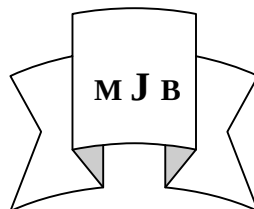


A Clinico-bacteriological Study of the Interrelation of *Corynebacterium urealyticum* and Struvite Urolithiasis

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Abstract

Three hundred twenty urine samples were collected from patients suffering urinary tract affection. Only alkaline sample were selected which formed 86 specimens yielded 8 isolates of *Corynebacterium urealyticum*. All the 8 isolates were identical regarding cultural characteristics and biochemical reactions. Results of the studied virulence factors expression by *Corynebacterium urealyticum* showed that urease was the most important factor in pathogenicity.

An experimental study was created to assess the ability Struvite stone formation by *Corynebacterium urealyticum* during its growth in human urine, it was found that there was gradual increase in urine pH with associated 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 human 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 form struvite crystals when incubated under similar experimental conditions.

The effects of the proton – pump inhibitors : omeprazole and lansoprazole on growth and pathogenicity of *Corynebacterium urealyticum* were examined, it was obvious that these compound markedly affected urease activity in a dose -dependent manner and the bacterial growth was significantly ($p < 0.001$) inhibited by an increasing concentrations of omeprazole.

الخلاصة

في هذه الدراسة تم إخضاع 320 عينة إدرار للعزل والتشخيص استحصلت من مرضى يعانون من التهابات المجاري البولية. عينات الإدرار قاعدية التفاعل كانت 86 عينة، وأعطت حصيلة 8 عزلات من بكتيريا *Corynebacterium urealyticum* جميعها امتلكت نفس الخصائص الزراعية والبايوكيميائية. إن دراسة القابلية للأمراضية لبكتيريا *Corynebacterium urealyticum* تشير لقلة إنتاجها لعوامل الضراوة حيث كان أهمها إنزيم اليوريز وينتج من قبل جميع العزلات أما قدرة *Corynebacterium urealyticum* على تكوين حصي الستروفايت الكلوي فأظهرت النتائج بأن كريستالات الستروفايت تكونت بعد مرور 24 ساعة من نمو البكتيريا في عينة إدرار زرعي مختبري مقارنة مع عدم قدرة *E. coli* على تكوين تلك الكريستالات في نفس الظروف المختبرية. أخيراً تم دراسة قدرة مثبطات مضخة البروتون : الاومبرازول والانسوبرازول على التأثير على نمو البكتيريا وقدرتها المرضية حيث وجد بأن التأثير التثبيطي كان معتمداً على الجرعة العلاجية بينما تأثر نمو البكتيريا بصورة واضحة ($P < 0.001$) بزيادة تركيز الاومبرازول.

Introduction

C*orynebacterium urealyticum* It is one of the more frequently isolated clinically significant corynebacteria from clinical specimens[1]. *Corynebacterium urealyticum* is a genitourinary

pathogen, has been cultured from the skin of hospitalized patients and from an inanimate hospital environment. The urea-splitting activity of *Corynebacterium urealyticum* plays an important role in its pathogenicity and explains the ability to live in an

alkaline environment . The strong urease activity results in an increase in urine pH , this alkaline pH not only has a toxic effect on the renal epithelium but also predispose to supersaturation of ammonium magnesium phosphate (struvite) , and ultimately to production of renal stone [2]. The struvite stone works as a nuclei for colonization of bacteria , causing more serious infection , increasing the incidence of pyelonephritis , alkaline encrusted cystitis and pyelitis , and recurrent urinary tract infections[3]. *Corynebacterium urealyticum* has been shown to be resistant to most antibiotics used for the treatment of urinary tract infection , vancomycin is commonly recommended until a specific antimicrobial sensitivity data are available [4] .

To our knowledge , there is no independent study concerning *Corynebacterium urealyticum* had been conducted in Iraq , this work aims to Isolate and identify *Corynebacterium urealyticum* associated with alkaline urine of patients with urinary tract infections. Study the in vitro stone formation ability by *Corynebacterium urealyticum*. Show the effect of the proton pump inhibitors: omeprazole and lansoprazole on the bacterium growth and pathogenic capacity .

Materials and Methods

Three hundred twenty patients attended the out – patients clinic , department of urology of Hilla general teaching hospital with symptoms of urinary tract infection underwent the study from October 2005 to April 2006 . 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 . Urine samples collected from all patients were sent

for general urine examination , urine culture & sensitivity .

The selected specimens were inoculated with the standard calibrated loop on to a Trypticase soya agar plates supplemented with 5% sheep blood and 1% Tween 80, those plates were incubated for 72 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,1990(5). 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 examination folloed by a series of biochemical tests to reach the final identification of *Corynebacterium urealyticum* .

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 (0, 4 , 8 , and 24 hours) of incubation .

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 and pathogenicity of *Corynebacterium urealyticum*

Results and Discussion

Because *Corynebacterium urealyticum* is highly associated with alkalinization of urine , all of the 320 samples were measured by a pH – strips , and of these , only 86 sample with a pH of 7.0 or higher were selected and cultured on a selective media for *Corynebacterium*

urealyticum . Seventy four alkaline urine specimens gave a positive culture . *Corynebacterium urealyticum* found in 8 samples , forming 10.8 % of the total 86 alkaline samples.

In this study , urinalysis results of urine specimens which later found to yield *Corynebacterium urealyticum* revealed that , most of the 8 samples were alkaline with mean pH of 7.5 , pyuria and struvite crystals were found in 5 specimens out of 8 (62.5 %) ,Where as haematuria detected in 6 of the 8 samples (75%).Table (1)

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 have been studied extensively by Griffith ,et ,al [6] using *Proteus mirabilis* and followed by Takebe ,et ,al [7] using *Ureaplasma urealyticum* .At the onset of the experiment , the urinary pH was about 5.5 for all the tested 3 samples .When *Corynebacterium urealyticum* grew in human urine , there was a slight elevation in the urinary pH at the first 4 hours , reaching about 6.2 at 8 hours and maximize after 24 hours up to 8.5 , however , this is not the case when *E.coli* grew at the same experimental conditions , as there was a very minor elevation in the urinary pH , where , the final reading was 6.0. On the other hand there was on changes in the pH of control urine.

Urine NH_4^+ concentration was found to be also affected In figure(1) . While *Corynebacterium urealyticum* grew in human urine , this was reflected on the NH_4^+ concentration , the urine used in this experiment priorly was tested and found to have a normal range of urea , ammonium concentration was about 15 mM/L and only slightly elevated at the first 4, however the increment rate resulted in

NH_4^+ concentration above 70 mM/L after an overnight period . Parallel with the growth of *Corynebacterium urealyticum* in the experimental urine ; *E. coli* although exceeded the rate of growth of *Corynebacterium urealyticum* , it did not change the pH , nor NH_4^+ concentration within the same pattern. The something was observed for the control urine as there was nonsignificant changes in the NH_4^+ concentrations through the 24 hours of incubation .

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 as illustrated in figure (2). An experiment represents a growth of *Corynebacterium urealyticum* in human urine with and without omeprazole & lansoprazole. the growth of *Corynebacterium urealyticum* in human urine resulted in a change in the primary pH & NH_4^+ concentration .the pH increased steadily at the 1st 4 hours and continued to do so with gradual increase in the rate till reaching the maximal level at 24 hours of incubation with 8.5 pH .When omeprazole and lansoprazole were added separately to experimental culture the PH started to increase after the 4th hour and remained increasing in a rate lower than reported for *Corynebacterium urealyticum* alone , till finally maximized after 24 hours of incubation at 6.6 pH culture containing omeprazole & 6.5 containing lansoprazole . *Corynebacterium Urealyticum* culture slightly increased the starting concentration of ammonium ion at the first 4 hours , reaching 30 mM/L at 8 hours, till 24 hours when it became 72 mM/L as its maximal reading . However , when

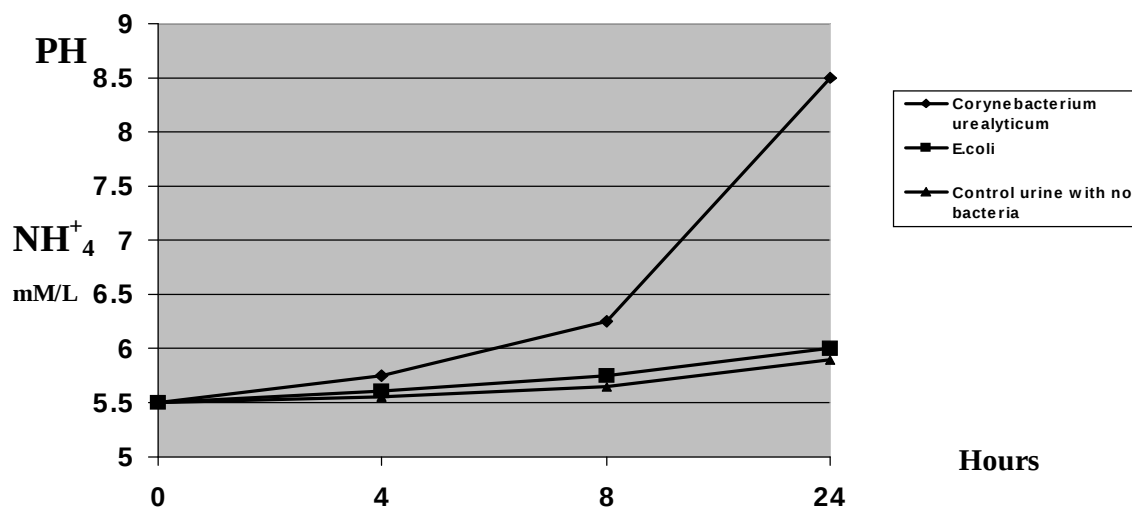
omeprazole and lansoprazole were added , the increment of the ammonium ion concentration was slower than that of *Corynebacterium urealyticum* alone and the steady changes at the first 4 hours gradually became faster through the 2nd 4 hours ,and continued to increase during an overnight incubation until 24 hours report which was 45 mM/L ammonium ion concentration of *Corynebacterium urealyticum* culture containing omeprazole & 42 mM/L for lansoprazole as shown in figure (2).

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Table (1) Urinalysis results of *Corynebacterium urealyticum* urinary tract infection.

Items of urinalysis	No . of patients/8	Percentage
Pus cells	5	62.5%
RBC _s	6	75%
Struvite crystals	5	62.5%
pH mean	7.5	



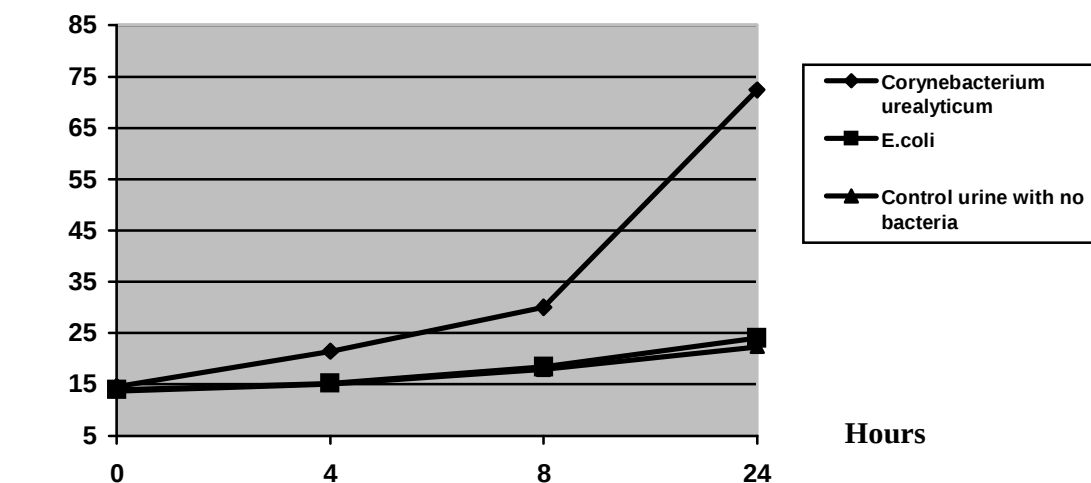
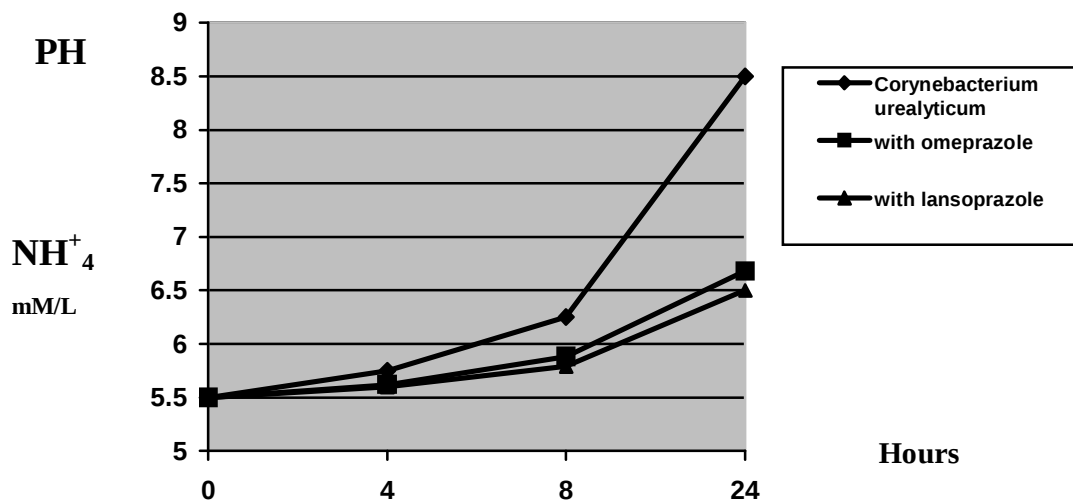


Figure 1 Effects of bacterial growth in human urine. Ammonium concentration (NH_4^+), and pH at 0,4 ,8 and 24 hours of incubation in the presence of *Corynebacterium urealyticum* , *E.coli* and control urine with no bacteria.



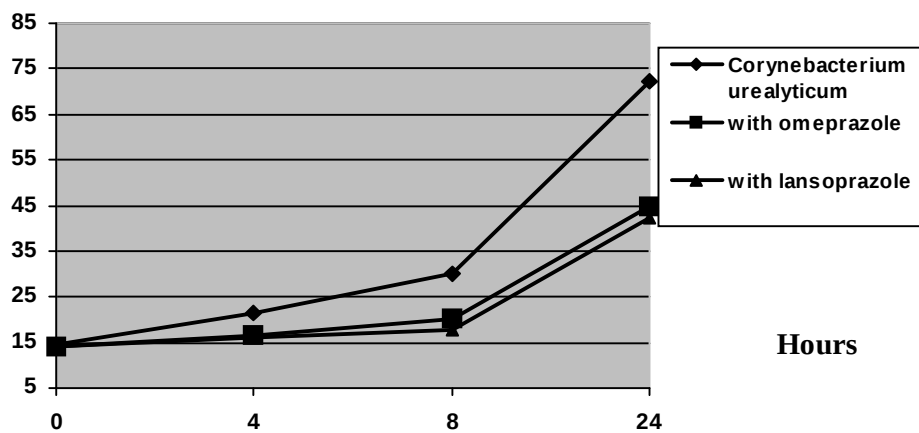


Figure 2 Effect of Omeprazole and Lansoprazole on pH and NH_4^+ change accompanied growth of *Corynebacterium urealyticum* in human urine.