterial mutants that have reduced ability to interact with antimicrobial agents but retain their full normal functions. The changes do not necessarily have to eliminate binding since the protein target molecule could be altered in such a way that,

although the antimicrobial agent binds it can no longer interfere with essential biochemical function. This type of resistance mechanism is most probable when the mutation affects a protein that in part of a multicomponent organelle such as the ribosome. (Barker, 1972).

Mutation may lead to resistance phenotypes by other indirect mechanisms. For example, if an antimicrobial agent binds to a componet that is present in the cell in limited quantity, over production of the component could titrate out the inhibitor. In this case, releif of inhibitor requires mutation in the regulatory genes that synthesis of vital component. Thus premotor. operator constitutive, or repressor negative mutation can effectively determine a resistance phenotype. Alternatively, over production of a protein can result from gene-dosage effects (gene amplication) by some mechanisms that produces multicopies of the required structural gene. A mutation may block the path way to a required biochemical intermediate whose synthesis is inhibited by an antimicrobial agent, in which case there is nothing to be inhibited. (Barker, 1972).

Inheritance

In discussing the development of resistance by inheritance, by far the most common determinants of drug resistance in bacteria are resistance plasmids (R plasmids). The existence of such extrachromosomal elements in bacteria

was unsuspected when antibiotics were first introduced. However, by the late 1950 in Japan, R plasmids were widspread in bacterial infections and they were detected in other parts of the world. (Falkow, 1975).

R plasmid mediated resistance is generally due to synthesis of protein which may enzymatically destroy the drug, modify the antibiotic to an innocuous form, or interact with the cell envelop to make it impermeable to the antibiotic. (Davis and Kagany, 1977)

R factor maydetermine resistance to one antibiotic, or they may carry resistance to 10 or more distinct antimicrobial agents (multiple resistance). R-plasmids are responsible for most of the drug resistance expressed by clinical isolates of gram negative bacteria. (Kenneth, et al., 1986).

Plasmids may be classified into two major classes, conjugative or non conjugative. Conjugative plasmid (or a sex factor is self transmissible from one cell to another. Its presence in a bacterial cell is usually manifested by synthesis of a proteinaceous cellular appendage called sex pillus as well as other plasmids mediated proteins which together provide for cell to cell transmission. Non conjugative plasmids do not have the inherent ability to initiate self transfer nor they transfer specific proteins. (Novick, 1969)

Extrachromosomal element play an important role in bacterial evolution. An abbreviated list of some plasmid determined functions is given below

Plasmid Determined Functions :

1- Replication, repair recombination.

2- Fertility.

3- Restriction and modification.

4- Resistance to antimicrobial agents.

5- Resistance to toxic metals and detergents.

6- Resistance to bacteriophage.

7- Metabolism of sugars and aromatic compounds.

8- Cell adhesion and attachment.

9- Virulence.

In addition to resistance to antibiotic, plasmid containing organism often have an advantage in their ability to grow on exotic nutrient. The distinctive feature of plasmid element is its physical separation from the chromosome of the host cell and its stable maintenance in this extrachromosomal state. (Lowbury, 1974).

Small multi-copy plasmids are invaluable tools for investigating many biological phenomena. First, they are ideal for the study of replication of supercoiled DNA in vitro and in vivo-secondary, gene expression can be analyzed in the absence of a background of chromosomal by segregation

of the plasmid into mincilles. Thirdly, the possibility of easily purifying large amount of supercoiled DNA is useful for the biochemical and DNA sequencing experiments. Using in vitro DNA recombination methods it is now possible to join prokaryotic or eukaryotic genes to plasmid replicons. This allows the application of plasmid techniques to the study of the expression of cloned genes. (Kusia, et. al. 1982)

Characteristics of Plasmids

Plasmids have been isolated from almost all genera of bacteria examined, although many of these plasmids are cryptic. R plasmids can be classified according to their molecular determinants that they carry. They may also differ in other respect that are important properties for their classification. A substatial proportion but not all, of the R-plasmids isolated from nature are transferable or conjugative, i.e they possess the sex factor activity necessary to initiate conjugation between plasmid carrying (R^+) and plasmid free (R^-) bacteria and to transfer the R^+ plasmid from one cell to another in the process. In some instances a transferable R-plasmid can also promote the transfer of non conjugative or non transferable plasmids that are present in the same host by a process known as mobilization. (Holloway, 1979)

Because of their propensity to infect new bacteria plasmids may be considered to be intracellular parasitic or commensal organelles and the bacteria in which they reside to be their hosts. The transfer range of a given plasmid relates to the range of bacteria to which it can be transferred by conjugation, whether or not is subsequently inherited in the transconjugant clone. The transfer range, which is usually much wider than the host range, therefore reflects the compatibility of the plasmid conjugal transfer system which is recipient ability of the conjugal pattern. (Mermod, et al., 1986).

Bacterial Properities Specified by Plasmid Determinants

Plasmid activities can be encoded in the following categories.

a) Resistance to noxious agents

Bacteria, like other living organisms are continually exposed to a range of noxious agents such as U.V irradiation and mutagenic chemicals, heavy metals, toxic organic compounds, antibiotics and lytic viruses (bacteriophages). Resistance to such agents is however, widespread and is frequently plasmid determined (Smith et. al. 1976; Foster, 1983). It should be emphasized that resistance to

antimicrobial agents utilized or produced in large quantities by man, either in medicine (e.g antibiotics & disinfectants), agriculture (e.g pesticides) or industry (e.g heavy metals) tends to be widely distributed. Plasmid mediated resistance to antibiotics is ubiquitous and the presence of single plasmid determinants for resistance to several antibioties (Foster 1983). Mercury compounds are used extensively in medicine as disinfectants and are also released in large quantities into the environment by industry. As a result, mercury resistance determinants are present in many plasmids, particulary antibiotic resistance plasmids (Foster 1983). Resistance to acriflavin in Staphylococcus (Ericson 1969) and to hexachlorophene in Pseudomonas (Sutton & Jacoby 1978), antiseptics that used in hospitals are mediated by antibiotic resistance plasmids.

b) Determinants that Increase Bacterial Competitiveness :

Plasmids are known to specify bacterial properties whose primary function would seem to be inhibition of growth of competing micro-organisms or promotion of growth of the host organism. Bacteriocins are protein antibiotics that generally have a narrow activity spectrum and kill bacteria closely related to the producing organism.

The name bacteriocin was introduced by Jacob et. al 1953 as a general term to define a class of antibiotic like substances produced by various species of bacteria and

distiguished from other antibiotics by their limited range of action and their chemical nature. Bacteriocins are active only against species of bacteria closely related to the strain producing them. They required a specific receptor site on the sensitive strain for their absorption and killing action. Strains producing colicins are immune to their own colicin. However (Ryan et. al. 1955) discovered an exceptional case where the bacteria were sensitive to the same colicin produced by them.

Reeves (1965) discussed the method of nomenclature of colicins. The individual colicins were refered to by the name of the producer strain followed by the type designation. For instance colicin K235-K is colicin of type K produced by E.coli K235. Colicinogenic factors (Col factors) had the same symbol, but are enclosed in parentheses, and the strain designation indicated the strain from which this C-factor was first derived. Thus E.coli K12 made colicinogenic originally derived from E. coli K235. The same principles of nomenclature are applied to the other bacteriocins.

Fredericq (1951) postulated that colicins and bacteriophages both appear in few instances of cross resistance between colicins and bacteriophages, suggesting a common receptor. Microcins are broad spectrum oligopeptide antibiotics. Bacteriocins and microcins are generally

plasmid determined (Hopwood, 1978).

Ferric iron is a mineral essential for bacterial growth and is normally available in the environment only in minute quantities. Some bacteria secrete a siderophore, a compound having an extremely high affinity for iron, and an uptake system for the siderophore iron complex, thereby depleting their environment starving other microbes that do not produce siderophores (Williams & Carbonetti 1985).

c) Adaptation to special ecological niches :

In order to profit from the parasitic mode a facultative pathogen must gain access to and colonize a target tissue, resist or avoid host defences relevant to that tissue, and ultimately damage the host (Timmis & Manning 1986)

Enterotoxigenic bacteria damage the host by releasing enterotoxins which act on the epithelium and cause fluid secretion (diarrhoea). There are two families of enterotoxins of gram -ve enteric bacteria : small molecular weight, heat stable polypeptide enterotoxins (ST), some of which activate guanylate cyclase in epithelial cells, and large molecular weight heat labile protein enterotoxins (choleratoxin) which activate adenylate cyclase. Enterotoxins are largely, though not exclusively, plasmid encoded in E.coli (Williams & Carbonetti 1985).

Whereas enterotoxigenic bacteria generally cause disease by intoxication, other bacteria invade the body. Shigella and entero invasive E.coli for example penetrate and multiply within large bowel epithelial cells, ultimately killing them and causing mucosal ulceration. The ability to invade and multiply in epithelial cells is always specified by a large plasmid that is readily lost during laboratory cultivation of these enteroinvasive bacteria (Sansonetti et al., 1986 and Timmis et al., 1986)

Once a pathogen has caused damage, inflammatory factors are induced and the invading organism is confronted with a battery of host defenses that include complement and phagocytes. Prolongation of the infection i. e the parasitic mode, requires bacterial resistance to these defenses. Resistance to complement and phagocytes may be mediated by a specific polysaccharide of LPS, acid C polysaccharide capsules, and outer membrane proteins of Gram negative bacteria (Timmis et al., 1985) and polypeptide capsules and fibrilar surface structures (M.proteins) of Gram positive bacteria. The O antigens of two Shigellae are partially or entirely plasmid determined (Timmis et. al., 1986). Thus plasmid determined factors can play key roles at various stages in infections.

Plasmids and the Survival of Bacteria

Bacterial plasmids may contribute to the fitness of a particular clone to survive in a specific environment. In many cases. however, the determinants that confer the advantage are unknown. The advantages to be gained from plasmid carriage appear to outweigh potential disadvantages imposed by an increased metabolic demand. For a bacterial cell growing in an environment into which antibiotics are periodically introduced possession of appropriate resistance genes is highly desirable and since the bulk of these are carried on R plasmids, plasmid carriage is also highly desirable. Hence it comes as no surprise to find that organisms recovered from hospital wards ,(O'Brien et al., 1980) Lee et al., 1986).

However, before considering the contribution plasmids make to bacterial cell survival it is appropriate first to consider plasmid survival. Are plasmids, in general, only maintained when they confer a selective advantage ? it has been proposed that plasmids are not particularly stable except when they confer a growth advantage, in that carriage imposes an unnecessary metabolic burden.(Godwin & Slater, 1979 and Helling et al., 1981).

Hence cells from which the plasmid has been eliminated have a slight growth advantage. This would be expected

rapidly to lead to a population of plasmidless cells. However, when mixture of organisms with and without plasmids have been inoculated, the plasmids carrying strains have competed effectively with the plasmidless strains (Meller et al., 1977, Jones et al., 1980 and Jones & Melling, 1984). It is certainly true that since well known laboratory constrents e.g (PACYC 184) Chang & Cohen 1978) are lost quite quickly from culture if they are not periodically reselected by growth on a medium containing an appropriate antibiotic (chloramphenicol, tetracycline). (Dodd & Bennett 1986).

Replication control mechanisms that regulate plasmid copy number in the cell ensure that the desirable plasmid complement is attained before the next round of cell division. This state persists only so long as expression of plasmid genes is efficient and the necessary host systems. such as DNA polymerase, are suitable. Experience indicates that plasmids are well adapted to the hosts in which they are found, although instability may be observed after transfer to another host, such as laboratory strain of E.coli. (Richmond and Sykes 1972)

Curing of R Plasmids

One of the most common features of plasmid is that they can be eliminated from host cells by various treatments. This process termed "curing" apparently results from inhibition of plasmid replication without parallel inhibition of chromosome replication, and as the result of cell division the plasmid is diluted out. Curing may occur spontaneously, but it is greatly increased by use of intercalating dyes such as acridine orange, acriflavine or ethidium bromide. (Hirota 1960, Hohn &Korn 1969; Yamagata & Vehida 1969 as well as by other treatments that affect DNA replication. Such treatment, may include thymine starvation (Clowes et. al 1965), ultraviolet, ionizing radiation, heavy metals (Hirota, 1956) other mutagens (Willets, 1967) as well as growth at elevated temperature (May et al., 1964 Terawaki et al., 1967, El Kerch and Plourde 1976 and Toama et al., 1983) are able to free or cure bacterial cells of plasmid DNA molecules.

Plasmid molecules, which exist as autonomously replicating circular DNA duplexes are eliminated by these agents either because of interference with their replication (acridines, ethidium bromide and novobiocin) or by alterations of their membrane attachment sites (Sodium dodecyl sulfate and elevated temperature) (Novick 1969).

Such effects on episomes were first demonstrated by Hirota and Ijima (1957) who found that acriflavin eliminated the F factor from F^+ cells of E coli and, therefore by Watanabe and Fukasawa (1961) who reported that acriflavine and acridine orange eliminated certain resistance determinants from the R factors of multiple drug resistance bacteria. It was shown five years later (Falkow, et. al 1966 & Rownad et. al 1968) that R factors are composed of DNA, the idea become apparent that the plasmid eliminating action of aminoacridines represent still another instants of genetic effects of complexing compounds. Bounchanud et al., (1969) reported that ethidium bromide eliminated certain resistance determinants from R factor.

Hahan & Cloke (1971) reported that ethidium bromide and acridine orange eliminated the resistance determinants for Kanamycin, chloramphenicol and ampicilline from an R factor in E. coli. Chloroquine and quinine exhibited the same activity while methylene blue was nonactive. Determinants of resistance to streptomycin and sulfadiazine were not cured by any of the compound. Curing provides circumstantial evidence for the existence of plasmids. It is also effective with plasmids that are non transmissible, however, since all curing agents are also mutagenic, it is essential to show that loss of genetic characteristic as a result of treatment with a curing agent is not due to mutation of chromosomal

gene. Usually curing occur at a much higher frequency than nutation and results in elimination of a large block of genes, hence the two process can be easily distinguished. Brock 1979)

Mitsuhashi et al., (1961) used acriflavin for the elimination of transferable R-factors from E.coli and Shigella strains .hey found that all markers for drug resistance were lost by treatment with acriflavin and the cells become sensitive to all drugs. It is also found that the frequency of elimination in Shigella was higher than in E.coli, Terawaki et al., 1967 investigated the elimination of Kanamycin resistance in E. coli strains . E. coli (R.Km) did not loose the Kanamycin marker at 25 °C and 37 °C. At 42, 7% loss of R. Km factor was noticed. Tomoeda et al., (1968) found that sodium dodecyle sulfate (SDS) eliminated R and F factors with very high efficiency from E.coli K12. Partial or complete loss of these genetic elements were observed.

Pinney and Smith (1973) found that R factor was cured from three strains of E.coli K12 by over night exposure to trimethoprim. Elimination was abolished in the presence of thymine indicating that curing is the result of induction of thymineless condition by trimethoprim.

Perea and Daza (1975) succeeded in the elimination of R factor plasmid from E.coli K12, E711, R19 and E.coli K12

E711 R38 with acridine orange and rifampicin (Bounchand et al., 1969). The most frequent loss factor for R_{19} was streptomycin (46.6% by spontaneous loss, 46% with acridine orange and 91.7% with rifampicin. The Kanamycin and streptomycin markers were spontaneously lost together in 41.7% and with neomycin in 8.3%, factor R38 lost the streptomycin marker spontaneously in 20.8% with acridine orange in 8.3% and with rifampicin in 98.4%.The total rate of loss in R19 was practically the same from spontaneous loss or from acridine orange or rifampicin. Factor 38, however, had a 100% loss with acridine orange and rifampicin and only 20% spontaneously.

Toame et al., (1983), tested 16 strains of E.coli for elimination of antibiotic resistance markers of R plasmid by subculturing E.coli strains in the presence of ethidium bromide and acriflavine, or at maximum temperature of 41 C. Partial and/or complete loss of resistance markers were observed with the frequency ranges of 20.8 - 44, 3.5 - 44 and 3.5 - 22.2% respectively. Such successful "Curing" occurred in 7(44%) out of 16 tested strains. Consequently, it is concluded that drug resistance in these 7 host strains is controlled by R plasmids.

MATERIAL AND METHODS

Materials and Methods

Subjects of the study :

Fifty patients (40 males and 10 females) aged above 50 years old, suffering from symptoms of urinary tract infections were studied as group A (Symptomatic cases). Also, 50 pateints (30 males and 20 females) not suffering from any symptoms of urinary tract infections were studied as group B (asymptomatic cases)

Specimen Taken

A mid stream urine sample was collected from each pateint in a sterile container under complete aseptic precautions.

Media Used

A- Media for urine culture :

- Cystine lactose electrolyte deficient medium (C.L.E.D). (B.B.L)
- Mac Conkey's agar. (oxoid)

B- Media for testing biochemical reactions :

- sugar media : glucose, lactose, maltose mannite and sucrose.
- peptone water
- Simmons citrate agar medium
- Nutrient broth
- Christensen's urea broth.

c- Calibrated disposable plastic loop that hold one microliter urine (Nunc)

Collection of Urine Samples

"according to Monica 1989"

- Male pateints were instructed to clean the periurethral region with soap and water and to collect mid stream samples in strile container.
- Female patients were instructed to clean the meatus with soap and water, to collect urine samples in sterile containers.
- The collected urine samples were subjected to direct macroscopical,microscpical and bacteriological study within one hour.

Bacteriological Study

[1]- Estimation of viable bacterial count :

The viable bacterial count was done using calibrated plastic disposable loop, (Nunc) where it was dipped in uncentrifuged urine and inoculated on C.L.E.D agar plate uniformely in different directions. The plates of cultured C.L.E.D agar were incubated for 24 - 48 hours at 37 C after which the growing colonies were counted and multiplied by 1000

Differential bacterial count = number of each type of colonies x 1000

Significant bacteriuria was considered where the viable count was over 10^5 bacteria per one millliliter urine (Kass et. al 1959).

[2]Urine Examination

Fresh centrifuged samples of urine were examined microscopically for pus cells. the presence of more than ten white cells per high power field indicated significant pyuria.

Moreover, Gram stained smears, from the centrifuged urine deposits were examined microscopically, the presence of more than five bacteria per oil immerssion field indicated significant bacteriuria. (Sacks and Abramson 1967)

[3]- Culture

The centrifuged urine deposits were inoculated on the following media

- Mac Conkey's agar
- C.L.E.D agar

incubation of the plates at 37^{o} C for 24 hours aerobically, and the growing colonies were examined as follows :

1- Naked eye examination for colonial characters.

2- Films stained with Gram stain to study the morphology of the organism.

3- Pure clutures were prepared on nutrient agar slopes for further identification by:

- Biochemical reactions,

- Indol production, citrate unilization, urea decomposition, methyl red reaction and Voges Proskauer's test, were done for Gram-ve bacilli (Cruckshank 1982).

- Oxidase test was used for identification of Pseudomonas pyocyanea. Tube coagulase test was performed for identification of Staphylococcus aureus.

4- Antibiotic sensitivity tests for the isolated organisms by disc diffusion medthod:

1- Emulsify several colonies of similar appearance of the test organism in a small volume of sterile peptone water, nutrient broth, or quarter strength Ringer solution.

2- Match the turbidity of the suspension against the turbidity standard which has a similar appearance to an overnight broth culture.

3- Using a sterile loop of about 4mm diameter, apply aloopful of the test organism suspension to the centre of the sensitivity testing plate.Use a sterile dry cotton wool swap to spread the inoculum evenly across the centre thrid of the plate.

4- Using a similar inoculation technique, inoculate an overnight broth culture of the control organism evenly across the upper and lower thirds of the plate, leaving a distance of no more than 5mm on each side of the test organism.

5- Allow the inocula to dry for a few minutes with the petridish lid in place.

6- Using sterile forceps, or a needle mounted in a holder, place the antimicrobial discs between the test and control inocula. Each disc should be pressed dowm on the medium and should not be moved once in place.

7- Within 30 minutes of applying the discs, incubate the plate aerobically at 35-37 C overnight.

8- Read the tests after checking that the bacterial growth of the test and control organisms is neither too heavy nor too light and the control inhibition zones measures 8-15 mm radius.Measure the radius of the inhibition zone, from the adge of the disc to the edge of the zone . The end point of inhibition is were growth starts. (Monica , 1989)

Antibiotic discs used :

	Disc used	Potency	Company
1 -	Nalidixic acid	30 ug	Bio-merieux
2 -	garamycin	30 ug	oxoid
3 -	vibramycin	30 ug	oxoid
4 -	Erythromycin	15 ug	oxoid
5 -	Sutrim	25 ug	oxoid
6 -	amoxicillin	25 ug	Bio-merieux
7 -	Rifocin	30 ug	oxoid
8 -	pencillin G	10 units	oxoid
9 -	Furadantin	30 ug	B.B.L
10-	chloramphenicol	30 ug	oxoid
11-	Streptomycin	10 ug	oxoid
12-	Norfloxacin	10 ug	oxoid
13-	Ceftriaxon CRD	30 ug	oxoid

[4] Serological identification of isolated Ecoli using Ecoli antisera by slide agglutinatin method.

[5] Plasmid Curing

Plasmids can be eliminated (cured) from bacteria by chemical and physical agents.

I- Physical agent "temperature"

Highly resistant isolates to different antibiotics were incubated into nutrient broth flasks (50 ml). The flasks were incubated at 42 °C, 45 °C for 24 hours with shaking.

Samples of 0.1 ml suspension from each flask were plated on nutrient agar plates at 37 °C for 24 hours, then the colonies were tested for resistance level to antibiotics. (May et. al, 1964 and Tera waki et. al 1967)

2- Chemical agent

Ascorbic acid : Cultures of isolates resistant to different antibiotics were grown in liquid nutrient broth supplemented with 100 and 300 ug ascorbic acid /ml medium over night with shaking. Samples of 0.1 ml from each ascorbic acid concentration were plated on nutrient agar plates.

The plates were incubated at 37 °C and the colonies from each ascorbic acid concentration were tested for their resistance to different antibiotic concentrations. (May et al.1964).

The antibiotic discs used to test the plasmid curing and their concentrations are :

Antibiotic	Concentrations
1- Garamycin	25, 50, 100, 200 ug/ml
2- Streptomycin	25, 50, 100, 200 ug/ml
3- Amoxicillin	25, 50, 100, 200 ug/ml