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Life Science Archives (LSA)

ISSN: 2454-1354

Volume – 9; Issue - 1; Year – 2023; Page: 2589 – 2597

DOI: 10.22192/lsa.2023.9.1.3

Research Article

MOLECULAR DETECTION OF VIRULENCE GENES AND MDR OF *Proteus mirabilis* ISOLATED FROM URINE OF DOGS WITH UTI

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Abstract

This present study was designed for molecular detection of virulence genes and MDR of *Proteus mirabilis* isolated from urine of dogs with UTI. 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. Three primers in this study were purchased from Bioneer, Korea to detect *Proteus* spp. virulence genes (*tetA, qepA* and *aadA1*). These primers were prepared according to the information of the company. Then PCR test was done. Antimicrobial sensitivity test was done for 14 different antimicrobials. The present study revealed that *tet*A presented in 4 isolates, *aad*A1 found in 2 isolates and only one isolates have *qep*A gene. The current results of antibiotics susceptibility testing showed that selected isolate was sensitive to levofloxacin, Azithromycin, ciprofloxacin and gentamycin, while it was resistant for another antibiotics. In conclusion, *Proteus mirabilis* which isolated from dogs with UTI were MDR for most antibiotics.

Article History

Received: 28.12.2022
Revised : 12.01.2023
Accepted: 09.02.2023

Key words: Molecular, *Proteus* sp., Dogs, MDR and Antibiotics.

1. Introduction

Members of the genus *Proteus*, which are Gram negative bacilli in the family Enterobacteriaceae, are often found in the digestive systems of both humans and animals (Hegazy, 2016). To put it simply, it is the epitome of a nosocomial infection that exploits medical care facilities (Jacobsen and Shirtliff, 2011). Current members of the *Proteus* genus include *P. mirabilis, P. vulgaris, P. penneri, P. hauseri, P. terrae*, and *P. cibarius*, as well as the genomic species that have yet to be named (Drzewiecka, 2016). Humans, canines, primates, swine, sheep, goats, racoons, cats, and rodents have all been shown to have *P. mirabilis* infections. It's thought to be part of the typical mammalian gut microbiota (Guentzel, 1996). Furthermore, both *P. mirabilis* and *P. vulgaris* are found in a variety of polluted environments, including water, sewage, and soil. Decomposition of the organic compounds of the animals is essential (Rózalski *et al.*, 1997).

Strains of Proteus from household pets that have developed resistance to antibiotics have been documented. They are able to survive in their natural habitats despite being exposed to antipathogenic substances, which contributes to

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their infectious character (Hola *et al.*, 2012). The failure of therapy due to antimicrobial resistance has a negative effect on the animal's well-being. Transmission of bacterial infections from pets to humans, especially those that have acquired resistance to antimicrobials, may have serious ramifications for human public health (Guardabassi *et al.*, 2004; Algammal *et al.*, 2020). ESBLs would lyse various lactam antimicrobial drugs, including Penicillin, different generations of cephalosporins, and carbapenems, which is why they are a major cause of ESBL resistance (Magiorakos *et al.*, 2012). These enzymes are able to cleave the -lactam ring, the chemical backbone of -lactam antibiotics (Fadare and Okoh, 2021).

Conjugated plasmids and transposons are the most prevalent carriers of tetracycline-resistant genes. Contrarily, the required genes are already present in the chromosomes of certain other strains (Guillaume, 2000). Tet genes provide resistance to tetracyclines *via* three main mechanisms: efflux pumps, ribosome protection, and enzymatic inactivation. The A.A.D. Aminoglycoside-3″-adenylyltransferase (AAD), encoded by a family of genes, promotes the activity of aminoglycosides (White and Rawlinson, 2001). From a veterinary and global public health perspective, it is crucial to be aware of bacterial resistance against the antimicrobials, specifically among the isolates of canine *Proteus* spp. (Harada *et al.*, 2014). Many researchers in Iraq work on isolation of this bacteria from these (Al-Samarrae, 2011; Sabeeh and Hatem, 2013). This study designed for molecular detection of virulence genes and MDR of Proteus mirabilis isolated from urine of dogs with UTI.

2. Materials and Methods

Genomic DNA of 19 *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 (ºC)	Ref.
tetA	F	GCTACATCCTGCTTGCCTTC	502	58	Rather *et al.* (2012)
	R	GCATAGATCGCCGTGAAGAG			
aadA1	F	GCAGCGCAATGACATTCTTG	282	60	Hollingshead and Vapnek. (1985)
	R	ATCCTTCGGCGCGATTTTG			
qepA	F	CTGCAGGTACTGCGTCATG	403	60	Cattoir *et al.* (2008)
	R	CGTGTTGCTGGAGTTCTTC			

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 25 µl. 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 (^{0}C)	Time	No. 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	

Table - 3: Antibiotic discs

Antibiotic	Concentration
Tetracycline	10 mcg
Levofloxacin	5 mcg
Clarithromycin	10 mcg
Amoxicillin+ Clavulanic acid	15 mcg
Clindamycin	20/10 mcg
Cephoxitin	2 mcg
Chloramphenicol	25 mcg
Azithromycin	30 mcg
Meropenem	10 mcg
Metronidazole	10 mcg
Ciprofloxacin	10 mcg
Cephazolin	20 mcg
Ceftriaxone	10 mcg
Gentamycin	25 mcg

A sterile swab was dipped into the standardized suspension of bacteria and excess fluid was expressed by pressing and rotating the swab firmly against the inside of the tube above the fluid level. Then, the swab was spreaded in all directions over the entire surface of the Muller Hinton agar, the inoculated plates were allowed to dry for 5 minutes. Then, the discs of antibiotics (Table - 3) were placed onto agar surface using a sterile forceps. after that the plates were inverted and incubated aerobically at 37 °C for 18 – 24 hrs (Quinn *et al.*, 2004).

After incubation, the zones of inhibition were measured by using electronic vernea depending upon a zone of inhibition reported.

3. Results and Discussions

The present study revealed that *tet*A (Figure 1) presented in 4 isolates, *aad*A1 (Figure 2) found in 2 isolates and only one isolates have *qep*A (Figure 3) (Table 4).

Table - 4: Number and percentages of isolates that carried different genes

Name of Gene	Number of Positive isolates	Percentages
*tet*A	4	21.1
*qep*A	1	5.3
*aad*A1	2	10.5

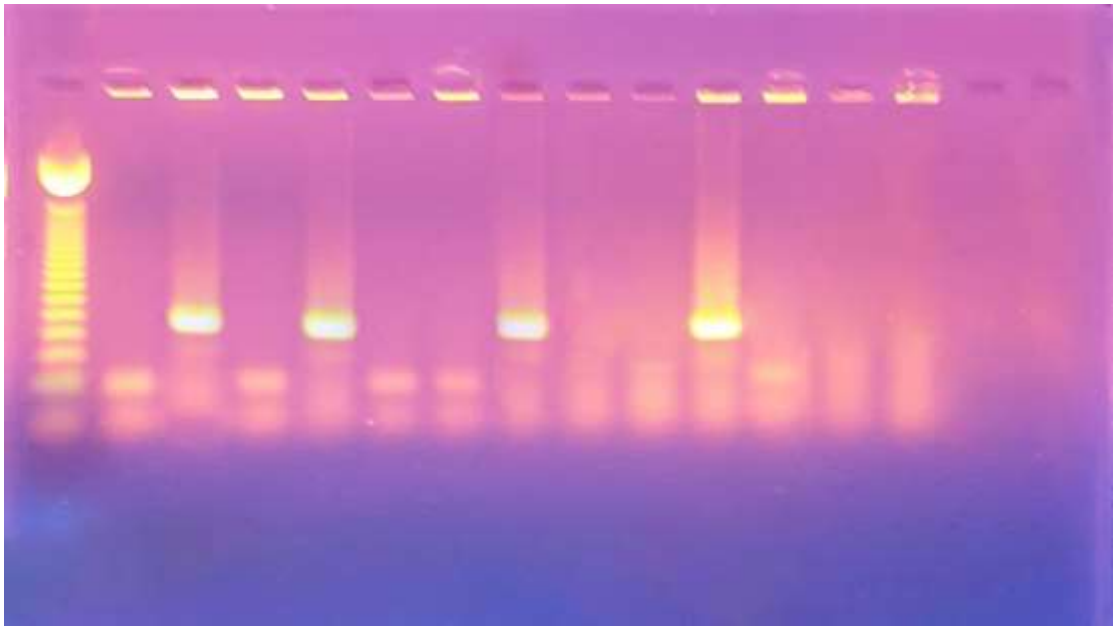

Figure – 1: Amplified *TetA* gene PCR product was electrophoresed on a gel at 70 volts for 90 minutes in 2 % Agarose, TBE (1x), and Ethidium bromide, 502 bp

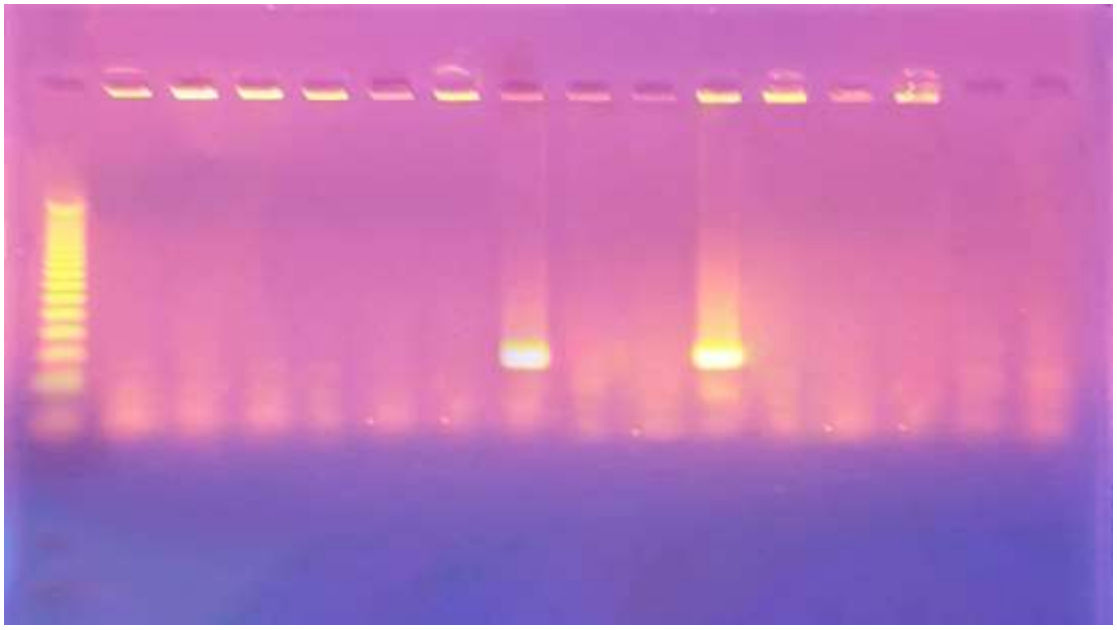

Figure – 2: Amplified *aadA1* gene PCR product was electrophoresed on a gel at 70 volts for 90 minutes in 2 % Agarose, TBE (1x), and Ethidium bromide, 403 bp

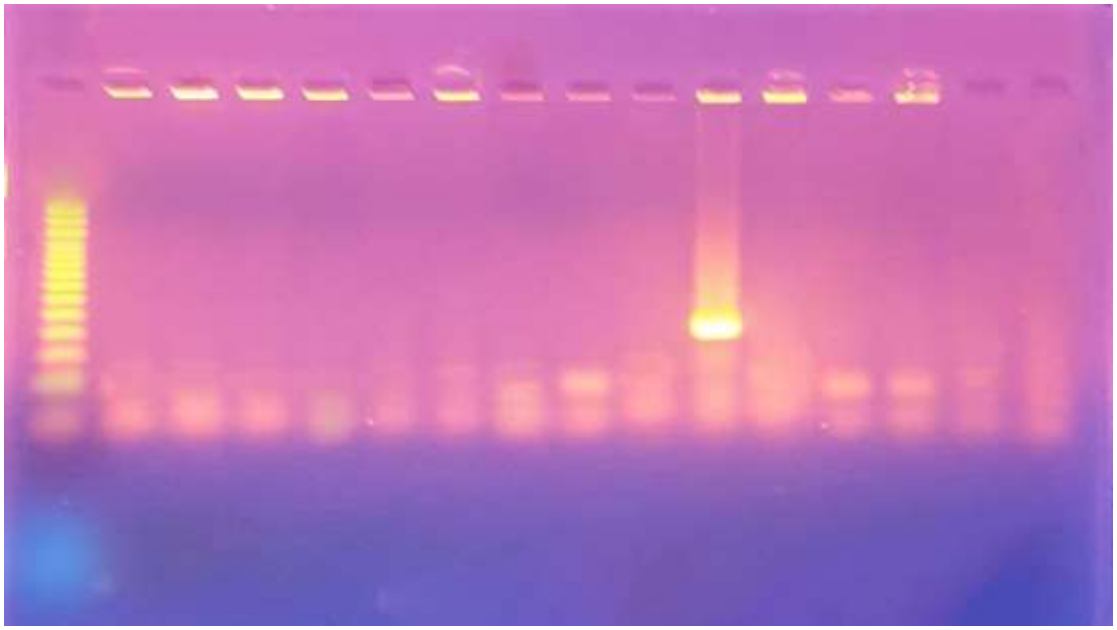

Figure – 3: Amplified *qepA* gene PCR product was electrophoresed on a gel at 70 volts for 90 minutes in 2 % Agarose, TBE (1x), and Ethidium bromide, 403bp

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 genes for resistance to antibiotics including aadA1, qepA and TetA were detected in *Proteus* spp. at different percentages. aadA1-encoding aminoglycoside resistance genes were found in 21.43 % (3/14) of *Proteus* spp. isolates (Doshi *et al.*, 2021), Additionally, Kobashi *et al.* (2007) isolated *Proteus* sp. strains from chicken excrement and found the *tetA* gene, which encodes their tetracycline resistance.

qepA gene was detected in the current study at a percentage 5.5 %, these were in agreement with previous results detected qepA gene harbored by 3 (4.4 %) of the *Proteus* spp. isolates (El-Kazzaz and Mahmoud, 2021).

Antibiotic susceptibility test for selected *Proteus mirabilis*

The current results of antibiotics susceptibility testing showed that selected isolate was sensitive to levofloxacin, Azithromycin, ciprofloxacin and gentamycin, while it was resistant for another antibiotics (Table – 5; Figure – 4 and Figure - 5).

Table – 5: Antibiotic susceptibility test for selected isolate of *Proteus mirabilis*

Antibiotic name	Resistant	Sensitive
Tetracycline	R	
Levofloxacin		S
Clarithromycin	R	
Amoxicillin + Clavulanic acid	R	
Clindamycin	R	
Cephoxitin	R	
Chloramphenicol	R	
Azithromycin		S
Meropenem	R	
Metronidazole		S
Ciprofloxacin		S
Cephazolin	R	
Ceftriaxone	R	
Gentamycin		S

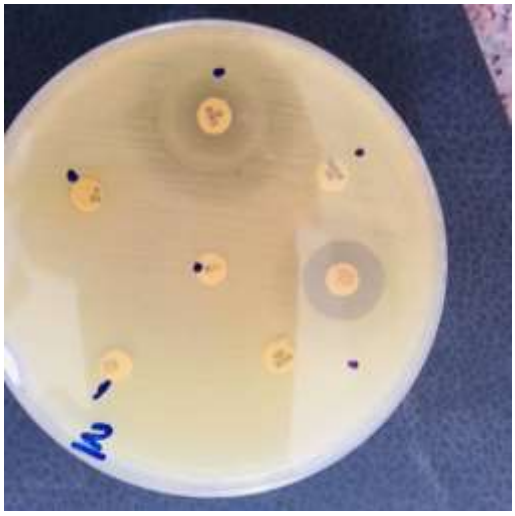

Figure - 4: Antibiotic susceptibility test for selected *Proteus mirabilis* isolate

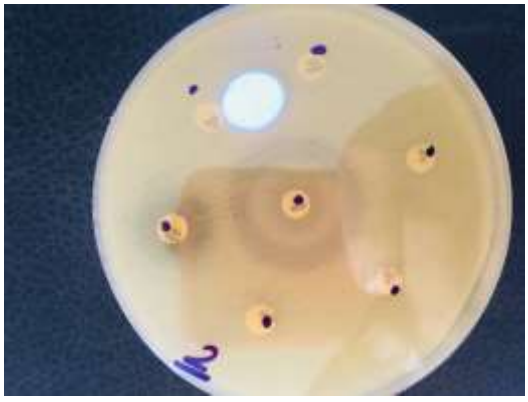

Figure – 5: Antibiotic susceptibility test for selected *Proteus mirabilis* isolate

Investigations were done on how various antibiotics affected Proteus isolates. These isolates interestingly displayed various susceptibilities to the antibiotics utilized in this investigation. Since the majority of the isolates exhibited resistance to three or more drugs, it has been determined that they were multidrug-resistant. Numerous integron- and plasmid-mediated antimicrobial resistance determinants may be found in certain Proteus species (Hall and Collis, 1998). According to this study's results, Proteus species have been found to be resistant to antibiotics by Newman et al. (2006), Mordi and Momoh (2009), Feglo et al. (2010), Bahashwan and El Shafey (2013), Kibret and Abera (2014), and Ahmed (2015). Although, the *Proteus* species are known to be susceptible to various medications, they currently seem to be resistant or less efficient. De Francesco *et al.* (2007) asserts that the etiology of drug resistance changes over time and may be brought on by the careless or incorrect usage of these antibiotics.

4. Conclusions

Proteus mirabilis which isolated from dogs with UTI were MDR for most antibiotics.

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Website	www.jpsscientificpublications.com
DOI Number	DOI: 10.22192/lsa.2023.9.1.3
Thomson Reuters Researcher ID	L – 5547 – 2016
ISI Impact Factor	4.206

How to Cite this Article:

Thaar Mohammed Najim, Atheer Abdlrazzaq Aldoori and Mustafa Salah Hasan. (2023). Molecular Detection of Virulence Genes and MDR of *Proteus mirabilis* Isolated from Urine of Dogs with UTI. *Life Science Archives*, 9(1): 2589 – 2597.

DOI: 10.22192/lsa.2023.9.1.3