Original Article

Molecular detection of *Pneumocystis jirovecii* using polymerase chain reaction in immunocompromised patients with pulmonary disorders in northeast of Iran

Parian M¹, Fata A¹, ²*, Najafzadeh MJ¹, Rezaeitalab F³

¹ Department of Parasitology and Mycology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
² Research Center for Skin Diseases & Cutaneous Leishmaniasis, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
³ Department of Internal Medicine, Imam Reza Hospital, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

*Corresponding author: Abdolmajid Fata, Department of Parasitology and Mycology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran. Tel: +98 9151160466; Email: fataa@mums.ac.ir

(Received: 7 March 2015; Revised: 4 June 2015; Accepted: 10 June 2015)

Abstract

**Background and Purpose:** Pneumocystis pneumonia, caused by *Pneumocystis jirovecii*, is a fatal disease threatening patients with AIDS or immunosuppression. Assessment of colonization in these patients is of great significance, since it can lead to severe pulmonary disorders. Considering the scarcity of published reports on *Pneumocystis jirovecii* isolates from patients in Mashhad, Iran, we aimed to evaluate pneumocystis colonization in individuals with different pulmonary disorders.

**Materials and Methods:** We used nested polymerase chain reaction (PCR) method to amplify mitochondrial large subunit-ribosomal ribonucleic acid (mtLSU-rRNA) gene in 60 bronchoalveolar lavage (BAL) samples, obtained from patients, referring to the Department of Internal Medicine (Pulmonary Diseases Section) at Imam Reza Hospital, affiliated to Mashhad University of Medical Sciences, Mashhad, Iran.

**Results:** DNA of *Pneumocystis jirovecii* was detected in 10 out of 60 BAL samples (16.66%) via nested PCR method.

**Conclusion:** According to the present findings, the colonization rate of *Pneumocystis jirovecii* was similar to the rates reported in other similar studies in Iran.

**Keywords:** Nested PCR, *P. jirovecii*

How to cite this paper:
Parian M, Fata A, Najafzadeh MJ, Rezaeitalab F. Molecular detection of *Pneumocystis jirovecii* using polymerase chain reaction in immunocompromised patients with pulmonary disorders in northeast of Iran. Curr Med Mycol. 2015; 1(2): 13-18. DOI: 10.18869/acadpub.cmm.1.2.13

Introduction

*Pneumocystis jirovecii* (abbreviated as *P. jirovecii*) is an opportunistic fungus, responsible for a severe pulmonary disease, known as pneumocystis pneumonia (PCP) [1]. PCP, caused by *P. jirovecii*, is regarded as one of the most frequent and serious complications, affecting immunocompromised patients such as those with HIV infection [2].

PCP is an important cause of morbidity and mortality among immunocompromised patients [3, 4]. Patients with malignancies, solid organ transplant recipients and patients treated by immunosuppressive agents are susceptible to this infection [5, 6, 7-13]. In addition, some other conditions such as diabetes and severe malnutrition increase the risk of PCP [14, 15].

Over the past decades, despite the decline in the number of PCP cases due to the use of highly active antiretroviral therapy (HAART) in HIV-infected patients, the incidence of this infection has remained high among patients with other conditions. Since colonization of the pulmonary tract with this fungus may promote the risk of infection, evaluation of colonization rate in immunocompromised patients is essential [14, 15].

Today, PCP can be diagnosed via different methods, depending on laboratory conditions, available resources, and type and quality of samples obtained from patients [16-18]. Specific staining methods such as methenamine silver, Giemsa, hematoxylin & eosin and fluorescence staining can be applied for the diagnosis of PCP [19-24]. Based on previous studies, the sensitivity of staining techniques, used for the diagnosis of PCP, varies between 70% and 90% in bronchoalveolar lavage (BAL) [22-26].

Today, molecular methods such as polymerase chain reaction (PCR) are being increasingly applied for the diagnosis of PCP,

Copyright© 2015, Published by Mazandaran University of Medical Sciences on behalf of Iranian Society of Medical Mycology and Invasive Fungi Research Center. This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial 4.0 International License (http://creativecommons.org/licenses/by-nc/4.0/) which permits copy and redistribute the material just in noncommercial usages, provided the original work is properly cited
To detect *P. jirovecii* in clinical samples, many studies have been conducted, using different gene targets and PCR techniques [28, 29]. However, since 1979, no studies have evaluated *P. jirovecii* isolates from patients with pulmonary disorders in Mashhad, Iran. The aim of this study was to isolate *P. jirovecii* from patients with pulmonary disorders by nested PCR method.

**Material and Methods**

**Patient population**

In the present study, 75 immunosuppressed patients, suspected of pneumonia, who were admitted to the Department of Internal Medicine (Pulmonary Diseases Section) at Imam Reza Hospital, affiliated to Mashhad University of Medical Sciences, Mashhad, Iran were recruited during September 2012 and March 2014.

60 patients were included in this study with at least three of the following symptoms: dry cough, dyspnea, fever, abnormal auscultation sound and new pulmonary infiltrates. The patients were considered to be immunosuppressed if they met one of the following criteria: bone marrow/solid organ transplantation, hematological malignancy, uncontrolled diabetes, malnutrition and receiving immunosuppressive treatment.

**Laboratory processing of specimens**

The obtained BAL samples were centrifuged at 4000 rpm for 10 min. Afterwards, the supernatant was removed and the pellet was resuspended in one-fifth of the supernatant. The final product was stored at a temperature of -20 °C for DNA extraction.

**DNA extraction and PCR amplification**

DNA extraction was performed, using Prime Prep Genomic DNA Isolation Kit (GenetBio, Korea), according to manufacturer’s instructions. DNA was eluted in 50 μL of elution buffer, which came with the kit, and was maintained at -20 °C.

**Primers**

PCR was performed for mitochondrial large subunit (mtLSU) ribosomal ribonucleic acid (rRNA) gene, using previously published primers (30). The used primers were as follows: pAZ102-E (5’-GAT GGC TGT TTC CAA GCC CA-3’) and pAZ102-H (5’-GTG TAC GTT GCA AAG TAC TC-3’) for external round PCR and pAZ102-X (5’-GTG AAA TAC AAA TCG GAC TAG G-3’) and pAZ102-Y (5’-TCA CTT AAT ATT AAT TGG GGA GC-3’) for nested round PCR.

**PCR and sequencing**

For first-round PCR, 2 μL of DNA was added to the reaction mixture, containing 2.5 mM MgCl<sub>2</sub>, 1 mM deoxyribonucleotide triphosphates (dNTPs), 1 mM primers and 1.8U Taq polymerase. PCR assay was carried out by an initial denaturation at 94 °C for 5 min, followed by 20 cycles of denaturation at 94 °C for 1 min, 56 °C for 1 min and 72 °C for 1 min in an ABI 2720 thermal cycler (Applied Biosystems, Foster City, CA).

For the nested round PCR, 2μL of 10-time diluted external-round product was added into the reaction mixture, containing 2.5 mM MgCl<sub>2</sub>, 0.5 mM dNTPs, 1 mM primers and 1.8 U Taq polymerase. PCR conditions for nested round PCR were as follows: initial denaturation at 94 °C for 5 min, followed by 20 cycles of denaturation at 94 °C for 1 min, at 59 °C for 1 min and 72 °C for 1 min.

PCR amplified products were run on 1.5% agarose gel, stained with ethidium bromide, and were visualized under an ultraviolet transilluminator. Positive and negative controls were included in each experiment. The positive control was the DNA extracted from BAL smears, which were positive, based on Giemsa staining and PCR experiments. On the other hand, sterile distilled water was used as the negative control in each run of the test. All positive PCR products of mtLSU-rRNA gene were sequenced. Sequences in this study were imported to MEGA software version 5 (http://www.megasoftware.net). Afterwards, they were adjusted and exported to Basic Local Alignment Search Tool (BLAST) of National Center for Biotechnology Information (NCBI) database for species recognition. For statistical analysis, Fisher’s exact test and independent samples t-test were performed, using SPSS version 16.
Molecular Detection of Pneumocystis jirovecii

Results

Among 99 samples transferred to the mycology laboratory, 39 samples were excluded due to positive culture for Mycobacterium tuberculosis, fungal contamination, and low concentration of DNA or incomplete information provided in the questionnaire. Among 60 patients enrolled in this study, 36 and 24 cases were male and female, respectively. It should be mentioned that two patients were from Turkmenistan and one originated from Afghanistan.

Regarding the risk factors, 16 patients had diabetes, 12 cases had been under long-term antibiotic therapy, 9 cases suffered from cancer, 8 cases had received chemotherapy, and 8 subjects had undergone major surgeries. Congenital immunodeficiency, hypereosinophilia syndrome, use of immunosuppressive drugs and an unknown form of immunodeficiency were the risks reported in four patients (Table 1).

Two primers, i.e., pAZ102-E and pAZ102-H in external round PCR and two primers, i.e., pAZ102-X and pAZ102-Y, in nested round PCR successfully amplified 346bp and 267bp fragments of P. jirovecii, related to 10 positive cases, respectively.

In terms of clinical symptoms, 44 patients (73.33%) had fever, and 55 cases (91.66%) presented with persistent dry coughs and wheezing. The molecular test (PCR) was performed on all samples, and 10 samples showed positive results for P. jirovecii (Figure 1).

The risk factors found in 10 confirmed cases of P. jirovecii were diabetes (n=3), chemotherapy (n=2), malignancy (n=2), extensive surgery (n=1), immunosuppressive drugs (n=1) and congenital immunodeficiency (n=1) (Table 1). According to the results, this infection was not significantly related to gender, age, acceptance or underlying diseases.

Table 1. Clinical information of the study population and PCR-positive samples

| Risk factors                          | PCR-positive | Total |
|---------------------------------------|--------------|-------|
| Diabetes                              | 3            | 19    |
| Chemotherapy                          | 2            | 8     |
| Malignancy                            | 2            | 9     |
| Extensive surgery                     | 1            | 8     |
| Congenital immunodeficiency           | 1            | 1     |
| Immunosuppressive drugs               | 1            | 1     |
| An unknown form of immunodeficiency   | 1            | 1     |
| Long-term antibiotic therapy          | 12           |       |
| Hypereosinophilia syndrome            | 1            |       |
| Total                                 | 10           | 60    |

Discussion

By the end of the Second World War, the first case of PCP was reported in an orphanage in Europe [31]. The most frequent cases of PCP have been observed among patients with AIDS, although other individuals with immunosuppression are also at risk of this infection. Different types of malignancies, congenital immune deficiency [9], diabetes [7], organ transplantation; malnutrition [3, 32-23] and immunosuppressive drugs [5, 20, 34] are the main predisposing factors for PCP.

The majority of studies have been performed on patients with AIDS and pulmonary disorders, whereas a limited number of studies have evaluated individuals, suffering from other types of immunodeficiencies. The present study was performed on patients with compromised immunity and pulmonary disorders. Considering the inclusion and exclusion criteria, 60 patients (24 females and 36 males) were included in the present research.

In the present study, the results of PCR and sequencing tests demonstrated that 10 BAL samples were colonized by P. jirovecii infection. Similarly, a study performed by Aoulay in 2004 in Paris showed the rate of positive samples to be 8.7%. In this study, it seems that prophylactic treatment was effective for decreasing the rate of infection [38]. In...
another study on a similar population in Iran, use of co-trimoxazole was an exclusion criterion, and \textit{P. jiroveccii} infection was reported in 12.60\% of cases [35].

Among 60 examined samples for \textit{P. jiroveccii}, one sample belonged to a four-year-old child with congenital immunodeficiency; however, the condition of this child was different from other patients. Considering the results reported in previous studies, the rate of this infection seems to be rather high in children. In this regard, in one study on hospitalized children in Denmark, the incidence of \textit{P. jiroveccii} infection was estimated at 16\% [36].

Among different immunocompromised patients, diabetic patients are not very susceptible to this infection. However, disorders resulting from uncontrolled diabetes considerably decrease the level of immunity [37]. The reason is the higher sensitivity of diabetic patients to viral and bacterial infections.

Diabetes mellitus is a condition which decreases the activity level of body cells, responsible for immunity [7]. These activities include infiltration, chemotaxis, phagocytosis and natural killer cell activities; however, impairment is reversible if the disease is controlled [37]. On the other hand, the high level of blood glucose may lead to weakened immune system, increased adherence of cell surface, impairment of humoral immunity and repression of superficial cell receptors [37].

According to epidemiological studies, the prevalence of PCP has been estimated at 10\% in different populations. This rate is insignificantly higher than the incidence rate of AIDS; therefore, it is necessary to follow-up patients with pulmonary disorders. In the present study, the rate of infection among diabetic patients was estimated at 18.8\%. \textit{P. jiroveccii} infection was also reported in type II diabetic patients [7].

In the present study, molecular examination of BAL samples, obtained from patients with malignancy, showed that only two samples (22.2\%) suffered from PCP; this finding was highly similar to the results reported by Khodadadi and colleagues [35]. Based on the findings of similar studies in different countries, the incidence of \textit{P. jiroveccii} infection was estimated at 16-22\% [38, 39]. Also, the main clinical signs and symptoms among patients in this study were chest pain, rales, fever and dry cough, which are common among patients with pneumocystis, as reported in similar studies.

Among 60 BAL samples examined in this study, only one direct Geimsa-stained smear was positive for \textit{P. jiroveccii}, while nested PCR confirmed the presence of \textit{P. jiroveccii} in 10 samples. This indicates that the sensitivity of nested PCR is 10 times higher than that of conventional methods. The sensitivity of molecular method has been confirmed by many researchers [30, 36, 40-44].

The present study was in fact the first report on \textit{P. jiroveccii} infection in Mashhad, Iran. However, this does not prove the absence of this infection before this study, since conventional methods are not sensitive enough to provide accurate results.

**Conclusion**

\textit{P. jiroveccii} infection is one of the causes of pulmonary disorders. Use of molecular methods is highly useful for detecting misdiagnosed infections. Infected patients can act as a source of infection for other susceptible individuals present in the same room or section. Therefore, identification of \textit{P. jiroveccii} infection is indicated for patients with pulmonary disorders.

**Acknowledgements**

This paper was extracted from a M.Sc. thesis (No. A-523) and a research project (No.910759), approved by the Research Committee and Deputy of Research at the School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran. The authors would like to thank the deputy of research for the financial support.

The authors would like to thank Mr. Mavvaji for his assistance and cooperation.

**Authors’ contributions**

M.P. obtained the specimens, performed all the tests and wrote the draft of the manuscript. A. F. designed and managed the research and edited the draft. MJ. N. supervised the research and F. R. referred the patients to the section.
All authors reviewed and approved the final manuscript.

Conflicts of interest
The authors declare no conflicts of interest.

Financial disclosure
Financial interests related to the materials of this study have been declared.

References
1. Aliouat-Denis CM, Martínez A, Aliouat el M, Pottier M, Gantois N, Dei-Cas E. The Pneumocystis life cycle. Mem Inst Oswaldo Cruz. 2009; 104(3):419-26.
2. Gottlieb MS. Pneumocystis pneumonia--Los Angeles. 1981. Am J public Health. 2006; 96(6):980-1.
3. Elvin K, Lidman C, Tynell E, Linder E, Bjorkman A. Natural history of asymptomatic and symptomatic pneumocystis carinii infection in HIV infected patients. Scand J Infect Dis. 1994; 26(6):643-51.
4. Morris A, Norris KA. Colonization by Pneumocystis jiroveci and its role in disease. Clin Microbiol Rev. 2012; 25(2):297-317.
5. Sulieman SE, Metjian TA, Zaoutis TE, Fisher BT. Pneumocystis pneumonia: epidemiology and options for prophylaxis in Non-HIV immunocompromised pediatric patients. Curr Fungal Infect Rep. 2014; 8(1):45-55.
6. Sepkowitz KA. Opportunistic infections in patients with and patients without acquired immunodeficiency syndrome. Clin Infect Dis. 2002; 34(8):1098-107.
7. de Boer MG, Brujinesteijn van Coppenraet LE, Gaasbeek A, Berger SP, Gelincck LB, van Houwelingen HC, et al. An outbreak of Pneumocystis jiroveci pneumonia with 1 predominant genotype among renal transplant recipients: interhuman transmission or a common environmental source? Clin Infect Dis. 2007; 44(9):1143-9.
8. Gianella S, Haebeler L, Joos B, Ledergerber B, Wuthrich RP, Weber R, et al. Molecular evidence of interhuman transmission in an outbreak of Pneumocystis jiroveci pneumonia among renal transplant recipients. Transpl Infect Dis. 2010; 12(1):1-10.
9. Vakil A, Okhotin H, Upadhyay H, Sherani K, Cervellione K, Fein A. Pneumocystis jiroveci Pneumonia (PJP) in a Renal Transplant Patient: Diagnosis and Clinical Management. Chest. 2013; 144(4):192A.
10. Eitner F, Hauser IA, Rettkowski O, Rath T, Lopau K, Pliquet RU, et al. Risk factors for Pneumocystis jiroveci pneumonia (PCP) in renal transplant recipients. Nephrol Dial Transplant. 2011; 26(6):2013-7.
11. Struijk GH, Gijsen AF, Yong SL, Zwinderman AH, Geerlings SE, Lettinga KD, et al. Risk of Pneumocystis jiroveci pneumonia in patients long after renal transplantation. Nephrol Dial Transplant. 2011; 26(10):3391-8.
12. McCaughan JA, Courtney AE. Pneumocystis jiroveci pneumonia in renal transplantation: time to review our practice? Nephrol Dial Transplant. 2012; 27(1):13-5.
13. Sassi M, Ripamonti C, Mueller NJ, Yazaki H, Kutty G, Ma L, et al. Outbreaks of pneumocystis pneumonia in 2 renal transplant centers linked to a single strain of pneumocystis: implications for transmission and virulence. Clin Infect Dis. 2012; 54(10):1437-44.
14. Sanno K, Hatanaka N, Yamagishi T, Kamemura H, Hirano Y, Kodaka N, et al. Pneumocystis pneumonia in a patient with type 2 diabetes mellitus. Intern Med. 2007; 46(4):1131-3.
15. Morrow BM, Samuel CM, Zampoli M, Whitelaw A, Zar HJ. Pneumocystis pneumonia in South African children diagnosed by molecular methods. BMC Res Notes. 2014;7:26.
16. Dei-Cas E. Pneumocystis infections: the iceberg? Med Mycol. 2000; 38 (Suppl):1):23-32.
17. Medrano FJ, Montes-Canó M, Conde M, de la Horra C, Respaldiza N, Gasch A, et al. Pneumocystis jiroveci in general population. Emerg Infect Dis. 2005; 11(2):245-50.
18. Mori S, Cho I, Sugimoto M. A cluster of Pneumocystis jiroveci infection among outpatients with rheumatoid arthritis. J Rheumatol. 2010; 37(7):1547-8.
19. Bandt D, Monecke S. Development and evaluation of a real-time PCR assay for detection of Pneumocystis jiroveci. Transpl Infect Dis. 2007; 9(3):196-202.
20. Baumann S, Reinfald M, Haghi D, Hofmann WK, Buchheidt D. Coinfection with Pneumocystis jiroveci and invasive pulmonary aspergillosis in an immunocompromised patient: a diagnostic challenge. Onkologie. 2013; 36(10):582-4.
21. Bytter A, Cabaret O, Foulet F, Cordonnier C, Costa JM, Bretagne S. Clinical significance of quantifying Pneumocystis jiroveci DNA by using real-time PCR in bronchoalveolar lavage fluid from immunocompromised patients. J Clin Microbiol. 2012; 50(2):227-31.
22. Grocott RG. A stain for fungi in tissue sections and smears using Gomori's methenamine-silver nitrate technic. Am J Clin Pathol. 1955; 25(8):975-9.
23. Harris JR, Marston BJ, Sangrujee N, DuPlessis D, Park B. Cost-effectiveness analysis of diagnostic options for pneumocystis pneumonia (PCP). Plos One. 2011; 6(8):e23158.
24. Mohammad-Ghaedehbin B, Arzanlou M, Habibzadeh S, Samani A, Saadat-Nazarian S, Najafi M. Comparison Of molecular and microscopy methods for identification of pneumocystis in bronchoalveolar lavage samples in ICU hospitalized patients. Iran J Publ Health. 2011; 40(1):17-9.
25. Mahmoodzadeh A, Haji A, Rezaianesh MR. Comparison of Gomori’s methenamine silver method with PCR technique on oral swab, bronchoalveolar
lavage and lung homogenate specimens in detection of pneumocystis. Iran J Parasitol. 2008; 3(2):21-5.
26. Pintozzi RL. Modified Grocott's methenamine silver nitrate method for quick staining of Pneumocystis carinii. J clin Pathol. 1978; 31(8):803-5.
27. Summah H, Zhu YG, Falagas ME, Vouloumanou EK, Qu JM. Use of real-time polymerase chain reaction for the diagnosis of Pneumocystis pneumonia in immunocompromised patients: a meta-analysis. Chin Med J. 2013; 126(10):1965-73.
28. Robberts FJ, Liebowitz LD, Chalkley LJ. Polymerase chain reaction detection of Pneumocystis jiroveci: evaluation of 9 assays. Diagn Microbiol Infect Dis. 2007; 58(4):385-92.
29. Torres J, Goldman M, Wheat LJ, Tang X, Bartlett MS, Smith JW, et al. Diagnosis of Pneumocystis carinii pneumonia in human immunodeficiency virus-infected patients with polymerase chain reaction: a blinded comparison to standard methods. Clin Infect Dis. 2000; 30(1):141-5.
30. Wakefield AE, Pixley FJ, Banerji S, Sinclair K, Moxon ER, Miller RF, et al. Detection of Pneumocystis carinii with DNA amplification. Lancet. 1990; 336(8713):451-3.
31. Morris A, Lundgren JD, Masur H, Walzer PD, Hanson DL, Frederick T, et al. Current epidemiology of Pneumocystis pneumonia. Emerg Infect Dis. 2004; 10(10):1713-20.
32. Masur H, Michelis MA, Greene JB, Onorato I, Stouwe RA, Holzman RS, et al. An outbreak of community-acquired Pneumocystis carinii pneumonia: initial manifestation of cellular immune dysfunction. N Engl J Med. 1981; 305(24):1431-8.
33. Phair J, Munoz A, Detels R, Kaslow R, Rinaldo C, Saah A. The risk of Pneumocystis carinii pneumonia among men infected with human immunodeficiency virus type 1. Multicenter AIDS Cohort Study Group. N Engl J Med. 1990; 322(3):161-5.
34. Roblot F, Le Moal G, Kauffmann-Lacroix C, Bastides F, Boutoille D, Verdon R, et al. Pneumocystis jiroveci pneumonia in HIV-negative patients: A prospective study with focus on immunosuppressive drugs and markers of immune impairment. Scand J Infect Dis. 2014; 46(3):210-4.
35. Khodadadi H, Mirhendi H, Mohebali M, Kordbacheh P, Zarrinfar H, Makimura K. Pneumocystis jirovecii Colonization in Non-HIV-Infected Patients Based on Nested-PCR Detection in Bronchoalveolar Lavage Samples. Iran J Public Health. 2013; 42(3):298-305.
36. Larsen HH, von Linstow ML, Lundgren B, Hogh B, Westh H, Lundgren JD. Primary pneumocystis infection in infants hospitalized with acute respiratory tract infection. Emerg Infect Dis. 2007; 13(1):66-72.
37. Fita A, Noori M. Another Look at the cases of Mucormycosis at Imam Reza Hospital. Med J Mashhad Univ Med Sc. 2006; 49(2):173-8.
38. Azoulay E, Bergeron A, Chevet S, Belne N, Schlemmer B, Menotti J. Polymerase chain reaction for diagnosing pneumocystis pneumonia in non-hiv immunocompromised patients with pulmonary infiltrates. Chest. 2009; 135(3):655-61.
39. Dimonte S, Berrilli F, D'Orazio C, D'Alfonso R, Placco F, Bordi E, et al. Molecular analysis based on mtLSU-rRNA and DHPS sequences of Pneumocystis jirovecii from immunocompromised and immunocompetent patients in Italy. Infect Genet Evol. 2013; 14:68-72.
40. Summah H, Zhu YG, Falagas ME, Vouloumanou EK, Qu JM. Use of real-time polymerase chain reaction for the diagnosis of Pneumocystis pneumonia in immunocompromised patients: a meta-analysis. Chin Med J. 2013; 126(10):1965-73.
41. Wilson JW, Limper AH, Grys TE, Karre T, Wengenack NL, Binnicker MJ. Pneumocystis jirovecii testing by real-time polymerase chain reaction and direct examination among immunocompetent and immunosuppressed patient groups and correlation to disease specificity. Diagn Microbiol Infect Dis. 2011; 69(2):145-52.
42. Mahmoodzadeh A, Hajia M, Rezaieimanesh MR. Comparison of Gomori's methenamine silver method with PCR technique on oral swab, bronchoalveolar lavage and lung Homogenate specimens in detection of pneumocystis. Iran J Parasitol. 2008; 3(2):21-5.
43. Tang YW. Molecular diagnostics of atypical pneumonia. Acta Pharmacol Sin. 2003; 24(12):1308-13.
44. Procop GW, Haddad S, Quinn J, Wilson ML, Henshaw NG, Reller LB, et al. Detection of Pneumocystis jiroveci in respiratory specimens by four staining methods. J clin Microbiol. 2004; 42(7):3333-5.