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Prevalence of *Proteus mirabilis* in Clinical Samples of UTI Patients with Rheumatoid Arthritis, Molecular Screening and Antibiotic Susceptibility Tests in Association with Interleukin-8 Analysis .

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Abstract: Rheumatoid arthritis (RA) is one of the more common autoimmune diseases, affecting individuals of middle age groups 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) and RA, the occurrence is found in females more than males, which mainly caused by microorganisms especially Gram-negative *P.mirabilis* bacterium aetiopathogenesis of RA which activate inflammation and production of cytokines and chemokines as IL-8 an important biomarker of the inflammatory immune response to pathogens. A total of 116 urine samples were collected from urinary tract infection patients with RA from different hospitals in Baghdad (Baghdad Teaching Hospital/ Medical City and Al-Nuaman Teaching Hospital) at a period of study from beginning of September 2023 to the end of March 2024. The clinical urine samples which collected were cultured on blood agar and MacConkey agar. Six of the specimen were identified as *P.mirabilis,* but only five isolated was confirmed depend on the automated VITEK2 system used for farther identification. Molecular methods using PCR technique targeting *16SrRNA*, the results indicated that only 5 isolates was diagnosed as *Proteus mirabilis*, further diagnosis using *UreC* gene was done, the results found that out of 6 samples only 2 were positive for identification *UreC.* The *P. mirabilis* samples were also characterized for antibiotic resistance and the results showed that *Proteus mirabilis* isolates1,2,3 and5 resists to 6 different antibiotics(Ampicilline/Sulbactum, Cefotaxime, Gentamicin, Tigecycline with a percentage reach(100%), but Rifampicin and Trimethoprim / Sulphamethazole with a prevalence rate of (80%) respectively. While all these isolates showed sensitivity to7-4 antibiotics Amikacin, Ceftazidime, Ceftolozane, Ciprofloxacin with a percentage of(100%),while Piperacillin/ Tazobactum, Meropenem and Imipenem showed moderately sensitivity with a rate ranging from (80-60%) respectively, with non significant differences ($P\geq0.05$). Haematological and immunological tests for RA patients to detect anti-pathogens antibody like Rheumatoid factor (RF+), also ESR, CRP and Anti-CCP tests were done. The results show positive reaction and increasing the levels of all these tests against pathogens. The level of IL-8 was measured and the mean level was elevated non significantly ($P\geq0.05$) in RA patients with UTI more than control (83.74pg/ml vs 74.09 pg/ml), also the level of IL-8 in RA patients' sera with UTI who infected by *P.mirabilis* is increased significantly, it gave mean level (126.3 pg/ml) in their serum.

Keywords: *Proteus mirabilis,* UTI, *UreC,* Antimicrobial susceptibility, Interleukin -8.

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory joint disease, which affects millions of people all around the world, with a prevalence rate ranging from 0.5 to 1%**.** RA is a disabling poly arthritic disease, which affects individuals of middle age groups and occurs three timesmore frequently in women than in men.(1,2,3). .RA is characterized by an inflammation of the synovium and a destruction of cartilage and bone**.** Scientific researches consider RA as an immune mediated disease that could possibly be triggered by an environmental (microbial) factor in a genetically susceptible individual extensive evidence based on the results of various microbial, immunological and molecular studies from different parts of the world, shows that a strong link exists between urinary tract infections (UTI) and RA, this has mainly caused by *P.mirabilis* microbes.(4,5,6).*Proteus mirabilis* is a Gram-negative facultative anaerobic rod shaped bacterium. It may found as part of the normal flora in intestine . This bacteria 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.(4,7,8). *Proteus 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. A UTI can occur anywhere in the Urinary tract which is made up of kidneys, ureters, urinary bladder, and urethra. UTI includes (urethritis, cystitis, pyelonephritis , epidydimitis, prostatitis, perinephritis) and it is considered as one among the most common infectious diseases that are widely seen among all age groups of individuals. (9,10,11). Most UTIs only involve the urethra and bladder, in the lower tract. However, UTIs can involve the ureter and kidneys, in the upper tract also.The bladder urothelium plays an important role in the host innate immune response to UTI, and the fact that women are more prone to getting UTIs, the role of estrogen’s effect on the urothelial defense mechanisms and the shorter distance from the urethral opening to the bladder, increases the chance that a potential uropathogen can rise to the bladder, multiply in the urine, and invade bladder wall or rise further to the kidneys causing UTI. Also it proposed that sub-clinical *Proteus mirabilis* urinary tract infections are the main triggering factors and that the presence of molecular mimicry and cross-reactivity between these bacteria and RA-targeted tissue antigens assists in the perpetuation of the disease process through production of cyto pathic auto-antibodie(12). Several studies show that exposure to specific strains of, *P.mirabilis* could play a role in early stage seropositive RA pathogenesis according to RF status (13). This bacterium is well-known for its urease enzyme production and distinctive ability to differentiate into elongated swarm cells(14). The bacterial *16S rRNA* gene encodes for the ribosomal RNA small subunit(15). It contains DNA sequences that are common to all bacteria and some that are unique to each species(16,17,18). Initial studies on bacterial taxonomy and phylogeny depend on the Sanger sequencing of the most common housekeeping marker which is the *16S rRNA* gene (19). 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 including toxins like hemolysin and its function of pore formation, biofilm and regulation of the pathogenicity (20,21). Ureas enzyme which causes kidney and bladder stones, as well as its ability to form crystalline biofilms on the outer surface and in the lumen of indwelling urinary catheters(22). The MR/P fimbriae are the most well-studied fimbriae of *P. mirabilis*, which are potentially involved in adhesion to theuroepithelium, contributes to the ability of an organism to establish infection in the urinary tract (23,24) in addition the role of cellular and humoral autoimmunity play role in the development of RA when *P. mirabilis* contact with host cells lead to stimulus release of urinary cytokines and chemokines like IL-8 which important apparatuses of the inflammatory immune retort to this pathogen.

This study aimed to investigate the prevalence of *P.mirabilis* bacteria in UTI patients with RA, detection of *UreC* virulence gene and examine the antibiotics sensitivity of this pathogen with measurement of IL-8 in RA patients’sera.

Materials and Methods

1.Urin Samples Collection

In this study, 216 urine specimens were collected from (100 healthy control and 116 Rheumatoid arthritis patients suffering from urinary tract infections with different age groups of both sex from different hospitals. Samples were collected from patients during the period from the beginning of September 2023 to the end of March 2024 from Baghdad Teaching Hospital/Medical City and Al Nuaman, Teaching Hospitals in Baghdad/Iraq.

2.Isolation and Identification of Bacterial Isolates

The urine samples of RA patients was cultured. Isolates were identified by methods of routine tests: depending on their morphology and physiology characteristics on the culture media and colony morphology on MacConkey , Nutrient agar and Blood agar. Biochemical tests including; catalase test, oxidase and ureas reaction and also VITEK-2 system used for more identification. The suspected bacterial isolates were re-inoculated on to MacConkey agar plates and then incubated over night at 37 C˚. A single colony was then taken and suspended in to solution. The turbidity of the bacterial suspension was adjusted with VITEK-2 densichek (Bio Merienx) to match the McFarland 0.5 standard in 0.45% sodium chloride (25). Then the VITEK-2(IDGNB) Kit(gram negative card) and the bacterial suspension tubes were manually loaded into the VITEK-2 system. Following steps on the software were done according to the manufacturer’s instructions (Bio Merieux, France) (26-27) .This system used to give good results for identification and confirmation with conventional methods (28).

3.Antimicrobial Susceptibility Testing

The antibiotic susceptibility tests was carried out for all the *P.mirabilis* isolates using VITEK-2 method in accordance to the recommendations of the manufacturer’s instructions. *Proteus mirabilis* isolates were tested for antibiotic sensitivity against 13 different antibiotics .Ampicillin/ Sulbactam, Amikacin, Pipercacillin/Tazobactum, Cefotaxime,Ceftazidime,Cefotrixone,Rifampicin,Imipenem,Gentamicin, Ciprofloxacin,Tigecycline,Meropenem, Trimethoprim/Sulphamethazolas.

4.Molecular Methods

1.DNA Extraction and PCR Protocol

The extraction of genomic DNA from *P. mirabilis* growth for all five VITEK-2 diagnosed isolates was carried out according to the protocol of (EasyPure-Genomic DNA Kit from

Transgene (China). Bacterial genomic DNA extracted from activated pure culture of *P. mirabilis* isolates were grown in brain heart broth for 24 hr. at 37 C°. (Qubit 4.0)instrument was used to detect the concentration of extracted DNA in order to detect the validity of samples for downstream application . Detection of DNA bands using agarose gel electrophoresis with concentration 1% and voltage 75 volt for 1hr.(12).

2.Detection of *P.mirabilis 16SrRNA* gene and *UreC* gene Using Conventional PCR Technique

Specific primers for*16SrRNA*were used for detecting the *P. mirabilis* bacteria in addition to *(UreC*) virulence gene, that related to urease production, as shown in (Table1). The PCR amplification mixture which used for detection of each gene include one NEB(England) Master Mix ,5X (12.5 µl), 3µl of DNA template, 1 µl (1 mM) of each forwarded and reversed primers and 7.5 µl of nuclease free water to complete the amplification mixture to 25µl (Table3). After preparing the reaction volume in PCR tube the mixture was spin down and the PCR tube placed in the PCR thermocycler. The amplification reactions was started according to the program described in (Table 2).

Table (1): The Primers Sequences and Size were Used for PCR Detection of genes

Genes name	Sequences (5→3) primer		Size (bp)	Ref.
16SrRNA P.mirabilis	**F**	**5'TTAGCTAGTAGGTGGGGTAAA'3**	**404**	**In this study**
	R	**5'AACCAGTTTCAGATGCAATTC'3**		
UreC	**F**	**5'CGAGCTGTTTCTTGAAATTGA'3**	**470**	**In this study**
	R	**5'CCTCTAACATGCGGTACATAT'3**		

Table(2): The PCR Program for (*16SrRNA* and *UreC*) Genes Amplification

Steps	Cycles	Genes amplification conditions			
		***16SRNA* Temp.⁰C**	**Time**	***UreC* Temp. ⁰C**	**Time**
InitialDenaturation	1	94	5.00 min	94	5.00 min
Denaturation	30 (16SRNA) 30 (*UreC*)	94	30 sec.	94	30 sec.
Annealing		48	45 sec	48	45 sec
Extension		72	45sec	72	45sec
FinalExtension	1	72	7 min	72	7 min

Table (3): Component of PCR Reaction for (1*6SrRNA* and *UreC*) genes

Component	Volume	Final Concentration
Master Mix , 5X	12.5 µl	1X
Forward primer	1 µl	1 Mm
Reverse primer	1 µl	1 Mm
DNA template	3 µl	25 ng
Nuclease free dH2O	7.5 µl	
Final volume	25 µl	

5.Haematological and Immunological Analysis

1.Blood Sample Collection

A total of 216 (116 RA patients with UTI and 100 apparently healthy control blood samples). Five ml of venous blood sample with drawn from each individual under aseptic technique the blood samples were collected from the same people whom urine samples were taken. ESR test was done by the Westergreen method and three milliliter of each samples were transferred to test tube, centrifuged at 25000 rpm for 10 minutes and separated serum was divided into eppendroff tubes and immediately frozen at -20 ºC till further use, for CRP ,anti-CCp and RF analysis (4).

2.Interleukin -8(IL-8) Level Measurment

The study included (58 RA patients with UTI and 30 apparently healthy control individual) to carried out the test. Serum separated from 2 ml of venous blood and examined using ELISA technique. The level of IL8, was determined for all samples included the five samples of patients sera with positive cultures of *P.m*irabilis in strict accordance with the instructions for each ELISA kit (ELK Biotechnology Co., Wuhan- Roche -Cobas, China).

Results and Discussion

Six isolates were suspected as *P.mirabilis* depend on their morphological characteristics of the colony, smooth colorless on MacConkey and Blood agar plate with swarming phenomena and distinct fishy odor on cultured media as shown in (fig1A+B). Recent study shown closely results with (Aneta Filipiak *et al.*,2020) (29). It was also found the isolate has inability to metabolize lactose and form smooth, pale, or colorless colonies (on MacConkey agar plate). In addition, biochemical tests were done, all 6 isolates gave negative result for oxidase, catalase and positive results for urease test. Out of 6 isolates, only 5 that diagnosed by VITEK-2 system.

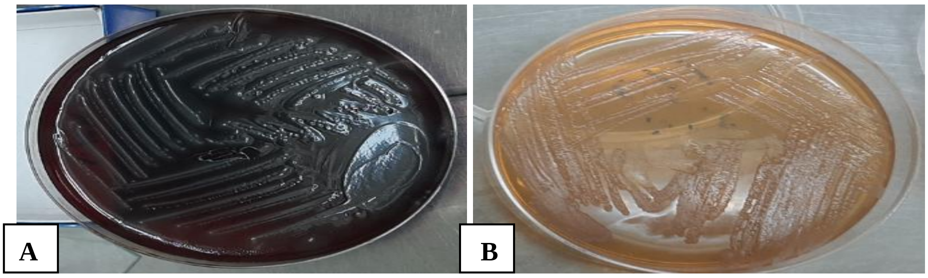

Fig.(1): The colonies of *Proteus mirabilis* on Blood agar (A) and MacConkey agar (B).

Antimicrobial Susceptibility Test

The information described in Table (4) explain the majority of the isolates showed multidrug resistance profiles. *Proteus mirabilis* isolate1,2,3 and5 resist to different antibiotics (Ampicilline/Sulbactum,Cefotaxime, Gentamicin,Tigecycline with a percentage reach(100%), but Rifampicin and Trimethoprim / Sulphamethazole with a prevalence rate of 80%) respectively. The results of the study agreed with what was informed as pathogens resistance to the antibiotic Ampicillin-Sulbactum and Cefotaxime was high as it the important antibiotics of this group (30), also they showed resistant for Gentamicin and Tigecyclin respectively. These observations are in agreement with studies of (31,32). In addition, they showed approximately intermediate resistance for Rifampicin and Trimethoprim-sulphamethazol, those findings were in agreement with that mentioned by (33). While all these isolates showed sensitivity with a percentage of(100%), to antibiotics(Amikacin, Ceftazidime, Ceftolozane,Ciprofloxacin, while Piperacillin/ Tazobactum, Meropenem and Imipenem showed moderately sensitivity with a rate ranging from 80-60% respectively, study agreed with the results that found the resistance pattern of the *P.mirabilis* was low rates and high susceptibility to the Amikacin, Piperacillin-Tazobactum, Ciprofloxacin , Ceftazidime and Ceftalozane antibiotics which was100-90% respectively(34). The result of this study agree with (35,36,37) that showed moderate sensitive of bacteria to the antibiotic Imipenem and Meropenem are the antibiotics of this group with high ability and effectiveness against Gram-negative bacteria. Nonetheless, the total of isolated bacteria were sensitive to Ciprofloxacin, which disagree with the results recorded by (38,39), while this result agrees with the researcher (40) with moderate resistant to Rrafampicin,Ttrimethoprim and Piperacillin who test the susceptibility against antimicrobial agents for the *P. mirabilis* pathogenic isolates.The isolates did not reach statistical significances differences with (P≥0.05), which varied in different studies. This may be due to limited numbers of isolates and the antibiotics-abuse in countries from which those isolates were isolated. Researches were confirmed that Multi-drug resistance was defined as resistance to at least 3 antibiotics. All the five *Proteus* species were found to be MDR. Species identification in agreement with Jitendra Kumar *et al*., 2013 (41). Therefore surveillance of antimicrobial resistance is essential in management and control of infections.

Table(4):The Result of Antibiotic Test for Each *Proteus mirabilis* Bacterial Isolates

Type of Isolates	**AMP**	**AMK**	**PRI**	**CIX**	**CRO**	**CAZ**	**RIF**	**CN**	**IPM**	**CIP**	**TGC**	**SXI**	**MEM**
P.mirabilis 1	R	S	S	R	S	S	R	R	S	S	R	R	S
P.mirabilis 2	R	S	S	R	S	S	R	R	I	S	R	R	S
P.mirabilis 3	R	S	S	R	S	S	R	R	S	S	R	R	S
P. mirabilis 4	R	S	I	R	S	S	I	R	I	S	R	I	I
P. mirabilis 5	R	S	S	R	S	S	R	R	S	S	R	R	S

Total Isolates =5 R=Resistance S=Susceptibility I= Intermediate

Ampiclline=AMP, Amikacin=AMK, Piperacillin/Tazobactum=PRL, Cefotaxime=CIX, Cefotrixon=CRO, Ceftazidime=CAZ, Rifampicin=RIF, Gentamycin=CN, Imipenem=IPM, Ciprofloxacin=CIP, Tigecycline=TGC, Trimethoprim/sulfamethoxazole=SXI, Meropenem=MEM.

Molecular Identification of *Proteus mirabilis*by*16SrRNA* gene and *UreC gene*.

The total genomic DNA was extracted from five bacterial isolates that diagnosed positively by VITEK-2 system.The concentration of DNA was measured by (Qubit4.0) instrument from Thermo fisher/USA). The results of gel electrophoresis showed the presence of DNA bands in the same level for all isolates in agaros gel, figure (3).

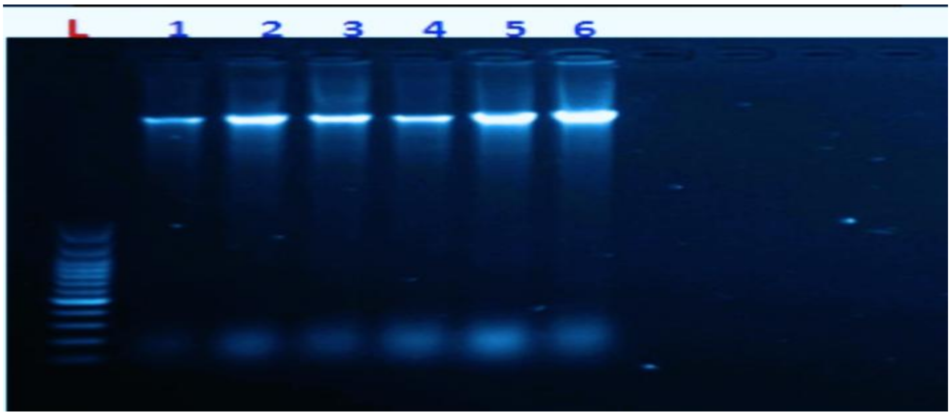

Fig.(3): Genomic DNA Extraction Electrophoresis for *P. mirabilis*(1%)agaros gel at 75 volt for 1 hr.

Further molecular diagnosis for *P.mirabilis* bacteria by using *16SrRNA* gene specific primer with molecular size 404 bp , and the results were detected by gel electrophoresis on 2% agarose and exposed to U.V light in which the results indicates that all 5 isolates give the same result for biochemical diagnosis, this technique was rapid and accurate with high identification genomic rate (100%) as indicated by (GhufranJ.*etall.*,2019) (42,4). Several studies referred to using of *16SrRNA* for identification of *P.mirabilis* isolated,*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 (43,44), figure (4).

Fig.(4): The Agarose Gel Electrophoresis(2%) of PCR Amplified Products for 16sRNA *gene* of *P.mirabilis* isolates. Lane L: for 1000bp DNA ladder, lanes (1-5): DNA of positive result with expected size 404 bp (2 % Agarose, 75V for 1 hr)..

Clear correlation was appeared between PCR techniques and culturing methods whereas all positive samples with culturing method done with conventional PCR for identification of all isolates of *P. mirabilis* depending on use of specific primers for *UreC* gene with molecular size (470bp), only two isolates exhibit the positive amplification results when the product of conventional PCR was detected by using gel electrophoresis as showing in figure (5) which closely with traditional methods and VITEK-2 system, therefore this study agree with a previous study by(4,10,45).The *UreC* was the most prevalent gene and was identified in two isolates. The *UreC* gene is responsible for the elevation of pH of urine and resulting in stone formation, the study compared with another study by (46,47) that *P.mirabilis* generates urease, which catalyzesthe hydrolysis of urea to ammonia and carbamate and elevates the pH of the urine to produce struvite or apatite crystals. Our results indicates the crucial role of *UreC* for the virulence of the *P.mirabilis* , regarding *UreC* gene a virulence factor, this result is in agreement with a previous study, indicating the presence of *UreC* approximately 40% among isolates obtained from patients with UTI (48,49).On the other hand, many studies demonstrate the high ability of *P. mirabilis* to produce urease (50,51) and the study of Laurel *et al*. (52) 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, Ureas $(NH_2)_2CO + 2H_2O$ $CO_2 \longrightarrow H_2O + 2NH_3$

Hence, it breaks down one molecule of urea to two molecules of ammonia and one carbon dioxide molecule, that causes non physiological 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. Urease also assists *P. mirabilis* to develop bacteriuria, cystitis, and kidney, and bladder stones (48,53). Results agree with the studies that pointed out *UreC* as a major gene, it encodes the large subunit responsible for the production of urease enzyme of the *Proteus mirabilis* (54).

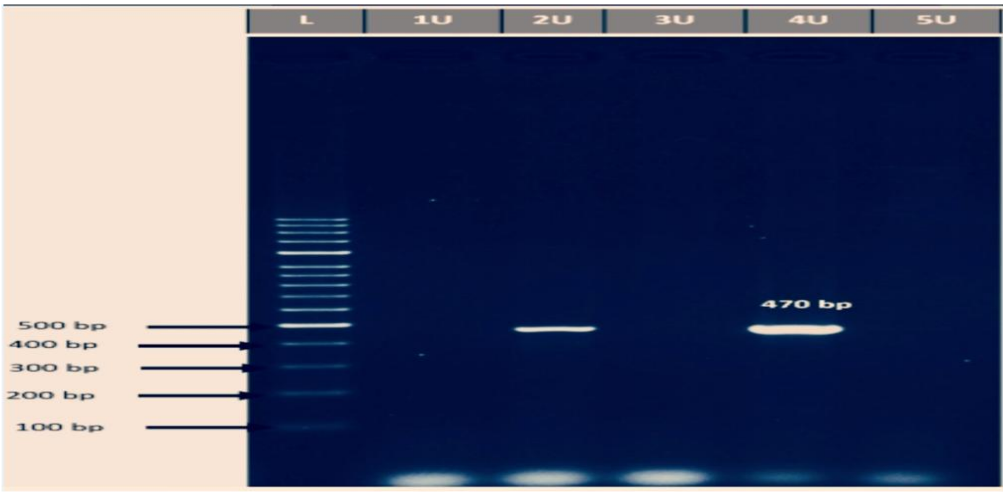

Fig.(5): The Agarose Gel Electrophoresis of PCR Amplified Products of *UreC gene* codify for urease enzyme of *P.mirabilis* isolates. Lane L: for 1000bp ladder, lanes (2,4): DNA of *P. mirabilis* positive result with expected size 470 bp (2 % Agarose, 75V for 1 hr).

Haematological and Immunological Analysis

1.Blood parameters tests

There is compelling evidence exists linking this microbe to RA, starting with recurrent sub-clinical Proteus UTIs and ending in the full development of RA(4). To prove the scientific logic of this possibility, many blood tests were done including (RF, ESR, CRP, RF and AcpA) all of these tests were high in the patients whom their urine are having *P.mirabilis* bacteria. Rheumatoid factor (RF) tests has shown that the pooled sensitivities of RF in the sera of RA patients infected with *P.mirabilis* as shown in Table (5A,5B) which agree with what indicated in previous study (4), among the study groups, it has been found that the mean level of the Erythrocyte Sedimentation Rate (ESR) was significantly higher between the case group than the controls (61.50mm/h vs16.97mm/h,T=36.668), respectively with a highly significant of ($P \leq 0.01$), but the mean value of the (ESR) was increased significantly according to the bacterial infection of *P. mirabilis* (65.0mm/h±3.31) Similarly, the mean level of C- reactive protein (CRP) was substantially higher in the cases group compared to the controls (39.57mg/L vs9.45mg/L,T=12.394), respectively with highly significant ($P \leq 0.01$), also the mean value of the (CRP) was elevated non significantly depend on the type of infected bacteria (75.83mg/L±13.22). Various metrics are utilized to measure the severity of rheumatoid arthritis (RA). These agreed with(55) that showed there was elevated (ESR, CRP and RF) as they that important in the diagnosis of inflammatory conditions .Although there is currently no clear consensus on when to administer one, the other, or both, the laboratory tests like the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) have been a crucial component of doctors' tools for many years. To measure acute disease activity among these tests, CRP has emerged as the most widely used serological marker (4,56). Likewise, all samples show positive results with rheumatoid factor test (RF), these findings by(23,57) has been shown that rheumatoid factor-(RF) positive patients with RA had significantly elevated levels of antibodies against *Proteus mirabilis*. In addition, the mean concentrations of anti-Citrullinated protein/peptide antibody (ACPA) were significantly higher among the cases group of rheumatoid arthritis than those of the rheumatoid arthritis free group (controls) (59.55 EU/mlvs19.20EU/ml,T=19.015) respectively with highly significant ($P \leq 0.01$), respectively, but the mean value of ACPA was increased non significantly among infected patients with *P. mirabilis* (80/66 EU/ml±9.28) results are listed in Table(5A,5B). Also, T. Rasheed *et al.*, 2017,showed the citrullinated proteins recognised by ACPA antibodies was found to be highly abundant and more active within the synovial joints of patients with RA(10).The relatively constant associations of certain autoantibodies such as RFs and ACPAs with RA have been linked to *Proteus* microbes and all these might be involved in the aeti-opathogenetic process and development of RA since they come before the start of disease symptoms and indicate a more severe disease course, therefore the combination of the two markers improves accuracy RA diagnosis(4).These extensive results provide a new approach to the treatment of this disease by combining the reduction of the Proteus microbial load together with the currently used biological approaches(10).

Table (5A): Mean Comparison of Haematological and Immunological Parameters Among Patients' Groups and Control (n=116 vs 100)

Cases	ESR	S.E.	CRP	S.E.	ACPA	S.E.
Patients	61.50	1.08	39.57	2.40	59.55	1.99
Control	16.97	0.54	9.45	0.37	19.20	0.72
T	36.668		12.394		19.015	
P-value	0.0001		0.0001		0.0001	
	(P≤0.01) Highly Significant		(P≤0.01) Highly Significant		(P≤0.01) Highly Significant	

Table (5B): Mean Comparison of Haematological and Immunological Parameters Among RA Patients Group with UTI (*P.mirabilis*) (n=116)

Cases	ESR	S.E.	CRP	S.E.	ACPA	S.E.
P.m.	65.0	3.31	75.83	13.22	80.66	9.28
T	2.28		1.182		1.012	
P-value	0.047		0.24		0.31	
	(P≤0.01) Highly Significant		P≥0.05: Non-Significant		P≥0.05: Non-Significant	

2.Interleukin-8 Levels Analysis.

The level of IL-8 differ significantly in RA patients sera with UTI according to their bacterial infection. The mean level of IL-8±SE increased non significantly (P≥0.05) in RA patients with UTI (83.74pg/ml±6.48vs 74.09 pg/ml±14.94), (P=0.15) as shown in (Table 6).

Table(6): Mean Comparison of IL-8 Among Study Groups

Cases	Conc.	S.E.	T-test	P-value
Patients =58	83.74	6.48	-1.454	0.15
Control =30	74.09	14.94		
P≥0.05: Non-Significant				

The mean level of IL-8 differ significantly in RA patients' sera who infected with *P. mirabilis* when it gave mean level (126.3 pg/ml) as shown in (Table7). Result is in agreement with a previous study by Tha'ir Abid D'asheesh *et al* ,(2020), which indicated a significant relationship between interleukin-8 (IL-8) production and urinary tract infection with *P.m*irabilis in RA patients (24,54).The level of IL-8 was influenced by bacterial virulence genes of *P.mirabilis* and associated with ESR, RF and CRP elevation. The results agree with Naba *et al*.,2017(55) that showed a cytokine responses to urinary tract infection vary with the severity of infection and the cytokine activation is influenced by a variety of host and bacterial variable. Also IL-8 a potent neutrophil chemotactic molecule, the presence of neutrophils in the urine by bladder epithelial cells is a hallmark of UTIs. However, their action may also lead to the local tissue damage (59).

Table(7): Mean Comparison of IL-8 Parameters Among Infected Groups with *P.mirabilis*

Cases	NO	Conc.pg/ml	LSD
P.mirabilis	**6**	**126.3**	**38.96**

The IL- 8 is a pro inflammatory cytokine which increased in reply to immune response for infected pathogens specially *P.m*irabilis. IL-8 is a chemokine reason to relocation of neutrophils to the apartment of irritation, causingthe pyuria. in infected persons. During advanced stages of UTI and levels of cytokines will be a rise in blood and urine(24).As a result of the outcomes indicate most of *P,mirabilis* isolates showed MDR to antibiotics and two out of five isolates have positive screening for urease production gene(*UreC*) by coincide with IL-8 levels elevation in patient sera. These agree with other researchs showed that uropathogen *Proteus mirabilis* rapidly invades the bladder urothelium , its frequently forms extracellular clusters which form foci of mineral and calcium ions deposition consistent with development of urinary stones, result to elicit a robust neutrophil response, there are two virulence factors required for cluster development urease, which is required for urolithiasis, and mannose resistant Proteus-like fimbriae.(23). Studies of Poland showed that *P.mirabilis* produces urease and hemolysin, which contributes to the formation of a crystalline biofilm, considered to be one of the most important virulence factors of *P.mirabilis* strains, along with their ability to swarm on a solid surface, the strains which were able to form a stronger biofilm had broader antimicrobial resistance profiles. It was also found that the strongest swarming motility correlated with susceptibility to most antibiotics(54). Likewise, research indicated there is relationship of tested antibiotic resistance was observed in *P. mirabilis* with the ability of strong and moderate biofilm production and formation in the isolates (47). Also Milan Chromek *et al*., (2005) found that all isolates had the urease activity complicated urinary tract Infections *P.mirabilis* induce uroepithelial cells production, elicits both innate and adaptive immune responses, although an efficient host defense against urinary tract infection is reliant upon an early activation of the innate immune response like IL-8. This responses characterized by the production of a number of proinflammatory mediators, including cytokines and chemokines. Bladder and kidney epithelial cells appear tobe a major source of interleukin-6 (IL-6) and interleukin-8 (IL-8) after infection with uropathogens, which areimportant in the development of local tissue damage Cytokine possesses a variety of proinflammatory functions, including activation of signals involved in neutrophils recruitment and production of acute phase proteins. IL-8 is a potent neutrophil chemotactic molecule and the appearance of neutrophils in the urine. Neutrophil recruitment to the site of infection has been shown to be critical for bacterial clearance from both the bladder and kidney, and the presence of neutrophils in the urine is a hallmark of UTI.(58,59). Therefore more investigation for hemolysin production virulence gene is required in other *P. mirabilis* isolates.

Conclusion

Molecular and immunological relation between the triggering aetiological factor, *Proteus mirabilis* antigens through MDR in recurrent UTIs, and damage targeted synovial tissue structures of RA patients in correlation of antibody-mediated cytotoxicity reactions and IL-8 production, RA patients should be treated early with anti-*Proteus* antibiotics as well as biological agents with restrictions for antibiotic prescription and susceptibility testing must be done before antibiotic dispensing to avoid irreversible joint damages in RA patients.

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