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Comparison of culture and PCR methods for detection of Mycoplasma pneumoniae,Chlamydia pneumoniae, and Legionella pneumophila

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Abstract

This study aims at comparing the culture and PCR techniques for the detection of Mycoplasma pneumoniae, Chlamydia pneumoniae, and Legionella pneumophila, which are important causes of atypical community-acquired pneumonia (CAP). this review article is a survey of the published articles consolidated from PubMed and Google Scholar on the topic. out of 24 articles from 1995 to 2022, 16 were selected and screened through the process. The evaluated culture and PCR methods were divided into 8 different types including culture, PCR, qRTPCR, real-time PCR, nested PCR, Duplex Real-Time PCR, multiplex real-time PCR, and in-house PCR. Among these methods, real-time PCR were the most accurate method for the detection of M.pneumonia. instead, the in-house PCR and Duplex Real-Time PCR have better results than The real-time PCR for the detection of c.pneumonia.
Our comprehensive survey showed that an in-house multiplex Real-Time PCR assay could result in reliable outcomes as a fast diagnosis method for all three bacteria.

Keywords: *mycoplasma, Legionella,Chlamydia,Diagnosis,Cultivation,Pneumonia*

Introduction

Background

Pneumonia is an infection that is known as the smallest prokaryotic microorganism and consists of an increased affinity for respiratory epithelial cells. It is swelling of the tissue lungs and lacks a cell wall(Tang et al., 2021). Several types of infection factors can cause bacterial pneumonia, Including mycoplasma pneumoniae, chlamydia pneumoniae, and Legionella pneumophila. Although they are important causes of atypical community-acquired pneumonia (CAP), rapid and sensitive diagnosis is difficult(Ishimaru et al., 2021). They are among the most frequent pathogens in the outpatient setting(Sattar & Sharma, 2018) and the main cause of respiratory tract infections in humans, especially children. These infections are a heavy burden to the world medical community(Zhao et al., 2020). most common types of community-acquired pneumonia (CAP) in children are caused by M.pneumonia annually(An et al., 2018; Lee et al., 2020). several studies attributed C.pneumoniae and M.pneumoniae to more than one-third of cases of CAP in children(Roh, Shim, & Chung, 2022). Respiratory Detection of these infections is preferably based on the PCR, serology, and isolation of the organism from specimens by cell culture(Roh et al., 2022). A wide range of extra-respiratory manifestations such as hematologic, gastrointestinal, renal, musculoskeletal, cardiovascular, immunological, dermatological, and neurological complications have been reported about these bacteria(Sattar & Sharma, 2018). In recent years, Culture is known as one of the most important standards for the detection of Mycoplasma pneumoniae,Chlamydia pneumoniae, and Legionella pneumophila infection, but low sensitivity (0.04-61%) and slow growth (up to 6 weeks) limit its employment in the clinical microbiology lab(Merida-Vieyra et al.). However, it is very time-consuming to manage some illness and infections caused by M. pneumoniae are common in most parts of the world, with outbreaks occurring every 4-7 years. The annual infection rate is estimated at 1.3% during epidemics and 50% during epidemics(Verkooyen et al., 1998). Legionella pneumophila. display a serious health risk for the elderly and especially, people with a weakened immune system or People with underlying diseases(Chambers, Slow, Scott-Thomas, & Murdoch, 2021). Also, some Legionella pneumophila has proved to cause pneumonia and the incidence of legionella infection is imagined to be underrated (Gonçalves, Simões, & Simões, 2021).

Clinical presentation of these infections may be indistinguishable from respiratory infections caused by viral and other bacterial agents. In addition, M.pneumoniae and C.pneumonia species are very slow and difficult to culture. M.pneumonia has also been included in pultipathogen panels for diagnosis of a diverse array of bacteria, fungal and viral agents capable of causing pneumonia (Diaz & Winchell, 2016).

their diagnosis rates are inadequate(Kerdsin et al., 2010).

Cultivation-based methods are time-consuming techniques with relatively low sensitivity and require facilities, as well as sufficient experience to interpret the results (J. W. Lim, Park, Tong, & Yu, 2020). Also, serological methods are a test with low sensitivity and aren't considered appropriate methods. in the past years, PCR-based detection methods have been developed to solve these problems(Loens, Ursi, Goossens, & Ieven, 2003; McMillan, 1998; Van Kuppeveld et al., 1992). this study aims to compare the culture and PCR methods for the recognition of Mycoplasma pneumoniae, Chlamydia pneumoniae, and Legionella pneumophila.

Methods

This systematic review is on culture and PCR methods for the detection of Mycoplasma pneumoniae, Chlamydia pneumoniae, and Legionella pneumophila. Our goal was comparison them together to find out the best method for the detection of Mycoplasma pneumoniae, Chlamydia pneumoniae, and Legionella pneumophila infection, considering the published articles on the topic.

Literature searches

Our literature was searched using the Web-based search engines: Google Scholar and Pubmed. we used the articles with the language restriction of English. A total of articles published from 1995 up to 2022, were considered for this review.

Results

24 articles were found and 8 articles were excluded, first considering the subject and abstract. Then, among them, selected 16 articles were screened in the process. In addition, we checked the articles'

reference lists to identify relevant studies. Also, we excluded repetitive articles, peer reviews, and textbooks from the articles list (Table 1).

Table 1 Diagnostic value of combining different methods for m.pneumonia

author	method	accuracy	sensitivity	specificity	Negative	Positive
Qu et al(Qu et al., 2013)	culture	73%	55.6%	90%	25%	75%
Zhang et al (Zhang, Zong, Liu, Ye, & Lv, 2011)	PCR	79%	62%	96%	21%	79%
Chaudhry et al (Chaudhry et al., 2013)	qRT-PCR	59%	34.6%	84.2%	81%	19%
Morozumi et al(Morozumi et al., 2006)	real-time PCR	97.7%	100%	95.4%	33.33%	66.67%
Lim and Chung(H.-K. LIM & CHUNG, 1999)	nested PCR	96.4%	92.8%	100%	93%	100%
Gullsby et al(Gullsby, Storm, & Bondeson, 2008)	Duplex Real-Time PCR	99%	100%	98%	-	-
Nummi et al(Nummi, Mannonen, & Puolakkainen, 2015)	multiplex real-time PCR	-	95%	-	50.56%	49.41%
	in-house PCR	-	-	-	-	-

Table 2 Diagnostic value of combining different methods for c.pneumonia

author	method	accuracy	sensitivity	specificity	Negative	Positive
Peeling et al(Peeling, 1995)	culture	75%	50%	100%	25%	75%
Verkooyen et al (Verkooyen et al., 1998)	PCR	90%	79.5%	100%	93.6%	100%
	qRT-PCR	-	-	-	-	-
Kuoppa et al(Kuoppa et al., 2002)	real-time PCR	82%	80%	83%	90%	70%
Kumar et al (Kumar, Kashyap, Kumar, & Kapoor, 2020)	nested PCR	68.84%	50%	87.67%	90%	10%

Gullsby et al(Gullsby et al., 2008)	Duplex Real-Time PCR	97%	93%	100%	7.14%	92.86%
Nummi et al (Nummi et al., 2015)	multiplex real-time PCR	-	95%	-	60.56%	39.44%
Chernesky et al (Chernesky et al., 2002)	in-house PCR	100%	54-94%	-	100%	100%

Table 3 Diagnostic value of combining different methods for Legionella pneumophila

author	method	accuracy	sensitivity	specificity	Negative	Positive
Pierre et al(Pierre, Baron, Yu, & Stout, 2017)	culture	90%	81%	99%	10%	90%
Pierre et al(Pierre et al., 2017)	PCR	56-100%	17-100%	95-100%	44-100%	56-100%
	qRT-PCR	-	-	-	-	-
Wilson et al(Wilson, Yen-Lieberman, Reischl, Gordon, & Procop, 2003)	real-time PCR	-	100%	100%	-	-
Devos et al(Devos, Clymans, Boon, & Verstraete, 2005)	nested PCR	66%	41%	90%	44%	56%
Yin et al(Yin et al., 2022)	Duplex Real-Time PCR	83%	94%	72%	17%	83%
Benitez et al (Benitez & Winchell, 2013)	multiplex real-time PCR	100%	100%	100%	100%	100%
Eble et al (Eble, Gehrig, Schubert-Ullrich, Köppel, & Füchslin, 2021)	in-house PCR	97%	99%	94%	-	-

In this review, we comprehensively surveyed 16 articles (see Table 1, Table 2, and table 3). The evaluated culture and PCR methods were divided into 8 different types including culture, PCR, qRTPCR, real-time PCR, nested PCR, Duplex Real-Time PCR, multiplex real-time PCR, and in-house PCR.

Among these methods, culture method showed that the accuracy (90%), sensitivity (81%), and specificity (99%) for Legionella pneumophila, accuracy (75%), sensitivity (50%), and specificity (100%) for C.pneumonia and accuracy (73%), sensitivity (55.6%), and specificity (90%) for M.pneumonia. performance of the culture method is different for the diagnosis of C.pneumonia, M.pneumonia, and Legionella pneumophila.

Discussion

The diagnosis of many infections with atypical pathogens such as mycoplasma pneumoniae infection, C.pneumoniae, or L.pneumophila is yet challenging. This is mostly a result of challenges faced on culture and also late results related to usual methods such as serology and culturing, which sometimes provide a retrospective diagnosis only. In the present research, the detection rate of various techniques for Mycoplasma pneumoniae, Chlamydia pneumoniae, and Legionella pneumophila was assessed and was compared the value of these diagnostic approaches.

Currently, multiple methods for laboratory detection of Mycoplasma pneumoniae,Chlamydia pneumoniae, and Legionella pneumophila infection exist which include culture, serological tests, and molecular diagnostic analyses such as real-time PCR, nested PCR, conventional PCR ant, etc. Culture is usually employed for antimicrobial sensitivity testing because it possesses high specificity. but low sensitivity (0.04-61%) and slow growth (up to 6 weeks) limit its use in the clinical microbiology laboratory(Loens, Goossens, & Ieven, 2010; Merida-Vieyra et al.; She et al., 2010; Vervloet, Marguet, & Camargos, 2007). However, it is very time-consuming to manage some illnesses and their diagnosis rates are inadequate. Also, serological tests are now commonly utilized for diagnostic approaches of Mycoplasma pneumoniae in the clinic and are known to be more sensitive in comparison to culture. However, Molecular diagnostics assays have higher sensitivity as compared to culture and serological tests and offer several advantages over the more traditional methods. molecular diagnostic assays.

In our study, the performance of molecular diagnostic assays, including culture, PCR, qRTPCR, real-time PCR, nested PCR, Duplex Real-Time PCR, multiplex real-time PCR, and in-house PCR have been compared. The real-time PCR and Duplex Real-Time PCR in this review were more sensitive and better than other methods for the detection of M.pneumonia respectively (Table 1). However, The Duplex Real-Time PCR is more accurate and has higher sensitivity than the Real-Time PCR method for the detection of M.pneumonia. Mycoplasma lacks a cell wall and is often isolated from respiratory and genitourinary mucosal surfaces(Metwally, Yassin, Essam, Hamouda, & Amin, 2014). growing M.pneumonia is very slow in culture and may take weeks to grow. Therefore, it is time-consuming and difficult to carry out culture analysis for routine diagnosis. In(Morozumi et al., 2006), a real-time PCR assay demonstrated a specificity of 95.4% and a sensitivity of 100% on all biological samples. This assay has been employed for evaluating new clinical specimens from newborns and kids (n=389) and adults (n=40). DNA extraction of samples was carried out with a commercially available EXTRAGEN ll kit and amplification was carried out with stratagene Mx3000P. In M.pneumpniae, the sequences of primers and MB probes were conserved in a single gene copy. Real-time PCR was done by utilizing 25l of real-time PCR master mix (TOYOBO Co, Osaka, Japan) and 0.15 l of primer and MB probe. Also, reaction mixtures were employed in wells(Morozumi et al., 2006).

The relative sensitivity and specificity of nested PCR assays are: : 92.8% and 100% for M.pneumonia (H.-K. LIM & CHUNG, 1999), 50% and 87.67% for C.pneumoniae(Kumar et al., 2020) and 41% and 90% for Legionella pneumophila(Devos et al., 2005). For detection of M.pneumonia, Cpneumoniae, and L.pneumophila, the culture method can take weeks. therefore, real-time PCR and nested PCR have been used, improving the sensitivity and specificity. The in-house PCR and Duplex Real-Time PCR were more sensitive and better than other methods for the detection of C.pneumonia, respectively (Table 2) The relative sensitivity of in-house PCR assay ranged from 54-94% for C.pneumonia and 99% for Legionella pneumophila. in(Gullsby et al., 2008), duplex real-time PCR assay, targeted the P1 adhesion gene for M.pneumoniae and the ompA gene for C.pneumoniae. then, in comparison to two conventional PCR assays targeting the ompA gene and 16S rRNA gene. sensitivity and specificity were 93% and 100%, respectively, for C. pneumoniae and 100% and 98%, respectively, for M. pneumoniae

The results of methods (PCRs and culture) for Legionella pneumophila are presented in Table 3. Among different methods, The multiplex real-time PCR and in-house PCR were more sensitive and better than other methods for the recognition of Legionella pneumophila, respectively (Table 3). Analytical sensitivity and specificity of the multiplex assay were 100% for Legionella pneumophila and confirmed by the use of a complete panel of 215 clinical and environmental isolates including fifty-two Legionella species and forty-four non-legionella strains(Benitez & Winchell, 2013). In addition, these assay allows simultaneous diagnosis and differentiation

of Legionella Pneumophila, and Legionella spp and is very faster (4h versus ≥ 8 days) and easier to interpret than culture. Among these methods, in-house PCR values were not found for M.pneumonia in our search results. researchers (Eble et al., 2021) tested in-house PCR assays under standardized conditions to detect Legionella pneumophila and Legionella spp.

the in-house PCR tests reported no false positives and The sensitivities of in-house PCR assays ranged from 54 to 94% for c.pneumonia. the accuracy, sensitivity, and specificity of in-house PCR were 97%, 99%, and 94% for Legionella pneumophila. Also, the accuracy and sensitivity of these methods were reported 100% and 54-94% for c.pneumonia. positive and negative values were 100% and 100%, respectively.

Each detection method for this bacterium has advantages and disadvantages. Although. The real-time PCR were the most accurate method for the detection of M.pneumonia but isn't more accurate and more sensitive than other methods for the detection of C.pneumonia.instead, the in-house PCR and Duplex Real-Time PCR have better outcomes than The real-time PCR for detection of c.pneumonia.

This research had some limitations, initially; the present research was a survey of the published articles, which did not, have data on clinical features. In addition, there was an absence of precise evaluation ways to measure the accuracy of survey results. Secondly, the unavailability of a gold standard diagnostic assay for all three bacteria resulted in difficulty to conclude to validate the most accurate and reliable approach.

Conclusion

Our review indicated that an in-house multiplex Real-Time PCR assay can result in a more dependable outcome as a fast diagnosis method for all three bacteria. this assay is may recognize microorganisms in a few hours, therefore assisting in managing infection. Ultimately, we expect that the in-house multiplex Real-Time PCR (IHMRT-PCR) will be expanded to a multi-objective method to detect several pneumoniae.

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Conflict of interest
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