Review

Advances in Epidemiological Studies of Herpes Zoster

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Abstract

Mycoplasma genitalium (Mg) commonly causes nongonococcal urethritis and cervicitis. Mg is a fastidious bacterium that poses difficulty in time-consuming isolation and culture. Lack of specificity for serological tests also hampers clinical research of Mg. With development of molecular biology, polymerase chain reaction tests, which exhibit high sensitivities and specificities, became primary tools for foundational and clinical studies of Mg.

Mycoplasma genitalium (Mg) is one of the important causes of male nongonococcal urethritis (NGU); high morbidity of Mg is especially observed in male patients with mycoplasma NGU^[1]. Simultaneously, Mg shows relationships with some female diseases, such as mucus cervicitis and pelvic inflammation. Therefore, as an important pathogen of sexually transmitted diseases, Mg significantly concerns researchers.

Isolation and Culture

Mg exhibits extremely slow growth because of harsh nutritional requirements. Difficulty arises from isolating cell-free Mg media from clinical specimens. For the first time in 1981, Tully *et al.* isolated two strains of Mg from urogenital tract specimens of male NGU patients by SP4 media. Afterward, few studies reported Mg culture. Then, researchers applied Vero cells to Mg culture. Jensen *et al.*^[2] used Vero cells to isolate and culture five bacterial strains from seven urine samples of male patients with failed azithromycin treatment and conducted in vitro drug sensitivity tests. Recently, Hamasuna *et al.*^[3] isolated and cultured Mg from first void urine (FVU) of male urethritis patients. However, the process from first vaccination to successful separation lasted nearly a year. This previous study showed that because of simplicity of SP4 culture media, initial culture costs one to three months with the use of urine

swab samples. Meanwhile, Vero cell cultivation only lasts for three weeks, significantly decreasing elapsed time. Isolation culture method is not suitable for clinically rapid detection because it is time consuming, and strains used easily die.

Serological Method

Mg and *Mycoplasma pneumoniae* (MP) possess similarities in structural characteristics and extensive antigen cross reaction. Nevertheless, application of serological method in clinical diagnosis lacks enough specificities. In recent years, many serological detection methods were established and applied in epidemiological studies. However, no serological method can be effectively used for diagnosis of clinical patients. Jurstrand *et al.*^[4] detected serum Mg antibody by lipid-associated membrane protein-enzyme immunoassay (LAMP-EIA); the antibody exhibited no cross reaction with other mycoplasmas. Although LAMP-EIA features high specificity, its detection results vary with time and several influence factors. Thus, difficulty arises from application of LAMP-EIA to clinical diagnosis.

Molecular Biology Method

DNA Probe Technology

Prior to development of polymerase chain reaction (PCR), DNA probe technology was used to study Mg. Sensitivity of

dot blot detection of Mg can reach 0.1 ng. Compared with culture method, DNA probe technology is more rapid and results in higher positive rates, simultaneously overcoming cross reaction of Mg serum and MP. However, DNA probe technology was gradually replaced by PCR because of its high cost, complex operation, and pollution of radioisotopes.

PCR Technology

To date, PCR is the most commonly used method in studying Mg. PCR can detect infinitesimal Mg DNA and features advantages, such as simple operation, rapid detection, high sensitivity, and specificity.

At present, PCR detection of Mg mainly aims at two gene targets, namely, MgPa gene and 16S rRNA gene. MgPa is the main adhesion protein on Mg cell surface and also serves as toxicity determinant and main antigen of Mg. Thus, MgPa becomes an increasingly gene target applied in nucleic acid amplification. MgPa-1/Mg-Pa-3 primers designed by Jensen *et al.* based on MgPa exhibit good specificity and sensitivity and are widely used in Mg infection diagnosis and study. Jense *et al.*^[5] compared sensitivity and specificity of two pairs of MgPa primers and two pairs of 16A rRNA. These researchers discovered that MgPa genes of Mg clinical isolates display sequence polymorphism to some extent. MgPa also possibly possesses base insertion mutations. 16S rRNA genes from separate sources showed 100% homology. Thus, 16S rRNA gene sequence is relatively conservative and stable and contains less polymorphism. In this study, primers designed also show suitability for studies of Mg infection. To date, the most used 16S rRNA primers include 16S-45F/MG16S-447R designed by Jensen *et al.* and 402 bp amplified fragments, whose sensitivities can reach six genomic copies^[5]. By comparing primers of MgPa and 16S rRNA, Jensen *et al.* observed higher sensitivity of 16S rRNA features than MgPa. Transcription mediated amplification (TMA) is similar to nucleic acid amplification by PCR. Wroblewski *et al.*^[6] applied TMA and PCR to detect Mg, and comparison results showed that the two methods are both sensitive to Mg detection. Mg genome is almost entirely known. Thus, in the future, additional gene targets may be discovered to optimize Mg detection methods.

Other detection methods were developed based on PCR. Mirnejad *et al.*^[7] used PCR restrictive fragment length polymorphism (RFLP) to simultaneously detect and identify Mg and ureaplasma urealyticum (Uu). By designing specific primers and restriction enzymes, PCR-RFLP provides a

simple, fast, and accurate method for Mg and Uu clinical tests and possible means for other genotyping studies. Samra *et al.*^[8] applied multiple PCR (mPCR) to simultaneously detect six common sexually transmitted disease pathogens, including Mg, significantly increasing detection efficiency. Other researchers detected various pathogens based on mPCR and reverse line blot hybridization (mPCR/RLB). McKechnie *et al.*^[9] performed mPCR/RLB to simultaneously detect 14 kinds of common urogenital canal disease pathogens, proving that mPCR/RLB is an accurate, fast, convenient, and an economical detection method. Bao *et al.*^[10] used fluorescence polarization method to detect four kinds of urogenital canal mycoplasmas. These scientists used asymmetric PCR to amplify DNA fragments on 16S rRNA of four mycoplasma species. Four specific fluorescent probes targeting different mycoplasma were hybridized to target genes in PCR products. The same researchers also used fluorescence polarization method to detect increased values of fluorescence polarization after hybridization to determine mycoplasma infection. The author also performed DNA sequence assay and discovered that fluorescence polarization can simultaneously detect various mycoplasmas. Compared with sequence assay, fluorescence polarization is simpler and more economical. Thus, this method may be used in detecting multiple urogenital tract pathogens.

Real-time Quantitative PCR

In real-time quantitative PCR, during PCR, through fluorescence signal, real-time detection of PCR is performed to obtain accurate and quantitative results of DNA templates. This procedure combines advantages of PCR and DNA probe hybridization. Compared with ordinary PCR, real-time quantitative PCR exhibits advantages of real-time detection, high sensitivity and specificity, demands of small sample, reduced pollution rate, and precise quantification. Probes and primers for 16S rRNA and MgPa gene segments were subsequently designed for real-time quantitative PCR detection of Mg. Edberg *et al.*^[11] first conducted a comparative study on ordinary 16S rRNA PCR, real-time quantitative 16S rRNA PCR, and real-time quantitative MgPa PCR, discovering higher sensitivity of real-time quantitative MgPa PCR than ordinary 16S rRNA PCR and real-time quantitative 16S rRNA PCR. Thus, real-time quantitative MgPa PCR is the most suitable clinical diagnostic method for Mg. Twin *et al.*^[12] also conducted a comparative study on real-time quantitative 16S rRNA PCR

and real-time quantitative MgPa PCR by using vagina swab samples in detecting Mg. These researchers noted that these methods showed good consistency and suitability for Mg detection using vaginal swabs.

Influence of Clinical Samples to Detection

In recent years, many nucleic acid amplifications were applied to Mg detection in patients. Some studies evaluated sensitivities of different types of samples. Past studies indicated higher positive rate of Mg in male FVU than in urine swab specimens. For female patients, positive rates of application of cervical swab and FVU specimens were simultaneously higher than that of FVU specimen. A research conducted by Wroblewski *et al.*^[6] considered that in female patients, vaginal swab was the specimen with highest sensitivity of Mg detection (positive rate of TMA reached 84%, and that of PCR totaled 91%), followed by cervical swab (TMA measured 60%, PCR was 53%), and urine (TMA totaled 58%, PCR reached 65%). Research results displayed that in Mg detection in female patients, vaginal swabs exhibit higher sensitivity than urine. Shipitsyna *et al.*^[13] showed higher Mg detection sensitivity of FVU than vagina swab for female patients (100% vs. 57%) and urine swab for male patients (83% vs. 75%). Lillis *et al.*^[14] simultaneously compared sensitivities of four kinds of specimens, such as vagina swab, cervical swab, urine specimen, and anal swab, to Mg detection. Results showed superiority of vaginal swab to urine and anal swab, coinciding with those obtained by Wroblewski. Edberg *et al.*^[15] discovered that cervical swab specimen carried out in FVU was more sensitive to Mg than FVU or that carried out in 2-SP buffer solution when testing female patients. In general, FVU may be the most sensitive specimen for male Mg tests. For female patients, test sensitivity can be significantly improved by using multiple clinical specimens. Different nucleic acid extraction methods also affect sensitivity of PCR; samples extracted by automated BioRobot M48 extraction kit are superior to those obtained by artificial Chelex method^[15]. Sample treatment after collection may also affect Mg detection. Specimens should be preferably observed within 24 h after collection. When samples require storing, observation should be accomplished immediately, and ideal storage is at 4 °C. A literature reported^[16] that using anhydrous gel-dried urine specimens may prolong distance of transportation under normal temperature without affecting specimen characteristics. Although many studies used previously

frozen specimens, cryopreservation temperature and long-time storage allow high false negative rates in detection in some specimens. Carlsen *et al.*^[17] discovered that compared with fresh clinical specimens, Mg DNA load of frozen specimens remarkably decreased, and detection sensitivity declined. Compared with frozen DNA extract, Mg DNA load and detection sensitivity of clinical specimens frozen at -20 °C declined significantly. In this regard, clinical specimens should be processed as soon as possible to avoid long-time frozen storage.

Conclusion

As an important pathogen of sexually transmitted disease, Mg bears significance in clinical and fundamental research for development of convenient detection methods with high sensitivity and specificity. Considering time consumption and low positive rates, isolation and culture are not suitable for routine detection of Mg. Antigen cross reaction of Mg and MP results in lack of specificity of serological methods. Thus, this method cannot be easily applied to Mg laboratory diagnosis. PCR method exhibits advantages of rapidness, fine sensitivity, and specificity and is also widely used in clinical studies. Compared with ordinary PCR, real-time quantitative PCR displays higher sensitivity and gradually became the primary measure for Mg detection.

Declarations

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No.

Competing interests

The author declares that he has no competing interest.

Authors' contributions

XM Gu made the literature analysis and wrote, discussed and revised the manuscript of this review.

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