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Detection of Legionella Pneumophila MIP and 16srRNA Genes in Kidney Transplant and Dialysis Wards by Polymerase Chain Reaction

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Abstract

Background: *Legionella* is a fastidious Gram-negative bacterium that is responsible for Legionnaires' disease. *Legionella* is a ubiquitous aquatic bacterium, especially in cooling systems. Several studies have investigated *Legionella* contamination in natural and man-made water resources. *Legionella* is resistant to chlorine and other disinfectants; thus, it is important to consider it in places where people with immunodeficiency are kept.
Objectives: The aim of this study was to detect the *Legionella pneumophila mip* gene in clinical samples, kidney transplants, and dialysis wards by the polymerase chain reaction (PCR) method.
Methods: In this study, 156 samples were taken from the kidney transplant and dialysis wards. DNA extraction was done. After confirmation of primers, PCR was performed to amplify *16srRNA* and *mip* genes. The PCR product was electrophoresed on agarose gel 1%.
Results: Among the samples, 23 samples were infected with *Legionella* (14.7%), of which 7 samples were identified for the *mip* gene (4.5%) and 16 samples for *16srRNA* (10.2%). The confirmation of the presence of these genes was done by sequencing. In serum, tissue, urine, hot water, and cold water samples were positive for the *16srRNA* gene (7.5%, 26.66%, 7.14%, 20%, and 6.66%, respectively). Among these samples, 50% of tissue samples, 25% of urine, and 33.33% of hot water were positive for the *mip* gene.
Conclusions: The presence of *L. pneumophila* in aqueous samples of transplant and dialysis wards is important. Therefore, rapid detection of this bacterium or the *mip* gene by a molecular method can play an important role in reducing infection and transplant rejection.

Keywords: *Legionella pneumophila*, *mip*, 16srRNA, PCR, Kidney Transplant, Dialysis

1. Background

Legionella is a ubiquitous aquatic bacterium like ventilation systems (1, 2). It causes sporadic and epidemic community-acquired pneumonia (CAP) or hospitalization in healthy and immunocompromised individuals (3, 4). *Legionella* strains cause 2 types of independent clinical diseases, including Legionnaires' disease and Pontiac fever, which is a self-limiting type (5, 6). According to the Centers for Disease Control (CDC), the prevalence of legionellosis in hospital settings ranges between 25% and 45% (7), and the mortality rate is 30% (8); in some hospital settings and sources, more than 40% have also been reported (9). The prevalence of *Legionella* in Iran is also 30 - 40%. (10). In Iran, the frequency of this bacterium has been studied in water samples of different parts of the hospital, and the technique used has been the culture and molecular method (10).

Aging, gender, smoking, alcohol use, underlying diseases (such as chronic lung disease, heart and kidney failure, and type 2 diabetes), inadequate antibiotic treatment, immunodeficiency, prolonged hospitalization, and kidney transplant are exacerbating factors (11).

The *mip* gene encodes a 24-kDa protein of MIP, inhibits phagolysosome integration into macrophage cells, and promotes intracellular survival of the bacterium (10, 12).

Also, the MIP protein can bind to FK506, which may also be effective in graft rejection (12). Various methods are currently being studied to control *Legionella* in the aquatic environments of the hospital. For this purpose, for the first time in this study, the identification of the *mip* gene DNA in urine is investigated that can rapidly and easily detect *Legionella* in the body. Rapid and accurate detection

of *Legionella pneumophila* is very valuable in transplant patients. In this study, kidney transplant and urine samples were used to identify the *L. pneumophila mip* gene, which is an interesting topic (12).

It should be noted that the BAL sample is also difficult and time-consuming and has a low sensitivity due to its polymerase chain reaction (PCR) inhibitors. Since there is a potential risk of *L. pneumophila* in transplanted patients with a low immune system, rapid detection of this bacterium or the *mip* gene by a molecular method can play an important role in preventing and reducing infection (13, 14).

2. Objectives

This study investigated the identification of the *L. pneumophila* mip gene in clinical and water samples of renal transplantation and dialysis wards of the selected hospital in Tehran by PCR.

3. Methods

3.1. Sampling

In this study, the kidney transplantation and dialysis wards of Baqiyatallah Hospital in Tehran were sampled. Hot and cold water faucets were collected as environmental samples (samples were collected in sterilized containers; we attempted to collect water with sediment). Clinical samples also included urine, a biopsy of kidney tissue, and blood.

3.2. Sample Preparation

The environmental samples were immediately transferred to the laboratory and centrifuged. Ten milliliters of environmental samples were poured into a 50-mL centrifuge tube and centrifuged at 12000 rpm for 15 minutes. After centrifugation, 9 mL of the supernatant of the 50-mL centrifuge tube was transferred into a glass, and 1 mL of the 50-mL centrifuge tube bottom with precipitates was used for DNA extraction.

Serum blood samples were separated by centrifugation at 3200 rpm for 10 minutes. Urine and kidney biopsy specimens were also centrifuged at 12000 rpm, and residual sediment and precipitated tissue were used for DNA extraction.

3.3. PCR

First, sequences for the *mip* gene and *16srRNA* gene of *L. pneumophila* were obtained from the NCBI site, and then primers were designed for the sequences analyzed by Gene Script online software. The sequences of these primers include *F-mip*: 5´- CAATGGCTGCAACCGATGCC -3´, *R-mip*: 5´- CCAATAGGTCCGCCAACGCT -3´ with Tm = 60 ^{oc} and Amplicon size 592 bp, *F-16srRNA*: 5´- AGGGTTGATAGGTTAAGAGC -3´, *R-16srRNA*: 5´- CCAACAGCTAGTTGACATCG -3´ with Tm=57 ^{oc} and Amplicon size 386 bp.

3.4. DNA Extraction

Nucleic acid was extracted according to the CinnaGen kit protocol (CinnaGen Company, Iran). PCR was performed as follows: PCR Master Mix 12.5 μL (1x), forward and reverse primer 1 μL (10 μmol), and template DNA 2 μL (50 ng) In the final volume of 25 μL.

PCR temperature program for *mip* and *16srRNA* genes in Corbett thermocycler was performed as follows: Initial denaturation at 94°C for one minute, Secondary denaturation at 94°C for 30 seconds, Annealing temperature at 58°C for 30 seconds, Extension temperature at 72°C for one minute, Final extension temperature at 72°C for five minutes with 35 cycles., and at the end of the reaction, the PCR product was electrophoresed on agarose gel 1% (15).

3.5. PCR Product Sequencing

Gene sequencing was performed by Fanavaran Gene Company. Sequencing was performed by the ABI Capillary System (Macrogen Research, Seoul, Korea), and the results of the open sequencing were monitored by Chromas software. Their BLASTs were performed in the EMBL/GenBank database (www.NCBI.nlm.NIH.gov/BLAST/).

3.6. PCR Sensitivity and Specificity

PCR sensitivity was performed with different dilutions of the genome. First, gene extraction was performed from the samples and then from this dilution: 10^{-1} to 10^{-8} dilutions were prepared as serial dilution. PCR was performed with all dilutions, and the last dilution was PCR.

PCR was performed on the primers of genomes other than *L. pneumophila*, such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Escherichia coli*, and *Klebsiella pneumonia*.

3.7. Statistical Analysis

The final analysis was performed using Excel software, and relevant charts were drawn. Bacterial sequences were also blasted at the NCBI site to confirm the identification of *L. pneumophila* strains.

4. Results

4.1. Specimen Analysis

In the present study, samples were collected from serum, tissue, urine, and environmental samples, whose specifications are given in Table 1.

4.2. PCR Results

The PCR results showed that out of 156 samples, 23 *Legionella* samples (14.7%) were identified, of which 7 samples were identified for the *mip* gene (4.5%) and 16 samples for *16srRNA* (10.2%).

The PCR results showed the frequency of *Legionella* based on 16srRNA and *mip* gene by the type of the sample in 23 *Legionella* samples. Results of PCR for 16srRNA gene in serum, tissue and urine samples were 7.5%, 26.66%, and 7.14% respectively and in hot water and cold water samples were 20%, and 6.66%, respectively. The result of PCR showed that the fifty percent isolates from the tissue sample, 25% urine, and 33.33% isolates of warm water were positive for the mip gene. The mip gene was not detected in serum and cold water samples.

4.3. Results of Sequencing 16srRNA and mip Genes

The results of *mip* gene sequencing showed 84% identity to the original genome.

4.4. Results of Sensitivity and Specificity

The dilution minimum that amplified DNA was 10^{-4}, and concentration was determined with an absorption spectrophotometer at 260 nm. The 1 pg/mol concentration was a dilution obtained as PCR sensitivity.

Specificity of PCR indicated that reaction was positive only for *L. pneumophila* and was negative for DNA of *P. aeruginosa*, *A. baumannii*, *E. coli*, and *K. pneumonia*.

5. Discussion

Legionella is a cause of acute and deadly pneumonia and can contaminate thousands of meters through aerosols and cause pneumonia (16, 17). The most common cause of death in patients is immunodeficiency (18).

Molecular methods make a reliable and rapid diagnosis of Legionnaires' disease (19, 20); in this regard, PCR is of great importance (21, 22). Several studies have reported that the sensitivity of this method is 100% (23). According to the fact that the MIP protein leads to intracellular survival of the bacterium, the *mip* gene was used for PCR. The *mip* gene was detected in 50% of tissue samples, while it was lower in urine (15%), hot water (33.33%), cold water (0%), and serum (0%).

Since the *mip* gene is a housekeeping gene, its expression is not affected by stress, disinfectant, or drug; thus, its presence is always with greater pathogenicity and inhibition of phagolysosome integration (12, 13).

In the present study, the 16srRNA gene of *L. pneumophila* was detected in 6.6% of cold water samples and 20% of hot water samples. The *mip* gene was not found in cold water but was 33.33% more than in hot water.

Borella et al. in a study of 119 hot water samples from Italian hotels showed that *Legionella* was present in 85% of the samples (24). In the present study, the prevalence of *Legionella* contamination in water and clinical samples was 14.7%.

In the study by Eslami et al., in the water supply system of Taleghani Hospital in Tehran, 34% of the samples were positive for *L. pneumophila* (25).

Moosavian and Dashti conducted a study on 150 water samples isolated from fish breeding pools, swimming pools, and cooling towers in Ahwaz, showing that 7.3% by culture were positive for *L. pneumophila* (26). In the present study, 20% and 6.66% of samples were positive for *Legionella* in hot and cold water, respectively. Detection with culture is time-consuming, but molecular methods are rapid.

Mirmohammadlo et al. conducted a study on 150 samples of water from 3 military hospitals in Tehran; *Legionella* frequency was reported in 37.3% of samples. The disparity in results between Mirmohammadlo et al. and the current study (14.7%) might be due to differences in sample size (27).

The *mip* gene has been used to diagnose *L. pneumophila* in clinical and environmental samples by various researchers (13, 28). In 1992, for the first time, *L. pneumophila* and *L. micdadei* were identified in bronchoalveolar lavage (BLA) samples by PCR (29). Therefore, among a total of 23 samples containing *L. pneumophila*, 7 samples (30.43%) were positive for the *mip* gene.

Hosseinidoost et al. investigated the presence of *Legionella* at Ekbatan Hospital in Hamadan. In this study, the *mip* gene and the PCR method were used for detection (30). In 2008, Mirkalantari et al. (31) detected *Legionella* isolates from BAL samples by culture and PCR in Iran. 4.2% of BAL specimens were positive by culture, and 6 (8.4%) were positive by PCR.

The results of studies with PCR indicated that this technique is suitable for detecting *L. pneumophila* (31-33).

In 2003, Wilson et al. used quantified PCR to detect the *L. pneumophila mip* gene (12).

In a study carried out in Iran by Bagheri et al., 50 environmental samples and 50 clinical samples (20 urine samples, 20 serum samples, and 10 tissue samples were analyzed). Fifty-four samples were positive for the *mip* gene.

Table 1. The Total Percentage of Samples by Location and Type of the Sample [a]

Serum Sample	Tissue Sample	Urine Sample	Environmental Sample (Cold Water)	Environmental Sample (Hot Water)	Total Sample Number
40 (71.4)	30 (19.2)	56 (16.6)	30 (19.2)	30 (19.2)	156

[a] Values are expressed as No. (%).

In total, 34 samples were positive for the *16srRNA* gene. Also, from 10 positive clinical samples, 2 urine, 2 kidney tissue and 6 serum samples were infected with Legionella pneumophila. In the present study, 14.4% of samples were positive for the *16srRNA* gene. The most positive samples were tissue samples and then hot water samples with a frequency of 26.6% and 20%, respectively. Cold water, serum, and urine had a frequency of 6.6%, 7.14%, and 7.5%, respectively (32).

Among the *16srRNA* positive samples, the *mip* gene was found in 30.43% of samples. There were differences in the frequency of the two studies, which may be due to differences in the number and type of samples studied.

The results showed that molecular methods could rapidly and accurately detect *L. pneumophila*. In dialysis and transplant wards, due to the presence of patients with immunodeficiency, the presence of *Legionella* is important for these patients.

In this study, the presence of the *mip* gene of this bacterium in the urine sample was identified, which is an interesting result.

5.1. Conclusions

It can be concluded that molecular methods play an important role in detecting *mip* and *16srRNA* genes in patients with immunodeficiency, especially in kidney transplantation and dialysis wards.

5.2. Limitations

One of the limitations of the research is the collection of kidney tissue samples, as well as the lack of financial support and high research costs.

Footnotes

Authors' Contribution: D. E., E. N., and M. E. conceived and designed the study, conducted research, provided research materials, and collected and organized data. D. E. analyzed and interpreted data. M. E. and D. E. wrote the initial and final draft of the article and provided logistic support. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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