



MOLECULAR STUDY OF *RPOB* GENE IN *PROTEUS MIRABILIS* ISOLATED FROM URINARY TRACT INFECTION FROM DIFFERENT HOSPITALS IN BAGHDAD

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Abstract

A total of 160 clinical specimens of catheterized urine collected from different hospitals in Baghdad including : Medicine city, Baghdad teaching hospital, Protect children specialist hospital and Central Child hospital, Al-Yarmook teaching hospital during the period from September 2017 to February 2018. Bacterial isolates were identified by a microscopic examination and diagnosed by using biochemical tests. These results were confirmed by identification using Api20E system.

The results showed that 103 samples were positive for bacteriological culture (64%), the percentage of *Proteus* spp. Was 52.4% (54) urine sample and percentage of *Proteus mirabilis* isolates was 74% (40 samples) (74%) while *Proteus vulgaris* was 25.9%(14 samples). The results also indicated that the isolation rate of *P. mirabilis* from females was (67%), which was higher than that of males (33%). Ten isolates of *P. mirabilis* were selected according to their sensitivity to antibiotics. Results revealed that all isolates were highly sensitive to Ciproflaxian (100%), Streptomycin (100%), Amikacin (90%), and Norfloxacin (90%) and Gentamycin (40%). On the other hand, these isolates were highly resistant to Tetracycline (100%), Trimethoprim/Sulphamethoxazole (60%), and Nalidixic acid (60%). Moderate level of resistance to Chloramphenicol (50%), and Tobramycin (20%).

The genetic study included an extraction of genomic DNA from 10 *P. mirabilis*, then detection of *rpoB* gene responsible for phylogenetic trait for these isolated which was amplified by using conventional PCR. All isolates gave positive results. The amplicons of *rpoB* gene of five isolates were randomly and sent for sequencing (Macrogen, Korea). Sequence analysis of *rpoB* genes showed 100% percent homology with genes of species *P.mirabilis*, registered in NCBI GenBank Database.

Key words : *Proteus mirabilis*, *rpoB* gene, urinary tract infections.

Introduction

Proteus is known as a nosocomial, opportunistic pathogen and is more common in community-acquired infections (Omoruyia and Evangelista, 2014). *Proteus* species (*P. mirabilis*, *P. vulgaris*, and *P. penneri*) are important pathogens of the urinary tract and primary infectious agent in patients with indwelling urinary catheters (Jacobsen *et al.*, 2008). Individuals suffering from urinary tract infections caused by *Proteus mirabilis* often develop bacteriuria, cystitis, kidney and bladder stones, and catheter obstruction due to stone encrustation, and acute pyelonephritis (Burall, 2004). Sequence analysis of the RNA polymerase β subunit encoding gene (*rpoB*)

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has been proposed as a novel tool for bacterial identification (Mollet *et al.*, 1997). *rpoB* sequencing is examined as a tool for intra-species discrimination of *P. mirabilis* clinical isolates.

Materials and Methods

A-Patients and specimens

160 Urine samples were collected from patients suffering from urinary tract infections: These samples taken from Medicine city, Baghdad teaching hospital and Central Child hospital for the period from September 2017 to February 2018.

B-Bacterial diagnosis

Isolation of *P. mirabilis* bacteria was performed by

a surface streak procedure on both blood and MacConkey agar using sterile loops and incubated at 37°C for 24 hours. Bacterial identification was made using biochemical test, and used AP20 system.

C. Antibiotic sensitivity test

All identified *P. mirabilis* isolates were tested for antibiotic susceptibility towards Nalidixic acid, Streptomycin, Amikacin, Norfloxacin, Tobramycin, Chloramphenicol, Tetracycline Trimethoprin / sulphamethazol, Gentamicin, and Amikacin antimicrobial agents by using Kirby-Bauer method. According to the clinical laboratories standard institute (CLSI, 2014), the susceptibility of tested isolates was detected depending on the size of inhibition zone formed by bacterial isolates.

D. Extraction of DNA:

DNA was extracted from ten isolates of *P. mirabilis* by using a commercial purification kit (Presto Mini Genomic DNA Kit, Geneaid, Thailand).

E. Amplification of *rpoB* gene by using conventional PCR

rpoB gene was amplified from 10 isolates of *P. mirabilis* by using specific primers (Table 1)

Table 1: Primers used in this study (Giammanco et al., 2012).

Gene	Primer sequence (5'-3')	Product size(bp)
<i>rpoB</i>	F:AACCAGTTCGCGTTGGCCTGG R:CCTGAACAACACGCTCGGA	1090 bp

Table 2: PCR program used for amplification *rpoB* amplification (Giammanco et al., 2012).

No.	Stages	Temperature (°C) and time		
1	Initial denaturation	95	4min	1 cycle
2	Denaturation	95	30 sec	30 cycles
3	Annealing	50	45 sec	
4	Extension	72	1 min	
5	Final extension	72	7 min	1 cycle

G- Sequence analysis of *rpoB* gene for genotyping of isolates.

The product of PCR amplification of *rpoB* gene in 10 isolates of *P. mirabilis* was confirmed by gel electrophoresis and purified by gel/ PCR DNA fragment extraction kit (Geneaid-Thialand). The sequencing of nucleotides was performed according to Macrogen Company, Korea by an automatic sequencer, DNA sequences were analyzed and similarity searches were carried out with the Basic Local Alignment Search tool (BLAST) in the National Center for Biotechnology Information (NCBI) website (<http://www.ncbi.nlm.nih.gov>).

Results and Discussion

Isolation and identification of *P.mirabilis*

Loopful amount from these samples were inoculated on MacConkey agar and blood agar and then incubated overnight at 37°C. A distinguishable swarming which is a unique characteristic for genus *Proteus* was observed, which is considered as confirmatory phenomenon for genus *Proteus* as previously described by (Liaw et al., 2000).

It was found that 103(64.3%) out of the total 160 samples collected gave positive results on MacConkey agar and Blood agar. These results were agreed with those reported by Al-Aabideen, (2005). Who found that the percentage of positive cultures of urine samples were 64.6%. However, such results were disagreed with those of Al-Kabby, (2007). Who found that percentage of positive culture of urine samples was 28.9%. The reason of the differences in percentage may be owed to differences in either size of samples or hospital locations as well as to the season and medications before sampling.

Result showed that 54 isolates belong to genus *Proteus* spp. from the 103 positive cultures, so the isolation percentage of *Proteus* from other bacteria of the UTI cases was 52.4%. This result was agreed with that of Ahmed, (2015) who found that *Proteus* isolates were representing 60% of the UTI cases tested. Nonetheless, this result disagreed with Adnan, (2014) who found that isolation percentage of *Proteus* occurrence (17.8%). From the previous results, *P. mirabilis* represent 74% (40 isolates) while *P. vulgaris* appeared only in 25.9% (14 isolates). The present results with previous study done in by Yassen and Khelkal, (2015) who mentioned that *P. mirabilis* performed 77% while *P. vulgaris* performed 23%.

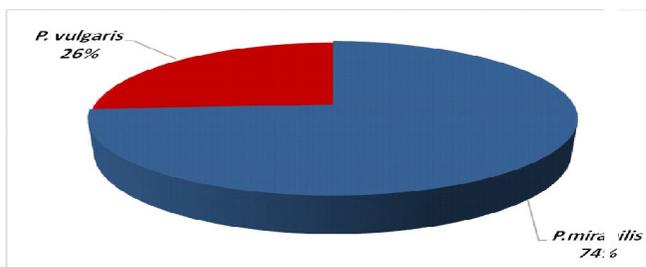


Fig. 1: Percentage of isolates of *P. mirabilis* as compared with *P. vulgaris* from patient suffering from UTI.

For confirmation of the biochemical results, the API 20E strips were used for Enterobacteriaceae identification containing 12 tests. The results revealed that the tested isolate was *P. mirabilis* as indicated in Fig. 2.



Fig. 2: API 20 E confirmatory test for *P. mirabilis* characterization.

Antibiotic susceptibility test

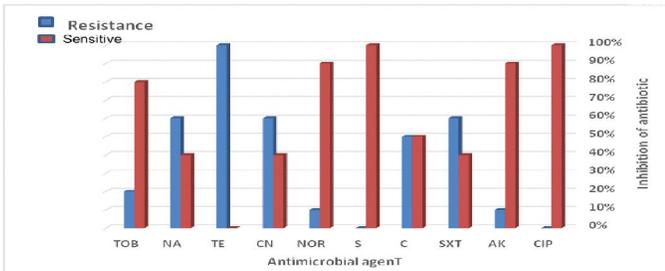


Fig. 2: Susceptibility of *P. mirabilis* to antibiotics.

In this study tested *P. mirabilis* isolates showed high sensitivity toward Ciprofloxacin (100%). These results were agreed with that reported by Saeed *et al.*, (2017) who showed that the susceptibility to Ciprofloxacin reaches 100%. High sensitivity to Streptomycin (100%) was also found which agreed to that reported by Habibu, (2014) who found that the susceptibility to this antimicrobial to 100%. *P. mirabilis* isolates showed high sensitivity to Amikacin reaches 90% these results were agreed to that reported by Al-Jumaily and Zgaer, (2016) who detected that the susceptibility to this antimicrobial was 92.6%. High Norfloxacin sensitive rate was also mentioned by Adnan *et al.*, (2014) who found that the *P. mirabilis* isolates were vary in their to sensitivity with percentage of 91.6%.

P. mirabilis show moderate sensitive to Gentamycin reaches 40% because it belongs to Aminoglycosides, which are powerful broad spectrum antimicrobials and are inhibitors of protein synthesis. Qaddoori *et al.*, (2015) demonstrated that the rate of susceptibility to gentamicin was (40%) which agree with result but were disagreed with result of Habibu, (2014) who found only 70% sensitive percentage

Only (20%) of the isolates showed resistant to Tobramycin. Kadhim *et al.*, (2014) was found that the *P. mirabilis* isolates were vary in their resistance to Tobramycin with a resistance percentage of 16.6%. But this result was disagreed with that of AL-Oqali, (2017) who found only (76.56%) resistance percentage. *P. mirabilis* showed (50%) resistance to chloramphenicol, which agreed with Zuhir and Alaubydi, (2016) who found that the *P. mirabilis* isolates were vary in their resistance to chloramphenicol with a resistance percentage of

62.5%. High resistance to tetracycline was found among the isolates 100%. Fadhail *et al.*, (2013) found that the *P. mirabilis* isolates were vary in their resistance to tetracycline which could reach to resistance percentage of 95.41%.

Isolates showed moderate resistance to Nalidixic acid (60%). Which agreed with Hussein, (2013) results who found that the *P. mirabilis* isolates were vary in their resistance to nalidixic acid with a resistance percentage of 69.2%. However, this result disagreed with Saeed, (2017) who found a resistance percentage 100%.

Results shown in figure 3 concerning trimethoprim-sulfamethoxazole indicate that *P. mirabilis* isolates developed moderate resistance reaches at 60% which agree with Al- Kazaz, and Al-Bassam, (2013) who reported a resistant percentage of 65%.

Amplification of *rpoB* by conventional PCR techniques

Amplification of *rpoB* gene was done using specific PCR primer. Result shown in figure 4 indicated successful amplification of the gene for all isolates as indicated by the presence of band with molecular size 1090 bp. Regarding *rpoB* gene these result are in agreement with a previous study by Mollet *et al.*, (1997) who isolated *P. mirabilis* from UTI samples and obtain the same molecular size band for *rpoB* gene in their isolates.

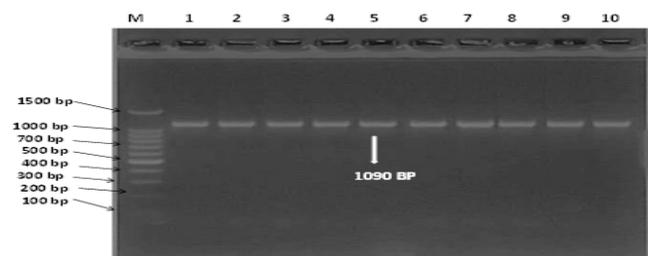


Fig. 3: Gel electrophoresis for amplification of *rpoB* gene of *P. mirabilis* Lane M (100 bp) ladder, Lines: 1-10 represent isolates 1, 3, 6, 8, 10, 11, 16, 18, 19 and 35 respectively. The product was electrophoresis on 1% agarose at 110 volt for 60 min.

Sequence analysis of *rpoB* gene

The sequence homology of *rpoB* gene was obtained by comparison with closest blast sequence in gene bank. Similarity was calculated using FASTA method. Data shown in table 3 indicate as much as 100% similarity of two isolated with that sequence of *P. mirabilis* AR-379. However, two isolate show 100% homology with that of *P. mirabilis* AR-CCG70746 and other isolate show 100% homology with that of *P. mirabilis* AR-379.

These results were in agreement with several other investigation who Mohammed, (2014) who showed

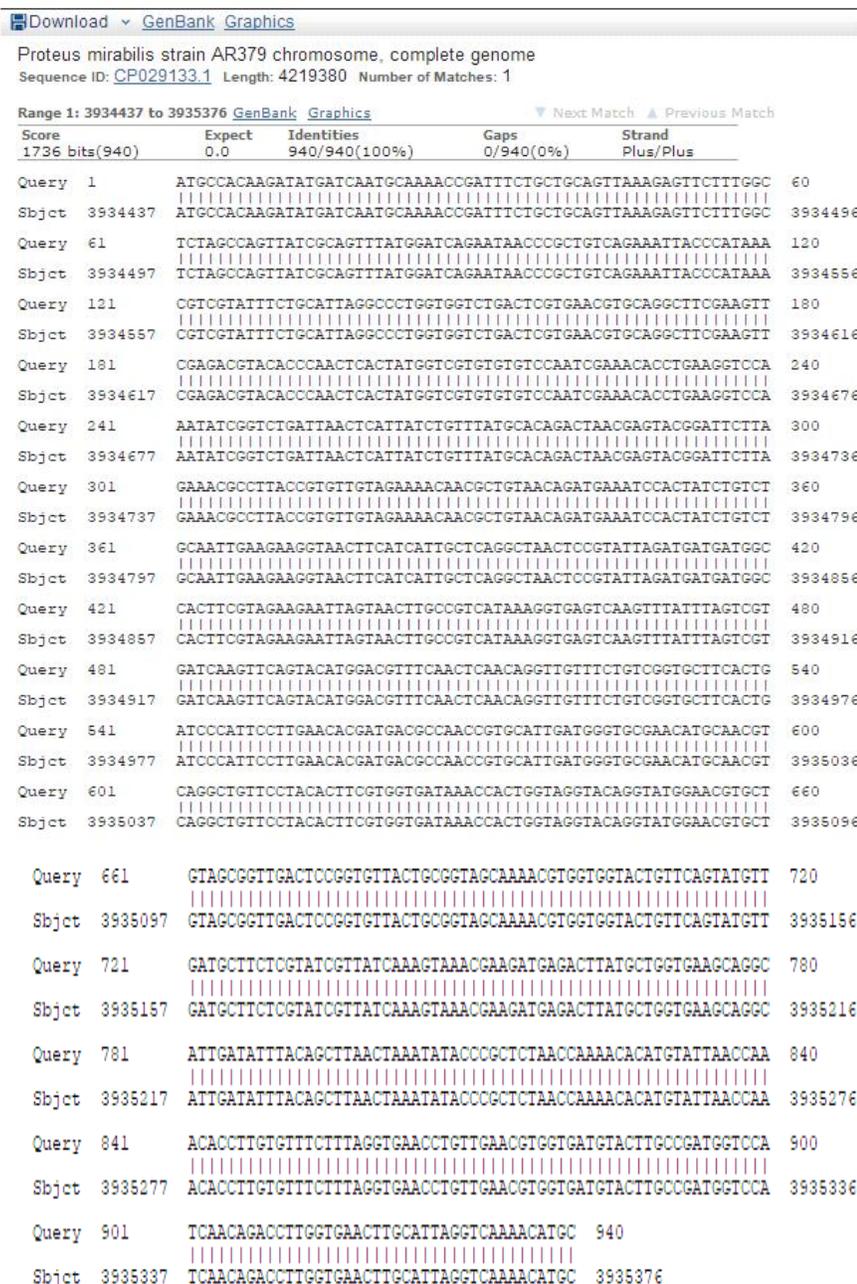


Fig. 4: Nucleotide sequences of the *P. mirabilis rpoB* gene as alignment with sequence of *rpoB* gene for AR-379 of Gen Bank.

Table 3: Average similarity percentage of *rpoB* sequence of *P. mirabilis* isolated from UTI patients in comparison to sequence obtained from NCBI blast.

No. of Local Isolate	NCBI Blast Alignment	<i>rpoB</i> Sequence Identity (%)
1	<i>P. mirabilis</i> AR-379	100%
3	<i>P. mirabilis</i> AR-CCUG70746	100%
6	<i>P. mirabilis</i> AR-GN2	100%
19	<i>P. mirabilis</i> AR-379	100%
35	<i>P. mirabilis</i> AR-CCUG70746	100%

sequence similarity to *P. mirabilis* strain ATCC 56283712 with 99% homology.

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