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Research Article

DETECTION OF *URER* AND *UREC* AMONG *PROTEUS MIRABILIS*

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ABSTRACT

Objective: This study aimed to investigate the correlation between *ureR* and *ureC* genes with the production of urease by *Proteus mirabilis*.

Methods: A total of 450 mid-stream urine samples have been collected from patients with urinary tract infection whom admitted to the hospitals in Annajaf Al-Ashraf province for consultancy during the period from October 2015 to February 2016.Out of 150 bacterial isolates, only 29 isolates were belong to *P. mirabilis* according to conventional methods (depending on microscopic and culturing examination as well as biochemical test) and molecular technique using *16SrRNA* gene.

Results: The results of phenotypic and genotypic detection of urease in *P. mirabilis* showed that all isolates were able to produce urease and possess *ureR* and *ureC* that encodes to urease by appearing of amplicon with molecular weight 359 and 533 bp, respectively, when electrophoresed on 1% agarose gel.

Conclusion: A correlation has been found between *ureR* and *ureC* genes with the production of urease by *P. mirabilis.*

Keywords: Urease, *Proteus mirabilis, ureR, ureC,* Polymerase chain reaction technique.

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INTRODUCTION

Proteus is a Gram-negative, anaerobic rods belongs to the family Enterobacteriaceae and has more than four species; most of *Proteus* species known to cause disease in humans are associated with opportunistic infections [1].

Proteus mirabilis is ubiquitous in the natural environment. It is involved in the disintegration of the organic matter; it is also normal flora of the human intestinal tract, together with *Escherichia coli* and *Klebsiella* spp. where the *E. coli* is the most common [2]. It is an opportunistic bacterial pathogen which under favorable conditions causes many diseases such as urinary tract infections (UTIs), and especially with complicated UTI [3]. Usually, they affect the upper part of urinary tract causing infections such as cystitis, urolithiasis (kidney or bladder stones), and acute pyelonephritis and occasional cases of neonates or infants meningitis, bacteremia, wound infections, septicemia, and rheumatoid arthritis [4]. Its causes diseases by two steps, the first one involve colonization at the site of infection using fimbriae, and second by Ali and Yousif [5]. *P. mirabilis* expresses several virulence factor involved in uropathogenesis such as adhesions, swarming motility, urease, hemolysin, proteases, and lipopolysaccharide endotoxins [6].

Urease is an important virulence factor in the pathogenicity of *P. mirabilis* which causes obstruction of indwelling urinary catheter and kidney and bladder stones [7]. This multimeric nickel-metalloenzyme is encoded by urea-inducible urease gene cluster (*ureDABCEFG*) [8]. Urease operon transcription is positively activated by *ureR* a dimer of identical 293 amino acid polypeptides that bind urea resulting in the binding of protein avidly to both *ureR* and *ureD* promoters, then RNA polymerase is activated by *ureR* and thus will initiate transcription [9]. Because of the important role of urease in the pathogenicity of *P. mirabilis*, this study was aimed at investigating to detect the correlation between *ureR* and *ureC* in urease production.

METHODS

Samples collection

About 450 mid-stream urine samples have been collected from patients suffering from UTI from both genders at different age groups. All samples were cultured on MacConkey agar and incubated for 24 hrs for primary isolation of *P. mirabilis.*

Identification of *P. mirabilis*

A suspected bacterial isolates that showed swarming phenomenon was further identified depending on conventional methods according to MacFaddin (2000) and molecular technique using *16SrRNA* gene by polymerase chain reaction (PCR) technique [8].

Phenotypic detection of urease

A slant of urease agar test tubes were inoculated with bacterial isolates by stabbing and streaking method then incubated at 37°C for 24-48 hrs change of media color from yellow to pink indicates a positive result [9].

Molecular experiment

Extraction of bacterial DNA

Extraction of template DNA from *P. mirabilis* isolates was carried out using boiling method as described by Sambrook and Russell (2001). Briefly, an overnight of brain heart infusion culture (10 ml) of bacterial isolates were centrifuged at 6000 rpm/10 minutes and the pellet was washed twice with *Sodium Chloride-Tris-EDTA, 1X solution pH 8.0, Fisher BioReagents* (STE) buffer (0.058 g of NaCl, 0.015 g of Tris base and 0.004 g of ethylenediaminetetraacetic acid [EDTA] in 10 ml of distilled water) then, 200 µl of STE buffer was added, mixed well and heated to boiling for 10 minutes. Later on, a lysate was transferred to a water bath at 80°C for 5 minutes then incubated on ice bath for 5 minutes. The mixture was centrifuge for 30 minutes at 15,000 rpm, and the supernatant was transferred to a new Eppendorf tube and mixed with 0.7 ml (V: V) of isopropanol and incubated at −4°C for overnight. The precipitated nucleic acid was recovered by centrifugation at

10,000 rpm/15 minutes, and the pellet was washed with 70% ethanol and drained off till no trace of ethanol was seen, then 200 µl of Tris-EDTA buffer was added to re-suspend the DNA.

Amplification of 16SrRNA gene

Monoplex PCR technique has been carried out to amplify *16SrRNA* using F-GAGTTTGATCCTGGCTCAG- and R-GGTTACCTTGTTACGACTT-. PCR mixture was used with a final volume of 20 µl consisting of 5 µl of master mix (2.5 U-iTag DNA polymerase, 2.5 mM deoxynucleotide triphosphates, ×1 reaction buffer and ×1 gel loading buffer), 2 µl of each forward and reverse, 5 µl of DNA template, and 6 µl of nuclease free water. PCR reaction was performed in PCR thermocycler (Biometra, USA) with the following conditions: 94°C for 2 minutes followed by 30 cycles of 94°C for 1 minute, 50.2°C for 1 minute, and 72°C for 1 minute with a final extension at 72°C for 5 minutes. The resulted amplicon was electrophoresis on 1% agarose gel stained with 0.5 µg/ml of ethidium bromide at 80 V for 1.5 hrs and the gel was visualized by ultraviolet transilluminator unit and photographed [10].

Amplification of ureR and ureC genes

Duplex PCR was used to amplify *ureR* and *ureC* genes using F-GCGGTTTATCACGAAGGGGT- and R-TGAGTGCGAAATTGCGATGG- for *ureR* (designed in this study) and F-CCGGAACAGAAGTTGTCGCTGGA- and R-GGGCTCTCCTACCGACTTGATC- for *ureC* [10]. PCR mixture with a final volume of 20 µl was prepared as described above. The conditions of amplification involved: 94°C for 2 minutes followed by 30 cycles of 94°C for 1 minute, 58°C for 1minute for annealing and 72°C for 1 minute, and 72°C for 5 minutes. The resulted amplicon was electrophoresis as mentioned previously.

RESULTS AND DISCUSSION

The initial diagnosis that based on microscopic examination and characteristic of bacterial colonies on selective and differential culture media showed that out of 150 bacterial isolates only 36 isolates were belong to *P. mirabilis* which appeared as Gram-negative bacilli (pinkish red) arranged as non-capsulated and non-spore forming short rods or bacilli, while growth characteristics showed swarming phenomenon on blood agar that looks like concentric rings rising from one center. It also showed a transparent or pale yellow color with no lactose fermentation colonies when cultured on MacConkey agar. The results of biochemical tests that followed to confirm the initial diagnosis revealed that all *P. mirabilis* isolates were positive to methyl red test, citrate utilization, catalase, and urease tests; while the results were negative for oxidase, Voges–Proskauer, indole, and lactose fermentation tests; results of triple sugar iron test showed Alk/A with positive hydrogen sulfide and gas production. The results of molecular identification showed that 29 isolates (19.33%) were belonged to *P. mirabilis* as the amplicon appeared with molecular weight 1500 bp as shown in Fig. 1.

The results of detection of *P. mirabilis* abilities to produce urease showed that all isolates (100%) are able to hydrolyze urea by production of urease. Furthermore, the results of duplex PCR technique for amplification of *ureR* and *ureC* revealed that all isolates 100% possess both *ureR* and *ureC* by appearance of amplicon with molecular weight 359 and 533 bp, respectively, when electrophoresed on an agarose gel stained with ethidium bromide as shown in Fig. 2.

UTIs are still commonly diagnosed in outpatients as well as in hospitalized patients [11]. *P. mirabilis* is a normal flora of the human intestinal tract [12], it is an opportunistic pathogen which under a favorable conditions causes UTI, and it is commonly associated with complicated UTI, and they are important causative agents in community-acquired and nosocomial UTI and associated with 8.50% of UTI [5,13].

This study revealed a variation in the results of biochemical tests that used for diagnosis of *P. mirabilis,* this may due to a variation in

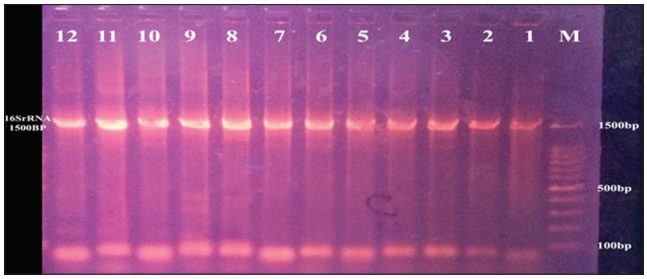

Fig. 1: Gel electrophoresis of polymerase chain reaction product of *16SrRNA* of *Proteus mirabilis* (amplicon with 1500 bp). Lane M: DNA marker (100 bp); Lane 1-12: Amplicon of *16SrRNA* of *P. mirabilis* (1% agarose, 80 V for 90 minutes)

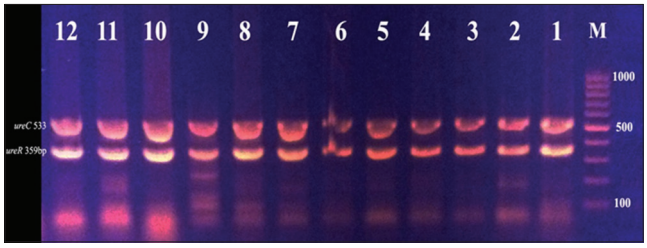

Fig. 2: Gel electrophoresis of duplex polymerase chain reaction product of *ureR* and *ureC* of *Proteus mirabilis* (amplicon with 359 and 533 bp, respectively). Lane M: DNA marker (100 bp); Lane 1-12: Amplicon of *ureR* and *ureC* of *P. mirabilis* (1% agarose, 80 V for 90 minutes)

metabolic activity of bacterial isolates such as their negative results to oxidase may indicating that isolates use other pathways other than cytochrome C oxidase for respiratory process [13].

Several studies referred to using of *16SrRNA* for identification of *P. mirabilis* isolated from ocular infections [14], cerebrospinal fluid [15,16] who used specific primers for *16SrRNA* gene to identify *P. mirabilis* and *Proteus vulgaris* that isolated from UTIs. *16SrRNA* was described as a high discriminatory power for identification of bacteria and to differentiate between closely related genera because it exists in almost all bacteria, often existing as a gene cluster or operon, and also the function of this gene has not changed over time [17].

On the other hand, many studies demonstrate the high ability of *P. mirabilis* to produce urease [18,19] and the study of Laurel *et al*. [20] found that all *P. mirabilis* isolates (100%) showed strong production of urease.

Urease catalyzes the hydrolysis of urea to ammonia and carbonate then break down to carbon dioxide and another molecule of ammonia, as the following equation,

$$(NH_2)_2CO + 2H_2O \xrightarrow{\text{Urease}} CO_2 + H_2O + 2NH_3$$

Hence, it breaks down one molecule of urea to two molecules of ammonia and one carbon dioxide molecule, that causes nonphysiological alkalization of urine as the pH rise, that result in sedimentation of urinary components such as Mg^{+2} and Ca^{+2} which are soluble at slightly acidic or neutral pH. As a result, struvite stones or carbonate apatite stones or both will form [21,22]. Urease also assists *P. mirabilis* to develop bacteriuria, cystitis, and kidney, and bladder stones [23].

A wide distribution of *ureR* and *ureC* among *P. mirabilis* was detected in several studies such as Lu *et al*. [24], MacFaddin [25], Mobley and Chippendale [26], and Mobley *et al*. [27]. Furthermore, Mobley and

Chippendale (1990) showed that all *P. mirabilis* isolated from different clinical source produced a high amount of urease phenotypically compared to other bacteria and there was a concordance between phenotypic and molecular detection of urease activity. In addition, Nicholson *et al*. [28] and Ali and Yousif (2015) found that *ureC* gene is very abundant in *P. mirabilis* bacteria.

UreR is a member of the *AraC* family of transcriptional regulators and contains both DNA- and urea-binding domains. Two regulators of urease transcription have been characterized, *ureR* and H-NS (histone-like nucleoid structuring protein) [29]. *UreR* gene is transcribed in the opposite direction of *UreDABCEFG*; *ureR* binds the promoters of *ureR* and *ureD* [30]. Transcription of the structural genes of urease is urea-inducible [31] where *ureR* acts as a positive regulator of urease activity and stimulates expression of urease genes in the presence of urea. H-NS is a negative regulator that represses *ureR* transcription. H-NS binds to the poly (A) tracts located in the intergenic region between *ureR* and *ureD* and inhibits transcription of *ureR* [32], so, *ureR* is required for basal urease activity in the absence of urea, for induction of urease by urea, and for virulence of *P. mirabilis* in the urinary tract [33].

Nicholson [34] demonstrated that the transcription of the urease operon is regulated by *ureR* a gene lies upstream from the seven accessory or structural genes and is transcribed from its own promoter in the direction opposite to the rest of the operon. Secondary regulation by a nitrogen regulatory system or catabolite repression does not appear. These data suggest that *ureR* governs the inducibility of *P. mirabilis* urease.

Urease gene cluster includes three structural genes, *ureA*, *ureB,* and *ureC*, in addition to four accessory genes, *ureD*, *ureE*, *ureF*, and *ureG*. Urease apoenzyme is comprised a trimeric complex of the trimer *ureABC* (that is, three copies of each subunit) [35]. Activation of this urease apoenzyme requires incorporation of nickel ions into the metallocenter located in *ureC* [36]. The accessory proteins *ureD*, *ureE*, *ureF*, and *ureG* coordinate activation of the functional urease enzyme by mediating incorporation of the nickel ions into the active site [37].

UreC encodes the large subunit responsible for the production of urease enzyme of the *P. mirabilis*, and it is very highly conserved among all species, so it regarded as a diagnostic feature of the bacteria of *P. mirabilis* [38,39]. Yet, it is considered in this study as virulence factor which had been detected using PCR in addition to *ureR.*

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