2. REVIEW OF LITERATURE

2.1. Reproductive Tract Infections

The control of reproductive tract infections (RTIs), especially sexually transmitted infections (STIs), is an urgent health priority in many countries (Hawkes et al., 2002). In a number of recent studies, it has been shown that bacterial and parasitic agents of STIs and RTIs, increase the release of virion particles in the semen and ulcers in the genital region and thus increase the risk of both acquisition and transmission of HIV in patients with STIs (Nessa et al., 2004). Kurth et al., 1995 have demonstrated that control of STIs through syndromic management in the general population in a rural area of Tanzania was able to reduce the serological incidence of HIV by 42%.

With an annual incidence of 340 million STI cases globally and many more endogenous and iatrogenic infections, reproductive tract infections (RTIs) are considered a global public health issue. In resource poor countries where 75% -85% of these new cases occurs, RTIs are among the five most common health problems leading to contact with the health system. RTIs entail a heavy toll on women, if untreated they can lead to pelvic inflammatory disease (PID), which can cause long-term sequelae, such as tubal infertility and ectopic pregnancy. RTI control in many resource-poor countries have been faced with logistical and methodological problems due to lack of standardized definitions of RTIs, lack of well-validated diagnostic criteria, lack of accurate laboratory tests, and lack of diagnostic equipment and skills. Therefore, the burden of RTIs may be substantially underestimated and is rarely on the list of public health priorities (Hng et al., 2009).

2.2. Prevalence of Candida spp.

2.2.1. Global prevalence

Rylander et al. (2004) carried out a study in the year 2000 at a single adolescent health centre in Stockholm, Sweden. In total, 220 sexually active women were examined and completed the questionnaire. The mean age of the women was 19 (range 12–22) years. C. albicans were isolated in 90 out of 219 samples and only 3 C. glabrata was isolated. The overall prevalence of candidal infections were 42.4%. Of
93 women with a positive *Candida* culture, 79 (85%) complained of current itching, discharge, fissures, or vulvar pain. Thus, only 14 (15%) had an asymptomatic *Candida* infection. Altogether, 6% of the participating women were asymptomatic *Candida* carriers. Past and present complaints of vulvar pruritus and discharge as well as clinical signs of vulvar erythema, fissures and abnormal discharge were significantly more common in women with a positive *Candida* culture than in those with a negative culture (p,0.001). A history of pain at intercourse was given by 52 (24%) of 217 women and was associated with a positive *Candida* culture (p,0.004). A history of recurrent candidiasis was also associated with pain at intercourse (p,0.01). There was a significant association between growth of *Candida* and frequent practice of oro-genital sex (p=0.02). A history of recurrent candidiasis was twice as common among women who had used oral contraceptives (OC) for at least 3 years (31%) compared to women who had used OC for a shorter period or not at all (14%).

Akinbiyi *et al.* (2008) determine the prevalence of *Candida albicans* and bacterial vaginosis in asymptomatic pregnant women in south Yorkshire, United Kingdom. One thousand and seventy-three (1073) consecutive women who attended the antenatal clinic at Barnsley, UK over a 15-month period were studied. The Prevalence of *Candida albicans* was 12.5% and the highest percentage of 65.7% of incidence occur in the age group of 21-30 years.

Khan *et al.* (2009) conducted a study in Pakistan. They stated that vulvovaginal candidiasis is the second most common cause of vaginitis in United States and the most common cause in Europe. It is an under reportable disease and prevalence estimates mainly on self reported histories or diagnosis by a clinician, without the benefit of microscopy or culture. Because of lack of specificity of clinical signs and symptoms, many of the women reported the diagnosis may have other conditions. On the other hand, a positive vaginal culture for *Candida* may reflect colonization in as many as 50% of healthy asymptomatic women. So, all the culture positive cases should be correlated clinically and other causes of vaginal discharge should be ruled out if culture reveals mixed or scanty growth of *Candida* spp. In this study, isolation of *Candida* was12% and it ranked second only to bacterial vaginosis in patients of vaginal discharge.
Hng et al. (2009) conducted a study in women seeking abortion in Vietnam. They are at increased risk of developing RTI complications. Among the study subjects, 54% of the women were diagnosed as having an RTI, including 3.3% with sexually transmitted infections. Endogenous infections were more prevalent. The prevalence of vulvovaginal candidiasis was 34% and the principle infectious microorganism was *Candida albicans*.

### 2.2.2. Prevalence in India

Neerja et al. (2006) studied four hundred women of reproductive age group (14-45 years) attending the Obstetrics and Gynecology outpatient department (OPD), Government medical college, Amritsar with reproductive tract complaints. Out of 400 patients, positive culture for *Candida* spp was obtained from 92 (23%) subjects. Of these 92 subjects, 76 (82.6%) showed normal vaginal pH (4.0-5.0) and 16 (17.4%) had the pH in excess of 5. Direct microscopy for yeast cells and mycelia was found to be positive in 78 (84.8%). Species identification of 92 cultures positive for *Candida* showed that 64 (69.57%) were *C. albicans*, 8 (8.7%) were *C. glabrata*, 6 (6.55%) each in *C. tropicalis* and *C. krusei*, and 4 (4.3%) each in *C. parapsilosis* and *C. guillirmondi*. Among the subjects, 248 were pregnant and *Candida* culture positivity was 28.2%. This was significantly higher (P<0.0001) than the culture positivity (7.9%) shown by 152 nonpregnant women. Thirty five women had a history of taking broad spectrum antibiotics like Ampicillin, Cephalosporins, Aminoglycosides or Quinolones two or more times during the year of the study and of them 15 (42.8%) were found to be culture positive for *Candida* spp. This was significantly higher (P=0.0059) than that observed in 77 (21.1%) of the 365 women who did not have a history of taking antibiotics during the same period. Women with parity of more than one and those using oral contraceptives also showed significantly higher rate of *Candida* culture positivity than women with parity one or nil (P=0.0119) and women not using oral contraceptives. On examination, vaginal erythema was found to be more common in women with positive culture than in women with negative culture.

Patel et al. (2006) conducted a study in the Women population aged 18-45 years living in North Goa district. A SPSS computer programme was used to select
3000 women randomly from the sampling frame during November 2001- May 2003. Of the 3000 randomly selected women, 2494 (83.1%) consented to participate in the study. The majority of participants were Hindu (74.6%) and most of the remainders were Christian (22.2%). 356 (14.3%) participants were unable to read or write. The majority of participants were homemakers (66.7%). *Candida* was not associated with any of the gender determinants. Endogenous infections were relatively common (BV 17.8%; *Candida* 8.5%), and sexually transmitted infections (STI) such as *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Trichomonas vaginalis* were infrequent (4.2%). Factors indicative of poverty and marginalization were associated with STIs and BV. Gender disadvantage, particularly spousal violence, was associated with BV, while concern about a husband’s extramarital relationships, an indicator of sexual risk, was associated with STI. Husband’s discharge was strongly associated with STI and a non-white vaginal discharge was associated with both STI and BV. Condom use and oral contraceptive use were associated with a reduced risk of BV.

Shethwala *et al.* (2009) conducted a study in 300 female sex workers from three different regions of Surat city. The prevalence of Candidiasis was 10.33%. 35 patients were infected with HIV and 17.4% (6 subjects) had co-infection with *Candida*. Gonococcal infection was not found in any of the commercial sex workers of Surat city, India.

Jindal *et al.* (2010) reported that the prevalence of Candidiasis was 4%. The maximum number of positivity was observed in the age group of 25 years. Vaginal discharge was the commonest symptom in the study. Eleven percent (11%) of these females had bacterial vaginosis and 4% had vulvovaginal candidiasis. It is need to have cost-effective strategies for the early diagnosis and treatment of STIs and for their prevention through information, education and behavior change.

Saikia *et al.* (2009) conducted the prevalence studies in Assam. Of 479 individuals, 186 (38.8%) had evidence of STD and 70 were positive for HIV. Most (64%) were in the age group of 15-30 years. Candidiasis was the most common finding on clinical examination (21.5%). A large proportion of the respondents were belonged to low socioeconomic status and a majority of them had formal education. A
high proportion of married individuals gave a history of extramarital sexual contact. The authors suggested that migration due to profession, an increasing number of women taking up jobs outside home, decline in joint family and increase tolerance to antisocial activities might have contributed to this phenomenon. The alarming high proportion of extramarital sexual relation needs further evaluation and analysis. A higher prevalence of candidiasis and viral infection was identified when compared to bacterial infections.

Rathod et al. (2012) examined the incidence, prevalence and risk factors for vulvovaginal candidiasis among a cohort of 898 women in south India (Mysore). The 898 women participating in the study contributed a total of 2551 study visits, with 800 women (89%) attending all three study visits and another 53 women (6%) attending two visits. Candida was detected in 885/2528 (35%) vaginal specimens tested on culture. Of the 885, 180 (20%) satisfied the case definition for diagnosis of vulvovaginal candidiasis, while the remaining 705 (80%) were considered asymptomatic infection. The prevalence of vulvovaginal candidiasis declined over the three study visits from 77/893 (9%) at baseline to 65/840 at three months (8%) and 38/795 (5%) at six months. The loss of vaginal Lactobacilli is the hypothesized mediator for the relationship between the receipt of antibiotics and the risk of vulvovaginal candidiasis.

2.2.3. Prevalence in Tamil Nadu

A community-based cross sectional study of RTIs was conducted by Prasad et al. (2005) during 1996–1997 among married women of 16–22 years of age in Tamil Nadu, India. Fifty-three percent of women reported gynecologic symptoms, 38% had laboratory findings of RTIs and 14% had clinically diagnosed pelvic inflammatory disease or cervicitis. According to laboratory diagnoses, 15% had sexually transmitted infections and 28% had endogenous infections. The prevalence of vulvovaginal candidiasis was 10% (45 out of 451). Multivariate analysis found that women who worked as agricultural laborers had an elevated likelihood of having a sexually transmitted infection, as did those married five or more years. Two-thirds of symptomatic women had not sought any treatment, the reasons cited were absence of
a female provider in the nearby health care center, lack of privacy, distance from home, cost and a perception that their symptoms were normal. Young married women in this rural Indian community have a high prevalence of RTIs but seldom seek treatment. Education and outreach are needed to reduce the stigma, embarrassment and lack of knowledge related to RTIs.

2.3. Prevalence of *Neisseria gonorrhoeae*

2.3.1. Global prevalence

Kahn *et al.* (2005) reported the *N. gonorrhoeae* prevalence in US juvenile detention centers. The prevalence was found to be 5.1% in females and 1.3% in males. Gonorrhoea prevalence was four times higher in females than in males. In females prevalence was three times higher in blacks compared to non-blacks and did not vary significantly by age group.

Mondal *et al.* (2008) analyzed the sexual behavior and sexually transmitted diseases (STDs) of street-based female sex workers (SFSWs) of Rajshahi city, Bangladesh. The socio-demographic profiles were studied among the SFSWs attending three drop in centers (DIC) named PIACT, PROVA, and Suraksha Madhumita in Rajshahi. 150 self-motivated and willing individuals were interviewed through a structured questionnaire to obtain obstetric histories and socio-demographic information. Among those SFSWs, 56.7% were infected with two or more pathogens of STDs such as gonorrhoea, chlamydia, herpes, syphilis, and trichomoniasis. The prevalence of gonorrhoea was 23.3%. They found a strong association between the prevalence of STDs among SFSWs and their socio-demographic profiles. Education is the most consistent and important determinant for prevention of STDs among all classes of people. Literate SFSWs have a lower incidence of STDs than illiterate SFSWs. Less than 2% of literate SFSWs have gonorrhoea compared to 34% of illiterate SFSWs. Older SFSWs who spent very little money for health purposes, those who had larger numbers of children, and those who used condoms inconsistently were observed to be at higher risk of STDs.
Huq et al. (2010) reported the prevalence of bacterial sexually transmitted infections among clients of brothel-based female sex workers (FSWs) in Jessore, Bangladesh. In this study, they collected a total of 156 condoms from clients of 13 sex workers during three consecutive days. From the condoms used by the clients, DNA was extracted from the seminal fluid and used for the diagnosis of *Neisseria gonorrhoeae*, *Chlamydia trachomatis* and *Trichomonas vaginalis* by polymerase chain reaction. The prevalence of *N. gonorrhoeae*, *C. trachomatis* and *T. vaginalis* among clients of FSWs was 4.5% (7/156), 2.5% (4/156) and 7% (11/156) respectively. This study provides information regarding men who used a condom during their encounter with FSWs. However, clients who did not use a condom with FSWs are more likely to have STIs than those who do. In conclusion, the results of the pilot study indicate that more than 12% clients of FSWs were positive for one or more bacterial STIs. Further studies among clients of FSWs are essential to understand the STI disease burden and sexual risk behavior among clients of FSWs and to design effective interventions targeting the bridge population.

Shahcheraghi et al. (2010) conducted a study in Karaj, Rajaii, Shahr, Varamin and Ewin female prisoners attending the clinic of prisons. The prevalence of gonorrhoea was found to be less than 0.1%. In comparison with other countries the prevalence of gonorrhoea in Iranian female prisoners is low. The detection of *N. gonorrhoeae* in prisoners and other people is so difficult because of taking antibiotics without prescription of doctors, giving antibiotics to prisoners for preventing the prevalence of contagious infection, using contraceptive methods such as condom and refusing for visiting doctors because of social reluctance of such disease.

Gibney et al. (2002) conducted a cross sectional study at Tejgaon truck stand, Dhaka, Bangladesh. The study group comprised 388 men working in the truck industry (drivers and helpers). Slightly over half (54%) were single. Almost two thirds were truck drivers and one third were helpers on trucks. The prevalence of gonorrhoea was 2.1%. There were only two co-infections with bacterial STD; one person had syphilis, gonorrhoea, and chlamydia and one person had both syphilis and gonorrhoea. Eleven of the subjects with HSV-2 also had a bacterial STD. High risk sexual behaviours for STD were common among the subjects. Condom use was very
low, with approximately 73% never using, 5% using once, almost 20% using occasionally, and 3% always or most of the time. The majority (54%) of them acknowledged having sexual relations with a female commercial sex worker (CSW) in the past year, and almost 40% had three or more sex partners in the past year. While 21% reported having a type of sexual relation (a “physical release”) with a male partner in their lifetime, only 7% had done so in the past year. For the 80 subjects who had ever had a male partner, sexual activities included anal sex (95%), oral sex (4%) and mutual masturbation (26%). Male partners of those 80 subjects included a friend (47.5%), a family member (5%), a male sex worker (15%), a neighbor (28.8%), and others (2.5%). The use of illicit drugs that are ingested or inhaled (particularly ganja and phensidyl) was common. Alcohol consumption was also frequent, though to a lesser extent. Slightly over one third of the subjects reported having ever been diagnosed with an STD.

2.3.2. Prevalence in India

Sharma et al. (2003) analysed a total of 2526 women attending the antenatal clinic in the department of obstetrics and gynaecology, Chandigarh. The women were divided into eight groups as pregnant women, contraceptive advice seekers, contraceptive users, women with infertility, women with leucorrhea and women with pelvic inflammatory diseases. The overall prevalence of RTI in various groups was 1.8. The infections were most common in pregnant women when compared to other groups. However, surprisingly Neisseria gonorrhoeae was not found in any of the women.

Ray et al. (2006) carried out a study at a regional STD centre in North India for a period of 15 years. Totally 78,617 subjects attended the STD centre including the voluntary counseling and testing centre (VCTC). The occurrence of gonorrhoea was 13%. They concluded that there was a gradual decline in the occurrence of new STI cases, a common observation in all government health facilities. The patients probably reported more to the private practitioners, expecting more confidentiality in dealing with these diseases. In the present study, most of the STI cases belonged to 21-30 years of age group. This is the sexually active group and at a high risk of being
behaviorally more vulnerable to STI acquisition. They generally have high number of sexual partners and more concurrent partnerships and changing partners more often among them than old age groups.

Shilpee et al. (2008) conducted a study in STI clinic in Delhi. The prevalence of gonococcal infection was 13%. Among them, 30% (4/13) of patients were found to be co-infected with HIV, Chlamydia and HBV. All these patients had recurrent episodes of gonococcal infections, although clinical manifestation was same as that of patients with gonococcal infection alone. Such recurrence of gonococcal infection in patients with multiple STIs highlights the vulnerability of such population in acquiring STIs and eventually becoming reservoir of drug-resistant organisms.

Bala et al. (2011) found that gonorrhoea rates in the present study dropped from 12.1% in 2004 to 6.4% in 2007. This drop may either be due to actual decreasing rate of gonorrhoea over the years or due to the fact that gradually less number of patients were reporting to the clinic because of easy availability of antimicrobials as a part of syndromic management of STIs in peripheral and private health setups. The maximum cases were observed in 21-30 year age group who were mostly unmarried and hence more prone to visit commercial sex workers. The data support earlier consensus that young adults and adolescents should constitute priority target group in STD control programme.

Das et al. (2011) conducted a study in Hyderabad and Mumbai from October 2008- May 2009 in female sex workers (FSWs). In their study, a maximum of 74.6% of participants had clinical symptoms or signs of STIs. The prevalence of Neisseria gonorrhoeae was found to be 14.1%. One in every four sex workers attending clinic services had laboratory confirmed cervical infection. Additionally there was a high prevalence of vaginal infections. The most common syndromes were vaginal discharge (78.5%) and lower abdominal pain (18.7%). Only 12 participants had genital ulcer disease syndrome. There was no variability in STI prevalence between street-based and home-based sex workers.
2.3.3. Prevalence in Tamil Nadu

Kumarasamy et al. (2008) determine the prevalence and incidence of STIs among South Indian men and women identified to be at increased risk for HIV infection. A total of 480 subjects at increased risk for HIV infection in a study carried out during August 2002 and December 2003 in Chennai, India. The most common prevalent STIs were Herpes simplex virus (HSV)-2, syphilis, and Trichomonas vaginalis. The prevalence of Neisseria gonorrhoeae was 2.0%. Among the study subjects, enrolled women participants with no schooling, participants with greater than four sex partners, and single participants (unmarried) were found to be at increased risk for HSV-2 infection. With the high incidence of STIs, prevention targeted on clinical management strategies among individuals practicing high risk behaviors may help to slow the continued spread of HIV in India. Due to the documented synergy between HIV and STIs, it has been suggested that the rapid diagnosis and treatment of STIs could serve as a cost-effective HIV prevention strategy. Additionally, sexual histories and partner management should be a standard component of clinical care at STI clinics and centers providing voluntary counseling and testing (VCT) services for HIV. They concluded that the prevalence rates of certain STIs, in this study, may be high enough to warrant universal screening.

2.4. Risk factors associated with reproductive tract infection

2.4.1. Candidiasis

Enweani et al. (2001) studied the effect of contraceptives on the prevalence of vaginal colonization with Candida spp in Edo state, Nigeria. High vaginal swabs were obtained from 500 volunteers who comprised 394 contraceptive users and 106 non-contraceptive users. The prevalence of Candida spp was 51.5% in contraceptive users and 40.6% in non-contraceptive users. There was a significant relationship between the type of contraceptive used and the prevalence of vaginal colonization. Age and marital status of the subjects sampled had significant relationship with the prevalence of vaginal colonization.
Reed et al. (2003) conducted a prospective cohort study includes 148 women with Candidal vulvovaginitis and 78 of their male sexual partners was conducted at two primary care practices in the Ann Arbor, Michigan, area. Thirty-three of 148 women developed at least one further episode of Candida albicans vulvovaginitis within 1 year of follow-up. Cultures of Candida spp from various sites of the woman (tongue, feces, vulva, and vagina) and from her partner (tongue, feces, urine, and semen) did not predict recurrences. Female factors associated with recurrence included recent masturbating with saliva (hazard ratio 2.66 [95% CI 1.17–6.06]) or cunnilingus (hazard ratio 2.94 [95% CI 1.12–7.68]) and ingestion of two or more servings of bread per day (p # 0.05). Male factors associated with recurrences in the woman included history of the male masturbating with saliva in the previous month (hazard ratio 3.68 [95% CI 1.24–10.87]) and lower age at first intercourse (hazard ratio 0.83 [95% CI 0.71–0.96]). Sexual behaviors, rather than the presence of Candida spp at various body locations of the male partner, are associated with recurrences of C. albicans vulvovaginitis.

Yusuf et al. (2007) evaluate the effect of contraceptives on prevalence of Candida spp on vaginal Candidiasis in Dhaka, Bangladesh. Among 350 women studied, Candida spp were isolated from 172 (49.1%) cases. From the total of 247 women of contraceptive users, Candida spp were isolated in 140 (56.7%) cases compared to 32 (31.1%) from 103 contraceptive users. Out of 172 Candida spp isolated, Candida albicans was the most frequently isolated pathogen. Among the women using contraceptives, the highest prevalence of 69.4% was observed in oral pill users as compared to those using injectables and IUCD in 12.2% and 2.1% respectively. This revealed that oral contraceptive pill was the most influencing contraceptives that cause vaginal candidiasis.

Xu et al. (2008) conducted a pilot study in a family medicine office including nonpregnant women aged 18 to 64 years who required >3 days oral antibiotics for nongynecological diseases. Age-matched (_5 years) women seen in the same clinic for noninfectious problems were recruited as controls. Eighty (44 in antibiotic group) women were recruited, 14 of 79 had asymptomatic vaginal Candida cultures positive at baseline. During follow-up, 10 of 27 women in antibiotic group were Candida
culture positive. In contrast, 3 of 27 women in the control group were *Candida* culture positive. The women in antibiotic group developed symptomatic VVC whereas none of the women in the control group developed vaginal symptoms. Approximately 22% of women developed symptomatic VVC during the 4 to 6 weeks after antibiotic therapy. They concluded that, the use of short courses of oral antibiotics seems to increase the prevalence of asymptomatic vaginal *Candida* colonization and incidence of symptomatic VVC.

Oviasogie *et al.* (2009) examined genital samples from pregnant women to determine the *Candida* spp and its predisposing factors. A total of 147 women (87 volunteer asymptomatic pregnant women and 60 asymptomatic non pregnant women) were examined. High Vaginal Swabs (HVS) were collected in pairs and demographic data (age of individual, age of pregnancy, occupation and use of contraceptive pill) on each subject were obtained alongside. Results revealed five *Candida* spp (*C. albicans, C. glabrata, C. tropicalis, C. stellatoidea, C. parapsilosis*) with 61.5, 17.9, 7.7, 5.1, 2.6 frequency of isolation respectively as against 73.7, 7.7, 1.4, 11.3, and 0.0 for control. Women in the second trimester of pregnancy had the highest occurrence (68.8 %) while the age groups 24- 30 and 31-37 years had the highest occurrence in the control (46.2 %) and in the pregnant ones (51.7 %) respectively. Traders had 68.8% and full-time housewives had 26.7 % of occurrence. More women who used contraceptives had *Candida* (58.3) as against non contraceptive users (35.3). The results reports that there are high rates of colonization of the vagina of pregnant women by *Candida*.

Akpan *et al.* (2011) investigating the effect of tight fitting underwear on the incidence of vulvovaginal candidiasis among non-pregnant women between aged 16-35 years. The study took place in seven health institutions between January and July 2009 in Uyo Metropolis, Nigeria. About 191(76.4%) of the 250 participants who were selected by sequential randomization met the inclusion criteria. Structured questionnaire were used to assess their regular patterns of dressing for the past one year and associated gynecologic problems. Vaginal smear and swabs were taken from each participant for microscopy and culture. High incidences of vulvovaginal candidiasis (76.8%) with its associated symptoms were observed among women who
regularly wore cotton tight/cotton underwear/pants (42.9%). It can be concluded that women who predominantly wear nylon tight and other synthetic underwear/pants are at a higher risk of vulvovaginal candidiasis.

2.4.2. Gonorrhoea

Workowski et al. (2008) suggested that essential and well-established component of gonorrhoea control is the screening of populations at risk for infection, because gonococcal infections among women are frequently asymptomatic, so screening of women at high risk is essential to detect reservoirs of infection. The U.S. Preventive Services Task Force recommends screening of *N. gonorrhoeae* in sexually active women (including pregnant women) and women at increased risk for infection. The most common risk factors of gonorrhoea includes age younger than 25 years, previous gonorrhoea infection or other STDs, new or multiple sex partners, inconsistent condom use, commercial sex work and drug use. Screening low-risk women and men is not recommended. Routine screening for gonorrhea and other common STDs is also recommended for sexually active men who have sex with men, including those with HIV infection.

Gazi et al. (2008) conducted a study among 250 brothel based sex workers (SWs) from June to November 2006 in Manisa and Izmir cities which are located in the Aegian region of Western Turkey. The study determines the prevalence of *C. trachomatis/N. gonorrhoeae* and HPV and to assess HIV/AIDS related knowledge, attitudes, behaviour and sexual practices among brothel based sex-workers. The prevalence of *C. trachomatis /N. gonorrhoeae* and HPV among sex-workers were 18.6% (37/187) and 9.7% (12/124) respectively. More than half of them had been working in this profession for more than 10 years and had very little education. They were either illiterate or primary-school graduates (77.9%). Only 62% of the women reported that they always used condom with clients. On the other hand, 29.1% of them used condoms frequently and only 8.5% occasionally used condoms. Subjects indicated that intercourse with a repeat/regular partner was the main reason for not using condom. Age or educational levels of SWs were not associated with use of
condoms. Surprisingly the overall knowledge on blood and sexual transmission of HIV/AIDS was high.

2.5. Socio-demographic characteristics and symptoms associated with RTIs

Jayandra et al. (2005) conducted a study amongst the commercial sex workers of red light area (RLA) of Surat. Among the study subjects, 80.5% of them had never attended a school. Except 3, all of the CSWs reported that they were married; however only three were living with their husband. 94.9% of them reported the use of condom all the times by them. 90% of them had more than two sexual partners (clients) per day. The range of number of different partners per day varied from 2 to 25, the mean being 5 clients per day. Lower abdominal pain was most common complaint (19.5%) followed by vaginal discharge (12.7). On external genital examination 52 (44.1%) CSWs had one or more clinical sign suggestive of STD.

Rao et al. (2006) surveyed ten villages having more than 90% tribal population of Jabalpur. The subjects were randomly selected. The sample size of 2100 was estimated for coverage. Married women aged 15 to 49 years were included in the study. A total of 2206 women from ten tribal villages were screened for the presence of various STDs. Their mean age was 30.7 years. Majority (72%) of the women were illiterate, while about 20% had received primary education. More than 75% women got married before the age of 18 years. A total of 172 women (7.8%) had at least one complaint suggesting RTI. The commonest complaint was vaginal discharge, followed by itching, dysmenorrhea, menorrhagia, irregular periods, burning micturition and dyspareunia. One hundred and seventy two women had at least one symptom suggesting RTI giving a community prevalence of 7.8%.

Panda et al. (2007) reported the prevalence of RTI/STI which was found to be 39.2% with a higher rural (44%) than urban (32%). Almost half of the symptom positive women were of 25-34 years of age. The commonest symptom of RTI/STD was vaginal discharge (91%) followed by lower abdominal pain (64%). Other common associated symptoms were backache (76%), vulval itching (51%) and burning during urination (34%).
Sharma and Gupta (2009) conducted a study with the objective of assessing the prevalence of various RTIs among married women in the reproductive age group of 15-44 years old in a rural area of the district of Sirmour (H.P.) during December 2001–March 2002. A total of 452 women were interviewed and 235 of them were found to be suffering from RTIs giving a prevalence of 51.9%. The trend in relation to age showed maximum prevalence in the 25–34 year old age group (63.6%). Divorcees and widows (n = 7) who were in small number did not report any non-marital sexual relationships and the prevalence of RTIs was less (28.6%) as compared with married women (52.4%). The prevalence of RTIs was found to be higher in illiterate women (72.2%) and it showed a decreasing trend with an increase in the level of education.

Jombo et al. (2010) carried out the study at the Microbiology laboratory of the Jos University Teaching Hospital (JUTH) Jos, Plateau state North central Nigeria. Vulvovaginal candidiasis is a common clinical finding among women especially the sexually active group, even though there has been a sustained increase in both the variety and potency of antifungal drugs over the past three decades. The study was therefore set up to ascertain the prevalence of vaginal candidiasis among women in Jos. Data generated from analysis of endocervical and high vaginal swab (ECS/HVS) specimens by the Microbiology laboratory of Jos University Teaching Hospital (JUTH) for a period of five years (July 1999 - June 2004) was compiled. Samples were collected, transported, stored and processed using standard laboratory procedures. Additional information was obtained from patients’ case notes in the records department. Results were analyzed using Epi Info 6 statistical software. The prevalence of Candida infection was found to be 29.1% (n = 2458); no isolate was recovered from those less than 10 years of age, while the peak age group of infection was 30 - 39 years 11.8% (n = 997); the age-group 20 - 49 years accounted for over 25% of the entire infections. Common clinical manifestations were no symptoms (24.7%), itching and rashes (29.4%). Due to the importance of the results, sex education workshops should be conducted for adolescents and young adults in order to educate them on the clinical importance of Candida infections.

Rabiu et al. (2010) conducted a descriptive cross sectional survey of 500 women attending the gynaecological outpatient and family planning clinics of the
Lagos State University Teaching Hospital was carried out between 1st June 2008 and 31st August 2008 using a pre-tested questionnaire. Gonorrhoea was the commonest RTI with the prevalence of 23.4% followed by syphilis (16%) and *Candida* (9.9%). Of all respondents, 57.7% chose vaginal discharge as a symptom of RTIs, 54.0% had vaginal itching and 32.4% had lower abdominal pain. Most of the respondents (77.2%) had heard of RTI’s. Toilet was the most perceived mode of contracting RTI’s (44.6%), followed by sexual intercourse and poor hygiene. Vaginal discharge was the commonest symptom of RTI’s named while inability to get pregnant was the commonest named complication. Majority of the respondent’s demonstrated poor overall knowledge of symptoms and complications of RTI’s. 37.4% of the respondents had experienced symptoms of RTI’s in the preceding six months. Vaginal discharge was the commonest symptom reported (21.8%) and the majority of those who reported symptoms sought medical treatment. Government health centers were the most visited health facilities for treatment. Even though most of the respondents have heard of RTI’s and sought treatment when symptomatic, they demonstrated poor overall understanding of the subject. There is need to educate women on preventive strategies, as RTI’s are often asymptomatic.

2.6. Virulence factors influencing the pathogenesis of microbes

2.6.1. Virulence factors of *Candida* spp.

Basu *et al.* (2003) recovered eighty-five isolates of *Candida* from 350 diverse clinical sources, viz. respiratory tract (sputum, bronchial washing, bronchoalveolar lavage, tracheal aspirate), blood, urine, high vaginal swab, skin and plastic devices, were studied in detail for their morphological and biochemical characters. Seven species of *Candida* were identified, viz., *C. albicans* (45.8%), *C. tropicalis* (24.7%), *C. parapsilosis* (10.5%), *C. krusei* (7.0%), *C. kefyr* (7.0%), *C. guilliermondii* (3.5%), and *C. glabrata* (1.1%). *C. albicans* was the predominant species isolated from all clinical specimens, except blood from which *C. krusei* was most frequently (38.4%) recovered. Out of 39 isolates of *C. albicans*, 26 (66.6%) and 19 (48.7%) exhibited strong proteinase and phospholipase activity respectively. There was a higher prevalence of proteinase producing strains amongst the vaginal and skin isolates than
that in urinary and respiratory isolates. Also a greater number of phospholipase producing strains was observed in the vaginal and urinary isolates than that in the respiratory and skin isolates.

Al-Fattani and Douglas (2006) made the chemical analysis for the matrix material which was extracted from biofilms of *C. albicans* and *C. tropicalis* and analysed chemically. Both preparations contained carbohydrate, protein, hexosamine, phosphorus and uronic acid. However, the major component in *C. albicans* matrix was glucose (32%), whereas in *C. tropicalis* matrix it was hexosamine (27%). Biofilms of *C. albicans* were more easily detached from plastic surfaces by treatment with the enzyme lyticase (β-1, 3-glucanase) than were those of *C. tropicalis*. Biofilms of *C. albicans* were also partially detached by treatment with proteinase K, chitinase, DNase I, or β-N-acetylglucosaminidase, whereas *C. tropicalis* biofilms were only affected by lipase type VII or chitinase. To investigate a possible role for the matrix in biofilm resistance to antifungal agents, biofilms of *C. albicans* were grown under conditions of continuous flow in a modified Robbins device (MRD). These biofilms produced more matrix material than those grown statically, and were significantly more resistant to Amphotericin B. Biofilms of *C. tropicalis* synthesized large amounts of matrix material even when grown statically and such biofilms were completely resistant to both Amphotericin B and Fluconazole. Mixed-species biofilms of *C. albicans* and a slime-producing strain of *Staphylococcus epidermidis* (RP62A), when grown statically or in the MRD, were also completely resistant to Amphotericin B and Fluconazole. Mixed-species biofilms of *C. albicans* and a slime-negative mutant of *S. epidermidis* (M7) were completely drug resistant only when grown under flow conditions. These results demonstrate that the matrix can make a significant contribution to drug resistance in *Candida* biofilms, especially under conditions similar to those found in catheter infections in vivo, and that the composition of the matrix material is an important determinant in resistance.

Shinobu et al. (2007) demonstrated that classical factors, like protease, phospholipase haemolytic activity and susceptibility to H$_2$O$_2$, do not have predominance in a specific genotype in relation to the virulence factors studied in vaginal secretion isolates of *C. albicans*. However, protease, phospholipase and
haemolytic activity were highly expressed, suggesting that these virulence factors are important in vaginal isolates. Generally, the different isolates expressed similar virulence potential, reinforcing the necessity for new studies and also that other factors related to the yeasts and the host such as adherence capacity must be involved in the development of the disease.

Das et al. (2008) revealed that in order to establish an infection, opportunistic pathogens have 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. They obtained one hundred and fourteen isolates of Candida spp from the samples and the isolates include 37 Candida albicans, 7 Candida glabrata, 5 Candida guilliermondii, 3 Candida kefyr, 45 Candida krusei, 5 Candida parapsilosis and 12 Candida tropicalis. Proteinase and Phospholipase assay was performed and the precipitation zone (Pz value) was determined. The percentage of isolates which produced detectable amounts of proteinase is 74.56% and isolates produced detectable amounts of phospholipase is 44.73%. They believe that production of both phospholipase and proteinase enzymes could be an important virulence factor for several Candida spp.

Karkowska-Kuleta et al. (2009) focused on major virulence factors of the most common fungal pathogens of humans Candida albicans, Aspergillus fumigatus and Cryptococcus neoformans. They concluded that the adherence to host cells and tissues, secretion of hydrolytic enzymes, phenotypic switching and morphological dimorphism were contributed to C. albicans virulence. At present, the majority of pathogenic fungi are susceptible to conventional antifungal treatment, but an increasing resistance to some antifungal drugs is a new, important problem in medicine. A way to avoid developing resistance is to use multidrug therapy or different antifungal agents, as well as to limit too frequent and uncontrolled usage of the newest category of drugs. It is also necessary to recognize the mechanism of pathogen–host interactions at the molecular level in order either to prevent the infection or to develop new strategies for therapy and new effective antifungal drugs.
Gil et al. (2010) were isolated 11 vaginal lactobacilli (LAB) isolates obtained from healthy patients to screen microorganisms with probiotic properties against Candida spp. The LAB were tested for their ability of auto-aggregation, coaggregation with C. albicans, C. glabrata, C. krusei, and C. tropicalis, adhesion to Caco-2 epithelial cells and production of lactic acid and hydrogen peroxide (H₂O₂). All lactobacilli isolates tested were able to autoaggregate (ranging from 25.3% to 75.4% assessed at 4 hours of incubation) and to co-aggregate with the four Candida spp into different degrees. Among them, L. crispatus showed the highest scores of coaggregation. The highest amount of lactic acid was produced by L. salivarius (13.9 g/l) followed by L. johnsonii (6.5 g/l), L. acidophilus (5.5 g/l) and L. jensenii (5.4 g/l). All isolates produced H₂O₂ but the highest levels (3 - 10 mg/l) were observed for L. acidophilus, L. crispatus, L. gasseri, L. johnsonii, and L. vaginalis. Only L. agilis, L. jensenii, L. johnsonii and L. ruminus were able to adhere to epithelial Caco-2 cells. Among the isolates evaluated, L. agilis, L. jensenii, L. johnsonii, and L. ruminus exhibited simultaneously several desirable properties as potential probiotic strains justifying future studies are encouraged to assess technological properties of those microorganisms for clinical use, including determination of their viability and stability in pharmaceutical preparations such as capsules resistant to gastrointestinal tract for oral intake and ovules/capsules for intravaginal administration.

Wang et al. (2010) developed an efficient computational framework, which integrates different kinds of data from genome-scale analysis, for global screening of potential transcription factors (TFs) controlling C. albicans biofilm formation. S. cerevisiae information and ortholog data were used to infer the possible TF-gene regulatory associations in C. albicans. Based on TF-gene regulatory associations and gene expression profiles, a stochastic dynamic model was employed to reconstruct the gene regulatory networks of C. albicans biofilm and planktonic cells. The two networks were then compared and a score of relevance value (RV) was proposed to determine and assign the quantity of correlation of each potential TF with biofilm formation. A total of twenty-three TFs are identified to be related to the biofilm formation, ten of them are previously reported by literature evidences. The results
indicate that the proposed screening method can successfully identify most known biofilm-related TFs and also identify many others that have not been previously reported. Together, this method can be employed as a pre-experiment screening approach that reveals new target genes for further characterization to understand the regulatory mechanisms in biofilm formation, which can serve as the starting point for therapeutic intervention of \emph{C. albicans} infections.

2.6.2. Virulence factors of \emph{Neisseria gonorrhoea}

\emph{Zhu et al.} (2002) stated that lipooligosaccharide (LOS) is a major virulence factor of the pathogenic \emph{Neisseria}. Nine \emph{lgt} genes at three chromosomal loci (\emph{lgt-1, 2, 3}) encoding the glycosyltransferases responsible for the biosynthesis of LOS oligosaccharide chains were examined in 51 \emph{N. gonorrhoeae} strains. DNA hybridization, PCR and nucleotide sequence data were compared to previously reported \emph{lgt} genes. Authors concluded that \emph{Neisseria} shares a common \emph{lgt} gene pool for biosynthesis of LOS. The \emph{lgt-1} and \emph{lgt-3} loci are hypervariable genomic regions in \emph{N. meningitidis}, whereas \emph{lgt-2} is conserved in both pathogenic \emph{Neisseria} spp. The typical polymorphisms of \emph{lgt} appear to have arisen through horizontal gene transfer and homologous recombination in addition to mutation events. This study about genetic diversity of the \emph{lgt} loci provides fundamental information for understanding the heterogeneity and antigenic variation of \emph{Neisseria} LOS.

\emph{Edwards and Apicella} (2004) explained that pili play a critical role in forming an initial attachment with host cells. Pili may also provide pathogenic mechanism by which non-motile gonococci are able to colonize and to ascend mucosal surfaces. Opa proteins are thought to contribute to the cellular tropisms exhibited by gonococci and are divided into two classes based on their ability to differentially recognize host cell surface molecules. The interaction of Opa with a target host cell may be modulated by specific LOS moieties expressed by a gonococcus. The oligosaccharide substitutions of LOS exhibit both inter and intrastrain variability. Interconversion of LOS oligosaccharides occurs spontaneously and is dependent upon the presence or absence of available substrates and enzymes involved in LOS biosynthesis. 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 presence of sialic acid on gonococcal LOS confers unstable resistance to the bactericidal action of normal human serum. Porin molecules trigger variable functional responses within host cells depending upon the particular porin and the host cell type under study. A unique feature ascribed to gonococcal porin is its ability to translocate into eukaryotic cell membranes, where it forms a voltage-gated channel that is modulated by host cell ATP and GTP.

2.7. Drug resistance in *Candida* spp.

2.7.1. Global Scenario

Richter *et al.* (2005) collected 564 vaginal yeast cultures from 429 vulvovaginitis clinic patients with signs and symptoms suggestive of candidal vulvovaginitis from January 1998 to March 2001 in University of Iowa. Antifungals and concentrations tested were Fluconazole (0.12 to 128 g/ml), Flucytosine (0.06 to 64 g/ml), Nystatin (0.06 to 64 g/ml), Itraconazole, Econazole, Clotrimazole, Miconazole, and Ketoconazole (all 0.007 to 8 g/ml). 420 *C. albicans*, 112 *Candida glabrata*, 30 *C.parapsilosis*, 12 *C.krusei*, 8 *Candida tropicalis* and 1 *Candida lusitaniae* were used to determine the antibiotic susceptibility test. Susceptibility test results for the 593 isolates revealed that resistance to Fluconazole (3.7%) or Flucytosine (3.0%) was infrequent. Resistance to itraconazole occurred among 16.2% of the isolates. The imidazoles were active against 94.3 to 98.5% of the isolates (MIC<sub>1</sub> g/ml). Nystatin MICs ranged from 1 to 16 g/ml, with a MIC inhibiting 90% of isolates (MIC<sub>90</sub>) of 4 g/ml. Fluconazole resistance (MIC<sub>64</sub> g/ml) was observed only among *C.glabrata* isolates (15.2%) and *C.krusei* isolates (41.7%; all *C.krusei* isolates should be reported as fluconazole resistant per NCCLS guidelines). Fluconazole susceptible-dose dependent (MIC, 16 to 32 g/ml) isolates were found among *C. glabrata* (51.8%), *C.krusei* (50%), *S.cerevisiae* (11.1%), and *C.parapsilosis* (3.3%). Itraconazole resistance was observed among *C.glabrata* (74.1%), *C.parapsilosis* (3.3%), *C. krusei* (58.3%), and *S. cerevisiae* (55.6%) isolates. Flucytosine resistance was noted among *C. albicans* (3.3%), *C. krusei* (8.3%), *C. tropicalis* (12.5%), *C. lusitaniae* (100%), and *Trichosporon* sp. (100%).
Rizvi et al. (2011) conducted a study in Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, over a period of two years. The cases selected for the study included symptomatic high-risk patients in whom no other aerobic pathogen was identified, from those *Candida* spp. were isolated repeatedly in pure culture, and those in which the infections were associated with clinical signs and symptoms. Seventy-six isolates of *C. albicans* from various clinical specimens were obtained from a total of 714 patients. A prevalence rate of *C. albicans* was 10.6%. The antifungal susceptibility pattern of the isolates showed that all (100%) isolates were sensitive to Nystatin, Amphotericin-B and Voriconazole in the concentrations tested. Fluconazole resistance was observed in 15 (19.8%) isolates. The MIC values of Fluconazole for the isolates ranged from 32 μg/ml (7%) to >64 μg/ml (93%). On the other hand, all (100%) isolates were sensitive to <0.06 μg/ml of Voriconazole. SDS-PAGE analysis of various isolates revealed at least 6 types of banding patterns, with the number of bands ranging from 5 to 18. The maximum number of isolates (34.2%) had a banding pattern with seven bands. Proteins with molecular weight of 22.5 kDa, 44.0 kDa, 60.5 kDa and 117.5 kDa were consistently present in the Fluconazole resistant isolates. The dendrogram of *Candida albicans* isolates showed that all the 15 Fluconazole-resistant isolates were grouped in two closely related clusters. The clusters of Fluconazole-resistant isolates were significantly unrelated to that of the Fluconazole-susceptible isolates. It was also seen that isolates from a common site tended to fall close together on cluster analysis.

Vandeputte et al. (2012) stated that antifungal drug resistance appears to be due to point mutations in either drug targets or transcription factors regulating actors of the resistance. In the near future high through put diagnostic tools could be used in the course of treatment of fungal infections in order to detect resistance and adjust therapeutic strategies accordingly before any clinical evidence and therefore allow a rapid adjustment of the antifungal treatment. The recent application of genome-wide studies to pathogenic fungi for both host-pathogen interactions and the biological study will hopefully encourage and facilitate the development of new effective therapeutic strategies. Such improvements in antifungal treatment may lead to a better clinical outcome.
2.7.2. Indian scenario

Mohanty et al. (2007) conducted a study in rural North India from May 2003 to April 2004. In recent years, there has been a significant increase in infections caused by non-albicans spp of Candida, particularly C. glabrata and C. tropicalis. We speculate this increasing detection of non-albicans Candida spp that is probably related to the widespread and inappropriate use of antimycotic treatments (self-medication, long-term maintenance treatments and repeated treatments for candidosis episodes). They concluded that majority of C. albicans isolates were susceptible to Fluconazole, its use may be continued for empirical therapy of uncomplicated candidal vulvovaginitis in the community.

2.8. Drug resistance in Neisseria gonorrhoea

Oladele et al. (2011) collected samples of 262 high vaginal swabs from patients attending Ogun State College of Health Technology, Ilese-Ijebu. The samples were screened for sexually transmitted diseases (STDs) and antimicrobial susceptibility pattern of the isolated pathogens. The prevalence of Neisseria gonorrhoeae was 0.38%. Age group 21 to 25 had the highest occurrence of pathogens. About 81.8% of the pathogens isolated showed resistance to five or more antibiotics while 6.81% showed resistance to one antibiotic. N. gonorrhoea had MIC ranging from 16 to 512 μg/ml when tested against Tetracycline, Ampiclause, Ampicillin, Gentamycin and Augmentin. N. gonorrhea had MBC of 32 μg/ml for Tetracycline and 512 μg/ml for Ampicillin, Ampiclause and Gentamycin. Most of the pathogens isolated in this study were multidrug resistant to antibiotics. Antibiotic resistance could be due to a gene transferred between bacteria in a horizontal fashion by conjugation, transduction or by transformation. Therefore, a gene for antibiotic resistance which had evolved from natural selection may be due to many antibiotic resistance genes reside on Plasmid that facilitate their transfer.

2.8.1. Global Scenario

Lawung et al. (2005) isolated 207 isolates of N. gonorrohea in Bangrak hospital. Among the 207 isolates, only 22 (10.6) isolates were susceptible to all antimicrobial agents including Penicillin, Ceftriaxone, Norfloxacin, Ofloxacin,
Ciprofloxacin and Spectinomycin. On the other hand, 148 (71.5%) isolates were resistant to Penicillin and all were positive for β-lactamase production. Their findings reveal the significant increase on the prevalence of multiple resistance determinants (Penicillin and Quinolones) coexisting in a single isolate. Quinolone resistance gene cannot be co-transferred with Ampicillin resistance plasmid to E.coli. In general Quinolone resistance involves chromosomal mutation that modifies the target DNA gyrase or accelerates the efflux to decrease Quinolone accumulation.

Farhi et al. (2009) conducted a study to detect the antimicrobial susceptibility of Neisseria gonorrhoeae in a venereology clinic in Paris between 2005 and 2007. They also detected the factors associated with Quinolone-resistant N. gonorrhoeae. Susceptibility of N. gonorrhoeae to five antibiotics was tested systematically. The rate of Quinolone resistant N. gonorrhoeae was 37.4% without significant association with gender, age, sexual behavior, past history of sexually transmitted diseases and susceptibility to other antibiotics. All N. gonorrhoeae were susceptible to Ceftriaxone and Spectinomycin.

Bhargava et al. (2010) detected 60% of Penicillin resistance in N. gonorrhoeae isolates isolated in Birgunj, Nepal. The isolation rate of penicillinase producing N. gonorrhoeae (PPNG) was 53.3% in the study and was higher than that reported in Tribhuvan university teaching hospital. This may be attributed to the lower socio-economic status and insufficient awareness among the people in the outskirts as compared to the capital of the country leading to indiscriminate use of antibiotics and delay in seeking medical treatment. The higher number of Penicillin resistant isolates was recovered from the age group 31-40 years when compared to other age group.

Kubanova et al. (2010) demonstrated that the level of resistance of N. gonorrhoeae to all antimicrobials used in the traditional gonorrhoea treatment (Penicillins, Tetracycline and Ciprofloxacin) is exceedingly high in Russia. However, between 2005 and 2008, all Russian isolates were susceptible to Ceftriaxone and still no gonorrhoea treatment failures were occur using Ceftriaxone of appropriate quality and dosage has been described for urogenital gonorrhoea worldwide. Nevertheless, in
Russia and in many other countries the MIC values of Ceftriaxone have increased. Resistance to azithromycin was also identified, dispersed in five of the seven FDs (range: 3.4% to 21% during 2007 and 2008), which may reflect the fact that Azithromycin is commonly used in these FDs. The resistance to Spectinomycin, which had not been identified in Russia in 2005 and is rare internationally, also increased during 2007 and 2008 (0.9%-7.2%), which may reflect a frequent use In conclusion, the present national RU-GASP survey emphasises that the antimicrobial resistance of \textit{N. gonorrhoeae} across Russia is exceedingly high and Ceftriaxone should be the first-line antimicrobial for gonorrhoea treatment. If there is no access to Ceftriaxone or in cases of severe beta-lactam antimicrobial allergy, Spectinomycin should be used.

Bokaeian \textit{et al.} (2011) demonstrated that gonococcal strains in the study revealed multi-resistance and could withstand atleast three classes of antibiotics totally or partially. In Zaheden, massive number of gonococcal strains which are quite resistant against Penicillin, Ciprofloxacin, Co-trimoxazole and Tetracycline were isolated. At the same time other antibiotics including Ceftriaxone, Spectinomycin and Cefexim are absolutely effective for treatment of gonorrhoea. 79% of the isolates showed resistance to Penicillin and among the resistant isolates 83.1% of the isolates produced penicillinase enzyme.

2.8.2. Indian Scenario

Bala \textit{et al.} (2007) observed a significant increasing trend of Penicillin and Ciprofloxacin resistance up to 2003 and 2004 respectively and subsequent decrease in resistant strains with a concomitant increase in less susceptible strains was observed. Tetracycline-resistant \textit{N. gonorrhoeae} increased significantly from 6.7% in 2002 to 22.9% in 2005. Only one isolate was resistant to Spectinomycin and nine isolates were less susceptible to Ceftriaxone during this 5 year period. Substantial proportions (23.3%) of strains were multiresistant.
Bala and Sood (2010) investigated the emergence and spread of Cephalosporin resistant and multidrug resistant *N. gonorrhoeae* strains, represents a worrying trend that requires monitoring and investigation. Routine clinical laboratories need to be vigilant for the detection of such strains and strategies for control and prevention could be reviewed and revised from time to time. It will be important to elucidate the genetic mechanisms responsible for decreased susceptibility and future resistance. There is also an urgent need for research of safe, alternative anti-gonococcal compounds that can be administered orally and have effective potency, allowing high therapeutic efficacy.

### 2.9. Molecular characteristics of drug resistance in *Candida* spp

Chong *et al.* (2003) were compare the genetic relatedness of sequential and single isolates of *Candida* strains from women with recurrent vaginal candidiasis (RVC) and *Candida* strains from women who had only one episode of infection within a 1-year period. In total, 87 isolates from 71 patients were cultured, speciated and genotyped by random amplification of polymorphic DNA (RAPD) analysis. Patients were categorized into three groups, namely those with a history of RVC from whom two or more yeast isolates were obtained (group A), a history of RVC from whom only a single isolate was obtained (group B) and a single episode of vaginal candidiasis within a 1-year period (group C). Six yeast species were detected: *Candida albicans*, *Candida glabrata*, *Candida lusitaniae*, *Candida famata*, *Candida krusei* and *Candida parapsilosis*. Interestingly, the prevalence of non-albicans spp was higher in group A patients (50 %) than in patients in groups B (36 %) or C (18·9 %). Eighty RAPD profiles were observed, with a total of 61 polymorphic PCR fragments of distinct sizes. Clustering analysis showed that, overall, the majority of patients in group A had recurrent infections caused by highly similar, but not identical, sequential strains [mean pairwise similarity coefficient (SAB) $\frac{1}{4} 0·721 \pm 0·308$]. The range of mean SAB values for intergroup comparisons for *C. albicans* isolates alone was 0·50–0·56, suggesting that there was no significant relatedness between strains from different groups. Genetic similarity of *C. albicans* isolates from patients in group A was lower than that of *C. albicans* isolates from patients in group C (mean SAB $\frac{1}{4} 0·532 \pm 0·249$ and $0·636 \pm 0·206$, respectively); this difference was
statistically significant (P < 0.036). These results demonstrate that the cause of recurrent infections varies among individuals and ranges between strain maintenance, strain microevolution and strain replacement; the major scenario is strain maintenance with microevolution. They also show that *C. albicans* strains that cause recurrent infections are less similar to each other than strains that cause one-off infections, suggesting that the former may represent more virulent subtypes.

Gauthier *et al.* (2003) stated that the *Candida albicans CDR1* and *CDR2* genes code for highly homologous ATP-binding cassette (ABC) transporters which are overexpressed in azole-resistant clinical isolates and which confer resistance to multiple drugs by actively transporting their substrates out of the cells. These transporters are formed by two homologous halves, each with an intracellular domain containing an ATP-binding site followed by a membrane-associated domain. Authors expressed Cdr1p and Cdr2p in *Saccharomyces cerevisiae* to investigate their functions. The two proteins were properly expressed and functional, as determined by Western blotting, drug susceptibility assays, and rhodamine efflux. Using total membrane proteins from these transformants, we showed that Cdr1p and Cdr2p bind to the photoreactive analogue of rhodamine 123, [125I] iodoaryl azidorhodamine 123 (IAARh123). IAARh123 photoaffinity labeling of membranes prepared from cells expressing either the N half or the C half of Cdr2p, or both, demonstrated that both halves contribute to rhodamine binding and can bind to rhodamine independently. Interestingly, Cdr1p was found to confer hypersusceptibility to FK520, an immunosuppressant and antifungal agent, whereas Cdr2p conferred resistance to this compound, uncovering a major functional difference between the two transporters. Furthermore, when administered in combination with azoles, FK520 sensitized cells expressing *CDR1* but not those expressing *CDR2*. Finally, they showed that Cdr2p confers hypersusceptibility to hydrogen peroxide and resistance to diamide, while Cdr1p has no effect against these oxidative agents. Taken together, their results demonstrate that, despite a high level of structural conservation, Cdr1p and Cdr2p exhibit major functional differences, suggesting distinct biological functions.

Mukherjee *et al.* (2003) evaluate the disruption of the *CDR1, CDR2*, or *MDR1* gene leads to altered abilities to form biofilms. They determined the metabolic
activities, dry weights, and viable cell counts of biofilms formed by mutant strains with these genes disrupted. Biofilms were formed on denture acrylic strips. The findings showed that efflux pump-deleted strains as well as the parent strain formed thick ECM-encased biofilms, showing similar morphologies when stained with Calcofluor white, a carbohydrate-binding dye. Biofilms formed by all the strains predominantly contained blastosporides, although some hyphae were also visible. These features were similar to those observed in biofilms formed by strain GDH2346, which was previously used to standardize the biofilm model. Moreover, biofilms formed by mutant and parent strains did not differ significantly in their metabolic activities as determined by using the XTT tetrazolium dye-based assay, or dry weights. To examine the correlation between cell density and metabolic activity, the numbers of viable cells in biofilms formed by the wild-type and mutant strains were determined. The results revealed that there is no difference between the viable-cell counts in 48-h biofilms formed by mutant and parent strains. Additionally, to ascertain that the disruption of genes does not lead to altered growth, the growth of these strains were also monitored and found no differences in their growth curves. Thus, the study concluded that biofilms formed by efflux pump-deleted strains were similar to those formed by the wild-type strain, indicating that any effects of these pumps on biofilm-associated drug resistance are not due to gross changes in biofilm structure or morphology and phase-specific mechanisms are operative in antifungal resistance of fungal biofilms.

Cannon et al. (2007) Exposure of *C. albicans* to antifungal drugs induces immediate phenotypic stress responses that may permit drug tolerance. This tolerance allows the selection, or evolution of stable stress responses that confer higher levels of resistance. All micro-organisms contain a plethora of genes that can potentially confer resistance to new environmental stresses. *C. albicans* is a diploid fungus with several gene families that have probably arisen by gene duplication. They discovered that alleles of CDR1 and CDR2 within the same strain differ functionally and that CDR2 alleles are continuing to evolve. Thus there are a large number of pumps with different substrate specificities that, if over-expressed or mutated, could confer resistance to novel antifungals with intracellular targets.
Shukla et al. (2007) found that *Candida albicans* drug resistance protein 1 (Cdr1p), an ATP-dependent drug efflux pump, contributes to multidrug resistance in *Candida*-infected immunocompromised patients. Previous cell-based assays suggested that Cdr1p also acts as a phospholipid translocator. They reconstituted the purified Cdr1p into sealed membrane vesicles. Comparison of the ATPase activities of sealed and permeabilized proteoliposomes indicated that Cdr1p was asymmetrically reconstituted such that ~70% of the molecules had their ATP binding sites accessible to the extravesicular space. Fluorescent glycerophospholipids were incorporated into the outer leaflet of the proteoliposomes, and their transport into the inner leaflet was tracked with a quenching assay using membrane-impermeant dithionite. We observed ATP-dependent transport of the fluorescent lipids into the inner leaflet of the vesicles. With 6 molecules of Cdr1p per vesicle on average, the half-time to reach the maximal extent of transport was ~15 min. Transport was reduced in vesicles reconstituted with Cdr1p variants with impaired ATPase activity and could be competed out to different levels by a molar excess of drugs such as Fluconazole and Miconazole that are known to be effluxed by Cdr1p. Transport was not affected by Ampicillin, a compound that is not effluxed by Cdr1p. Our results suggest a direct link between the ability of Cdr1p to translocate fluorescent phospholipids and efflux drugs. We note that only a few members of the ABC superfamily of *Candida* have a well-defined role as drug exporters; thus, lipid translocation mediated by Cdr1p could reflect its cellular function.

2.10. Molecular characteristics of Drug resistance in *Neisseria gonorrhoeae*

2.10.1. Plasmid mediated resistance

Bhalla et al. (1998) determine the antibiotic susceptibility and plasmid profile of all *Neisseria gonorrhoeae* strains (PPNG and non-PPNG) isolated from May 1995 to March 1996 in Lok Nayak Hospital, New Delhi, India. Fifty consecutive isolates of *N. gonorrhoeae* recovered from 48 men with urethritis and two women with endocervicitis, attending the STD clinic were included in this study. All the PPNG strains in the present study harboured the 4.4 MDa Asian type β lactamase plasmid along with the 25.2 MDa plasmid encoding for high level tetracycline resistance. The
2.6 MDa cryptic plasmid was present in 40 (80%) gonococcal isolates, 10 (25%) of them showed the concatameric 7.8 MDa plasmid. Four plasmid profiles could be distinguished among the 40 isolates that harboured plasmids. All TRNG isolates had the 25.2 MDa plasmid but two plasmid patterns could be seen among the 14 TRNG strains. Four (28.5%) of the TRNG strains also exhibited reduced sensitivity to Norfloxacin (MIC >1 μg/ml QRNG). All four PPNG isolates harboured the 4.4 MDa Asian type of penicillinase encoding plasmid along with the 25.2 MDa high level tetracycline resistance plasmid.

Moodley et al. (2001) collected the N. gonorrhoeae isolates from patients attending the city health STD clinic in Durban, South Africa. The prevalence of PPNGs doubled from 1995 to 1997 and has remained at 30% since then. The doubling of the PPNG prevalence in 1997 was associated with a decrease in the DPNGs (P < 0.001) and an increase in PEN-S in that year. In 1998/99 and 1999/00, a significant increase in the DPNGs was seen and this was accompanied by a decrease in PEN-S strains. There was a dramatic increase in the number of strains exhibiting high-level resistance to tetracycline (TRNG) between 1997 (3%) and 1998/99 (51%). This increase was counteracted by a reduction in CRMNGT in 1998/99. A doubling of the prevalence of CMRNGT was accompanied by a marked decrease in the number of strains fully susceptible to tetracycline in 1999/00 (7%). All 1995 strains had MICs of ciprofloxacin ≤0.007 mg/L, but only 71% fell in this category in 1999/00. In addition, over 10% of strains demonstrating MICs of ≥0.03 mg/L were seen in 1998/99 and 1999/00. The same trend was observed with Ofloxacin but at higher MIC levels. The susceptibility to Erythromycin also changed over this period. The MICs in 1997 ranged from ≤0.007 to 0.5 mg/L, but these values changed to 0.03–4 mg/L for the 1999/00 strains. The data for Azithromycin (available for 1999/00 only) showed a similar distribution of MICs when compared with erythromycin for the same period. No resistance to Ceftriaxone was detected. However, an increase in MICs was observed with 50% of strains in 1999/00. A similar pattern was observed for Spectinomycin, as evidenced by the increase in MICs with 28% of strains exhibiting MICs ≥ 16 mg/L in 1997, 52% in 1998/99 and 100% in 1999/00. Two strains showed a marked decrease in susceptibility to the drug with an MIC of 64 mg/L. Furthermore,
widespread use of these antimicrobials in the community may offer a selective advantage to the development of resistance. The implications of this are far reaching and the local susceptibility trends of *N. gonorrhoeae* need to be monitored constantly to direct therapy.

Ahmed et al. (2010) determined the presence of Penicillinase producing *N. gonorrhoeae* (PPNG) and Tetracycline resistant *N. gonorrhoeae* (TRNG) among the *N. gonorrhoeae* isolates by PCR. Approximately 14% of the isolates were PPNG in 1997 compared to 44% in 2006. Of the isolates from 1997, 20% were TRNG compared to 86% in 2006. None of the isolates was both PPNG and TRNG in 1997 and 1998 compared to 42% in 2006. More than 90% of the isolates from 1997 to 2006 carried 3.2-MDa African type PPNG plasmid. However, 4.5% (8/175) and 4.5% (5/131) of the PPNG isolates of 2002 and 2003 respectively carried a Toronto-type plasmid respectively. Of the TRNG isolates, 11% (2/18), 2% (1/49), 7.5% (9/119), 3.2% (6/186), and 2% (5/252) in 1998, 1999, 2000, 2002, and 2003 carried 1600-bp American type *tetM* gene in the 25.2-MDa conjugative plasmid respectively, and the remaining TRNG isolates carried 700 Dutch type *tetM* genes in the 25.2-MDa conjugative Plasmid. More than half of the isolates were resistant to three drugs including Ciprofloxacin, the first-line therapy for gonorrhoea. They suggested that the knowledge of antimicrobial susceptibility of *N. gonorrhoeae* is a prerequisite for the proper treatment and control of gonococcal infection.

### 2.10.2. Chromosomal mediated resistance

Tiejun et al. (2009) detected the Fluoroquinolone resistance among *Neisseria gonorrhoeae* isolates from Shanghai, China. Ninety five per cent (76 of 80) of isolates were resistant, 3.75 per cent (3of 80) intermediate resistant, and 1.25 per cent (1 of 80) were sensitive to fluoroquinolone drug ciprofloxacin. Sequencing and RFLP analysis of *gyrA* and *parC* revealed that all resistant isolates had dual mutations of S91F and D95A/G/N in *gyrA*. Some isolates had an extra mutation within *parC* either of D86N, S87N or E91A/G. Mutation patterns for *gyrA* and *parC* were significantly (*P*<0.05) associated with MICs level. They concluded that mutations of S91F and D95A/G/N in *gyrA* combined with S87N in *parC* were 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 maker for Quinolone-resistance prediction in Shanghai, P.R. of China.

2.11. Diagnosis and control of RTIs

Roy *et al.* (2005) evaluated and compared the cost and disease outcomes associated with 4 strategies, identified from current practice and consultations with experts, for diagnosing and treating gonorrhea in women. All 4 strategies ensured that PID did not develop in 99% of all treated patients, regardless of the assumed prevalence of gonorrhea. This finding means that the costs per patient treated are almost the same as the cost per patient successfully treated (i.e., costs per patient with no PID or sequelae) and that relative costs are central in determining cost-effectiveness. The overall conclusion from our model is that decisions regarding changes in drug therapies used for gonorrhea treatment require several types of data. Both prevalence of gonorrhea and prevalence of ciprofloxacin-resistant gonococcal strains must be considered. Since prevalence data are dynamic and population-specific, ongoing collection of such data is necessary to allow informed decision making to take place.

Bala *et al.* (2005) aimed to compare the results of two methods of susceptibility testing, minimum inhibitory concentration (MIC) values by E test with disc diffusion results by Australian Gonococcal Surveillance Programme (AGSP) method in *N. gonorrhoeae* isolates. They conclude, both the above methods were easy to perform, gave reproducible results, did not involve use of specialized techniques or equipment and could be performed in any routine diagnostic laboratory. MIC testing was more reliable as it has the advantage of producing a more defined estimation of the level of resistance in less sensitive strains as reported for Ceftriaxone in the present study, and also from China. On the other hand, disc diffusion method (AGSP) was simple, cost-effective (Indian rupees 6 for one antibiotic disc testing, Indian rupees 170 for one E test strip) and therefore, was more feasible in routine diagnostic laboratories. As there is a need for ongoing monitoring of antimicrobial susceptibility in *N. gonorrhoeae* in resource poor countries like India, disc diffusion method remains the method of choice for routine testing.
Ison et al. (2006) obtain information on the comparability of methods for the laboratory diagnosis of bacterial sexually transmitted infections (STIs) that contribute to the surveillance data in the European Union (EU) and Norway. The methodology used for the isolation and identification of *N. gonorrhoeae* did show variation but all the techniques used were appropriate for the laboratory diagnosis of *N. gonorrhoeae*. Resistance to therapeutic agents for gonorrhoea, such as penicillin and ciprofloxacin, has been increasing in many parts of the world and presents a major threat to the control of gonorrhoea because there are limited alternative agents available and a vaccine is not a realistic possibility.

Savicheva et al. (2007) stated that for laboratory diagnosis of *N. gonorrhoeae*, culture was suitable for most type of specimens, inexpensive in comparison with NAATs, allows antibiotic susceptibility testing and phenotypic or genotypic characterization with high sensitivity and specificity. A culture of gonococci can be affected by various factors, e.g., treatment of the patient with antimicrobials as well as local application of gynaecological lubricants, irrigations, and spermicidal agents. Cotton, calcium alginate, and dacron swabs used for sampling can all be toxic for gonococci. This is due to the fact that unsaturated fatty acids in cotton fibres, chlorine-based bleaching agents, resin in wooden shafts, and glue used to connect swabs to the shaft may be toxic for gonococci. Consequently, direct inoculation on culture plates is ideal for reducing the time of exposure to possible toxic substances. Otherwise, the choice of appropriate swabs may be crucial, and charcoal-coated swabs should be used if charcoal is not included in the transportation medium. Ingredients such as starch, charcoal and blood of accurate transportation media can act as adsorbing agents and therefore increase the capacity of gonococci to survive during the transport.

Marot-leblond et al. (2009) stated that the clinical symptoms of vulvovaginal candidiasis (VVC) are nonspecific, and misdiagnosis is common, leading to a delay in the initiation of antifungal treatment. They evaluated a new immunochromatography test (ICT), the CandiVagi assay (SR2B, Avrille, France), for the rapid diagnosis of VVC. This test, employs an immunoglobulin M antibody directed against the β-1, 2-mannopyranosyl epitopes found in the yeast cell wall, and was compared with direct
microscopic examination and culture of vaginal swabs. Two-hundred five women were investigated, including 130 women with symptomatic vaginitis and 75 asymptomatic controls. The sensitivities of microscopic examination, culture, and ICT for the diagnosis of VVC were 61%, 100%, and 96.6%, respectively, while the specificities of the three methods were 100%, 82%, and 98.6%, respectively. ICT had a negative predictive value of 98.6%, a positive predictive value of 96.6%, and an efficiency of 98%. ICT provided a rapid result and a better compromise between sensitivity and specificity than conventional microscopy and culture for the diagnosis of VVC. The authors concluded that this easy-to-perform diagnostic test will be useful to practitioners treating women with symptoms of vaginitis.

Lowe et al. (2009) estimate the accuracy of the clinical diagnosis of the three most common causes of acute vulvovaginal symptoms (bacterial vaginosis, candidiasis vaginitis, and trichomoniasis vaginalis) using a traditional, standardized clinical diagnostic protocol compared to a DNA probe laboratory standard. The sample was 547 active duty U.S. military women (43% Army, 54% Navy, and 3% other branches) who presented for healthcare with vulvovaginal symptoms. The presenting symptom was abnormal discharge of a wide range of descriptions (278, 50.8%), itching/irritation (182, 33.3%), malodor (57, 10.4%), vulvar burning (23, 4.2%), and miscellaneous others (7, 1.3%) including vulvar pain and vaginal discomfort. Of this sample, 535 participants' had complete data sets for the relevant clinical and DNA probe laboratory standard test. For each single infection, the clinical diagnosis had a sensitivity and specificity of 80.8% and 70.0% for bacterial vaginosis; 83.8% and 84.8% for candidal vaginitis; and 84.6% and 99.6% for Trichomoniasis vaginalis when compared to the DNA probe standard.

Babic and Hukic (2010) included female patients visited Gynaecology-Obstetrical Clinics at the University of Sarajevo and female patients who visited Gynaecology Department at the General Hospital in Sarajevo. The study included total of 447 women, divided into two groups. Test group included 203 pregnant women in the last trimester of pregnancy, while control group included 244 non-pregnant women in the fertile period. Age of female patients in both groups was within the range of 20 to 38. From the test group, 59.1% (120/203) showed negative...
using the microscopic examination, while 40.9% (82/203) were observed as positive. From the control group, 76.2% (186/244) of microscopic findings were determined as negative, while 23.8% (58/244) were observed as positive. Positive microscopic findings were more frequent in the test group, as compared to the control group. Test group showed 46.8% (95/203) positive cultures and 53.2% (108/203) negative cultures. From the control group showed 25.4% (62/244) positive cultures while 74.6% (182/244) correlated to negative cultures. The results indicate that gravidity is one of the risk factor for the vaginal candidosis.

Usharani et al. (2011) stated that PCR is expensive and not available at all places, so speciation of *Candida* can be done by using Hicrome *Candida* differential base, modified (HIMEDIA), growth on CMA for appearance of Chlamydsospore formation, growth at 45°C, growth on Pal’s agar.

Rad et al. (2012) identify the spp of vaginal *Candida* isolates by using multiplex PCR. 191 isolates from patients admitted to Mahdieh hospital, Tehran, Iran were identified in this study. The vaginal swab specimens were cultured on Sabouraud Dextrose Agar. The ITS1 region between the 18S and 5.8S rRNA genes and a specific DNA fragment within the ITS2 region were amplified. *C. albicans* and *C. glabrata* were the most common species isolated from the specimens. Among vaginal samples, 89.7% contained only one species of *Candida* and 10.3% contained more than one species of *Candida*. The prevalence of different species of *Candida* was as follow: *C. albicans* (65.1%), *C. glabrata* (13.1%), *C. tropicalis* (6.2%), *C. krusei* (4%), *C. guillermondii* (0.6%), *C. parapsilosis* (0.6%), mixed infection of *C. glabrata* and *C. albicans* (5.7%), *C. parapsilosis* and *C. albicans* (1.1%), *C. krusei* and *C. albicans* (0.6%), *C. albicans* and *C. tropicalis* (0.6%), *C. glabrata* and *C. tropicalis* (0.6%), *C. krusei* and *C. tropicalis* (0.6%), *C. krusei* and *C. glabrata* (0.6%), and a combination of *C. glabrata*, *C. krusei*, and *C. albicans* (0.6%). A mix of *C. glabrata* and *C. albicans* was the most common mixed infection isolated from the samples. The analysis revealed a significant positive association between older age and infection with *C. glabrata* isolates. Multiplex PCR is a fast, yet reliable method to identify *Candida* species. *C. albicans* and then *C. glabrata* are the two most common causes of vulvovaginal candidiasis. The number of mixed fungal infections is higher among Iranian population compared to international reports.