ORIGINAL RESEARCH

Molecular diagnosis of Mycoplasma spp. Arthritis by PCR

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Abstract

Background: Arthritis is one of the most common inflammatory diseases worldwide. It is characterized by symptoms such as systemic inflammation and autoantibody production. The molecular mechanisms in pathogenesis of arthritis are not fully understood. Studies show that many microorganisms, including Mycoplasmas, play a role in arthritis. The PCR method is a fast and accurate molecular method for the detection of Mycoplasma genus. The main objective of this study is the detection of Mycoplasma spp arthritis by PCR method.

Methods: In this study, 70 samples of synovial fluid collected from Shariati hospital. DNA samples were extracted by phenol-chloroform standard method. Using several Mycoplasma standard strains and 16S rRNA gene target optimized PCR test of Mycoplasma spp. Sensitivity and specificity test were performed on the basis of standard methods and then performed on the DNA extracted of samples.

Results: PCR product was amplified by 272 bp and was observed on 2% gel electrophoresis. Specificity test with DNA of other microorganisms showed 100% specificity of these primers. The limit of detection was evaluated 100 copy/reaction. From 70 samples of synovial fluid, 2 samples (3%) were positive and 68 cases (97%) were negative.

Conclusion: This study showed that a number of infectious arthritis are Mycoplasma spp at the same time, and the PCR technique can be used as a sensitive and accurate way of early detection of Mycoplasma spp arthritis.

Keywords: Mycoplasm spp; PCR, Joint fluid;Diagnosis; Arthritis

Introduction

Mycoplasma refers to the Greek word Mykes, called mushrooms and plasma. For the first time it was used in the 1950's to describe the PPLO. This term was first used to describe the grown form of Mycoplasma mycoides, but after a short time it was used for all PPLOs of human and animal origin identified at that time (1).

Arthritis is the most common joint disease worldwide with many causes. Some of these causes are basically inflammatory, such as inflammatory arthritis and rheumatoid arthritis, although the cause of osteoarthritis was primarily attributed to destruction of the joint, associated with little inflammation. After many years of research it was found that in spite of any treatment to eliminate the causes of joint destruction, the disease continued and the inflammation process became even more important in this disease (2). Bacterial or septic arthritis is one of the most common and most important infectious arthritis and medical emergencies, especially in children. Therefore, prompt diagnosis and timely drug and surgical treatments may reduce the likelihood and severity of permanent injuries and prevents damage to the growth plate and synovium in children (3). Arthritis actually refers to more than 110 types of rheumatoid arthritis, which affect joints, muscles, tendons and even skin and internal organs.

In a study conducted in 2010, two cases of infectious arthritis have been associated with Mycoplasma hominis after knee replacement (4). Another study of bacterial arthritis has described Mycoplasma hominis, after Staphylococcus aureus and Streptococci, as a cause of infectious arthritis (5, 6). In another study on synovial fluid in 24 patients with arthritis, positive Mycoplasma pneumonia was reported in 19 cases (79%). In this study, all 6 patients with inflammatory and non-inflammatory arthritis, i.e. 100%, and 8 out of 10 patients with osteoarthritis, i.e. 80%, were reported positive in terms of Mycoplasma pneumonia (7). In another study by Petrov on blood and joint blood samples of 418 patients with rheumatoid arthritis, Mycoplasma arthritis (20.5%, 15.5%), Mycoplasma fermentans (15.6%, 13.2%) and Chlamydia trachomatis (18.4%, 13.2%) were reported in blood and fluid samples, respectively.

Diagnosis of arthritis is based on blood tests and radiology. Infection arthritis in joints is associated with many diagnostic challenges in most cases and identification and treatment require a combination of identification methods to identify the exact type of arthritis. Diagnostic methods currently include: 1) characteristics and symptoms, 2) analytical results, 3) microbiological tests.

The culture-based microbiology is very time consuming to detect and diagnose mycoplasma. The discovery and invention of molecular techniques have created faster ways to trace pathogenic bacteria. The polymerase chain reaction technique, based on the natural phenomenon of DNA replication in cells, theoretically provides the possibility to trace even a single bacterial cell in a sample. Using this technique, a copy of the gene turns into over billions of copies within a few hours, making it easier to track them later. Various types of PCR methods have been used to trace genomic DNA of bacteria and have had good results as well (9). Infectious arthritis is one of the major emergencies of infectious medicine and rheumatology by which most young people get infected. Therefore, the correct management of the disease needs to up-to-date information about arthritis and methods for diagnosis and treatment. Various studies have been conducted based on PCR-based molecular probe methods for detecting human and animal mycoplasmas. Fast diagnosis and no need to bacterial culture, considering bacteria complex food needs and their slow growth,

are advantages of this method (10). The aim of this study was rapid molecular diagnosis of mycoplasma arthritis by PCR.

Materials and Methods

In this study, the species belonging to the Mollicutes included: Mycoplasma pneumonia (NCTC 10119), Mycoplasma arginini, Mycoplasma hyorhinis, Mycoplasma orale, Mycoplasma synoviae, Mycoplasma gallinarum (Razi 1967), Mycoplasma gallisepticum (Razi 1976), Mycoplasma ovipneumoniae (Razi 1986), Mycoplasma agalactiae (Razi 1964), Ureaplasma urealyticum (Razi, 1990), and Ecoleplasma Laid Levi was extracted from standard DNA strain by DNG-PLUS. In this study, 70 synovial fluid specimens of those suffering from arthritis confirmed by a specialist were collected from rheumatology ward of Shariati hospital. Boiling+Phenol/chloroform carried out DNA extraction of the synovial fluid specimens. The compounds required for polymerase chain reaction in 25 µl were prepared as follows: 5 µl of template DNA, 1 µl of each forward and reverse primers, 2.5 µl of PCR buffer (10X) (sinaclon), 0.75 µL MgCl2 with a concentration of 50 mM (sinaclon), 0.5 µl of the mixture of dNTP (10mM) (sinaclon) and 0.3 µL of Taq DNA Polymerase (sinaclon) and 14 µL of sterilized deionized double-distilled water was used to calibrate the volumetric flask. The thermal program used and optimized was: 93 ° C for 20 seconds, 60° C for 20 seconds, and finally 72° C for 30 seconds, and the replication was performed in 40 cycles. The PCR product, with the desired size (272 bp), besides the size of the marker and positive and negative controls, on Agarose gel 1.5%, were examined by SYBR safe (sinaclon) in the system "Gel Documentation" (Mager-science). To test the sensitivity of the primer pairs used in this test, different dilutions were prepared from the suspension of Mycoplasma arginini with a specific CFU, and their DNA was extracted. Finally, the PCR test was performed on samples of the specified number. DNA of some organisms such as human, mice, Mycobacterium tuberculosis, Pseudomonas aeruginosa, Salmonella typhi, and Staphylococcus aureus carried out specificity test. An optimized PCR test was performed on all specimens in several rounds along with positive and negative controls.

Results

PCR technique was optimized using MGSO and GPO-3 primers and DNA of various Mycoplasmas such as Mycoplasma pneumonia, Mycoplasma arginine, Mycoplasma hyorhinis, and Mycoplasma orale. This PCR, with DNA of all tested Mycoplasmas, produced 272 bp products (Figure 1).

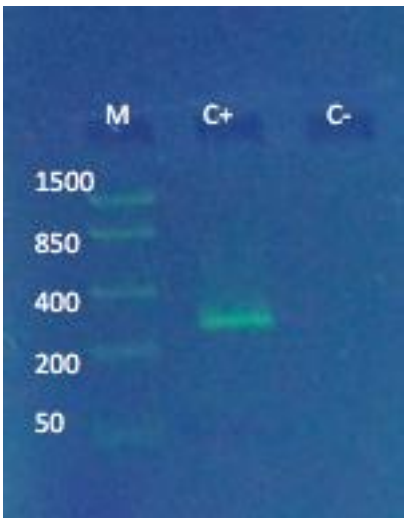

Figure 1. Optimized PCR test using primers GPO-3, MGSO: Column M, Size Marker (1Kb DNA Ladder bioflux); Column 1, Positive control; Column 2, Negative control.

The test sensitivity was evaluated by diluting the mycoplasma culture with a specific colony-forming unit. It was shown that the sensitivity of this test is 100 copies per test (Figure 2).

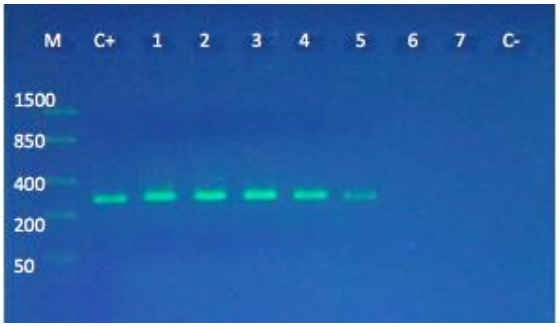

Figure 2. Optimized PCR sensitivity test: M column, size marker (LOW Range DNA Ladder Thermoscientific); the first column is positive control; column 1: 10^6CFU, column 2: 10^5CFU, Column 4: 10^3CFU, Column 5: 100 CFU, Column 6: 10 CFU, Column 7: 1 CFU, Column 8: negative control. The specificity test showed that the primers used did not produce any unwanted product with DNA of non-mycoplasma bacteria such as Mycobacterium tuberculosis, Pseudomonas, Salmonella typhimurium, Staphylococcus aureus, as well as human and mouse DNA (Figure 3).

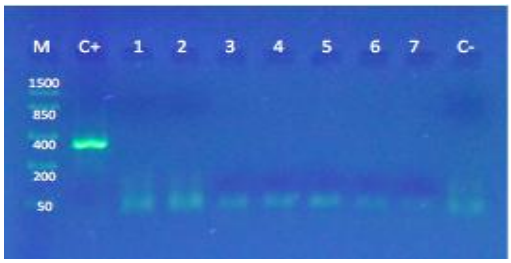

Figure 3. Optimized PCR specificity test: M column, size marker (LOW Range DNA Ladder Thermoscientific); the next column is positive control; column 1: human DNA; column 2: mouse DNA; column 3: Mycobacterium tuberculosis; column 4: Pseudomonas aeruginosa; column 5: Staphylococcus aureus; column 6: Salmonella typhimurium; column 7: Hepatitis B virus and C-: negative control. Mycoplasma contamination was sought in 70 samples of synovial fluid by PCR. Of these, 2 contaminated specimens (3%) were detected through duplication of the correct piece.

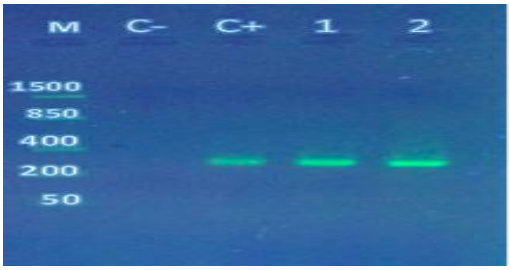

Figure 4. M: size marker (low range DNA Ladder- Thermo scientific); C-: negative control; C+: positive control (272 bp); 1 and 2: positive samples.

Discussion

Septic arthritis is one of the most common and important infectious and emergency arthritis, and rapid diagnosis and early onset of therapeutic and surgical treatments are necessary. In the absence of treatment, it will cause the possibility of permanent damage to the growth plate and synovium. It usually improves with early diagnosis and treatment. However, full recovery lasts for weeks or months. Delay in treatment may result in injury and destruction of the joint and loss of its movements, or permanent disability of the joint and blood infections, which may ultimately lead to joint replacement. Infectious (septic) arthritis is inflammation in a joint caused by any joint infection, which is common in larger joints, such as the hip joint, or those exposed to the shot like knees or hand joints (11).

Infectious arthritis might be caused by bacterial infection in the joints or a bacterial infection common with other factors such as fungal or mycobacterial infection. Every year in the United States about 20,000 arthritis patients are diagnosed. This increase in disease may be due to an increase in the elderly population. Also, Boston and Taiwan reported an increase in infectious arthritis from 8% to 27%. Factors such as age, diabetes, alcoholism, and skin lesions may increase the risk of infectious arthritis (12).

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improves with early diagnosis and treatment. However, full recovery lasts for weeks or months. Delay in treatment may result in injury and destruction of the joint and loss of its movements, or permanent disability of the joint and blood infections, which may ultimately lead to joint replacement.

This study is important because many articles have addressed the disease and in all the articles it is pointed out that the timely diagnosis of the condition is very important in its treatment because failure in timely diagnosis and treatment associates with irreparable consequences for the patient. If treatment of the disease is delayed, it will cause cartilage and bone destruction and disability and the joint may be lost forever or lead to the blood infection that is very dangerous. Even if the disease is very severe and treatment is not done, it leads to death in some cases. The mortality rate of infectious arthritis has been reported at around 5% to 15% over the past 25 years (13). Diagnosis is performed by counting blood cells, blood culture and infectious synovial fluid culture, and imaging of the joint (radiography) (14).

The exact cause of arthritis is unknown (15) but there are several reasons or grounds for it to be mentioned, including age (16), gender, genetic background (17), environmental factors, stress (18), and various infectious agents such as Mycobacterium tuberculosis (19), Coccilia bourne (20), some intestinal bacteria such as Escherichia coli, Proteus vulgaris (21), Klebsiella pneumonia, Salmonella typhi, Shigella dysenteriae, some oral anaerobic bacteria, such as Porphyromonas gingivalis (22), T. forsythensis, Prevotella intermedia, Mycoplasmas (23 and 24) including Mycoplasma pneumonia, Mycoplasma hominis, Ureaplasma urealyticum, Mycoplasma fermentas (25), Mycoplasma arthritis (26), Chlamydia trachomatis, Staphylococcus aureus (27) Streptococcus pyogenes, Gonococcus, Hemophilus influenza (28) and even fungal and viral infections. In another study on synovial fluid of 24 patients with arthritis, Mycoplasma pneumonia was reported in 19 cases (79%) (29). In another study on synovial fluid of 131 patients with Rheumatoid Arthritis, using genus-specific primer, 70 cases were Mycoplasma positive, i.e. 53.4%, which is significant compared with the current study, i.e. 3% of all samples (30). This may be given the study of Lei Zhang et al., because of the difference between results of the PCR test on adult samples compared to children that were more accurate in adults and also better results of real-time PCR than conventional PCR (31). This suggests that owing to the abundance of Mycoplasma species, a method with a proper function, which can detect all species in a single time unit is necessary. Another study by Hadi (2011) mentioned 10% results (32). Also a study by Petrov et al. on the blood and synovial fluid of 218 RA patients indicated the presence of M. arthritidis in 20.5% of the patients' blood and 15.9% in their joints fluid samples (33).

In studies conducted by Kuo et al. (2017) detection of M. synoviae infection by PCR in patients with arthritis was very fast and accurate and using common sequences like the 16SRNA, it brought even more rapid and accurate performance (34). In studies by Haier (1999) on blood serum of 28 patients with arthritis, 8 cases (28%) were reported Mycoplasma fermentans positive, 5 cases (18%) were Mycoplasma pneumonia positive, 6 cases (18%) Mycoplasma hominis positive, and one case (3%) was Mycoplasma penetrans positive (35).

In another study on synovial fluid of 24 patients with arthritis, using Nested PCR, 19 cases (79%) were Mycoplasma pneumonia positive (36). The positive results of this study were more than the results of the present study, associated with significant differences. However first, methodology of the study, second, the number of samples examined, and third, the geographic areas in these two studies were different.

Various studies based on PCR-based molecular detection methods (37) were carried out to identify human (38) and animal (39) mycoplasmas that a fast diagnosis and no need to bacterial culture, given complex food and slow growth of bacteria, are among advantages of these methods (40).

Identification of bacterial DNA in synovial fluid of arthritis patients was done by Tena et al. (2001). The study was carried on 22 patients with arthritis. Using the PCR method

and special primers, the bacteria DNA was detected in the synovial fluid and studies have shown that presence of bacteria in the synovial fluid exacerbates the disease symptoms. The advantages of the PCR method is being fast and you do not need to fresh samples and the tests can be done even after taking an antibiotic or even you can use archived samples as well. This method can even detect microorganisms that have recently been identified and there is still no way to detect them. The high cost and contamination of the samples tested, which results in false positive results, are of disadvantages of this method (41).

According to Leng Z and He Q, sensitivity of the PCR test in the synovial fluid is weak, for the presence of reaction inhibitors such as DNase and protease may cause false negative results. Therefore, in PCR, little amount of mycoplasma DNA would not be measurable (42 and 43) and other PCR methods such as nested PCR or RT –PCR are used to improve sensitivity of PCR test in the synovial fluid. However, they have not been developed so far to detect Mycoplasma genus.

PCR is currently used successfully to detect a wide range of infections caused by viruses, parasites and bacteria such as mycoplasmas (44). In this study, the serological and PCR methods had the same results. The wrong answer of serological tests is due to the antibiotic application. In fact, the high antibody titre after 3 to 4 months of infection and cross-reactivity of antibodies leads to different results of serological and PCR tests (45 and 46). In the study of Kunita et al. (1989) the PCR method showed more sensitivity than blotting, hybridization and culture methods (47).

Conclusion

In PCR, there are several factors that can make this method more efficient to achieve better results. In this study, the PCR method was used for rapid detection of Mycoplasma arthritis. Two out of 70 samples tested were positive (3%). This study showed that a percentage of infectious arthritis could be resulted from Mycoplasma. Consequently, the molecular technique of PCR is a fast, sensitive, and accurate method compared with other methods of detecting Mycoplasmas in synovial fluid.

Conflict of interests

Authors declare no conflict of interest.

References

1. Wolfgang-Joklik K, Hilda-Willett P, Dennis-Bernard A. Zinsser Microbiology. 20th ed. Norwalk: Appleton & Lange; 1992.pp 617-22.
2. Mathews CJ, Weston VC, Jones A, Field M, Coakley G. 2010, Bacterial septic arthritis in adults. Lancet ,375(9717):846-855.
3. Leiting V, Kleven S. Preparation of a heterogeneous conjugate to detect Mycoplasma iowae by immunofluorescence. Avian Diseases. 2000:697 -700.
4. lee JH,lee JH,lee NY,Ha CW,Chung DR,Peck KR. Korean JLab Med.2009 Apr:29(2):135-9.
5. OHL CA. Infectious arthritis of native joints. In:mandell GL, Bennett JE, Dolin R. Principles and practice of infectious diseases. 6th ed. Philadelphia: churchillivingstone; 2005. p. 1311-2.
6. Madoff L, Thaler S. Infectious arthritis. In: Kasper D,FauciA, LongoA.Harrisons Principle of internal medicin 16th ed.New York:McGraw-Hill: 2005.p.2050-4
7. Johnson SM, Bruckner F, Collins D. Distribution of Mycoplasma pneumoniae and Mycoplasma salivarium in the synovial fluid of arthritis patients. Journal of Clinical Microbiology. 2007;45(3):9537
8. Petrov A. Frequency of different infectious agents persistence in mononuclear leukocytes of blood and synovial fluid in patients with rheumatoid arthritis. Likars' Ka Sprava/Ministerstvo Okhorony Zdorov'ia Ukrainy. 2004(5-6):28-32.
9. Hong Y, Berrang ME, Liu T, Hofacre CL, Sanchez S, Wang L, Maurer JJ. 2003. Rapid detection of Campylobacter coli, C. jejuni and Salmonella enterica on poultry carcasses by using PCRenzyme-linked immunosorbent assay. Appl Environ Microbiol; 69(6): 3492-3499.
10. Chaudhry R, Sharma S, Javed S, Passi K, Dey A, Malhotra P. Molecular detection of Mycoplasma pneumoniae by quantitative real-time PCR in patients with community acquired pneumonia. The Indian Journal of Medical Research. 2013;138(2):24.
11. Zeman MN, Scott PJ. Current imaging strategies in rheumatoid arthritis. Am J Nucl Med Mol Imaging. 2012;2(2):174-220.
12. Hyung J. Cho, Leah A. Burke, Mikyung Lee, 2014, Septic Arthritis , Hosp Med Clin 3 2014 494-503.
13. Goldenberg DL. Septic arthritis. Lancet. 1998;351(9097):197-202.13
14. Clements JN. Treatment of rheumatoid arthritis: A review of recommendations and emerging therapy. Formulary. 2011;46(12):532.
15. Paliard X, West SG, Lafferty JA, Kappler J, Marrack P, Kotzin B. Evidence for the effects of a superantigen in rheumatoid arthritis. Science. 1991;253(5017):325-9.
16. Innala L, Berglin E, Möller B, Ljung L, Smedby T, Södergren A,et.al. Age at onset determines severity and choice of treatment in early rheumatoid arthritis: a prospective study. Arthritis Research & Therapy. 2014;16(2):R94.
17. Kim HK, Joo JS, Lee HY, Kwon JT, Sohn DR, Hong SJ, et al. Association study of polymorphisms between the Radixin gene and rheumatoid arthritis in a Korean population. Genetics And Molecular Research: GMR. 2014;13(2):3697-703.
18. De Brouwer SJ, van Middendorp H, Stormink C, Kraaimaat FW, Joosten I, Radstake TR, et al. Immune responses to stress in rheumatoid arthritis and psoriasis. Rheumatology (Oxford, England). 2014 (In press).
19. Aoki S, Yoshikawa K, Yokoyama T, Nonogaki T, Iwasaki S, Mitsui T, et al. Role of enteric bacteria in the pathogenesis of rheumatoid arthritis: evidence forantibodies to enterobacterial common antigens in rheumatoid sera and synovial fluids. Ann Rheum Dis. 1996:55(6):3639.
20. Schoffelen T, Kampschreur L, van Roeden S, Wever P, den Broeder A, Nabuurs-Franssen M, et al. Coxiella burnetii infection (Q fever) in rheumatoid arthritis patients with and without anti-NFα therapy. nnals of the Rheumatic Diseases. 2014 (In press).
21. McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N Engl J Med. 2011;365(23):2205-19.

22. Ogrendik M. Rheumatoid arthritis is linked to oral bacteria: etiological association. Mod Rheumatol. 2009;19(5):453-6
23. HakkarainenK,Turunen H, Miettinen A, Karppelin M, Kaitila K, Jansson E. Mycoplasmas and arthritis. Ann Rheum Dis. 1992;51(10):1170-2.
24. Hoffman RW, O'Sullivan FX, Schafermeyer KR, Moore TL, Roussell D, Watson-Mckown R, et al. Mycoplasma infection and rheumatoid arthritis. Analysis of their relationship using immunoblotting and an ultrasensitive polymerase chain reaction detection method. Arthritis & Rheumatism. 1997;40(7):1219-28
25. Stewart SM, Duthie JJ, Mackay JM, Marmion BP, Alexander WR. Mycoplasmas and rheumatoid arthritis. Ann Rheum Dis. 1974;33(4):346-52
26. Petrov A. Frequency of different infectious agents persistence in mononuclear leukocytes of blood and synovial fluid in patients with rheumatoid arthritis. Likars' Ka Sprava/Ministerstvo Okhorony Zdorov'ia Ukrainy. 2004(5-6):28-32.
27. Ataee RA, Hedaiatich M, Khanshan RM, Hosein M. Molecular Screening ofStaphylococcal Enterotoxin Type P Encoding Gene From Clinical Isolate. *Jundishapur Journal of Microbiology*. 2013;6(5):e6365
28. Borriello SP, Murray PR, G F. Mycoplasma in: Topley & Wilson's Microbiology & Microbial Infections. 10th.ed. 2005;PP.1958-2005
29. Johnson SM, Bruckner F, Collins D. Distribution of Mycoplasma
30. Reza Golmohammadi, Ramezan Ali Ataee, Gholam Hossein Alishiri , Reza Mirnejad , Ali Najafi , Mehdi Tate , Davoud Esmaieli, Nematollah Jonaidi-Jafari. Molecular Diagnosis of *Mycoplasma pneumoniae* in Synovial Fluid of Rheumatoid Arthritis Patients. Iranian Journal of Medical Microbiology. 2014;8(1);p1
31. Lei Zhang,Zhi-Yong Zong, Yan-Bin Liu,Hui Ye and Xiao- Ju Lv, Indian J Med Res. . 2011 Sep; 134(3):270-280
32. Hadi N, Kashef S, Moazzen M, Pour MS, Rezaei N.Survey of *Mycoplasma pneumoniae* in Iranian childrenwith acute lower respiratory tract infections. Braz J Infect Dis 2011-15-97-101
33. Petrov A. Frequency of different infectious agents persistence in mononuclear leukocytes of blood and synovial fluid in patients with rheumatoid arthritis. Likars' Ka Sprava/Ministerstvo Okhorony Zdorov'ia Ukrainy. 2004(5-6):28-32.
34. Hung- Chih Kuo,Dan-Yuan Lo , Chiou-Lin Chen, Yun-Long Tsai, Jia-Fong Ping, Chien-Hsien Lee, Pei-Yu Alison Lee, andHsiao-Fen Grace Chang. Rapid and sensitive detection of *Mycoplasma synoviae* by an insulated isothermal polymerase chain reaction-based assay on a field-deployable device.Poult Sci. 2017 Jan; 96(1): p1.
35. Haier J, Nasralla M, Franco AR, Nicolson GL. Detection of mycoplasmal infections in blood of patients with rheumatoid arthritis. Rheumatology. 1999;38(6):5049.
36. Johnson SM, Bruckner F, Collins D. Distribution of Mycoplasma pneumoniae and Mycoplasma salivarium in the synovial fluid of arthritis patients. Journal of Clinical Microbiology. 2007;45(3):953-7
37. Loens K, van Loon A, Coenjaerts F, van Aarle Y, Goossens H, Wallace P, et al. Performance of different mono-and multiplex nucleic acid amplification tests on a multipathogen external quality assessment panel. Journal of Clinical Microbiology. 2012;50(3):977-87.
38. Mirnejad R, Amirmozafari N, Kazemi B. Simultaneous and rapid differential diagnosis of Mycoplasma genitalium and Ureaplasma urealyticum based on a polymerase chain reaction-restriction fragment length polymorphism. Indian Journal of Medical Microbiology. 2011;29(1):33-36
39. Righter DJ, Rurangirwa FR, Call DR, McElwain TF. Development of a bead-based multiplex PCR assay for the simultaneous detection of multiple Mycoplasma species**.** Vet Microbiol. 2011;153(3-4):246-56
40. Chaudhry R, Sharma S, Javed S, Passi K, Dey A, Malhotra P. Molecular detection of Mycoplasma pneumoniae by quantitative real-time PCR in patients with

community acquired pneumonia. The Indian Journal of Medical Research. 2013;138(2):2440(8):p1
41. Pacheco-Tena C,Alvarado De La Barrera c, Lopez-Vidal Y, Vazquez-Mellado J, Richaud-Patin Y,Amieva RL, LIorente L, Martinez A,Zuniga J,Cifuentes-Alvarado M,.Burgos-Vargas R. Bacterial DNA in synovial fluid cells of patients with juvenile onset spondyloarthropathies.Rheumatology (Oxford). 2001Aug; 40(8):p1
42. Leng Z\ Kenny GE\ Roberts M[Evaluation of thedetection limits of PCR identi_cation of *Mycoplasma pneumoniae* in clinical samples[Mol Cell Probes1994.8:125-30.
43. He Q\ Marjama_ki M\ Soini H\ Mertsola J\ Viljanen MK[Primers are decisive for sensitivity of PCR[Biotech1994.17:82-7
44. Kunita S, Terada E, Goto K, Kagiyama N (1990)Sensitive detection of *Mycoplasma pulmonis* byusing the polymerase chain reaction. *Experimental Animals* 39,104-7.
45. Cassell GH, Davis JK, Simeka JW, Lindsey JR, CoxNR, Ross S, Fallon M (1986) Mycoplasmal infections: Disease pathogenesis implications forbiomedical research, and control. In: *Viral and mycoplasmal infections of laboratory rodents*(Bhatt PN, Jacoby RO, Morse ill HC, New AE, edsl. New York: Academic Press Inc, pp 87-130.
46. Simecka JW, Davis JK, Cassell GH (1987) Specificversus nonspecific immune response in murine respiratory mycoplasmosis. *Israel Journal ofMedical Sciences* 23,485-9.
47. Kunita S, Terada E, Ghoda A, et *al.(* 1989)A DNAprobe for specific detection of *Mycoplasma pulmonis. Experimental Animals* 38,215-19.