Molecular Study of Proteus mirabilis Isolated From Urinary Tract Infections in Erbil City

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Abstract

During the period of 1st of April to15th of August 2012, forty five isolatesof *Proteus mirabilis* were identified among 600 samples taken from patients with urinary tract infection from different hospitals (Rapareen Pediatric, Rizgary, Hawler Teaching, West Emergency and Zheen International hospitals) in Erbil City. Isolates were identified from urine sample by using cultural, morphological and biochemical characteristics. Furthermore, the identity of the isolates confirmed by VITEK 2 systems. The isolates appeared to be varied in their resistance; Imipinime (IMP) was the most effective antibiotic against isolates of *P. mirabilis* and the resistance rate of the isolates for Ciprofloxacin(CIP) were 8.8%, 11% for Aztreonam(AT), 11% for Fosfomycin(FOX), 15.5% for Norfloxacin(NOR), 17.7% for Tobramycin(TOB), 20% for Amikacin(AK), 22% for Piperacilin(PI), 26% for Ceftriaxone(CTR), 28.8% for Cefotaxime(CTX), 33% for Nalidixic acid(NA), 35.5% for Gentamicin(GEN), 40% for Cephalothin (CEP),44% for Chloramphenicol(C), and 62% for Ampicillin(AMP) respectively. Out of 45 *P. mirabilis* isolates, 12(26.6%) were ESBL producers, while 33(73.3%) were non ESBL producers. The plasmid profile of three isolates (P6, P32 and P40) that showed resistance to most antibiotics and one isolate (P16) that was sensitive to all antibiotics were conducted by using gel electrophoresis, the results revealed that two isolates (P6 and P32) have one band, the other (P40) revealed two bands with molecular weigh more than 10 Kbp for all three isolates, while the isolate P16 contain no band. Three isolates (P32, P38 and P40) that revealed resistance to most antibiotics were chosen for transformation process and the results showed that the resistance genes for amikacin, ampicillin, chloramphenicol, ceftriaxone, cefotaxime, gentamicin, and tobramycin were located on plasmid DNA for P32 isolate, while for P38 isolate, the ampicillin, chloramphenicol, gentamicin, , piperacilin and tobramycin located on plasmid. On the other hand, the resistance genes for ampicillin, chloramphenicol, cephalothin, ceftriaxone, cefotaxime, gentamicin, , piperacilin and tobramycin were located on plasmid DNA for P40 isolates.The results of transformation confirmed by gel electrophoresis, and showed that plasmids of P32 and P38 and both plasmid in P40 had been transformed successfully. All *P. mirabilis* isolates were screened for the presence of (*ureC*) on genomic DNA using polymerase chain reaction (PCR) assay. Results showed that all isolates were positive for the existence of *ureC* virulence gene.

Key words: ***P.mirabilis,*****Antibiotic resistance, proteolytic activity**, **Transformation, *ure C* gene**

INTRODUCTION

Urinary tract infections (UTIs) are one of the most common bacterial infections encountered in medical practice affecting people of all ages and account for significant morbidity and health care cost (Kose*et al*., 2007). UTIs also contribute the most common nosocomial infections in many hospitals, and accounts for approximately 35% of all hospital acquired infections. Majority of UTIs are not life threatening and do not cause any irreversible damage. However, when the kidneys are involved, there is a risk of irreparable tissue damage with an increased risk of bacteremia (Hvidberg*et al*., 2000). UTIs are one of the most prevalent extra-intestinal bacterial infection, the Enterobacteriaceae, were the most frequent pathogens detected, causing 84.3% of the UTIs (Gales *et al.*, 2000). The most frequent etiological agents causing (UTIs) are Gram- negative bacteria belong to the Enterobacteriaceae family; *P. mirabilis* is the third most common cause of complicated UTI and the second most common cause of catheter-associated bacteriuria in long term catheterized patients (Warren, 1997 and Adamus-Bialek*et al*., 2013). *P. mirabilis* becomes a significant problem mostly in individuals that have vulnerable immune systems and are in danger of nosocomial transmission, such as hospitalized patients (Farkosh*et al*., 2008). It possesses a host of potential virulence factors that aid its pathogenesis. The two major ones, flagella and urease, have been investigated most thoroughly. Urease is responsible for the formation of bladder and kidney stones and at later stage of infection could facilitate the colonization of the urinary tract. Also it mediates the hydrolysis of urea into ammonia and carbon dioxide and is the most potent virulence factor of *P. mirabilis* (Gygi*et al*., 1995). The constant increase in the antibiotic resistance of clinical bacterial strains has become an important clinical problem (Adamus-Bialek*et al*., 2013). The accelerated emergence of antibiotic resistance among the prevalent pathogens is the most serious threat to the management of infectious diseases (Kotra*et al*., 2002). The evolution and spread of various mechanisms of antimicrobial resistance among common human pathogenic members of Enterobacteriaceae is of increasing concern and lead to narrowing of available therapeutic options (Boucher *et al*., 2009). The extended-spectrum β-lactamase producing bacteria are typically associated with multiple drug resistance because plasmid responsible for ESBL production frequently carries gene encoding resistance to other classes of antibiotics. Therefore, antibiotic option in the treatment of ESBL producing organism are extremely limited (Paterson and Bonomo, 2005), this phenomenon is mainly due to the presence of resistance plasmid or R-factor which may be passed from one bacterium to another or to other strains of *P. mirabilis* by transformation process. A tight relationship between bacterial cells harboring plasmids and their drug resistance profile was known (Maltezou, 2009). Plasmids allow the movement of genetic material, including antimicrobial resistance genes between bacterial species and genera . Virulence factors are encoded by genes located on the chromosome or on the plasmids. The location of virulence factors on such genetic mobile elements may facilitate the spread of virulence properties within bacterial communities (Silva and Mindoca 2012). Ttherefore this study are concerned with isolation and identification of *P. mirabilis* from patients suffering from UTIs.Study the antimicrobial resistance patterns of the isolated *P. mirabilis* to different antibiotics.Determination of the frequency of ESBLs production among the *P. mirabilis* isolates, Characterization of the plasmid DNA profile pattern of four isolates using gel electrophoresis technique, Determination of the site of genes encoding resistance to

antibiotics of the most resistant isolates by performing genetic transformation, and determination of the presence of virulence gene (*UreC*) in *P. mirabilis* isolates using polymerase chain reaction (PCR) assay.

Materials and Methods

Patients and sample collection

Six hundred urine samples were collected from patients with symptomatic urinary tract infection attended from different hospital in Erbil City (Rapareen Padiatric, Rizgary, Hawler Teaching, West Emergency and Zheen International hospitals), during the period of 1st April to15th August 2012 .Bacterial isolates were identified by performing morphological, cultural, biochemical tests, and Vitek 2 system .

Antibiotic susceptibility test

To study the effect of different antibiotics on the isolates of *P. mirabilis*, Mueller-Hinton agar was used as growth media (Wayne, 2005).Antibiotic resistance patterns of the isolates were determined using the Disc diffusion (Kirby Bauer) method;bacterium inoculate was adjusted to 0.5McFarland standard of Clinical and Laboratory standards institute (CLSI, 2007). The test inoculums were spread onto Mullur-Hinton agar using a sterile cotton swab. The tested antimicrobial agents were aseptically placed on the inoculated Muller Hinton agar and incubated overnight. The zones of inhibition were measured and interpreted according to (CLSI, 2007).

Detection of Extended spectrum beta lactamase (ESBL) activity

The ESBL activity was investigated by double disc synergy method; the tested inoculums (adjusted to 0.5 McFarland turbidity) were spread onto Muller-Hinton agar using sterile cotton swabs. A disc of augmentin AMC was placed on Muller-Hinton agar, then discs of CTX, CAZ and AT was kept around it, at distance ranging between 16-and 20 mm from the augmentin disc (center to center). The plate was incubated at 37°C overnight. Distance between the discs was required to be suitably adjusted for each isolate in order to accurately detect the synergy. The organisms were considered to be ESBL producer when the zone of inhibition around any of the expanded spectrum cephalosporin discs showed a clear-cut increase towards the augmentin disc (Collins *et al*., 2004).

Detection of proteolytic activity on skim milk agar

Bacteria to be tested for protease activity were either streaked or spot inoculated onto skim milk agar and incubated at 37°C for 24-72 hours. Protease activity was identified as a distinct clearing on the surface of the milk around the colonies (Wassif*et al.,* 1995).

Gelatin Hydrolysis

Certain bacteria are able to hydrolyze gelatin by secreting a proteolytic enzyme called gelatinase. Gelatin liquefaction can be tested by stabbing nutrient gelatin deep tubes. Following incubation, the cultures are placed in a refrigerator or ice bath at 4°C until the bottom resolidifies (Madigan *et al.,* 2012).

Plasmid DNA Extraction

Laboratory Protocol

Plasmid DNA was extracted and purified from 5ml overnight culture of the selected isolates of the *P. mirabilis* grown in LB broth medium containing 100µg/ml Ampcillin using a plasmid DNA purification kit, according to the manufacturer's

instructions. A single bacterial colony was used to inoculate 5 ml LB, which was incubated overnight in a shaker incubator at 37^{o} C. A volume of 3 or 5 ml of each isolate in an eppendorff tube was centrifuged for 30 sec at 13,000 rpm and the supernatant discarded. The bacterial pellet resuspended by vortexing in 250 µl of resuspention buffer (RNase A solution was added), until no clumps of the cell pellet remain.Two-hundred and fifty µl of lysis buffer added to resuspended cells and the tube closed and gently mixed by inverting the tube several times without vortexing.Three-hundred fifty µl of neutralization buffer was added and gently mixed by inverting the tube several times. Resuspended cells centrifuged at 13,000 rpm for 10 min at 4^{o} C, then a column inserted into collection tube.After centrifugation, supernatant transferred promptly into the column, and centrifuged at 13,000 rpm for 60 sec. The column removed from the collection tube, the filtrate in collection tube discarded. The spin column placed back in the same collection tube. Five hundred µl of washing buffer A added and centrifuged at 13,000 rpm for 60 sec. The column removed from collection tube, the filtrate discarded in collection tube, and then placed the spin column back in the same collection tube. Seven-hundred µl of washing buffer B was added, centrifuged at 13,000 rpm for 60 sec. The filtrate discarded in collection tube and column placed back in the same collection tube, Centrifuged at 13,000 rpm for 60 sec to dry the filter membrane.The column put in a clean and sterile eppendorf. Fifty µl of elution buffer added to the upper reservoir of the column, and let stand for 1 min. Then, the tube centrifuged at 13,000 rpm for 60 sec.The purified DNA plasmid used immediately in downstream applications or store at -20 ^{o}C (Manufacturer protocol).

Plasmid profile

The extracted plasmids were electrophoresed in 0.7% agarose gel with Tris-borate ethylene diamine tetra-acetic acid (TBE), Agarose gel electrophoresis was used to separate DNA fragments according to size, 0.7% (w/v) agarose gel was made by adding 0.7 gm of agarose to 100 ml of 1X TBE buffer solubilized by heating at boiling temperature in microwave oven for 2 min, then the agarose was left to cool down at 55 ° C before pouring in a tray to solidify. A comb was placed near one edge of gel, and gel was left to harden. After gel solidification, the comb was removed gently and the gel was soaked in a gel tank contain 1X TBE buffer and the placement of the gel in the tank should be in a way that the wells located on the negative (cathode) pole. The amount of TBE buffer had to be sufficient to cover about 2-3 mm of the gel, 1 kb DNA ladder (Fermentas) was used as molecular size marker of DNA fragment, 3µl of loading buffer was mixed with 10µl plasmid DNA extract, and then samples were added carefully to individual wells.After loading the samples into wells, the power supply was set on 45 volts for 15min. Then the voltage changed to 75 volts and the gel was run for 90 min. until the bromophenol blue dye migrated to the other end of the gel. The gel was immersed in ethidium bromide (0.5 µg/ml of D.W.) for 30-45 min. The gel was visualized by UV-trans illuminator and then photographed (Sambrook and Russell, 2001).

Determination of location of genes conferring antibiotic resistance

To determine the location of antibiotic resistance genes in *P. mirabilis*, the purified plasmid from *P. mirabilis* (resist to antibiotics) transformed to sensitive strain of *E. coli* DH5α(it is a bacterial strain that does not contain the plasmid used in this study and is a derivative of *E. coli* K12). Competence can be induced by chemical treatments such as ice-cold $CaCl_2$ treatment followed by a brief heat shock (Casali and Preston, 2003).

Preparation of competent cells

To make the cured cultures competent, 5 ml of LB broth was inoculated with a single colony of *E. coli* DH5α (whose plasmid is manipulated genetically, plasmidless), incubated with shaking (100 rpm) for 24 hours at 37^{o} C,then 1 ml of bacterial culture was added to 50 ml LB broth, incubated with shaking at 37°C for 3-4 hrs. The bacterial growth was monitored by measuring the optical density (OD_{650}) with spectrophotometer. Logarithmic-phase cultures of *E. coli* typically have an (OD_{650} of 0.5–0.7). The cells were harvested by centrifugation at 5,000 rpm for 10 min, the supernatant discarded, and then resuspended. One ml of ice-cooled 0.1 M $CaCl_2$ added to thepellet, and then 39 ml of the same solution was added. The resuspended cells left on ice for 30 min, centrifuged for 10 minutes at same velocity, the supernatant discarded, and the bacterial pellet resuspended in 2.5 ml of ice-cold $CaCl_2$ (Casali and Preston, 2003).

Transformation of Competent Cells

Ten µl of prepared plasmid DNA from *P. mirabilis* isolates plated on LB agar plates and incubated at 37^{o}Cfor 24 hours to ensure that they are not contaminated with the bacterial cells. Two-hundred µl of bacterial suspension transferred into a sterile eppendorf tube containing 5µl of plasmid DNA and mixed gently. For each set of transformations, negative controls prepared that consist of competent cells without DNA were plated on LB agar plate with AMP. The transformation mixture placed on ice for 30 min, and then placed in water bath at 42°C for 90 second without shack the tubes. After heat shock, the transformation mixture was put on ice for 5 min. The transformation mixture transferred to a suitably sized tube containing one and a half ml of LB broth, and incubated with shaking at 37^{o}C for 1 hour. Following incubation, the mixture aseptically transferred to eppendorf tube and centrifuged at 14,000 rpm for 1min. Then, the supernatant discarded, and the pellet resuspended in small amount of remaining supernatant. Finally, the resuspended pellet spread on selective LB agar plates. All the plates were incubated at 37 ^{o} C for 24 hours (Casali and Preston, 2003).

Polymerase chain reaction assay (PCR)

DNA extraction

Three to five ml overnight culture in a 1.5 or 2 ml micro centrifuge tube harvested by centrifugation for 10 min at 10000 rpm, the supernatant discarded.The pellet resuspended in 180 µl of digestion solution, 20 µl of proteinase k solution was added and mixed thoroughly by vortexing or pipetting to obtain a uniform suspension. The sample incubated at 56 ^{o} C for ~ 30 min in shaking water bath, until the cells are completely lysed.Twenty µl of RNase solution was added then mixed by vortexing and the mixture incubated for 10 min at room temperature. Two-hundred µl of lyses solution was added to the sample, mixed thoroughly by vortexing for about 15 sec until homogeneous mixture was obtained.Four-hundred µl of 50% ethanol was added and mixed by pipetting or vortexing.The prepared lysate transferred to Gene JET™ genomic DNA purification column and inserted in a collection tube. The column centrifuged for 1min at 6,000 rpm. The collection tube then discharged containing the flow- through solution. The Gene JET™ genomic DNA purification column placed into a new 2 ml collection tube.Five hundred µl of wash buffer I (with ethanol added) was added then centrifuged for 1 min at 8,000 rpm. The flow-through discarded and the purification column placed back into the collection tube.Five hundred µl of wash buffer II (with ethanol added) was added to the Gene JET™ genomic DNA purification column, Centrifuged for 3 min at maximum speed (≥ 12,000 rpm).Two-hundred µl of

elution buffer was added to the center of the Gene JET™ genomic DNA purification column membrane to elute genomic DNA. Incubated for 2 min at room temperature and centrifuged for 1min at 8000 rpm.The purified DNA immediately discarded in downstream applications or stored at -20 ^{o} C.

DNA amplification

PCR was performed in a 25µl of reaction volume. PCR was used to detect the urease (*UreC*)gene in the genomes of the *P. mirabilis* isolates. The *UreC*primers used were forward 5'-GTTATTCGTGATGGTATGGG-3' and reverse: 5'-ATAAAGGTGGTTACGCCAGA-3'. The PCR cycles were: denaturation at 95°C for 1 min, annealing at 52°C for 1 min, and extension at 72°C for 1 min, repeated 35 times (Stankowska*et al*., 2008).

Detection of PCR product

The amplified products were visualized by ethidium bromide staining after gel electrophoresis of 7 µl of the final reaction mixture in 1.2% agarose. 100 bp DNA ladder (Gene dire) was used as molecular markers (Sambrook and Russella, 2001).

Results and discussion

Collection of *P. mirabilis* isolates

Forty-five isolatesof *P. mirabilis* were identified among 600 urine samples from patients with urinary tract infection from different hospitals (Rapareen Pediatric, Rizgary, Hawler Teaching, West Emergency and Zheen International hospitals) in Erbil City, during the period of 1st of April to15th of August 2012.

Identification of *P. mirabilis* isolates

The identification of *P. mirabilis* isolates performed according to the following steps:

Morphological identification

It grows well on MacConkey agar with optimum growth at 37˚C and form several sorts of discrete light brown colony indicating of not lactose fermentation .Also shows characteristics of swarming on the blood agar surface which appears as concentric rings of growth emanating from a single colony or inoculum .

Smear preparation

Under the light microscope, *P. mirabilis* appears as straight Gram-negative rods. Its cells are appearing as non-capsulated and non-spore forming .

Biochemical identification

The biochemical tests for all bacterial isolates were positive for citrate, catalase, motility and urease production test, but they were negative for oxidase test. On Kligler Iron Agar medium, all isolates of *P. mirabilis* under study produced a red (alkaline) slant and a yellow (acidic) butt reaction due to the non-lactose fermentation and glucose fermentation with H2S production. Furthermore, Vitek 2 system was performed to support above results and to be confirmed that the isolates were *P. mirabilis*. The proteolytic activity of 45 isolates of *P. mirabilis* was determined on skim milk agar and 42 (93.3%) isolates of *P. mirabilis* produce proteolytic activity after incubation for 24 hrs at 37 ˚ C . One isolate appered to be non-proteolytic at this stage, but on incubation for a further 24 hrs , clear zones of proteolysis were formed.Our results agree with Senior (1999) who found that 94% of *P. mirabilis* isolated from urine had protease positive.The secreted proteolytic enzymes of most isolates of *P. mirabilis* degraded both gelatin and casein (milk). However, few isolates

degraded gelatin but not casein. The inability of some *P. mirabilis* that degrade gelatin but not casein is believed to be the outcome of weaker protease production and a less sensitive substrate. Senior (1999) demonstrated that the mobility and number of the protease bands on gel containing gelatin or casein were the same for strains that degraded both gelatin and casein as for those that degraded gelatin but not casein suggests that the protease produced by the former group of strains.

Antibiotic resistance pattern of *P. mirabilis* isolates

Antibiotic test for 45 isolates of *P. mirabilis* was done against 15 antibiotics (AK, AMP, AT, C, CEP, CIP, CTR, CTX, FOX, GEN, IMP, NA, NOR, PI and TOB).The following table indicated that all the isolates of *P. mirabilis* revealed different resistance rate to most antibiotics used and resistance percent was 62 % for AMP, 44% for C, 40% for CEP, 35.5% for GEN, 33% for NA, 28.8% for CTX, 26% for CTR, 22% for PI, 20% for AK, 17.7% for TOB, 15.5% for NOR, 11% for AT and FOX, while the lowest percent 8.8% was to CIP, and all isolates were sensitive for IPM.

Table(1): Sensitivity of *P. mirabilis* isolates to different antibiotics and percentage of resistance

No. of isolates	% of resistance	Antibiotics at final concentration														
		amikacin	ampicillin	aztreonam	Chloramph.	cephalothin	ciprofloxacin	ceftriaxone	cefotaxime	fosfomycin	gentamycin	Imipinime	Nalidixic acid	norfloxacin	piperacillin	tobramycin
1	20	S	S	R	R	S	S	R	S	S	S	S	S	S	S	S
2	0	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
3	13.33	S	S	S	R	S	S	S	S	S	S	S	R	S	S	S
4	13.33	S	S	S	R	S	S	S	S	S	S	S	R	S	S	S
5	0	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
6	53.33	S	R	R	R	R	S	R	R	R	S	S	S	S	R	S
7	46.66	S	R	R	S	R	S	R	R	R	S	S	S	S	S	R
8	33.33	S	R	R	S	R	S	R	S	R	S	S	S	S	S	S
9	20	S	R	S	S	S	S	S	S	S	R	S	R	S	S	S
10	40.	S	R	S	S	S	S	S	S	R	R	S	R	R	S	S
11	20	R	S	S	S	S	S	R	S	S	S	S	R	S	S	S
12	13.33	S	S	S	S	S	S	R	S	S	S	S	R	S	S	S
13	6.66	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
14	6.66	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
15	6.66	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
16	0	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
17	26.66	S	S	S	R	S	S	S	R	S	S	S	R	R	S	S
18	40	S	R	S	S	R	S	S	R	S	R	S	R	R	S	S
19	33.33	S	R	S	S	S	R	S	S	S	R	S	R	R	S	S
20	0	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
21	13.33	S	R	S	S	S	R	S	S	S	S	S	S	S	S	S
22	13.33	S	S	S	R	S	S	S	S	S	S	S	S	S	R	S
23	13.33	S	R	S	R	S	S	S	S	S	S	S	S	S	S	S
24	20	S	R	S	S	R	S	S	S	S	S	S	R	S	S	S
25	26.66	R	S	S	R	R	S	S	S	S	S	S	R	S	S	S
26	6.66	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S
27	0	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
28	0	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

29	40	S	R	S	S	R	S	R	R	S	R	S	S	S	R	S
30	46.66	S	R	S	R	R	S	S	R	S	R	S	R	S	R	S
31	33.33	S	R	S	S	R	S	R	R	S	R	S	S	S	S	S
32	60	R	R	S	R	R	S	R	R	S	R	S	S	S	R	R
33	26.66	R	R	S	S	S	S	S	S	S	R	S	S	S	S	R
34	13.33	S	R	S	R	S	S	S	S	S	S	S	S	S	S	S
35	33.33	S	R	S	S	R	S	S	R	S	R	S	S	S	R	S
36	46.66	R	R	S	R	R	S	S	R	S	R	S	S	S	S	R
37	40	S	R	S	R	R	R	S	S	S	R	S	S	R	S	S
38	53.33	R	R	S	R	R	S	R	R	S	S	S	R	S	S	R
39	13.33	S	S	S	R	S	S	S	S	S	S	S	S	R	S	S
40	86.66	R	R	S	R	R	R	R	R	R	R	S	R	R	R	R
41	0	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
42	20	R	R	S	S	S	S	S	S	S	R	S	S	S	S	S
43	46.66	S	R	R	R	R	S	R	R	S	S	S	S	S	R	S
44	46.66	S	R	S	R	R	S	S	S	S	R	S	R	S	R	R
45	46.66	R	R	S	R	R	S	S	S	S	R	S	S	S	R	R
% of resis .Isol ates		20	62	11	44	40	8	26	28	11	35	0	33	15	22	17

R= Resistant, S = Sensitive

IMP was the most effective antibiotic against isolates of *P. mirabilis* and 100% of these isolates were susceptible to IPM, 33% of the isolates were resistant to NA, Regarding to C resistance, 44% of isolates was resistant to it.In the current study, it has been shown that the resistance raised to the third generation of cephalosporin 3GC such as CTX and CTR, and the results were 28.8% and 26% for CTX and CTR respectively. Low resistance pattern to CIP reported with a percentage of 8.8%. High resistance pattern for AMP reported with a percentage of 62%, Twenty-two percent of the isolates were resistant PI, The resistance of *P. mirabilis* isolates to GEN was 35.5, On detecting the resistance of *P*. *mirabilis* for FOX and AT, it was found that 11% of the isolates were resistant to both. IMP was the most effective antibiotic against isolates of *P. mirabilis*, this result was agree with Luzzaro *et al.,* (2006), Dalela (2012) and Adamus-Bialek*et al.,* (2013). They reported that all *P. mirabilis* isolated from different clinical sources were sensitive for IMP. This may be attributed to the inability of *P. mirabilis* to produce enzymes that degrade or inactivate this antibiotic. Therefore, IMP is the most active drug for the treatment of UTIs causing by *P. mirabilis*. AL-Hamadan *et al.,* (2007) found that 20 % of *P. mirabilis* isolates were resistant to NA. Yah *et al.,* (2007) revealed that 17.5% of *P. mirabilis* isolates were resistant to NA, Soliman (2006) who mentioned that 36% of the isolates were resistant to C, These results were agreed with Karim (2010) who mentioned that 31.25% of the isolates resistant for the 3GCs. this was agreed with Torzewska*et al.,* (2005) and Li *et al.,* (2012) who found that the resistance for CEP was 33% and 35.2% respectively, Karim (2010) who found that the resistance to CIP was 10% and 6.25%, respectively. This may be due to the efficiency of this antibiotic in eradication of infections caused by Gram negative bacilli, this result agreed with Yah *et al.,* (2001) who revealed that 19.6% of the isolates were resistant to NOR, similar result obtained by Torzewska *et al.,* (2005) who found the resistance to AMP as 56%, This was explained by the overuse of antibiotics especially AMP in Erbil Hospitals, and also to the missuses of these antibiotic as they are prescribed without sensitivity test (Decre et al., 2002). These results agree with Li *et al.,* (2012) who found that 16.6%

of the isolates resistance to AK. The mechanism of action of quinolones (include NA) and fluoroquinolones includes (CIP and NOR) is the inhibition of type II topoisomerase enzymes, which involved in the bacterial DNA replication. Two mechanisms had been found to determine resistance to fluoroquinolones and quinolones. The most important of these mechanisms are the accumulation of mutations in the bacterial enzymes targeted by fluoroquinolones DNA gyrase and DNA topoisomerase IV (Robicsek*et al.,* 2006). Another mechanism is the decreased intracellular antibiotic accumulation by efflux pumps (Poole, 2005). Resistance against PI is primarily mediated by a structural change of the penicillin binding proteins (leading to lower affinity of the antibiotics) or by bacterial production of enzymes cleaving the beta-lactam ring. Other mechanisms are decreased permeability or active transportation via efflux pumps (Chambers, 2005).Resistance to aminoglycosides (i.e. GEN, AK, KA and TOB) can be mediated by bacterial production of aminoglycoside modifying enzymes, decreased uptake due to membrane permeability, transport or efflux, point mutations at the drug target, and methylation enzymes changing the target (Poole, 2005).The widespread antibiotic usage exerts a selective pressure that acts as a driving force in the development of antibiotic resistance (Levy, 1992 and Virve*et al.,* 2004). The high resistance of the bacterial isolates in this study to different antibiotics may be related to the presence and dissemination of plasmids within heterogeneous population of these bacteria (Brooks *et al.,* 2001), which can transfer between genera and species of bacteria lead to the prevalence of resistance by conjugation and transformation (Dionisio*et al.,* 2002). In addition to weakling immunity system in some human due to poor nutrition or heredity factors makes bacteria to be more resistant (Sharma *et al.,* 2009). Another reason of resistance in *P. mirabilis* may be attrebuted to mutation in the structure of bacterial cell wall and leads to lacking of porins, also consider as reason for resistance of bacteria to antibiotic especially to β- lactam antibiotics (Martinez-Martinez, 1999).

Detection of ESBL production in*P. mirabilis*

All isolates of *P. mirabilis* were tested for extended- spectrum β-lactamase (ESBLs) production, they were determined that 26.67% showed ESβLs positive as detected by double disc synergic test.

Table (2): Number and percentage of ESBL producing in *P. mirabilis* isolates

Production of ESBLs	P. mirabilis isolates	
	Number	Percentage %
Positive	12	26.67
Negative	33	73.33
Total	45	100

Results of our study were agree with the results obtained by Okesola and Adeniji (2010) and Al- Duliami*et al.,* (2011) which were 37.1% and 33.3% respectively. Other results reported by Bonnet *et al.,* (1999), Chanal*et al.,* (2000), Spanu*et al.,* (2002) and Yong *et al.,* (2005) where the ESβLs prevalence were 14.3%, 14.2%, 16.3% and

17% respectively for *P. mirabilis* isolated from different clinical source.The spread of ESBL producing bacteria has become strikingly rapid worldwide, indicating that continuous monitoring systems and effective infection control measures are absolutely required (Kiratisin*et al*., 2008). Spanu*et al.,* (2002) revealed that 16.3% of *P. mirabilis* isolates were ESBL positive and 98% of these appeared to be TEM-type (Temoniera), the expression of TEM -type enzymes is frequently associated with resistance to aminoglycosides and fluoroquinolones. The observation that these isolates express mainly TEM-type enzymes may have significant therapeutic implications, since *P. mirabilis* has been recognized as an important cause of morbidity in hospitalized patients (De Champs *et al.,* 2000).An ESBL resistance mechanism was mediated by the production of metallo β-lactamase, lack of antibiotic penetration due to mutations in the porins or due to the loss of certain outer membrane proteins and the efflux pumps (Singhal*et al.,* 2005, Walsh et al., 2005 and Noyal*et al.,* 2009).Plasmids have crucial role in the dissemination of resistance genes like ESBL. The most predominant plasmid-mediated β-lactamases found in the clinical isolates of *P. mirabilis* are TEM-derived ESBLs, non-TEM and non-SHV (sulfhydryl variable) derivatives (Emery and Weymouth, 1997).The worldwide dissemination of ESBLs is a typical example of evolution of bacterial resistance that required major revisions in testing and reporting susceptibility to ESBLs. The random and over use of β-lactam antibiotics might stimulate the bacterial population to develop different defense mechanisms as production of ESBL enzymes (Bonnet, 2004).

Plasmid profile of *P. mirabilis*

Three isolates that exhibited the highest resistance toward antibiotics designated as P6, P32 and P40 and one isolate that was sensitive to all antibiotics designated as P16 were selected for plasmid profile.After preparing of plasmid DNA content from the chosen bacterial isolates, ten µl plated on sterilized LB agar in order to examine their purity and the results showed that no contamination of prepared plasmid DNA detected.Extracted plasmids analyzed by gel electrophoresis on 0.7% agarose gel as shown in figure (1). The results revealed that P6 which represented by lane 2 and P32 by lane 3 have one band with molecular weight more than 10 kb, while P40 which represented by lane 4 revealed two bands with molecular weigh more than 10 Kbp and lane 5 which represent P16 was plasmidless. Diverse plasmid pattern was detected in *P. mirabilis* isolates; this explains the difference in sensitivity of the isolates towards the different antibiotics. According to previous studies, it is well known that most Gram negative bacteria are harboring plasmids that are responsible for antibiotic resistance (Helling *et al.,* 1981and Virve *et al.,* 2004).Pearson *et al.,* (2008) determined genomic sequences of uropathogenic*P. mirabilis*, the results revealed that have a single plasmid consisting of 36,289 bp. Other investigators (Stankowska et al., 2008) also detected two plasmids harbored by some of analyzed strains of P. mirabilis that was size of 6 and 93kb.The study of Yah *et al.,* (2007) showed that some of the isolates had plasmid bands that ranged from ≤ 0.55 kbp to ≥ 1.14 kbp. This indicates that plasmids allow the movement of genetic materials, including antimicrobial resistance genes between bacterial species and strains. The multiple copies of plasmid bands might have resulted from covalently close circular, open circular or linear forms of the same plasmid that might migrated at different rates on agarose gel electrophoresis.R-plasmids have been detected in many members of the Enterobacteriaceae. The antibiotic resistance conferred by plasmids broadly reflect the agents commonly used against their bacterial hosts, whereas resistance to tetracycline and to certain penicillins and aminoglycosides occurs on plasmids from bacteria of either Gram type. Plasmids may also confer resistance to

other antimicrobial agents. These include heavy metal ions such as mercury (Robinson and Tuovinen, 1984).

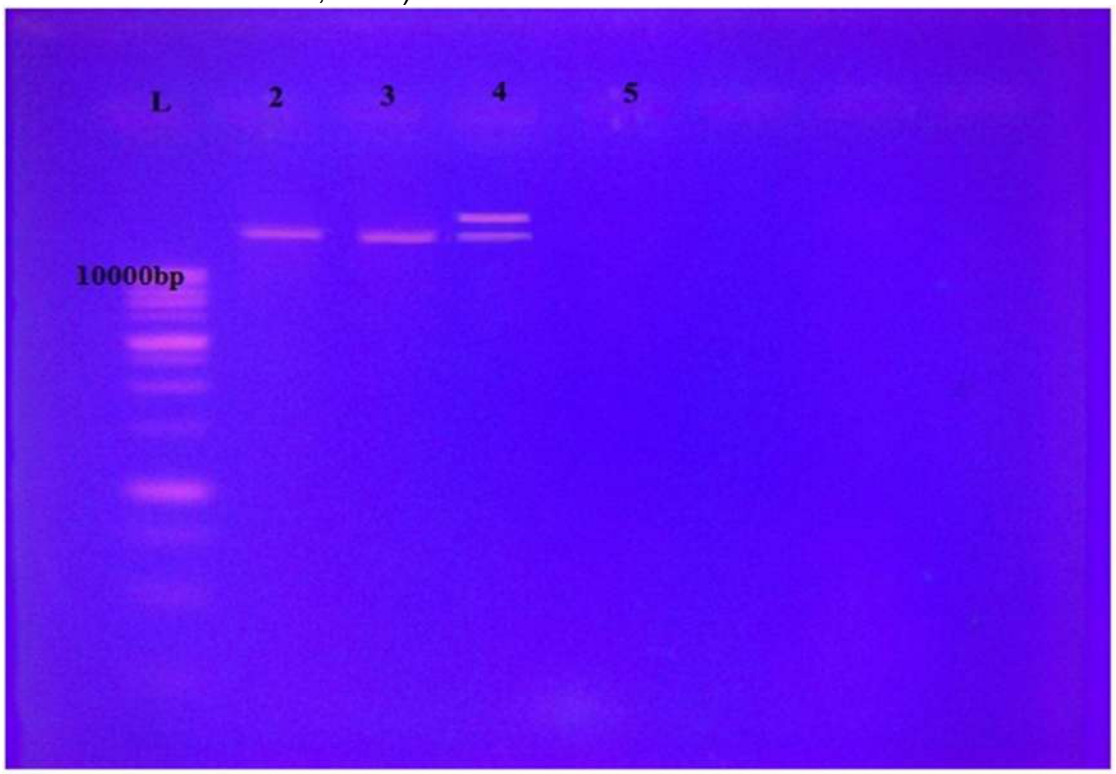

Figure (1): Plasmid profile of four tested *P. mirabilis* isolates
Lane 1 10 000 bp DNA ladder . Lane 2 Plasmid content of P6 . Lane 3 Plasmid content of P32.
Lane 4 Plasmid content of P40 . Lane 5 Plasmid content of P16 (plasmidless).
All tested isolates were harboring plasmids except P16 and this may partly explain by that this isolate sensitive to all antibiotics understudy, however, the same pattern was observed with plasmid isolated from P6 and P32, While P40 that was resistant to most tested antibiotics have two plasmids.

Genetic site determination of the antibiotic resistance genes in *P. mirabilis* by genetic transformion

The process of genetic transformation is depended on the ability of laboratory *E. coli* DH5α strain, which have known genotype to be transformed from sensitive strain to resistant one for some antibiotics under study after treating with CaCl2 and exposing to the prepared plasmid DNA from chosen bacterial isolates using heat shock according to the method of (Casali and Preston, 2003). Extracted plasmid DNA from the three isolates (P32, P38 and P40) that represents the most resistant isolates to antibiotics under study which shown in table (3) was plated on LB agar to ensure either it is contaminated or not, after incubation period, there was no growth which means that the extracted plasmid DNA is not contaminated during the extraction process. Table (4) shows *E. coli* DH5α (after comfirming its not proteus that usuually produce swarming) had the ability to receive purified plasmid DNA from *P. mirabilis* isolates and transformed successfully.Ten colonies of *E. coli* DH5α transformants were chosen to be purified on LB agar media. This step is essential to ensure the stability of resistance phenotype to antibiotics in transformed colonies and regular

segregation of these plasmids after purification, and then these colonies were subjected to antibiotic resistance test using Kirby-Bauer method and the main result were recorded in table (4).

Table (3): Antibiotic resistance pattern of tested isolates before transformation

Bacterial Isolates & Laboratory strain	Antibiotics													
	AK*	AMP	AT	C	CEP	CIP	CTR	CTX	FOX	GEN	IPM	NOR	PI	TOB
DH5-α	S	S	S	S	S	S	S	S	S	S	S	S	S	S
P32	R	R	S	R	R	S	R	R	S	R	S	S	R	R
P38	R	R	S	R	R	S	R	R	S	S	S	S	S	R
P40	R	R	S	R	R	R	R	R	R	R	S	R	R	R

Table (4): Antibiotic resistance pattern after transformation of E. coli DH5α with purified plasmid from *P. mirabilis*

Bacterial Isolates & Laboratory strain	Antibiotics													
	AK*	AMP	AT	C	CEP	CIP	CTR	CTX	FOX	GEN	IMP	NOR	PI	TOB
DH5-α	S	S	S	S	S	S	S	S	S	S	S	S	S	S
P32	R	R	S	R	S	S	R	R	S	R	S	S	S	R
P38	S	R	S	R	S	S	S	S	S	S	S	S	S	R
P40	S	R	S	R	R	S	R	R	S	R	S	S	R	R

From the table (4), it's clear that extracted plasmid DNA from P32, P38 and P40 transferred successfully to *E. coli* DH5α strain, in which the transformant colonies of *E. coli* DH5α showed resistance for AK, AMP, C, CTR, CTX, GEN and TOB for P32, for AMP, C, and TOB for P38, for AMP, C, CEP, CTR, CTX, GEN, PI and TOB for P40, respectively. Figure (2) shows the plasmid profile of transformant cells of *E. coli* DH5α strain, after transformation with purified plasmid from P32, P38 and P40 isolates and the results revealed that one plasmid in P32, P38 and both plasmids in P40 had been transformed successfully with molecular weight of more than 10 Kbp.

In the present study, variation in resistance of *E. coli* DH5α transformant colonies to different antibiotics observed, this might be indicated that the antibiotic resistant genes located on different plasmid fragments which were known as r-determinant. However, in general R-plasmids consist of two major fragments, the RTF-Tc which were carrying several numbers of genes specialized for replication process, copy number of plasmid and bear the genes which were responsible for the resistance to tetracycline only, and the r-determinant which contains all genes that were responsible for the resistance and confirmed antibiotics resistance (Clowes, 1972).Also the differences of DH5α transformant colonies after testing its resistance to different antibiotics may be related to the size of plasmid DNA species in the bacterial isolates under study and their entrance to the host depends on their size (Puhler and Timmes, 1984).Furthermore, the genes responsible for conferring resistance to antibiotic used are located on plasmid DNA except for CEP and PI for

P32, for AK, CEP, CTR and CTX for P38, and for AK, CIP, FOX and NOR for P40, which seems to be chromosomally located or on large plasmid in which cannot enter DH5α acting as recipient. Yang et al., (1998) reported that multidrug resistance plasmids may carry genes encoding resistance to other antibiotics such as aminoglycosides. Brown *et al.,* (2003) have reported that horizontal gene transfer is a factor in the occurrence of antibiotic resistance in clinical isolates and suggested that the high prevalence of resistance to a particular antibiotic does not always reflect antibiotic consumption as previously suggested by others (Nwanze *et al.,* 2007).Al-Otraqchi (2006) reported different transforming colonies for *E. coli* isolates and he concluded that all genes responsible for confirming resistance to Ampicillin, Erythromysin, Chloramphenicol, Lincomycin, Nalidixic acid, Teracycline and Gentamicin were located on plasmid DNA, while those confeing resistances to Streptomycin and Trimethoprim might be chromosomally encoded.Khder (2008) performed transformation process for *P. mirabilis* isolated from urine, wound and burn, and revealed that the resistance genes of (Chloramphenicol, Doxicillin, Erythromycin, Gentamycin, Kafalexin, Lincomycin, and Penicillin) in P7 isolate and the resistant genes of all tested antibiotics from P23 isolate are not chromosomally coded.Nanakaly (2013) performed transformation process for uropathogenic *E.coli* and she found that the resistance genes for Ampicillin, Nitrofurantion, Cefotaxime, Amoxcillin-clavulanic acid, Ceftriaxone, Chloramphenicol and Sulfomethaxazole-trimethoprim were located on plasmid DNA for E3, while those for Ciprofloxacin, Cephalothin, Amikacin and Gentamicin seemed to be located on chromosomal DNA. For E25 isolate, the resistance genes for Ampicillin, Nitrofurantoin, Cefotaxime, Amoxcillin-clavulanic acid, Sulfomethaxazole- trimethoprim and Chloramphenicol were located on plasmid DNA and those for Ciprofloxacin, Cephalothin, Amikacin, Gentamycin and Ceftriaxone were located on chromosomal DNA. Also, the resistant genes for Ampicillin, Ceftriaxone, Nitrofurantion and Cefotaxime were located on plasmid DNA for E34 isolate, while the resistance genes for Ciprofloxacin, Amikacin, Cephalothin, Gentamicin, Amoxcillin-clavulanic acid and Sulfomethaxazole-trimethoprim were chromosomal encoded for that isolate.

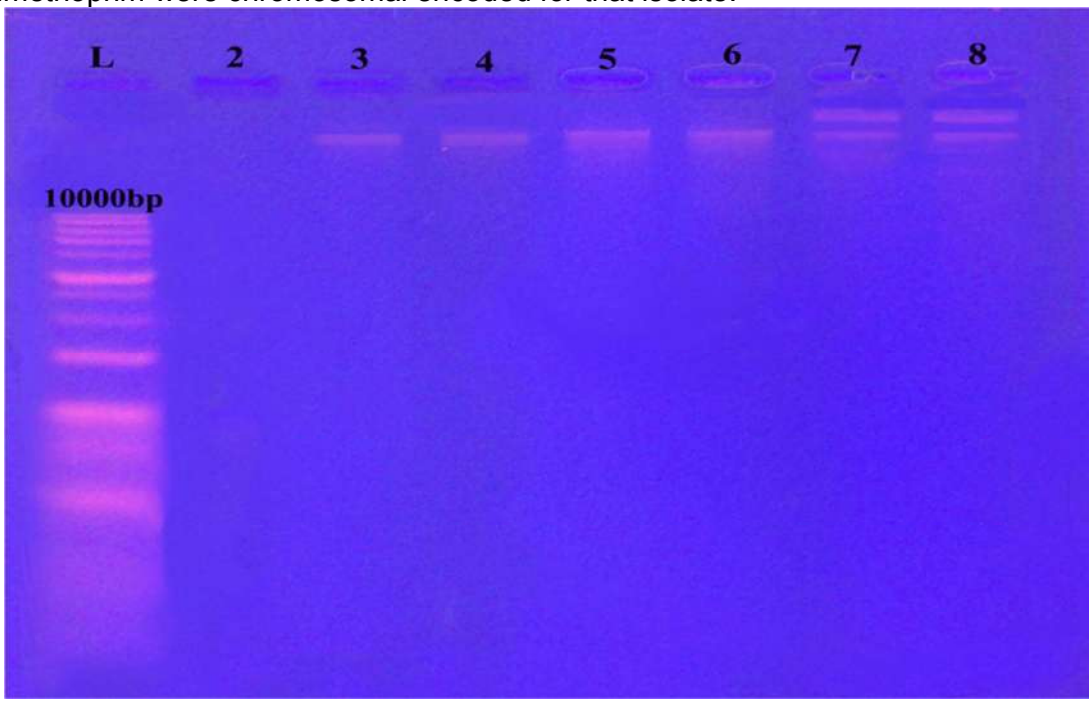

Figure (2): Plasmid profile of transformant colonies

Lane 1 10 000 bp DNA ladder Lane 2 E. coli DH5α laboratory strain
Lane 3 Plasmid content of P32
Lane 4 P32 transformant colonies
Lane 5 Plasmid content of P38
Lane 6 P38 transformant colonies
Lane 7 Plasmid content of P40
Lane 8 P40 transformant colonies

Detection of *UreC*gene among *P. mirabilis* isolates using PCR technique

After extraction of genomic DNA from *P. mirabilis* isolates using (gene JET™ Genomic DNA purification kit) polymerase chain reaction (PCR) and gel electrophoresis were carried out in order to determine the presence of *UreC* gene in the DNA of *P. mirabilis* isolated from patient with UTIs. PCR was performed on a pure DNA template (free from RNA contamination) of each bacteria isolate separately for amplification of *UreC* gene via the use of specified oligonucleotide primers that flanked DNA sequence to be amplified. PCR also needed dNTP which are the four nucleotide triphosphate, and needed thermo-stable polymerase with magnesium ions in the buffer. The reaction was carried out by temperature (denaturation temperature) was applied to separate (melt) each strand of the double helical DNA, then temperature is lowered to let primers anneal to the template and finally the temperature was set at 72 o C which was optimum for the enzyme taq polymerase that extends the primers by incorporating the dNTP. After amplification of *UreC* gene in each bacterial samples separately, PCR products of all bacterial samples were visualized the amplified products by ethidium bromide staining after gel electrophoresis to detect the presence of the gene in the sample. Results in figure (3) indicate that *P. mirabilis* isolates which contain *UreC* gene, exhibit positive PCR products on gel which all of the isolates contained that gene Moreover, the produced band is of 330 bp, which represents the presence of *UreC* gene.

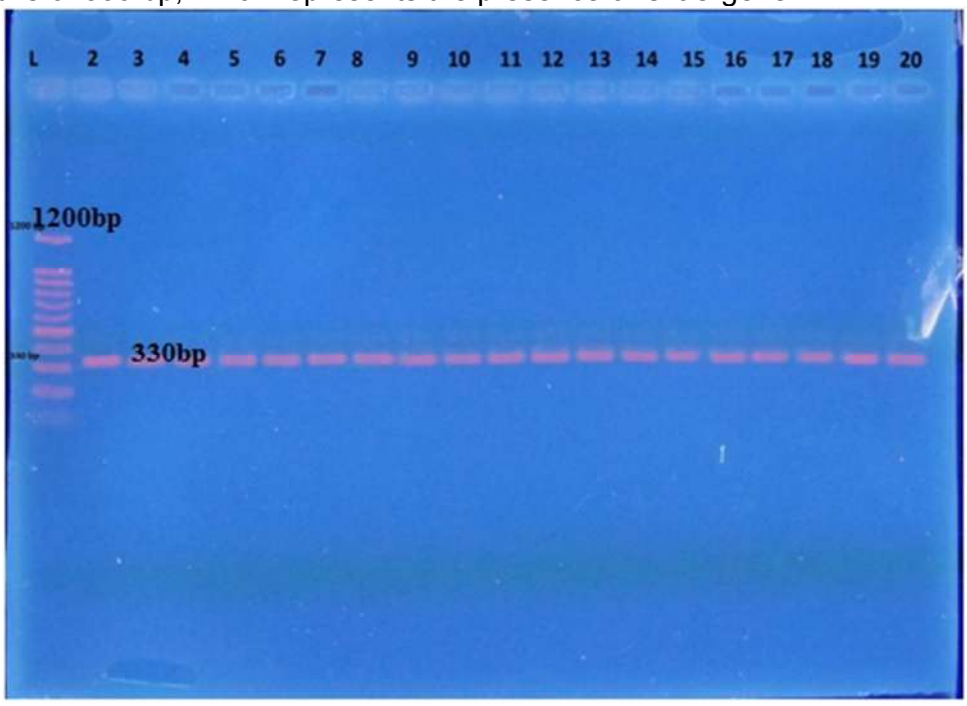

Figure (3): Products of polymerase chain reaction performed with *UreC* Primers and the specified *P. mirabilis* DNA
Lane 1: 100 bp DNA ladder
Lane 2-20: Amplified PCR product of *UreC* gene

All the patients with symptomatic urinary tract infectionh were positive for *ure c* which indicate a good relation between such gene and urinary tract infection. Results of this stydy were similar to previous reports by Stankowska *et al.,* (2008) who performed PCR to confirm the presence of urease gene in twelve *P. mirabilis*. Their results revealed that all isolates contain *UreC* in patient with UTI. The persistence of a *P. mirabilis* infection is compounded by the ability of this organism to cause the formation of urinary stones and encrust indwelling catheters, indeed the formation of stones around the organism can make antibiotic treatment ineffective and stone formation requires urease (Mobley and Hausinger, 1989). Urease is the cytoplasmic nickel-containing enzyme which catalyzes the hydrolysis of urea into carbon dioxide and ammonia and raises the environmental pH, and mediates precipitation of normally soluble polyvalent ions from the urine, specifically precipitation of magnesium, ammonium, phosphate, and calcium ions results in formation of the struvite and carbonate hydroxyapatite crystals that comprise urinary stones (Burall *et al.,* 2004).These results showed that urease has been demonstrated to be a significant virulence factor in *P. mirabilis* isolates. These results are in agreement with other investigations. Mobley and Chippendale (1990), Gendlina et al., (2002), Rozalski et al., (2007), Jones et al., (2007), El- Baghdady et al., (2009) and Al-Dulami et al., (2011) demonstrated that all *P. mirabilis* isolated from different clinical source produced high amount of urease phenotypically compared to other bacteria. There is a concordance between phenotypic and molecular detection of urease activity.The genes encoding *P. mirabilis* urease are *UreDABCEFG.* The active enzyme denoted by *UreABC,* which is activated upon the insertion of nickel ions. *UreC* contains the nickel metallocenter. UreD, UreE, UreF, and UreG are accessory proteins that contribute to assembly of the active complex and insertion of the nickel ion into the metallocenter (Jones and Mobley, 1989; Park et al., 1994 and Mobley et al., 1995). Two regulators of urease transcription have been characterized, *UreR* and H-NS (histone-like nucleoid structuring protein). *UreR* is a member of the AraC family of transcriptional regulators and contains both DNA- and urea-binding domains (Nicholson et al., 1993). The *UreR* gene is transcribed in the opposite direction of *UreDABCEFG;UreR*binds the promoters of *UreR* and *UreD* (D'Orazio et al., 1996). Transcription of the structural genes of urease is urea-inducible (Island and Mobley 1995).*UreR* acts as a positive regulator of urease activity and stimulates expression of the 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* (Poore and Mobley, 2003).

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پوختە

لێکۆڵینەوەیەکی گەردیلەیی و مایکرۆبایلۆجی بۆ بەکتیریای *Proteus mirabilis* جیاکراوە لەهەوکردنی ڕێڕەوەکانی میز لە شاری هەولێر

لە ماوەکانی نێوان 1 ی نیسان تاوەکو 15 ی ئابی ساڵی 2012، چل و پێنج جیاکراوەی بەکتریا *Proteus mirabilis* دەست نیشان کرا لەنێو 600 نموونە کە لە ئەو نەخۆشانە وەرگیرابوون کە هەوکردنی بۆری میزیان هەبوو لە چەند نەخۆشخانەیەکی جیاواز (نەخۆشخانەکانی ڕاپەڕینی منالآن، ڕزگاری، هەولێری فێرکاری، فریاکەوتنی ڕۆژئاوا و ژین) لە شاری هەولێر، جیاکراوەکان دەست نیشان کران لە میز بە بەکارهێنانی سیفاتی کێڵگەیی، تایبەتمەندی ڕوخساری و کارلێکە کیمیا زیندەگیەکان، سەرەڕای ئەوەش، بۆ زیاتر دەست نیشان کردنی جیاکراوەکان سیستەمی ڤایتەک بەکارهێنرا.تاقیکردنەوەی هەستیاری بۆ گشت جیاکراوەکان ئەنجامدرا بە بەکارهێنانی پازدە دژە زیندەیی کە پێکهاتبوون لە ئەمیکاسین، ئەمپسیلین، ئەزتریۆنام، کلۆرامفینکۆڵ، سیفالۆسین، سپرۆفلۆکساسین، سیفتریاکزۆن، سیفۆتاکزیم، فۆسفۆمایسین، جنتامایسین، ئیمیپێنیم، نالیدیکسیك ئەسید، نۆرفلۆکساسین، پاییەرسیلین و تۆبرامایسین. ئیمیپێنیم کاریگەرترین دژە زیندەیی بوو بۆ جیاکراوەکانی *P. mirabilis*. ڕێژەی بەرگری جیاکراوەکان بۆ دژە زیندەییەکان بریتی بوو لە 8.8٪ بۆ سپرۆفلۆکساسین، 11٪ بۆ ئەزتریۆنام و فۆسفۆمایسین، 15.5٪ بۆ نۆرفلۆکساسین، 17.7٪ بۆ تۆبرامایسین، 20٪ بۆ ئەمیکاسین، 22٪ بۆ نۆرفلۆکساسین، 26٪ بۆ سیفتریاکزۆن، 28.8٪ بۆ سیفۆتاکزیم، 33٪ بۆ نالیدیکسیك ئەسید، 35.5٪ بۆ جنتامایسین، 44٪ بۆ کلۆرامفینکۆڵ و 62٪ بۆ ئەمپسیلین.تاقیکردنەوەی ئەنزیمی دڕێژە شەبەنگی بێتا لاکتامی (ESBL) بۆ گشت جیاکراوەکان ئەنجام درا لە ڕێگای تێستی دوو پەپکی هاوکاری (DDST) بە بەکارهێنانی (ئەمۆکسیکلاڤ، ئەزتریۆنام، سیفۆتاکزیم و سیفتازیدیم). لە هەر 45 جیاکراوەکەی *P. mirabilis*, 12 (26.6٪) دروستکەری ئەنزیمی بیتالاکتامن، بەڵام 33 (73.3٪) تونای دروست کرنی ئەم ئەنزیمەیان نەبوو. پرۆفایلی پلازمید هەڵبژێردرا بۆ سێ جیاکراوە (P6, P32 and P40) کە بەرزترین بەرگریان نیشاندا بۆ زۆربەی دژە زیندەییەکان و جیاکراوەیەك (P16) کەهەستیاربو بۆ گشت دژە زیندەییەکان بە بەکارهێنانی gel electrophoresis، بەرەنجامەکان ڕوونیان کردەوە کە دوو جیاکراوە تەنها یەك بلازمیدیان هەبوو بەڵام ئەویتریان دوو بلازمیدی هەبوو کە بارستایی گەردییەکەیان زیاتربو لە 10 کیلۆ تفتی دووانی،بەلام جیاکراوە P16هیچ بلازمیدی تیدانەبوو.سێ جیاکراوە (P38، P32، و P40) هەڵبژێردران کە بەرزترین بەرگریان نیشاندا بۆ زۆربەی دژە زیندەییەکان بۆ کرداری گواستنەوە و دیاریکردنی شوێنی جینی بەرپرس لە بەرگری دژە زیندەییەکان، وەچەی تاقیگەیی *Escherichia coli* DH5-α لەگەڵ پوختەکراوی پلازمیدی ترشە ناوکی کەم ئۆکسجین لەو سێ جیاکراوە بەکارهێنرا. کرداری گواستنەوە بە سەرکەوتووی ئەنجامدرا و ئەنجامەکانیش پیشانیاندا کەوا جینی بەرگر بۆ ئەمیکاسین، ئەمپسیلین، کلۆرامفینکۆل، سیفتریاکزۆن، سیفۆتاکزیم، جنتامایسین، نالیدیکسیك ئەسید و تۆبرامایسین کەوتوونەتە سەر پلازمید بۆ جیاکراوەی P32، بەڵام بۆ جیاکراوەی P38، جینی بەرگر بۆ ئەمپسیلین، کلۆرامفینکۆڵ،جنتامایسین، نالیدیکسیك ئەسید، پاییەرسیلین و تۆبرامایسین کەوتوبووە سەر پلازمید. لەلایەکی ترەوە، جینی بەرگر بۆ ئەمپسیلین، کلۆرامفینکۆڵ، سیفالۆسین، سیفتریاکزۆن، سیفۆتاکزیم، جنتامایسین، نالیدیکسیك ئەسید، پاییەرسیلین و تۆبرامایسین کەوتبوە سەر پلازمید بۆ جیاکراوەی P40. هەموو جیاکراوەکانی*P. mirabilis*پشکنێران بۆ بوونی جینی بەرپرس لە ئەنزیمی شیکەرەوەی یۆریا (*UreC*)لەسەر جینۆمی ترشە ناوکی کەم ئۆکسجین بە بەکارهێنانی تەکنیکی PCR. بەرەنجامەکان ڕوونیان کردەوە کە هەموو جیاکراوەکانئەنزیمی شیکەرەوەی یۆریایان هەبوو.

الخلاصة

دراسة جزيئية وضد حياتية لبكتيريا *proteus mirabilis* المعزولة من التهاب المجارى البولية

خلال الفترة من 1 أبريل الى 15 أغسطس لعام 2012، تم تشخيص خمسة واربعون من العزلات *Proteus mirabilis* من بين 600 عينات من المرضى الذين يعانون من التهاب المسالك البولية من مستشفيات مختلفة (مستشفى راثةرين للأطفال، رزطارى، هولير التعليمية، الطوارئ الغربي و ذين) في مدينة اربيل، تم تشخيص العزلات من البول باستخدام الخصائص المزرعية، المورفولوجية والبيوكيميائية .وعلاوة على ذلك، فإن هوية من العزلات أكده من خلال النظام الفايتك.وقد تم اختبار الحساسية للمضادات الحيوية لجميع العزلات باستخدام المضادات الحيوية التي كانت خمسة عشر وتضمنت (أميكاسين، الأمبيسلين، ازتروماناام، كلورامفينيكول، سيفالوثين، سيبروفلوكساسين، سيفترياكسون، سيفوتاكسيم، فوسفوميسين، جنتاميسين، الإميبينيم، وحامض نالديكسيك، النورفلوكساسين، بايبرسيلين، وتوبراميسين. كان IMPالمضاد الحيوي الأكثر فعالية ضد عزلات *P. mirabilis* ، وكان معدل مقاومة العزلات تجاه هذه المضادات الحيوية 8.8٪ لـ CIP، و 11٪ لـ AT ، 11٪ FOX J، و 15.5٪ لـ NOR، 17.7٪ لـ TOB، 20٪ لـ AK، 22٪ لـ NOR، 26٪ لـ CTR، 28.8٪ لـ CTX، 33٪ لـ NA، 35.5٪ لـ GEN، 44٪ لـ و 62٪ لـAMP.اجربت اختيار انتاج انزيم بيتا لاكتميز واسعة الطيف باستعمال اختيار مزدوج القرص التآزر (DDST) باستخدام (الأمبيسلين+حامض كلافيولونك، ازتروماناام ، سيفوتاكسيم و، سيفتازيديم). ففي جميع خمسة و اربعون العزلة *P. mirabilis* ، كانت 12 (26.6٪) منتجة لنزيم ESBL ، بينما 33 (73.3٪) كانوا غير منتجين لـ ESBL.تم دراسة المحتوي البلازميدي لثلاث عزلات وهى P16, P32, P40و التي أظهرت مقاومة عالية لمعظم المضادات الحيوية وعزلة رقم P16 والتى كانت حساسة لجميع المضادات باستخدام الترحيل الكهربائي للهلام، وكشفت النتائج أن اثنين من العزلات لها حزمة واحد والعزلة الثالثة لها حزمتين مع وزن جزيئي اكثر من 10Kbفي حين لم تحتوي عزلة p16 اية بلازميدات.وقد تم اختيار ثلاث عزلات (32، 38 و 40) والتي أظهرت مقاومة عالية لمعظم المضادات الحيوية لعملية التحول وتحديد مكان وجود الجينات المقاومة للمضادات الحيوية، وذلك باستخدام سلالة مختبرية *Escherichia coli* DH5-α مع الحمض النووي البلازميد المنقية لهذه العزلات .أجريت عملية التحول بنجاح، وأظهرت النتائج أن الجينات المقاومة لـ (أميكاسين, الأمبيسلين، كلورامفينيكول، سيفترياكسون، سيفوتاكسيم، جنتاميسين, حامض نالديكسيك وتوبراميسين) كانت موجودة على البلازميد لعزلة 32، في حين العزلة 38، فإن الجينات المقاومة ضد (الأمبيسلين، كلورامفينيكول، ، جنتاميسين, حامض نالديكسيك، بايبرسيلين وتوبراميسين) تقع على البلازميد. من ناحية أخرى تم تحديد موقع الجينات المقاومة لـ (الأمبيسلين، كلورامفينيكول، سيفالوثين ، سيفترياكسون، سيفوتاكسيم, ، جنتاميسين, حامض نالديكسيك, بايبرسيلين وتوبراميسين) على البلازميد لعزلة 40 وكانت واقعة علي البلازميد.تم فحص جميع العزلات *P. mirabilis* لوجود الجين اليورياز (*UreC*) على الحمض النووي باستخدام تفاعل البلمرة المتسلسل (PCR) مقايسة .وكانت جميع العزلات موجبه لوجود جين *UreC* الفوعة.