CHAPTER II
LITERATURE REVIEWS

Chlamydia trachomatis

Biology

The Chlamydia species are distinguished from all other microorganisms by a unique growth cycle, and are placed in their own family (Chlamydiaceae). They are obligate intracellular parasites that replicate within the cytoplasm of host cells and the elementary body (EB) is adapted for extracellular survival and for initiation of infection. After adhesion to the eukaryotic cell, the EB enters the cell by endocytosis and stays in an intracellular vacuole, which develops to a so-called inclusion. Subsequently, the EB changes to a metabolically active and dividing form called the reticulate body (RB), which is adapted for intracellular multiplication. There are three Chlamydia species pathogenic to humans: *C. pneumoniae, C. psittaci* and *C. trachomatis.* All of these express a genus-specific lipopolysaccharide (LPS) antigen on the surface, which causes cross-reactions between the three species in diagnostic tests based on Chlamydia LPS antigen (56). Serological variants (serovars) A–C of *C. trachomatis* preferably colonise the eye and cause trachoma, serovars D–K preferably colonise the genital tract and cause genital infections, and L1–L3 cause lymphogranuloma venereum (LGV). *C. pneumoniae* and *C. psittaci* are etiological agents mostly of respiratory infections.

Some recent genome sequencing studies have provided us with a new understanding of *C. trachomatis* (57, 58). The first sequenced *C. trachomatis,* serovar D, genome consisted of a 1,042,519 base-pair chromosome (GenBank accession no. AE001273) and a 7,493 base-pair plasmid (58). Analysis of this genome showed that *C. trachomatis* comprises 894 likely protein- coding genes. Counterparts of enzymes characterised in other bacteria were identified in *C. trachomatis* to account for the minimal requirements for DNA replication, repair, transcription and translation. The

inclusion membrane (Inc) proteins and the polymorphic membrane proteins (Pmp) found occupy 12% to 19% of the Chlamydia-specific genomic sequences and are, according to present knowledge, unique to Chlamydia. The Chlamydia organism expresses a major outer membrane protein (MOMP) that is surface-exposed and forms the basis for classification of different serovars of *C. trachomatis*. The *omp1* gene, which codes for the MOMP, is present in all three human pathogenic Chlamydia species and is used for genotype determinations of *C. trachomatis.* The gene contains five conserved domains and four variable domains (VDI to VDIV) that vary considerably between the species (59, 60).

Life cycle and Pathogenesis

The *Chlamydiae* have a complex life cycle that involves two forms of the organism: EB, which is the nonreplicating extracellular infective form, and the RB, which is the intracellular replicative form. The life cycle begins when an elementary body adheres to and is endocytosed into a host transitional or columnar epithelial cell. Once inside the host cell, the elementary body transforms into a reticulate body. In the growth phase, the reticulate bodies undergo binary fission within vacuoles called inclusions. The reticulate bodies then reorganize into elementary bodies through DNA condensation. After 48-72 hours, the elementary bodies are released from the infected cell to initiate infection of adjacent cells or transmission to another person. *C. trachomatis* infects the superficial mucosa (squamocolumnar and columnar epithelial cells) of the urinary tract, reproductive tract, conjunctiva, gastrointestinal tract, and respiratory tract. It is now believed that part of the tissue damage caused by chlamydial infections is due to an immunologic or hypersensitivity mechanism. Despite this, the majorities of infections are asymptomatic and may persist for months to years (61).

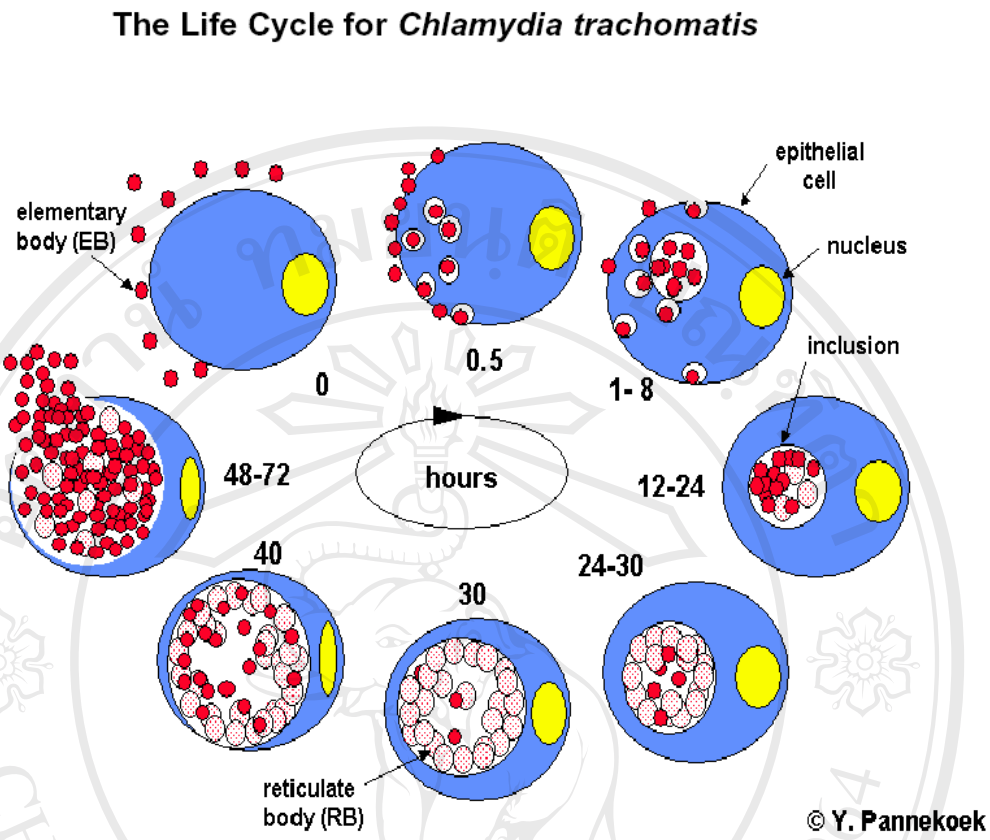

Figure 1 Life cycle of *C. trachomatis*

Source:http://www.chlsmydiae.com/chlamydiae/docs/biology/biol_devreg.htm

Epidemiology

Incidence

Each year, an estimated 3-5 millions cases of sexually transmitted chlamydial infection occur in the U.S. with the highest infection rates seen in women under age 25. In 2000, over 700,000 cases were reported to the Centers for Disease Control (CDC), for a rate of 257.5 cases per 100,000 persons (62). This was nearly double the number of gonorrhea cases reported. Reported rates of chlamydia increased dramatically in the U.S. during the 1990s, in part due to increased screening, expanded reporting requirements, and improved diagnostic technology. Chlamydial infections have a wide geographic and socioeconomic distribution. Nearly every state

in the country has a rate over 150 cases per 100,000 populations. In Thailand has a rate 20-40 cases per 100,000 populations.

Prevalence

The prevalence of chlamydial infections varies according to the clinic setting, age, and gender (63). Among young women, high positivity rates are seen in public STD clinics (10-30%), juvenile corrections (15-30%), school-based clinics (10-15%), family planning clinics (5-10%), managed care organizations (5-10%), and prenatal clinics (5-10%) (64, 65). Among men, high positivity rates are seen in STD clinics (10-20%), juvenile corrections (5-9%), and military recruits (4%) (66, 67).

Risk Factors

Young age and female gender are the most important risk factors for chlamydial infection. Additional risk factors identified for chlamydial infection include new or multiple sex partners, inconsistent use of barrier contraceptives, use of oral contraceptives, cervical ectopy, douching, and black race and low socioeconomic status (68-70).

Transmission

Chlamydial infection is highly transmissible, with 65-70% of exposed sex partners concurrently infected (71). Initially, transmission was thought to be more efficient from an infected man to his female partner, however, recent studies have demonstrated that transmission rates may be similar between genders. Based on very limited data, orogenital contact seems inefficient for transmission of chlamydial infections, although well-designed transmission studies are difficult to conduct for ethical reasons.

Clinical Manifestations

Urogenital infection in women

In the minority of women with chlamydia who have clinical findings, the spectrum of urogenital disease in women includes, but is not limited to, cervicitis, urethritis, bartholinitis, pelvic inflammatory disease (PID), and perihepatitis (4). Symptomatic women may complain of vaginal discharge, abnormal vaginal bleeding,

abdominal or pelvic pain, dysuria, or dyspareunia. The pelvic exam may be normal or reveal cervicitis, cervical motion tenderness (CMT), or uterine or adnexal tenderness.

Cervicitis

Among women with urogenital chlamydial infection, the cervix is the most common site of infection (75-80%). Although the majority of women with cervical chlamydial infection are asymptomatic, up to 30% have evidence of mucopurulent cervicitis (MPC) on speculum exam. MPC is characterized by a purulent or mucopurulent exudate visible in the endocervix or on an endocervical swab (positive swab test). Cervical friability (spontaneous or easily induced endocervical bleeding of the cervix) is also a sign of MPC. An increased number of white cells on a Gram stain of cervical discharge or exudate is no longer considered a criterion for diagnosis, as it has poor predictive value and has not been standardized.

Urethritis

Some patients may present with an acute urethral syndrome or urethritis characterized by dysuria and demonstration of fewer than 10^5 bacteria/ml of urine. Among women with urogenital infection, the urethra is a frequent site of infection (50-60%).

Pelvic Inflammatory Disease (PID)

An estimated 1 million women develop PID every year in the U.S. PID is the leading cause of preventable infertility in the U.S. Up to 40% of women with untreated chlamydial infection will develop PID (72). Due to tubal scarring, 20% of these women will progress to develop infertility, 18% will develop chronic pelvic pain, and 6% will have an ectopic pregnancy. The risk of infertility doubles with each episode of PID (73, 74).

Although various organisms have been associated with the development of PID, chlamydia has been demonstrated to infect the fallopian tubes or endometrium in over half of cases. The infection generally begins in the lower genital tract and ascends to the fallopian tubes to produce salpingitis. Infection in the tubes can also lead to tubo-ovarian abscess, which carries a risk of rupture and bleeding. The time required for spread from cervix to upper tract is still unclear. Lower abdominal pain, cervical motion tenderness, and adnexal or uterine tenderness in a sexually active woman suggest a diagnosis of PID. Chlamydia also causes “silent PID”, inflammation

without symptoms, that is associated with increased risk for infertility and ectopic pregnancy. Treatment for PID includes antibiotic regimens that provide empiric coverage for chlamydia, gonorrhea, anaerobes, and other likely pathogens (75).

Perihepatitis (Fitz-Hugh-Curtis Syndrome)

This rare syndrome is characterized by right upper quadrant pain, nausea, vomiting, fever, and normal transaminases. About 70% of perihepatitis cases are associated with chlamydia. PID may not be clinically evident. Although thought to be caused by direct spread of the organism from the infected fallopian tubes to the capsule of the liver, hematogenous and lymphatic spread may also occur. Inflammation of the liver capsule may lead to intra-abdominal adhesions.

Urogenital infections in men

Over 50% of urogenital chlamydial infections in men are asymptomatic. When symptoms are present, nongonococcal urethritis (NGU) is the most common manifestation of chlamydial infection. Other manifestations of disease in men include epididymitis, proctitis, and Reiter's syndrome (4). Chlamydial infections among men readily respond to treatment with antibiotics and rarely produce long-term medical sequelae.

Urethritis (Non-Gonococcal Urethritis)

Symptomatic men with NGU typically present with mucoid urethral discharge and dysuria. Symptoms of NGU are often mild and develop slowly over a few days. The incubation period is estimated to be 5-10 days, but may be longer. NGU in men is defined on the Gram stain of a urethral specimen by the presence of at least 5 polymorphonuclear leukocytes (PMNs) per oil immersion field (1000x) in the absence of intracellular Gram-negative diplococci (GNDC). Chlamydial organisms are not visualized on Gram stain. If Gram stain is unavailable, the diagnosis of urethritis can be supported by a positive leukocyte esterase test or the presence of at least 10 PMNs per high power field (400x) on first-void urine.

Epididymitis

Sexually transmitted epididymitis is most commonly seen in men less than 35 years of age, and 70% of cases are attributable to chlamydia. *N. gonorrhoeae* also can cause epididymitis. Epididymitis can be caused by non-STD pathogens, mainly *E. coli* and *Pseudomonas*. These infections occur more commonly in men over the age

of 35 and in men who engage in insertive anal intercourse. Overall, it is an uncommon complication, occurring in less than 2% of men with urogenital chlamydial infections. Patients typically present with unilateral scrotal pain, fever, and epididymal tenderness or swelling. Clinical examination findings include epididymal or testicular tenderness, swelling, and mass. Urethritis may be a clinical feature of the disease. The similarity in presentation to testicular torsion warrants consideration of diagnostic studies to rule out this urologic emergency. In correlation with exam findings, confirmed NGU is presumptive of chlamydial epididymitis. A positive test for Chlamydia from the urethra or the epididymal aspirate is diagnostic. Empiric treatment for epididymitis provides coverage for chlamydia, gonorrhea and other potential pathogens (75).

Anorectal infection (proctitis)

Proctitis is seen almost exclusively in homosexual men engaging in receptive anal intercourse; however, women also are susceptible. Rectal infections are generally asymptomatic but may cause symptoms characteristic of proctitis (4). Proctitis is manifested by rectal discharge, bleeding, tenesmus, and pain during defecation. If caused by non-LGV strains, proctitis is usually mild. Anoscopy reveals patchy mucosal friability and mucopurulent discharge in the distal rectum. A rectal Gram stain with at least 1 PMN without intracellular GNDC is presumptive of chlamydial proctitis, and a positive test for chlamydia is diagnostic.

Conjunctivitis

Ocular or ophthalmic infections may result from exposure to infectious genital secretions during sexual contact or by autoinoculation. Ocular disease usually presents as unilateral eye discomfort and hyperemia, with or without mucopurulent discharge. The conjunctiva often has a follicular appearance. Pre-auricular lymphadenopathy and otitis media may develop. Only 1% of persons with proven genital infection have ocular manifestations of infection while over 50% of persons with ocular infection have concurrent genital infection (76). Treatment with the same recommended oral regimens as for genital infections is required.

Lymphogranuloma veneeum

Lymphogranuloma venereum (LGV) is a sexually transmitted infection that is caused by *C. trachomatis* serovars L1, L2, and L3. Disease usually presents as painful

inguinal adenopathy up to 6 weeks after exposure to infection. In two-thirds of cases, the adenopathy is unilateral and may progress to become fluctuant and suppurative. Constitutional symptoms such as fever, chills, meningismus, myalgias or arthralgias also may be present. This syndrome presents most commonly in persons ages 20- 30 years, and the highest prevalence of disease remains in Africa, Asia, and South America. Fewer than 200 cases per year were reported in the U.S. in the last several years (77). LGV serovars are also associated with proctocolitis. Signs and symptoms include severe rectal pain, discharge, hematochezia, fever, and lymphadenopathy. Anoscopy is markedly abnormal with lesions extending into the colon. If untreated, this form of proctocolitis may lead to bowel obstruction. Treatment of LGV consists of a 3-week course of doxycycline (75).

Reiter's syndrome

Reiter's syndrome is a post-inflamatory autoimmune disease that occurs almost exclusively in men. Over 80% of persons with Reiter's syndrome are positive for the HLA-B27 phenotype. Other organisms, such as *Shigella, Salmonella, Yersinia*, and *Campylobacter* also have been associated with Reiter's syndrome, however, chlamydia accounts for up to 70% of cases with non-diarrheal disease (78). The disease occurs 3-6 weeks after a urogenital infection. The classic presentation involves conjunctivitis, urethritis, arthritis, and mucocutaneous skin lesions. Less common manifestations include keratoderma blenorrhagica and circinate balanitis. Most cases resolve completely within 2-6 months, but may last more than a year. Fifteen percent of cases will develop disease recurrence. Among men with NGU, 1-3% will develop Reiter's syndrome. Symptoms generally respond to nonsteroidal anti-inflammatory agents.

Neonatal infections

Nearly two-thirds of neonates born to infected mothers will develop chlamydial colonization after delivery. *C. trachomatis* can cause conjunctivitis and pneumonia in infants (83). Approximately 18-50% of colonized newborns will develop conjunctivitis, and 11-20% will develop pneumonia. Exposed infants can also develop asymptomatic infections of the oropharynx, genital tract, and rectum. Detecting maternal chlamydial infection through routine prenatal screening and adequately treating before delivery prevents neonatal infections.

Inclusion Conjunctivitis

C. trachomatis is the most common cause of neonatal conjunctivitis in the U.S. Neonatal chlamydial conjunctivitis appears 5-14 days after birth and is characterized by hyperemia and mucopurulent discharge. Laboratory confirmation of *C. trachomatis* is required to differentiate the organism from other potential pathogens. Conjunctival cells (not just exudate) should be present on specimens being tested. Culture and non-culture tests can be used. Ocular prophylaxis does not prevent infection to the newborn. Furthermore, prophylaxis aimed at the eye will fail to prevent direct infections or colonization by chlamydia at other sites, such as the vagina, rectum, oropharynx, nasopharynx, and lung.

Pneumonia

C. trachomatis is the etiologic agent in up to 20% of cases of infant pneumonia. Subacute, afebrile pneumonia due to chlamydia generally develops at age 1-3 months, and is characterized by a repetitive staccato cough with tachypnea, hyperinflation, and bilateral diffuse infiltrates on a chest x-ray. Wheezing is rare and peripheral eosinophilia is sometimes observed. Fifty percent of newborns will have a history of conjunctivitis at the time of diagnosis. Untreated newborns may develop a protracted course of illness that may include apneic spells, asthma or obstructive airway disease. Although culture of nasopharyngeal specimens can be used to detect chlamydia, the presence of the organism in the nasopharynx is not diagnostic for pneumonia. High IgM titers are the best indicators of chlamydial pneumonia.

Laboratory diagnosis

Laboratory confirmation of infection should be conducted for all patients suspected to have chlamydial infection. The diagnostic methods to detect chlamydial infection have changed significantly in the past few years. Currently available methods include tissue culture, detection of antigens using direct fluorescent antibody (DFA) or enzyme immunoassay (EIA), and detection of nucleic acid sequences by probe hybridization, enzymatic amplification, and hybrid capture (18). Because chlamydia is an intracellular bacterium, specimen collection has focused on obtaining columnar epithelial cells from the affected sites such as the endocervix or the urethra.

Newer nucleic acid amplification technologies can detect even small fragments of chlamydial nucleic acid in urine or in vaginal swabs. Of note, blood in the specimen may interfere with test performance.

Tissue Culture

Tissue culture has been the gold standard diagnostic test because of its superior test performance characteristics compared with antigen detection and nucleic acid probe hybridization technologies. However, this status is being challenged because of its low sensitivity compared with new amplification technologies. The sensitivity of cell culture is variable (75- 85%) but the specificity is nearly 100%. Because of the high specificity, culture is still recommended as the detection method for suspected cases of child sexual abuse. Culture can be used for all anatomical sites. Tissue culture is labor and time intensive, and the successful recovery of organisms using this method requires strict attention to rigorous specimen handling and transport procedures. Swabs with wooden shafts should be avoided, as they can be toxic to chlamydia. Periodic proficiency testing in laboratories is necessary to ensure specimens are processed according to recommended procedures. In addition, quality assurance testing should be performed to assess the adequacy of collection of the appropriate cell types for testing (i.e., columnar cells from the endocervix, urethra, rectum, or conjunctiva) (18).

Antigen Detection Methods

Both direct fluorescent antibody (DFA) and enzyme immunoassay (EIA) have been used for antigen detection. These tests detect organisms by immunologic methods and do not require live organisms. They are not approved for all anatomical sites. Although the sensitivity and specificity of DFA (e.g., *MicroTrak*®) is dependent upon proper interpretation by a skilled microscopist, the test has a sensitivity of 70%-75% and a reported specificity of 95-99%. DFA is the only method where specimen adequacy can be evaluated as part of reading the test result because the presence of columnar cells can be determined while reviewing the slide for evidence of chlamydia. EIA (e.g., Syva EIA), has a sensitivity of 50- 75% and a specifi city of 95-99%. This sensitivity makes EIA unsatisfactory for screening asymptomatic populations. To elicit better test performance characteristics from EIA technologies,

confirmatory testing, verification assays, negative gray zone testing, and periodic quality assurance for specimen adequacy should be performed (18, 79).

Non-Amplified Probe Hybridization

Non-amplified nucleic acid probe tests (Gen- Probe *PACE 2*®) are commonly used because of the ease of automation and reduced expense. Furthermore, this test offers the advantage of being able to detect both *C. trachomatis* and *N. gonorrhoeae* from the same swab specimen. To elicit best test performance characteristics from nucleic acid probe hybridization technologies, confirmatory testing, verification assays, negative gray zone testing, and periodic quality assurance for specimen adequacy should be performed (18, 79).

Nucleic Acid Amplifi cation Tests

Nucleic acid amplification tests (NAATs) provide excellent sensitivity (90-95%) and specificity (98-100%). The amplification tests currently on the market are *LCx*® (Abbott, LCR), *Amplicor*® and *COBAS*® (Roche, PCR), *AmpCT*® and *APTIMA*® (Gen-Probe, TMA), and *ProbeTec*® (Becton-Dickinson, SDA). These tests identify up to 30% of chlamydial infections that would be missed by other methods (22). These tests offer the option of detecting *N. gonorrhoeae* from the same specimen, however, not all NAATs have complete FDA clearance for gonorrhea testing on all collection sites. Further, the increase in sensitivity has enabled the use of noninvasive specimen collection, such as first-voided urine and self-collected vaginal swab. These noninvasive methods eliminate the need for painful urethral swabs in men and pelvic examinations in women, particularly adolescents, thus, significantly increasing patient acceptability (80). For these reasons, urine specimens are the test of choice for testing male patients and for screening women in settings where a pelvic exam is not indicated. Amplification tests have not been FDA-approved for pharyngeal and rectal specimens. Contamination at the time of collection (i.e., the clinic site) or at the laboratory may result in false positive results. Because the amplification process is enzyme-dependent, the presence of inhibitors (e.g., blood) may cause false negative results; however, some assays have internal controls and methods of minimizing inhibition. Amplified tests cost more than nucleic acid probe and other tests.

Signal Amplification

The Digene *Hybrid Capture 2*® uses a signal amplified nucleic acid hybridization assay to identify chlamydial infections. This test has a sensitivity somewhat less than NAATs and offers the option of detecting *C. trachomatis* and *N. gonorrhoeae* from the same specimen. Because of its recent introduction into the market, experience with this assay is limited.

Other Tests

Chlamydia serology is of no value in the diagnosis of acute uncomplicated urogenital infections. High background prevalence and infrequent rises and falls in IgG and IgM make test results less meaningful. Serology is useful in the diagnosis of LGV and neonatal pneumonia. Rapid clinic-based tests for chlamydia, e.g., *QuickVue*®, *Clearview*®, Biostar *OIA*®, Abbott *Testpack*®, are not recommended in clinical settings because their overall performance is inferior to currently available laboratory-based tests. In particular, they have a low sensitivity of approximately 50% (18).

Treatment

The current CDC recommendations for the treatment of uncomplicated chlamydial infection include azithromycin 1 gram orally in a single dose or doxycycline 100 mg orally 2 times a day for 7 days (75). The results of clinical trials indicate that azithromycin and doxycycline are equally effective (95%) in eradicating infection (81). These investigations were conducted primarily in populations in which adherence to a 7-days regimen was good. Azithromycin has prolonged bioavailability, wide tissue distribution, high intracellular concentration, and high activity against chlamydia. However, it is more expensive than doxycycline. Azithromycin is now approved for use in persons less than 15 years of age. In populations with poor compliance with treatment or minimal follow-up, azithromycin may be more cost effective because it provides a single dose, directly observed therapy (82). Doxycycline has the advantage of low cost and a longer history of extensive use, but has the disadvantage of a longer course and resultant problems with adherence in certain populations (84).

Neisseria gonorrhoeae

Biology

The obligate human pathogen *N. gonorrhoeae* belongs to the Neisseria genus of the bacterial family Neisseriaceae. The two primary pathogenic Neisseria species, *N. gonorrhoeae* and *N. meningitidis*, are genetically and morphologically similar. However, molecular, cellular and biochemical differences exist, which probably reflect the different diseases that the two species are causing (85, 86). Thus, *N. gonorrhoeae* mostly colonises the urogenital tract and causes gonorrhoea, while *N. meningitidis* preferably colonises the throat/upper respiratory tract and in some cases is the cause of meningitis and/or septicaemia. The chromosome of the *N. gonorrhoeae* strain FA1090 was recently determined to consist of 2,153,944 bp (GenBank accession no. AE004969, http://www.genome.ou.edu/gono.html). One cryptic plasmid, several b-lactamase-encoding plasmids and different conjugative plasmids may also be carried by *N. gonorrhoeae* strains. Horizontal genetic exchange, through transformation and conjugation, frequently occurs between *N. gonorrhoeae* strains and also with other similar species, mostly commensal Neisseria or *N. meningitidis* (87–90). A random recombination between chromosomal genetic loci, most probably mediated by transformation, is presumed to be frequent in nature, and consequently the population structure of *N. gonorrhoeae* is fully sexual, i.e. panmictic or non-clonal (87). The frequent horizontal genetic exchange in combination with mutations causes a high level of genotypic and phenotypic variability, which is important for evasion or adaptation to the immune response of the host and for development or spread of antibiotic resistance mechanisms. These properties make the bacteria effective in persisting without severely damaging the host, i.e. in producing mildly symptomatic or asymptomatic infection (25), and this also stresses the importance of using adequate methods for diagnosis and characterization of this highly variable pathogen.

Life cycle and Pathogenesis (91)

Gonorrhea in adults is almost invariably transmitted by sexual intercourse. The bacteria adhere to columnar epithelial cells, penetrate them, and multiply on the basement membrane. Adherence is mediated through fimbriae and opa (P.II) proteins. although nonspecific factors such as surface charge and hydrophobicity may play a role. Fimbriae undergo both phase and antigenic variation. The bacteria attach only to microvilli of nonciliated columnar epithelial cells. Attachment to ciliated cells does not occur.

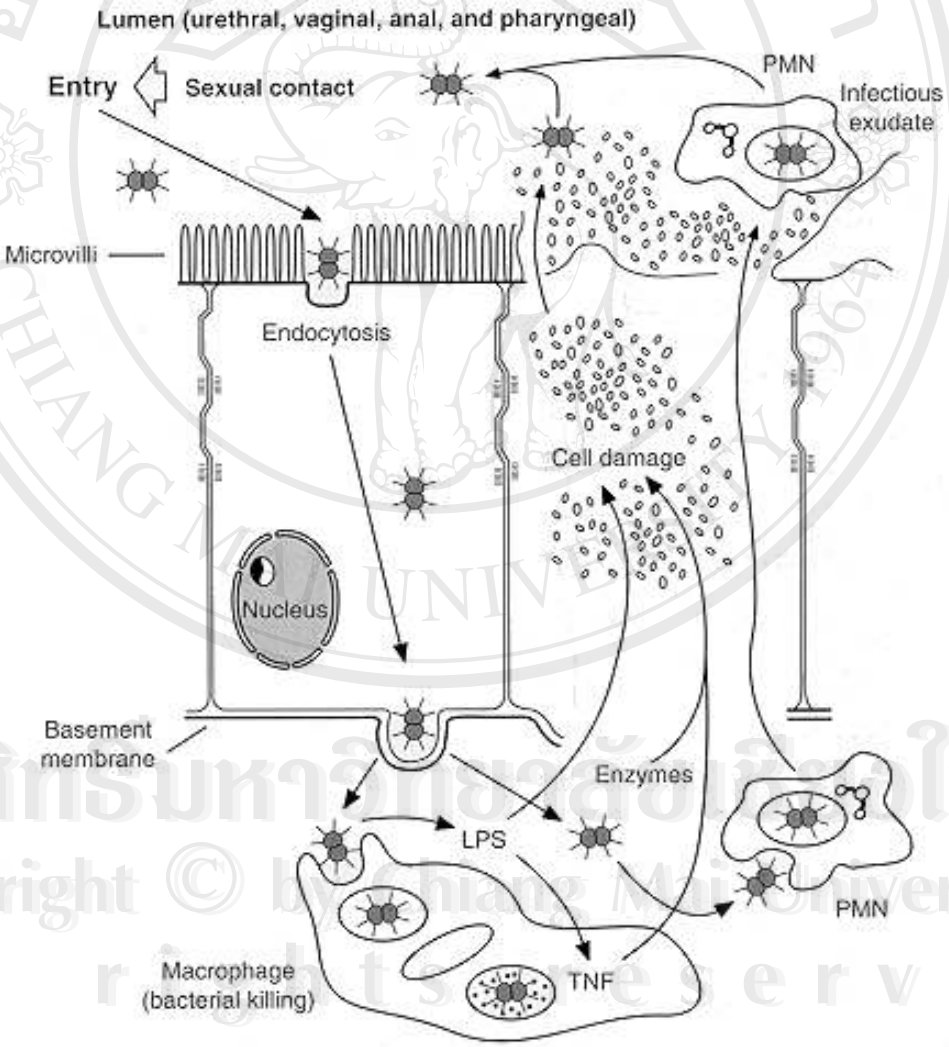

Figure 2 Pathogenesis of uncomplicated gonorrhea
source: http://www.Textbookof bacteriology.net/

Most of the information on bacterial invasion comes from studies with tissue culture cells and human fallopian tube organ culture. After the bacteria attach to the nonciliated epithelial cells of the fallopian tube, they are surrounded by the microvilli, which draw them to the surface of the mucosal cell. The bacteria enter the epithelial cells by a process called parasite-directed endocytosis. During endocytosis the membrane of the mucosal cell retracts and pinches off a membrane-bound vacuole that contains the bacteria. The vacuole is transported to the base of the cell, where the bacteria are released by exocytosis into the subepithelial tissue. The neisseriae are not destroyed within the endocytic vacuole, but it is not clear whether they actually replicate in the vacuoles as intracellular parasites.

A major porin protein, P.I (Por), in the outer membrane of the bacterium is thought to be the invasin that mediates penetration of a host cell. Each *N. gonorrhoeae* strain expresses only one type of Por; however, there are several variations of Por that partly account for different antigenic types of the bacterium.

N. gonorrhoeae can produce one or several outer membrane proteins called Opa (P.II) proteins. These proteins are subject to phase variation and are usually found on cells from colonies possessing a unique opaque phenotype called O+. At any particular time, the bacterium may express zero, one, or several different Opa proteins, and each strain has 10 or more genes for different Opas. Rmp (P.III) is an outer membrane protein found in all strains of *N. gonorrhoeae.* It does not undergo antigenic variation and is found in a complex with Por and LOS. It shares partial homology with the OmpA protein of *Escherichia coli.* Antibodies to Rmp, induced either by a neisserial infection or by colonization with *E. coli,* tend to block bactericidal antibodies directed against Por and LOS. In fact, anti-Rmp antibodies may increase susceptibility to infection by *N. gonorrhoeae.*

During infection, bacterial lipooligosaccharide (LOS) and peptidoglycan are released by autolysis of cells. Both bacterial polysaccharides activate the host alternative complement pathway, while LOS also stimulates the production of tumor necrosis factor (TNF) that causes cell damage. Neutrophils are immediately attracted to the site and feed on the bacteria. For unknown reasons, many gonococci are able to survive inside of the phagocytes, at least until the neutrophils themselves die and release the ingested bacteria.

Neisserial LOS has a profound effect on the virulence and pathogenesis of *N. gonorrhoeae.* The bacteria can express several antigenic types of LOS and can alter the type of LOS they express by some unknown mechanism. Gonococcal LOS produces mucosal damage in fallopian tube organ cultures and brings about the release of enzymes, such as proteases and phospholipases that may be important in pathogenesis. Thus, gonococcal LOS appears to have an indirect role in mediating tissue damage. Gonococcal LOS is also involved in the resistance of *N. gonorrhoeae* to the bactericidal activity of normal human serum. Specific LOS oligosaccharide types are known to be associated with a serum-resistant phenotypes of *N. gonorrhoeae.*

N. gonorrhoeae can utilize host-derived N-acetylneuraminic acid (sialic acid) to sialylate the oligosaccharide component of its LOS, converting a serum-sensitive organism to a serum-resistant one. Organisms with nonsialylated LOS are more invasive than those with sialylated LOS but organisms with sialylated LOS are more resistant to bactericidal effects of serum. There is also antigenic similarity between neisserial LOS and antigens present on human erthyrocytes. This similarity to "self" may preclude an effective immune response to these LOS antigens by maintaining the immunotolerance of the host.

N. gonorrhoeae is highly efficient at utilizing transferrin-bound iron for in vitro growth; many strains can also utilize lactoferrin-bound iron. The bacteria bind only human transferrin and lactoferrin. This specificity is thought to be, in part, the reason these bacteria are exclusively human pathogens.

Strains of *N. gonorrhoeae* produce two distinct extracellular IgA1 proteases which cleave the heavy chain of the human immunoglobulin at different points within the hinge region. Split products of IgA1 have been found in the genital secretions of women with gonorrhea, suggesting that the bacterial IgA1 protease is present and active during genital infection. It is thought that the Fab fragments of IgA1 may bind to the bacterial cell surface and block the Fc-mediated functions other immunoglobulins.

Occasionally, as described above, invading *N. gonorrhoeae* enter the bloodstream causing a Gram-negative bacteremia which may lead to a disseminated bacterial infection. Asymptomatic infections of the urethra or cervix usually serve as

focal sources for bacteremia. Strains of *N. gonorrhoeae* that cause disseminated infections are usually resistant to complement and the serum bactericidal reaction. This accounts for their ability to persist in the bacteremia. In Gram-negative bacteremias of this sort, the effect of bacterial endotoxin can be exacerbated by the lyis of bacterial cells which may simply liberate soluble LPS.

Virulence Factors (91)

Like the other pyogenic bacteria, *N. gonorrhoeae* has a wide range of virulence determinants, although it does not produce any exotoxins. The first stages of infection, involving adherence and invasion, are mediated by surface components of the gonococci. The bacterium first attaches to epithelial cells by means of its fimbriae, specifically N-methylphenylalanine (Type 4) pili, the main subunit of which is PilE. After initial attachment, the bacteria enter a second stage of binding mediated by the outer membrane protein P.II (also known as Opa) which is needed for tight binding and invasion of epithelial cells. Also, P.II from one bacterium will bind to LOS of an adjacent bacterium, which allows for the construction of a microcolony which may be functionally analogous to a biofilm. However, the invasion of a cell involves a single bacterium, not whole microcolonies. *N. gonorrhoeae* also produces an IgA1 protease that probably plays a role in the colonization stage.

The outer membrane porin of *N. gonorrhoeae* P.I (also known as Por) is equivalent to the ompC and ompF porins of *E. coli* that are involved in the passage of solutes through the outer membrane. However, P.I apparently has a role in virulence that allows the gonococci to survive inside of phagocytes. Purified P.I has been shown to inhibit the ability of phagocytes to kill ingested bacteria.

The lipooligosaccharide (LOS) of the outer membrane is thought to be responsible for most of the symptoms of gonorrhea. Gonococcal LOS triggers an intense inflammatory response. Subsequent activation of complement, attraction and feeding by phagocytes, and the lysis of the phagocytes themselves, contributes to the purulent discharge. The local production of TNF, elicited by LOS, is thought to be the main cause of damage to the fallopian tubes. In addition, in strains that cause systemic infection, LOS binds sialic acid from the serum forming a microcapsule of

sialylated LOS, which allows the gonococci to resist the host immune response and serum bactericidal reaction.

Table 1. Surface components of *N. gonorrhoeae* that may play a role in virulence

Designation	Location	Contribution
PilE	major fimbrial protein	initial binding to epithelial cells
P.II (Opa)	outer membrane protein	contributes to invasion
P.I (Por)	outer membrane porin	may prevent phagolysosome formation in neutrophils and/or reduce oxidative burst
LOS	outer membrane lipooligosaccharide	elicits inflammatory response, triggers release of TNF
P.III (Rmp)	outer membrane protein	elicits formation of ineffective antibodies that block that block bactercidal antibodies against P.I and LOS
Tbp1 and Tbp2	outer membrane receptors for transferrin	iron acquisition for growth
Lbp	outer membrane receptor for lactoferrin	iron acquisition for growth

Nonsialyated LOS and P.I (Por) on the bacterial surface are known to be effective targets for bactericidal antibodies. However, if antibodies produced against P.III (also known as Rmp) react with their antigenic site on the gonococcal surface, the effect is to block bactericidal antibodies against LOS and P.I and to protect the bacterium from complement-mediated lysis.

Finally, *N. gonorrhoeae* has a well-developed iron acquisition system that permits it to extract iron from its host during growth, which is necessary to support bacterial invasion. Basically, the bacterium is able to form two transferrin receptors (Tbp1 and Tbp2) and one lactoferrin receptor (Lbp) in its outer membrane, which are induced under low-iron conditions, and which are able to directly extract iron from transferrin and lactoferrin, respectively. The proteins can also extract iron from heme and hemoglobin.

Epidemiology (92, 93)

N. gonorrhoeae was second in frequency only to *C. trachomatis* among reported communicable infections in worldwide. The age distribution of *N. gonorrhoeae* infections is similar to that for *C. trachomatis* infections. In the US the reported rate is approximately 240 cases per 100,000 populations. In Thailand has a rate 20-40 cases per 100,000 populations. However, the incidence has declined considerably in the modern West in recent years. The rate of gonorrhea is much higher in African Americans compared to other racial groups and is much higher in the rural southeastern United States and in inner cities, presumably because of an association with socioeconomic and behavioral factors. Internationally, disease rates are unknown for most developing countries. In much of Western Europe, rates approximate those in the United States. The incidence is substantially lower in most European countries, and indigenous gonorrhea has virtually been eliminated in Sweden. The highest incidence of gonorrhea and its complications occurs in developing countries. The median prevalence of gonorrhea in unselected populations of pregnant women has been estimated to be 10% in Africa, 5% in Latin America, and 4% in Asia.

Risk factors

All sexually active populations are at risk, and the level of risk rises with the number of sexual partners and the presence of other sexually transmitted diseases (STDs). The highest rates of gonorrhea are found in young (15-30 years) unmarried persons and in groups of low educational and socioeconomic status. Infection in children is a marker for child sexual abuse.

Transmission

Gonococcal infection usually follows mucosal inoculation during vaginal, anal, or oral sexual contact or perinatally. Sexual contact, the risk of transmission of *N. gonorrhoeae* from an infected woman to the urethra of her male partner is approximately 20% per episode of vaginal intercourse and rises to 60-80% after 4 or more exposures. In contrast, the risk of male-to-female transmission approximates 50-70% per contact, with little evidence of increased risk with more sexual exposures. Transmission through penile-rectal contact is fairly efficient. Persons who have unprotected intercourse with new partners frequently enough to sustain the infection are defined as core transmitters. Neonatal infection may follow conjunctival inoculation during birth or direct infection through the scalp at the sites of fetal monitoring electrodes.

Clinical Manifestations (91)

The disease gonorrhea is a specific type of urethritis that practically always involves mucous membranes of the urethra, resulting in a copious discharge of pus, more apparent in the male than in the female. The first usage of the term "gonorrhea", by Galen in the second century, implied a "flow of seed". For centuries thereafter, gonorrhea and syphilis were confused, resulting from the fact that the two diseases were often present together in infected individuals. Paracelsus (1530) thought that gonorrhea was an early symptom of syphilis. The confusion was further heightened by the classic blunder of English physician John Hunter, in 1767. Hunter intentionally inoculated himself with pus from a patient with symptoms of gonorrhea and wound up giving himself syphilis!. The causative agent of gonorrhea, *N. gonorrhoeae,* was first described by A. Neisser in 1879 in the pustular exudate of a case of gonorrhea. The organism was grown in pure culture in 1885, and its etiological relationship to human disease was later established using human volunteers in order to fulfill the experimental requirements of Koch's postulates.

Gonorrheal infection is generally limited to superficial mucosal surfaces lined with columnar epithelium. The areas most frequently involved are the urethra, cervix,

rectum, pharynx, and conjunctiva. Squamous epithelium, which lines the adult vagina, is not susceptible to infection by the *N. gonorrhoeae.* However, the prepubescent vaginal epithelium, which has not been keratinized under the influence of estrogen, may be infected. Hence, gonorrhea in young girls may present as vulvovaginitis. Mucosal infections are usually characterized by a purulent discharge.

Uncomplicated gonorrhea in the adult male is an inflammatory and pyogenic infection of the mucous membranes of the anterior urethra. The most common symptom is a discharge that may range from a scanty, clear or cloudy fluid to one that is copious and purulent. Dysuria (difficulty in urination) is often present. Inflammation of the urethral tissues results in the characteristic redness, swelling, heat, and pain in the region. There is intense burning and pain upon urination. Endocervical infection is the most common form of uncomplicated gonorrhea in women. Such infections are usually characterized by vaginal discharge and sometimes by dysuria. About 50% of women with cervical infections are asymptomatic. Asymptomatic infections occur in males, as well. Males with asymptomatic urethritis are an important reservoir for transmission and are at increased risk for developing complications. Asymptomatic males and females are a major problem as unrecognized carriers of the disease, which occurs in the U.S. at an estimated rate of over one million cases per year.

In the male, the organism may invade the prostate resulting in prostatitis, or extend to the testicles resulting in orchitis. In the female, cervical involvement may extend through the uterus to the fallopian tubes resulting in salpingitis, or to the ovaries resulting in ovaritis. As many as 15% of women with uncomplicated cervical infections may develop pelvic inflammatory disease (PID). The involvement of testicles, fallopian tubes or ovaries may result in sterility. Occasionally, disseminated infections occur. The most common forms of disseminated infection are a dermatitis-arthritis syndrome, endocarditis and meningitis.

Rectal infections (proctitis) with *N. gonorrhoeae* occur in about one-third of women with cervical infection. They most often result from autoinoculation with cervical discharge and are rarely symptomatic. Rectal infections in homosexual men usually result from anal intercourse and are more often symptomatic. Partners must be treated as well to avoid reinfection.

Ocular infections by *N. gonorrhoeae* can have serious consequences of corneal scarring or perforation. Ocular infections (ophthalmia neonatorum) occur most commonly in newborns who are exposed to infected secretions in the birth canal. Part of the intent in adding silver nitrate or an antibiotic to the eyes of the newborn is to prevent ocular infection by *N. gonorrhoeae.*

Laboratory Diagnosis (93)

Laboratory diagnosis of gonococcal infections depends on identification of *N. gonorrhoeae* at an infected site.

Isolation through culture

This is the diagnostic standard and should be used whenever practical. A single culture on most selective media has a sensitivity of 95% or more for urethral specimens from men with symptomatic urethritis and 80-90% for endocervical infection in women. Simultaneous inoculation on selective and nonselective media may provide the highest yield. Although the urethra is commonly infected in women with gonorrhea, culturing urethral specimens does not materially increase the diagnostic yield except in women who lack cervices because of hysterectomy. Patients with possible disseminated gonococcal infection (DGI) should have culture samples taken from all possible mucosal sites (ie, pharynx, urethra, cervix, rectum) and from blood and synovial fluid. Rectal and pharyngeal specimens are inoculated onto selective medium only. When collecting specimens in males, any discharge present at the meatus can be easily recovered for examination. If no discharge is present at the meatus, urethral material must be recovered by inserting and rotating a small swab 2-3 cm into the urethra. A calcium alginate or Rayon swab on a metal shaft is recommended. When collecting specimens in women, the exocervix is first wiped of exudate. A swab is then placed into the external os and rotated for several seconds. However, take care to avoid contact with vaginal mucosa or secretions. In patients who may have DGI, all possible mucosal sites should be cultured (eg, pharynx, cervix, urethra, rectum), as should blood and synovial fluid (in cases of septic arthritis). Three sets of blood cultures should also be obtained. Specimens from any mucosal site should be inoculated immediately in selective media for

gonorrheal organisms, such as modified Thayer-Martin, or on chocolate agar at room temperature, which should be incubated in an enriched carbon dioxide environment. The growth of typical oxidase-positive colonies that consist of gram-negative diplococci strongly suggests gonorrhea.

Smears with Gram's stain

In men, the diagnosis of urethritis can be performed using either of 2 methods of Gram staining. The first is via a urine sample. Preferably, examine the patient at least 2 hours after micturition or before their first morning void. The patient should provide a first-morning void, and the first 10-15 mL of the urine is saved. The urine is centrifuged so that the sediment may be analyzed microscopically under high power or oil immersion. The presence of 10 or more polymorphonuclear leukocytes (PMNs) seen under high power is suggestive of urethritis. The second method is a Gram stain of urethral exudate. The presence of 4 or more PMNs per oil-immersion field is diagnostic for urethritis. In symptomatic males, Gram staining of urethral exudate has a sensitivity of 90-98% and a specificity of 95-98%(93). However, in asymptomatic males, the sensitivity of the Gram's stain is only 60%(93). Therefore, culture studies are recommended if an asymptomatic gonococcal infection is suggested. The presence of typical gram-negative intracellular diplococci after Gram stain establishes a diagnosis of gonorrhea. If these organisms are not observed, the patient is said to have nongonococcal urethritis. Results are considered equivocal if typical morphotypes not associated with neutrophils are present or if cell-associated but morphologically atypical organisms are observed. A simple Gram stain is probably the method of choice for the detection of gonorrhea in symptomatic males because it is much less expensive and much more rapid than the Gen-Probe method.

In women with positive results from cervical cultures, the Gram stain results from the endocervix are 50-60% sensitivity and 82-97% specificity(93. Also, the presence of more than 10 PMNs per high-power field on an endocervical smear is consistent with cervicitis. In women who lack cervices because of hysterectomy, use urethral culture to make the diagnosis. No available serologic test is sufficiently sensitive and specific to merit use for screening or diagnostic purposes.

Imaging Studies:

Ultrasound or CT scan for PID, pelvic ultrasound or CT scan images may show thick dilated fallopian tubes or abscess formation. PID is uncommon in pregnancy. Therefore, ultrasound should be used to help rule out ectopic pregnancy whenever a pregnant patient has signs and symptoms of possible PID. In current practice, vaginal ultrasonography and CT scan help to define the cause of pelvic pain syndromes.

Other Tests:

Various tests can be used, if available, to detect the antigen or the genome of gonococci in exudates. Fluorescein-conjugated monoclonal antibodies for direct fluorescence microscopy can be used to detect antigen. Enzyme-linked immunoassays for the detection of gonococcal antigen with polyclonal antigonococcal antibodies can also be used. Polymerase chain reaction tests for gonococcal DNA amplification can be used, although they are quite expensive and do not contribute much in most settings. Ligase chain reaction tests for the presence of gonococcal DNA are also becoming available. These tests are highly specific and extremely sensitive, but they are expensive.

Treatment (91)

The recommended treatment for uncomplicated infections is a third-generation cephalosporin or a fluoroquinolone plus an antibiotic (e.g., doxycycline or erythromycin) effective against possible coinfection with *C. trachomatis*. Sex partners should be referred and treated. The current CDC Treatment Guidelines recommend treatment of all gonococcal infections with antibiotic regimens effective against resistant strains. The recommended antimicrobial agents are ceftriaxone, cefixime, ciprofloxacin, or oflaxacin.

PCR Amplification

Despite the development of numerous alternative methods over the years, PCR and PCR-derived techniques remain the most widely used methods of NAA (94). PCR is based on the ability of DNA polymerase to copy a strand of DNA (95-97). The enzyme initiates elongation at the 3' end of a short (primer) sequence bound to a longer (target) strand of DNA. When two primers bind to complementary strands of target DNA, the sequence between the two primer binding sites is amplified exponentially with each cycle of PCR. Each cycle consists of three steps: (i) a DNA heat denaturation step, in which the double strands of the target DNA are separated; (ii) a primer-annealing step, in which primers anneal to their complementary amplification target sequences at a lower temperature; and (iii) an extension reaction step, in which DNA polymerase extends the target sequences between the primers. At the end of each cycle, which consists of the above three steps, the PCR products are theoretically doubled (Fig.3). The whole procedure is carried out in a programmable thermocycler. Generally, 30 to 50 thermal cycles result in detectable amounts of the target sequence originally present in less than 100 copies, with potential sensitivity to the single-copy level (98).

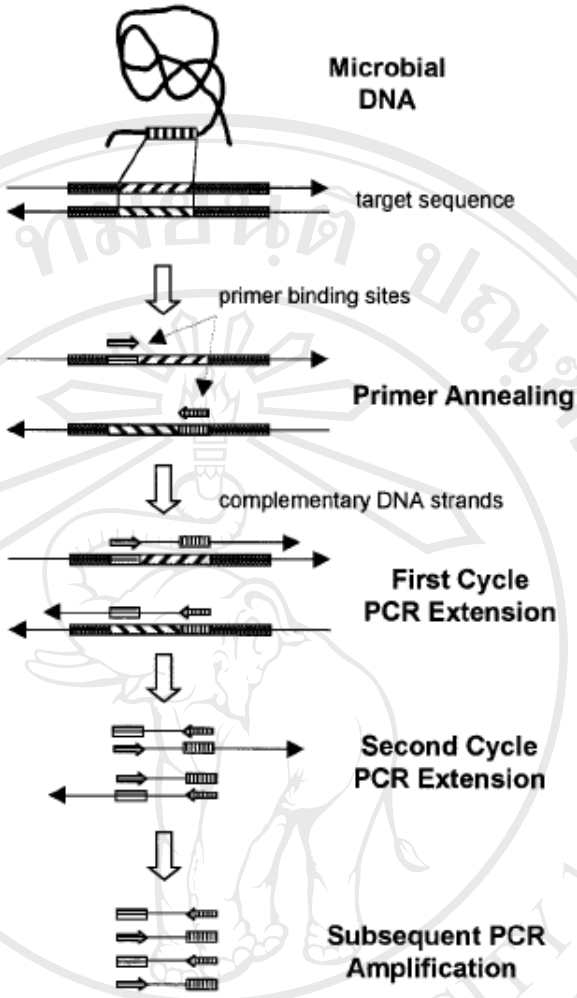

Figure 3. Steps of the PCR reaction. A DNA target is required. RNA pathogens can also be amplified with a prior reverse transcriptase enzyme step. The key components are two primers (small DNA segments or oligonucleotides) specific for the target region and a thermostable DNA polymerase. With alternating temperatures the PCR technique denatures target strands, anneals primers and amplifies the strand by replicating the coordinate strand from the primed complexes, creating double strand nucleic acid products called amplicons. The cycle is repeated 25 to 40 times until large quantities of amplified product are produced. The amplicon can be detected either by separation on an agarose gel under the influence of an electric current or by an enzyme immunoassay color detection method.

Multiplex PCR

Multiplex PCR is an amplification reaction in which two or more sets of primers specific for different targets are introduced in the same tube, allowing multiple target sequences to be amplified simultaneously (Fig.4) (94, 99). Primers used in multiplex reactions must be designed carefully to have similar annealing temperature and to lack complementarity, in order to avoid dimerization. Extensive empirical testing is often needed. Coamplification of multiple targets can be used for different purposes. For diagnostic purposes, multiplex PCR can be used for detecting internal controls or for detecting multiple pathogens in a single specimen (100-102). Quantitative competitive PCR, a variation of multiplex PCR, can be used to quantify the amount of target sequence in a specimen (103,104). Multiplex PCR assays play a larger role in human and cancer genetics, in which target nucleic acid is not limiting. However, development of multiplex PCR assays for detection of infectious organisms is more complicated and can result in lower sensitivity

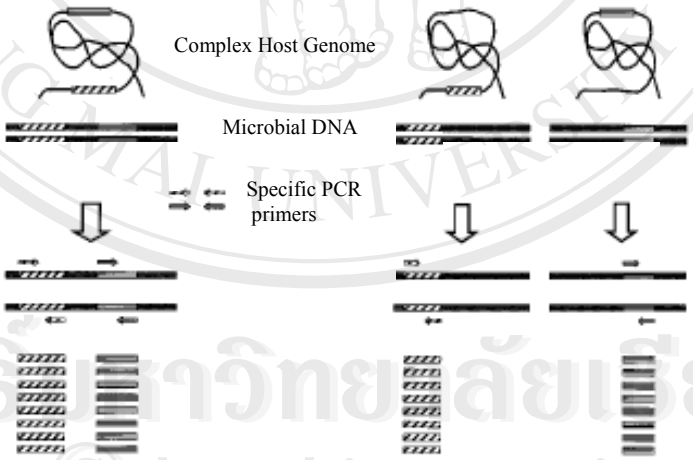

Figure 4. Features of multiplex PCR. Multiplexing involves the running of multiple specific PCR reactions simultaneously in the same sample tube to test for different DNA or RNA templates. Several sets of oligonucleotide primers are added to the reaction to generate several sets of different PCR products.

Nested PCR

Nested PCR Designed mainly to increase sensitivity nested PCR uses two sets of amplification primers (Fig.5) (105). One set is used for the first round amplification, consisting of 15 to 30 cycles. Products of the first reaction are then a subjected to a second round of amplification with another set of primers specific for sequence within the product of the first primer pair (105,106). Nested PCR is highly sensitive due to the large total cycle number. It is theoretically more specific than amplification using the same number of cycles with a single primer set, because the amplicon from the first round of amplification must contain hybridization sites for the second primer pair; amplification by the second primer set verifies the specificity of the first PCR reaction. The major disadvantage of nested amplification is the high risk of contamination incurred during transfer of first-round amplification products to a second tube. This transfer step can be avoided either by physically separating the two amplification mixtures with a layer of wax or oil (107) or by designing the second primer set with an annealing temperature substantially higher than that of the first (108).

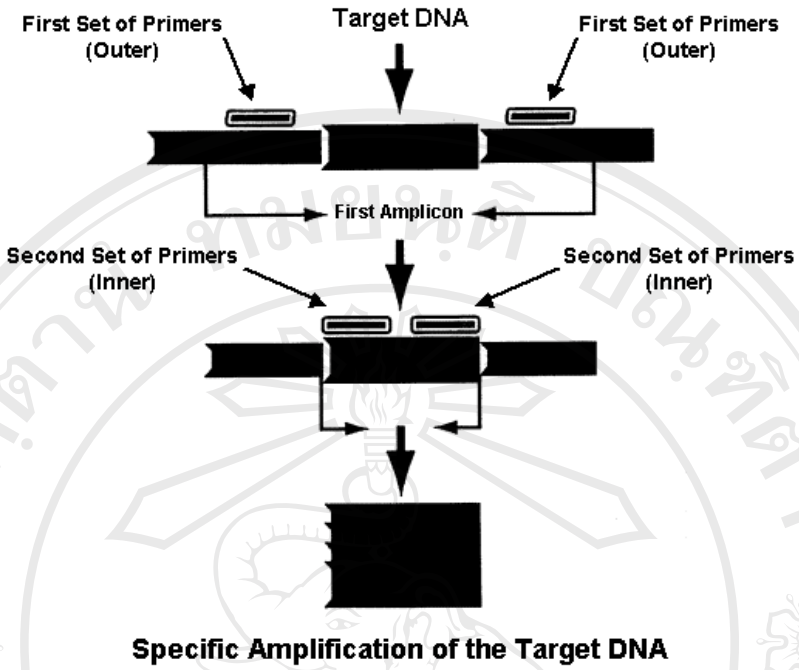

Figure 5. Illustration of nested PCR. The target DNA sequence of one set of primers (termed "inner" primers) is located within the target sequence of the second set of primers (termed "outer" primers). In practice, a standard PCR reaction is first run with the patient sample using the "outer primers". Then a second PCR reaction is run with the "inner primers" using the product of the first reaction as the amplification target. This procedure increases the sensitivity of the assay by reamplifying the product of the first reaction in a second reaction. The specificity of the assay is increased because the inner primers amplify only if the first PCR reaction yielded a specific product.

Recent studies have demonstrated that molecular amplification assays such as PCR and ligase chain reaction (LCR) have high sensitivity and specificity for detection of either gonorrhea or chlamydia in a variety of sample types including urethral and endocervical swabs and urine (45, 49-55). However, they require separate processing and amplification techniques for each pathogen. In this study we evaluated the In-house multiplex single-tube nested PCR (M-SN PCR) for *N. gonorrhoeae* and *C. trachomatis* that simultaneously detects both pathogens in a single amplified in different types of genitourinary specimens.

Several recent studies demonstrated that PCR has higher sensitivity than culture for the detection of these two organisms in clinical specimens. The sensitivity of culture has been estimated to range from 50-85 % in different laboratory setting compared to that of PCR assay. The over all sensitivity of PCR to range from 95-100 % in gold standard specimens; urethral swab and endocervical swab, 70-90% in vaginal swab and urine. For multiplex PCR, in 1995 Mattony JB, et al. (131) have developed and reported sensitivity of 10 fg of *C. trachomatis* and *N. gonorrhoeae* DNA (equivalent to 1 to 2 genome copies). The sensitivity of M-PCR for detecting *C. trachomatis* was 100% (22 of 22 specimens). Sensitivity of M-PCR for *N. gonorrhoeae* was 92.3% (12 of 13 specimens). The specificity of M-PCR was 100% for both *C. trachomatis* (178 of 178 specimens) and *N. gonorrhoeae* (187 of 187 specimens).

Noninvasive screening options, such as urine testing or self-collected dry vaginal swabs could eliminate some of the barrier to screening and detection for *C. trachomatis* and *N. gonorrhoeae* infections. Noninvasive methods are clearly prefered by patients and could substantially increase the acceptability and convenience of screening in a variety of settings. Several newly developed nucleic acid amplification tests that use noninvasive sample have been evaluated.

Others studies have reported that self-collected vaginal swab can be used successfully to diagnose sexually transmitted infections, eliminating the need for a clinician and a pelvic examination for specimen collection (132-135). These swabs have performed as well as or better than clinician-obtained endocervical swabs for diagnosis of either *N. gonorrhoeae* or *C. trachomatis* by DNA amplification assays. In 2002, Gaydos et al. (136) reported sensitivity and specificity for detection of *N. gonorrhoeae* in dry vaginal swabs versus wet vaginal swabs was 88.9 % vs. 96.3% and 98.3% versus 98.2% respectively. And sensitivity and specificity for detection of *C. trachomatis* in dry vaginal swabs versus wet vaginal swabs was 91.3 % vs. 94.6% and 99.3% versus 99.3% respectively. Additionally, they reported sensitivity and specificity for detection of *N. gonorrhoeae* by culturation was 63.0% and 100%, *C. trachomatis* by enzyme immunoassay (EIA) was 72.9% and 99%. Our results confirmed their results and indicated that self-collected dry vaginal swabs tested by

In-house M-SN PCR performed better than the currently used methods such as culturation for *N. gonorrhoeae* and EIA for *C. trachomatis.*

The need to develop acceptable, better, and more easily available techniques for diagnosing STD for all high-risk populations is significant. Five of the top 10 reportable diseases in worldwide are STD, and sequelae include pelvic inflammatory disease, infertility, and cervical cancer (137). Additionally, STD is associated with increased risk for human immunodeficiency virus acquisition (138, 139). One objective of this study was to investigate whether a self-administered vaginal swab would be acceptable to women and urine specimens would be acceptable to men.