MOLECULAR DETECTION OF *UREC, HPMA, RSBA* AND *MRPA* GENES OF *PROTEUS MIRABILIS* URINARY TRACT INFECTION IN PATIENT WITH RHEUMATOID ARTHRITIS

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ABSTRACT

This study was aimed to detect of four important virulence genes (*UreC, HpmA, rsbA* and *mrpA*) from *Proteus mirabilis* bacteria isolated and do the important four blood test (ACPA, RF, ESR, CRP) for patient with urinary tract infection and rheumatoid arthritis in Baghdad/Iraq. Fifty (50) clinical urine samples were collected and cultured on blood agar and macckongy agar. Ten of the samples were identified as *P.mirabilis* and the automated system VITEK 2 used to complete the identification. Molecular methods using PCR technique targeting *UreC, HpmA, rsbA* and *mrpA* genes, using PCR technique and the results were *UreC* found in 90% of the samples, *HpmA* 90%, *rsbA* 100% and *mrpA* 80%. The *P. mirabilis* samples were also characterized for antibiotic resistance and the result showed that the majority of samples were show high resistant to Cefotaxime 90%, while the highest susceptibility was against Impenem antibiotic 70%.

Key words: automated system, targeting , PCR . blood culture bacteria infection,

مجلة العلوم الزراعية العراقية -2020 :51(عدد خاص):245-251 الحمداني والهاشمي

كشف عن جينات *UREC, HPMA, RSBA* AND *MRPA* GENES OF *PROTEUS MIRABILIS* التي تصيب المسالك البولية والتهاب المفاصل الرثوي

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المستخلص

تهدف الدراسة الى الكشف عن اربع مورثات ضراوة مهمة *UREC, HPMA, RSBA* AND *MRPA* من بكتريا *P*roteus mirabilis من المرضى الذين يعانون من التهاب المسالك البولية والتهاب المفاصل الرثوي في بغداد – العراق . جمعت خمسون عينة ادرار وزرعت على اوساط مختلفة , و عشرة عينات منها تم تشخيصها على ا نها نفس البكتريا . اعتمادا على الفحزصات الكيموحيوية التقليدية بالاضافة الى نظام الـ VITTEK-2 . من جهة اخرى تم جمع نماذج الدم من المرضى لاجراء الاختبارات التالية (ACPA , RF , CRP , ESR)استعملت الطرائق الجزيئية باستعمال تقنية السلسلة النتبلمرة المستهدفة لجينات *UREC, HPMA, RSBA* AND *MRPA* و كانت النتائج ان مورث Urec قد وجد في 90% من العينات و HpmA في 90% و RsbA في 100% و MrpA كان في 80% من عينات البكتريا المعزولة . خضعت العينات ايضا الى فحص الحساسية حيث اشارت النتائج ان غالبية العزلات اظهرت مقاومة لمضاد Cefotaxime 90% في حين اعلى نسبة حساسية كانت تجاه المضاد الحيوي Impenem 70%

كلمات مفتاحية: عينات، نماذج الدم، عينات، مضادات، اختبارات

***Received:11/7/2019, Accepted:3/10/2019**

INTRODUCTION

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) and RA, this has mainly caused by *P.mirabilis* bacterium (10). *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. 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. A UTI can occur anywhere in the Urinary tract. It 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). 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 (17), including toxins like hemolysin and its function of pore formation, biofilm and regulation of the pathogenicity (14). Urease enzyme which causes kidney and bladder stones (2) . The MR/P fimbriae are the most well-studied fimbriae of *P. mirabilis*, which are potentially involved in adhesion to the uroepithelium, contributes to the ability of an organism to establish infection in the urinary tract (15). The *rsbA* gene was regulator of swarming behavior that encodes a sensory, RsbA function may as a protein sensor of environmental conditions. It stimulated bio film formation and Extracellular polysaccharide formation (11 , 12). This study was aimed to detect of four important virulence genes (*UreC, HpmA, rsbA* and *mrpA*) from *Proteus mirabilis*

MATERIALS AND METHODS

A total of 50 urine samples were collected from patients with UTI and RA. Samples were collected from patients during the period from October 2018 the end of August 2019 From the Medicine City/Baghdad Teaching Hospital, Medicine City/ Teaching Laboratory, private laboratories in Baghdad/Iraq. The urine of these 10 patients were identified by methods of routine tests: colony morphology (blood and macckongy agar) , biochemical tests including; catalase test, oxidase reaction, urease test and also VITEK 2 system used for more identification.

Blood tests

Five millilitres of venous blood has been collected and divided into parts, some putted into Erythrocyte sedimentation rate (ESR) tube and the remained putted in a clean plain tube and allowable to clot at 37 C˚ for 10 minutes (for ESR test). Then centrifugeing at 2500 rrpm for 5 mine. until serum separaeted. Serum is storeed at 20 C˚ until used for the other three tests (RF, CRP, and ACPA).

Antimicrobial susceptibility

The antibiotic susceptibility tests was carried out for all the *P.mirabilis* isolates using Mueller-Hinton method to measure the zones of inhibition in accordance with the recommendations of clinical and laboratory standards institute . The antibiotics used were: (F): Nitrofuranton (100mcg/disc), (AK): Amikacin (10mcg/disc), (GM): Gentamicin (10mcg/disc), (APX): Ampicillin/cloxacillin (30mcg/disc), (C): CeftaCloramphenicol (30mcg/disc), (CTX): Cefotaxime (30mcg/disc), (IMP): Imipenem (10 µg/disc), (RP): Rafampicin (2µg/disc).

Molecular detection

DNA was extracted from activated pure culture of *P.mirabilis* bacteria using genomic DNA Bacteria Kit (geneaid). Detection of DNA bands using Agarose gelelectrophoresis (1%). Then conventional PCR were used in this study to detect the presence of the important virulence genes that related with causing UTI and RA *(UreC, HpmA, rsbA* and

mrpA) of the *P.mirabilis* bacteria using specific primers as shown in Table 1.

Table 1. The Optimum Condition of Detection Gene

Genes Name	primer Sequences	Size (bp)	Conditions	Ref.
Urec	F: CCG GAA CAG AAG TTG TCG CTG GA R: GGG CTC TCC TAC CGA CTT GAT C	533	94°C 3min 94 °C 1min 63°C 30sec40x 72°C 1min 72°C 7min	(D'Orazio SE, *et al., 1996*)
rsbA	F: TTG AAG GAC GCG ATC AGA CC R: ACT CTG CTG TCC TGT GGG TA	467	94°C 5min 94°C 60sec 58°C 45sec35x 72°C 1min 72°C 7min	(Badi, S. *et al.*, 2014)
hpmA	F: GTT GAG GGG CGT TAT CAA GAG TC R: GAT AAC TGT TTT GCC CTT TTG TGC	709	95 ^{0} C 5min 1x 95 ^{0} C 30sec 62^{0} C 30sec 30x 72 ^{0} C 20 sec 72 ^{0} C 5min 1x	(Cestari *et al.*, 2013)
16sRNA	F: GTG TAG CGG TGA AAT GCG R: ACG GGC GGT GTG TAC AA	700	95 ^{0} C 5min 1x 95 ^{0} C 30sec 62^{0} C 30sec 30x 72 ^{0} C 20 sec 72 ^{0} C 5min 1x	(Brause, 2003)
mrpA	F: TTC TTA CTG ATA AGA CAT TG R: ATT TCA GGA AAC AAA AGA TG	565	94°C 3min 94°C 30sec 40°C 30sec40x 72°C 30sec 72°C 7min	(Sosa, *et al.*, 2006)

Protocol

The PCR amplification mixture which used for detection n of each gene includes FIREPol® Master Mix 5X (4 µl), 5 µl of DNA template, 1 µl (1 mM) of each forwarded and reversed primers and 9 µl of nuclease free water to complete the amplification mixture to 20µl, (Table 2).

Table 2. PCR mixture components for each gene

Component	Volume	Final Concentration
FIREPol® Master Mix , 5X	**4 µl**	**1X**
Forward primer	**1 µl**	**1 µM**
Reverse primer	**1 µl**	**1 Mm**
DNA template	**5 µl**	**25 ng**
Nuclease free dH2O	**14 µl**	
Final volume	**25 µl**	

After preparing the reaction volume in PCR tube the mixture was spin down and then PCR tube placed in the PCR thermo cycler and the amplification reactions was started according to the program described in Table 1.

RESULTS AND DISCUSSION

A total of 50 urine sample of patient with UTI and RA that admitted to different hospitals in Baghdad/Iraq during the period, from October 2018 to August 2019, from both gender with age ranged from 30-85 years old. All colonies were diagnosed in the lab as *P.mirabilis* due to characteristic swarming motility on blood agar, and inability to metabolize lactose (on MacConkey agar plate) (Fig. 1). Also *P. mirabilis* produces a very distinct fishy odor. in another hand all 10 isolates gave negative result for oxidase, catalase and positive results for urease test.

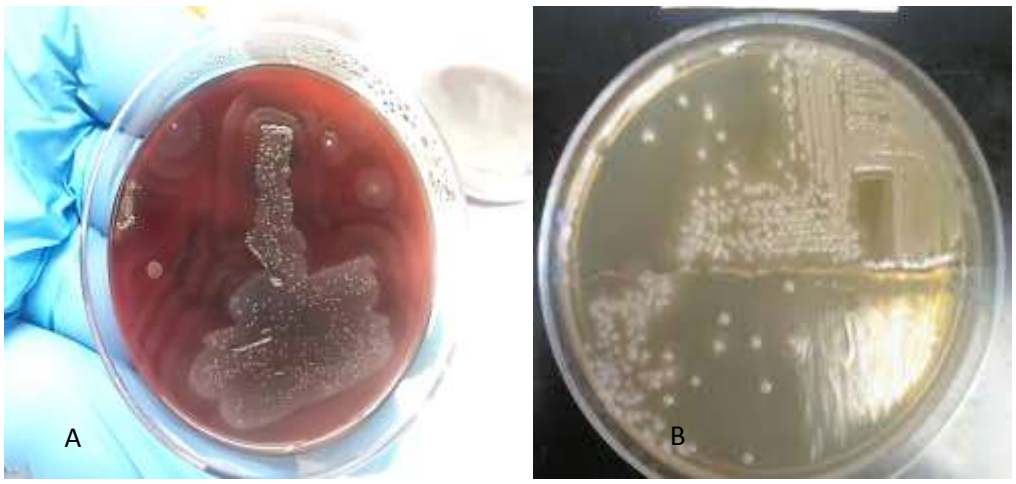

Fig. 1. *Proteus mirabilis* on Blood agar (A) and macconkey agar (B).

The resistance pattern to nine antimicrobials disks was shown in fig.(2). It shows high resistance reach to 70% against Gentamycin & Nitrofuranton, Cefotaxime 90%, while the highest susceptibility was to the Imipenem antibiotic which was 70%.

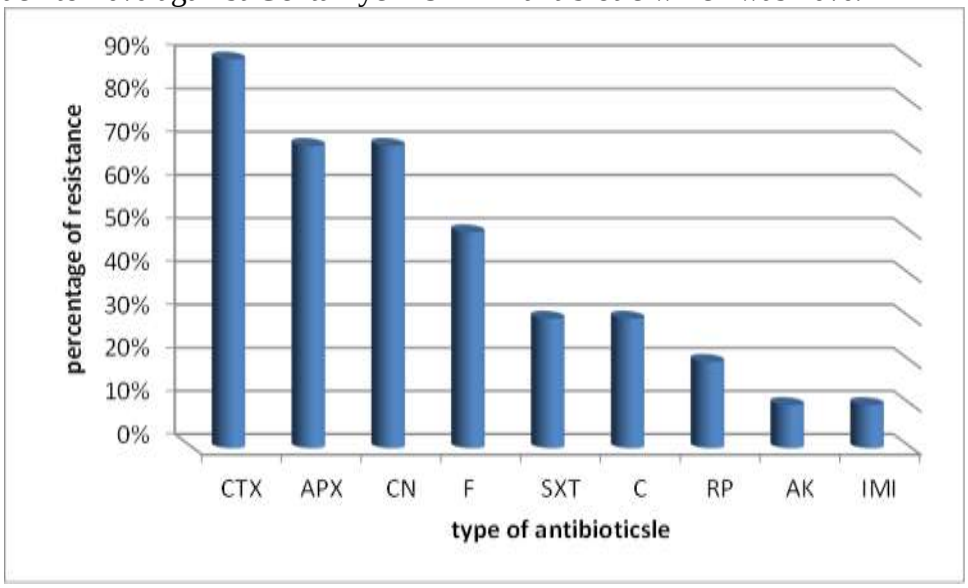

Fig 2. Show the resistance of bacteria to a variety of the antibiotic

The extraction of chromosomal DNA was done, from all 10 isolates that diagnosed by VITEK 2 system. The results of gel electrophoresis showed the presence of DNA bands in the same level for all isolates in agaros gel (Fig. 3). Then, the concentration and purity of extracted DNA was measured by QuantiFluor® (Promega, USA).

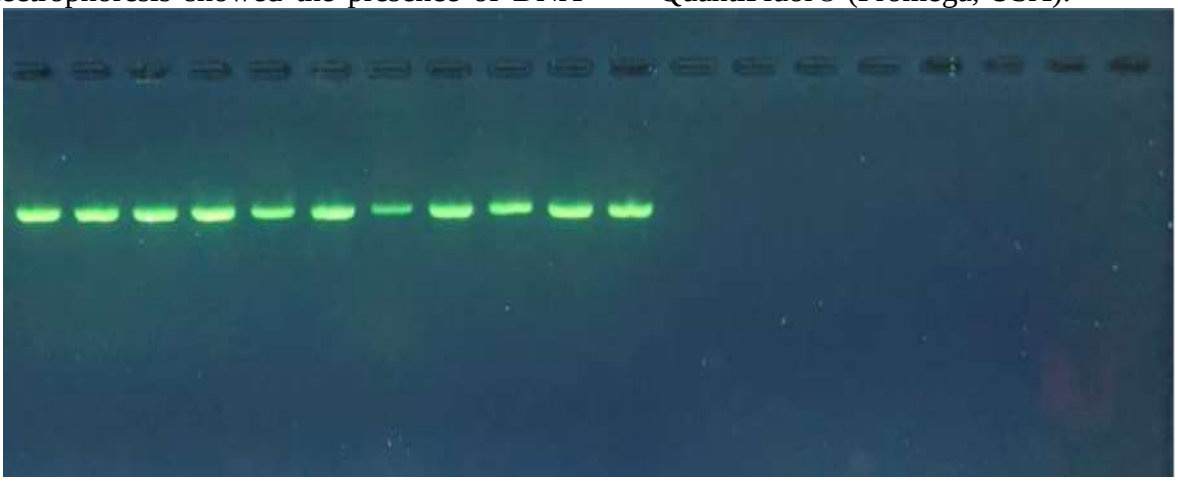

Fig. 3. Genomic DNA extraction electrphorasis (1% agarose gel at 75 volt for 30min).

Further molecular diagnosis for *P.mira bilis* bacteria by using 16sRNA gene specific primer, and the results were detected by electrophoresis on 2% agarose and exposed to U.V light in which the results indicates that all (10) isolates give the same result for biochemical diagnosis. (Fig. 4).

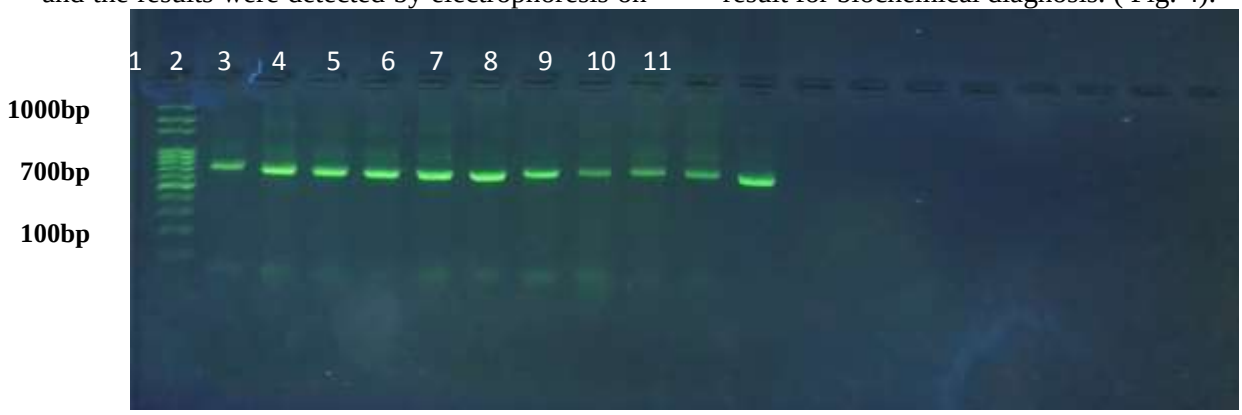

Fig. 4 . 16sRNA (of size 700bp) extraction electrphorasis on 2% agarose gel at 75 volt for 30min

Approximately nine of all the isolated (10) samples gave positive results for *UreC & HpmA* genes *(*except one sample). The results of *UreC* and *HpmA* gene with molecular size 533bp and 709bp respectively were shown in Fig. 5 and 6 The gene α-hemolysin is encodes by two genes, *hpmA* and *hpmB*, that regulate the HpmA (166 kDa) proteins. α-hemolysin is responsible for damaging tissue and activating when its N-terminal peptide has cleaved, so the results can be activating *HpmA* (140 kDa) (8 , 12)

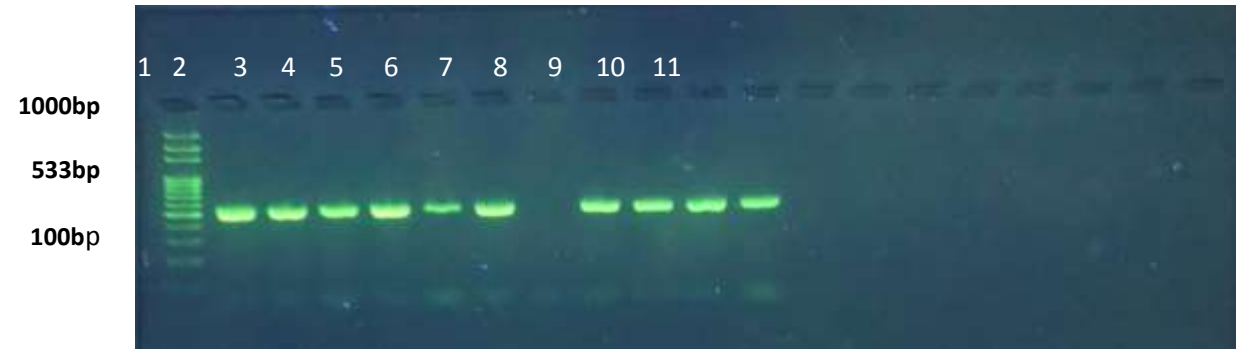

Fig. 5. Agarose gel electrophoresis (2% agarose, 75 V for 1:45 hour) of *UreC* gene PCR products (533bp) codify for urease enzyme of *P.mirabilis* isolates

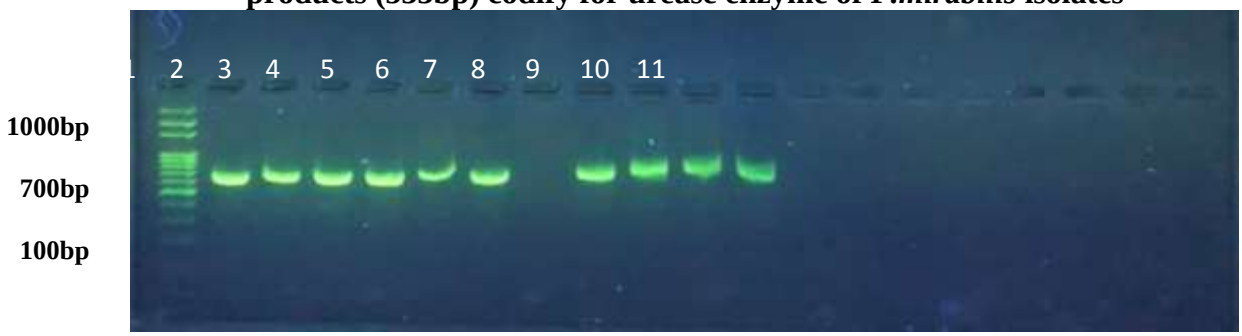

Fig.6. Agarose gel electrophoresis (2% agarose, 75 V for 1:45 hour) of *hpmA* gene PCR products (709bp) codify for hemolysin A of *P.mirabilis* isolates.

While the result of gel electrophoresis for amplification PCR product showed that the presence of bands of *rsbA* in all the samples, which means that the primer of *rsbA* gene bind specifically to complement sequence within DNA template (Fig.7).

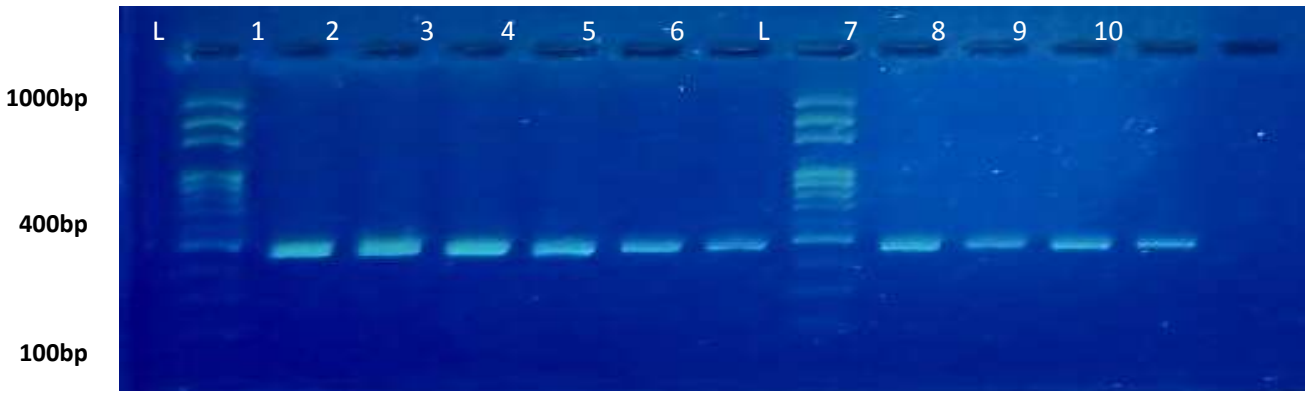

Fig.7. Agarose gel electrophoresis (2% agarose, 75 V for 1:45 hour) of *rsbA* gene PCR products (467bp) of *P.mirabilis* isolates

For *mrpA* genes detection, a primer of 565bp were used and out of the ten *p.mirabilis* isolates eight of them were carrying this gene (Fig. 8).

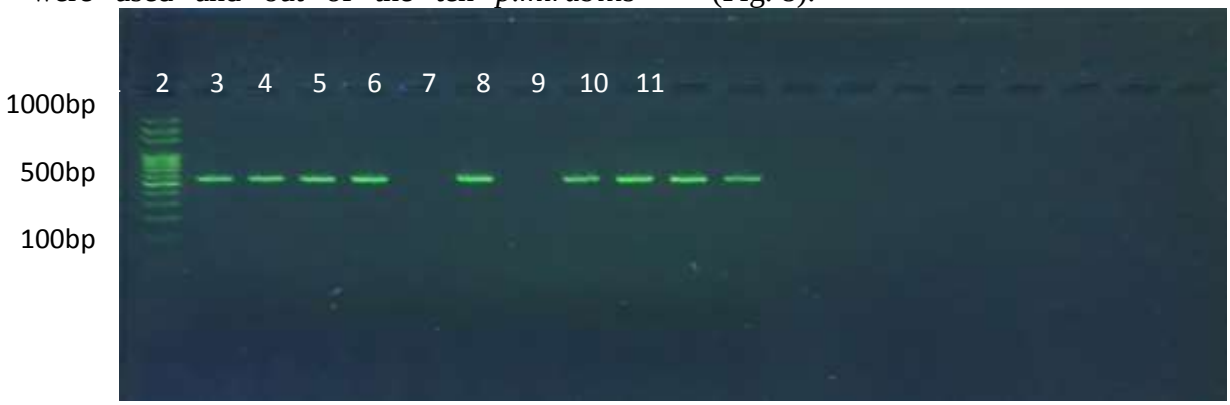

Fig. 8 . Agarose gel electrophoresis (2% agarose, 75 V for 1:45 hour) of *mrpA* gene PCR products (565bp) codify for fimbria of *P.mirabilis* isolates.

Based on the results of this study carried out in relation to Proteus microbes table (3), 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 (13).

Table 3. Represented the results of blood tests for the 10 samples isolates

Samples no.	Gender	Age	Types of bacteria	ESR (mm/hr)	RF	CRP (Mg/dl)	ACPA (U/ml)
1	**Female**	**76**	***P.mirabilis***	**73**	**+**	***60***	***67***
2	**Male**	**72**	***P.mirabilis***	**62**	**+**	***94***	***68***
3	**Female**	**83**	***P.mirabilis***	**81**	**+**	***80***	***72***
4	**Female**	**73**	***P.mirabilis***	**33**	**+**	***52***	***45***
5	**Female**	**82**	***P.mirabilis***	**39**	**+**	***48***	***43***
6	**Female**	**67**	***P.mirabilis***	**41**	**+**	***63***	***47***
7	**Male**	**78**	***P.mirabilis***	**67**	**+**	***80***	***33***
8	**Female**	**59**	***P.mirabilis***	**52**	**+**	***73***	***49***
9	**Female**	**66**	***P.mirabilis***	**61**	**+**	***62***	***70***
10	**Female**	**61**	***P.mirabilis***	**70**	**+**	***78***	***81***

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