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ROLE OF *ENTEROBACTER CLOACAE* ISOLATED FROM URINARY TRACT INFECTION IN STONE FORMATION

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ABSTRACT

In our previous study, we detect (8) isolates of *Enterobacter cloacae* from UTI deepened on phenotypic and under molecular level. Production of urease enzyme by urea agar method, urease extracellularly and stone formation from *Enterobacter cloacae* isolates were detected, the results showed all bacterial isolates 8(100%) produced urease enzyme and struvite stone formation.

KEYWORDS: Enterobacteriaceae, *Enterobacter cloacae,* stone formation, urease.

INTRODUCTION

Enterobacter is a genus of common Gram-negative, facultatively anaerobic, rod-shaped, non-spore-forming bacteria of the family Enterobacteriaceae. Several strains of these bacteria are pathogenic and cause opportunistic infections in immunocompromised (usually hospitalized) hosts and in those who are on mechanical ventilation. The urinary and respiratory tracts are the most common sites of infection. The genus *Enterobacter* is a member of the coliform group of bacteria. It does not belong to the fecal coliforms (or thermotolerent coliforms) group of bacteria, unlike *Escherichia coli*, because it is incapable of growth at (44.5°C) in the presence of bile salts. Some of them showed quorum sensing properties as reported before.[1] Urinary tract infections (UTIs) are among the most common infectious diseases occurring in either the community or healthcare setting. Uncomplicated UTIs typically occur in the healthy adult non-pregnant woman, while complicated UTIs (cUTIs) may occur in all sexes and age groups and are frequently associated with either structural or functional urinary tract abnormalities. Examples include foreign bodies such as calculi (stones), indwelling catheters or other drainage devices, obstruction, immunosuppression, renal failure, renal transplantation and pregnancy.[2] The most important and common problem of urinary system is the formation of renal stones.[3] Struvite stones (also known as "infection stones", urease or triple-phosphate stones), form most often in the presence of infection by urea-splitting bacteria which degrade urea into ammonium and carbon dioxide, thus providing a major substrate for the development of a large, branched renal calculus. It was assumed that struvite stones account for 5-30% of all urinary stones.[4] Struvite stone has a chemical composition of magnesium, ammonium phosphate, with or without calcium carbonate. *Proteus mirabilis*, *Proteus vulgaris*, and *Morganella morganii* are the most common organisms isolated; less common organisms include *Ureaplasma urealyticum*, and some species of *providencia*, *Klebsiella*, *Serratia*, and *Enterobacter*.[5] These infection stones are commonly observed in people who have factors that predispose them to urinary tract infections, such as those with spinal cord injury and other forms of neurogenic bladder, ileal conduit urinary diversion, vesicoureteral reflux, and obstructive uropathies. They are also commonly seen in people with underlying metabolic disorders, such as idiopathic hypercalciuria, hyperparathyroidism, and gout. A urease positive bacteria through decomposition of urea and production of ammonium hydroxide can result in an increase in urine pH.[1-4] This increase not only has a toxic effect on the renal epithelium but also predispose to super saturation of ammonium-magnesium phosphate and apatite carbonate, therefore, leads to crystallization and deposition of crystals and ultimately to production of renal stones.[6] Ammonium magnesium phosphate (struvite) stones work as nuclei for colonization of bacteria[7], causing more serious infections, increased incidence of pyelonephritis, alkaline incrusted cystitis and recurrent urinary tract infections.[3] The increase in urine pH, which occurs in the presence of some bacteria is among factors predisposing infectious stone formation. It is shown that urease is necessary to split urea to ammonia and CO_2, leading to formation of ammonia ions and development of alkaline urine both of which are preconditions for the formation of struvite and carbonate apatite crystals.[8]

Aims of The Study

Our study aimed to detected of production of urease enzyme extracellularly and stone formation from *Enterobacter cloacae* isolates from UTI.

MATERIAL AND METHODS

Isolation of bacteria: according to previous study of.[9]

Production of urease extracellularly

1. Each bacterial isolate was grown on three tubes containing Stuarts urea broth, the first tube was free urea, the second tube was supplemented with 1% urea and the latter was supplemented with urea and tween 80.
2. The growth was then separated by filtration
3. The supernatant (filtrates) was subjected for detection urease activity in urea agar base
4. The positive filtrates were than subjected to show the ability of extracellular urease to form struvite stone.
5. All tubes were incubated at the same incubation conditions.
6. Ammonium concentration (indophenol method, appendix), turbidity and pH were determined at the beginning of the experiment and after 4,8 and 24 hours of incubation.
7. Sediment was examined at the same intervals and crystals, if any were identified both macro and microscopically.[10]

Urea agar method

Urease is an enzyme that breaks the carbon-nitrogen bond of amides to form carbon dioxide, ammonia and water. The preparation of media by sterilize urea base agar by autoclave, then cooled it to (50˚C) and add urea supplement to it, then poured it in sterile tubes, for test inoculated it by bacterial culture ,then incubate for (24-48 hours) at (37˚C). When reading the result of test the increased of medium PH result from urea break down and ammonia releasing. this pH change is detect by pH indicator that turned pink in basic environment. A positive test of urease indicate by pink medium, no change in media color mean negative reaction.[11]

***In Vitro* Stone Formation**

1. 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 *E. cloacae* aerobically at (37^{o}C) for (24) hours in brain heart infusion broth which was enriched with (1%) Tween (80) and (10%) serum. (1ml) of (1:10) dilution in human urine of an overnight culture of *E. cloacae* was inoculated in to (9ml) of sterile urine.
2. *E. coli* (certified to be non-urease producer) was inoculated in the same mentioned above producer done for the overnight culture of *E. cloacae.*
3. All inocula gave a final count of about 10^{7}CFU/ml.

4. A control of (10ml) of urine (from the same person) was also studied.
5. All tubes were incubated at the same incubation conditions (at (37°C) for overnight aerobically).
6. Ammonium concentration (indophenol method; appendix), turbidity and pH were determined at the beginning of experiment and after (4,8) and (24) hours of incubation.
7. Sediment was examined at the same intervals and crystals, if any, were identified both macro- and microscopically.[10]

RESULTS AND DISCUSSION

Urease production and struvite stone Formation

Enterobacter cloacae isolates were found to be urease positive in urea agar method, production of urease extracellularly. Urine samples obtained from healthy individuals were used to show the ability of bacteria to produce struvite stone. It was found that struvite stone formed by all isolates due to their ability to produce urease. These results were showed in Table (1), (2) and Figure (1). These results are correlated with those obtained with.[12] who found that the *Enterobacter cloacae* have ability to produce struvite stones in urine because of their ability to produce urease. Studies showed that both urea and urease must be present for bacteria to form stones.[13] Struvite crystals form only in the presence of alkalinity induced by urease or ammonium hydroxide. Alkalinity induced in urine by sodium hydroxide brings about crystallization of apatite but not struvite.[14] Bacterial urease, has been implicated as a contributing factor in the formation of urinary and kidney stones. Our results indicate that *E. cloacae* can induce the formation of struvite stone in urine samples in vitro and this formation occurs as a result of the ability of these isolates to produce extracellular urease. The formation of urinary stones results from the pH rise that occurs in urine due to the release of ammonium ions from urea as a result of activity of bacterial ureases. The stones are a mixture of struvite and carbonate apatite which precipitate at pH > 6.5.[15] The ability of *E. cloacae* to form stone was investigated through testing the pH and crystal in human urine inoculated with *E. cloacae*, *E. coli*, and controlled urine without bacteria at (0, 4, 8), and (24) hours of incubation. A gradual increase in the turbidity was noticed until the 4th hours when the rate of proliferation become faster, increasing the microbial population to the maximum when examined after an overnight incubation. When the *E. cloacae* grew in human urine, there was the elevation in the urine pH at the first (4) hours was (7), reaching about (8.5) at (8) hours and maximized after (24) hours up to (9.5), this effect not observed with the *E. coli* that grew at the same conditions, as it was expected, the final pH reading for *E. coli* was (6).

On the other hand, there were no changes in the pH control of urine free of bacteria. The amount of crystals seen microscopically increased gradually parallel with that of both pH and turbidity reaching to the maximum number after (24) hours of incubation when a white sediment appeared at the bottom of the tube at the time. There were no sediment observed in the case of both *E. coli* and control urine after an overnight incubation. The formation of struvite stone by urease producing bacteria was previously studied by other investigators such as[16] in *Proteus mirabilis*, and[17] in *Corynebacterium urealyticum*. The present study has confirmed that urease play a major role in stone formation and this point is very important in recommended to find medication for urease activity inhibition to prevent the stone formation. The ammonia that is generated by urease activity raises the pH of the urine resulting in calcium and magnesium phosphate crystal formation within the biofilm matrix.[15]

Table 1: production of stone formation by *Enterobacter cloacae*

test	E_1	E_2	E_3	E_4	E_5	E_6	E_7	E_8	Percentage %
Production of Stone formation	+	+	+	+	+	+	+	+	100

Table 2: production of urease by *Enterobacter cloacae*

test	E_1	E_2	E_3	E_4	E_5	E_6	E_7	E_8	Percentage %
Production of urease by agar method	+	+	+	+	+	+	+	+	100
Production of urease extracellular	+	+	+	+	+	+	+	+	100

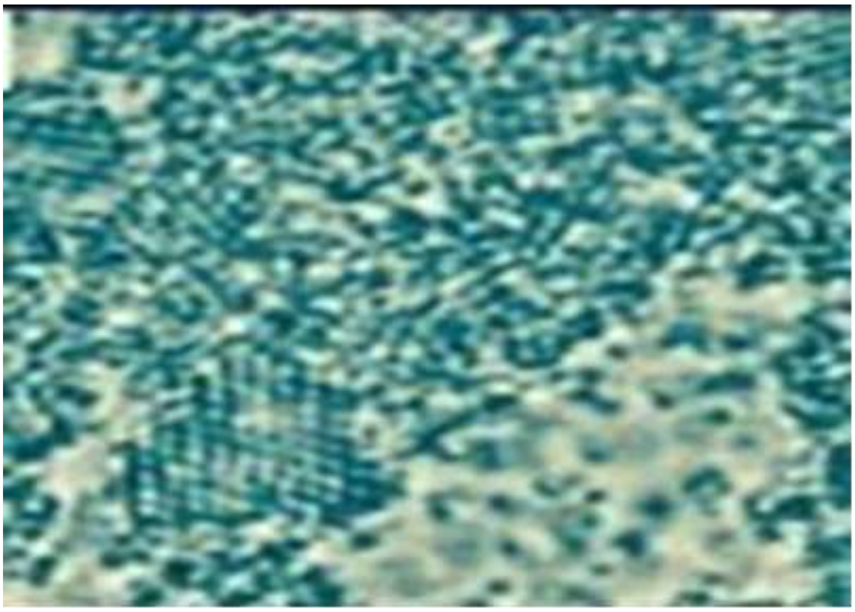

Figure 1: Stone formation by *Enterobacter cloacea.*

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