INTRODUCTION
1. INTRODUCTION

1.1. Reproductive tract infections (RTIs)

In both men and women, there are several potential sites of infection. Reproductive tract infections affect the external genital region and the reproductive organs. In females, infections in the area of the vulva, vagina, or cervix are referred to as lower reproductive tract infections and infections in the uterus, fallopian tubes, and ovaries are considered as upper reproductive tract infections. For males, these infections are at the penis, testicles, urethra or the sperm tube. The three types of reproductive tract infections are endogenous infections, iatrogenic infections and the more commonly known sexually transmitted infection and have its own specific causes and symptoms. Reproductive tract infections were caused by a bacterium, virus, fungus or other organism. Some reproductive tract infections are easily treatable and may be cured. But some of the infections are more difficult and non curable such as AIDS (WHO, 2001).

Reproductive tract infections (RTI) including sexually transmitted diseases (STD) are increasingly recognized as a major cause of morbidity in India. Reproductive tract infections (RTIs) represent a vast reservoir of infection among women of reproductive age especially in developing countries. The annual incidence of sexually transmitted diseases (STDs) in India is estimated at 5 per cent, i.e., approximately 40 million new infections occur every year in the country. Lower reproductive tract infection is a common, but neglected health problem in women during their reproductive age. Women suffer from reproductive morbidities for a long time because of the prevailing ‘culture of silence’ (Panda et al., 2007). Reproductive tract infections entail a heavy toll on women; if untreated can cause serious consequences of infertility, ectopic pregnancy, cervical cancer, menstrual disturbances, pregnancy wastage and low birth weight babies. The presence of RTI’s especially ulcer causing STI’s may enhance the acquisition and transmission of the Human Immunodeficiency Virus (Rabiu et al., 2010). The risk of becoming HIV infected after a single sexual exposure is increased 10-30 fold in the presence of a genital ulcer (Acharya et al., 2006). Female RTI’s usually originate in the lower
genital tract as vaginitis or cervicitis and may produce symptoms such as abnormal vaginal discharge, genital pain, itching and burning feeling with urination.

1.2. Types of Reproductive tract infections

1.2.1. Endogenous infections

Endogenous Infections are probably the most common reproductive tract infections worldwide. They result from an overgrowth of organisms normally present in the vagina. Endogenous infections include bacterial vaginosis and candidiasis. These can easily be treated and cured. Endogenous infections are common and are influenced by environmental, hygienic, hormonal and other factors like co-existent of diabetes and immunocompromised state like AIDS (NACO, 2011).

1.2.2. Iatrogenic infections

Iatrogenic Infections are caused by a bacterium or other micro-organism, when introduced into the reproductive tract through a medical procedure such as menstrual regulation, induced abortion and insertion of intra uterine devices (IUD) or during childbirth. This can happen due to unsterilized surgical instruments used during the procedure, or if an infection that was already present in the lower reproductive tract is pushed through the cervix into the upper reproductive tract. Iatrogenic infections are more commonly seen among the population with high prevalence of STI/RTI, where health care providers do not have the training or supplies to perform procedures safely. Postpartum and post abortion infections are also more common where medical services and follow-up care are not provided safely (NACO, 2011).

1.2.3. Sexually transmitted infections

Sexually Transmitted Infections are caused by viruses, bacteria, or parasitic micro-organisms that are transmitted through sexual activity with an infected partner. About 30 different sexually transmitted infections have been identified, some of which are easily treatable and some are non curable. Sexually transmitted infections (STI) such as syphilis, gonorrhoea and chancroid spread more rapidly in places where
communities are disrupted, migrant labor is common and commercial sex networks are active. Though the STI are infectious diseases, however, STI transmission depends mainly on human behavior. A person with many sexual partners is much more likely to acquire a STI than a person with one partner. They also have more opportunity to infect others. In fact, most STI transmission occurs within a small part of the population that has multiple sex partners. This does not mean, however, that the rest of the community is not at risk for STI infection (NACO, 2011). WHO estimates that each year there are over 340 million new cases of sexually transmitted infections in which 75-85% occur in developing countries. In India alone, 40 million new cases emerge each year.

1.3. Microbiology of Reproductive tract infections

Worldwide, the most common lower reproductive tract infection is vaginitis. Infection of the cervix may be caused by a variety of pathogens, particularly *Neisseria gonorrhoeae* and *Chlamydia* (Das et al., 2011). Infections of the cervix are considered more severe than vaginitis because they commonly result in upper reproductive tract infection with serious consequences. The microbiology of vaginitis has been studied frequently and the most common types reported are *Gardnerella vaginalis*, *Trichomonas vaginalis* and *Candida* spp (Al Quaiz, 2000). The migration of infections into the upper reproductive tract, including the uterus, fallopian tubes and ovaries are often a direct complication of lower reproductive tract infections, particularly sexually transmitted diseases. The sexually transmitted infections are also caused by *Haemophilus ducreyi*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Treponema pallidum*, *HIV*, *Herpes Simplex virus* (HSV), *Human Papilloma virus* (HPV) and *Hepatitis B* virus (HBV) (Thappa and Kaimal, 2007).

The most common reproductive tract infections are candidiasis and gonorrhoea. Now it’s becoming a major public health challenge, due to the high incidence of these two infections accompanied by the increasing threat of drug resistance (WHO, 2012). Candidiasis which is an endogenous infection caused by the yeast *Candida* spp and gonorrhoea which is a sexually transmitted disease caused by a Gram negative bacterium *Neisseria gonorrhoeae*. 
1.4. Reproductive tract infections caused by Fungi and Bacteria

1.4.1. Fungal reproductive tract infection - *Candida*

*Candida* is a common colonizer of the human gastrointestinal, respiratory and reproductive tracts. These yeasts are associated with diseases ranging from superficial or mucocutaneous Candidiasis to life-threatening systemic infections (Mirhendi *et al.*, 2005). Even in the modern advances in medicine, there is a rise in the incidence of fungal infections especially those due to *Candida* spp. *Candida* spp are the second most common cause of vulvovaginitis worldwide. However, 20–50% of women have *Candida* spp in their vaginal flora without showing any clinical symptoms. *Candida*
*albicans* is the most common and clinically relevant species that accounts for 85–90% of Vulvovaginal candidiasis (VVC). However, there has been a significant trend towards the emergence of other species such as *Candida glabrata*, *Candida krusei*, and *Candida parapsilosis* which ironically show more resistance to the first line antifungal treatments (Rad *et al.*, 2012).

1.4.2. Historical perspective of *Candida*

In “Of the Epidemics,” Hippocrates described oral candidiasis (around 400B.C.) as “mouths affected with aphthous ulcerations”. In 1665, Pepys Diary reported “a patient hath a fever, a thrush and a hiccup” (Martin and Jones, 1940) perpetuating the idea that oral thrush originates from the host. Mycologists accepted this perception as late as the early 1900s where Castellani quoted previous accounts of thrush as “morbid secretions of the oral mucosa”. However, a few clinicians and mycologists swayed popular belief towards the idea of an infectious agent causing thrush. In 1771, Rosen von Rosenstein defined an invasive form of thrush (Calderone, 2002). In 1839, Langenbeck was first recognizing a fungus in a patient with typhoid fever. Oropharyngeal and esophageal thrush with pseudomembranes were found at autopsy. “Under the microscope magnified, the pseudomembranes consisted of an immense number of fungi” He describes in detail about septate hyphae, branched pseudohyphae and blastoconidia. In 1844, J.H. Bennett observed a similar fungus in the sputum and the lungs of a patient with a pneumothorax and criticized the conclusion by Lagenbach. Bennett concluded that the disease was “indicative of great depression of the vital powers and impairment of the nutritive functions of the economy. Two years later, Berg explicitly concluded that thrush was caused by a fungus and stated that “descriptions of the disease unsupported by demonstration of the fungus could not substantiate the diagnosis”. He was able to reproduce the infection in healthy children and thereby confirmed his hypothesis that the fungus caused the infection. Though, Langenbeck (1839) first documented the fungus associated with thrush, he failed to make the direct connection.
1.4.2.1. Classification of yeast *Candida*

In 1847, the distinguished French mycologist, Charles Philippe Robin, classified the fungus as *Oidium albicans* (*albicans* means “to whiten”) to name the fungus causing thrush. Hill and later Martin and Jones misclassified *Candida* into the genus Monilia, a genus containing fungi that commonly grow in plants. Subsequently, clinicians erroneously referred to the etiology of thrush as “Monilias” despite the fact mycologists had already elucidated the morphological differences between the fungus associated with thrush and the fungus in the genus *Monilia*. Christine Berkhout and others noted these differences, particularly the ability of this fungus to infect humans. The Botanist Christine Marie Berkhout reclassified it under the current genus *Candida* and described it in her doctoral thesis at the University of Utrecht in 1923. *Candida* is derived from Latin where *toga candida* was a white robe worn by Roman Senators (Mc Cool). Berkhout’s taxonomy was later heralded by the prominent French mycologists, Maurice Langeron and Paul Guerra, as “…the beginning of the rational systematics of the non-ascosporogenous yeasts”. However, it was not until 1954 that the Eighth Botanical Congress officially endorsed the binomial *Candida albicans* as the *nomen conservandum* formally ending the 200 year long uncertainty over the etiology and taxonomy of *Candida*. Currently, there are some 200 species within the genus *Candida*. These yeast-like cells are anamorphic (sexual imperfect) fungi belonging to the form-class Blastomycetes. They are characterized by their polymorphic nature and ability to produce budding yeast cells (blastoconidia), mycelia, pseudomycelia, and blastospores. Of the nearly 200 species, six species such as *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, and *C. lusitaniae* are most commonly associated with human infection (Barnett, 2004).

1.4.3. Pathogenic mechanism and virulence factors of *Candida*

Vulvovaginal candidiasis (VVC) is caused by the transformation of the yeasts from coloniser status (asymptomatic) to infectious agents (symptomatic vaginitis). Local and systemic factors can determine the transformation of *Candida* from a commensal to a pathogenic organism. The line between its status as yeast and hyphae is very thin and as the host cell becomes immunocompromised, it becomes active and
starts secreting several enzymes (Bhat et al., 2011). Overgrowth of Candida may be the result of the inhibition of vagina’s normal flora, decrease in local cellular immunity, change in metabolic and tropic environment of vagina, and unknown mechanisms. In order to establish an infection, Candida has to evade the immune system, survive, divide in the host environment, and spread to other tissues. Proteinase and phospholipase secretion has been implicated as potential virulence factors for some Candida spp responsible for infections (Das et al., 2008). Though this disease rarely threatens the life, its consequences include serious pathogenesis, loss of work force, and costs. Currently, antifungal drugs are increasingly used both as preventive and treating agents, which in turn causes species resistant to drug (Bahodoran et al., 2010). Constitutional factors such as pregnancy, oral estrogen contraception, antibiotic medication, immunodepression and a persistent intestinal carriage may be implicated in recurrent vulvovaginal mycosis. Recurrent vulvovaginal mycosis has recently been included in the Centers for Disease Control and Prevention's classification of AIDS for stage B (Warszawski et al., 1996).

All pathogenic microorganisms have developed mechanisms that allow successful colonization or infection of the host. As a result, most pathogens, including Candida spp, have developed an effective battery of putative virulence factors and specific strategies to assist in their ability to colonize host tissues, cause disease, and overcome host defences. The virulence factors expressed or required by Candida spp to cause infections may vary depending on the type of infection, the site and stage of infection, and the nature of the host response (Naglik et al., 2003). The important virulence factors found to influence the pathogenesis mechanisms are biofilm formation, secreted aspartyl proteinase production, phospholipase production, resistance to hydrogen peroxide and haemolytic activity.

1.4.3.1. Biofilm formation

Biofilm formation plays an important role in the pathogenicity of Candida albicans. Biofilm can serve as reservoirs for the cells to continually seed infection. Moreover, Candida biofilm cells are much more resistant than free-living planktonic cells to many antifungal agents. As a result, the biofilm-specific property of Candida
cells has developed recent interests in the study of biofilm structure, physiology, and regulation. Biofilms are defined as surface-associated communities of cells surrounded by an extracellular matrix and displaying phenotypic features that differ from their planktonic counterparts. The development of *Candida* biofilm can be divided into four sequential steps. First, the yeast cells adhere to a foreign substrate (host tissue or medical device). Second, the yeast cells proliferate across the substrate surface and pseudohyphae and hyphae begin to develop. Third, the extracellular matrix is produced and the network of pseudohyphae and hyphae cells is embedded within this matrix. Biofilm will then mature into a complex three-dimensional structure. Finally, the progeny biofilm cells disperse to enable remote surfaces to be populated (Wang *et al.*, 2010).

1.4.3.2. Secreted Aspartyl Proteinase

The extracellular proteolytic activity for *Candida* spp is due to aspartyl proteinase enzymes (SAP: Secreted Aspartyl Proteinase) whose expression depends on both the yeast strain and the environment. SAPs are secreted by pathogenic *Candida in vivo* during infection and there is a correlation between virulence and the level of proteinase production in both the clinical isolates and laboratory strains of *Candida*. The enzymes are able to degrade a number of important defensive host proteins such as immunoglobulins and complement (Mishra *et al.*, 2007). Proteinases are highly virulent in nature enabling invasion of host tissues. They also assist the yeast in evading the host defense mechanisms. SAP proteins also help in digesting molecules for their nutrition, digesting host cell walls for adhesion and invasion, and affecting the host immune system to resist antimicrobial attack. The proteins are between 35-50 kDa in size and form the majority of extracellular proteins of *Candida* spp (Bhat *et al.*, 2011).

1.4.3.3. Phospholipases

Phospholipases are hydrolytic enzymes associated with membrane damage of the host cells, adherence and penetration. Invasion on host cells by microbes entails penetration and damage of the outer cell envelope. It helps the organisms to injure,
invade, and egress from various host cells. Some of the phospholipases may remove processed antigens from the surface of antigen-presenting cells. The secretion of phospholipases was first detected by Costa et al., 1967 and confirmed by Werner, 1966. *C. albicans* is the major species secrete phospholipases (Ibrahim et al., 1995).

**1.4.3.4. Sensitivity to Hydrogen Peroxide**

In women of childbearing age, the vaginal ecosystem is dominated by *Lactobacillus* spp. Lactobacilli modulate the vaginal microbiota by different mechanisms such as hydrogen peroxide production. *Candida* spp was more resistant to hydroxyl radicals generated by photolysis of H$_2$O$_2$. A catalase activity assay demonstrated that *Candida* spp had stronger catalase activity and could be one of the reasons for the resistance of the fungus to photolysis of hydrogen peroxide (Nakamura et al., 2010). Growth of majority of *Candida* spp will be controlled by the presence of Lactobacillus, resulting in the situation of equilibrium or colonization.

**1.4.3.5. Hemolytic property**

Hemolysin is another putative virulence factor thought to contribute to candidal pathogenesis. In particular, the secretion of hemolysin followed by iron acquisition facilitates hyphal invasion in disseminated candidiasis (Malcok et al., 2009). Haemolytic activity is associated with the ability of pathogenic organisms to acquire iron from mammalian cells, a critical stage in the establishment of an infection (Shinobu et al., 2007). *Candida* spp exhibits hemolytic activity when cultured on glucose-enriched blood agar. The hemolytic activity is detected in intact cells particularly in hyphae and also secreted into the culture medium. This hemolytic activity is associated to a mannoprotein residing in its mannan moiety (Mishra et al., 2007).

**1.4.4. Pathology of Candida**

The *candida* spp attached to the mucosal surface of vaginal tract, multiply in the vaginal epithelial cell and colonize. The epithelial invasion of *Candida* spp was takes place after overcoming the barrier of the epithelial mucosa. Then the fungi
entering the connective tissue of lamina propria, bridging tissue cellular defense mechanisms and penetrate into the vascular bed with subsequent dissemination and produce lesions. The emergence of symptoms was due to inflammatory reaction of tissue response to invasion of *Candida* spp, as well as various virulence factors of the *Candida* spp (Odds, 1998). Vaginal cell receptivity varies among individuals, but all strains of *Candida* adhere to either exfoliated vaginal and buccal epithelial cells or mucosal surfaces, through the yeast surface mannoprotein. It is suggested from in vitro studies that germ tube and mycelium formation facilitates vaginal mucosal invasion. Exogenous and endogenous factors may enhance germination and resulted in symptomatic vaginitis, or inhibit germination. Increased proteinase secretion may be a result of the transformation from the blastoconidium or colonization phase to the germinated invasive vaginitis stage or occur as an independent virulence factor. It is reported that hereditable spontaneous switching may occur spontaneously *in vivo* also. Colonizing yeasts with a change in environment can transform to a more virulent phase. Colonization rates vary from 10-25%, and the critical issue understands the process of asymptomatic colonization to symptomatic vaginitis, which is unclear. Inflammation may be caused by direct hyphal invasion or inducing symptoms of allergic reaction without identification of a specific event. Precipitating factors are pregnancy, where estrogens enhance yeast mycelium formation, or high levels of reproductive hormones. High oral contraceptive use, uncontrolled diabetes, during or following use of antimicrobial agents, and use of poorly ventilated clothing facilitates the *Candida* infection (Sobel, 1989).

Local immunity is more important than that in the systemic circulation for host defense against vaginitis. Women suffer from an immunological abnormality that predisposes them to recurrent vulvovaginal candidiasis. Because of the increased incidence of mucosal candidiasis in individuals with depressed cell-mediated immunity (CMI), defects in CMI are viewed as a possible explanation for recurrent vulvovaginal candidiasis. In humans, it was revealed that protection against vaginitis coincides with a non-inflammatory innate presence, whereas symptomatic infection correlates with a neutrophil infiltrate in the vaginal lumen and elevated fungal burden. Thus, instead of vulvovaginal candidiasis being caused by a putative deficient
adaptive immune response, it is now being considered that symptomatic vaginitis is caused by an aggressive innate response (Fidel, 2007).

1.4.5. Clinical manifestations of Candida

The changes in the vaginal microenvironment are generally necessary for Candida to induce pathological changes associated with clinical symptoms. Depending on the age, locality and socio-economic status, the frequency of vaginal yeast isolates has been reported 5 to 48.4% in healthy women (Gross et al., 2007). Carriage rate of Candida spp tends to increase with age. Generally, women are said to be present with clinical symptoms of vulvovaginal candidiasis when the high vaginal yeast count is greater or equal to 105 colony forming unit/ml of vaginal fluid (Carlson et al., 2000). The infection is characterized by intense vulval and vaginal irritation which often gives rise to inflammation, soreness, redness and white spots in and around the genital tract. Clinical manifestations such as thick cheesy white, curd-like vaginal discharge, painful urination and painful sexual intercourse are associated with the infection (Akpan et al., 2011).

1.4.6. Epidemiology of Candidiasis

1.4.6.1. Global Scenario

Vulvovaginal candidiasis is a common infection among women worldwide. In Europe it is the primary cause of vaginal infections where as in the United States, Candida spp is now the second most common cause of vulvovaginal candidiasis (Kaufman et al., 1989). In Austria, the prevalence of vulvovaginal candidiasis was 30.43%. Among the positivity, C. albicans prevalence was 87.9%. Non-albicans Candida was detected in 12.1%, mainly C. glabrata. Patients aged 21-40 years were significantly more prone to suffer from vulvovaginal candidiasis compared with other age-related groups (Paulitsch et al., 2006). In North East Brazil, the prevalence was found to be 20% (Oliveira et al., 2007). In a study conducted in Belzium, the prevalence of candidiasis was 20.1%. C. albicans was isolated most frequently (68.3%), followed by C. glabrata (16.3%) and C. parapsilosis (8.9%) (Bauters et al., 2002). Worldwide, the frequency of C. albicans varies between 45% to 90%. In West
Indies, the prevalence of vulvovaginitis was 32% and *C. albicans* accounts for 78% of infections (Jackson et al., 2005).

### 1.4.6.2. Indian scenario

In India, the prevalence of candidiasis was found to be 4% in rural Punjab (Jindal et al., 2010), 23% in Delhi (Goswami, 2000), 20.47% in Aligarh (Ahmad et al., 2009) and 21.5% in Assam (Saikia et al., 2009). For the female population until recently, vaginal candidiasis was often ignored or treated as an insignificant problem. In addition, many mental and emotional problems are found to be associated with vaginitis infection (Sobel et al., 1998). The prevalence of *C. albicans* in pregnant women was 12.5% and the highest percentage of 65.7% was in the age group of 21-30 years. In a community-based cross-sectional study conducted by Prasad et al., 2005 reported the 10.0% prevalence of vulvovaginal candidiasis in Tamil Nadu.

### 1.4.7. Problems faced with Vulvovaginal Candidiasis

Vulvovaginal candidiasis is an infection affecting millions of women worldwide every year, principally affecting the vulva and vagina and is caused by yeasts *Candida* that normally inhabit the vaginal mucosa (Dota et al., 2011). Candidal vulvovaginitis is one of the most common diagnoses made by physicians caring for women. The incidence of vulvovaginal candidiasis suggest approximately two-thirds of women experience at least one episode during their lifetime and nearly 50% of women have multiple episodes (Richter et al., 2005) and 5% will suffer recurrent vulvovaginal candidiasis (RVVC). Although it is rarely life threatening, the costs of the disease to patients and society are great, including medical evaluation costs, physical disability, psychological stress, and concern about the possibility of a more serious diagnosis (Reed et al., 2003). Vaginal discharge, the major sign of vaginitis, is the most common presenting feature in women attending sexually transmitted diseases clinics (Mirza et al., 1983). These pathogenic species of *Candida* derive their importance not only from the severity of their infections but also from their ability to develop resistance against antifungal. Widespread and prolonged use of azoles has led to the rapid development of the phenomenon of multidrug resistance (MDR), which
poses a major hurdle in antifungal therapy. Trends in spectrum and antimicrobial susceptibility of yeast species causing vulvovaginitis represent an interesting issue. The confirmatory diagnosis based on culture is not routinely performed nor generally advised in many regions, because all procedures done are expensive and time consuming. Several facts account for not performing routine cultures and antifungal susceptibility procedures in developing countries (Dias et al., 2011).

1.4.8. Factors influencing Vulvovaginal Candidiasis

Some of the factors which predispose women to vaginal candidiasis are change in pH, use of oral contraceptives, tight clothing and personal hygiene. However, it is reported that there is increase in occurrence of vaginal candidiasis during pregnancy, due to increased levels of hormones such as estrogen and steroid hormones (Oviasogie et al., 2009). In addition, many mental and emotional problems are associated with vaginitis. Oro-genital sex was an independent risk factor for the growth of Candida (Rylander et al., 2004). The other documented risk factors are antibiotics, chemotherapeutics, age etc. Vaginal candidiasis occurs in the presence of factors that increase the virulence of Candida and as a result of the reduction in local defence mechanisms (Babic and Hukic, 2010).

1.4.9. Laboratory diagnosis of Vulvovaginal Candidiasis

The diagnosis of vulvovaginal candidiasis is based on microscopic observation and culture. They are tedious and labor-intensive, and so their use for the analysis of large numbers of samples is impractical and costly. The laboratory investigations are further limited by their reliance on selective media, and many bacterial populations are refractory to cultivation. In recent years, cultivation-independent methods based on analysis of 18S rRNA gene sequences directly extracted from samples have been used to overcome these limitations and are widely employed to explore microbial diversity in various habitats (Zhou et al., 2009). For genital infections such as vulvovaginal candidiasis, diagnosis by molecular methods is poorly established (Guerra et al., 2008). As molecular diagnostic methods are rapid and reliable, Polymerase chain reaction- Random Fragment Length Polymorphism can used for the
diagnosis of etiologic agent of vulvovaginal candidiasis. A multiplex Polymerase chain reaction method was used to identify the *Candida* spp which seemed to be a reliable, rapid and cost effective technique since it only requires PCR components and a commercial DNA extraction kit. Another advantage of Multiplex PCR method is its ability to identify more than one species in a single specimen (Rad *et al.*, 2012).

### 1.4.10. Prevention and treatment of Candidiasis

Current National and International guidelines for recurrent vulvovaginal candidiasis include obtaining accurate diagnosis and using suppression and maintenance (oral or vaginal) therapy. This involves initially high doses of antifungal agents usually for 2 weeks, followed by long term weekly or monthly therapy. A vaginal Imidazole (eg. Clotrimazole 1%) or Nystatin, intravaginally, at night; Fluconazole 50mg orally, once daily or Itraconazole capsules 100 mg orally, once daily (Watson and Pirotta, 2011).

### 1.4.11. Antifungal resistance in *Candida*

Over the next decade, antifungal resistance may become an increasingly crucial determinant of the outcome of antifungal treatment. The factors contributing to antifungal resistance includes, virulence, size of fungal population, pharmokinetic, dose, nature and drug interactions, immune status, presence of foreign bodies and hematological malignancy (Agha *et al.*, 2010). Azoles are antifungal drugs frequently used for treatment of VVC and such therapy can be complicated by the emergence of drug resistant yeasts. Resistance to azole antifungals was reported in the late 1980s in *C. albicans* after prolonged therapy with Miconazole and Ketoconazole. Recently, antifungal resistance results in Biofilm- associated infections. *In vitro* studies on the cell wall of Fluconazole-susceptible and resistant *C. albicans* strains detected altered distribution of cell wall glucan-associated proteins and these effects may be stably incorporated into the cell wall upon acquisition of resistance (Angiolella *et al.*, 2002). Microorganisms develop mechanisms to counteract the fungicidal or fungistatic effects of all antifungal classes that are based on three major mechanisms, namely, reducing the accumulation of the drug within the fungal cell, decreasing the affinity of
the drug for its target, and modifications of metabolism to counterbalance the drug effect (Vandeputte, 2012). Azoles-resistant species have arisen in vivo and in vitro that show changes in the target enzyme lanosterol 14-a-demethylase, in expression of multidrug efflux pumps, or in both. The use of over-the-counter (OTC) products, which became available in the early to mid-1990s for self-treatment of vaginitis plays an important role in drug resistance of Candida. Many of the OTC drugs are azole-based, therefore frequent or prolonged use of these products has the potential to select for widespread drug resistance. In fact, cross-resistance between OTC drugs (Miconazole, Clotrimazole, and Tioconazole) and Fluconazole has been observed in clinical isolates of Candida spp (Mathema et al., 2001).

1.4.12. Molecular mechanism of drug resistance in Candida

In all fungal species, ERG11 is the gene encoding ERG11p or lanosterol 14α-demethylase, an essential enzyme for ergosterol synthesis. Antifungal drug resistance has been associated with point mutations, alterations in ergosterol biosynthetic pathway and increased levels of expression of the ERG11 gene. Fluconazole and other azoles resistance has also been associated with point mutations of the ERG11 gene. These mutations result in conformational changes that reduce effective binding between azoles and their target (Casalinuovo et al., 2004). Evidence is accumulating that changes in other enzymes in the ergosterol biosynthetic pathway can also contribute to resistance (Casalinuovo et al., 2004). Drug efflux from the cells is another component of resistance in Candida spp, as overexpression of two types of efflux pump has been correlated with antifungal resistance. They are ATP-Binding Cassette (ABC) transporters and the major facilitators superfamily (MFS). The ABC transporter genes CDR1 and CDR2 encode ATP dependent efflux pumps that are overexpressed in many azole resistant isolates. Deletion of these genes results in hypersensitivity to azoles. The major facilitator gene MDR1 encodes a pump that uses the proton motive force at the membrane to transport drugs and other compounds across the plasma membrane. Overexpression of this pump is also associated with resistance, and deletion results in hypersensitivity toazole drugs (White et al., 2002). The asexual and diploid nature of C. albicans complicates the characterization of gene expression in antifungal drug resistance. The studies show that other factors may
contribute to Fluconazole resistance development such as chromosome copy number, loss of heterogeneity, gene disruption at definite loci and other genetic strategies (De Backer and Dijck, 2003).

1.5. Reproductive tract infections caused by Bacteria

1.5.1. Bacterial reproductive tract infection – *Neisseria gonorrhoeae*

*Neisseria gonorrhoeae* is a Gram negative diplococcus that commonly infects the mucous membrane lined by columnar or cuboidal and non-cornified epithelial cells. Infection may cause cervicitis, urethritis, proctitis, pelvic inflammatory disease, pharyngitis and conjunctivitis. Chronic pelvic pain, ectopic pregnancy, infertility, stillbirths, prematurity, low birth weight in infants and increased susceptibility to HIV infection are possible sequelae (Siu and Kwan, 2011). This organism was first identified in 1879 by Albert Neisser and is one of the oldest infections known to man and is efficiently transmitted through unprotected sexual intercourse. Gonorrhoea, known colloquially as the clap and the drip, is most frequently spread during sexual contact. It can also be transmitted from the mother's genital tract to the newborn during birth, causing ophthalmia neonatorum and systemic neonatal infection. Complications were highly prevalent in the pre-antibiotic era and, to a lesser extent, still occur today as a result of delayed access to effective, or dispensing of inappropriate antibiotics (Lewis, 2007).

1.5.2. Gonorrhoea: A Historical Perspective

*Neisseria gonorrhoeae* infection can be found in biblical text within the Book of Leviticus and in ancient Chinese writings, making gonorrhea one of the oldest diseases known to humans. Hippocrates referred to acute gonorrhea as “strangury” obtained from the “pleasures of Venus” in the fourth and fifth centuries B.C (Sparling, 1999). It was not until A.D130 that Galen, who mistakenly confused the purulent discharge associated with gonococcal urethritis with semen, introduced the term gonorrhea, which means “flow of seed” after the Greek words gonor (seed) and rhoia (flow). The most notable account of *N. gonorrhoeae* infection is found in the personal diary of James Boswell, the famous biographer of Samuel Johnson. Boswell described the possible consequence of asymptomatic infection in women, especially in his wife, who never exhibited symptoms of gonococcal infection, but lost four of
her nine pregnancies. Neisser described the causative agent of gonorrhoea in purulent exudates from the genital tract and conjunctiva in 1879; however, it was not until 1882 that Leistikow and Loffler finally cultivated the gonococcus (Edwards and Apicella, 2004).

1.5.3. Pathogenesis and Virulence factors of *Neisseria gonorrhoeae*

To survive the host immune response, *N. gonorrhoeae* has developed complex strategies to avoid host defense mechanisms. It includes antigenic and phase variation of Opa proteins, Pili, and lipooligosaccharide (LOS) in the cell wall of *N.gonorrhoeae*. The multiple antigenic variants which are produced by the Pilin protein during infection, helps the gonococci to escape from the host immune mechanism (Seifert *et al.*, 1994). Other mechanisms which help the gonococci to escape from host immune system is masking of gonococcal antigen (sialylation of LOS which prevents the binding of bactericidal antibodies and the activation of complement), the similarity of terminal LOS sugars to host glycolipids, release of IgA1 proteases and blocking antigen by the binding of antibodies to a reduction modifiable protein (Rmp).

![Pathogenesis of Neisseria gonorrhoeae](image)

**Fig : 2 - Pathogenesis of Neisseria gonorrhoeae**
Rmp is physically associated with Por on the outer membrane of gonococci and is highly immunogenic for noncomplement-fixing antibodies. Antibodies directed against Rmp bind to the Por-Rmp complex, and block the effective deposition of complement fixing anti-Por antibodies (Rice, 1989). Therefore women infected with \textit{N. gonorrhoeae} who produced Rmp antibodies had an increased risk of salpingitis.

1.5.3.1. Pili

Initial attachment of gonococci to the surface of columnar epithelial cells is mediated by pili which are filamentous outer membrane appendages composed of multiple subunits, the most important of which is pilin or pilE which forms the pilus fiber (Scheuerpflug \textit{et al.}, 1999). Pili play a central role in localized neisserial infection by mediating the selective primary adherence to secretory (non-ciliated) epithelial cells and possess additional binding functions, which may contribute to gonococcal colonization of mucosal surfaces (Wang \textit{et al.}, 1998). Pilus has an affinity towards the complement regulatory protein called CD46. It is a human-specific transmembrane protein which is expressed by all nucleated cells. Pili modulate host cell signaling mechanisms to aid the invasion of epithelial cells by gonococcus. It plays an important role in initiating infection and anti-pilin antibodies can block the binding of \textit{N. gonorrhoeae} with epithelial cells. Pili negative variants are usually non-infectious (Kellogg \textit{et al.}, 1963).

1.5.3.2. Opa proteins

The gonococci obtain a secondary tight contact with epithelial surfaces via the phase-variable, colony opacity-associated (Opa) proteins. Opa proteins contribute to the cellular tropisms exhibited by gonococci and are divided into two classes, Opa\textsubscript{50} proteins and Opa\textsubscript{52} proteins. Opa\textsubscript{50} proteins that recognize host cell heparin sulfate proteoglycans (HSPG) and Opa\textsubscript{52} proteins that recognize members of the carcinoembryonic antigen-related family of cell adhesion molecules (CEACAM or CD66). However, Vitronectin and fibronectin functions as bridging molecules between the gonococcus and its target HSPG receptor. More than 95% of Opa expressing \textit{N. gonorrhoeae} and clinical isolates of \textit{N. gonorrhoeae} are able to bind
CEACAM1 suggesting that an Opa-CEACAM association can occur in vivo. The infected women who had anti-opa antibodies had a lower risk of gonococcal salpingitis (Edwards and Apicella, 2004).

1.5.3.3. Lipooligosaccharide (LOS)

The pathogenic Neisseria possess the outer membrane LOS molecules which lack the repeating O-antigen sugar that comprises the polysaccharide side chain of LPS. This property differentiates the Neisseria from other Gram negative LPS. The oligosaccharide substitutions of LOS exhibit both interstrain and intrastrain variability (Apicella et al., 1987). Interconversion of LOS oligosaccharides occurs spontaneously and is dependent upon the presence or absence of available substrates and enzymes involved in LOS biosynthesis. The spontaneous conversion of oligosaccharide determinants can change the manner in which the gonococcus associates with host tissues and hence, can potentially alter the course of gonococcal disease. LOS oligosaccharide side chains terminate in epitopes that mimic sugar moieties of mammalian glycosphingolipids. This helps the gonococcus to avoid the immune response and allows the bacterium to use host-derived molecules that normally associate with the resembled structure. Some LOS structures can serve as acceptor molecules for sialic acid deposition. LOS sialylation is mediated by gonococcus-encoded sialyltransferase that is present in the gonococcal outer membrane. The gonococcus lacks the ability to synthesize CMP-N-acetylneuraminic acid and must parasitize this substrate from its human host. The presence of sialic acid on gonococcal LOS confers unstable resistance to the bactericidal action of normal human serum (Nairn et al., 1988).

1.5.3.4. Porin

Gonococci become engulfed in a process known as parasite-directed endocytosis. Following attachment to mucosal cells, parasite-directed endocytosis may involve activation of acidic sphingomyelinase and appears to be enhanced by protein I or Por (Putten et al., 1998). Porin, water-filled channel through which small molecules transverse the gonococcal outer membrane and porin molecules trigger
variable functional responses within host cells depending upon the particular porin and the host cell type. A unique feature ascribed to gonococcal porin is its ability to translocate into eukaryotic cell membranes (Blake and Gotschlich, 1981). Porin can initiate the apoptosis by inducing a calcium influx in epithelial cells and may play a role in the cytotoxicity observed in fallopian tube organ culture (FTOC) and in the shedding of epithelial cells which occurs in vivo during mucosal infection. Gonococci also produce several proteases, peptidases and phospholipases which may play a role in pathogenesis (Todar, 2004).

1.5.4. Pathology of Gonorrhoea among Genders

1.5.4.1. In Men

Gonorrhoea infection in men most commonly occurs as an acute urethritis. It is due to the inflammatory response directed against infecting gonococci. Very less percentage of men will develop asymptomatic gonococcal urethritis (Sparling, 1999). The common symptom of symptomatic gonococcal disease in men is the presence of a purulent discharge, which is associated with PMN influx and shedding of urethral epithelial cells. During the incubation period gonococci are quiescent and cannot be cultured from the urethra for up to 40 hrs. After the initiation of infection, a purulent exudative process begins. In men, gonococci enter urethral epithelial cells that give a protective environment for survival and multiplication and infected epithelial cells are subsequently shed from the mucosal surface to the urethral lumen. The chemokine interleukin-8 (IL-8), cytokine IL-6 and tumor necrosis factor alpha (TNFα) are prevalent within the urethral lumen helpful for the progressive gonococcal disease. These conditions may potentially initiate the inflammatory response associated with gonococcal urethritis by triggering PMN influx and subsequently might potentiate the clinical symptoms associated with disease. During infection, the gonococci are found within PMNs and urethral epithelial cells. The interaction of gonococci with PMNs is dependent upon the presence of Opa proteins (Shafer and Rest, 1989). Opa proteins facilitate a gonococcus-PMN interaction and promote intracellular survival of gonococci by sequestering pyruvate Kinase as a means to acquire pyruvate which is required for gonococcal viability. The ability of gonococci to acquire iron (in the form
of lactoferrin or transferrin) from its sole human host is required for successful colonization in male urethra. The intimate association between the urethral epithelium and the gonococcus is achieved through the interaction of the asialoglycoprotein receptor (ASGP-R) and gonococcal LOS, a major constituent of the gonococcal cell membrane. Sialylation of the LNnT epitope enhance the ability of gonococci to invade primary urethral epithelial cells and epithelial cell lines and to be phagocytosed by neutrophils. Neuraminidase is also present on the surface of professional phagocytic cells, which are generally abundant in the urethral lumen under conditions favoring the progressive gonococcal disease.

1.5.4.2. In Women

The majority of gonococci transmitted from men to their partners have sialylated LOS, but the presence or absence of sialic acid on LOS does not influence the interaction of the gonococcus with primary cervical epithelial cells. Within the lower female genital tract, sialylated gonococci might become modified to enhance disease transmission to men. That is, neuraminidases produced by the vaginal microflora can potentially remove sialic acid from sialylated gonococci. Cervical epithelia also produce neuraminidase; however, the specificity of this enzyme to cleave endogenous or exogenous substrates exhibits cyclic variability. The level of sialic acid found within the microenvironment of the cervix also exhibits cyclic variation. In contrast to the inflammatory response generated predominately with gonococcal infection of the male urethra, 50 to 80% of women with lower genital tract N. gonorrhoeae infection are asymptomatic and 70 to 90% of women with disseminated infection lack signs of genital tract involvement (Hook and Hansfield, 1999). Within the lower female genital tract, the cervical epithelia provide a source of alternative pathway complement activity. Within minutes of infection in primary cervical epithelial cells, complement protein C3b is deposited on the lipid A portion of gonococcal LOS and is rapidly inactivated to iC3b. The receptor CR3 serves as the primary receptor for N. gonorrhoeae adherence and helps in the invasion of the ectocervix and endocervix.
Binding of gonococcal pilus to the I domain of CR3 probably allows the gonococcus to overcome the electrostatic repulsion between its own cell surface and that of the cervical cell and may juxtapose the gonococcus at the cervical cell surface. Binding of the gonococcus to CR3 requires the cooperative action of iC3b bound to the gonococcal surface in conjunction with gonococcal porin and pilus. Opa proteins do not appear to be required for adherence to or invasion of primary cervical epithelial cells. Gonococci are then internalized within macropinosomes. Gonococcal phospholipase D (NgPLD) promote the infection of primary cervical epithelial cells and augment signaling events that trigger CR3 mobilization to the surface of primary cervical cells. It also modulates cervical cell signal transduction events leading to membrane ruffling. Ascending infection of the uterus and fallopian tubes may occur as a consequence of hormonal changes that alter the mucosal epithelia, molecules available for gonococcal use, and virulence factors expressed by the gonococcus. One of the factors like menses is associated with an increased risk to women for PID and for disseminated infection. C3 production by the cervical epithelium exhibits cyclic variability, and the highest levels of C3 are detected during menses. The ability of gonococci to use heme, hemoglobin, and haptoglobin-hemoglobin is also postulated to be responsible for the increased risk observed for women to develop PID and disseminated disease during menses.

Approximately 45% percent of women with gonococcal cervicitis will develop an ascending infection, the prerequisite to PID. Ascent to the upper female genital tract might be facilitated through the ability of gonococci to exhibit twitching motility, in conjunction with hormonal changes which influence the expression of complement and molecules serving as gonococcal receptors within the female genital tract. Conversely, expression of the lutropin receptor (LHr) increases in an ascending manner from the endometrium to the fallopian tubes, and expression is up regulated at the time of menses. It is speculated that the LHr serves as a receptor for gonococcal invasion of endometrial and fallopian tube epithelia. The interaction of the gonococcus with the LHr increases the invasive character of the gonococcus. Human chorionic gonadotropin (hCG), a natural ligand for LHr, can competitively inhibit the association of gonococci with FTOC and Hec1B cells, presumably by interfering with
an LHR-gonococcus interaction. Gonococci also possess a surface molecule that mimics hCG. The LHR is also present on the human uterus, placenta, deciduas and fetal membranes thus a gonococcus-LHR interaction occurring on deciduas and placental membranes could potentially result in severe complications of disease and contribute to the increased risk of spontaneous abortion associated with *N. gonorrhoeae* infection. Gonococcal adherence to fallopian tube epithelia occurs selectively on nonciliated cells and ciliated cells of the fallopian tube epithelia that are subsequently shed. If left untreated, complete loss of ciliary action can occur. (Edwards and Apicella, 2004).

1.5.5. Clinical manifestations of Gonorrhoea

The clinical symptoms of *N. gonorrhoeae* and their different subtypes depend on the antigenic characteristics of the respective surface proteins. Certain subtypes are able to evade serum immune responses and are more likely to lead to disseminated (systemic) infection. Infection of the lower genital tract, the most common clinical presentation, primarily manifests as male urethritis and female endocervicitis. Infection of the pharynx, rectum, and female urethra occur frequently but are more likely to be asymptomatic or less symptomatic. Retrograde spread of the organisms occurs in as many as 20% of women with cervicitis, often resulting in pelvic inflammatory disease (PID), with salpingitis, endometritis, and tubo-ovarian abscess. Retrograde spread can lead to frank abdominal peritonitis and to a perihepatitis known as Fitz-Hugh-Curtis syndrome. Long-term sequelae of PID, such as tubal factor infertility, ectopic pregnancy, and chronic pain, may occur in up to 25% of affected patients. Epididymitis or epididymo-orchitis may occur in men after gonococcal urethritis. Lower genital infection is a risk factor for the presence of other sexually transmitted diseases (STDs), including Human Immunodeficiency Virus (HIV).

Conjunctivitis can occur in adults, as well as children, following direct inoculation of organisms (usually as a result of hand-eye inoculation in adults) and can lead to blindness. Disseminated gonococcal infection (DGI) occurs following approximately 1% of genital infections. Patients with DGI may present with symptoms of rash, fever, arthralgias, migratory polyarthritis, septic arthritis,
tendonitis, tenosynovitis, endocarditis, or meningitis. Symptoms may appear within 2 to 10 days after exposure to an infected person—even longer for women (up to 3 weeks). Generally 30% to 40% of infected women do not exhibit any symptoms. Thus it is possible to be infected with gonorrhea and not know about the infection. The most frequently observed symptoms are infection and irritation of the vagina and cervix, the need to urinate often, itching and burning of the vagina, thick yellow or green discharge and bleeding between menstrual periods.

1.5.6. Epidemiology of Gonorrhoea

Gonorrhoea is one of the classical sexually transmitted infections (STIs) with humans as the only host. Despite a sharp decline in the incidence of gonococcal infection in developed countries during the last decade, gonorrhoea still remains one of the most common STIs in developing countries. The prevalence of gonococcal infection varies greatly among countries in the developed and developing world where it continues to be a major public-health problem (Ahmed et al., 2010)

1.5.6.1. Global scenario

According to a global estimate from World Health organization (WHO), around 62 million new cases occurred in 1995 and the highest rate was found in South and Southeast Asia, Sub Saharan Africa and South and Central America (Bhargava et al., 2010). However, in the world, the incidence of gonorrhoea has been reported between 4.6 to 64.7% (Hansen et al., 2003). The isolation of *N. gonorrhoeae* from STDs victims varies from 4.6 to 64.7 % throughout the world (Bokaeian et al., 2011). *N. gonorrhoeae* infection is the second most common notifiable disease in the United States, with 3, 58, 366 cases reported in 2006. However, reported cases probably represent an underestimate of the actual disease burden because of under diagnosis and under reporting, it is estimated that there were approximately 718, 000 incident gonorrhoea cases in 2000 (Workowski et al., 2008).

In the United Kingdom (UK), the gonococcal infection shows a clear increase from 1957 onwards, reaching a peak in the early seventies and then decreasing. In Denmark and Sweden, there was a decline in the incidence of gonorrhoea
(De Schryver and Meheus, 1990). In Australia, the number of cases of gonorrhoea declined from a peak of 6,599 in 1982-1983 to 1121 in 1990-1991 showing a reduction of 83% (AGSP, 1993).

1.5.6.2. Indian scenario

In India, the prevalence of gonorrhoea ranged from 0 to 19.1%. The study conducted in Hyderabad and Mumbai female sex workers, the occurrence was found to be 14.1% (Das et al., 2011). The prevalence rate was varied in different parts of the country. In Chandigarh, the prevalence was nil (Sharma et al., 2003). A study conducted by Ray et al., 2006 in North India and Shilpee et al., 2008 in Delhi reported the incidence of 13%. Kumarasamy et al., 2008 in Tamil Nadu observed the prevalence rate of 2% in South Indian Men and Women.

1.5.7. Problems faced with Gonorrhoea

Gonorrhoea, a disease of public health importance, not only leads to high incidence of acute infections and complications but also plays a major role in facilitating Human Immunodeficiency Virus (HIV) acquisition and transmission. This is thought to result from an increase of viral load in the semen or cervico-vaginal fluids from those co-infected with gonorrhoea and HIV, and to increase in the number of target cells for HIV in the inflammatory exudates present in sexually transmitted diseases. Gonorrhoea is an easily curable STI. However undetected, untreated infections can lead to complications. Also, asymptomatic patients, unaware of their infection, may serve as a reservoir of infection to their partners (Bala and Sood, 2010). Antibiotic resistance may also be a serious problem when women contract sexually transmitted infections (STI), especially gonorrhoea. An estimated 3 million treatment failures due to resistant gonorrhoea occur each year in the world and will incur an additional cost of US$ 500 million. When treatment guidelines recently had to be changed in the US, due to increasing resistance to ciprofloxacin, the cost of treatment increased fivefold (React facts, 2008). The problem is further compounded by the emergence of resistance to antimicrobial agents that are commonly used against *N. gonorrhoeae*, making the treatment expensive and prolonged. Resistance to
antimicrobial agents has resulted in morbidity and mortality from treatment failure. The multidrug resistant strains of *N. gonorrhoeae* isolates are now emerging in many countries. In European countries the decrease in the incidence of gonorrhoea accompanies by changes in the epidemiology of the disease and an increase of MDR *N. gonorrhoeae* isolates (Van Duynhoven, 1999).

1.5.8. Factors influencing gonococcal infection

Gonococcal infections among women are frequently asymptomatic so screening of women at high risk and detecting factors influences the infection is essential to identify the reservoirs of infection. The risk factors of gonorrhoea vary greatly depend upon the population characteristics. The risk factors are multiple or anonymous sex partners, black race and young age (Barry *et al*., 2008). Younger age could reflect more sexual activity, more partners, and possibly less knowledge and experience with STI prevention, including negotiating condom use (Nguyen *et al*., 2008). Illiteracy, first sexual intercourse before the age of 20 years, past history of STI and no treatment taken is considered as risk factors as per the study conducted by Jayandra *et al*., 2005. Prostitution and drug usage was also considered as the important risk factors for gonorrhoeal infection (Workowski *et al*., 2008).

1.5.9. Laboratory diagnosis of Gonorrhoea

In women with acute gonorrhoea, bacteriological confirmation is dependent on culture evidence. Gram stain has a sensitivity of approximately 50% for gonococcal cervicitis. The Gram stained smear is too insensitive and yields high percentage of false negatives; it may also give false positives due to the presence of saprophytic *Neisseria*, which on smear are morphologically indistinguishable from *N. gonorrhoeae* (Basaca-Sevilla *et al*., 1980). At present, culture is the preferred laboratory test. However, if the specimens require long transportation times or have been exposed to extreme temperatures, culture is less sensitive than the nucleic acid methods. The nucleic acid tests, especially the semi-automated and automated systems, are less labour intensive than culture methods and allow high throughput processing of clinical specimens. The processing time for nucleic acid methods is
shorter than for culture methods (Ng and Martin, 2005). Molecular-based methods are rapid, reliable and effective for genital specimen screening measures, especially when applied to areas of high disease prevalence. However, clinical and analytical sensitivity for some commercial systems decreases dramatically when testing urine samples. Moreover, cross reactivity to non-pathogenic Neisseria spp has been documented for selected methods. Therapeutic decisions may increasingly become compromised owing to a lack of cultured N. gonorrhoeae for antimicrobial susceptibility testing. In vitro experiments suggest that transcription-mediated amplification has greater analytical sensitivity than the other molecular-based methods now available (Munson et al., 2009).

1.5.10. Prevention and treatment of Gonorrhoea

The usage of latex condoms during sexual intercourse, avoidance of sexual contact with high-risk partners, treat the infected sexual partners or have them tested before having sexual relations are the preventive measures that should be followed for gonorrhoea. CDC recommends Ceftriaxone 250 mg intramuscularly in a single dose, or Cefexime 400 mg orally in a single dose plus Azithromycin 1 g orally in a single dose or Doxycycline 100 mg orally twice a day for 7 days for treatment of uncomplicated gonococcal infections of the cervix, urethra and rectum for the treatment (Siu and Kwan, 2011).

Only two vaccines for gonococci have entered into clinical trials. The first was a crude killed whole cell vaccine and the second was a purified Pil vaccine. There was no evidence of protection with the crude killed whole cell vaccine even though the vaccine was said to be well tolerated and induced an antibody response in over 90% of vaccine recepients. The purified Pil vaccine showed no protection against a heterologous strain expressing antigenically variant Pil in the naturally acquired infections. Recent vaccine development efforts have focused on the female mouse model of genital gonococcal infections (Zhu et al., 2011).
1.5.11. Antibacterial resistance in *Neisseria gonorrhoeae*

Effective antibiotic treatment is the most essential component which is used to control gonorrhoea. The development of resistance in *N. gonorrhoeae* to multiple antimicrobial agents challenges this component of gonorrhoea control. Multiple classes of antimicrobials had been used for treatment of *N. gonorrhoeae* in the past 60 years. Sulphonamides, Penicillins, Tetracyclines and Fluoroquinolones were the treatment options in the past but are no longer efficacious now (Siu and Kwan, 2011).

![Fig: 3 - Mechanism of antibiotic resistance](image)

Sulphanilamides were used for gonococcal treatment after their introduction in 1936, but their efficacy was short-lived because of the rapid emergence of resistance by 1945 (Kampmeier, 1983). Penicillin became the recommended antimicrobial regimen for the next 40 years. The progressive decline in susceptibility initially associated with chromosomally mediated resistance (exhibited by a stepwise increase in resistance) and later by the acquisition and spread of plasmids containing genes for penicillinase production required serial increases in the recommended dose of...
intramuscular procaine penicillin (with probenecid) from 50,000 units in 1945 to 4.8 million units by the early 1970s. In 1985 because of emerging Penicillin resistance, Ceftriaxone became a recommended regimen for the treatment of uncomplicated gonococcal infections. At the same time, Tetracycline resistance (both plasmid and chromosomally mediated) was spreading to the extent that Tetracycline was no longer a viable treatment option. By 1989, resistance to Penicillin and Ceftriaxone was sufficiently widespread and was no longer effective. Ciprofloxacin was used as an alternative treatment option (CDC, 1989). By 1993, on the basis of data regarding high efficacy, safety and convenience as single dose therapies, oral fluoroquinolones (Ciprofloxacin, Ofloxacin) were recommended as oral regimens for gonorrhoea treatment, (Workowski et al., 2008). The organism acquires resistance by spontaneous mutation or by acquisition of new DNA via conjugation or transformation, and resistance may, thus, be chromosomal or plasmid-mediated (Dillon and Yeung, 1989). A single organism can have both the mechanisms of resistance, and resistance to multiple antibiotics is often common (Ahmed et al., 2010). In this regard, emergence of new strains of penicillinase-producing gonococci (PPNG) as well as other strains, which are all resistant against certain antibiotics including, Spectinomycin, Tetracycline, Ciprofloxacin and Cefexim have created massive concern in the world of medicine (Handsfield et al., 2005).

1.5.12. Molecular mechanism of drug resistance in Neisseria gonorrhoeae

The genetic background for the evolution of antimicrobial resistance in gonococci is either chromosomal mutation or the acquisition of R plasmids. Strains with mutations in chromosomal genes emerged in the late 1950s. Two types of chromosomal resistance have been described. The first type is drug specific; it is due to single-step mutation to high level resistance. The second type involves mutations at several chromosomal loci. The combination of mutations at these various loci determines the level of resistance and the pattern of resistance. In 1976, a new aspect of Penicillin resistance was recognized when strains that had acquired plasmids coding for the production of β-lactamase appeared simultaneously in England and the United states. In 1985, the gonococcus acquired another R plasmid that coded for high-level resistance to Tetracycline (Lind, 1997). Certain acquired plasmids and
genetic mutations enhance virulence. TEM-1–type beta-lactamase (penicillinase) affects penicillin binding and efflux pumps and confers resistance to penicillin. *TetM* protects the ribosome and confers resistance to tetracycline. Alterations in *gyrA* and *parC* genes result in fluoroquinolone resistance by efflux activation and decreased antibiotic cell permeation.

Emergence and spread of quinolone resistant *N. gonorrhoeae* may have been accelerated by heavy use of the quinolones and it involves chromosomal mutation that modifies the target DNA gyrase or accelerates the efflux to decrease quinolone accumulation. Resistance to penicillin can be mediated by chromosomal change to affect the penicillin binding proteins and by production of β-lactamase via episome. Both chromosomally mediated and penicillinase plasmid mediated resistance is widespread. The resistance to Spectinomycin usually occurs by chromosomal mutation resulting in high level resistance (Lawung *et al.*, 2005). In the plasmid profiling the most frequent plasmid pattern was the combination of 4.5, 2.6 and 24.5 MDa plasmids. The conjugative plasmid is highly prevalent and the size was 24.5 MDa (Palomares *et al.*, 1990). A mutation of S91F and D95A/G/N in *gyrA* combined with S87N in *parC* was the most prevalent mutation pattern of fluoroquinolone resistant *N. gonorrhoeae* isolates. This mutation pattern was associated with a high level of quinolone resistance (MIC >16.0 μg/ml) which can serve as a marker for quinolone-resistance prediction (Tiejun *et al.*, 2009).

1.6. Need of Research

Reproductive tract infections (RTIs) generally seen as a ‘silent’ epidemic and is one of the major public health problems causing a significant proportion of gynecological morbidity and maternal mortality in developing countries. Studies on RTI in India indicate higher rate of prevalence varying from 19-71% which can be attributed to different socio-cultural practices and taboos prevailing in different communities. Though both men and women suffer with RTIs, their consequences are far more devastating and widespread among women. Amongst women, RTIs often go undiagnosed and untreated and when left untreated they lead to complications and an increased risk of HIV transmission. The gynecology morbidity was high among the female because they keep secret about their gynecological problems. This is due to the
humiliation for many women because they are considered unclean. Therefore the disease burden was usually underestimated. Control of reproductive tract infections is very tedious in resource-poor countries and faces with logistical and methodological problems. Further the emergence of drug resistance among the frequently encountered clinical pathogen that causes RTIs are worsened which reinforces the need for an epidemiological study and its surveillance of its susceptibility to antimicrobials against the etiological agents. This problem was usually faced during the treatment of vulvovaginal candidiasis and gonorrhoea which is the most common reproductive tract infections. Close monitoring of the antimicrobial susceptibility on all the isolates of Candida and N. gonorrhoeae in developing countries is essential in an environment of rapidly changing resistance patterns, So as to provide guidance for appropriate case management. Therefore, the present study was aimed to describe the disease burden, associated risk factors, identification and characterization of changing antimicrobial resistance pattern of candidiasis and gonorrhoea with the following objectives.

OBJECTIVES:

- To study the prevalence rate of vulvovaginal candidiasis and gonococcal infections among ICTC patients in Namakkal District, Tamilnadu.
- Isolation and characterization of Candida spp and Neisseria gonorrhoeae from ICTC patients attended Primary Health Centre, Sendamangalam, Tamil Nadu.
- To assess the antibiogram pattern of Candida spp and Neisseria gonorrhoeae isolates against the selected antifungal and antibacterial drugs.
- Characterization of virulence factors from Candida spp such as biofilm formation, Aspartyl proteinase production, Phospholipase production, Hemolytic activity and susceptibility to Hydrogen peroxide solution.
- Identification and characterization of the azole resistance gene in Candida spp.
- To amplify and sequence 28S rRNA gene for Candida spp.
- To determine the plasmid mediated resistance for drug resistant Neisseria gonorrhoeae.
- To amplify and sequence the tbpB gene of Quinolone resistant Neisseria gonorrhoeae isolate.