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Letter to the Editor

Efficacy of PCR Analysis of *Mip, Doth* and *Gspd* Genes with Culture in Detection of *Legionella pneumophila*

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Dear Editor-in-Chief

Legionella microorganism is ubiquitous and found worldwide naturally in rivers, streams, springs of hot water, swimming pools, tanks, water piping networks, cooling tower and conditioning systems (1). This bacterium causes sporadic and epidemic cases of community-acquired pneumonia (CAP) in healthy and immunocompromised from hospital or community settings (2). Studies showed that 3% to 8% of all CAP are possibly caused by *Legionella* spp. where 85% of those caused by *L. pneumophila* (3). Two independent clinical diseases caused by *Legionella* species include; legionellosis that is a severe form of pneumonia and another one is Pontiac fever; a self-limiting flu-like disease (4). We aimed to investigate the efficacy of PCR analysis of *mip,* dotH and gspD genes with culture in the detection of L. pneumophila.

In this cross-sectional study during 2016, 100 samples (50 of clinical samples and 50 samples from hospital water) were collected. Detection and identification of *Legionella* isolates was performed using microbiological methods and biochemical tests. Samples treated with a solution of N HCL-KCL2 and then incubated in 56 °C for 12 min. Then, DNA of them was extracted. And PCR technique was performed for the detection of genes. To design primers of selected genes, all genome sequences were identified in the genome databases, then assembled and analyzed, and primers designed with Gene Runner software after design, selected primers were blasted by BLast N to compare the sequence of primers with existing GenBank records. The primers sequences were as follows; F-t4ss:5'-GTGTGGTGTAGGCTGGTTTG-3', R-t4ss: 5'-CTAACCCAGAAGTGCCGATT-3', F-mip: 5'-AAAGGCATGCAAGACGCTAT-3'; R-mip: 5'-GTATCCGATTTTCCGGGTTT-3, F-16srRNA: 5'-AGGGTTGATAGGTTAAGAGC-3', R-16srRNA: 5'-CCAACAGCTAGTTGACATCG-3'; F-t2ss: 5'- GGGCATTAGTGGCCTTAGAA-3', R-t2ss: 5'CTCCACGAGGTGACGATATG-3'. Then data statistically analyzed using SPSS (Chicago, IL, USA) software trough Chi- square test.

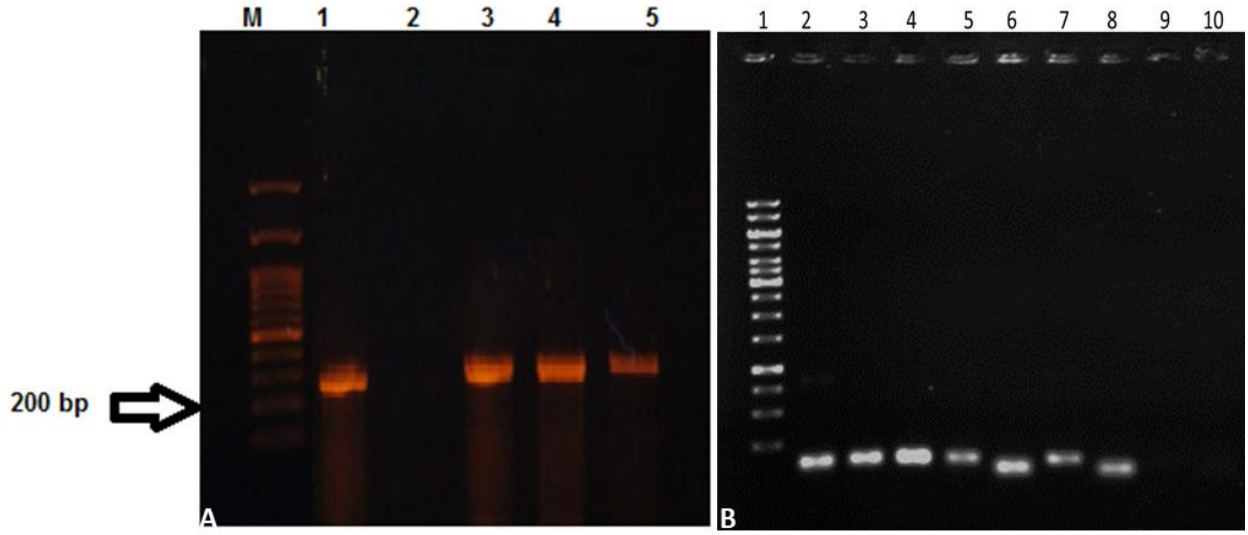

Fig. 1A: PCR image of *mip* gene of *Legionella* spp., on the 1% gel electrophoresis. Line M: Marker 50 bp, line 1: It corresponds to positive control, line 2: It is related with negative control and line 3, 4 and 5: Product size 242 bp of *mip* gene in tested samples. B: PCR image of *dotH* and *gspD* genes of Legionella spp., on the 1% gel electrophoresis. Line1: Marker 50 bp, well 2: Positive control for *gspD* gene, well 3: environmental sample for *gspD* gene, well 4: negative control, well 5: Clinical specimen for *gspD* gene, well 6: positive control for *dotH* gene and wells 7 and 8: Environmental samples for the *dotH* gene

Based on the results of culture, 14(14%) isolates of *Legionella* were recovered from clinical and water samples. PCR results showed 64(64%) out of 100 samples were positive for each of *mip* and *dotH* genes. Of these 64 positive samples for *dotH* genes (24 and 40 cases belonged to the clinical and water samples, respectively). Among the 64 samples were positive for *mip* gene, 42 and 22 cases belonged to the water and clinical samples, respectively (Fig. 1). Furthermore, 53(53%) of samples were positive for the *gspD* gene, of which 23(43.4%) of samples were from clinical and remaining from water samples.

There were some limitations to separate *Legionella* from samples by culture include; a long incubation period and *Legionella* growth is overshadowed by fast-growing organisms (5), as well as presence of living *Legionella* that doesn't have the power to grow on the media, so, all species of *Legionella* are not detectable by culture (6). Therefore, it is imperative that despite the importance and high sensitivity of culture in isolation of this organism, in addition to the culture, PCR technique can be also used to detecting this bacterium. For the first time in this study *dotH* and *gspD* genes was used alongside with *mip* gene by PCR technique for diagnosis of *Legionella*, and according to the obtained findings, sensitivity rate of two genes was comparable, so can use of them as promising genes in rapid detection of *Legionella* from different samples.

Results showed the prevalence of three genes; *mip, dotH* and *gspD* is high using PCR, so can use of these genes in PCR as a rapid detection method accompanying with culture for diagnosis of *Legionella*.

Conflict of interest

The authors declare that there is no conflict of interest.

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