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Syndromic Testing for Sexually Transmitted Infection: Current and Future Demand

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Abstract

Sexually transmitted infections (STIs) are a major global public health problem, with a significant social burden worldwide. Accurate and appropriate diagnosis and treatment of STIs are important for preventing the transmission of STIs as well as major health consequences of untreated STIs, such as infertility and certain cancer. For the diagnosis of STIs, the application of conventional culture and immunoassays is limited by their low sensitivity and long turnaround time. Nucleic acid amplification tests for STIs allow for syndromic tests for multiple pathogens simultaneously and show high sensitivity with a short turnaround time. This review discusses the characteristics of commercially available multiplex molecular platforms and the features needed in next-generation syndromic tests for STIs.

Keywords

Sexually transmitted infection
Nucleic acid amplification test
Syndromic test

1. Introduction

The World Health Organization (WHO) estimated that 374 million new treatable sexually transmitted infections (STIs) occurred in 2020, and by pathogen, *Trichomonas vaginalis* (TV) (156 million), *Chlamydia trachomatis* (CT) (129 million), *Neisseria gonorrhoeae* (NG) (82 million), and *Treponema pallidum* (TP) (7.1 million) [1]. In an effort to combat this growing epidemic, WHO published a five-year public health strategy for STIs as one of the Sustainable Development Goals in 2016. According to the report, the goal is to end STIs as a public health threat by 2030 [2]. Therefore, proper early diagnosis of STIs and screening of asymptomatic carriers is important to prevent complications of untreated infections and prevent transmission.

Classical microscopy and culture are ideal methods for diagnosing and treating STIs, but depending on the species, culture conditions can be challenging or impossible [3-5]. Immunoassays detect antigens of the

pathogen or antibodies produced in the body by the infection. These immunoassays have the advantage of being simple and easy to use [6]. However, they do not apply to all STIs, some antigen tests have low sensitivity, and antibody tests have limitations such as not being able to differentiate between past and current infections [7,8]. Recently, nucleic acid amplification tests have been developed to screen for STIs, and various diagnostic methods using these tests have begun to be commercialized. In particular, nucleic acid amplification tests have relaxed requirements for transporting specimens compared to culture tests, contributing to the improvement of public health through screening for STIs [9,10]. Multiplex PCR tests based on these nucleic acid amplification tests are playing a crucial role in improving the diagnosis and treatment of patients by detecting pathogens and resistance genes for a group of diseases with similar symptoms at once [11]. This symptom-based diagnosis, in which infectious diseases with similar symptoms are tested at once to accurately and quickly identify the causative pathogen, is called 'syndromic testing' and is currently being used to diagnose bloodstream infections, respiratory infections, gastrointestinal and central nervous system infections [12-14].

The WHO first recommended symptomatic diagnosis and treatment for the management of STIs in 1991, and typical symptoms include vaginal or urethral discharge, genital ulcers, and lower abdominal pain [15]. Therefore, the utility of syndromic testing as a symptom-based pathogen diagnostic method is likely to increase with the development of point-of-care tests, the use of different specimen types and self-reported samples, and the introduction of antibiotic-resistance genetic analysis. This review will evaluate nucleic acid amplification test-based syndromic testing, which has been developed for the diagnosis of STIs and is currently in commercial use, and discuss the development of the next generation of syndromic testing.

2. Syndromic testing for sexually transmitted infection: current and future demand

2.1. Targets

The syndromic tests currently available for the diagnosis of STIs are mostly nucleic acid amplification-based and vary in the type of specimen, number of targets detected, number of panels or tubes used, and type of internal control. Specimen types include urine, cervical or vaginal secretions, and some tests also use extragenital specimens such as anorectal and pharyngeal specimens. Kits have been developed and are available for the detection of as few as two and as many as 14 targets. The Alinity m STI assay (Abbott Molecular Inc., Des Plaines, IL, USA) consists of a single panel of four pathogens: CT, NG, TV, and *Mycoplasma genitalium*. This test has the advantage that nucleic acid extraction from the specimen, nucleic acid amplification, and reading are all automated and performed within 2 hours using the Alinity m Instrument (Abbott Molecular Inc.) [16,17]. The Alinity m Instrument is capable of testing 300 specimens in 8 hours, and its large size makes it useful for hospitals or trusts with centralized laboratories. Most test kits detect the above four pathogens as standard, and kits that test for six pathogens usually detect *Mycoplasma hominis* and *Ureaplasma urealyticum* in addition to the above four pathogens [18], and the care GENE™ STD-12 detection kit (WELLS BIO Inc., Seoul, Korea), which can detect 12 pathogens, can detect *Candida albicans*, *Gardnerella vaginalis*, TP, *Ureaplasma parvum*, and Herpes simplex virus 1/2 in addition to the above six pathogens [19]. This increase in the number of pathogens that can be detected by a single test can reduce the number of specimens required for diagnosis and the number of single tests, and therefore the associated healthcare costs. The ability to identify the pathogen causing an infection in a single test can lead to faster diagnosis and treatment, improving the quality of healthcare. However, in some cases, multiplex nucleic

acid amplification tests may suffer from performance degradation since the test is performed for multiple pathogens under the same amplification conditions. Therefore, the type and number of pathogens that can be detected by syndromic testing should be selected by considering the cost-effectiveness of the test, the target population (asymptomatic screening population or symptomatic patients), and the testing location (primary, secondary, or tertiary care hospital).

2.2. Extragenital specimen

The US Centers for Disease Control and Prevention (CDC) guidelines for the treatment of sexually transmitted diseases, published in 2010, also emphasize the importance of testing for extragenital specimens in a specific population (men who have sex with men) [20]. In response to the growing need for testing extra-genital specimens, the APTIMA Combo 2 Assay (Gen-Probe Inc., San Diego, CA, USA) and Xpert® CT/NG (Cepheid, Sunnyvale, CA, USA) assay were the first few FDA-approved nucleic acid amplification tests for extra-genital specimens from the anus and pharynx in May 2019. A meta-analysis of the performance of Xpert® CT/NG (Cepheid) according to different specimens showed no significant difference in sensitivity and specificity between CT and NG for urogenital and anal specimens [21]. When looking at the actual detection rates of CT and NG in extra-genital specimens, in women, CT was 1%–3% / 7%–17% / 5%–3% in pharyngeal/anal/genital specimens, respectively, and NG was 1%–2%/ 0%–3%/ 1%–2%, respectively [22]. As such, it is thought that expanding the range of specimens available for the diagnosis of STIs may increase the probability of detection of the pathogen in some populations and reduce the probability of transmission in asymptomatic carrier form [22].

2.3. Point-of-care testing

Currently, point-of-care tests for the diagnosis of STIs are developed and commercially available, with most providing detection results in 25 to 90 minutes [23]. The existing point-of-care tests include immunoassays to detect antigens and antibodies and molecular biological diagnostic methods based on nucleic acid amplification tests, most of which are aimed at detecting one to two pathogens and have high sensitivity and specificity [24]. Point-of-care testing is favored for the diagnosis of STIs because most patients with STIs have difficulty returning to the clinic for confirmation of test results, which often leads to loss of follow-up and treatment failure [25]. Reflecting the importance of point-of-care testing, the CDC's 2020 Morbidity and Mortality Weekly Report (MMWR) suggests that the ideal system of care for STIs is to provide results and linkage to treatment on the same day as the office visit [26]. In addition, the Sexually Transmitted Diseases Diagnostic Initiative (SDI) under the umbrella of the WHO has identified the ASSURED criteria (A = affordable, S = sensitive, S = specific, U = user-friendly, R = robust and rapid, E = equipment free, D = deliverable to those who need them) as the basic criteria that point-of-care tests should meet [27].

In a survey of 256 healthcare workers involved in STIs, CT was selected as the highest priority pathogen to be included in point-of-care tests, and high sensitivity (90%–99%) was the most important consideration when selecting a point-of-care test [28]. Several currently developed and used point-of-care tests can detect CT, NG, TV, TP, and human immunodeficiency virus alone or in combination. For example, the Xpert® CT/NG (Cepheid) test is an automated molecular diagnostic method based on nucleic acid amplification testing that reports results within 90 minutes using real-time polymerase chain reaction. The Xpert® platform is very easy to operate, as all the nucleic acid extraction and gene amplification processes take place in the cartridge once the sample is mixed with buffer and the cartridge is mounted on the instrument. Suitable samples for the test are vaginal secretion samples collected by swabs and urine samples from women or men. Recently, non-genitourinary specimens, such as rectal swabs or pharyngeal specimens, have been used to validate performance [29]. In a recent meta-analysis, the

sensitivity/specificity of CT and NG for urine specimens was reported to be 90%/100% and 94%/100%, respectively, while the sensitivity/specificity of CT and NG for vaginal secretions was reported to be 91%/99% and 96%/100%, respectively [21].

The Xpert® CT/NG test requires a total of 90 minutes, which is a significant reduction in test time compared to the existing nucleic acid amplification test, but it is a long time for patients to wait for results after being seen in the clinic [30]. Therefore, the *io*® CT/NG assay (binx health, Boston, MA, USA) was approved by the US FDA in August 2019, which can be tested after collecting the specimen in the office and the results can be checked within 30 minutes [31]. Data from clinical studies of this method have reported sensitivity/specificity of 96.1%/99.1% and 100%/99.9% for CT and NG, respectively, for vaginal discharge samples [32]. The point-of-care platforms developed and commercialized to date are limited to specific pathogens, and most point-of-care tests in Korea are performed in laboratories rather than clinics, which has the disadvantage of increasing the time required for testing. The use of point-of-care testing platforms for syndromic testing can be considered by shortening the test time, simplifying the test process, and expanding the detection targets.

2.4. Detection of drug-resistant pathogen

CT, NG, and TP are antibiotic-resistant strains of sexually transmitted pathogens; however, due to the misuse and overuse of antibiotics, their antibiotic resistance is increasing. In particular, multidrug-resistant *N. gonorrhoeae* has become a global concern and has been included in the CDC's list of "five resistant strains that pose a threat to public health" [33]. The emergence of NG resistant to penicillin and doxycycline led to the inclusion of fluoroquinolones (FQs) in standard treatment guidelines for NG. However, with the emergence of NG resistant to FQ in the 1990s, the prevalence of resistant strains increased dramatically, with FQ-resistant NG exceeding 5% in many countries and FQ resistance exceeding 90% in 10 countries [34,35]. In response, the CDC removed FQ from its standard treatment guidelines for NG in 2007 [36], and more recently, NG with drug resistance to extended-spectrum cephalosporin and azithromycin, which have replaced FQ, has also increased [37,38].

Antibiotic resistance is also increasing against *Ureaplasma* spp. and *Mycoplasma* spp. which are among the causative agents of STIs. *Ureaplasma* spp. are broadly divided into *U. parvum* and *U. urealyticum* and are reported to colonize the genitourinary tract in up to 80% of healthy adult women [39]. However, some cause genital infections that require treatment (urethritis, endometritis, prostatitis, vaginitis), and infections, especially in pregnant women, have been reported to increase the risk of miscarriage, stillbirth, chorioamnionitis, and preterm birth [40,41]. As these *Ureaplasma* spp. do not have a cell wall, glycopeptide or β-lactam antibiotics are ineffective; therefore, tetracyclines, macrolides, which are inhibitors of protein synthesis, or FQs, which inhibit nucleic acid replication, are used as therapeutic agents. However, improper use of antibiotics has led to the development of acquired resistance mutations in *Ureaplasma* spp. and their frequency is gradually increasing [42]. In a recent literature reported from China, the frequency of resistance of *Ureaplasma* spp. to ofloxacin was reported from 24.1% in 1999 to 71.9% in 2019 [42]. However, the reporting of this resistance frequency varies by region and is thought to be a result of country-specific antibiotic use regulations [43]. *M. genitalium* can cause non-gonococcal urethritis in men, cervicitis in women, and pelvic inflammatory disease [44,45]. Similarly, the lack of a cell wall makes it naturally resistant to β-lactam antibiotics, and azithromycin, a member of the macrolide family, is used as a first-line treatment, with moxifloxacin, a member of the FQ family, recommended in addition [46,47]. It has been widely reported that the resistance of *M. genitalium* to azithromycin is associated with point mutations at positions 2058 and 2059 (*Escherichia coli* numbering) of the V region of the 23S rRNA gene [48]. In addition, resistance to moxifloxacin is known to be caused by amino acid mutations at S83

and D87 (*M. genitalium* numbering) of the amino acids in the ParC protein. In some studies, variants in the *gyrA* gene have also been reported to be associated with moxifloxacin treatment failure [49]. As these genetic variants associated with antibiotic resistance mechanisms have been identified, testing methods to identify these variants have been developed and some are in the process of being commercialized.

The ResistancePlus® GC assay (SpeeDx Pty. Ltd., Sydney, Australia) combines the detection of NG with the presence of FQ-resistant bacteria. The test detects NG by checking the *opa* gene and the *porA* pseudogene, and resistance to ciprofloxacin is determined by the presence of the S91F mutation in the *gyrA* gene. A large-scale clinical device evaluation in 20 European countries showed that the test had a sensitivity of 98.6% and a specificity of 100% for detecting NG, and reported a sensitivity and specificity of 99.8% relative to phenotypic susceptibility for detecting GyrA S91WT/S91F [50]. Regarding the detection of resistance mutations in *M. genitalium*, there are currently several commercially available molecular genetic-based tests, and the detection of resistance to FQ is mainly based on the presence or absence of mutations in ParC. The sensitivity for detecting FQ resistance ranged from 91.8%–94.7% and the specificity was 100%, although the results varied depending on the test method and literature [51-53]. In addition, a test for detecting macrolide-resistant *M. genitalium* has also been developed, which identifies resistance by checking for mutations in the 23S rRNA gene region. This test was reported to have a sensitivity of 100% and a specificity of 99.2% in one study [53].

The development and commercialization of tests to identify antibiotic resistance in major STI causative agents could improve the effectiveness of treatment and prevent the further development of antibiotic-resistant bacteria through the correct use of antibiotics. However, many of the current tests are available internationally and are limited to a few major pathogens. In addition to expanding the detection targets with syndromic testing, it is necessary to check the antibiotic resistance of the detected targets together to ensure the correct use of antibiotics and improve the therapeutic effectiveness.

3. Conclusion

Syndromic testing for effective diagnosis and treatment of STIs can be an important tool for early diagnosis and treatment of symptomatic infections and asymptomatic carriers. However, while it has the advantage of detecting multiple pathogens at once, its clinical validity needs to be validated due to the detection of colonizing organisms. Treatment of colonized bacteria that do not require treatment has the risk of leading to the emergence of antibiotic-resistant bacteria and unnecessary increases in healthcare costs. Therefore, when syndromic testing is used for diagnosis in clinical practice, appropriate judgment and interpretation by clinicians considering patient symptoms and pathogen characteristics are important.

Disclosure statement

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