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MOLECULAR DETECTION OF UREASE AND HEMOLYSINE GENES IN PROTEUS MIRABILIS BACTERIA ISOLATED FROM URINE OF RHEUMATOID ARTHRITIS PATIENTS IN NAJAF PROVINCE

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Abstract: *P. mirabilis* bacteria have a significant role in causing urinary tract infection which can be defined as the inflammatory response of urothelium to bacterial invasion, which is usually associated with bacteriuria and pyuria. According to the suggestion that *Proteus* microbes could possibly be involved in the etiopathogenesis. *Proteus mirabilis* was the commonest bacteria isolated in this study 15 (51%) followed by *E. coli* in 6 (20.4), *Klibsiella spp*.5 (17%) and *Staphylococcus saprophiticus* 3 (10.3%). All isolates of ***P. mirabilis*** appeared highest rate of resistance (100%) in the case of Cefotaxime, Amoxicillin, Ampicillin. And moderately resistance to Netilmicin (71.4%). All isolates presented high sensitivity (100%) to Meropenem, Levofloxacin, and Ciprofloxacin. All isolates15 (100%) appeared the presence of urease and hemolysine genes by using PCR technique.

Keywords: Molecular, Detection, Arthritis Patients

Introduction

Proteus mirabilis is a Gram-negative, facultative anaerobic rod shaped bacterium. It may found as part of the normal flora in intestine. This organism is not usually a pathogen, but does become a problematic when it comes into contact with urea in the urinary tract, So that, the infection can spread to the other parts of the body (AL-Hamadani et al ., 202). This bacterium is well-known for its urease enzyme production and distinctive ability to differentiate into elongated swarm cells. *P. mirabilis* bacteria have a significant role in causing urinary tract

infection which can be defined as the inflammatory response of urothelium to bacterial invasion, which is usually associated with bacteriuria and pyuria (Hussien,2016). Since Proteus mirabilis had many virulence factors that were important for inflicting UTIs, these factors had an importance role to make an infection in different areas of the urinary tract (Stankowska et al., 2008), including toxins like hemolysin and its function of pore formation, biofilm and regulation of the pathogenicity (Schaffer &Pearson,2015). Urease enzyme which causes kidney and bladder stones (Al-Duliami et al., 2011). Rheumatoid arthritis RA is one of the more common autoimmune diseases, affecting approximately 1% of the population worldwide. The exact cause of RA is not known; however, initiation of disease seems to cause by an interaction among genetic susceptibility, environmental triggers, and chance. Different immunological and microbiological studies results support that there could be a link between urinary tract infections (UTI) mainly caused by *P.mirabilis* bacterium and RA (Gibofsky et al.,2014).

Infectious agents have been suspected as potential triggers of RA for a long time, and the search is still in progress. The suggestion that *Proteus* microbes could possibly be involved in the etiopathogenesis of RA and large number of studies have shown that antibodies against *P. mirabilis* were significantly elevated among RA patients but not in healthy control (Ebringer et al., 2003). Furthermore, in an earlier study increased antibodies to *P. mirabilis* only, but not to four viruses; *influenza, adenovirus, rubella* and *parvo- virus,* and autoantigens were observed in RA patients in comparison to healthy controls. (Deighton et al., 1992) .The association of RFs with increased *anti-Proteus* antibody titres in patients with RA could be.explained on the basis that these anti-IgG auto-antibodies are produced secondarily as the result of B cells stimulation by exogenous and microbial antigens. RFs, however, are more likely to be involved in immune complex-mediated damage in RA, especially in those patients with associated 'vasculitis possibly through activation of the complement system. (. Kato ,2000).

This study was aimed to detect of two important virulence genes (UreC& HpmA) from *Proteus mirabilis* isolated from urine of RA patients.

The Objective of this study include:

1. Isolation and identification of *Proteus mirabilis* bacteria from urine of RA patients by using convention methods and vitek system.
2. Phenotypic detection of *Proteus mirabilis* some virulence factors.
3. Detection of antibiotic susceptibility of *Proteus mirabilis* isolates to the most 7 antibiotic used for treatment
4. Molecular detection of urease (*UreC,*) and hemolysin (*HpmA*) genes as important virulence genes in in *Proteus mirabilis* isolated from RA patients by using PCR technique.

2.1 General characteristic of *Proteus mirabilis*

Proteus mirabilis is a member of the Enterobacteriaceae family and is a highly motile bacterium. Unlike the other members of Enterobacteriaceae, new phylogenetic tree classification based on shared core proteins, ribosomal proteins, and four multilocus sequence analysis proteins placing *Proteus* within a new *Morganellaceae* family (Adeolu *et al.*, 2016).

Proteus bacilli are dimorphic bacteria. when grown in aliquid medium, the cells display swimming behavior and have a distinct morphology; i.e., they are motile, peritrichous flagellated(6 to 10 flagella per cell) rods, 1.0 to 2.0 mm in length.These bacilli, referred to as

swimmer cells, are similar in manyaspects of their physiology to other members of the family Enterobacteriaceae. When transferred to a solid medium, Proteus bacilli undergo morphogenesis to swarmer cells and swarm over the surface of solid medium. This kind of growth of Proteus rods on solidified nutrient medium is termed the swarming phenomenon (Eberl *et al.*, 1999).

P. mirabilis is well known for its ability to produce urease, which generates ammonia and elevates the pH of the urine to >7.2.5 Calcium and magnesium crystallization in the urine of alkaline pH blocks the catheter lumen and causes acute urinary retention and the development of bacteriuria and other ascending infections6

P. mirabilis can be found in a wide variety of environments, including soil, water sources, and sewage, but it is predominantly a commensal of the gastrointestinal tracts of humans and animals (2, 3). While the bacterium is capable of causing a variety of human infections, including those of wounds, the eye, the gastrointestinal tract, and the urinary tract, it is most noted for infections of the catheterized urinary tract, known as catheter-associated urinary tract infections (Sanmarti et al., 2009)

P. mirabilis is not a common pathogen that causes urinary tract infections (UTIs) in normal hosts(Stankowsks et al., 2008). In contrast, P. mirabilis is isolated relatively frequently in complicated UTIs, such as those that present in patients with functional or anatomical abnormalities, especially patients with urolithiasis or a chronic indwelling urinary catheter(Cestari et al., 2013).

2.3 pathogenesis and virulence factors

The pathogenic virulence factors of Proteus has been includ fimbriae; flagella; enzymes: urease, (hydrolyzing urea to CO2 and NH3); proteases degrading antibodies, deaminase amino acid; tissue matrix proteins and proteins of complement system; iron acqusition systems and toxins: hemolysins, Proteus toxin agglutinin (Pta), and lipopolysaccharide (LPS) endotoxin (Nielubowicz &Mobley2010).

The hemolytic activity produced by P. mirabilis is associated to hemolysin HpmA. This hemolysin is associated to the cell, calcium- independent, former of pores, encoded by two genes, hpmA and hpmB, that regulate the HpmA (166 kDa) and HpmB(63 kDa) proteins, respectively. HpmA hemolysin is responsible for tissue damage and is activated when its N-terminal peptide is cleaved, resulting in active HpmA(140 kDa). HpmB is responsible for HpmA activation and transport .

Clearly, *P. mirabilis* possesses an impressive arsenal of virulence factors (Fig. 1). Urease is a critical feature of this species, but the bacterium also expresses a startling number of fimbriae and other adhesins. The most well-studied fimbria is the mannose- resistant *Proteus*-like (MR/P) fimbria, whose expression is phase variable. As well, a variety of potent toxins and proteas es compound virulence. Similar to other members of the *Enterobacteriaceae*, *P. mirabilis* carries numerous secretion systems, including types I, III, IV, V, and VI.

The important virulence factor of *Proteus mirabilis* includes

1. Adhesions

   Attachment to the target cell may be the first essential step in the establishment of any pathogen in the host. Fimbria-mediated adherence has been suggested to contribute to pathogenesis of Proteus spp. Proteus mirabilis 's bacterial adhesion is a key step in colonizing and developing infections, which is done by fimbriae (Hasan,2020). This bacteria attaches to and swarms across the surface of urinary catheters to gain a foothold

in the urinary tract (Jones et al., 2005).

fimbriae are surface appendages sometimes called pili which are shorter and finer than flagella and they are composed of structural protein subunits termed pillins. There are two classes of pili: ordinary pili which play a role in the adherence to host cells and sex pili which are responsible for the attachment of bacterial cells to each other during conjugation(Brooks et al., 2007)).

There are five common fimbriae type that implicated in infection as following:

A. **Mannose-resistant / Proteus-like (MR/P) fimbriae:** This kind of fimbria is the most appropriate form in Proteus mirabilis**B.**

B. **B.Uroepithelial cell adhesion (UCA/NAF) fimbriae:**

This type of fimbriae is taking the form of long flexible rods, the UCA/NAF fimbriae have an essential adhesion role to the uroepithelial cells also it has an important role in the colonization of the urinary tract (Jiang et al.,2018).

C. Ambient-temperature fimbriae (ATF): In the ambient way of Proteus mirabilis, the ATF fimbriae are important (Hasan, 2020b).

D. Proteus mirabilis fimbriae (PMF):

The role of PMF fimbriae that shown is in old studies is introducing in the bacterial cells colonizing of urinary tract lower part but not the kidneys, recently, the studies proved that the role of PMF fimbriae is vital in the colonization of bacterial cells to bladder and kidneys (Sarshar et al.,2020).

E. Proteus mirabilis P-like pili (PMP) fimbriae: PMP fimbriae were first identified from a dog with urinary infection by Proteus mirabilis strain (Debnath et al.,2018).

1. Motility

The motility is the most important virulence factor in Proteus mirabilis that influence in invading and spreading of infection in urinary tract parts (Kuan et al.2014).The infection begins with the invasion of the periurethral region, then it travels through the urethra and progresses to the bladder, other areas of the urinary tract (Hickling et al.,2017).

The presence of flagella on the surface of pathogenic and opportunistic bacteria has been thought to facilitate the colonization and dissemination from the initial site (Shirtliff and Leid, 2009).

Swarming is a multicellular differentiation phenomenon that allows a population of bacteria to migrate on a solid surface in a coordinate manner. It is important in movement of Proteus species to new locations and most probably helps them in the colonization of microorganisms. (Verstraeten et al., 2008).

2. Toxins and Enzymes

- Hemolysin: Hemolysin is a toxin that enters the target membrane of the eukaryotic cell and causes pores that trigger ion efflux and then cell disruption (Cabezas et al.,2017). Hemolysin promotes bacterial infection spread in the kidney and pyelonephritis develops in

ascending UTIs (Los et al.,2013).Proteus mirabilis hemolysin genes are a two-part secretion (hpmA and hpmB) (Etxaniz et al.,2020).

-Proteus toxic agglutinin (Pta): Proteus toxic agglutinin is a protein that is designated as the outer membrane autotransporter that mediates aggregation of the cell and includes the catalytic α-domain that can lyse the kidney and bladder cells (Gupta et al.,2019). The Proteus mirabilis negative pta gene had reduced pathology, as well as a serious

colonization defect in urine, kidneys, and spleen (Engel et al.,2007).

B. Urease: Urease is significant in Proteus mirabilis pathogenesis, which catalyzes stones formation in the kidney and bladder or inhibits the urinary tract (Flannery et al.,2009). Urease is extremely important for Proteus mirabilis pathogenesis (Ranjbar et al.,2015). This enzyme catalyzes the formation of stones in the kidney and bladder or encrusts or obstructs the urinary tract (Armbruster et al.,2017).The cluster of urease genes (ureRDABCEFG) codifies the multimeric nickel metallo enzyme, which hydrolyzes urea into ammonia and dioxide, increases the PH, and induces multipurpose urinary ions.

Methods

2.1 Sample collection

A total of 50 urine samples were collected from patients with RA. Samples were collected from patients during the period from October 2021to the end of March 2022 From the Medicine City/Al-Sadder Teaching Hospital in AL-Najaf/Iraq. The urine of all patients were cultured on different media colony morphology (blood and macckongy agar) , biochemical tests including; catalase test, oxidase reaction and urease test were used for more identification.

2.2 Preparation of Buffer and Chemical solutions:

2.2.1 McFarland‟s Standard Solution No.0.5 (1.5 X 10^8) cell/ml The 0.5 McFarland standard tube was prepared by adding 0.5 ml of a 1.175% (wt/vol) barium chloride dihydrate ($BaCl_2.2H_2O$) solution to 99.5 ml of 1% (vol/vol) H_2SO_4. The turbidity standard was liquated into test tubes identical to those used to prepare the inoculum suspension. The McFarland standard tubes were sealed with parafilm to prevent evaporation and stored for up to 6 months in darkness at room temperature. A ccuracy of the density of a prepared 0.5 McFarland standard was checked by using a spectrophotometer.

Results and Discussion

Out of of 50 urine sample were collected from patient (39 female and 11 male)with UTI and RA that admitted to Medicine City/Al-Sadder Teaching Hospital in Najaf/Iraq during the period from October 2021 to the end of March 2022 from both gender with age ranged from 30-70 years old. *Proteus mirabilis* was the commonest bacteria isolated in this study 15 (51%) followed by *E. coli* in 6 (20.4) , *Klibsiella spp*.5 (17%) and *Staphylococcus saprophiticus* 3 (10.3%).

All *P.mirabilis* colonies gave biochemical result as in table (4.2). swarming motility on blood agar, and inability to metabolize lactose (on MacConkey agar plate) (Fig. 4.1). Also P. *mirabilis* produces a very distinct fishy odor. in another hand all 15 isolates gave negative result for oxidase, catalase and positive results for urease test.

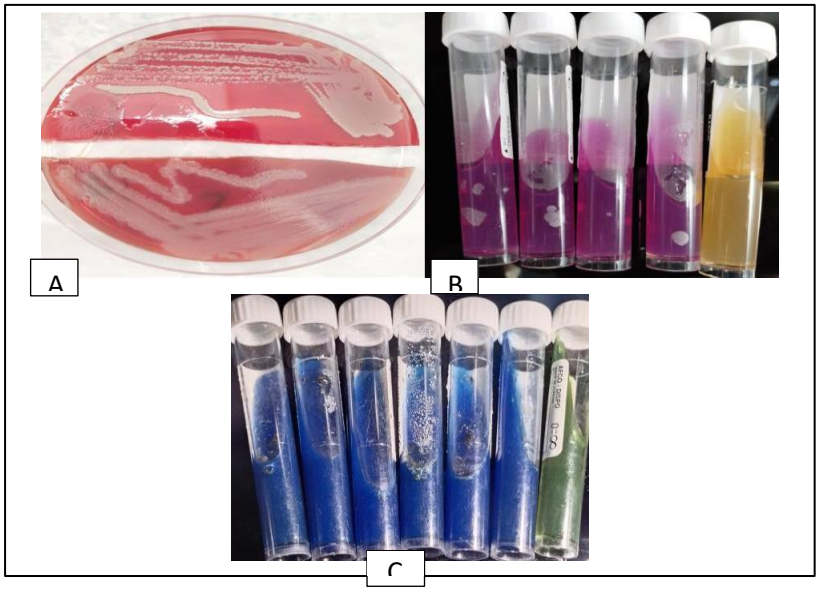
Table (4.1): Distribution of bacteria isolated from urine of RA patients

Bacterial isolates	No.	%
Proteus mirabilis	15	51

Escherichia coli	6	20. 4
Klebsiella spp.	5	17
Staphylococcus *Saprophiticus*	3	10. 3

Table(4.2) Biochemical result of *Proteus mirabilis* isolated in this study

Test	Result
Oxidase	-
Catalse	-
Urease	+
Indole production	
Methyl red	
Voges-Proskauer	
Simmons Citrate	+
H2S production	+
Motility	+
Kligler agar	



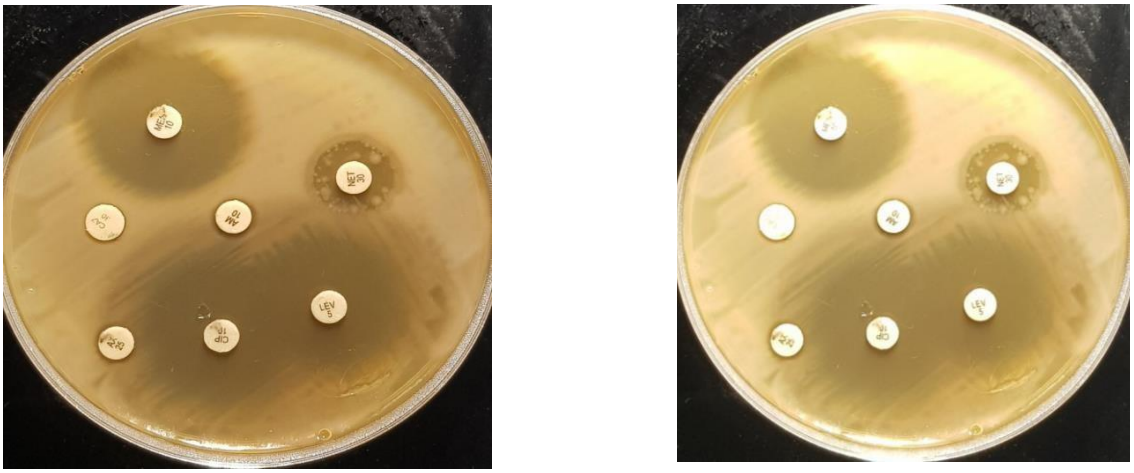
Figure(4.1):A- *P. mirabilis* on blood agar B-urease production

4.3 Antibiotic susceptibility of P. mirabilis

Table (4-3) show the resistance of *Proteus mirabilis* to 7 antimicrobial agents by using Kirby-Bauer disk diffusion method (Bauer et al., 1966). The results were interpreted according to the

diameter of inhibition zones and compared with inhibition zones determined by CLSI (2021), and to decide the susceptibility of bacteria to antimicrobial agent whether being resistant.

In this study *P. mirabilis* showed a different susceptibility towards antibiotics. All isolates of *P. mirabilis* appeared highest rate of resistance (100%) in the case of Cefotaxime, Amoxicillin, Ampicillin. And moderately resistance to Netilmicin (71.4%). All isolates presented high sensitivity (100%) to Meropenem , Levofloxacin, and , Ciprofloxacin as in figure (4.1) and table (4.3)

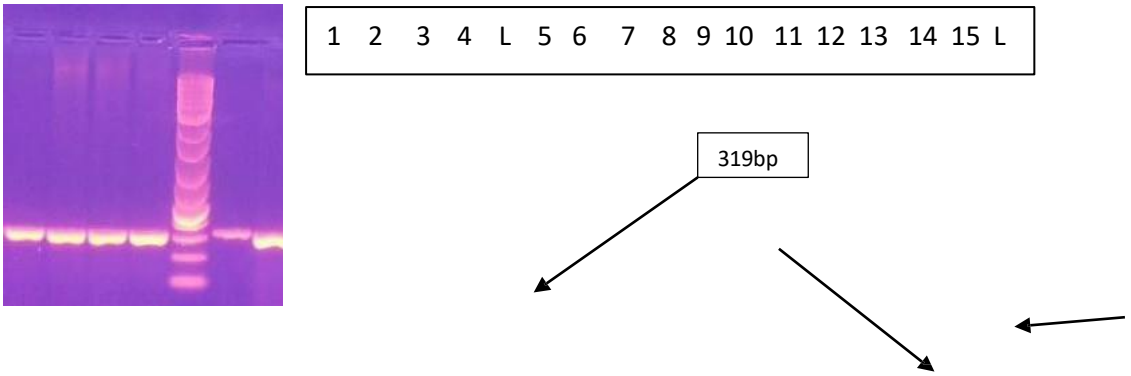


Figure(4.1): Antibiotic susceptibility of P.mirabilis to 7 different antibiotic.

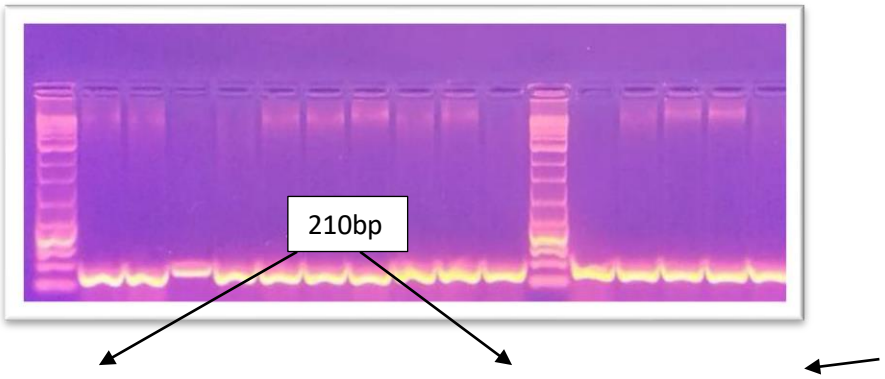
Table (4.3): Antibiotic susceptibility of P.mirabilis to 7 different antibiotic.

Antibiotics	Symbole	Resistance NO.(%)	Sensitivity No.(%)
Meropenem	MEM	0	15(100%)
Levofloxacin	LEV	0	15(100%)
Ciprofloxacin	CIP	0	15(100%)
Netilmicin	NET	4(26.6%)	11(71.4%)
Cefotaxime	CTX	15(100%)	0
Amoxicillin	AX	15(100%)	0
Ampicillin	Am	15(100%)	0

4.3 PCR detection of hemolysine and urease genes



Figure(4.3): **PCR amplified products of *urease* gene of the *P. mirabilis* using the primers with expected size 319bp.**
Lane (L), DNA marker (100bp ladder) .
Lane (1,2 ,3,4,5,6,7,8,9,10 ,11,12, 13,14,15) No. of amplify of *urease* gene



Figure(4.4): **PCR amplified products of *hemolysin* gene of the *P. mirabilis* using the primers with expected size 210bp.**
Lane (L), DNA marker (100bp ladder) .
Lane (1,2 ,3,4,5,6,7,8,9,10 ,11,12, 13,14,15) No. of amplify of *hemolysin* gene

Figure (4.3)and (4.4)shown that all isolates15 (100%) appeared the presence of urease and hemolysine genes. α-hemolysin is responsible for damaging tissue and activating when its N-terminal peptide has cleaved. Based on the results of this study carried out in relation to Proteus microbes (figure4.3, 4.4) .

Molecular similarity was observed between the "EQKlRRAA" amino acid sequences present in the RA-associated HLA-DR molecules and the "ESRRAL" amino acid motif present in hemolysins of

P. mirabilis. (Gibofsky et al.,2014) Subsequently, another molecular homology was discovered between the "IRRET" pentapeptide present in Proteus urease and the "LRREI" amino acid sequences found in type XI collagen, which is a component of hyaline car- tilage and present predominantly in the small joints. Reciprocal immunological cross-reactivity has been demonstrated between Proteus and HLA-DR4 pep- tides, (Wilson et al., 1995) and elevated antibody levels against the synthetic peptides from these cross-reactive mole- cules were found among RA patients from England, Japan and Norway(Tiwana et al.,1999). It could

be said that compelling evidence exists linking this microbe to RA, starting with recurrent sub-clinical Proteus UTIs and ending in the full development of RA. To prove the scientific logic of this possibility, many blood tests was done including (ESR, CRP, RF and AcpA) all of these tests was high in the patients whom their urine are having P. mirabilis bacteria. A meta- analysis has shown that the pooled sensitivities of ACPA and RF are similar, but ACPA positivity is more specific for RA than IgM RF, IgG RF, or IgA RF positivity Furthermore, the "shared epitope" EQRRAA and type XI collagen sequence LRREI each contain an arginine doublet which could be acted upon by (PAD) enzymes during inflammatory episodes and to produce further quantities of CCPs. There is thus a clear link between Proteus bacteria and the presence of AcpA in the early stages of RA (Schaffer et al,2015).

Conclusion

In this study concluded that *P. mirabilis* was the commonest bacteria isolated from urine of RA patients. All *P. mirabilis* isolates have higher sensitive to Meropenem, Levofloxacin, and Ciprofloxacin and higher resistant to Cefotaxime, Amoxicillin, mpicillin. And moderately resistance to Netilmicin. All isolates presented urease and hemolysine genes.

References

[1] Adeolu, M.; S. Alnajar,; S. Naushad, and R . S. Gupta(2016). Genomebased phylogeny and taxonomy of the „Enterobacteriales": proposal for Enterobacterales ord. nov. divided into the families terobacteriaceae, Erwiniaceae fam. nov., Pectobacteriaceae fam. nov., Yersiniaceae fam. nov., Hafniaceae fam. nov., Morganellaceae fam. nov., and Budviciaceae fam. nov. Int J Syst Evol Microbiol 66:5575– 5599

[2] Al-Duliami, A.; N.G..Nauman,; A. S.Hasan, and Z. H. Al-Azawi, 2011.Virulence factors of Proteus mirabilis isolated from patients Otitis media in Baquba and its Peripheries. Diyala. J Med;1(1):69-75

[3] Badi, S. A., J. Nowroozi, and A. A. Sepahi,2014. Detection luxS, qseC and rsbA genes' band in Proteus mirabilis and Escherichia coli isolated frozm urinary tract infections. pajoohandehjournal.19(3):142-147

[4] Brause, B. D. 2003. microbes and antibiotics. In: the adult knee. Callaghan jj, Rosenberg AG, Rubash HE, Simonian PT,Wickiewicz tl (ed). Vol 11. Lippencott Williams and Wilkins, Philadilphia, 1493-1499

[5] Cestari, S. E.; M. S.Ludovico,; F. H.Martins,. S.P.D.; Rocha, W.P. Elias, and J.S. elayo, 2013. Molecular Detection of HpmA and HlyA Hemolysin of Uropathogenic Proteus mirabilis. Curr Microbiol., 67:703–

[6] D"Orazio, S. E.; V. Thomas, and C. M. Collins, 1996. Activation of transcription at divergent urea-dependent promoters by the urease gene regulator UreR. Mol Microbiol, 21(3):643-55.

[7] Dattelbaum, D. J.; V. C. Lockatell,; E.D.Johnson, and T. L. Mobley 2003. UreR, the transcriptional activator of the Proteus irabilis urease gene cluster, is required for urease activity and virulence in experimental urinary tract infection. J Infect Immun, 71:1026-30

[8] Gibofsky, A.; MD; JD; FACP and FCLM,2014. Epidemiology, Pathophysiology, and Diagnosis of Rheumatoid Arthritis: A Synopsis. The American journal of managed care

.vol. 20, NO.70

[9] Henderson, I. R.; F.; Navarro-Garcia, M.; Desvaux, R. C. Fernandez, and D. Ala'Aldeen, 2004. Type V protein secretion pathway: the utotransporter story. Microbiology and molecular biology reviews, 68(4): 692-744

[10] Hussein, S. 2016. Biochemical and Genetic Study of Virulence Factor Hemolysin of Proteus mirabilis Isolated from Urinary Tract Infection

[11] Liaw, S.; H. Lai, and W. Wang, 2004. Modulation of Swarming and Virulence by Fatty Acids through the RsbA Protein in Proteus mirabilis Infection and Immunity,Dec. 72(12): 6836–6845

[12] Manos, J. and R. Belas, 2006. The Genera Proteus, Providencia, and Morganella. Prokaryotes. 6:245–269.

[13] Sanmarti, R.; E.; Graell, M. L..Perez,; G.; Ercilla, O.; Vinas, J. A. Gomez-Puerta, et al.2009. Diagnostic and prognostic value of antibodies against chimeric fibrin/filaggrin citrullinated synthetic peptides in rheumatoid arthritis. Arthritis Res Ther, 11:135

[14] Schaffer, J. N.; and M. M. Pearson, 2015. Proteus mirabilis and urinary tract infections. Microbiology spectrum, 3(5).

[15] Sergio P.; R S.; ocha1; Jacinta, Pelayo and P. Waldir, Elias, 2007. Fimbriae of uropathogenicProteus mirabilis. FEMS Immunol Med Microbiol (51) 1–7

[16] Sosa, V.; G. Schlapp, and P. Zunino, 2006.Proteus mirabilis isolates of different origins do not show correlation with virulence attributes and can colonize the urinary tract of mice. Microbiology. 152, 2149–2157

[17] **MaccFadin**,J.K. (2000). Biochemical test for identification of medical bacteria. (3rd ed .). Lippincott Williams and Winkins . Awolter Klumer Company . Philadelphia Baltimor .New York.

[18] Tiwana H, Wilson C, Alvarez A, Abuknesha R, Bansal S, Ebringer A. Cross-reactivity between the rheumatoid arthritis- associated motif EQKRAA and structurally related sequences found in Proteus mirabilis. Infect Immun 1999;67:2769-75.

[19] Takeuchi F, Kosuge E, Matsuta K, Nakano K, unaga , Juji T, et a!. Antibody to a specific HLA-DR~1 sequence in Japanese patients with rheumatoid arthritis. Arthritis Rheum 1990;33:1867-8.

[20] Dybwad A, Forre 0, Sioud M. Increased serum and synovial fluid antibodies to immunoselected peptides in patients with rheumatoid arthritis. Ann Rheum Dis 1996;55:437-41.

[21] **Brook,** G., Carroll, K., Butel, J. and Morse, S. (2010). Jawetz, melnik and aldelbergs. Medical Microbiology 25th ed. Lang Medical books McGraw-Hill Companies, USA.

[22] **Clinical and Laboratory Standards Institute.**(2020). Performance standards for antimicrobial susceptibility testing; Twenty-First Informational Supplement . CLSI document M02-A10 and M07-A8. Clinical and Laboratory Standards Institute, Wayne, PA.

[23] **Bauer**, A.W.; Kirby, W.M.M.; Sherris, j.s.; and Turk, M. (1966).Antibiotic susceptibility testing by a standardized single disk method .