A Clinico-bacteriological Study Of the Interrelation of *Corynebacterium urealyticum* and Struvite Urolithiasis

A thesis

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By

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دراسة سريرية و بكتريولوجية حول

علاقة جرثومة

Corynebacterium urealyticum

و تكوين حصى المجاري البولية

اطروحة

مقدمة إلى كلية الطب في جامعة بابل

كجزء من متطلبات نيل درجة الماجستير

في علم الأحياء المجهرية الطبية

أعدت من قبل

ايفاد كريم عبد الشبلي

بكالوريوس طب وجراحة عامة

١٤٢٦ هـ ٢٠٠٦ م

بسم الله الرحمن الرحيم

وَلَقَدْ أَنزَلْنَا إِلَيْكُمْ آيَاتٍ مُّبَيِّنَاتٍ وَمَثَلاً مِّنَ الَّذِينَ خَلَوْا مِن قَبْلِكُمْ وَمَوْعِظَةً لِّلْمُتَّقِينَ

صدق الله العظيم

سورة النور/ الآية ٣٤

Declaration

We certify that this thesis was prepared under our supervision at the University of Babylon as partial requirements for the degree of M.Sc. in Microbiology…

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Dedication

To …

My Parents

My husband

My son, Ali

To whom I own everything

IFAD

Acknowledgment

Praise is to " ***ALLAH*** " and to his prophet " ***MOHAMMED*** " because this research has been completed under their benediction.

Sincerely and greatly I feel urged to present my deepest affection and gratitude to my supervisor **Dr. Mohammed Sabri Abdul- Razak** for his invaluable support, patience , guidance, encouragement, enlightment and advice throughout conducting this research.

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الخلاصة :

في هذه الدراسة تم إخضاع ٣٢٠ عينة إدرار للعزل والتشخيص استحصلت من مرضى يعانون من التهابات المجاري البولية والمراجعين إلى مستشفى الحلة التعليمي العام الفترة من تشرين الأول ٢٠٠٥ وحتى ابريل ٢٠٠٦ .

عينات الإدرار ذات التفاعل القاعدي كانت ٨٦ عينة . أعطت حصيلة ٨ عزلات لبكتريا Corynebacterium urealyticum جميعها امتلكت نفس الخصائص الزراعية والبايوكيمياوية . أنواع أخرى من البكتريا والفطريات تم عزلها أيضا .

سريرياً ،المرضى المصابون بالتهابات المجاري البولية بهذه البكتريا غالباً ما يكونون خالين من الأعراض العامة لالتهابات المجاري البولية .حرقة البول ، الم الظهر واسفل البطن والادرار الدموي هي اعراض محتملة ولكن هذا لا يكون منطبقا على الحمى . عموماً فان وجود كريستالات الستروفايت ورائحة الامونيا في الإدرار من العلامات المؤكدة لانتان المجاري البولية بتلك البكتريا .

حيث Corynebacterium urealyticum تمت دراسة العوامل المشجعة للاصابة بـ وجد بان الاستعمال المسبق للمضادات الحيوية والتهابات المجاري البولية السابقة يزيد من الاستعداد للإصابة بها . وتم تقييم تأثير بعض العوامل الأخرى كالرقود في المستشفى وقثطرة المجاري البولية والعجز الكلوي والديلزة .

Corynebacterium urealyticum دراسة تأثير بعض المضادات الحيوية على بكتريا أظهرت مقاومتها لأغلب المضادات المستخدمة حيث تراوحت نسبة المقاومة من ١٠٠% للامبسلين ،السيفوتاكزيم ،السفكزيم ،التريميثوبريم إلى ٨٧.٥% مقاومة ضد الاموكسيسلين ، ٧٥% مقاومة ضد الارثرومايسين ،الازثرومايسين والجنتامايسين ، ٦٢.٥% ضد

الاميكاسين ، ٥٠% ضد السبروفلوكساسين بينما أظهرت الدراسة إن أكثر المضادات الحياتية فعالية هو الفانكومايسين بنسبة مقاومة ١٢.٥% فقط .

أظهرت قلة Corynebacterium urealyticum ان دراسة القابلية الامراضية لبكتيريا إنتاجها لعوامل الضراوة حيث كان أهمها إنزيم اليوريز وينتج من قبل جميع العزلات وكذلك الحال لإنزيم البروتيز الخارجي ، بينما إنزيم الالكلاين فوسفتيز ينتج من ٧٥% من هيمولايسين ينتج من قبل عزلة واحدة فقط ،في حين ان β – العزلات ، ووجد بان إنزيم جميع العزلات كانت غير قادرة على انتاج كل من البكتريوسين والسدروفورات .

على Corynebacterium urealyticum و خلال دراسات مختبرية تمت دراسة قدرة تكوين حصى الستروفايت عند زرعها و إنماؤها في إدرار بشري حيث ظهر ان الازدياد و ايون الامونيوم للإدرار البشري كان مصحوبا بتكوين كريستالات pHالتدريجي في أل التي درست قابليتها على تكوين تلك E.coli الستروفايت مقارنة مع عدم قدرة ال الكريستالات خلال نموها تحت نفس الظروف المختبرية .

أخيراً تمت دراسة قدرة مثبطات مضخة البروتون : الاومبرازول واللانسوبرازول على وقدرتها الامراضية حيث Corynebacterium urealyticumالتأثير على نمو بكتريا وجد بان التأثير التثبيطي كان معتمداً على الجرعة العلاجية بينما تأثر نمو البكتريا بصورة بزيادة تركيز الاومبرازول (P < ٠.٠٠١) واضحة .

مفاتيح الكلمات :

Corynebacterium urealyticum , الإدرار قاعدي التفاعل ،حصى المجاري البولية ، انتانات المجاري البولية .

ABSTRACT

Three hundred twenty urine samples were collected from patients suffering from urinary tract infection who were admitted to Hilla General Teaching Hospital since October ٢٠٠٥ to April ٢٠٠٦.

Only alkaline samples were selected which formed eighty six specimens yielded ٨ isolates of *Corynebacterium urealyticum* . All the ٨ isolates were identical regarding cultural characteristics and biochemical reactions. Other types of bacteria and yeast were also isolated.

Clinically , patients with UTI due to this bacteria can be totally asymptomatic or they present with symptoms of dysuria , suprapubic discomfort , flank pain . Haematuria can be a presenting symptom . Otherwise , fever is inconstant , and passage of stones and ammonia odor of the urine are strong suggestive evidence of UTI due to *corynebacterium urealyticum* .

The predisposing factors were evaluated in this study. It was found that the previous use of antibiotics and previous UT١ due to organisms other than *Corynebacterium urealyticum* were the most important factors while hospitalization ,catheterization , renal failure and dialysis were other factors .

The antibiogram results of *Corynebacterium urealyticum* revealed its wide range of antibiotic resistance,١٠٠٪ resistence rate

reported for ampicillin , cefotaxime, cefixime and trimethoprim , ٨٧.٥٪ to amoxicillin, ٧٥٪ to azithromycin , clarithromycin and

gentamicin ,٦٢.٥ % to amikacin, ٥٠ % to ciprofloxacin , the most effective antibiotic was vancomycin with only ١٢.٥ % resistance.

Results of the studied virulence factors expression by *Corynebacterium urealyticum* showed that urease was the most important factor in pathogenicity, it was produced by all of the isolates , extracellular protease also was produced by all of them , alkaline phosphotase expressed by ٧٥ % of the isolates , *β*-haemolycin produced only by one isolate, both bacteriocin and siderophores were not produced by any of the isolates.

An experimental study was created to assess the ability of *Corynebacterium urealyticum* to form struvite stones during its growth in human urine . It was found that there was a gradual increase in urine pH with associating elevation of ammonium ion concentration accompanied by bacterial growth and struvite crystals which appeared grossly as a white precipitate at the bottom of the testing tube after an overnight incubation in sterile urine . This observation was compared to the failure of *E.coli* and control urine to increase the urine pH or ammonium concentration in sterile urine

specimens , together with the inability to form struvite crystals when incubated in similar experimental circumstances .

The effects of the proton – pump inhibitors: omeprazole and lansoprazole on growth and pathogenicity of *Corynebacterium urealyticum* were examined . It is obvious that these compound markedly affected urease activity in a dose-dependent manner and the bacterial growth is significantly ($p<٠.٠٠١$) inhibited by an increasing concentrations of omeprazole .

Key words :

Corynebacterium urealyticum , alkaline urine , struvite urolithiasis , UTI .

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CFU	Colony forming unit
CDC	Centre for Disease Control & prevention
EMB	Eosin Methylene Blue
GUE	General urine examination
HPF	High Power field
MR	Methyl – Red reagent
NH_4^+	Ammonium ion
PPIs	Proton-pump inhibitors
RBCs	Red blood cells
Spp.	Species
TMP-SMX	Trimethoprim – sulfamethoxazole
TSI agar	Triple Sugar Iron agar
UTI	Urinary tract infection
VP	Voges – Proskauer
WBCs	White blood cells

١.١ Introduction

Corynebacterium urealyticum (formerly known as *corynebacterium* group D٢ or CDC group D٢) is an aerobic , catalase positive, gram positive rods which shows resistance to multiple antibiotics (Pitcher*,et.al.*,١٩٩٢) . It is one of the most frequently isolated clinically significant corynebacteria from clinical specimens (Funke and Bernards , ٢٠٠٣) . It began to signal its existence in the Forties , and was described for the first time in ١٩٧٤ by a team of the CDC of Atlanta , but it was not until ١٩٨٥ when it began to be studied with thoroughness (Soriano and Garcia , ٢٠٠٢).

Many species of corynebacteria are part of the normal flora of the skin and mucous membranes,*Corynebacterium urealyticum* which is a genitourinary pathogen (Roksha*,et.al.,*٢٠٠٣) has been cultured from the skin of hospitalized patients and from an inanimate hospital environment . This prevalence is sex related and it seems to predominate more in the perigenital region than in other areas (Soriano and Garcia, ٢٠٠٢)

Somestudieshave been done concerning the pathogenicity of *Corynebacterium urealyticum* and its role in the etiology of urinary tract infection and urolithiasis (Aguado,*et.al.*,١٩٩٣; Bordji,*et.al.*,٢٠٠٤ and Frassetto,*et.al.*,٢٠٠٤) .Theurea splittingability of*Corynebacterium urealyticum* plays an important role in its

pathogenicity and explains its ability to live in an alkaline environment .The strong urease activity results in an increase of urine pH . This alkaline pH not only has a toxic effect on the renal epithelium but also predisposes the supersaturation of struvite , and ultimately the production of renal stone (Burne,*et.al.*,٢٠٠٠) . The struvite stone works as a nucleus for colonization of bacteria , causing more serious infection , increasing the incidence of pyelonephritis , alkaline encrusted cystitis and pyelitis , and recurrent urinary tract infections (Emmons,*et.al*.,٢٠٠٤)

Corynebacterium urealyticum is an etiological agent in alkaline encrusted cystitis which is a very severe lower urinary tract infection that is difficult to treat . It is a chronic inflammatory condition of the urinary bladder , described for the first time by Francois in ١٩١٤ as a localized ulcerative inflammation with deposition of struvite on the bottom and on the walls of an ulcer, or a bladder that already harbor some form of inflammation (MacGregor,٢٠٠٠) . At present , most of the cases of alkaline encrusted cystitis are caused by *Corynebacterium urealyticum* (Meng,٢٠٠٥).

Corynebacterium urealyticum has been shown to be resistant to most antibioticsused for the treatment of urinary tract infection, vancomycin is commonly recommended until a specificantimicrobial sensitivity data are available (Meng,٢٠٠٥). The ability of this bacterium to resist a wide range of antibiotics is the result of many

different mechanisms one of which is the production of enzymes that destroy the drug like β-lactamase , adenylating , phosphorylating , and acetylating enzymes ; also there is a loss or alteration of specific proteins that serve as a binding sites or receptors in the susceptible strains (Laurence,*et.al*.,١٩٩٧) .

Up to our knowledge , there is no documented study concerning *Corynebacterium urealyticum* has been conducted in Iraq .

Thus , this work will take this issue as its task , it tries to :

١. **Isolate and identify *Coynebacterium urealyticum* associated with alkaline urine of patients with urinary tract infections.**
٢. **Examine some factors or attributes that may play a role in the pathogenicity.**
٣. **Show the effect of some antibiotics in vitro .**
٤. **Study the in vitro stone formation ability by *Corynebacteriun urealyticum*.**
٥. **Show the effect of the proton pump inhibitors : omeprazole and lansoprazole on the bacterium growth and pathogenicity.**

١.٢ Literature Review

١.٢.١ *Corynebacterium urealyticum* characteristics

Corynebacterium(from the Greek, koryne: meaning " club ", and "bakterion"," little rod ") *urealyticum* " ureolytic" is a gram positive , non- acid fast, catalase positive , oxidase negative, strict aerobic, asporogenic rods that are lipophilic and nonmotile . The cell wall is weaker at the ends allowing the organism to assume a club-shape ; during cell -division the daughter-cells remain attached "snapped" on one side forming L´s and V´s, this arrangement and the club-shape of these cells suggests Chinese character (MacGregor,٢٠٠٠).

The gram-staining morphology shows gram positive coccal or coccobacillary forms (pleomorphic ٠.٥-١μm) that may resembles streptococci. This morphology is often a cuneiform with slightly curved rods, sides not parallel, sometimes slightly wider ends (Funke and Bernards,٢٠٠٣). The morphology varied depending on the age of culture, the culture medium , and the degree of subculturing . Also, the bacterium typically contains granules of polyphosphate (volutin), often at the poles, are referred to as metachromatic granules (Collee ,*et.al.*,١٩٩٦) .

On trypticase soya agar with ٥٪ sheep blood and ١٪ Tween ٨٠, the colonies are slow growing (require incubation for ٤٨–٧٢ hours) small (< ١ mm at ٢٤ hours) , opaque , gray to white , glistening , non-pigmented , and usually non-hemolytic. Blood or serum containing media is required for growth (Marshall, *et.al.*, ١٩٨٧).

The key biochemical reactions that differentiate *Corynebacterium urealyticum* from other corynebacteria species are the strong urease activity , inability to reduce nitrate or readily ferment most carbohydrates (MacGregor, ٢٠٠٠) .

The cell wall structure contains meso–diaminopimelic acid (m–DAP) as the diamino acid as well as short chain mycolic acid , arabinose , and galactose . Palmitic , oleic , and stearic acids are the main cellular fatty acids .Tuberculostearic acid can also be found in *Corynebacterium* cell wall (Funke and Bernard, ٢٠٠٣) .

Corynebacterium urealyticum, like most species of corynebacteria is a normal flora of the skin and the mucous membranes , not only of man , but also of some other mammals . It seems much more frequent in the skin of women than of men , and in both sexes , it predominates more in the perigenital region than in other areas . It is not infrequent to isolate this bacteria from fomities and from the air of rooms where in colonized individuals exist (Soriano and Garcia, ٢٠٠٢).

Twelve different ribotypes were obtained to *Corynebacterium urealyticum* that were variable in their host predominance (human or animals) . Human–related ribotypes are significantly more antibiotic resistant , ribotyping appears to be the most useful tool for strain characterization (Neito,*et.al.*,٢٠٠٠).

Most recent systems of microbial diagnosis correctly identified *Corynebacterium urealyticum* (Simoons-smith,*et.al*.,٢٠٠٠) , with its two versions of data base ; API – system easily did that . Pitcher,*et.al.*,١٩٩٢ successfully separated *Corynebacterium urealyticum* from other species of the genus corynebacteria on the basis of DNA –base composition and DNA –DNA hybridization values .

١.٢.٢ Natural habitat & mode of transmission

The habitat of *Corynebacterium urealyticum* is not known with certainty, this bacteria seems to form part of the normal cutaneous flora ,with high affinity to urinary apparatus specially to distal urethra mucous membranes (Euzeby,٢٠٠٣).

For a long time, *Corynebacterium urealyticum* was considered as a simple cutaneous contaminant or colonizer with little potential

pathogenicity , yet in the recent few years, Balci*,et.al.*,٢٠٠٢ focused theattentiononthepathogenic significances of opportunistic bacteria including *Corynebacterium urealyticum* as there are increasing reports of its association with various infections in patient with normal and hypoimmunity conditions, this is supported by studies of Mikucka,*et.al.*,١٩٩٧ after decades of confusion about the clinical significance, considering the endo and exogenous nature of infection by this bacteria .

The methodsoftransmissionof*Corynebacterium urealyticum* are not completely understood. Transmission from patient to patient , from a colonized hospital staff to patient , and from environmental contamination to patients all are recognized . Neito,*et.al.*,٢٠٠٠ isolated this bacteria from the skin of hospitalized patients, he suggested that it could be transmitted nosocomially, and when an outbreak of nosocomial infection described , a very high prevalence may be found .

No racialpredilectionfor *Corynebacterium urealyticum* infection wasreported and fortunatelymanyof the infections are treatable with antibioticsbut inmultidrug resistant cases transmission of plasmid responsible for the resistance may be important (Frassetto,*et.al.*,٢٠٠٤).

١.٢.٣ Incidence of *Corynebacterium urealyticum* infections

Corynebacterium urealyticum has been recognized as an aetiological agent in an increasing numbers of incidence of serious infections among hospitalized and immunocompromized individuals , infections are often nosocomial , occur in patients with defected host defense , exposure to broad spectrum antibiotics and prolonged hospitalization time (Neito,*et.al*.,١٩٩٦) . After decades of confusion about *Corynebacterium urealyticum* clinical significance , it is now clear that this bacterium commonly colonize before infection , and most infections are acquired during hospitalization (Balci,*et.al.*,٢٠٠٢).

The length of hospitalization period did not significantly contribute to the incidence of skin colonization by *Corynebacterium urealyticum* . It is possible , however , that in such a situation in which a nosocomial spread of this microorganism occurred, a very high incidence rate may be found (Soriano,*et.al.*,١٩٨٨).

Infection with *Corynebacterium urealyticum* occurs in already fragile hosts in whom any additional problems produce at least some morbidity (Frassetto*,et.al.*,٢٠٠٤) .

Immunocompromised patients appear to have higher colonization rate than healthy persons and may have a greater chance of developing an infection after being colonized (Bordji,*et.al.*,٢٠٠٤).

١.٢.٤ Antibiotic Resistance

Corynebacterium urealyticum is generally considered to be resistant to multiple antibiotics .The first published case of infection by this organism was caused by a sensible stock to many antibiotics , in the Eighties , most of the isolated stocks offered resistance to numerous antibiotics, including the β– lactams , macrolids , aminoglycosides and sulfonamides (Zapardiel,*et.al.*,١٩٩٧) . Nevertheless , a property that have been lost progressively , probably because many of the patients with infection by this bacteria have received numerous antibiotics including fluoroquinolone (Soriano and Garcia,٢٠٠٢) .

It is suggested that several external factors could enhanced the appearance of multiresistant strain of *Corynebacterium urealyticum* which could arise from susceptible cutaneous strain ,these factors are unknown but hospitalization and previous use of antibiotics could play a role in the appearance of resistance (Garcia-Bravo*,et.al.*,١٩٩٥).

Most recent studies show an alarming rate of antibiotic resistance by *Corynebacterium urealyticum* , resistance to β- lactams

,clindamycin,erythromycin ,azithromycin , ciprofloxacin , and gentamicin is quite frequent , with vancomycin , doxycycline , and fusidic acid being the agents that are most effective in vitro . Antimicrobial Susceptibility tests may be of utility when prescribing antibiotics in cases in which the present organism is involved .In clinical infections; resistance to many antibiotic is increasing , and so , determination of their susceptibility results may be necessary in order to obtain the best therapeutic goals . The ultimate therapeutic regimens must be chosen according to the in vitro results , thelocation of infection , and previous clinical experience (Camello,*et.al.,*٢٠٠٣).

Soriano,*et.al.,*١٩٨٨ hypothesizedthat since the perineum has been another skin site more heavily colonized by this bacteria ;the antibiotic resistance of *Corynebacterium urealyticum* may start in the gastrointestinal tract and subsequently spread to skin sites .

Corynebacterium urealyticum is almost always multidrug resistant , but rare penicillin-susceptible strains have also been described (Funke and Bernard,٢٠٠٣).

Generally *Corynebacterium urealyticum* is resistant to all of the penicillins and combination of penicillin and β- lactamase inhibitors, also it is resistant to all cephalosporins including the most recently developed ones , and those effective against other gram – positive bacteria . The only exception were to cefoxitin .There is a scanty

knowledge about the mechanism of resistance of *Corynebacterium urealyticum* for most antibiotics , there has been no explanation for the lack of activity of β – lactams in general or for the activity of cefoxitin , which is although scant ,but higher than that of other β-lactams (Garcia – Rodriquez,*et.al.*,١٩٩١) .

Macrolids other than erythromycin show activity similar to that obtained with erythromycin with clarithromycin being the most active , may be due to its greater intrinsic activity . Resistance to macrolids – lincosamide antibiotics is probably due to target modification , however , resistance from drug inactivation also occurs , yet , other non-well defined mechanisms may have been present there (Soriano,*et.al.*,١٩٩٨)

Quinolones were shown to be more active than cephalosporins against *Corynebacterium urealyticum ,* nevertheless , the activity of fluorinated quinolones is irregular and on the whole it is lower than that previously reported , but as could be expected , quinolones with the higher intrinsic activity and those more active against gram positive bacteria were among the most effective against *Corynebacterium urealyticum* (Garcia – Diez,*et.al.*,١٩٩١) .

Of quinolones , ciprofloxacine is superior to norfloxacine administrated at the same dose , the reason could be that ciprofloxacin not only showed more intrinsic in vitro activity against *Corynebacterium urealyticum* but also achieved higher level in serum

and urine (Soriano,*et.al.*,١٩٩١) .The quinolones concentration may be lower in urine of female than male , nevertheless , the best therapeutic results obtained with ciprofloxacin were dose-dependant and the excellent therapeutic results obtained with teicoplanin suggest that higher level in serum correlates better with therapeutic success than do higher level in urine , where quinolones may be less active (Soriano,*et.al*.,١٩٩١).

Aminoglycosides are generally ineffective against *Corynebacterium urealyticum* , although , amikacin may have a value , this is due to its resistance to most aminoglycoside inactivating bacterial enzymes than is gentamicin (Garcia-Bravo,*et.al.*,١٩٩٥) .

Tetracyclines , have irregular activity against *Corynebacterium urealyticum* , doxycycline is the most effective , however their bacteriostatic activity may impair their usefulness in patients with infections caused by this bacteria , who are frequently immunosuppressed , and the higher urinary pH caused by this bacteria may also impair the activity of this group of antibiotic (Garcia – Diez,*et.al*.,١٩٩١).

Corynebacterium urealyticum is universally very susceptible to glycopeptides , both vancomycin and teicoplanin show similar activity , but the pharmacokinetic character of teicoplanin and the possibility of intramuscular administration can make it a valuable alternative to vancomycin for the treatment of urinary tract infection caused by this

pathogen (Garcia – Rodriquez,*et,al.*,١٩٩١) . A high resistance rate , even for vancomycin and to most other known antibiotics have been found , yet , no teicoplanin – resistant case is present (Hernandez,*et.al.*,٢٠٠٦).

Rifampicin although show a very wide antibacterial range , it is not very active against *Corynebacterium urealyticum* but the use of this antibiotic alone hinders by the possibility of development of one step mutation leading to resistance (Garcia–Rodriquez,*et.al.*,١٩٩١) .

Although *Corynebacterium urealyticum* isolates were previously found to be susceptible to many antibiotics , more recent isolates have developed a multiantimicrobial resistance . Neito,*et.al.*,٢٠٠٠ and Frassetto,*et.al.*,٢٠٠٤ both hypothesized that a transmission of plasmid responsible for the multidrug resistance may be important .

١.٢.٥ *Corynebacterium urealyticum* attributes that may influence virulence

The microorganisms possess many attributes or factors that enable them to initiate infection or disease , these are called "virulence factors " . Certain virulence factors may be produced in vivo and not in vitro , and vice versa (Leslie,*et.al*.,١٩٩٨).

The ability of a microorganism to adhere , penetrate the normal mechanical barrier and resist the chemical barrier of the skin and mucous membranes , survive and grow , is a prerequisite for both the indigenous (opportunistic) parasite and the true pathogen to initiate infection (MacGregor,*et.al*.,٢٠٠٠).

The concept of bacterial virulence or pathogenesis in the urinary tract infers that " *not all bacterial species are equally capable of inducing infections* " , *the more compromised the natural defense mechanisms , the fewer virulence requirements of bacterial strain to induce infection*; this is supported by the well documented in vitro observation that bacteria isolated from patient with complicated urinary tract infection frequently fail to express virulence factors (Naber,*et.al.*,٢٠٠٦) .

١.٢.٥.١ Urease

Urea is excreted in the urine of mammals as a detoxification product . Human urine contains ٠.٤ – ٠.٥ M urea , which results in an annual release of ١٠ kg of urea per adult .Urease (urea amidohydrolase **)** catalyzes the hydrolysis of urea to yield ammonia and carbamate , which spontaneously hydrolyze to form carbonic acid and a ٢nd molecule of ammonia .

Urea + water → ammonia + carbamate

Carbamate + water → ammonia + carbonic acid

At physiological pH , the carbonic acid proton dissociates and the ammonia molecule equilibrates with water to be protonated resulting in a net increase in pH (Mobely and Housinger,١٩٨٩).

Carbonic acid → hydrogen ion + carbonate

٢ ammonia + water → ٢ ammonium ion +٢ hydroxide ion

١.٢.٥.١.١ ROLE OF UREASE IN PATHOGENESIS:

Bacterial urease is involved in the pathogenesis of many clinical conditions , it is directly associated with the following:

١- Infection induced stones :

They account for about ١٥- ٢٠ % of all urinary stones ,they are a mixture of struvite and carbonate apatite . When ammonia is released by the action of microbial urease , the pH increases leading to crystallization of stones . In man , *Proteus mirabilis* is the most common organism implicated in stone formation . Recent studies show that *Corynebacterium urealyticum* is an important pathogen involved in struvite stone formation (Thoumas,*et.al.*,٢٠٠٤).

٢- Acute pyelonephritis :

This disease which results from bacterial infection is characterized by interstitial inflammation and tubular necrosis , urease elaborated by urea – splitting microorganisms appears to contribute significantly to tissue damage , inflammation and cell invasion (Hertig,*et.al.,*٢٠٠٠) . *Corynebacterium urealyticum* is responsible for the picture of acute and chronic cystitis (including encrusted cystitis) , pyelonephritis , pyeloureteritis and sepsis . In patient with kidney transplant it can produce encrusted pyelitis and other pathologies that may lead to the loss of organ (Soriano and Garcia , ٢٠٠٢)

٣- Host defence mechanisms :

The defense mechanisms against bacteria are compromised in the kidney tissue by inactivation of complement , the kidney tissue inactivated the fourth component of complement and this attributed to the release of ammonia by the activity of renal glutaminase . A parallel was demonstrated between the quantity of ammonia librated and the extent of anticomplementary effect , it is suggested that ammonia generated by urease producing organisms probably also contribute to the anticomplementary effect (Mobely and Housinger,١٩٨٩).

٤- The indwelling catheters :

The catheterized urinary tract appears to offer an excellent nick for colonization by urease producing organism leading to urinary catheter obstruction specially in long term urinary catheters (Dominguez-Gil,*et.al.*,١٩٩٩) . It is believed that *Corynebacterium urealyticum* adheres to all catheter materials (specially to urinary catheters) in greater numbers than do other bacteria (Soriano,*et.al.,*١٩٩٣) .

١.٢.٥.١.٢ PHYSIOLOGY OF MICROBIAL UREASE :

A- Urease regulation :

Ammonia , a product of urea – hydrolysis , is the preferred nitrogen source among enteric bacteria and it can be assimilated into a variety of nitrogenous compounds via glutamine which is synthesized by addition of ammonia and glutamate , a reaction catalyzed by glutamine synthetase . It was earlier hypothesized that synthesis of many enzymes related to nitrogen metabolism including urease was regulated by glutamine synthetase , however this proposal was found later to be incorrect . Other bacterial species appear to respond directly to substrate urea as an inducing agent , a third class of urease appear to be produced constitutively and synthesis is not affected by addition , or limitation of ammonina , urea , or other nitrogenous compounds (Mobely and Housiger,١٩٨٩) .

B- Cellular localization of urease :

Although urease is occasionally described as an extracellular enzyme , some studies indicated a periplasmic or membrane – bound location , while other considerable evidence proposed that the enzyme is cytoplasmic in both bacteria and yeast (Mobely and Housinger,١٩٨٩) .

١.٢.٥.١.٣ UREASE INHIBITORS :

Microbial urease inhibitors have potential values in controlling urolithiasis , they can be used as probes to unravel the enzyme mechanism .

Several urea analogues (substrate analogues) have been examined as urease inhibitors including alkylated urea , thiourea , hydroxyurea and numerous hydroxamic acids . Hydroxyurea is both an inhibiter and substrate of microbial urease ; addition of this compound to a sample of urease result in a rapid inhibition followed by a slow recovery of activity as hydroxyurea is hydrolyzed . Hydroxamic acids are good metal chelators , thus the mechanism of inhibition have been generally assumed to involve binding to the active site nickel ion (Soriano,*et.al.*,١٩٨٧).

Phosphoroamides possess a tetrahedral geometry that may mimic an intermediate state in enzymatic catalysis , thus acting as transition state analogue . While recent studies with urease have indicated that fully protonated phosphoric acid is a competitive inhibitor , little inhibition at neutral pH because partially deprotonated , phosphate binds poorly to the enzyme . Other urease inhibitors such as thiol , thiol – reactive reagents , boric acid and boronic acid all are shown to be a competitive inhibitors to urease enzyme while the kinetic mechanism of the inhibition by fluoride is still not fully characterized (Kobashi,*et.al.*,١٩٦٢) .

The proton – pump inhibitors (benzimidazole group) : omeprazole , lansoprazole and analogues :

The inhibitory mechanism by the proton – pump inhibitors against urease and the (H^+/ K^+) – ATPase has been suggested to be due to blocked SH – residues on the cystien residues which is essential for the activity of both the urease and the ATP –ase . Nagata*,et.al.,*١٩٩٥ suggested that the inhibition is independent of the urea concentration , and the potent bacterial urease may represents a potential target for the benzimidazoles , while the inhibition of this enzyme may provide one explanation for the antibacterial property of this group of compounds . Nevertheless , Mirshahi,*et.al.,*١٩٩٨ have found that bacterial urease activity is competitively inhibited by omeprazole in a dose – dependent manner , and urease enzyme exhibits a hyperbolic kinetic with respect to urea concentration , the rate of urea degradation is proportional to the concentration of urease , and urease activity of bacteria grown in the presence of omeprazole is lower than that found in controlled samples , a Line Weaver – Burk analysis indicated that bacteria grown in the presence of omeprazole has K_m value for urease higher than control , and the extent of inhibition is proportional to omeprazole concentration .

١.٢.٦ Other factors that may influence virulence of *Corynebacterium urealyticum*

١.٢.٦.١ Alkaline phosphatase

It is a hydrolase enzyme (catalyze the non specific hydrolysis of phosphonoesters) . As the name suggests , it is more effective in an alkaline environment (Ching,*et.al*.,١٩٩٨) . Although the actual purpose of this enzyme is still not fully understood ; the simple hypothesis is that it is a means for the bacteria to generate free phosphate group for uptake and use is supported by the fact that alkaline phosphatase is usually only produced by the bacteria during phosphate starvation (Dixon,١٩٩٩) .

Alkaline phosphatase is usually found in all living cells and it is one of the master control switchers that regulate virtually all types of cellular activities . To turn cellular activity on , an enzyme called kinase attaches or tags a phosphate molecule to a specific amino acid in the cell , to turn cellular activity off , another enzyme called protein tyrosine phosphatase , removes or snips off the phosphate molecule (Penny and Huddy,١٩٦٧) . It is believed that the parasitic phosphatase has a role in infection because it is produced more

during infection (Miniatis,*et.al.*,١٩٨٢) . *Corynebacterium urealyticum* is able to produce alkaline phosphatase enzyme , although variably , depending on the source of isolation (whether human or animal) or the type of infection . There is a strong evidence suggesting the important role of alkaline phosphatase in pathogenesis of *Corynebacterium urealyticum* has been proposed by Neito,*et.al.*,٢٠٠٠.

١.٢.٦.٢ Extracellular protease

Proteolytic enzymes represent a class of enzymes that catalyze important proteolytic steps in infection cycle of number of pathogenic microorganisms , they assist the hydrolysis of large polypeptide into smaller peptides and amino acids (Beynom and Bond,١٩٨٩) . Numerous proteases are produced by microorganisms owing to their broad biochemical diversity and their susceptibility to genetic manipulation and depending on the species of producer or the strain , even belonging to the same species . Several proteases are produced by the same strain under various cultural conditions (Pollack,*et.al.*,٢٠٠٠) .

Protease is involved in the virulence of many gram positive bacteria including *Corynebacterium* species (Roksha,*et.al.*,٢٠٠٣) .

A study done in Iraq by Alwash,٢٠٠٦ concerning benign prostatic hyperplasia and chronic prostatitis revealed the role of protease enzyme which acts as a spreading factor facilitating bacterial invasiveness in the urinary infection caused by *Corynebacterium urealyticum* .

١.٢.٦.٣ Bacteriocines

Bacteriocines are grouped under the term toxin and provide a means of defense against other microorganisms in the same environment (Leslie*,et.al.*,١٩٩٨). They are generally a proteinaceous agents , yet sometimes complexes with lipids , carbohydrates or other distinctive proteins (Nissen – Meyer*,et.al.,*١٩٩٢).

١.٢.٦.٤ Haemolycins & Siderophore

Haemolycins are enzymes that destroy red blood cells and other cells of various sources , they play a major role in the iron acquisition . *Corynebacterium urealyticum* have been found to be haemolycin producer ; although inconstantly, a β-haemolycin was produced in up to ١٦ % of strains studied by Tilton,*et.al.*,١٩٩٢. While siderophore are compounds that chelate iron and promote its transport as a soluble complex . A plasmid – determined

siderochrom plays a major role in the invasiveness of some pathogenic bacteria (Nassif,*et.al*.,١٩٨٩) .

١.٢.٧ Clinical aspects of *Corynebacterium urealyticum* infections:

١.٢.٧.١ Alkaline urine

The urine pH is normally acidic (about pH:٦) and may swings from acid to alkaline and back during the course of a day . Infection with urea- splitting bacteria such as *Proteus* species and *Corynebacterium urealyticum* causes an alkaline urine .

Urine alkalinity is one of the most common urinary problems , this condition is generally more common in women and it seems to be more often due to urine alkalosis rather than from any other causes . As long as the urine is slightly acid (pH ٦ or less) most bacteria can not proliferate , because the acidic medium is not conducive to such growth . On the other hand , if the urine exhibits a pH above ٦ (pH of ٧ , ٨ and even ٩ is possible) it then provides an acceptable media for bacterial growth . Otherwise , a strong alkaline

urine can be itself , even in the absence of infection , a sufficient factor to initiate an irritation to the urinary tissues (Graff, ١٩٨٣).

Alkaline urine does not always indicate the presence of a urea – splitting organisms . During the day , the rush of acid into the stomach after a meal creates a relative alkalinization of the rest of the body , resulting in what is called an " alkaline tide " in the urine following meals . Besides , several pathological conditions may also cause an alkaline urine , including the chronic use of diuretics , excess aldosterone , and excessive vomiting of gastric contents without lower gastrointestinal contents (Ying,٢٠٠٥).

According to the German reference Teedrogen,٢٠٠١ sodium bicarbonate in baking soda will cause an alkaline pH urine , but only for a short period of time when the buffering system of the body is kept in balance via the excretion of excess alkalinity through the urine . Foods may also contribute to the alkaline urine , the conventional texts in both the U.S. and Europe imply that a low protein , high fruit and vegetable diet specially citrus may lead to alkalinization of urine .

١.٢.٧.٢ Interrelation between Urinary tract infection and alkaline urine

Since ١٩٩٢ *Corynebacterium urealyticum* is known to be an agent of urinary infection . The infection occurs in a predisposed ground (immunosuppression , catheterization , probes ,urinary tract infection with other pathogen , ..etc) . In patients is presented with leukocyturia (pyuria) , haematuria , alkaline urinary pH containing many bacteria and ammonium – magnesium phosphate crystals , *Corynebacterium urealyticum* is responsible for chronic and recurring UTI_s , occurring among patients who are (emaciated , immunocompromized , diabetic , with vesicle problems , ...etc) often for a long time hospitalization , carrying a probe or having underwent instrumental intervention or surgical operations (Soriano,*et.al*.,١٩٨٥) .

The patients present with alkaline urine containing struvite crystals and in the presence of preliminary lesion of the vesical mucous membrane ; encrusted cystitis (chronic and ulcerative ignition of the bladder accompanied by struvite deposit on the mucous membranes) and even pyelitis or pyelonephritis are possible to be observed (Euzeby,٢٠٠٣).

Urinary tract infection due to *Corynebacterium urealyticum* is opportunistic and it requires three conditions to proceed:

١. A clinical context with immunosuppression or prolong antibiotic therapy .

٢. A urologic interventions either surgical or endoscopic responsible for contamination of the urinary tract .

٣. An inflammatory or neoplastic lesion , preexisting on the urothelium providing a favorable environment for stone encrustation (Thoumas,*et.al.*,٢٠٠١)

Corynebacterium urealyticum is characterized by its ability to produce urease and tolerance of high urinary pH (alkaline urine) , these properties enable it to grow and colonize in urinary tract (Bsci,٢٠٠٠) . The bacterial urease transforms urea into ammonia , this activity explains the modification of urinary pH which becomes alkaline leading to formation of struvite crystals which injures the glycosaminoglycan layer which in turn increases the bacterial adherence and enhances the formation of renal stone and encrustation of the urinary catheters (Naber,*et.al.*,٢٠٠٦).

Alkaline encrusted cystitis which is a severe chronic infection of the bladder , was first described at the beginning of the twentieth century by Francois , ١٩١٤ . More recently alkaline encrusted pyelitis has been reported particularly in renal transplantation (Aguado,*et.al.*,١٩٩٣) but also in native kidney (Meria,*et.al.*,١٩٩٨) . These infections are caused by urea- splitting organisms and are characterized by stone encrustation in the wall of the urinary tract. Although numerous bacteria have a positive urease activity , *Corynebacterium urealyticum* is frequently the origin of this disease

which is nosocomial and occurs in immunocompromised and debilitated patients (Bailiff,*et.al.*,٢٠٠٣). They usually present with dysuria and suprapubic pain , inconstant fever , macroscopical haematuria with elimination of stones , and an ammonia odor of the urine , are strongly indicative of this disease , while urine analysis is characterized by alkaline pH , pyuria , haematuria , and struvite crystals (Thoumas,*et.al.*,٢٠٠١).

١.٢.٧.٣ Struvite stone formation

One of the most important and common problem of urinary system is the formation of renal stones . Identification of renal stones has a history of ٢٠٠٠ years . Factors like nutrition , environmental conditions , sex , genetics , and presence of urease positive bacteria have been reported to play a role in the formation of renal stone (Bordji,*et.al.*,٢٠٠٤) .

Struvite calculi account for up to ٣٠٪ of all urinary stones worldwide , they are also known as triple- phosphate , infective , and urease – stones , they are found more frequently in women and patients older than ٥٠ years (Meng,٢٠٠٥) . The normally soluble polyvalent ions become supersaturated as the pH increases from ٦.٥ to ٩ which occurs when ammonia is released by the microbial urease – catalyzed urea hydrolysis with consequent crystallization and stone formation (Naber,*et.al.*,٢٠٠٦).

In addition to microbial ureolytic activity it is speculated that organic substances contribute to the formation of the struvite stone matrix . Griffith,*et.al.*,١٩٨٨ suggested that host mucoproteins from the urothelial surface may serve as a nidus for stone formation .

Bacterial glycocalyx may also play an important role in struvite stone maturation . Mclean,*et.al.*,١٩٨٥ and Nickel,*et.al.*,١٩٨٥

proposed that the glycocalyx forms an initial surface for adherence of the infecting bacteria , urease activity then causes struvite and apatite precipitation and entraps the resultant crystals as well as host mucoproteins , the bacteria within this matrix are protected from antibiotics action , resulting in a persistent infection , further stone growth and potentially sever renal damage (Mobely and Housinger.,١٩٨٩).

Treatment of struvite stones depends on the eradication of the urinary infection and the complete removal of the stone that contains the urea-splitting bacteria , local acidification of urine , urease inhibitors and vancomycin will inhibit stone growth by preventing formation of ammonium-magnesium phosphate crystals (Hertig,*et.al.*,٢٠٠٠) .

٢.١ PATIENTS & MATERIALS

٢.١.١ Patients

Three hundred twenty patients attended the out – patients clinic , department of urology at Hilla general teaching hospital with symptoms of urinary tract infection underwent the study for the period October ٢٠٠٥ to April ٢٠٠٦ . Urine samples collected from all patients & sent for general urine examination , urine culture & sensitivity .

٢.١.٢ Specimens Collection

Mid – stream , clean – catch urine samples were collected in sterile screw – capped glass tubes, alkaline samples were selected according to the reading of a pH-strip . The patients were first

instructed about how to collect the specimens correctly . The samples were transferred to the laboratory, as soon as possible , for examination.

٢.١.٣ Laboratory instruments

The laboratory instruments used in this study are shown in Table (٢.١).

Table (٢.١) Laboratory Instruments

No.	Instrument	Company
١	Autoclave	Stermite , Japan
٢	Incubator	Memmert , Germany
٣	Sensitive electron balance	A & D , Japan
٤	Distillatory	GFL , Germany
٥	Centrifuge	Hermle , Japan
٦	Oven	Memmert , Germany
٧	Refrigerator	Concord , Italy
٨	Millipore filter	Satorins membrane filter Gm ,BH ,W. , Germany
٩	Light microscope	Olympus , Japan
١٠	Micropipette	Oxford , USA
١١	pH – meter	Hoeleze & Cheluis kG , Germany
١٢	Spectrophotometer	Bauseh & Lomb
١٣	Inoculating loop	Japan
١٤	Inoculating needle	Japan
١٥	Benson burner	Germany

٢.١.٤ Chemical & Biological materials :

A – Chemical materials :

The chemical & biological materials used in this study are shown in Table (٢.٢).

Table (٢.٢) Chemical & Biological materials

No.	Chemical materials	company
١	NaCl , NH_4Cl , $MgSO_4$, $CaCl_2$, KOH , KH_2PO_4 , Na_2HPO_4 , K_2HPO_4.	Merk –Damstad
٢	α - naphthol , easculin , trichloroacetic acid , tetramethyl – ρ- para phenylene – diamine dihydrochloride , chloroform	B.D.H.
٣	H^2O^2, glucose , ٩٩٪ ethanol alcohol , urea- solution , kovac's reagent	Fluka chemika ,Switzerland .
٤	Antibiotic discs	Oxoid –England
٥	Omeprazole powder	Ajanta pharma ,India
٦	Lansoprazole powder	Brownx & burk ,London ,U.K.

B-Culture media :

The culture media used in this study are shown in Table (٢.٣)

Table(٢.٣) Culture media

No.	Media	company
١	Blood agar base , Brain – heart infusion agar , Brain – heart infusion broth , agar –agar , MacConkey agar , Peptone broth , Müller-Hinton infusion agar	Mast
٢	Nutrient agar , Nutrient broth	Oxoid
٣	Urea agar base , Simon citrate agar , Triple sugar iron agar , MR –VP broth, Trypticase soya agar	Diffco - Michigan

٢.٢ METHODS

٢.٢.١ The preparation of reagents

١. Oxidase Reagent

This reagent was prepareddirectly bydissolving ٠.١g of tetramethyl –ρ- paraphenylene diaminedihydrochloride in ١٠ ml of distilled water,to be store in a dark container. Every time used ,the reagent has been freshly prepared (Baron*,et.al.,*١٩٩٦).

٢. Catalase Reagent

This reagent is prepared by adding ٣٪ of (٣٧٪ w/v) H٢O٢ to ١٠٠ ml of distilled water , to be stored in a dark container (Baron*,et.al.,*١٩٩٦).

٣. Methyl Red Reagent

٠.١ gm of methyl red was dissolved in ٣٠٠ ml of ٩٩٪ ethanol and then , the volume was completed to ٥٠٠ ml by distilled water (MacFaddin,٢٠٠٠) .

٤.Barritt's Reagent of Voges Proskauer test

A-٥ gm of α - naphthol was dissolved in ١٠٠ ml of ٩٩٪ ethanol alcohol ,stored in a dark container in cool place.

B- ٤٠ gm of KOH was dissolved in ١٠٠ ml of distilled water (Collee,*et.al.,*١٩٩٦).

٥. Kovac´s reagent

Itwaspreparedbydissolving٥ gmof (ρ - dimethyl aminobenzaldehyde)in ٧٥ ml of amyl alcohol,then ٢٥ ml of concentrated HCl was added, thisreagent wasusedforthe detectionofindole (MacFaddin,٢٠٠٠).

٢.٢.٢ The Preparation Of Media

١. M٩ Media

٦g of Na_2HPO_4 , ٣g of KH_2PO_4 , ٠.٥g of NaCl and ١g of NH_4Cl are dissolved in ٩٥٠ ml of distilled water with ٢٪ agar , then they sterilized into autoclave , after cooling the mixture to ٥٠ ° C , ٢ ml of ١M of $MgSO_4$, ١٠ ml of ٢٠٪ glucose and ٠.١ ml of ١ M of $CaCl_2$ (all of them were sterilized separately by filtration) were added to it ,then the volume was completed to ١٠٠٠ ml (Miniatis,*et.al.*,١٩٨٢) .

٢.Easculin Media

Nutrient agar was prepared , then ١.٤ gm ferric citrate and ٤g easculin were added, the volume was then completed to ١٠٠٠ ml , then were poured into tubes and were sterilized into the autoclave , slants of media were made (Baron,*et.al.*,١٩٩٦).

٢.٢.٣ The Preparation of Solutions

The preparation of ρ – nitrophenyl phosphate solution :

٠.١ mg of ρ-nitrophenyl phosphate powder was dissolved in ٩٩ ml of sterile distilled water by which the volume was completed to ١٠٠ ml , this solution used for the detection of phosphatase production (Penny & Huddy,١٩٩٦).

٢.٢.٤ Staining

٢.٢.٤.١ Gram stain :

This stain was used to differentiate gram negative from gram –positive bacteria in steps declared by Baron,*et.al.*,١٩٩٦ .

٢.٢.٤.٢ Albert stain :

١. This stain was used for the identification of *Corynebacterium urealyticum* microscopically . The steps of staining were as follows:
٢. A heavy smear of bacterial isolate was prepared on glass slide , allowed to get dry without heat fixation .
٣. Albert stain was applied for ٣-٥ minutes , then it was washed with water .
٤. Albert iodine was then applied for ١ minute , washed with water and allowed to dry in air . Later the slide was examined under the microscope to show bacilli with numerous metachromatic granules (Benson,١٩٩٨)

٢.٢.٥ General Urine examination

The urine specimens were collected as aseptically as possible in sterile tubes . Mid-stream specimens were taken from the patients and in those where catheter was inserted , urine specimens were obtained at the time of changing the catheter , through a clean one , then the standard urinalysis was carried for each specimen to determine :

١. the color of urine .
٢. the turbidity .
٣. the reaction by using a sensitive pH – indicator of a known pH – range .
٤. sugar and protein content .
٥. the microscopical content by evaluating the sediment of the centrifuged specimen for:

- number of pus cells per high power field.

- number of RBC_s per high power field.

- number of epithetial cells per high power field.

- number and type of casts and crystals.

٢.٢.٦ Isolation and Identification of *Corynebacterium urealyticum*

Because *Corynebacterium urealyticum* was associated with alkalinization of urine , the pH of all specimens were initially measured by a pH –strips and only specimens with pH of ٧.٠ and higher were cultured for *Corynebacterium urealyticum* . The incubation period required ٤٨ hours or longer before growth was detected , that was due to the slow growth rate of the organism . Furthermore , the isolation was enhanced with the use of specific selective media thus , techniques for the optimum recovery of the organism would certainly increase the expense of processing urine specimens .

The selected specimens were inoculated with the standard calibrated loop onto a Trypticase soya agar plates supplemented with ٥٪ sheep blood and ١٪ Tween ٨٠ , those plates were incubated in ٥٪ $CO_٢$ for ٧٢ hours , then were examined for the presence of small , opaque , white – grayish , usually non -haemolytic colonies . The gram- positive bacilli were further identified according to the protocol of Coyle and Lipsky,١٩٩٠ (Ryan and Murray,١٩٩٤) . In addition to gram – stain , a specific stain had been used (Albert stain) to give a clue to the diagnosis of *Corynebacterium urealyticum* .

The microscopical examinations were followed by a series of biochemical tests to reach the final identification of *Corynebacterium urealyticum*.

٢.٢.٧ Biochemical Tests

١.Catalase test

Catalase is an enzyme that catalyses the release of oxygen from hydrogen peroxide . A small amount of bacterial growth was transferred by a sterile wooden stick onto the surface of a clean dry glass slide , one drop of ٣٪ $H_٢O_٢$ was added to it , the formation of gas bubbles indicated the positive result (Collee,*et.al.*,١٩٩٦).

٢.Oxidase Test

The test depends on the presence of certain bacterial oxidases that would catalyze the transport of electrons between electron donors in the bacteria and a redox dye (tetramethyl – ρ - phenylene-diamine dihydrochloride) the dye was reduced to a deep purple color . A piece of filter paper was saturated in a petri dish with oxidase reagent (freshly prepared) , a small portion of the bacterial colonies was spread on the filter paper by a wooden stick . When the color of the smear turned from rose to purple , the oxidase test was positive (Baron,*et.al.*,١٩٩٦).

٣. Coagulase Test

Several colonies of bacteria growth were transferred with a loop to a tube containing ٠.٥ ml of plasma . The tube was covered to prevent evaporation and was incubated at ٣٧°C overnight . The test was read by tilting the tube and observing the clot formation in the plasma . Negative test results in the plasma remained free-flowing with no evidence of a clot (Collee,et.al.,١٩٩٦).

٤. Indole Test

Some bacteria decompose the amino acid tryptophane , which accumulated in the medium and tested for by a colorimetric reaction with ρ - dimethylenebenzaldehyde . ١٪ of tryptone broth solution was prepared in tube , then it was sterilized into an autoclave , after that the broth was inoculated by a loopful of ١٨ hr. bacterial growth & was incubated for ٤٨-٧٢ hours at ٣٧° C . Indole test was done by adding ٦-٨ drops of kovac's reagent (p-dimethylaminobenzaldehyde in amyl alcohol) . The positive reaction was characterized by formation of red – colored ring at the top of the broth , while formation of yellow – colored ring indicated a negative result (MacFaddin,٢٠٠٠) .

٥. Methyl –Red Test

This test employed to detect the production of sufficient acid during the fermentation of glucose that was shown by a change in the color of methyl red indicator . The test was performed by preparing (MR – VP broth) with ٥ ml in each tube . The tubes were inoculated with bacterial colonies , then incubated for ٢٤ hours at ٣٧°C . After that ٦-٨ drops of methyl –red reagent was added . The change of color to orange- red indicated a positive result (MacFaddin,٢٠٠٠).

٦. Voges – Proskauer Test

This test was used to detect the production of acetyle methyl carbinol from carbohydrates fermentation , or its reduction products. It was performed by preparing MR –VP broth ٥ ml in each tube , inoculation with bacteria colonies was done , then followed by incubation for ٢٤ hours at ٣٧°C , after that ١٥ drops of ٥٪ α - naphthol (reagent A) were added followed by ١٠ drops of ٤٠٪ KOH (reagent B) shaken well and allowed standing up for ١٥ minutes before considering the reaction as negative . When positive, the culture turned red at the surface of the broth and the color spread gradually throughout the tube . The positive result indicated a partial analysis of glucose which produced acetyl methyl carbinol or its reduction product (Collee,*et.al.*,١٩٩٦).

٧.Citrate Utilization Test

After sterilization of Simmon`s citrate medium by autoclaving , the bacterial colonies were inoculated and incubated for ٢٤ – ٤٨ hours at ٣٧ °C , the change of the color of medium from green to blue with streaks of growth indicated positive results , while unchanged original green color with no growth indicated a negative results (Benson,١٩٩٨).

٨. Nitrate Reduction Test

This is a test for the presence of the enzyme nitrate reductase which causes the reduction of nitrate in the presence of a suitable electron donor , to nitrite which can be tested for by an appropriate colorimetric reagent . The bacterial colonies were inoculated and incubated for ٣ days , then ٠.١ ml of the test reagent added to the test culture . A red color developed within a few

minutes indicating the presence of nitrite (positive result), the negative results indicated by addition of zinc dust which gives a red color due to nitrate reduction (Collee*,et,al,*,١٩٩٦) .

٩. Urease Test

The presence of urease enzyme can be tested for by growing the organism in the presence of urea and testing for alkali (NH_3) production by means of a suitable pH indicator . The urea agar base was sterilized by autoclave , allowed to get cool to ٥٠ ^{o} C , urea solution (previously sterilized by filtration) was then added , a deep slope of the medium was made in a sterile tubes then inoculated by the bacterial colonies and incubated at ٣٧^{o}C , and examined after ٤ hours and an overnight incubation , no tube being reported negative until after ٤ days incubation .Urease positive cultures changed the color of the indicator to purple – pink (Collee,*et.al.*,١٩٩٦).

١٠. Easculin Test

The organisms were grown in easculin slants for ٢٤ hours at ٣٧ ^{o}C . The dark brown color indicated a positive result , unchanged color was a negative result (Baron,*et.al.*,١٩٩٦).

١١. Motility test

The semisolid media was dispended in test tubes with ١٠ ml in each tube and leaving the set in vertical position then the bacterial colonies were inoculated by stabbing singly down the centre of the tube to about half the depth of the medium . The cultured tubes were incubated at ٣٧° C and were examined after ٦ hours , ١ and ٢ days . Non-motile bacteria had generally confined to the stab line and given sharply defined margins with leaving the surrounding medium clearly transparent , while motile bacteria were typically given diffuse hazy growth that spread throughout the medium rendering it slightly opaque (MacFadden,٢٠٠٠).

١٢. Triple Sugar Iron (TSI) Test

This test determined the ability to ferment carbohydrates and to produce hydrogen sulfide . The bacterial colonies were inoculated on TSI medium slants by stabbing and streaking , then they were incubated at ٣٧°C for ٢٤-٤٨ hours . The change of the color of the medium from orange – red to yellow was due to carbohydrates fermentation while the formation of hydrogen sulfide gave a black – colored precipitate at the bottom (MacFaddin,٢٠٠٠).

٢.٢.٨ Haemolycin Production

Haemolycin production was shown on blood agar media . The results were obtained after the incubation of the non-inoculated plates for ٢٤ house at ٣٧ ° C to exclude any contamination of blood . Then the organisms were inoculated at that blood agar plates and incubated again for ٢٤ hours at ٣٧ ° C . Any haemolysis presence could be detected around the colonies (either α or β) (DeBoy,*et.al.*,١٩٨٠) .

٢.٢.٩ Siderophore Production

M٩ media were prepared and supplemented with ٢٪ agar, after sterilization in autoclave and cooling to ٥٠°C , ٠.٢٥ % g /l glucose (sterilized by filtration) and ٢٠٠ µm of dipyridyle were added to it , then the organisms were inoculated into this media and incubated for ٢٤ hours at ٣٧ ° C . Presence of growth meant positive results (Nassif,*et.al.*,١٩٨٩).

٢.٢.١٠ Production of Extracellular Proteases

This method was carried out by using M٩ media supplemented with ٢٪ agar . After sterilization in autoclave and cooling to ٥٠ ̊C , ٠.٢٥ gm/l glucose (sterilized by filtration) was added , then the medium was supported by ١٪ gelatin , inoculated and incubated for ٢٤- ٤٨ hours at ٣٧ ̊C , then ٣ ml of trichloroacetic acid (٥٪) was added to precipitate the proteins. The formation of transparent area around the colonies indicated the positive results (Benson,*et.al.*,١٩٩٨).

٢.٢.١١ Production of Alkaline Phosphatase

Detection of alkaline phosphatase production depended on the hydrolysis of ρ-nitrophenyl phosphate (PNP) as follows:

١. ٠.٥ ml of ٠.١ % PNP was added to the test tube
٢. Each tube was inoculated with a heavy inoculum of the tested bacteriagrown on blood agar
٣. The tubes were incubated for ٤ hours at ٣٧ ^{O}C .

If the color changed to yellowish , it would mean the liberation of ρ-nitrophenol which indicated the positive results (Penny and Huddy,١٩٦٧).

٢.٢.١٢ Bacteriocin Production

Detection of Bacteriocin production depended upon a procedure that had been developed by Abbot and Shannon ,١٩٥٨ :

١. A median streak of the test strain with a vertical line was done on Trypticase soya agar and then incubated at ٣٧ ^{o}C for ٤٨ hours to allow the bacteriocin to spread around the growth line.
٢. At the second day , a sensitive strain (s) was (were) inoculated on a nutrient agar and incubated at ٣٧^{o}C to the next day .
٣. At the ٣rd day , the petri – plate cover of the streaked plate was covered by a filter paper impregnated with chloroform in an upright position , then plate culture was inverted upon its cover for ½ hour , then the culture was scrapped by a sterilized glass slide in to a disinfecting vessel and the plate culture was exposed to chloroform vapors , leaving the plate open for ١ hour to remove the chloroform vapor .
٤. The inoculated sensitive strain (s) (which had grown on nutrient agar) was streaked crossing the original scrapped streak line on Trypticase soya agar plate culture and

incubated at ٣٧°C for an overnight . The bacteriocin production was scored as growth inhibition at the median streak line .

٢.٢.١٣ Antibiotics Sensitivity Test

Antibiotic diffusion test (the Kirby–Bauer susceptibility test) was modified and used as follows :

١. It was performed by using a pure culture of previously identified bacterial organism . The inoculum to be used in this test was prepared by adding growth from ٥ isolated colonies grown on blood agar plates to ٥ ml of broth , this culture was then incubated for ٢ hours to produce a bacterial suspension of moderate turbidity . A sterile swap was used to obtain an inoculum from the standardized culture , this inoculum was then swapped on Müeller – Hinton plate .

٢. The antibiotic discs were placed on the surface of the medium at evenly spared intervals with flamed forceps or a disc applicator , incubation was usually for an overnight at ٣٧°C.

٣. Antibiotics inhibition zones were measured using a caliper , zone size was compared to standard zones (from the supplying manufactures) to determine the susceptibility or resistance of organism to each antibiotics (MacFaddin,٢٠٠٠).

Table(٢.٤) The Antibiotics Discs Potency

No.	Antibiotic	Disc Potency µg / disc
١	Amikacin	٣٠
٢	Ampicillin	١٠
٣	Amoxicillin + clavulanic acid	٢٠ ١٠
٤	Azithromycin	١٥
٥	Cefixime	٥
٦	Cefotaxime	٣٠
٧	Ciprofloxacine	٥
٨	Clarithromycin	١٥
٩	Gentamicin	١٠

١٠	Trimethoprime + sulphamethoxazole	١.٢٥ ٢٣.٧٥
١١	Vancomycin	٣٠

٢.٢.١٤ In Vitro Stone Formation by *Corynebacterium urealyticum*

١. A fresh urine sample was obtained from a volunteer with no history of urinary stones or urogenital infectious diseases , sterilized by Seitz filtration . The stone formation ability was detected by growing *Corynebacterium urealyticum* aerobically at ٣٧°C for ٢٤ hours in brain heart infusion broth enriched with ١٪ Tween ٨٠ and ١٠ % serum . ١ml of ١: ١٠ dilution in human urine of an overnight culture of *Corynebacterium urealyticum* was inoculated in to ٩ ml of the sterile urine .

٢. *E.coli* (certified to be non urease- producer) was inoculated in the same mentioned above procedure done for the overnight culture of *Corynebacterium urealyticum* .

٣. All inocula gave a final count of about $١٠^{٧}$ CFU /ml .

٤. A control of ١٠ ml of urine (from the same person) was also studied .

٥. All tubes were incubated at the same incubation conditions .

٦. Ammonium concentration (indophenol method ;appendix) , turbidity and pH were determined at the beginning of experiment and after ٤ , ٨ and ٢٤ hours of incubation .

٧. Sediment was examined at the same intervals and crystals , ifany , wereidentified both macro- and microscopically (Soriano,*et.al.*,١٩٨٦).

٢.٢.١٥ The Effect of omeprazole and lansoprazole on *Corynebacterium urealyticum* stone formation ability

The same procedure described for the stone formation with *Corynebacterium urealyticum* was modified as :

١. A tube containing a fresh urine within it and *Corynebacterium urealyticum* had grown for an overnight .

٢. A ٢nd tube containing , in addition to the contents of the ١st tube, omeprazole (٠.٨ mg/ml) .

٣. ٣rd tube containing in addition to the contents of the ١st tube, lansoprazole (٠.٢ mg/ml) .

٤. A control ٤th tube contained only urine .

٥. All tubes were incubated aerobically at ٣٧°C for ٢٤ hours .

٦. Ammoniumconcentration(indophenol method) , pH , turbidity and crystals were determined at the beginning of the experiment and again after ٤ , ٨ and ٢٤ hours . Crystals had been looked for both macro- & microscopically .

٢.٢.١٦ The effect of omeprazle on the growth rate of *Corynebacterium urealyticum*

١. *Corynebactericum urealyticum* was grown aerobically at ٣٧°C for ٢٤ hours in brain heart infusion broth enriched with ١٪ Tween ٨٠ and ١٠ % serum .

٢. ٧ samples were prepared each contained about the same concentration of *Corynebacterium ureaylicum* count($١٠^{٧}$ CFU/ml) with different concentrations of omeprazole (٠, ٠.٢ ,٠.٤ ,٠.٦ ,٠.٨ ,١.٠ ,١.٢ mg/ml)

٣. In order to determine the number of viable count of the organism ; ٠.٢ ml was removed from each tube after a serial dilutions with normal saline and poured on the surface of Trypticase soya agar plates .

٤. Then incubation was for ٢٤ hours at ٣٧ °C.

٥. After that , the growth rate of *Corynebactrium urealyticum* with and without omerprazole was investigated .

٣.١ CLINICAL SIGNIFICANCE OF ALKALINE URINE

Urine pH is used to classify urine as either a dilute acid or alkalinesolution . pH ٧ is the point of neutrality on the pH scale , the higher the numbers , the greater the alkality of the urine and vice versa .

Urine pH is an important screening test for renal disease . Some bacteria responsible for UTI make the urine more alkaline , because these bacteria split urea into ammonia and other alkaline waist products . Hagar and Magath , ١٩٢٥ found that the formation of renal stones is related to the urine pH ; in an alkaline urine , struvite and calcium phosphate crystals become stones .Patients being treated for renal calculi are frequently have a manipulated diet or medications to change the pH of the urine so that kidney stones will not be formed . In contrast to the inhibitory effect of acidic urine , alkaline urine may provide a suitable environment for bacterial growth .

The diurnal variation of the urinary pH is highly correlated to the acid – base status of the body which is an important mechanism the body uses to maintain a constant body pH , this system is kept in balance via the excretion of excess alkali through the urine (Teedrogen,٢٠٠١).

Although alkaline urine is generally regarded as a biochemical sign ofthe urinary infection by urea-splitting bacteria , many non-infectious physiological conditions may be associated with alkaline urine in the absence of urease producing bacteria , of these the dietary (drinking citrus foods or eating baking soda) and the chronic use of drugs as diuretics may be significantly important (Ramfrez,*et.al*.,٢٠٠٢).

٣.٢ ISOLATION OF MICROORGANISMS FROM ALKALINE URINE OF PATIENTS WITH UTI

In the present study , ٣٢٠ urine samples were obtained from patients suffering of urinary tract infection , attending the out patients clinic , department of urology in Hilla teaching hospital for a period from October , ٢٠٠٥ to April , ٢٠٠٦ .

Because *Corynebacterium urealyticum* is highly associated with alkalinization of urine , all of the ٣٢٠ samples were measured usinga pH – strips , and of these , only eighty six samples with a pH of ٧.٠ or higher were selected and cultured on a trypticase soya agar media for *Corynebacterium urealyticum* .

Seventy four alkaline urine specimens gave a positive culture , otherwise , the negative cultures were attributed to the presence of non- cultivable organisms , such as *Ureaplasma urealyticum* , Mycobacteria or viruses ; or the presence of a non – infectious conditions may be implicated there , such as a physiological alkalinity of the urine following specific foods , drugs , or a pathological state as in inflammation , neoplasm or others .

All of the seventy four positive cultures gave a pure bacterial colonies of single organism .

Table (٣.١) Types of isolated microorganisms from an alkaline urine of patients with UTI

Isolated microorganisms	No. of isolates			%
	Male	Female	Total	
***Proteus* spp.**	١١	٢١	٣٢	٣٧.٢٪
***Klebsiella* spp.**	٤	١٠	١٤	١٦.٢%
Corynebacterium urealyticum	٢	٦	٨	٩.٣%
***Staphylococcus* spp.**	١	٥	٦	٦.٩ %
***Morganella* spp.**	١	١	٢	٢.٣%
***Enterococcus* spp.**	٠	٢	٢	٢.٣%
***Enterobacter* spp.**	١	٠	١	١.١٦%
***Moraxella* spp.**	٠	١	١	١.١٦%
Yeast	١	٧	٨	٩.٣%
Positive cultures	٢١	٥٣	٧٤	٨٦٪
Negative cultures	٧	٥	١٢	١٤٪
Total	٢٨	٥٨	٨٦	١٠٠٪

The results of this study are represented in Table (٣.١) which shows that the most commonly isolated organism from the alkaline urine samples was *Proteus* species forming ٣٧.٢% (٣٢ samples) , followed by *klebsiella* species ١٤ isolates (١٦.٢ %) , while both yeast and *Corynebacterium urealyticum* found in eight samples , forming ٩.٣ % of the total ٨٦ alkaline samples . *Staphylococcus*

species isolated from six specimens (٦.٩ %) , while two isolates (٢.٣%) of *Enterococcus* species and *Morganella* species, and only one isolate (١.١٦%) of both *Enterobacter* and *Moraxella* species were found.

Among all other organisms , *Proteus* spp. plays the most important role in increasing the urine pH . Bordji,*et.al.*,٢٠٠٤ recommended a vaccination against *Proteus mirabilis* infection to prevent colonization and urolithiasis . While Emmons,*et.al.*,٢٠٠٤ pointed out that calculi related to urinary tract infection most commonly occur in patients who experience a recurrent urinary tract infection with *Proteus* species , *Klebsiella* species , and *Pseudomonas* species.

Klebsiella species have been found to be increasingly prevalent in many recent surveys about UTI (Mehdie,٢٠٠٠) , most commonly in catheterized patients (Yassin,١٩٨٨) with a prevalence rate exceeded ١٧٪ .

The concept that UTI plays a role in urolithogenesis is a long standing one , the presence of urea- splitting bacteria , most commonly *Proteus* and *klebsiella* species , are usually associated with struvite stone formation with a potential source of a significant morbidity (Meng,*et.al.*,٢٠٠٥) .

Infection with coryneform bacteria is more frequent in patients after renal transplant , these with neoplasm , AIDs, catheterized patients or long term antibiotic therapy , in addition to babies and elderly . It is characteristic that in urine of patients with bacteriuria with this organism , we could detect struvite crystals and alkaline reaction due to the activity of urease produced by this organism . The results of this study about *Corynebacterium urealyticum* isolation are supported by Mikucka,*et.al.*,١٩٩٧ who isolated this bacterium in ٩٪ from patients with UTI , it was also conducted that the coryneform identification and the evaluation of its role as a pathogenic factor still pose a problem , while the cooperation of the microbiologist and the clinicians seems to be of vital importance. *Corynebacterium urealyticum* is highly associated with renal struvite stones formation ; Bordji,*et.al.*,٢٠٠٤ isolated this bacterium from ١١.٣٨٪ patients with renal symptoms affection attended Tehran hospitals, while the French hospitals revealed ٨.١٪ *Corynebacterium urealyticum* of urine specimens from highly selected patients in a study done by De Briel,*et.al.*,١٩٩١ . Camello ,*et.al.*,٢٠٠٣ suggested that *Corynebacterium urealyticum* is a well – established human pathogen exhibiting resistance to several antibiotics , it accounted for ٥.٥٢٪ of isolates of corynform from various sites of patients in Brazil teaching hospitals .

About the role of *Staphylococcus* spp. , Guirguitzova,*et.al.*,٢٠٠٢ showed that ٢٣٪ of complicated UTI_s were due to coagulase – negative staphylococci , this supports the results of this study which may be explained as that the opportunistic infections are usually caused by endogenic flora

originated from physiological flora ; kazmierczak,*et.al.*,٢٠٠٥ proposed that both of coagulase negative staphylococci and *Corynebacterium urealyticum* establish a kind of symbiological equilibrium on the surface of human skin .

Enterococcus species . a known urea – splitting bacteria , isolated by Sakran,*et.al.*,٢٠٠٣ from ٣٪ of patients with recurrent urinary tract infections , a mixed infection with *Corynebacterium urealyticum* also may occur as documented by Mikucka,*et.al.*,١٩٩٧. The pathogenic potential of coagulase – negative staphylococci and the *enterococci* is controversial ; under certain circumstances , as the presence of stones or foreign bodies , *Staphylococcus* spp . can be a relevant pathogen , otherwise it is not so common in complicated UTI (Naber,*et.al.*,٢٠٠٦).

The isolated *Morganella* spp. in this work confirms the results arrived at by Abdul – Razak,٢٠٠٤ who isolated *Morganella morgani* from ٨ % of alkaline urine samples , it was noticed that urinary tract infection by this pathogen is usually characterized by a relapse and remission , and that the urease enzyme produced by this bacterium is constitutively expressed like that of *Proteus* spp. and *Corynebacterium urealyticum* .

Studies concerning alkaline urine and urinary infections are very scanty , the difference between the rate of isolation of *Corynebacterium urealyticum* and an alkaline urine may be explained on the basis of the selection of patients and the use of selective media which help culture of this organism from all urine samples .

٣.٣ URINALYSIS IN *CORYNEBACTERIUM UREALYTICUM* URINARY TRACT INFECTION

The occurrence of alkaline urine is infrequent when compared to acidic urine , specially when it associates with urinary infection and urinary stone , giving a hint to the presence of urea – splitting bacteria . Hence , *Corynebacterium urealyticum* is characterized by a strong urease production .The pH of all of the ٣٢٠ urine specimens were measured by a pH –strips and only specimens with pH of ٧ or higher (which were ٨٦ specimens) were selected and cultured for *Corynebacterium urealyticum* .

Urinalysis frequently provides an information suggestive of infection with *Corynebacterium urealyticum* , these are alkaline urine , pyuria , haematuria , and the presence of struvite crystals (Graham and Galloway,٢٠٠٢) . Soriano and Garcia,٢٠٠٢ considered that the occurrence of these

signs is not always together , while the integrity of leukocytes seems to be very affected by the urine alkalinity .

In this study , urinalysis results of urine specimens which later found to yield *Corynebacterium urealyticum* revealed that most of the eight samples were alkaline (٧٥٪) with mean pH of ٧.٥ , pyuria and struvite crystals were found in five specimens out of eight (٦٢.٥٪) whereas haematuria detected in six of the eight samples (٧٥٪).

Table (٣.٢) Urinalysis results of *Corynebacterium urealyticum* urinary tract infection.

Items of urinalysis	No . of patients/٨	Percentage
Pus cells	٥ / ٨	٦٢.٥٪
RBC_S	٦ / ٨	٧٥٪
Struvite crystals	٥ / ٨	٦٢.٥٪
pH mean	٧.٥	

These results confirm those of Lutwick,١٩٩٨ in a study concerning *Corynebacterium urealyticum* , it was found that , most of urine samples were characterized by gross haematuria , with mucus and strong odor of ammonia , while encrustation by struvite crystals in the urine may occur .

Bordji,*et.al.*,٢٠٠٤ proposed that , although *Corynebacterium urealyticum* is highly correlated with alkaline urine , it still can live in neutral or even in an acidic environments , in the present study it has been found that two isolates of this bacteriA isolated from urine specimens with range pH of ٥-٧ (Table ٣.٣).

Table (٣.٣) The frequency of *Corynebacterium urealyticum* according to the pH of the urine

Range of urine pH	No . of *Corynebacterium urealyticum* isolates
٥-٦	١
٦-٧	١
٧-٨	٢
٨-٩	٤
Total	٨

When urinalysis is suggestive or a patient has clinical evidence of bacteriuria with a negative standard culture , physician

must consider *Corynebacterium urealyticum* and request the

communication for the clinical microbiology laboratory to specifically look for this bacterium (Ritter,*et.al.,*٢٠٠٤).

٣.٤ CLINICAL FEATURES OF URINARY INFECTION WITH *CORYNEBACTERIUM UREALYTICUM*

In this work , it was observed that , the clinical features of patients who were found to have a positive culture for *Corynebacterium urealyticum* included suprapubic pain or discomfort which was found in five patients of the total eight , macroscopical haematuria and dysuria found in four patients while flunk pain and fever in only two patients .

Table (٣.٤) Clinical Features of patients with positive cultures of *Corynebacterium urealyticum* urinary tract infection

Symptom	No . of patients/٨
Suprapubic discomfort	٥/٨
Flunk pain	٢/٨
Macroscopic haematuria	٤/٨
Dysuria	٤/٨
Symptoms free cases	٢/٨

*total no. of patients is eight

It was also observed that only two patients out of the eight were free of symptoms , this result supports that of Soriano*,et.al.*,١٩٩١ who found that urinary tract infection with *Corynebacterium urealyticum* oftenly gives a clinical picture in about ٦٠ % of the patients only , with some form of urologic disturbance symptoms , while Aguado*,et.al.*,١٩٨٧ noticed that about two thirds of the patients in his study had urinary tract symptoms , it was also revealed that the existence of previous lesions in the bladder favors the development of serious urinary symptoms . Meng,*et.al.*,٢٠٠٥ proposed that even in advanced infection with *Corynebacterium urealyticum* , ٢٥ % of patients may remain free of symptoms .

On the other hand patients with struvite stones can be totally asymptomatic , encrusted cystitis usually presents with dysuria and suprapubic discomfort , while encrusted pyelitis can be minimally symptomatic for long period , otherwise , fever is inconstant, macroscopic haematuria with elimination of stones and an ammonia odor of the urine are strongly indicative of urinary infection by *Corynebacterium urealyticum* . A clinical consequence such as acute obstruction or loss of function of the affected kidney may also be present (Thoumas,*et.al*.,٢٠٠٤) .

Meng,*et.al.*,٢٠٠٥ in a study about urinary infection with *Corynebacterium urealyticum* showed that , the clinical presentation of patients with struvite stones can be variable , infection may result in pyelonephritis or perinephric abscess , symptoms include flank pain classic for renal

colic , low grade fever , urinary symptoms (frequency , dysuria) and haematuria (gross or microscopical) , however struvite stones are rarely manifest as a solitary ureteral calculi with acute renal colic (Sofras,*et.al.*,١٩٨٨).

٣.٥ RISK FACTORS ASSOCIATED WITH *CORYNEBACTERIUM UREALYTICUM* URINARY TRACT INFECTION

To determine the role of some factors that may predispose to infection with *Corynebacterium urealyticum* , all the eight patients with positive culture of this bacterium were evaluated , and the results are illustrated in Table(٣.٥) .

Table (٣.٥) Risk Factors associated with *Corynebacterium urealyticum* urinary tract infection

Risk Factor	No. of isolates/٨	%
Previous use of antibiotics	٧/٨	٨٧.٥ %
Previous urinary tract infection	٧/٨	٨٧.٥ %
Hypoimmunoe conditions (diabetes mellitus , pregnancy , heart disease , …etc)	٤/٨	٥٠ %
Hospitalization	٤/٨	٥٠ %
Catheterization	٣/٨	٣٧.٥ %
Renal failure and dialysis	٢/٨	٢٥ %

*total number of patients is eight (٨)

Among those patients it was found that the previous use of antibiotics and the previous UTI played the most important role in causation of UTI by this bacterium , both of them were found in seven patients out of the total eight (٨٧.٥ %) . Followed by hospitalization which was found in four patients (٥٠٪) . Immunosuppressive conditions also was found in four patients ; two of them were diabetic , one pregnant woman , and only one patient with heart disease . On the other hand ,

catheterization was found in three patients (٣٧.٥٪) while renal failure and dialysis found in only two patients (٢٥٪) .These result support those of MacGregor,٢٠٠٠ who found that risk factors also may include urologic manipulation and previous urologic diseases that created a vesicle ground .

The results arrived at by Garcia–Bravo,*et.al*.,١٩٩٥ are also in line with this work's results about the importance of hospitalization and previous use of antibiotics as risk factors in *Corynebacterium urealyticum* infections . It was proposed that , the percentage of resistance to most of the used antibiotics in UTI is significantly higher in *Corynebacterium urealyticum* isolates from hospitalized patients than from those who are nonhospitalized , otherwise , the frequency of resistance among patients who received antibiotics in the previous month is higher than those who did not receive antibiotics, implicating the antibiotic pressure in hospital environment .

In patients with diabetes mellitus , pregnancy , heart problem , malignancies , AIDs , and other conditions that may be associated with hypoimmunity the incidence of UTI in general was found to be increased , this finding confirmed that of Naber,*et.al*.,٢٠٠٦ who found that in immunosuppression , specially diabetes mellitus , the antibacterial defense mechanisms are adversely affected with increased risk of acute pyelonephritis by opportunistic infections originating in the lower urogenital tract .

It was already noticed that pregnancy predisposed to bacteriuria and a large proportion of pregnant ladies may be asymptomatic . It is possible that the increased incidence of asymptomatic bacteriuria is related in part to ureteral dilatation which occurs during pregnancy (Emmons,*et.al*.,٢٠٠٤).

The daily incidence of bacteriuria ; once catheter is placed , is ٣-١٠ % , while ٩٠-١٠٠ % of patients with long- term catheterization develop bacteriuria . Catheters inoculate organisms into the bladder and promote colonization by providing a surface for bacterial adhesion and causing mucosal irritation . Soriano*,et.al.,*١٩٩٣ and Emmons*,et.al*.,٢٠٠٤ both found that *Corynebacterium urealyticum* , as does *Proteus* spp. , adheres to all catheter materials in greater numbers than do other bacteria .

Also , in this study, the occurrence of urinary tract infections with *Corynebacterium urealyticum* was found to be increased in patients with chronic renal failure , this confirmed similar results of Emmons,*et.al.*,٢٠٠٤ who attributed the high incidence to the host defense modification specially in patients on maintenance haemodialysis , while Naber,*et.al*.,٢٠٠٦ attributed it to the infrequent voiding , low urinary flow rate , and urinary concentration defects associated with renal impairment .

٣.٦ IDENTIFICATION OF *CORYNEBACTERIUM UREALYTICUM*

Corynebacterium urealyticum is a gram – positive , catalase positive , oxidase negative rod . It is strict aerobic , non acid – fast , asporogenic , lipophilic and nonmotile . It assumes a club shape (although may be pleomorphic pleogram positive) , it's arrangement suggests Chinese character (MacGregor,٢٠٠٠) .

This morphology is variable , depending on the age of the culture , the culture medium , and the degree of subculturing . Metachromatic granules (volutin granules) typically found at the poles (Collee,*et.al.*,١٩٩٦) .

On Trypticase soya agar enriched with ٥٪ sheep blood , ١٪ Tween ٨٠ , the colonies are slow growing , small , gray to white , glistening , non pigmented and usually nonhaemolytic . Blood and serum containing media are required for growth (Marshall*,et.al.,*١٩٨٧) .

The key biochemical reactions that differentiate *Corynebacterium urealyticum* from other corynebacteria species are ; strong urease activity , inability to reduce nitrate or readily ferment most carbohydrates (MacGregor,٢٠٠٠) .

Table (٣.٦) The diagnostic features of *Corynebacterium urealyticum* .

No.	Test	Result
١.	Gram stain	Gram positive bacilli,Chinese character.
٢.	Albert stain	Metachromatic granules at the poles.
٣.	Growth on Trypticase soya agar with ٥٪ sheep blood and ١٪ Tween ٨٠	Small, gray- white, non pigmented , glistening , usually non haemolytic colonies
٤.	Oxidase	Negative
٥.	Urease	Positive
٦.	Catalase	Positive
٧.	Nitrate reduction	Negative
٨.	CHO - Glucose Fermentation: Fructose Lactose	Negative Negative Negative
٩.	Easculin hydrolysis	Negative
١٠.	Alkaline phosphatase	Positive
١١.	Motility	Negative
١٢.	Coagulase	Negative

*this table is performed according to MacFaddin,٢٠٠٠ .

٣.٧ INVESTIGATION OF THE VIRULENCE ASSOCIATED FACTORS OF *CORYNEBACTERIUM UREALYTICUM*

The integrity of the natural defense mechanisms in a compromised host is probably important to determine the expression of virulence factors that initiate infection . The concept of bacterial virulence in the urinary tract infers that not all bacterial species are equally capable of inducing infections . This is supported by the well documented hypothesis that bacteria isolated

from a compromised host or patient with complicated UTI frequently fail to express virulence factors(Naber,*et.al*.,٢٠٠٦) .

The pathogenicity of *Corynebacterium urealyticum* in causing urinary tract infection and its ability to produce virulence factors is still a matter of confusion , studies concerning this are sparse and generally undocumented . In the present study , we examined the capacity of *Corynebacterium urealyticum* to show some virulence factors that are involved in its pathogenicity in the urinary apparatus.

A great part of the pathogenicity of *Corynebacterium urealyticum* in the urinary tract is attributed to the elaboration of a powerful urease (Soriano,*et.al*.,١٩٨٧).

Table (٣.٧) Virulence factors produced by *Corynbacterium urealyticum*

Virulence factor	No. of isolates with positive result
Urease	٨
Alkaline phosphatase	٦
Extracellular protease	٨
Haemolycin	١
Bacteriocin	-
Siderophore	-

- total no. of isolates is eight(٨)

In this work , it was found that ,all the isolates of *Corynebacterium urealyticum* were urease producer , this result is supported by Soriano and Garcia,٢٠٠٢ who regarded the rapid urease reaction as a useful clue to the potential identification of the organism . At the time of urinary infection , the very strong and rapid urease activity of *Corynebacterium urealyticum* is responsible for the alkalinization of urine and consequently struvite stone formation and urolithiasis. Euzeby,٢٠٠٣ proposed that , taking into account its spectrum of sensitivity ; treatment of infection by *Corynebacterium urealyticum* with antibiotics is not easy , yet , not effective long term , otherwise the surgical ablation of the vesicle

encrustation and most importantly , pharmacological neutralization of urease may give a better outcome (Vasquez,*et.al.*,٢٠٠٤).

Although , most of the studies about *Corynebacterium urealyticum* considered its urease production , no search dealt with its ability to express bacteriocin or siderophore , and very few publications are found on haemolycin , protease and alkaline phosphatase of this bacterium .

In the present study , we investigated *Corynebacterium urealyticum* virulence related factors , and it was found that bacteriocin and siderophore were not produced in vitro at all , this may indicate that the more compromised the host defense mechanisms are , the fewer virulence factors are present , otherwise , other alternative pathways adopted by this organism to replace the action of those virulence factors may be implicated as an explanation for the lack of bacteriocin and siderophore expression (Tagg,١٩٩٢).

Many of the published studies about *Corynebacterium urealyticum* indicated its inability to produce haemolycin , in this study it was found that only one isolate of the eight , was able to produce β-haemolycin when the bacterium grew on blood containing medium . This result is confirmed by Tilton,*et.al.*,١٩٩٢ who found that ١٦ % of *Corynebacterium urealyticum* isolates included in his study were able to produce β- haemolycin .

In this study , alkaline phosphatase and extracellular protease were found to be produced in vitro by *Corynebacterium urealyticum .* Alkaline phosphatase accepted to be produced variably by *Corynebacterium urealyticum* , depending on the cultural conditions , source of isolation and type of infection .This result goes with that of Neito,*et.al.*,٢٠٠٠ who suggested the important evidence of the role of alkaline phosphatase in the pathogenesis of this bacterium in urinary apparatus .

On the other hand , extracellular protease is regarded as an important virulence factor produced by many gram-positive bacteria, it acts through the degradation of proteins in the host tissues thus it facilitates invasiveness and spread of the organism and contributes to the pathogenesis of infection cycle (Rae,*et.al.*,١٩٩٨) .

A study conducted in Iraq by Alwash,٢٠٠٦ concerning benign prostatic hyperplasia and chronic prostatitis also revealed the ability of *Corynebacterium urealyticum* to produce alkaline phosphatase and extracellular protease in vitro , focusing on the importance of these factors in the virulence of the bacteria .

٣.٨ ANTIBIOTICS RESISTANCE BY *CORYNEBACTERIUM UREALYTICUM*

Most recent studies show an alarming rate of antibiotic resistance by *Corynebacterium urealyticum* . The sensitivity tests may be of utility when prescribing antibiotics in case in which the present organism is involved and the determination of these susceptibility results may be necessary in order to obtain the best therapeutic goals (Camello,*et.al.*,٢٠٠٣) .

The results represented in Table (٣.٨) declare the antibiogram profile of eleven antibiotics investigated on *Corynebacterium urealyticum* . The overall picture reflects the high resistance rate of this bacterium , nearly most of the drugs are found to be ineffective , with ١٠٠٪ of resistance against ampicillin , cefotaxime , cefixime and combination of trimethoprime – sulphamethoxazole , and only one isolate of the eight shows a moderate susceptibility to amoxicillin – clavulanic acid (٨٧.٥٪ of resistance) , while gentamicin , azithromycin , and clarithromycin are effective only against two (٢٥٪) of the isolates . The frequency of resistance to amikacin is ٦٢.٥ % and ciprofloxacin is ٥٠٪ , yet , the most effective antibiotic is vancomycin with only ١٢.٥٪ resistance .

Table (٣.٨) The effect of some antibiotics on isolates of *Corynebacterium urealyticum*

Antibiotic	Isolate no .								Resistance rate
	١	٢	٣	٤	٥	٦	٧	٨	
Ampicillin	-	-	-	-	-	-	-	-	١٠٠٪
Amikacin	-	+	-	+	+	-	-	-	٦٢.٥٪
Azithromycin	+	-	-	-	-	+	-	-	٧٥٪
Amoxicillin-clavulanic acid	-	-	-	+	-	-	-	-	٨٧.٥٪
Cefotaxime	-	-	-	-	-	-	-	-	١٠٠٪
Cefixime	-	-	-	-	-	-	-	-	١٠٠٪
Ciprofloxacin	+	-	-	+	-	+	+	-	٥٠٪
Clarithromycin	-	+	+	-	-	-	-	-	٧٥٪
Trimethoprim + SMX	-	-	-	-	-	-	-	-	١٠٠٪
Gentamicin	-	-	-	-	-	+	-	+	٧٥٪
Vancomycin	+	-	+	+	+	+	+	+	١٢.٥٪

N. B. + = sensitive , -= resistant

The results of this study confirm those of previous publications , showing high incidence of multidrug resistance among infections caused by *Corynebacterium urealyticum* .

There is a little knowledge about the mechanism of resistance of *Corynebacterium urealyticum* to most known antibiotics , Soriano,*et.al.*,١٩٩٥ results are similar to the results obtained in this work about the resistance of this organism to all of the penicillin's , they also found a scant effect of amoxicillin – clavulanic acid , the only β-lactamase inhibitor , yet , giving no explanation for the lack of activity of $\beta-$lactams in general or for the activity of amoxicillin – clavulanic acid against *Corynebacterium urealyticum* .

Data of this study shows a complete ineffectiveness of the investigated cephalosporin's against *Corynebacterium urealyticum* , this is in line with a study by Funke,*et.al.*,١٩٩٦ who pointed to the

high resistance rate of this microorganism to all of cephalosporin's including the most recently developed agents and those active against other gram- positive bacilli .

The hundred percent resistance rate of *Corynebacterium urealyticum* to trimethoprime – sulphamethoxazole in this study agrees with Marshall,*et.al.*,١٩٨٧; Garcia – Rodriquez,*et.al.*,١٩٩١ and Euzepy,٢٠٠٣ who reported the complete non explanable resistance of *Corynebacterium urealyticum* to this drug .

The high frequency of resistance of *Corynebacterium urealyticum* to macrolids reported in the present work agrees with the explanation given by Soriano,*et.al.*,١٩٩١ as it occurs through two mechanisms : a target modification and drug inactivation , otherwise other non-well defined mechanisms may also be present , while they attributed the activity of clarithromycin (which is the most effective macrolid against *Corynebacterium urealyticum*) to its high intrinsic activity .

The activity of quinolones against *Corynebacterium urealyticum* was extensively studied by Soriano,*et.al.*,١٩٩٠ concentrating on the fluorinated quinolones including ciprofloxacin which is widely used in the treatment UTI , they compared between the serum : urine levels of the drugs and its in vitro activity , and it was found out that high serum levels correlate better with therapeutic success than do high urine levels . This explains our results about the low activity of ciprofloxacin against *Corynebacterium urealyticum* . Nevertheless ciprofloxacin concentration may be lower in urine of female than male and the best therapeutic results are dose – dependent .

The antibiogram data of the present study concerning aminoglycosides action confirm those of Garcia-Bravo,*et.al.*,١٩٩٥ . Aminoglycosides are generally ineffective against *Corynebacterium urealyticum* and the ineffectiveness of most of aminoglycosides inactivating bacterial enzymes against amikacin explains its superiority upon gentamicin .

Soriano and Garcia,٢٠٠٢ showed that , the sensitivity of *Corynebacterium urealyticum* to aminoglycosides and the fluoroquinolones have been lost progressively , because many of the patients with infection by this bacterium have received numerous antibiotics including ciprofloxacin and gentamicin .

Finally ,it is generally agreed that the most effective in vitro antibiotic against *Corynebacterium urealyticum* are the glycopeptides , vancomycin show high activity and is recommended as a drug of choice for the treatment of UTI caused by *Corynebacterium urealyticum* (Hernandez,*et.al.*,٢٠٠٦) .

In spite of the fact that *Corynebacterium urealyticum* isolates were previously found to be susceptible to many antibiotics the most recent strain are characteristically multirug resistant . Neito*,et.al.*,٢٠٠٠ and Frassetto,*et.al.*,٢٠٠٤ Both hypothesized that a transmission of a plasmid responsible for the multidrug resistance may be important .

The environmental pH affects the lipid solubility and diffusibility of the antibiotics , consequently it alters absorption , distribution and elimination . pH – dependent bidirectional transport across membranes have been observed for many drugs including the weak acidic and basic one (Ramfrez,*et.al*.,٢٠٠٢) .

Urinary pH varied between the extremes of acid and alkaline ; thus the amount of drug reabsorbed from the renal tubular lumen by passive diffusion can be strongly affected by the prevailing urine pH (Garcia – Rodriquez ,*et.al*.,١٩٩١)

Taking into consideration the resistance of *Corynebacterium urealyticum* to many antibiotics , elevation of the urine pH may produce unfavorable conditions that may reduce the effectiveness of most of antibiotics (Bodji,*et.al.*,٢٠٠٤).

٣.٩ IN VITRO STONE FORMATION BY *CORYNEBACTERIUM UREALYTICUM*

Struvite stones are thought to develop in urinary tract infected with urea splitting bacteria , the bacterial urease hydrolyzes urea , leading to hyperammonuria and alkalinization of urine with consequent crystallization of struvite . This theory has been studied extensively by Griffith*,et.al.*,١٩٧٣ using *Proteus mirabilis* and recently by Takebe,*et.al.*,١٩٨٤ using *Ureaplasma urealyticum* .

In the present study , the stone forming ability of *Corynebacterium urealyticum* was investigated through an experiment testing the ammonium concentration , pH , crystals , and the optical density of human urine inoculated with *Corynebacterium urealyticum* , *E.coli* , and controlled urine with no bacteria at ٠ , ٤ , ٨ , and ٢٤ hours of incubation .

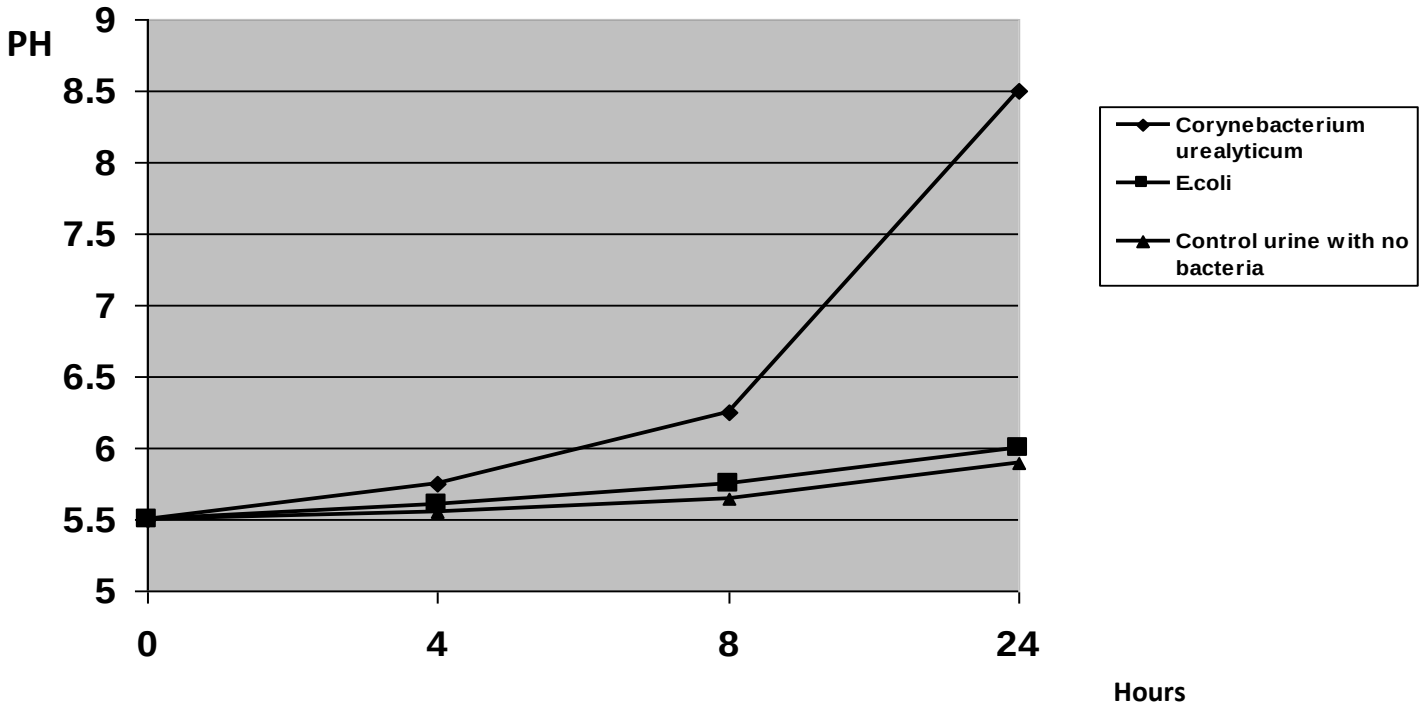
When human urine was inoculated with *Corynebacterium urealyticum* and *E.coli* ; a gradual increase in the cell density was noticed until the ٤th hour when the rate of proliferation became faster , increasing the microbial population to the maximum when examined after an overnight incubation . *Corynebacterium urealyticum* grew in human urine proliferated steadily in a rate lower than that reported for *E.coli* , it is generally agreed that *E.coli* is rapidly multiplying organism if compared to the slowly growing *Corynebacterium urealyticum* (Walkden,*et.al*.,١٩٩٣).

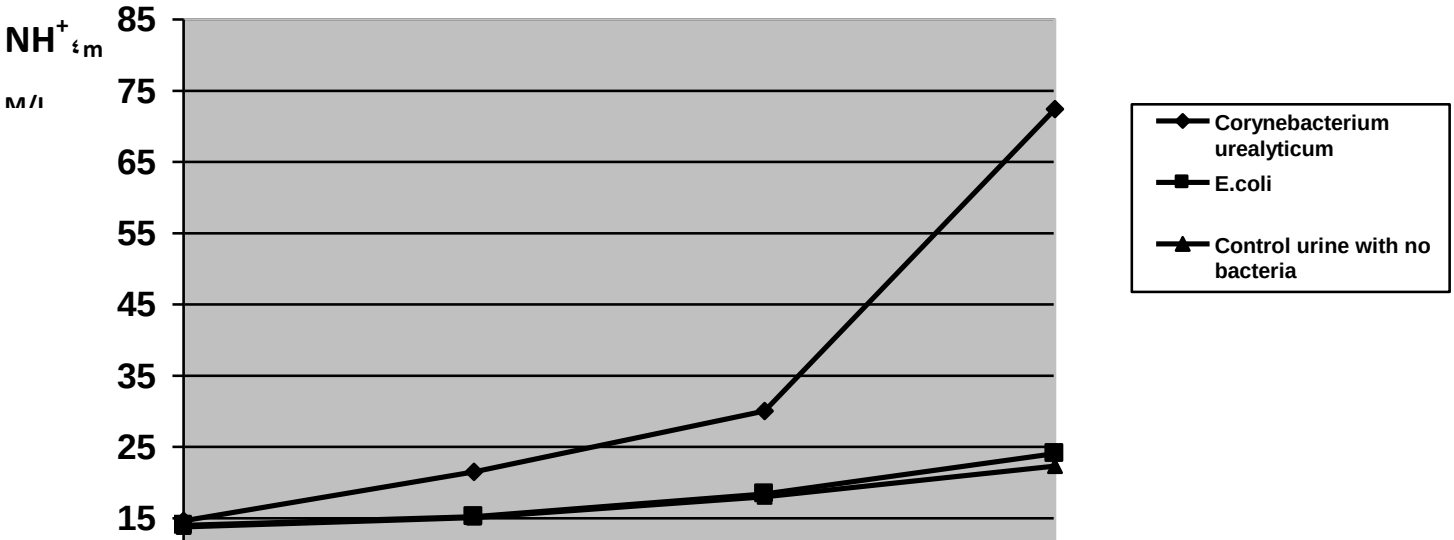
The final reading of optical density for *Corynebacterium urealyticum* at ٢٤ hours of incubation was obviously lower than that reported for *E.coli* . However , non-inoculated control urine gave the minimal changes of optical density readings during the whole period of experimental study .

During the experimental bacterial growth in human urine , the primary urine pH , ammonium ion (NH_4^+) and crystals were measured , and remeasured again at ٤ , ٨ and ٢٤ hours of incubation . The pH changes observed in this study are represented in Figure (٣.١).

At the onset of the experiment , the urine pH was about ٥.٥ for all the three tested samples . When *Corynebacterium urealyticum* grew in human urine , there was a slight elevation in the urine pH at the first ٤ hours , reaching about ٦.٢ at ٨ hours and maximized after ٢٤ hours up to ٨.٥ ,

however , this is not the case when *E.coli* grew at the same experimental conditions , as it was expected , there was a very minor elevation in the pH , whereas , the final reading was ٦.٠ . On the other hand there were no changes in the pH of control urine which was free of bacteria during the period of study.





Hours

Figure (٣.١) Effects of bacterial growth in human urine. Ammonium ion concentration ($NH^+_٤$), and pH at ٠,٤ ,٨ and ٢٤ hours of incubation in the presence of *Corynebacterium urealyticum* , *E.coli* and control urine with no bacteria.

Urine $NH_٤^+$ concentration was found to be also affected In figure (٣.١) . While *Corynebacterium urealyticum* grew in human urine , this was reflected on the $NH_٤^+$ concentration , the urine used in this experiment priorly was tested and found to have a normal range of urea , ammonium ionconcentration was about ١٥ mM/L and only slightly elevated at the first ٤ hours, however , after the ٢nd four hours of incubation , the concentration of ammonium ion was approximately found to be doubled , the increment rate resulted in $NH^+_٤$ concentration above ٧٠ mM/L after an overnight period . Parallel with the growth of *Corynebacterium urealyticum* in the experimental urine ; although *E.coli* exceeded the rate of growth of *Corynbacterium urealyticum* , it did not change the pH , nor did it change $NH^+_٤$ concentration within the same pattern . The overall increase in the concentration of $NH^+_٤$ associated with the growth of *E.coli* was non significant if compared to that produced by *Corynebacterium urealyticum* growth , the same finding was observed for the control urine which was free of bacteria , as there was no significant changes in the $NH^+_٤$ concentrations through the ٢٤ hours of incubation .

The resultant changes of the pH and $NH^+_٤$ induced by bacterial growth in human urine was consequently associated with crystallization of struvite , they are represented in Table (٣.٩) .

Table (٣.٩) struvite crystals formed in human urine associated with bacterial growth .

Bacteria	No. of crystals / Hpf microscope at zero time	٤ hours	٨ hours	٢٤ hours
Corynebacterium urealyticum	٠-٢	٢-٤	٤-٨	١٠-١٥
E.coli	٠-٢	٠-٢	٠-٢	٠-٢
Control urine with no bacteria	٠-٢	٠-٢	٠-٢	٠-٢

Struvite stones formation associated with urinary infection by *Corynebacterium urealyticum* is thought to be as a consequence of hyperammonuria and alkalinization of urine associated with this bacterium growth (Rodman,١٩٩٩) . The numbers of crystals seen microscopically increased gradually parallel with that of both the pH and $NH^{+}_{٤}$ concentration , reaching a maximum number after ٢٤ hours of incubation when a white sediment appeared at the bottom of the tube at that time . However , as it was expected , *E.coli* growth resulted in no increase in the number of crystals seen microscopically as in the case of other non urease producer organisms , and urine inoculated with no bacteria did not alter the number of crystals at all . There was no sediment observed in case of both *E.coli* and the control urine after an overnight incubation .

The observed data in the present study is compatible with that of Soriano*,et.al.*,١٩٨٦ who examined the ability of *Corynebacterium urealyticum* to induce urinary stones in a rat model which strongly supported most of Koch's postulate .

The results of this work also agree with Takebe,*et.al.*,١٩٨٤ in a study about the stone formation by *Ureaplasma urealyticum* who observed the correlation between the struvite stone formation and the increment of pH and $NH^{+}_{٤}$ concentration when a urea splitting bacteria grew in human urine .

Corynebacterium urealyticum is a relatively fastidious microorganism and is probably less virulent than other gram negative bacilli usually involved in urinary tract infection , urease enzyme produced by this bacterium plays a major role in the pathogenesis of struvite stone formation , and it is agreed to be the direct cause of hyperammonuria and alkalinization of urine seen in UTI caused by this bacterium.

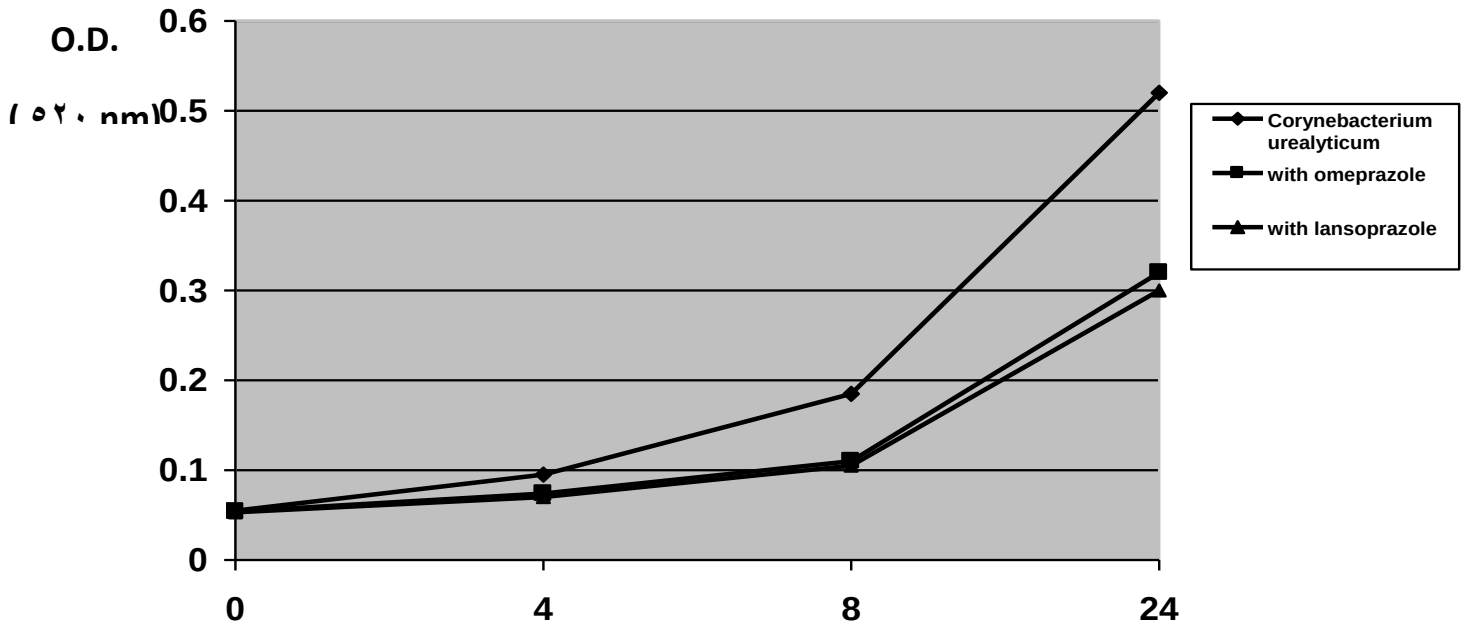
٣.١٠ EFFECTS OF OMEPRAZOLE & LANSOPRAZOLE ON THE STONE FORMATION BY *CORYNEBACTERIUM UREALYTICUM*

The present study is conducted to examine whether the proton –pump inhibitors (omeprazole & lansoprazole) can be useful as chemotherapeutic agents against diseases caused by *Corynebacterium urealyticum* and also to evaluate the antibacterial activity of this group of compounds .

The following data obtained from an experiment represents a growth of *Corynebacterium urealyticum* in human urine with and without omeprazole & lansoprazole to evaluate the effects of these compounds on the growth of *Corynebacterim urealyticum* as presented in Figure (٣.٢) .

At the onset of the inoculation of bacteria into the experimental broth culture of urine , there was a low grade increase of the cells density as a part of the accommodation of the bacteria to the new environment , mean while the rate of proliferation increased after ٤ hours of incubation and the microbial population density reached its maximum reading after ٢٤ hours of incubation .

When omeprazole (٠.٨ mg/ml) and lansoprazole (٠.٢ mg/ml) were added separately to the growing culture , from the start , a consistent and significant reduction in the population density was

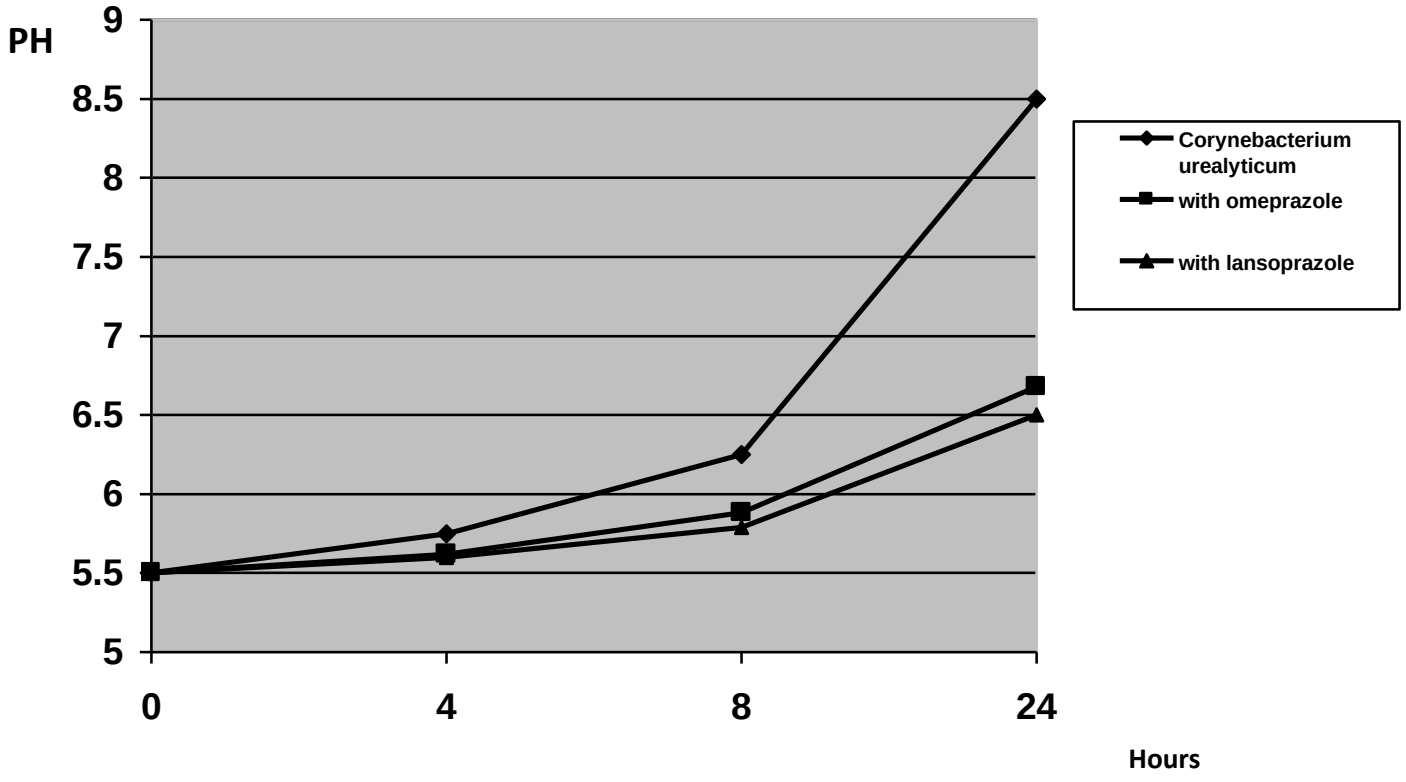


Hours

Figure (٣.٢) Growth of *Corynebacterium urealyticum* in human urine with & without omeprazole & lansoprazole .

observed , the rate of proliferation was slow if compared to the broth culture containing *Corynebacterium urealyticum* alone . After ٢٤ hours , it was obvious that the addition of omeprazole and lansoprazole markedly affected the growth of the bacterium .

The growth of *Corynebacterium urealyticum* in human urine resulted in a change in the primary pH & $NH^{+}_{٤}$ concentrations as shown in Figure (٣.٣) .The pH increased steadily at the first ٤ hours and continued to do so with gradual increase in the rate till reaching the maximal level at ٢٤ hours of incubation with ٨.٥ pH .



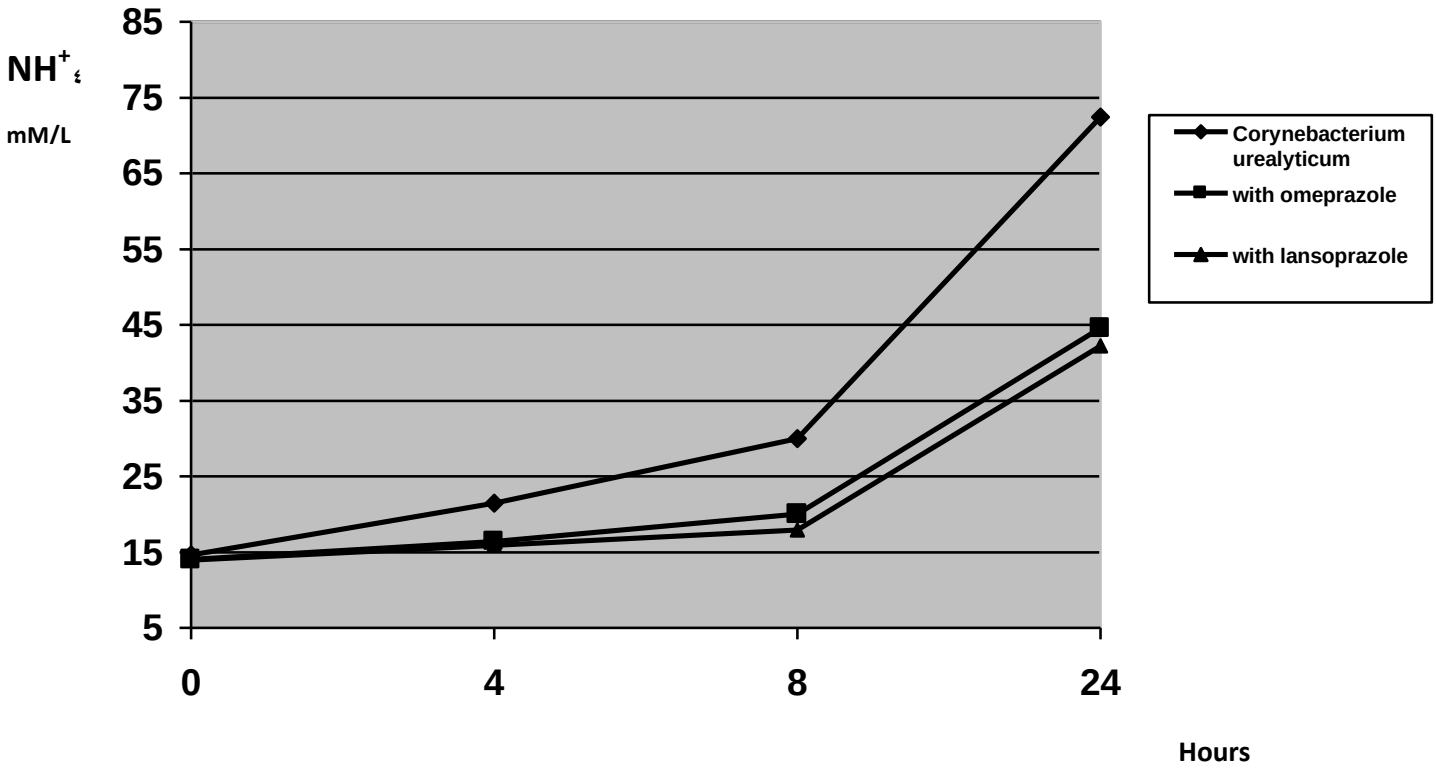


Figure (٣.٣) Effect of Omeprazole and Lansoprazole on pH and $NH^{+}_{٤}$ change accompanying growth of *Corynebacterium urealyticum* in human urine.

On the other hand , when omeprazole and lansoprazole were added separately to experimental cultures the pH started to increase after the ٤th hour and remained increasing in a rate lower than that reported for *Corynebacterium urealyticum* alone , till finally maximized after ٢٤ hours of incubation at ٦.٦ pH for the culture containing omeprazole & ٦.٥ pH for lansoprazole .

Corynebacterium urealyticum experimental culture slightly increased the starting concentration of ammonium ion at the first ٤ hours , reaching ٣٠ mM/L at ٨ hours, till ٢٤ hours when it became ٧٢mM/L as its maximal reading . However , when omeprazole and lansoprazole were added separately , the increment of the ammonium ion concentration was slower than that of *Corynebacterium urealyticum* alone and the steady changes at the first ٤ hours gradually became faster through the ٢nd ٤ hours , and continued to increase during an overnight incubation until ٢٤ hours report which was ٤٥ mM/L ammonium ion concentration of *Corynebacterium urealyticum* culture containing omeprazole & ٤٢mM/L for lansoprazole .

The reported changes involved the pH and ammonium ion concentration were also associated with a noticeable increase in crystals formation . Table (٣.١٠) represents the microscopical number of crystals formed by *Corynebacterium urealytium* with and without omeprazole and lansoprazole.

Table (٣-١٠) Struvite crystals formation in human urine inoculated with *Corynebactrium urealyticum* with and without omeprazole & lansoprazole.

Bacterium	Omeprazole	Lansoprazole	No. of crystals / Hpf microscope at hour : zero	٤	٨	٢٤
Corynebacterium urealyticum	–	–	٠-٢	٢-٤	٤-٨	١٠-١٥
Corynebacterium urealyticum	٠.٨ mg/ml	–	٠-٢	٠-٢	٢-٤	٤-٨
Corynebacterium urealytiurm	–	٠.٢ mg/ml	٠-٢	٠-٢	٢-٤	٤-٨

Microscopically the number of crystals increased slowly during the first few hours , however , it was doubled after ٨ hours of incubation in urine containing *Corynebacterium urealyticum* alone . This , yet , was not enough for the crystals to be seen grossly only until ٢٤ hours of incubation when a precipitate appeared at the bottom of the experimental tube.

Nevertheless, the addition of omeprazole land lansoprazole found to be markedly affecting the crystal formation in such a way that the formation of crystals diminished and the microscopical increment was insufficient to induce a gross precipitate even after ٢٤ hours of incubation.

The data represented in this work confirms that of Nagata,*et.al.,*١٩٩٥ in a study involving the growth inhibition of *Ureaplasma urealyticum* by the proton pump inhibitors who noticed that , the benzimidazole proton pump inhibitors such as omeprazole and lansoprazole markedly inhibit the activities of ureases from Jack-been as well as from bacteria such as *Helicobacter pylori* , *Proteus* spp . , *Providencia rettigeri* and *Klebsiella pneumonea* , proposing that the inhibitory activity of the

proton pump inhibitors against ureases from various sources are as potent as (or even more active than) that of other urease inhibitors such as hydroxamic acid , hydroxyurea and thiourea which have been established to be potent and specific urease inhibitors since ١٩٦٢ by Kobashi,*et.al.,*١٩٦٢.

In the present study , the degree of the inhibitory activity of the proton- pump inhibitors did not change at different concentrations of urea , by other words it was independent on urea concentration . This result supports that of Nagata,*et.al.*,١٩٩٥ who demonstrated the mechanism of inhibition of urease enzyme by the proton pump inhibitors as to be due to the blocked SH-residues of the cysteine in the active site which is essential for the optimal activity of the enzyme . This inhibition is suggested to be independent of the substrate concentration .

Results of this study also agree with Kühler,*et.al.*,١٩٩٥ in a study about the structure – activity relationship of omeprazole and analogues as urease inhibitors , who demonstrated the presence of two Lewis-acid nickel ions and more importantly a reactive cysteine residue in the active site of the enzyme .

Takeuchi,*et.al.*,١٩٧٧ have also reported the significant amino acid sequence similarity between microbial ureases suggesting a common urease gene and thus similar active site , while Kühler,*et.al.*,١٩٩٥ hypothesized that omeprazole and analogues covalently modify cysteine residues on the active site of both urease and the H^+/K^+ -ATP ase of the parietal cells in the stomach .

The data presented in this work are strongly compatible with those of Mirshahi,*et.al.*,١٩٩٨ who have demonstrated an optimal inhibitory action of the proton pump inhibitors omeprazole and lansoprazole in vitro when used at concentration of ٠.٨ mg/ml and ٠.٢mg/ml respectively .

On the other hand , observation by Mirshahi,*et.al.*,١٩٩٨ suggested that the potent bacterial urease may represent a potential target for the benzimidazole proton pump inhibitors , and that inhibition of this enzyme may provide one explanation for the antibacterial properties of this group of compounds . Anyway , evidence derived from the in vitro studies is inconclusive , suggesting a mechanism of inhibition that is unrelated to competition for the benzimidazole group against the bacterial urease , consequently , there is a need to elucidate more fully the nature of the effect of omeprazole and analogues on urease – producing bacteria in vitro .

٣.١١ EFFECT OF OMEPRAZOLE ON THE GROWTH OF *CORYNEBACTERIUM UREALYTICUM*

The hydrolysis of urea by urease is considered to play a major role in the energy metabolism of urease producing microorganisms by promoting ATP – synthesis . In this study it was investigated whether omeprazole in different concentrations can affect the growth rate of *Corynebacterium urealyticum* .

It was observed that omeprazole significantly affected the bacterial growth when added to the culture media of *Corynbacterium urealyticum* in an increasing concentrations , this is illustrated in Figure (٣.٤) as a negative correlation .

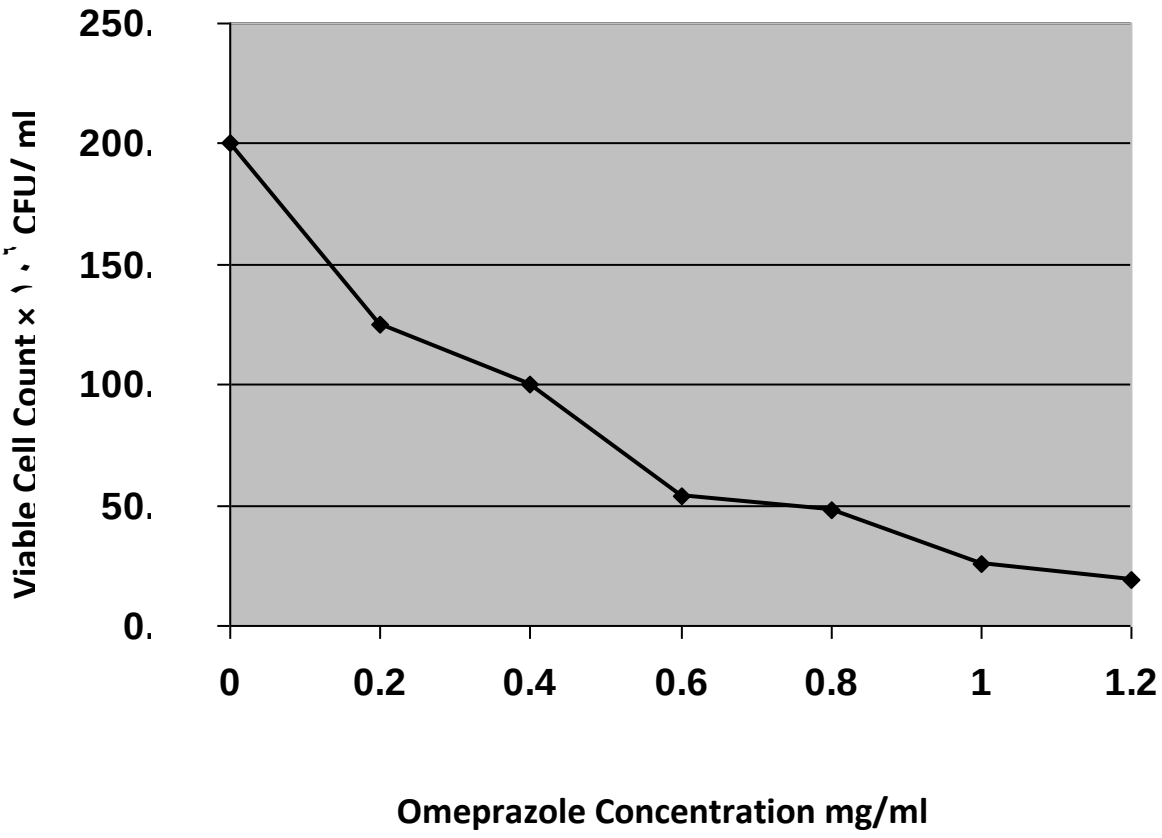


Figure (٣.٤) Effect of omeprazole on the growth of *Corynebacterium urealyticum.*

The presence of omeprazole in the environment where *Corynbacterium urealyticum* grew was found to have an inhibitory action on the microbial population proliferation , as shown in Table (٣.١١) , only ٥٠٪ of the population and colonies remained with ٠.٤mg/ml omeprazole concentration , the microbial growth diminished to less than ٢٤٪ with the increment of the concentration of omeprazole to ٠.٨ mg /ml in the culture media , this relationship was markedly demonstrated with higher concentrations , that only ٩.٥ % of the initial colonies remained viable with the addition of ١.٢ mg / ml omeprazole to the culture media .

From these results it seems that there was a dose dependent correlation between the growth of *Corynebacterium urealyticum* and omeprazole concentrations , that viable cell count was obviously affected by increasing concentrations of omeprazole , this is supported by a study of Nagata,*et.al.*,١٩٩٥ who demonstrated the growth inhibitory activity of the proton- pump inhibitors against *Ureaplasma urealyticum* growing culture .

Mirshahi,*et.al.*,١٩٩٨ also supported this study, by observing a dose dependent correlation between *H.pylori* growth and omeprazole , it was also noticed that , when omeprazole was added to the growth culture from the start , the exponential growth was only maintained for few hours before the microbial population quickly declined .

Table (٣.١١) Effect of omeprazole on the growth rate of *Corynebacterium urealyticum.*

Omeprazole concentration mg/ ml	**Viable count of *Corynebacterium urealyticum* CFU/ ml**	**Percentage of the remaining colonies**
٠.٠	٢×١٠^{٨}	١٠٠٪
٠.٢	١.٢٥×١٠^{٨}	٦٢.٥٪
٠.٤	١×١٠^{٨}	٥٠٪
٠.٦	٥.٤×١٠^{٧}	٢٧٪
٠.٨	٤.٨×١٠^{٧}	٢٤٪
١.٠	٢.٦×١٠^{٧}	١٣٪
١.٢	١.٩×١٠^{٧}	٩.٥٪

Correlation Coefficient =- ٠.٩٤٥ , significant at ٠.٠٠١ level

Since a great part of the pathogenicity of *Corynebacterium urealyticum* in the urinary apparatus belonges to the elaboration of the powerful urease , attempts have been made for its pharmacological neutralization , although clinical studies do not , yet , some exist which have allowed to evaluate the effectiveness of this drug against *Corynebacterium urealyticum* , it is our opinion to join the proton pump inhibitors as a chemotherapeutic agents in the treatment of infections caused by this bacteria .

٤.١ Conclusions

The results obtained from this work may extrapolate the following conclusions :

١. Urinary tract infections associated with alkaline urine are usually caused by urease – producing organisms .

٢. Selection of urine samples with alkaline pH , pyuria , haematuria and struvite crystals increase the chance of recovery of *Corynebacterium urealyticum*..

٣. The incidence of culture *Corynebacterium urealyticum* from an alkaline urine of patients with UTI is ٩.٣% .

٤. *Corynebacterium urealyticum* is characteristically multidrug resistant to most known antibiotics used in UTI , the glycopeptide vancomycin remains the most effective one .

٥. Infection with *Corynebacterium urealyticum* usually occurs in a predisposed patients with multiple risk factors including hospitalization , previous use of antibiotics , catheterization , previous UTIs with other organisms , immunosuppressive diseases , renal failure and dialysis , transplantation .

٦. Urease enzyme produced by *Corynebacterium urealyticum* plays the major role in the pathogenesis of uropathy .

٧. The most important pathology induced by *Corynebacterium urealyticum* infection in the urinary apparatus is struvite urolithiasis and the resultant encrusted uropathy .

٨. The proton-pump inhibitors : omeprazaole and lansoprazole may have a noticeable inhibitory effect on *Corynebacterium urealyticum* growth and urease activity .

٤.٢ Recommendations

Several recommendations can be offered by this study as follows:

١. Study of the occurrence of alkaline urine in conditions other than UTI .

٢. Study of the prevalence of *Corynebacterium urealyticum* as a pathogen in diseases other than UTI .

٣. Molecular study about *Corynebacterium urealyticum* including its virulence factors expression and the multidrug resistance state .

٤. Prolongation of incubation period and the use of selective media to enhance isolation of *Corynebacterium urealyticum* from suspected patients .

٥. Avoidance of antibiotic pressure in the hospitalized patients .

٦. Technobiological and enzymological study of *Corynebacterium urealyticum* urease in comparison to other microbial and Jack bean ureases .

٧. More studies are required to fully explain the exact action of the proton –pump inhibitors against the microbial ureases .

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Measurement of urine ammonia by indophenol's method:

The ammonia formed from urease action reacts with phenol in the presence of hypochlorite to form indophnol which with alkali gives a blue colored compound .Na- nitroprusside acts as a catalyst, increasing the rate of reaction , the intensity of the color obtained and its reproducibility .

Procedure :

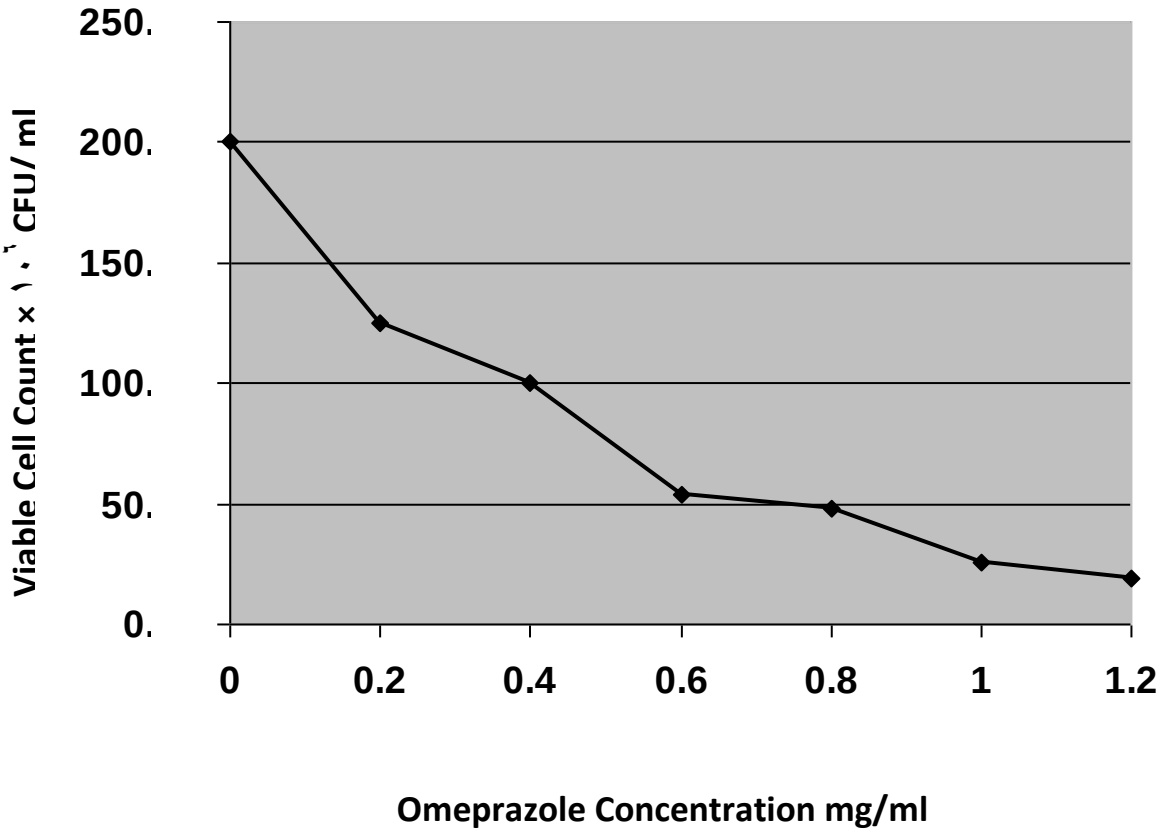
Measure ٢ ml of phenol –nitroprusside reagent and add ٠.٠٤ ml of fresh urine culture , in the test tube , To the standard tube add ٢ ml of phenol –nitroprusside reagent and then add ٠.٠٤ml of standard ammonia solution .To the blank take ٢ ml of phenol –nitroprusside reagent and ٠.٠٤ ml of distilled water .

Add to each tube ٢ ml of alkaline hypochlorite solution and place in water bath for ٣ minutes at ٥٥^{o}C , then keep at ٣٧^{o}C for ٢٠ minutes . Read the test & standard against the blank at ٦٣٠ nm .

The effect of some antibiotics on isolates of *Corynebacterium urealyticum*

Antibiotic	Isolate no .								Resistance rate
	١	٢	٣	٤	٥	٦	٧	٨	
Ampicillin	-	-	-	-	-	-	-	-	١٠٠٪
Amikacin	-	+	-	+	+	-	-	-	٦٢.٥٪
Azithromycin	+	-	-	-	-	+	-	-	٧٥٪
Amoxicillin-clavulanic acid	-	-	-	+	-	-	-	-	٨٧.٥٪
Cefotaxime	-	-	-	-	-	-	-	-	١٠٠٪
Cefixime	-	-	-	-	-	-	-	-	١٠٠٪
Ciprofloxacin	+	-	-	+	-	+	+	-	٥٠٪
Clarithromycin	-	+	+	-	-	-	-	-	٧٥٪
Trimethoprim + SMX	-	-	-	-	-	-	-	-	١٠٠٪
Gentamicin	-	-	-	-	-	+	-	+	٧٥٪
Vancomycin	+	-	+	+	+	+	+	+	١٢.٥٪

N. B. + = sensitive , - = resistant



Effect of omeprazole on the growth of *Corynebacterium urealyticum.*