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ORIGINAL RESEARCH

Molecular study of genetic diversity in uropathogenic *Proteus* spp using RAPD-PCR

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Abstract

Objective: *Proteus* species are the most important cause of urinary tract infections after *Escherichia coli*. Due to the increasing prevalence of this pathogen and the emergence of antibiotic-resistant strains, this study aims to investigate the genetic diversity between *P. vulgaris* and *P. mirabilis* isolates isolated from patients with UTI.

Materials and Methods: 260 samples were collected from October to January 2021 from men and women in different age distributions. *P. vulgaris* and *P. mirabilis* species were identified using routine biochemical and microbiology methods. After determining their antibiotic resistance pattern, the genetic relationship of the selected strains was determined using the RAPD-PCR technique.

Results: The antibiotic resistance pattern investigation results showed that all the isolated strains were sensitive to nalidixic acid, cephalothin, ciprofloxacin, and nitrofurantoin. In contrast, all the strains had 100% resistance to tetracycline and penicillin. 34 *P. vulgaris* isolates were classified into two clusters, A and B. Cluster B predominated among *P. vulgaris* isolates, and the isolates in this cluster had the most similarities. In comparison, cluster A contained just two isolates. Also, 22 isolates of *P. mirabilis* were placed in 4 clusters (A-D). The most prevalent sizes of DNA banding patterns identified were 300, 400, 600, and 1000 base pairs.

Conclusion: These findings revealed the genetic variety of the investigated clinical *P. mirabilis* and *P. vulgaris* isolates. RAPD-PCR was shown to be a suitable fingerprinting technology for *Proteus* spp. which may be employed for epidemiological investigations and infection control measures.

Keywords: *Proteus* spp., Urinary tract infection, RAPD-PCR, Genotyping

Introduction

Proteus spp. are Gram-negative rods in the *Enterobacterales* order and the *Morganellaceae* family [1]. These species are found in the typical bacterial ecology of the human and animal digestive tracts and in soil and water. Their existence is thought to be due to fecal contamination [2]. *Proteus* species are considered the third cause of hospital-associated infections [3]. *Proteus* spp. Includes six species: *P. mirabilis*, *P. vulgaris*, *P. penneri*, *P. cibarius*, *P. terrae*, and *P. hauseri*, as well as three genomospecies 4, 5, and 6. *P. vulgaris*, *P. mirabilis*, and *P. penneri* are frequent infections afflict immunocompromised people [4]. *P. mirabilis* is the most often detected species from clinical samples, with 90% originating from urinary tract infections (UTIs) but also respiratory, eye, ear, nose, skin, burn, meningoencephalitis, osteomyelitis, and wound infections [5]. *P. mirabilis* is one of the most prevalent causes of UTIs after *E. coli*, particularly in patients with anatomical abnormalities in the urinary system or using catheters or other urinary tools. Cystitis and acute pyelonephritis are typical *P. mirabilis*-related UTIs [6]. In addition, *P. mirabilis* infections produce urinary stones, which may cause significant injury to the bladder and kidneys and obstruct urinary catheters [7]. According to reports, the majority of UTIs caused by *P. mirabilis* are the result of catheter use. It is known that *P. vulgaris* is oxidatively harmful, non-spore-forming, non-capsulated, and capable of causing wound infections, UTIs, bloodstream infections, burn infections, and respiratory tract infections [8]. *P. vulgaris* has swarming motility, which may be viewed as a wave-like movement over the surface of a solid agar media. *P. vulgaris* rods form a crystalline biofilm on the surface of urinary catheters, obstructing the catheter and causing severe repercussions for patients, such as acute pain with urinary retention or incontinence [9]. Several mechanisms, including urease and protease synthesis, fimbria-mediated bacterial adhesion to the uroepithelium, flagella-mediated swarming motion, expression of outer-membrane proteins, hemolysin, and ion acquisition, contribute to the pathogenicity of these bacteria [9,10].

Reports of epidemics caused by *P. mirabilis* and *P. vulgaris* show the need to develop appropriate epidemiological investigative methodologies [11]. Identifying possible infection origins requires reliable detection, classification, and subtyping of strains for efficient epidemiological surveillance and monitoring [12]. *Proteus* strain genotyping may aid in identifying particular pathogens in a given host and act as a guideline for determining the source of infection in epidemiological investigations. Several traditional typing approaches, such as plasmid typing, serotyping, antibiogram, and phage typing, are expensive and time-consuming but cannot distinguish among highly associated strains; thus, there is a growing emphasis on developing and applying molecular techniques [13]. Random Amplified Polymorphic DNA (RAPD) - PCR has attracted much interest in epidemiological investigations because of its simplicity, speed, sensitivity, repeatability, and low cost (no need for expensive specialized instrumentations) [14]. RAPD-PCR is a powerful technique for the definitive molecular identification of bacteria. Several studies have suggested this technique for screening many isolates due to its high efficiency [15]. RAPD techniques are PCR reactions, but a small (8–12mers) single primer may bind randomized to several DNA sequences in the bacterial genome to generate repeating electrophoresis patterns useful for genotypic distinction [16]. Since *Proteus* species are one of the most important causes of nosocomial infections, especially in people who use catheters, it is essential to understand the transmission of *Proteus* strains from an epidemiological point of view to monitor the spread of strains. Therefore, we exposed clinical *P. mirabilis*, and *P. vulgaris* isolates to the RAPD-PCR test to assess this approach's efficiency, reliability, and discriminatory capacity for epidemiological research of this pathogen and the genetic diversity among the isolates.

Materials and Methods

Study sample

In a cross-sectional investigation, random urine samples from 260 individuals with UTIs were obtained from different hospitals in Shahrekord, Iran, between October and January 2021. Urine samples were collected using

clean-catch midstream urine. Blood agar (Merck, Germany) and MacConkey agar (Merck, Germany) cultures were used to identify bacteria isolated from UTIs. Then all the samples were detected utilizing traditional microbiological and biochemical tests, such as catalase and urease production, citrate consumption, motility, gas production from glucose, gelatin hydrolysis, H2S production, Methyl Red (MR), nitrate reduction, potassium cyanide growth, and glucose and glycerol fermentation. After identifying the isolates as *P. mirabilis* and *P. vulgaris*, they were kept at -20 °C on TSB (trypticase soy broth) with 15% glycerol for subsequent use [17].

Antibiotic susceptibility testing

The Kirby– Bauer disk diffusion technique was used to determine antibiotic resistance following Clinical Laboratory Standards Institute standards (CLSI 2021). The following antibiotics were employed: Penicillin (P, 10µg/disk) 10µg, Tetracycline (TE,30 µg/disk), Streptomycin (S, 10 µg/disk), Chloramphenicol (C, 30 µg/disk), sulfamethoxazole-Trimethoprim (SXT, 1.25/23.75 µg/disk), Gentamycin (GM, 10 µg/disk), Enrofloxacin (NFX, 5 µg/disk), Nalidixic acid (NA, 30 µg/disk), Cephalothin (CF, 30 µg/disk), Ciprofloxacin (CIP, 5 µg/disk), Trimethoprim (TMP, 5 µg/disk), Nitrofurantoin (E/M, 300 µg/disk), Ampicillin (AM, 10 u/disk) [18].

DNA extraction

Following the manufacturer's instructions (Sinaclon, Iran), bacterial genomic DNAs were obtained: In microtubes, 100 µl of sterile PBS (phosphate-buffered saline) was infused with 100 µl of bacteria using a sterile needle. Each tube received 400 µl of lysis buffers, which were then shaken to combine. 300 µl of precipitation buffers were added and stirred slowly. The whole mixture of microtubes was transferred to special filter tubes and centrifuged for one minute at 13,000 rpm. Then, 400 µl of washing buffer (1) was added to the filter pipes and centrifuged at 13,000 rpm for one minute. Next, 400 µl of washing buffer (2) was passed to filter pipes and centrifuged at 13,000 rpm for one minute, and this was done again. Next, filter pipes were centrifuged at the same speed without adding any material, and dry filters were taken and deposited in sterile microtubes. Then 50 µl of a dilution buffer that had been heated in a thermoblock at 65 °C for 3-5 minutes was added to the filter pipes and centrifuged at the same speed. Subsequently, filters were discarded, and the tube containing bacterial DNA was frozen at -20 °C in preparation for the next phase of investigations [19].

RAPD-PCR used for genotyping detection

From 127 isolates of *P. vulgaris* and 72 isolates of *P. mirabilis* isolated from 260 investigated samples, 34 isolates of *P. vulgaris* and 22 isolates of *P. mirabilis* were selected for genotyping by the RAPD-PCR method. These isolates from both males and females and from different age groups were randomly selected. To do this, 5 µL of the DNA sample was added to 20 µL of PCR amplification mixture comprising 1.2 µL dNTPs (8 mM), 2 µL of 5 × PCR buffer, 1.2 µL MgCl2 (3 mM), 7.3 µL of DNase/RNAse free distilled water (Qiagen, Germany), 0.3 µL of 1 U Taq Polymerase and 1.5 µL forward and reverse primers. Amplification reactions were conducted using a random primer (5'-GGGTGGGTAA-3') manufactured by CinnaGen Company and a thermal cycler (Flexcycler2, Germany) [3]. The amplification protocol consisted of an initial denaturation stage at 95 °C for 5 minutes, 30 × 90 °C for 30 seconds; 55 °C for 30 seconds; extension at 72 °C for two minutes; and a final extension at 72 °C for ten minutes. To analyze the PCR product, 1% electrophoresis gel stained with safe stain (DNA Safe Stain, CinnaGen, Iran) was used in a TBE buffer. Under transilluminator UV light (Uvitech, UK), gels were observed. A 100 bp DNA ladder (Thermo Fischer Scientific, USA) was employed as a molecular weight marker.

Statistical analysis

For genotyping data analysis, the similarity matrix between strains was then produced by matching simple and dice similarity indices using GelClust and Fig Tree tools. UPGMA was used to plot the associated dendrograms, which were then grouped using an 80% matching coefficient.

Results

***Isolation rate of Proteus* spp**

This study showed that out of 260 collected samples, *P. vulgaris* was detected in 127 (48.84%) samples and *P. mirabilis* in 72 (27.

69%) samples. Table 1 shows a significant relationship between gender (male and female) and infection rate. This study showed that the rate of UTIs with both *Proteus* spp. is higher in women than men (**Table 1**). Also, this study investigated the percentage of *Proteus* spp. according to different age groups. According to the obtained results, the infection rate of *Proteus* spp. increased with age.

Table 1. Distribution of *Proteus* species isolated by age and sex.

Age groups (year)	No. samples	No. positive *P. vulgaris* (%)			No. positive *P. mirabilis (%)*		
		No. positive *P. vulgaris* (%)	Male	Female	No. positive *P. mirabilis* (%)	Male	Female
<10	32	3 (9.37%)	-	3 (100.00%)	4 (12.50%)	1 (25.00%)	3 (75.00%)
10-20	52	6 (11.53%)	2 (33.33%)	4 (66.67%)	9 (17.30%)	-	9 (100.00%)
20-30	64	22 (34.37%)	2 (9.09%)	20 (90.91)	16 (25.00%)	2 (12.50%)	14 (87.50)
30-40	38	26 (68.42%)	3 (11.53%)	23 (88.47%)	14 (36.84%)	4 (28.57%)	10 (71.43%)
40-50	28	20 (71.42%)	4 (20.00%)	16 (80.00%)	10 (35.71%)	3 (30.00%)	7 (70.00%)
50<	46	41 (89.13%)	5 (12.19%)	36 (87.81%)	19 (41.30%)	4 (21.05%)	15 (78.95%)
Total	260	127 (48.84%)	16 (12.59%)	111 (87.41%)	72 (27.69%)	14 (19.44%)	58 (80.56%)

Antibiotic susceptibility pattern

All *P. vulgaris* and *P. mirabilis* isolates were exposed to an antibiotic sensitivity test using the disk diffusion method against 13 antibiotics. The results of antimicrobial sensitivity tests (**Table 2**) demonstrated that chloramphenicol, enrofloxacin, nalidixic acid, cephalothin, ciprofloxacin, and nitrofurantoin antibiotics were the most effective antibiotics against *P. vulgaris and P. mirabilis* in the male group. Antimicrobial resistance of *Proteus* spp. isolates was most robust against tetracycline (100 %) and penicillin (100 %) in both male and female isolates, as shown in **Table 2**. According to the obtained results, women showed more antibiotic resistance than men. *P. vulgaris* had the highest antibiotic resistance to gentamycin (80.18%), trimethoprim (71. 17%), and sulfamethoxazole-Trimethoprim (63.06 %) in the female group, while the lowest antibiotic resistance belonged to nitrofurantoin (7.02%) and chloramphenicol (8.10 %). The results also revealed that *P. mirabilis* was resistant to the following antibiotics: gentamycin, ampicillin, and trimethoprim, with percentages of 72.41%, 68.96%, and 67.24% in the female group, respectively.

Table 2. Antimicrobial sensitivity pattern of *Proteus* spp. to various antibiotics.

Antibiotics	*P. vulgaris*		*P. mirabilis*	
	Male (16)	Female (111)	Male (14)	Female (58)
P10*	16 (100%)	111 (100%)	14 (100 %)	58 (100 %)
TE30	16 (100%)	111 (100%)	14 (100%)	58 (100%)
S10	5 (31.25)	61 (54.96%)	5 (35.71%)	31 (53.44%)
C30	2 (12.5%)	9 (8.10%)	2 (14.28%)	4 (6.89%)
SXT25	4 (25%)	70 (63.06%)	4 (28.57%)	34 (58.62%)
GM10	9 (56.25%)	89 (80.18%)	9 (64.28%)	42 (72.41%)
NFX5	2 (12.5%)	62 (55.85%)	2 (14.28%)	31 (53.44%)
NA30	2 (12.5%)	62 (55.85%)	2 (14.28%)	27 (46.55%)
CF30	2 (12.5%)	37 (33.33%)	5 (35.71)	18 (31.03%)
CIP5	2 (12.5%)	61 (54.95%)	2 (12.5%)	31 (53.44%)
TMP5	3 (18.75%)	79 (71.17%)	3 (21.42%)	39 (67.24%)
F/M300	2 (12.5%)	8 (7.02%)	2 (14.28%)	7 (50.00%)
AM10	7 (43.75%)	81 (72.97%)	4 (6.89%)	40 (68.96%)

*In this table P10= penicillin (10 u/disk); TE30= tetracycline (30 µg/disk); S10= streptomycin (10 µg/disk); C30= chloramphenicol (30 µg/disk); SXT= sulfamethoxazole-Trimethoprim (1.25/23.75 µg/disk); GM10= gentamycin (10 µg/disk); NFX5= enrofloxacin (5 µg/disk); NA30= nalidixic acid (30 µg/disk); CF30= cephalothin (30 µg/disk); CIP5= ciprofloxacin (5 µg/disk); TMP5= trimethoprim (5 µg/disk); F/M300= nitrofurantoin (300 µg/disk); AM10= ampicillin (10 u/disk).

RAPD-PCR molecular typing

From 127 isolates of *P. vulgaris* and 72 isolates of *P. mirabilis* isolated from 260 examined samples, 34 isolates of *P. vulgaris* and 22 isolates of *P. mirabilis* were selected for genotyping. The characteristics of the selected isolates are shown in the **table 3**.

Table 3. *Proteus* spp. isolates isolated for RADP-PCR.

P. vulgaris		*P. mirabilis*		
Age group	Male	Female	Age group	Male	Female
<10	0	1	**<10**	0	1
10-20	2	0	**10-20**	0	2
20-30	1	7	**20-30**	1	4
30-40	1	6	**30-40**	1	5
40-50	1	4	**40-50**	3	0
50<	2	9	**50<**	0	5
Total	7	27	**Total**	5	17

The isolates' genetic profiles were then evaluated using RADP-PCR. This method produced 2-10 amplification bands with sizes ranging from 300 to 1500 bp. *P. vulgaris* isolated was classified into two clusters (A and B) (**Fig 1A**). Considering the similarity above 80%, the isolates with the highest degree of similarity were placed in one group. Since most isolates had the same banding pattern, cluster B was the dominant cluster with a minimum similarity of 74% and the highest similarity of 100%. In addition, strains 14 and 9 displayed identical banding patterns and were assigned to the A cluster (**Fig 1A**). Isolate 33 had the slightest resemblance with other *P. vulgaris* isolates. In addition, isolates 1, 2, 3, 4, 5, 6, and 7 had the highest degree of similarity with other isolated strains.

RAPD-PCR with primer OPZ08 was used to identify the genetic patterns of 22 *P. mirabilis* isolates identified. Each sample's bands were counted. According to dendrogram analysis, RADP-PCR classified the isolates into four (A-D) clusters with 80% similarity (**Fig 2A**). According to the data, strains 8, 12, 18, and 19 were not identical to any *P. mirabilis* isolates. Isolates 3, 4, 10, and 15 had 100% similarity with each other. In addition, 13 and 14 isolates had the most significant similarity. Isolates 20, 21, and 22 with the lowest degree of similarity were reported in subgroup A (**Fig 2A**). Also, the highest degree of similarity, equal to 79.2%, was written

between 34 *P. vulgaris* with all isolates of *P. mirabilis* except isolate 16 (**Fig 3**).

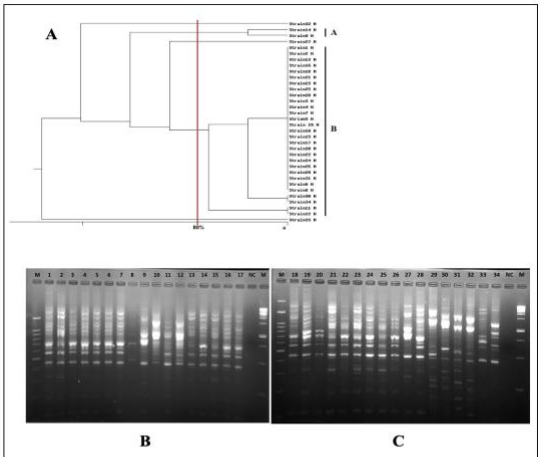

Figure 1. RAPD-PCR results for 34 uropathogenic *P. vulgaris* isolates. (A) The dendrogram was created using the RAPD-PCR fingerprinting of 34 clinical isolates of *P. vulgaris*. Isolates were divided into two groups (A, B). (B, C) PCR gel electrophoresis of *P. vulgaris* isolates in the RAPD analysis (Lane M, left= 100bp DNA Ladder, Lane M, right= 1kb DNA Ladder, Lane NC= Negative control sample, Lanes 1-34= *P. vulgaris* selected isolates)

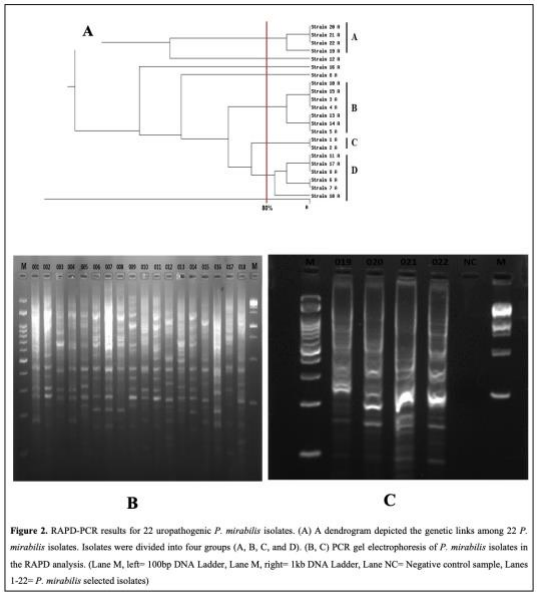

Figure 2. RAPD-PCR results for 22 uropathogenic *P. mirabilis* isolates. (A) A dendrogram depicted the genetic links among 22 *P. mirabilis* isolates. Isolates were divided into four groups (A, B, C, and D). (B, C) PCR gel electrophoresis of *P. mirabilis* isolates in the RAPD analysis. (Lane M, left= 100bp DNA Ladder, Lane M, right= 1kb DNA Ladder, Lane NC= Negative control sample, Lanes 1-22= *P. mirabilis* selected isolates)

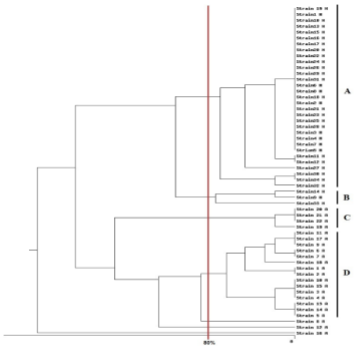

Figure 3. RAPD-PCR results for 56 uropathogenic *Proteus* spp. isolates. (A) The dendrogram was created using the RAPD-PCR fingerprinting of 56 clinical isolates of *P. vulgaris* and *P. mirabilis*. Isolates were divided into 4 groups (A-D).

Discussion

Proteus species are known as commensal microbes of the human digestive system, varying in abundance depending on location. Clinically, they are most often known as a cause of urinary tract infections. *Proteus* spp. is the third most common cause of hospital-acquired infections and the third most common source of community-acquired illnesses [20, 21, 22]. *P. mirabilis* and *P. vulgaris* are often isolated from urine because they are prevalent causes of catheter associated UTIs [22]. This research describes the antimicrobial resistance and genotyping of bacteria isolated from urinary tract infections (UTIs) in Iranian hospitals. The prevalence of multidrug resistance is a current concern for urinary tract infections (UTIs) and a significant obstacle in treating UTIs. Growing antimicrobial resistance and the increase in the frequency of multidrug-resistant bacteria that cause UTIs have become serious issues for the public's health due to the irrational and improper use of antimicrobials and the widespread presence of antibiotics in the food chain [23]. The present research examined the antimicrobial susceptibility profile and phylogenetic grouping of *P. vulgaris* and *P. mirabilis* isolates associated with community-acquired UTI (n=260). Additionally, we investigated the link between these factors. Because of the physical and structural variations between the sexes, our study found that the prevalence of UTIs was much higher in females than in males. These findings aligned with similar studies on UTIs [24,25].

According to the results obtained from this study, *P. vulgaris* and *P. mirabilis* isolates in both male and female groups were 100% resistant to penicillin and tetracycline antibiotics. The findings are comparable to prior research on UTIs [22,26]. Resistance to several routinely prescribed antibiotics, such as ampicillin, trimethoprim, gentamycin, and co-trimoxazole; was considerably more excellent among female UTI isolates than male UTI isolates. This was the case when compared to isolates from patients with a urinary tract infection. In addition, *Proteus* isolates in the female group were sensitive to chloramphenicol. Our study found that the prevalence of *Proteus* spp. was not constant across all age groups further subdivided by

gender (Table 1). For example, *P. vulgaris* was less common in male subjects aged 20 years and 60 (33.33% and 12.19%, respectively) and more common in female patients aged 20 years and 30 (87.41%). The incidence of *P. mirabilis* was reported to be most significant (100%) in young females aged 10 to 20. Enrico Magliano et al. did research with a patient group similar to ours (both males and females of any age). Their results showed that urinary tract infections caused by *Escherichia coli* were less common in young and older men, while the highest prevalence was in women aged 15 years and older [27]. Our findings supported the theory that hospital-acquired infections are more resistant than community-acquired infections due to selection pressure, inconsistent antibiotic use, etc. Another objective in determining the antimicrobial susceptibility profile of the *P. vulgaris* and *P. mirabilis* isolates was to test for compatibility among these strains. Similarities between the *P. vulgaris* and *P. mirabilis* isolates might be revealed by analyzing their antibiotic susceptibility data. Antibiotic resistance patterns did not consistently exhibit sufficient strain-to-strain heterogeneity to categorize samples into distinct groups [28]. It takes genetically based strategies to distinguish between isolates that differ slightly in their resistance profiles. There has been increased research into how to trace the origin of microbes, leading to the development of several genotyping-based techniques [28].

Bacterial isolates have been compared and studied using several different typing methods. However, PCR-based approaches are often used because of their accuracy, rapidity, reproducibility, sensitivity, specificity, and reliability when analyzing DNA fingerprints [29, 30]. Among several methods, RAPD-PCR stands out for its specificity and sensitivity in identifying bacterial isolates due to its characteristics. The RAPD technique may successfully identify genetic changes across isolates and type a broad range of bacterial species. Numerous bacteria, including *Vibrio*, *Shigella*, *Staphylococcus aureus*, *Salmonella* spp., and many more, have been molecularly typed using this method [31]. The RAPD-PCR methodology proved more successful and sensitive than other techniques for fingerprinting and distinguishing Proteus strains. In bacterial epidemiology, determining genetic similarity between bacteria is essential for evaluating the risk of cross-infection. This has led to the widespread adoption of the RAPD-PCR technique because of its ease of use, speed, low cost, and reproducibility [31].

The RADP-PCR uses shorter primers (8-12 nucleotides long) with optional sequences that bind to nonspecific locations on microbe template DNA. The amplification of repeating portions of template DNA aids in constructing a unique profile for bacterial identification [32]. In order to better understand the clonal variability and phylogenetic closeness of clinical *Proteus* isolates, we decided to use RAPD-PCR since it is a straightforward and inexpensive genotyping tool for differentiating different strain types. The current study, 260 urine samples were collected from UTI patients over three months. 34 *P. vulgaris* and 22 *P. mirabilis* isolates were collected using microbiological and biochemical methods and then genotyped. The RAPD-PCR method was used to obtain DNA fingerprinting patterns after gel electrophoresis. *P. mirabilis* and *P. vulgaris* are fully typeable since all 56 isolates produced banding patterns. DNA banding patterns indicated that 300, 400, 600, and 1000 base pairs were the most prevalent sizes. Using the banding patterns generated by RADP-PCR, the UPGMA application created a dendrogram. Accordingly, 34 isolates of *P. vulgaris* were placed in two clusters (A, B), and 22 isolates of *P. mirabilis* were identified in 4 groups (A-D). There are 29 *P. vulgaris* isolates with the same resistance genotype and belonging to the same clone type B. The presence of this cluster provides evidence for the spread of clone type B *P. vulgaris* isolates worldwide. Genomic similarities were found in preliminary comparisons between *P. mirabilis*, and *P. vulgaris* isolates using the (5'-GGGTGGGTAA-3') primer. Using this primer, it has been determined that only one *P. vulgaris* isolate is similar to all *P. mirabilis* isolates. Isolates that share a cluster's genomic profile may also have a common mechanism for resisting antibiotics. The discriminating index was used to evaluate the level of discrimination achieved by this method. This numerical indicator illustrates the typical probability of distinguishing two unrelated strains as different types. In this study, RADP-PCR was shown to have an 80% discriminative index.

Similarly, research comparing and using various PCR-based approaches to establish the epidemiologic relatedness of *P. mirabilis* have been conducted. Michelim et al. employed 33 isolates, comprising 29 *P. mirabilis* isolates, one *E. coli* isolate, one Hafnia alvei isolate, and two *Morganella morganii* isolates. *E. coli*, *M. morganii*, and *H. alvei* were differentiated from each other and Proteus strains after being subjected to PCR-based typing techniques such as RAPD, ERIC-PCR, and BOX-PCR [33]. Using the RADP-PCR technique, Lamees A. Abdul–Lateef investigated the relationship between 18 clinical isolates of *P. vulgaris*. Their results show that analysis of *P. vulgaris* genomes from diverse samples revealed that *P. vulgaris* strains are not specific for infection [3]. Likewise, the RAPD approach was used to characterize 187 *E. coli* strains obtained from urine samples of inpatients and outpatients. The results showed that the isolated *E. coli* strains have high genetic diversity because each cluster has its characteristics [12]. In another investigation, Sosa et al. used the ERIC-PCR approach to evaluate the genetic diversity of *P. mirabilis* isolates. All collected isolates were typed using this approach, and the DNA fingerprint revealed 4 to 17 amplification bands with widths ranging from 400 to 4000 bp, indicating the genetic variety of their isolates [34].

The RAPD-PCR approach was employed to categorize *Proteus* strains genetically, one of the most significant and prevalent bacteria. The investigation of isolates in multiple subgroups suggests the sufficient differentiation power of this technique in genotype differentiation. We could categorize the 56 isolates investigated using RAPD-PCR and a primer sequence. The current study's findings demonstrated that the RAPD-PCR technique is a straightforward, rapid, and low-cost tool for describing the genetic diversity of several *Proteus* spp., including *P. vulgaris* and *P. mirabilis* strains. More research on samples from other hospitals is suggested, as is a comparison of the RAPD-PCR approach to newer molecular technologies.

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Conflict of interest

Author declares no conflict of interest.

References:

1. Girlich D, Bonnin RA, Dortet L, Naas T. Genetics of acquired antibiotic resistance genes in Proteus spp. Frontiers in Microbiology. 2020 Feb 21;11:256.
2. Hamilton AL, Kamm MA, Ng SC, Morrison M. Proteus spp. as putative gastrointestinal pathogens. Clinical Microbiology Reviews. 2018 Jun 13;31(3):e00085-17.
3. Abdul-Lateef LA. Genotyping Diversity of Proteus vulgaris by using RAPD-PCR and Study of Some Factors on the Activity of Bacteria. European Journal of Molecular & Clinical Medicine. 2020;7(2):182-93.
4. O'Hara CM, Brenner FW, Miller JM. Classification, identification, and clinical significance of Proteus, Providencia, and Morganella. Clinical Microbiology Reviews. 2000 Oct 1;13(4):534-46.
5. Pishbin Z, Mehrabian S. Molecular analysis of Proteus mirabilis isolated from urine samples using ERIC-PCR. Gene Reports. 2020 Jun 1;19:100637.
6. Yuan F, Huang Z, Yang T, Wang G, Li P, Yang B, Li J. Pathogenesis of Proteus mirabilis in catheter-associated urinary tract infections. Urologia Internationalis. 2021;105(5-6):354-61.
7. White AN, Learman BS, Brauer AL, Armbruster CE. Catalase Activity is Critical for Proteus mirabilis Biofilm Development, Extracellular Polymeric Substance Composition, and Dissemination during Catheter-Associated Urinary Tract Infection. Infection and Immunity. 2021 Oct 1;89(10):e00177-21.
8. Ioannou P, Vougiouklakis G. Infective endocarditis by Proteus species: a systematic review. Germs. 2020 Sep;10(3):229.
9. KishanRaj S, Sumitha S, Tang TH, Citartan M, Chinni SV. Comparative genomic identification and characterization of npcRNA homologs in Proteus vulgaris. Journal of Biosciences. 2021 Dec;46(4):1-0.
10. Sarshar M, Behzadi P, Ambrosi C, Zagaglia C, Palamara AT, Scribano D. FimH and anti-adhesive therapeutics: a disarming strategy against uropathogens. Antibiotics. 2020 Jul 10;9(7):397.
11. Armbruster CE, Smith SN, Johnson AO, DeOrnellas V, Eaton KA, Yep A, Mody L, Wu W, Mobley HL. The pathogenic potential of Proteus mirabilis is enhanced by other uropathogens during polymicrobial urinary tract infection. Infection and Immunity. 2017 Jan 26;85(2):e00808-16.
12. Amiri HS, Ranjbar R, Sohrabi N, Khamesipour F. Molecular typing of uropathogenic Escherichia coli strains isolated from patients by REP-PCR. Современные технологии в медицине. 2017;9(3 (eng)):71-5.
13. Almasian-Tehrani N, Alebouyeh M, Armin S, Soleimani N, Azimi L, Shaker-Darabad R. Overview of typing techniques as molecular epidemiology tools for bacterial characterization. Cellular, Molecular and Biomedical Reports. 2021 Aug 1;1(2):69-77.
14. Mahmoud MF, Fathy FM, Gohar MK, Awad WM, Soliman MH. Genotyping of Pseudomonas aeruginosa Strains Isolated from Surgical Site Infected Patients by RAPD-PCR. Systematic Reviews in Pharmacy. 2020;11(12):1998-2005.
15. Ayhan K, Coşansu S, Orhan-Yanıkan E, Gülseren G. Advance methods for the qualitative and quantitative determination of microorganisms. Microchemical Journal. 2021 Jul 1;166:106188.
16. Zare S, Derakhshandeh A, Haghkhah M, Naziri Z, Broujeni AM. Molecular typing of Staphylococcus aureus from different sources by RAPD-PCR analysis. Heliyon. 2019 Aug 1;5(8):e02231.
17. Bahashwan SA, El Shafey HM. Antimicrobial resistance patterns of Proteus isolates from clinical specimens. European Scientific Journal. 2013 Sep 30;9(27).
18. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 31th ed. CLSI supplement M100Ed31E. Wayne, PA: Clinical and Laboratory Standards Institute, 2021.
19. Mahmmudi Z, Emami A, Gorzin A. Genotyping of Pseudomonas aeruginosa strains isolated from burn patients by RAPD-PCR molecular technique with primers 287. International Journal of Advanced Biotechnology and Research. 2016;7(2):102-10.
20. Shaaban M, Elshaer SL, El-Rahman A, Ola A. Prevalence of extended-spectrum β-lactamases, AmpC, and carbapenemases in Proteus mirabilis clinical isolates. BMC Microbiology. 2022 Dec;22(1):1-3.
21. Zafar U, Taj MK, Nawaz I, Zafar A, Taj I. Characterization of Proteus mirabilis Isolated from Patient Wounds at Bolan Medical Complex Hospital, Quetta. Jundishapur Journal of Microbiology. 2019 Jul 31;12(7):e87963.
22. Fallah F, Parhiz S, Azimi L. Distribution and antibiotic resistance pattern of bacteria isolated from patients with community-

acquired urinary tract infections in Iran: a cross-sectional study. International Journal of Health Studies. 2019 Jul 10;4(2): ISSN 2423-6594. Available at: <https://ijhs.shmu.ac.ir/index.php/ijhs/article/view/631>
23. Van Duin D, Paterson DL. Multidrug-resistant bacteria in the community: trends and lessons learned. Infectious Disease Clinics. 2016 Jun 1;30(2):377-90.
24. Orrett FA. Urinary tract infections in general practice in a rural community in South Trinidad. Saudi Medical Journal. 2001 Jun 1;22(6):537-40.
25. Kalal BS, Nagaraj S. Urinary tract infections: a retrospective, descriptive study of causative organisms and antimicrobial pattern of samples received for culture, from a tertiary care setting. Germs. 2016 Dec;6(4):132.
26. Mirzaei A, Nasr Esfahani B, Raz A, Ghanadian M, Moghim S. From the urinary catheter to the prevalence of three classes of integrons, β-lactamase genes, and differences in antimicrobial susceptibility of Proteus mirabilis and clonal relatedness with Rep-PCR. BioMed research international. 2021 Jun 10;2021: 9952769.
27. Magliano E, Grazioli V, Deflorio L, Leuci AI, Mattina R, Romano P, Cocuzza CE. Gender and age-dependent etiology of community-acquired urinary tract infections. The Scientific World Journal. 2012 Jan 1;2012: 349597.
28. MRAIDI, Dunya A.; LAFTA, Inam J. RAPD-PCR and phylogenetic analysis of E. coli isolated from human and cattle urinary tract infections. *Iraqi Journal of Veterinary Sciences*, 2021, 35: 59-66.
29. Drzewiecka D. Significance and roles of Proteus spp. bacteria in natural environments. Microbial Ecology. 2016 Nov;72(4):741-58.
30. Wasfi R, Hamed SM, Amer MA, Fahmy LI. Proteus mirabilis biofilm: development and therapeutic strategies. Frontiers in Cellular and Infection Microbiology. 2020 Aug 14;10:414.
31. LIN, Tiehao, et al. Review on molecular typing methods of pathogens. *Open journal of medical microbiology*, 2014, 4.03: 147.
32. Gholami D, Emruzi Z, Noori AR, Aminzadeh S. Advances in bacterial identification and characterization: methods and applications. Advanced Research in Microbial Metabolites and Technology. 2019 Nov 1;2(2):119-36.
33. Michelim L, Muller G, Zacaria J, Delamare AP, Costa SO, Echeverrigaray S. Comparison of PCR-based molecular markers for the characterization of Proteus mirabilis clinical isolates. Brazilian Journal of Infectious Diseases. 2008;12:423-9.
34. Sosa V, Schlapp G, Zunino P. Proteus mirabilis isolates of different origins do not show correlation with virulence attributes and can colonize the urinary tract of mice. Microbiology. 2006 Jul 1;152(7):2149-57.