International Journal of Humanities Social Science and Management (IJHSSM)
Volume 3, Issue 1, Jan.-Feb. 2023, pp: 388-396 www.ijhssm.org

A Review: Gonorrhoea

K. Malleswari*
Nalanda Institute of Pharmaceutical Sciences
Kantepudi(V), Sattenapalli(M), Guntur (Dt), 522438

D. Rama Brahma Reddy
Nalanda Institute of Pharmaceutical Sciences
Kantepudi(V), Sattenapalli(M), Guntur (Dt), 522438

K. Venkateswarlu
Nalanda Institute of Pharmaceutical Sciences
Kantepudi(V), Sattenapalli(M), Guntur (Dt), 522438

Date of Submission: 21-01-2023 Date of Acceptance: 04-02-2023

ABSTRACT:
The bacterium Neisseria gonorrhoeae causes the sexually transmitted infection (STI) gonorrhoea, which has an estimated global annual incidence of 86.9 million adults. Gonorrhoea can present as urethritis in men, cervicitis or urethritis in women, and in extragenital sites (pharynx, rectum, conjunctiva and, rarely, systemically) in both sexes. Confirmation of diagnosis requires microscopy of Gram-stained samples, bacterial culture or nucleic acid amplification tests. As no gonococcal vaccine is available, prevention relies on promoting safe sexual behaviours and reducing STI-associated stigma, which hinders timely diagnosis and treatment thereby increasing transmission. Single-dose systemic therapy (usually injectable ceftriaxone plus oral azithromycin) is the recommended first-line treatment. However, a major public health concern globally is that N. gonorrhoeae is evolving high levels of antimicrobial resistance (AMR), which threatens the effectiveness of the available gonorrhoea treatments. Improved global surveillance of the emergence, evolution, fitness, and geographical and temporal spread of AMR in N. gonorrhoeae, and improved understanding of the pharmacokinetics and pharmacodynamics for current and future antimicrobials in the treatment of urogenital and extragenital gonorrhoea, are essential to inform treatment guidelines. Key priorities for gonorrhoea control include strengthening prevention, early diagnosis, and treatment of patients and their partners; decreasing stigma; expanding surveillance of AMR and treatment failures; and promoting responsible antimicrobial use and stewardship. To achieve these goals, the development of rapid and affordable point-of-care diagnostic tests that can simultaneously detect AMR, novel therapeutic antimicrobials and gonococcal vaccine(s) in particular is crucial.

KEY WORDS: Gonorrhoea, Gonorrhoea diagnosis, STDs Guidelines, Gonorrhea treatment, gonococcal antimicrobial resistance.

I. INTRODUCTION

The sexually transmitted infection (STI) gonorrhoea remains a major public health concern globally. The aetiological agent of gonorrhoea, the bacterium Neisseria gonorrhoeae (the gonococcus), generally causes mucosal infections of the urogenital tract, predominantly infecting columnar and transitional epithelia, although it can also attach to the stratified squamous epithelium of the ectocervix[1,2] . Such N. gonorrhoeae infections most frequently result in urethritis in men and cervicitis in women, but urethritis in women is also observed[3,4] . This obligate human host-adapted pathogen was described for the first time by Albert Neisser in Gram-stained microscopy of urethral discharge in 1879 (ref.[5]). N. gonorrhoeae is a diplococcal (that is, it is typically composed of two joined cells with the adjacent sides flattened, resulting in a characteristic kidney or coffee bean appearance on microscopy), Gram-negative microorganism; it belongs to the bacterial class Betaproteobacteria and the family Neisseriaceae, and has been co-evolving with its human host for centuries. The family Neisseriaceae comprises the genus Neisseria and

International Journal of Humanities Social Science and Management (IJHSSM)
Volume 3, Issue 1, Jan.-Feb. 2023, pp: 388-396 www.ijhssm.org

other genera such as Kingella and Eikenella[6–8] . The Neisseria genus currently consists of at least 23 species, of which about half are humanrestricted species, some are animal-restricted and some can be isolated from mucosal surfaces in both humans and animals[8] . N. gonorrhoeae is genomically, morphologically and phenotypically closely related to the other pathogenic Neisseria species, Neisseria meningitidis, which is typically carried as a commensal in the (naso) pharynx of 10–15% of the general population but occasionally causes fatal septicaemia and/or meningitis[6,8–10.] N. gonorrhoeae is also related to several other commensal Neisseria species that reside particularly in the pharynx. Despite containing many of the pathogenicity and virulence factors of N. gonorrhoeae and N. meningitidis, the commensal Neisseria species, from which these two pathogenic Neisseria species have evolved, do not normally cause pathology[9] as they are unable to induce substantial polymorphonuclear leukocyte (PMNL)-based inflammation and lack several additional factors and mechanisms of interacting with host molecules, cells and tissues[(11)]. The pathogenesis and pathophysiology of N. gonorrhoeae have been studied for decades; however, detailed knowledge regarding many fundamental properties is lacking.

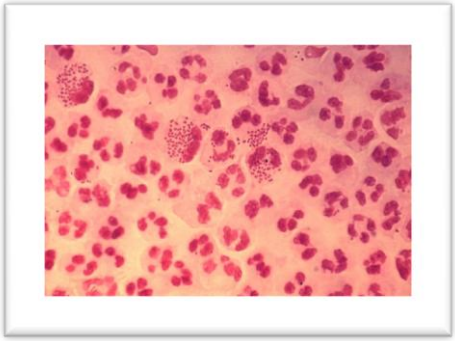


Fig No 1: Gonorrhea

GONORRHEA: HISTORICAL OUTLOOK

History of gonorrhea dates back to the history of mankind. In this review, we have attempted to provide an overview of the historical aspects of the disease.

Gonorrhea is one of the oldest sexually transmitted infections (STIs) known to humankind. There is some conflict of opinion regarding its exact origin, but according to the general consensus, the disease has been present from the ancient times.[12] A disease resembling gonorrhea was described by the Chinese emperor, Huang Ti (2600 BC) in his textbook.[13] It is believed that the mention of "an issue of seed" in the Book of Leviticus in the Old Testament and the precautions suggested refers to this disease.[12] Gonorrhea was termed "strangury" by Hippocrates (460–375 BC) who claimed that it resulted from the "pleasures of Venus."[13] Celsus (25 BC–50 AD) was well aware of gonorrhea and its complications. He used to catheterize patients with urethral strictures.[13] The Greek physician, Galen (131–200 AD) coined the term "gonorrhea" and he referred to it as "an unwanted discharge of semen" (gono: seed, rhea: flow).[14]

The disease was also referred to as "The Clap," referring to the "clapping" sensation experienced by the infected person during urination. Others believe that the name is derived from the ancient treatment of "clapping" an infected penis on either side with a big book to remove pus. Some others are of the opinion that the word "The Clap" is derived from French brothels, known as "Les Clapiers," where the disease was quite rampant.This name translates as "rabbit huts," referring to the small huts in which the prostitutes lived. In those days, men were considered as victims and women as the cause. The basic biology of the female reproductive tract was mistakenly thought to breed diseases since it was believed to provide adequate warmth and moisture for microbial growth.[15]

Throughout the history, wars were associated with outbreaks of STIs. Historical evidence suggests that the Roman soldiers fighting with Julius Caesar (100–40 B.C) suffered from gonorrhea. STIs including gonorrhea had caused many deaths during the Crimean war (A.D 1854–1856).[16]

Although the actual cause remained obscure, English parliament passed a law to halt the spread of "the perilous infirmity of burning" so as to ensure that gonorrhea was brought to a decline and ultimately removed from the society.[17] This remains the earliest known legal records of the disease and dates back to around 1161 AD.

The French king Louis IX passed a similar law in 1256 AD.[18] Confusion regarding the relation between gonorrhea and syphilis arose with the arrival of syphilis in Europe in the late 15th century. Great surgeons such as Ambroise Pare and John Hunter considered gonorrhea and syphilis to be manifestations of the same disease.

SIGNS AND SYMPTOMS

Gonorrhea infections of mucosal membranes can cause swelling, itching, pain, and the formation of pus.The time from exposure to symptoms is usually between two and 14 days, with most symptoms appearing between four and six

International Journal of Humanities Social Science and Management (IJHSSM)
Volume 3, Issue 1, Jan.-Feb. 2023, pp: 388-396 www.ijhssm.org

days after infection, if they appear at all. Both men and women with infections of the throat may experience a sore throat, though such infection does not produce symptoms in 90% of cases. Other symptoms may include swollen lymph nodes around the neck.Either sex can become infected in the eyes or rectum if these tissues are exposed to the bacterium.

WOMEN

Half of women with gonorrhea are asymptomatic but the other half experience vaginal discharge, lower abdominal pain, or pain with sexual intercourse associated with inflammation of the uterine cervix. Common medical complications of untreated gonorrhea in women include pelvic inflammatory disease which can cause scars to the fallopian tubes and result in later ectopic pregnancy among those women who become pregnant.[19]

MEN

Most infected men with symptoms have inflammation of the penile urethra associated with a burning sensation during urination and discharge from the penis. In men, discharge with or without burning occurs in half of all cases and is the most common symptom of the infection.This pain is caused by a narrowing and stiffening of the urethral lumen.The most common medical complication of gonorrhea in men is inflammation of the epididymis. Gonorrhea is also associated with increased risk of prostate cancer.

CAUSES:

Gonorrhea is caused by the bacterium Neisseria gonorrhoeae. Previous infection does not confer immunity – a person who has been infected can become infected again by exposure to someone who is infected. Infected persons may be able to infect others repeatedly without having any signs or symptoms of their own.

1.1.1.1 **RISK FACTORS**

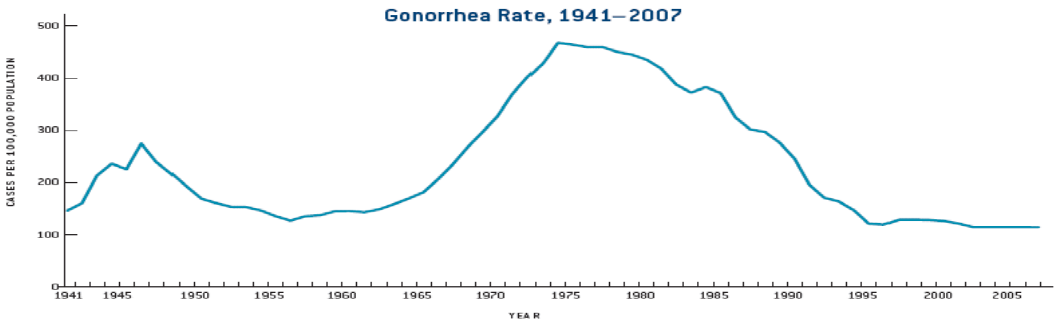
It is discovered that sexually active women younger than 25 and men who have sex with men are at increased risk of getting gonorrhoea. (20)

Other risk factors include:

- Having a new sex partner
- Having a sex partner who has other partners
- Having more than one sex partner
- Having had gonorrhea or another sexually transmitted infection.

EPIDEMIOLOGY

In 2016, the WHO estimated that there were 86.9 (95% uncertainty interval 58.6–123.4) million incident global cases of gonorrhoea (global prevalence 0.9%) among adults 15–49 years of age(21) (Fig. 2). The epidemiological diversity of gonorrhoea manifests itself in the variability of the geographical distribution and the prevalence among certain populations; determinants of such variability include sexuality and sexual orientation, socioeconomics, demographics, geographical and cultural ramifications (including stigma and taboos), and access to and quality of sex education, prevention, testing and diagnostics, as well as political commitment in the provision of health services(22–23)



Gonerrhea rates, united states 1941-2007

Mechanisms/pathophysiology

The bacterium N. gonorrhoeae

Growth and metabolism. N. gonorrhoeae is a fastidious organism that is sensitive to many environmental factors such as oxygen, nonphysiological temperatures, desiccation and the presence of toxic substances (such as many fatty acids), among others(24); thus, the bacterium does not survive for long outside the human host, and is difficult to culture (Box 1). Many strains have

incomplete biosynthetic capabilities for amino acids, presumably because amino acids and other important nutrients are readily obtained from the human host. Iron (which is essential for bacterial growth) is acquired from the host by binding iron-containing host proteins such as transferrin, lactoferrin and haemoglobin at the bacterial surface and stripping these molecules of iron that is then delivered to the bacterial cytoplasm(25). Owing to the broad range of oxygen levels within different niches of the male and female urogenital tracts, it is possible that N. gonorrhoeae encounters aerobic, microaerobic, and anaerobic conditions within the host, and the bacteria are able to grow in all these conditions(26).

GENETICS

Using WGS, it has been shown that the modern gonococcal population is not as old as previously thought and has been shaped by antimicrobial treatment of STIs as well as other infections, leading to the emergence of two major genomic lineages, one multidrugresistant and one multidrug-susceptible, with different evolutionary strategies(27). N. gonorrhoeae has a single circular chromosome between ~2.1 and 2.3 megabase pairs (~2,200–2,500 protein-coding sequences), which exists as diploid, homozygous, chromosomes(28,29).

In addition, N. gonorrhoeae can acquire additional DNA via horizontal genetic transfer (HGT), the noninherited external acquisition of new genetic material from another bacterium. HGT occurs mainly by type IV pilus-mediated DNA transformation (uptake of DNA from the environment and subsequent incorporation into the genome). N. gonorrhoeae is naturally competent for transformation during its entire life cycle, but transformation only occurs at high frequency between cells of N. gonorrhoeae and other Neisseria species. Approximately 80% of isolates carry a chromosomal insertion called the gonococcal genetic island, which has genes similar to those carried on the conjugal plasmid (that is, genes involved in conjugation — the DNA transfer between bacteria by cell-to-cell contact). However, in N. gonorrhoeae these conjugation gene products act to secrete chromosomal DNA into the medium that is then available for DNA transformation. Pilus-mediated DNA transformation provides efficient transport of DNA into the bacterial cell and DNA uptake sequences are highly represented in Neisseria genomes (~1,900–2,000 copies per genome)(30,31). This efficient transformation is one reason why AMR determinants efficiently spread from cell to cell. Notably, this ability of N. gonorrhoeae to transfer DNA between strains makes clonal analysis difficult because alleles are not stably linked and led to the creation of the multilocus sequence typing system to characterize bacterial lineages by the DNA sequence type of several defined and more conserved housekeeping genes(32). Multilocus sequence typing systems are now available for many different bacterial species(33). Furthermore, this reassortment of alleles suggests that mixed-strain gonorrhoea infections are common(34,35), although widely unrecognized, as most clinical laboratories analyse and save single colonies when culturing isolates, probably underestimating the incidence of mixed infections. Ideally, multiple colonies should be tested. Nearly all gonococcal strains contain a cryptic plasmid (with no defined functions); many contain a plasmid encoding a penicillinase (mostly TEM-1 or TEM-135 β-lactamase), which results in high-level penicillin resistance, and conjugative plasmids, which sometimes carry tetM causing high-level tetracycline resistance, although these plasmids are not as prevalent as reported for many other bacterial species. Several penicillinaseencoding plasmids of different size have been described in N. gonorrhoeae and named according to their epidemiological origin, such as the widely spread and most common African, Asian and Rio/Toronto plasmids. Different conjugative gonococcal plasmids carrying tetM have also been described, the most common being the American tetM plasmid and the Dutch tetM plasmid(36). In addition, several double-stranded and single-stranded bacteriophage gene islands have been annotated within the N. gonorrhoeae genome, but no isolated bacteriophage that can infect and lyse the bacteria has been found(37). Colonization determinants. N. gonorrhoeae shares many colonization determinants with other human-restricted Neisseria species that rarely cause infection. The factors required to establish a host niche include the type IV pilus, the opacity protein family (Opa proteins), the porinPorB, efflux pumps and metal transport systems (Fig. 3). N. gonorrhoeae probably has to compete with the resident microbiota for colonization, but little is known about how different resident commensal organisms may limit or cooperate with N. gonorrhoeae during colonization. Gonococcal pili are required for efficient mucosal colonization (typically of nonciliated columnar epithelia) and carry out many functions, including initial adherence to host cells and tissues, self-adherence and adherence to other N. gonorrhoeae cells, a means to crawl along mucosal surfaces called twitching motility,

International Journal of Humanities Social Science and Management (IJHSSM)
Volume 3, Issue 1, Jan.-Feb. 2023, pp: 388-396 www.ijhssm.org

protection from PMNL killing mechanisms(38), and HGT by DNA transformation(39). Clinical isolates of N. gonorrhoeae are always piliated, but quickly lose pilus expression in laboratory culture through a variety of mechanisms, showing that pilus expression is under strong selective pressure during infection. The Opa proteins mainly act as adhesins that bind to a variety of receptors found on many different cells and tissues(40) and mediate more intimate attachment and initiation of microcolony formation. Most Opa proteins bind to one or more human carcinoembryonic antigenrelated cell adhesion molecules (CEACAMs), a family of surface-exposed proteins. Opa proteins only bind to human forms of these proteins, and a few Opa proteins also bind to heparansulfate proteoglycans. Although some Opa–CEACAM interactions lead to cell signalling events, such as induction of the oxidative burst from PMNLs, most Opa interactions seem to be important for adherence to cells and tissues(41). All Gram-negative bacterial porins (transmembrane channel proteins) act to allow small molecules access to the periplasm. The N. gonorrhoeaeporin (PorB) is one of the most abundant proteins in the outer membrane; it increases attachment, is then translocated to the host cell mitochondria and impairs the ability of phagocytes to kill the bacteria. Other important properties include resisting the action of complement factors, modulating apoptosis, invasion of host cells and involvement in AMR(42,43). N. gonorrhoeae expresses up to five efflux pump systems: MtrC–MtrD–MtrE, MacA–MacB–MtrE, NorM, FarA–FarB–MtrE and MtrF(44,45). These export pumps have varying narrow or extensive substrate specificity and have many roles in pathogenesis, including removing toxic molecules encountered during infection, such as fatty acids and cationic peptides, and removing antimicrobials from the cell (that is, acting as AMR determinants). Finally, there are three iron acquisition systems in the envelope of N. gonorrhoeae, and each can strip iron from a human protein that is designed to sequester iron from pathogenic organisms. There is an acquisition system for transferrin (TbpA–TbpB), one for lactoferrin (LbpA–LbpB) and one for haem (HpuA–HpuB), which can be found, for example, in haemoglobin.

DIAGNOSIS, SCREENING AND PREVENTION

Clinical presentation and diagnosis

The incubation period for urogenital gonorrhoea ranges from ~2 days to 8 days (46). The clinical manifestations of gonorrhoea are variable and differ markedly in men and women. At least 90% of men with gonococcal urethritis are symptomatic, presenting with obvious urethral discharge and dysuria, a fact that permits the application of syndromic diagnosis (based on a set of symptoms and signs that are characteristic of a clinical manifestation) in many settings as both a time-saving and cost-saving measure. For men with symptomatic urethritis, Gram stain may be used to support symptom evaluation. By contrast, laboratory-based diagnostic tests have a more important role for gonococcal detection in asymptomatic men, women and in patients of all genders for extragenital (rectal and pharyngeal) infections, which are mostly asymptomatic or present with nonspecific symptoms. Although ~40% of women with gonococcal cervicitis may report abnormal vaginal discharge, this symptom is unreliable for syndromic diagnosis of gonorrhoea, as many other equally or more common genitourinary infections in women (for example, bacterial vaginosis, trichomoniasis and vaginal candidiasis) may cause the same symptoms. Microbiological diagnosis of gonorrhoea can be challenging, as many regions do not have a laboratorybased diagnostic capability and rely on syndromic management algorithms to guide empirical antimicrobial treatments. Microbiological diagnosis is performed by the detection of Gram-negative diplococci in stained smears using microscopy, culture of N. gonorrhoeae and/or nucleic acid amplification tests (NAATs) detecting N. gonorrhoeae DNA or RNA.

PREVENTION

As with most sexually transmitted diseases, the risk of infection can be reduced significantly by the correct use of condoms, not having sex, or can be removed almost entirely by limiting sexual activities to a mutually monogamous relationship with an uninfected person.

Those previously infected are encouraged to return for follow up care to make sure that the infection has been eliminated. In addition to the use of phone contact, the use of email and text messaging have been found to improve the re-testing for infection.

Newborn babies coming through the birth canal are given erythromycin ointment in the eyes to prevent blindness from infection. The underlying gonorrhea should be treated; if this is done then usually a good prognosis will follow.

International Journal of Humanities Social Science and Management (IJHSSM)
Volume 3, Issue 1, Jan.-Feb. 2023, pp: 388-396 www.ijhssm.org

TREATMENT

Antibiotics are used to treat gonorrhea infections. As of 2016, both ceftriaxone by injection and azithromycin by mouth are most effective. However, due to increasing rates of antibiotic resistance, local susceptibility patterns must be taken into account when deciding on treatment. Ertapenem is a potential effective alternative treatment for ceftriaxone-resistant gonorrhea.

Adults may have eyes infected with gonorrhoea and require proper personal hygiene and medications. Addition of topical antibiotics have not been shown to improve cure rates compared to oral antibiotics alone in treatment of eye infected gonorrhea. For newborns, erythromycin ointment is recommended as a preventative measure for gonococcal infant conjunctivitis.

Infections of the throat can be especially problematic, as antibiotics have difficulty becoming sufficiently concentrated there to destroy the bacteria. This is amplified by the fact that pharyngeal gonorrhoea is mostly asymptomatic, and gonococci and commensal Neisseria species can coexist for long time periods in the pharynx and share anti-microbial resistance genes. Accordingly, an enhanced focus on early detection (i.e., screening of high-risk populations, such as men who have sex with men, PCR testing should be considered) and appropriate treatment of pharyngeal gonorrhoea is important.

TRADITIONAL DIAGNOSTIC METHODS MICROSCOPY.

In resource-limited settings, light microscopy of Gram-stained samples is often the only method available to diagnose infection with N. gonorrhoeae presumptively. The sensitivity and specificity of the Gram stain, which tests for the presence of characteristic Gram-negative diplococci within PMNLs, can vary substantially between studies and depends upon the specime the highest sensitivity and specificity were reported with urethral swab samples from symptomatic males (89% to >98% and >95%, respectively),(47,48), whereas the sensitivity was as low as 40–50% in urethral specimens from asymptomatic males, and in endocervical or urethral specimens from women,(49,50). This difference can probably be explained by a reduced bacterial load, particularly in these urethral samples, and by the presence of many other bacterial species in the endocervical samples. Gram stain is not suitable for the diagnosis of N. gonorrhoeae from pharyngeal specimens (because other Neisseria species with similar morphology are prevalent in the oral and nasopharyngeal cavity) or rectal specimens (which have a sensitivity ≤40%)(51,52). A methylene blue staining method is an alternative to the Gram stain, and similar high sensitivity and specificity were reported for diagnosing gonococcal urethritis in men(53).

Culture.

Prior to the introduction of NAATs, culture (Table 1) of the organism was the gold standard and this remains the only diagnostic method available in some settings as it is a low-cost method. Culture also remains recommended for test-of-cure for treatment failure, in cases of sexual abuse and to evaluate PID.Furthermore, complete AMR testing can only be accomplished if N. gonorrhoeae is cultured. Culture performance is dependent upon factors such as anatomical site of the cultured sample, method of specimen collection, media and conditions used to transport the sample to the diagnostic centre, on selective and/or selective culture media,conditions of incubation,and species confirmatory tests.(53-56)

REFERENCE

[1]. Edwards, J. L., Shao, J. Q., Ault, K. A. &Apicella, M. A. Neisseria gonorrhoeae elicits membrane ruffling and cytoskeletal rearrangements upon infection of primary human endocervical and ectocervical cells. Infect. Immun. 68, 5354–5363 (2000).

[2]. Evans, B. A. Ultrastructural study of cervical gonorrhea. J. Infect. Dis. 136, 248–255 (1977).

[3]. Barlow, D. & Phillips, I. Gonorrhoea in women. Diagnostic, clinical, and laboratory aspects. Lancet 1, 761–764 (1978).

[4]. Schmale, J. D., Martin, J. E. Jr &Domescik, G. Observations on the culture diagnosis of gonorrhea in women. JAMA 210, 312–314 (1969).

[5]. Quillin, S. J. & Seifert, H. S. Neisseria gonorrhoeae host adaptation and pathogenesis. Nat. Rev. Microbiol. 16, 226–240 (2018).

[6]. Elias, J. F. & Vogel, U. in Manual of Clinical Microbiology 12th edn Vol. 1(eds Carroll, C. C. et al.) 640–655 (American Society for Microbiology, 2019).

[7]. Adeolu, M. & Gupta, R. S. Phylogenomics and molecular signatures for the order Neisseriales: proposal for division of the order Neisseriales into the emended family Neisseriaceae and Chromobacteriaceae fam. nov.Antonie van Leeuwenhoek 104, 1–24 (2013).

[8]. Tønjum, T. & van Putten, J. in Infectious Diseases 4th edn (eds Cohen, J., Powderly, W. G. & Steven M. Opal) 1553-1564 (Elsevier, 2016).
[9]. Liu, G., Tang, C. M. & Exley, R. M. Non-pathogenic Neisseria: members of an abundant, multi-habitat, diverse genus. Microbiology 161, 1297–1312 (2015).
[10]. Johnson, A. P. The pathogenic potential of commensal species of Neisseria. J. Clin. Pathol. 36, 213–223 (1983).
[11]. Seifert, H. S. Location, location, location — commensalism, damage and evolution of the pathogenic Neisseria. J. Mol. Biol 431, 3010–3014 (2019).
[12]. King A, Nicol C, Rodin P. Gonorrhoea. In: Venereal Diseases. 4th ed. Great Britain: The Whitefriars Press Ltd.; 1980. p. 172-7.
[13]. Lee KC, Ladizinski B. The clap heard round the world. Arch Dermatol2012; 148:223.
[14]. Fiumara NJ, Wise HM, Many M. Gonorrhoeal Pharyngitis. N Engl J Med 1967; 276:1248-50.
[15]. Gruber F, Lipozencic J, Kehler T. History of venereal diseases from antiquity to the renaissance. ActaDermatovenereol Croat 2015; 23:1-11.
[16]. Morgan MK, Decker CF. Gonorrhea. Dis Mon 2016; 62:260-8.
[17]. Baarda BI, Sikora AE. Proteomics of Neisseria gonorrhoeae: The treasure hunt for counter measures against an old disease. Front Microbiol2015; 6:1190.
[18]. Riffenburgh RH. Regression and correlation methods. In: Statistics in Medicine. 2nd ed., Ch. 24. Amsterdam: Elsevier; 2006. p. 447-86.
[19]. Sanger WW. The History of Prostitution. New York: Harper and Brothers; 1910.
[20]. LaCroix P. The History of Prostitution. Vol. 2. New York: MacMillan; 1931.
[21]. Rowley, J. et al. Chlamydia, gonorrhoea, trichomoniasis and syphilis: global prevalence and incidence estimates, 2016. Bull. World Health Organ. 97, 548–562P (2019).
[22]. Adler, M., Foster, S., Richens, J. &Slavin, H. Sexual Health and Care. Sexually Transmitted Infections, Guidelines for Prevention and Treatment. Health and Population Division Occasional Paper 136 (Overseas Development Administration, London, 1996).
[23]. Dallabetta, G. A., Laga, M. &Lamptey, P. R. Control of Sexually Transmitted Diseases: A Handbook for the Design and Management of Programs (AIDSCAP/Family Health International, 1996).
[24]. Rohde, K. H. & Dyer, D. W. Mechanisms of iron acquisition by the human pathogens Neisseria meningitidis and Neisseria gonorrhoeae. Front. Biosci. 8, d1186–d1218 (2003).
[25]. Cole, J. A. Legless pathogens: how bacterial physiology provides the key to understanding pathogenicity. Microbiology 158, 1402–1413 (2012).
[26]. Sanchez-Buso, L. et al. The impact of antimicrobials on gonococcal evolution. Nat. Microbiol. 4, 1941–1950 (2019). This genomics paper provides evidence that the modern gonococcal population is not as old as previously anticipated and has been formed by antimicrobial treatment, leading to the emergence of one multidrug-resistant lineage and one multisusceptible lineage with different evolutionary strategies.
[27]. Tobiason, D. M. & Seifert, H. S. The obligate human pathogen, Neisseria gonorrhoeae, is polyploid. PLoS Biol. 4, 1069–1078 (2006).
[28]. Unemo, M. et al. The novel 2016 WHO Neisseria gonorrhoeae reference strains for global quality assurance of laboratory investigations: phenotypic, genetic and reference genome characterization. J. Antimicrob. Chemother. 71, 3096–3108 (2016).
[29]. Goodman, S. D. &Scocca, J. J. Identification and arrangement of the DNA sequence recognized in specific transformation of Neisseria gonorrhoeae. Proc. Natl Acad. Sci. USA 85, 6982–6986 (1988).
[30]. Berry, J. L., Cehovin, A., McDowell, M. A., Lea, S. M. &Pelicic, V. Functional analysis of the interdependence between DNA uptake sequence and its cognate ComP receptor during natural transformation in Neisseria species. PLoS Genet. 9, e1004014 (2013).
[31]. Bennett, J. S. et al. Species status of Neisseria gonorrhoeae: evolutionary and epidemiological inferences from multilocus sequence typing. BMC Biol. 5, 35 (2007).
[32]. Maiden, M. C. et al. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc. Natl Acad. Sci. USA 95, 3140–3145 (1998).
[33]. Goire, N. et al. Mixed gonococcal infections in a highrisk population, Sydney, Australia 2015: implications for antimicrobial

resistance surveillance? J. Antimicrob. Chemother. 72, 407–409 (2017).
[34]. Martin, I. M. &Ison, C. A. Detection of mixed infection of Neisseria gonorrhoeae. Sex. Transm. Infect. 79, 56–58 (2003).
[35]. Unemo, M. & Shafer, W. M. Antibiotic resistance in Neisseria gonorrhoeae: origin, evolution, and lessons learned for the future. Ann. NY Acad. Sci. 1230, E19–E28 (2011).
[36]. Piekarowicz, A. et al. Characterization of the dsDNA prophage sequences in the genome of Neisseria gonorrhoeae and visualization of productive bacteriophage. BMC Microbiol. 7, 66 (2007).
[37]. Stohl, E. A., Dale, E. M., Criss, A. K. & Seifert, H. S. Neisseria gonorrhoeae metalloprotease NGO1686 is required for full piliation, and piliation is required for resistance to H2O2- and neutrophil-mediated killing. mBio 4, e00399–13 (2013).
[38]. Biswas, G. D., Sox, T., Blackman, E. & Sparling, P. F. Factors affecting genetic transformation of Neisseria gonorrhoeae. J. Bacteriol. 129, 983–992 (1977).
[39]. Dehio, C., Gray-Owen, S. D. & Meyer, T. F. The role of neisserialOpa proteins in interactions with host cells. Trends Microbiol. 6, 489–495 (1998).
[40]. Sadarangani, M., Pollard, A. J. &Gray-Owen, S. D. Opa proteins and CEACAMs: pathways of immune engagement for pathogenic Neisseria. FEMS Microbiol. Rev. 35, 498–514 (2011).
[41]. Deo, P. et al. Outer membrane vesicles from Neisseria gonorrhoeae target PorB to mitochondria and induce apoptosis. PLoSPathog. 14, e1006945 (2018).
[42]. Massari, P., Ram, S., Macleod, H. &Wetzler, L. M. The role of porins in neisserial pathogenesis and immunity. Trends Microbiol. 11, 87–93 (2003).
[43]. Madico, G. et al. Factor H binding and function in sialylated pathogenic Neisseriae is influenced by gonococcal, but not meningococcal, porin. J. Immunol. 178, 4489–4497 (2007).
[44]. Olesky, M., Zhao, S., Rosenberg, R. L. & Nicholas, R. A. Porin-mediated antibiotic resistance in Neisseria gonorrhoeae: ion, solute, and antibiotic permeation through PIB proteins with penB mutations. J. Bacteriol. 188, 2300–2308 (2006).
[45]. Shafer, W. M. et al. in Efflux-Mediated Antimicrobial Resistance in Bacteria (eds Li, X. Z., Elkins, C.&Zgurskaya, H.) 439-469 (Adis, Cham, 2016).
[46]. Hagman, K. E. et al. Resistance of Neisseria gonorrhoeae to antimicrobial hydrophobic agents is modulated by the mtrRCDE efflux system. Microbiology 141, 611–622 (1995).
[47]. Lee, E. H. & Shafer, W. M. The farAB-encoded efflux pump mediates resistance of gonococci to long-chained antibacterial fatty acids. Mol. Microbiol. 33, 839–845 (1999).
[48]. Bignell, C. &Unemo, M., European STI Guidelines Editorial Board. European guideline on the diagnosis and treatment of gonorrhoea in adults. Int. J. STD & AIDS 24, 85–92 (2012).
[49]. Ghanem, K. G. Clinical manifestations and diagnosis of Neisseria gonorrhoeae infection in adults and adolescents UpToDate.com https://www.uptodate. com/contents/clinical-manifestations-and-diagnosisof-neisseria-gonorrhoeae-infection-in-adults-andadolescents/print (2019).
[50]. Ison, C. A. Laboratory methods in genitourinary medicine. Methods of diagnosing gonorrhoea. Genitourin. Med. 66, 453–459 (1990).
[51]. Unemo, M. &Ison, C. in Laboratory diagnosis of sexually transmitted infections, including human immunodeficiency virus (edsUnemo, M. et al.) 21–54 (World Health Organization, 2013). This comprehensive chapter describes biological sampling, different methods for laboratory detection and antimicrobial susceptibility testing of N. gonorrhoeae.
[52]. Taylor, S. N., DiCarlo, R. P. & Martin, D. H. Comparison of methylene blue/gentian violet stain to Gram's stain for the rapid diagnosis of gonococcal urethritis in men. Sex. Transm. Dis. 38, 995–996 (2011).
[53]. Papp, J. R. S. J., Gaydos, C. A. & Van Der Pol, B. Recommendations for the laboratory-based detection of Chlamydia trachomatis and Neisseria gonorrhoeae — 2014. MMWR Recomm. Rep. 63, 1–19 (2014).
[54]. Dillon, J. R. Sustainable antimicrobial surveillance programs essential for controlling Neisseria gonorrhoeae superbug. Sex. Transm. Dis. 38, 899–901 (2011).
[55]. Starninosss, S. D., J. R. Laboratory manual: identification and antimicrobial susceptibility testing of Neisseria gonorrhoeae 2nd edn Co-ordinating Centre for the Gonococcal Antimicrobial Susceptibility Surveillance

Program in Latin America and the Caribbean (2002).

[56]. Starnino, S. & Dillon, J. R. in Laboratory diagnosis of sexually transmitted infections, including human immunodeficiency virus (edsUnemo, M. et al.)199–218 (World Health Organization, 2013).