

Research Article

ISOLATION AND MOLECULAR DETECTION OF *Proteus* spp. FROM DOGS SUFFERED FROM URINARY TRACT INFECTION

Thaar Mohammed Najim^{1*}, Atheer Abdrazzaq Aldoori¹ and Mustafa Salah Hasan²

¹College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq

²College of Veterinary Medicine, University of Fallujah, Fallujah, Iraq

Abstract

This investigation was designed for isolation and molecular detection of *Proteus* spp. from Dogs suffered from Urinary tract infection. All samples of urine from dogs and puppies were used for isolation of this bacteria, samples plated on Blood agar and MacConkey agar media, then biochemical tests were done for identification of bacteria. The VITEK 2 system was used for confirmation of isolates. It was very important step to complete PCR assay, which was used to check the extracted DNA by loading the eluted DNA by Agarose gel electrophoresis. Three primers in this study were purchased from Bioneer, Korea to detect *Proteus* spp. virulence genes (*ureR*, *ZapA*, *mrpA*). Then PCR test was done. Two hundred urine samples were taken from all dogs, 127 females and 73 males, these samples were taken from veterinary clinics in Fallujah, Ramadi, Tikrit and Aden square veterinary hospital and stray dogs from Karmah, Saqlawiyah, Sicher. Samples were primarily identified through its cultivation, on MacConkey agar, Blood agar due to presence of swarming phenomena, Gram stain and different other Biochemical tests were also done. Twenty-six isolates were suspected by primary identification, then after, the isolates were confirmed by VITEK2 device, the results of VITEK test confirmed that 19 isolates were *Proteus mirabilis*. Females were affected significantly higher than male, and also pet were affected more than stray dogs. The current study showed that age group 4 - 6 months was higher than another age groups. The number and percentages of infection was significantly higher in age group 1 - 3 months than another age groups and pet dogs was more infected than stray dogs. The present study revealed that all *Proteus* isolates possess *ureR* gene, while *zapA* and *mrpA* were presented in 6 isolates.

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1. Introduction

In the Veterinary literature, bacterial cystitis and sub-clinical bacteriuria have both been referred to as Urinary tract infections (UTIs). Both clinical and subclinical manifestations are included in terms like Urinary tract infection and

reinfection. More specifically, bacterial cystitis or urethrocystitis refers to bacteriuria that has been confirmed by urine culture, together with clinical symptoms of lower urinary tract inflammation include stranguria, pollakiuria, and gross hemorrhage, as well as urinary cytological evidence of inflammation (Weese *et al.*, 2011). Currently, sub-clinical bacteriuria in the veterinary

*Corresponding author: Thaar Mohammed Najim,

literature is defined as culture-documented bacteriuria without the aforementioned clinical signs of lower urinary tract inflammation, with the presence or absence of pyuria, which is not thought to preclude asymptomatic bacteriuria in humans (Wan *et al.*, 2014).

According to numerous studies, older female dogs are more likely to develop bacterial urinary tract infections (UTI), which suggests that this is a significant risk factor (Wong *et al.*, 2015). Regardless of gender, the average age of diagnosis is about 7 - 8 years (Hall *et al.*, 2013).

As a newly discovered opportunistic pathogen that causes nosocomial infections in both people and animals, *Proteus* spp. has grown in significance (Jacobsen and Shirliff, 2011; Adams-Sapper *et al.*, 2012). They have been linked to the development of chronic otitis and urinary tract infections (UTIs) in companion animals (Zamankhan Malayeri *et al.*, 2010; Marques *et al.*, 2019). There is evidence to suggest that *P. mirabilis*-related UTIs in people will result in the same strain of bacteria in their stools (Schaffer and Pearson, 2015). Similarly, the presence of *Proteus* spp. in the feces of dogs is linked to a higher risk of urinary tract infections brought on by the same bacterium (Harada *et al.*, 2014).

Proteus spp. is the second most frequent Enterobacteriaceae identified in companion animals with UTI, after *E. coli*, which is the primary cause of UTI (Moyaert *et al.*, 2017). Concerns are raised about the potential for companion animals to serve as human reservoirs for resistant bacteria due to the rising antimicrobial resistance trends in bacteria isolated from companion animals (Pomba *et al.*, 2017; Marques *et al.*, 2019). In Portugal, reports of *P. mirabilis* that produces CMY-2 and is commonly Multidrug-resistant (MDR) and resistant to third-generation cephalosporins are rising (Marques *et al.*, 2019). According to Gaastra *et al.* (1996), companion animals with

UTI caused by *P. mirabilis* have significant bacterial loads in their urine and feces, which raises the possibility that resistant strains of *P. mirabilis* might spread into the home environment of the owner.

Many researchers in Iraq work on isolation of this bacteria from these (Al-Samarrae, 2011; Sabeeh and Hatem, 2013). This investigation was designed for Isolation and Molecular detection of *Proteus* spp. from dogs suffered from urinary tract infection.

2. Materials and Methods

All samples of urine from dogs and puppies were used for isolation of this bacteria, samples plated on blood agar and MacConkey agar media, then biochemical tests were done for identification of bacteria (Quinn *et al.*, 2004).

The VITEK 2 system is a new fully automated system to provide rapid and accurate identification and susceptibility testing results for the most clinical isolates, identification was made on the bases of biochemical reactions, and determinations were made by applying an algorithm to the growth kinetics monitored by VITEK 2 system (Funke *et al.*, 1998) and the procedure was performed according to the manufacturer's instructions.

Genomic DNA of *Proteus* spp. isolates were extracted by using (Presto™ Mini g DNA Bacteria Kit Geneaid. USA). The purity and concentration of extracted DNA was measured using Nanodrop spectrophotometer. It was very important step to complete PCR assay, which was used to check the extracted DNA by loading the eluted DNA by Agarose gel electrophoresis. Six primers in this study were purchased from Bioneer, Korea to detect *Proteus* spp. virulence genes. These primers were prepared according to the information of the company (Table - 1).

Table - 1: The Primers with their Sequences and product size

Primers	Primer sequence (5' to 3')		Product size (bp)	Melting temperature (°C)	Reference
<i>ureR</i>	F	GGTGAGATTTGTATTAATGG	225	58	Zhang <i>et al.</i> (2013)
	R	ATAATCTGGAAGATGACGAG			
<i>zapA</i>	F	ACCGCAGGAAAACATATAGCCC	540	59	Ali and Yousif (2015)
	R	GCGACTATCTTCCGCATAATCA			
<i>mrpA</i>	F	ACACCTGCCCATATGGAAGATACTGGTACA	550	40	Barbour <i>et al.</i> (2012)
	R	AAGTGATGAAGCTTAGTGATGGTGATGGTGATGAGAG TAAGTCACC			

For detecting virulence genes of *Proteus spp.* by PCR, the PCR amplification mixture which used for detection these gene includes master mix (12.5 µl), 2 µl of template DNA, 2 µl of each forwarded and reversed primers and 6.5 µl of nuclease free water to complete the amplification mixture to 25µl.

The Eppendorf PCR tubes which containing the mixture were transferred to Thermocycler and started the program for amplification as shown in the Table - 2 below.

Table - 2: PCR program for detection of Virulence genes

Step	Temperature (°C)	Time	Number of cycles
Initial denaturation	94	5 min.	1
Denaturation	94	60 sec.	35
Annealing	-	-	
Extension	72	60 sec.	
Final extension	72	10 min.	1
Hold	4	10 min	

3. Results and Discussions

Isolation and identification of *Proteus spp.* from dogs

Two hundred urine samples were taken from all dogs, 127 females and 73 males, these samples were taken from veterinary clinics in Fallujah, Ramadi, Tikrit and Aden square veterinary hospital and stray dogs from Karmah, Saqlauiah, Sicher.

Samples were primarily identified through its cultivation, on MacConkey agar, Blood agar due to presence of swarming phenomena (Figure 1), Gram stain and different other biochemical tests were also done. Twenty-six isolates were suspected by primary identification, then after, the isolates were confirmed by VITEK2 device, the results of VITEK test confirmed that 19 isolates were *Proteus mirabilis*.



Figure – 1: Swarming of *Proteus* spp.

The isolates of *Proteus*, on the other hand, stood out from other Enterobacteriaceae by being pale and unable to ferment lactose on MacConkey agar, as well as by exhibiting their swarming motility. *Proteus* cells looked Gram negative, bacilli, and non-spore forming under the microscope. While *Proteus* spp. had negative findings for indole synthesis, oxidase, and lactose fermentation, it produced good results for urease, citrate, and catalase. In a test of sugar fermentation that resulted in gas generation and the development of a black precipitate, this species generated an alkaline slant (red) with an acid bottom (yellow) (H₂S production) (Markey *et al.*, 2013).

Survey study

The Table - 4 shows that females were affected significantly higher than male, and also

pet were affected more than stray dogs. Many authors found that female dogs were affected than male (Waki *et al.*, 2009; Decôme *et al.*, 2020), while only one author found that prevalence of UTI in male than female (El-Tarabili *et al.*, 2022). Bacteria are often found in greater concentrations from the mid to distal urethra, although in healthy dogs, these organisms seldom cause UTI (Jagger, 2010), Bacterial ascent is prevented by the high-pressure region in the middle of the urethra and spontaneous urethral contractions, decreased epithelial receptor sites as a result of epithelial morphological variations also aid in reducing bacterial colonization in the proximal and mid urethra. In contrast to female dogs, male dogs longer urethra and antibacterial prostatic secretions are hypothesized to lower the incidence of UTI (Hall *et al.*, 2013).

Table – 4: Samples and percentages of infection by *Proteus* spp. from both sexes, stray and pet dogs

Sex	Number of Stray (%)	No. of Pet (%)	Total (%)	Number and percentages of infection
Male	7 (30.43)	58 (32.77)	65 (32.5)	7 (36.8)
Female	16 (69.57)	119 (67.23)	135 (67.5)*	12 (63.2)
Total	23 (11.5)	177 (88.5)**	200	19 (9.5)

The current study showed that age group 4 - 6 months was higher than another age groups (Table - 5).

Table – 5: Age groups of dogs in both sexes, stray and pet dogs

Age groups	No. of Stray (%)		No. of Pet (%)		Total
	Male	Female	Male	Female	
1 - 3 months	3	8	6	17	34
4 - 6 months	1	2	26	39	68
7 - 12 months	2	3	13	27	45
1 - 2 years	0	1	4	12	17
3 - 4 years	1	1	7	13	22
5- 10 years	0	1	2	11	14
Total	23 (11.5)		177 (88.5)		200

The number and percentages of infection was significantly higher in age group 1-3 months than another age groups and pet dogs was more infected than stray dogs (Table - 6).

Table – 6: Number and percentages of infections in both sexes, stray and pet dogs

Age groups	No. of Stray (%)		No. of Pet (%)		Total
	Male	Female	Male	Female	
1 - 3 months	1	1	1	3	6 (31.6) **
4 - 6 months	0	1	2	1	4 (21)*
7 - 12 months	0	0	1	2	3 (15.8)*
1 - 2 years	0	0	0	1	1 (5.3)
3 – 4 years	0	1	1	0	2 (10.5)
5 - 10 years	0	0	1	2	3 (15.8)*
Total	4 (21.1%)		15 (78.9%)		19

The current study showed that the percentages of infection by *Proteus* spp. was 9.5 %, this result was in agreement with results of Gatoria *et al.* (2006) who found that the percentages of UTI in dogs was 6.3 %, also the results compatible with results of Ling *et al.* (2001) who found that prevalence of *Proteus* spp. from canine UTI cases was 9.3 %, and Moyaert *et al.* (2017) documented a percentages of *Proteus* UTI infection was 11 %, while the results was disagreed with results of Marcia *et al.* (1995) who reported higher percentages of *Proteus* spp. from dogs which was 15 %, also, Waki *et al.* (2009) found higher percentages of proteus from canine UTI. The current results showed that the infection with *Proteus* spp. were higher in ages less than 1 year and mainly at age group 1 - 3 months, these results were agreed with results of Féria *et al.* (2001) who reported that the main age of infection with *Proteus* spp. infection in the urinary system of dogs was less than I year, while another showed

reverse results and reported that percentages of infection with *Proteus* spp. was increased by 20 % for each year increase in age and 27 % for each day increase (Féria *et al.*, 2002). The results of this study may be explained by the young age of the newly arrived animals, their weakened immune system (due to the stress of shipping), and the disruption of the natural microbial population in their gastrointestinal tracts caused by feed deprivation, a sudden change in diet, or stress. Also, the prevalence of *Proteus* spp. was highly in pet dogs than stray, these may be due to close similarity *P. mirabilis* strains may infect both companion animals and people, the prevalence of clusters comprising strains from humans and companion animals suggests that *P. mirabilis* is a zoonotic disease. These findings highlight the potential reservoir and transmission roles that companion animals may have in the spread of uropathogenic *P. mirabilis* to humans and vice versa (Marques *et al.*, 2019).

Molecular study

The present study revealed that all *Proteus* isolates possess *ureR* gene (Figure - 2), while

zapA (Figure - 3) and *mrpA* (Figure - 4) were presented in 6 isolates (Table - 7).

Table - 7: Number and percentages of isolates that carried different genes

Name of Gene	No. of Positive isolates	Percentages
<i>ureR</i>	19	100 **
<i>zapA</i>	6	31.6
<i>mrpA</i>	6	31.6

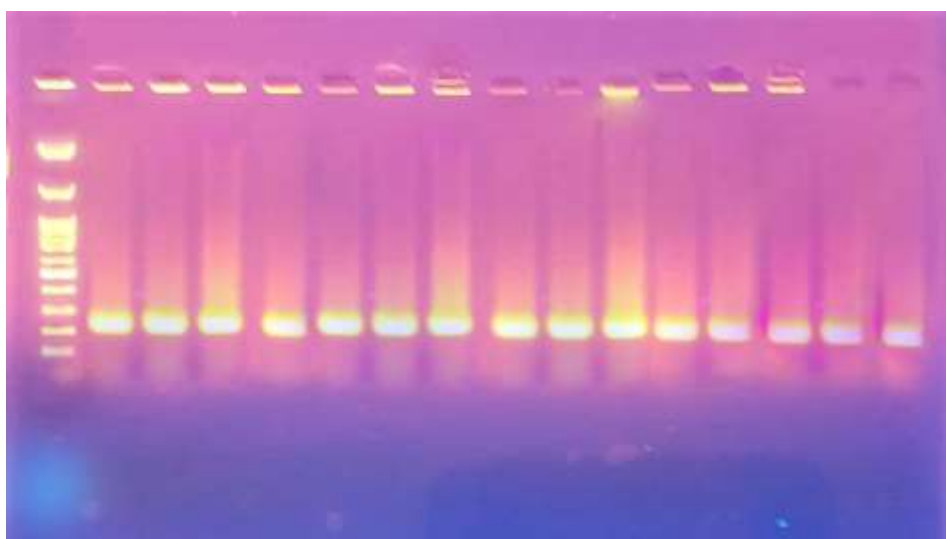


Figure – 2: Amplified *UreR* gene PCR product was electrophoresed on a gel at 70 volts for 90 minutes in 2 % Agarose, TBE (1x), and Ethidium bromide, 225 bp

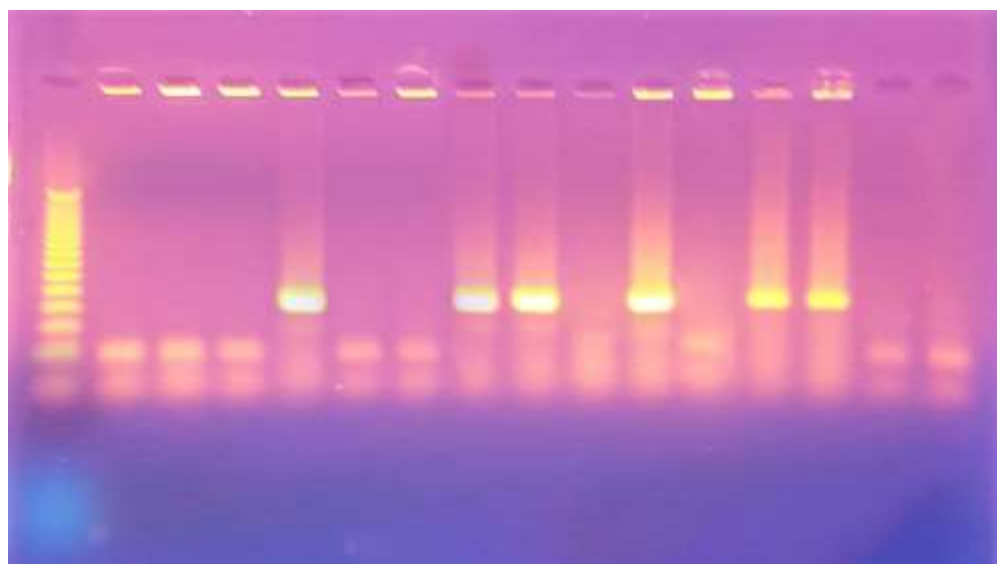


Figure - 3: Amplified *ZapA* gene PCR product was electrophoresed on a gel at 70 volts for 90 minutes in 2 % agarose, TBE (1x), and Ethidium bromide, 540 bp

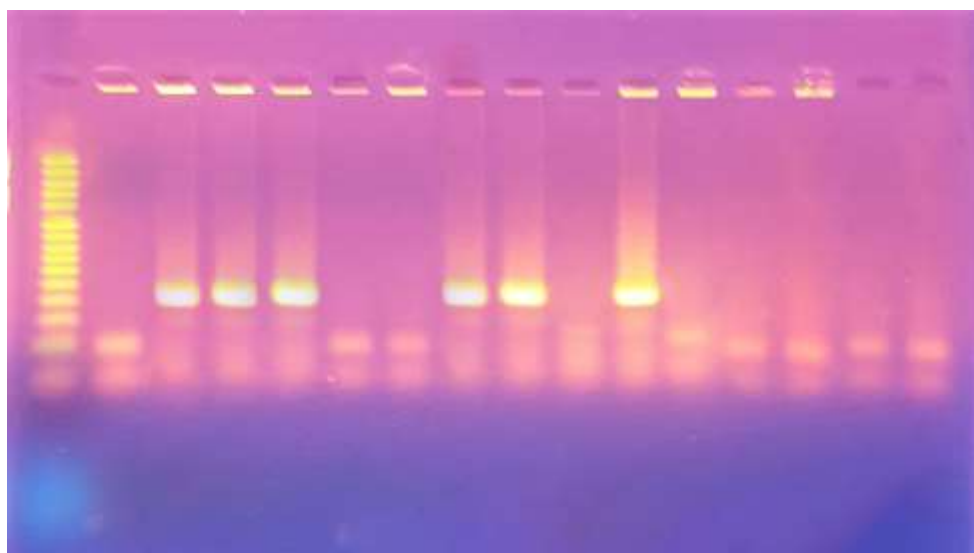


Figure - 4: Amplified *mrpA* gene PCR product was electrophoresed on a gel at 70 volts for 90 minutes in 2% agarose, TBE (1x), and ethidium bromide, 550bp

Modern molecular approaches provide the right instruments to study microorganisms in their natural environments. As long as there are sufficient commonalities at the genome level, the strain's variances in biochemical characteristics do not prevent it from belonging to a genus. In order to distinguish between *Proteus* spp. Strains - especially those from the novel genomospecies, for which biochemical separation is impossible molecular approaches must be used (Drzewiecka, 2016).

The most significant virulence factors are encoded by the genes *zapA*, *mrpA*, and *ureR*, which have also been reported to be more prevalent in earlier investigations (Abbas *et al.*, 2015, Alsherees *et al.*, 2016). The *ureR* genes on the *ure* operon are in charge of producing the urease enzyme, and prior research identified *ureC* as a key gene that contributes to urease synthesis (Li and Mobley, 2002). Protease is produced by the *zap* operon, which is represented by the *zapA*, *zapB*, *zapC*, and *zapD* genes. *ZapA* is particularly crucial for controlling IgA protease expression during the differentiation of swimmer cells into swarmer cells (Walker *et al.*, 1999).

The *P. mirabilis ureR* amplification in this research was 100 %, indicating that *ureR* occurs more often than the other genes. It shows how

important *ureR* is to *Proteus* sp. pathogenicity. These results are consistent with a prior study's findings on the presence of the *ureR* gene in around 96.6 % of isolates taken from UTI patients (Ali and Yousif, 2015). However, the prevalence of *mrpA* and *ZapA* genes in *P. mirabilis* was inconsistent with other investigations, which found that clinical *P. mirabilis* isolates included 30 % (Alsherees *et al.*, 2016) and 100 % (Ali and Yousif, 2015) of both genes, respectively. The *mrpA* gene is the most significant gene among those studied, despite having a low percentage of prevalence in earlier research owing to its involvement in a number of pathogenic variables (Abbas *et al.*, 2015).

4. Conclusions

- *Proteus mirabilis* was isolated in significant percentage from urine of dogs.
- The isolates were recorded in female than male and especially in pets and with age less than 1 year.
- The molecular results showed that all *Proteus* isolates were possess *ureR* gene and with a lesser extent another virulence genes.

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