Chapter 7

Microbicides for the Prevention of HPV, HIV-1, and HSV-2: Sexually Transmitted Viral Infections

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Additional information is available at the end of the chapter

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Abstract

Sexually transmitted diseases (STDs) can be transmitted through genital-genital, oro-genital, or anogenital contacts and remain to be a public health concern worldwide. Approximately one million people around the world are believed to be newly infected with sexually transmitted infections (STIs) each day. Numerous causative agents including bacteria, viruses, protozoa, yeast, and fungi are responsible for STIs; however, viruses exhibit more serious risks, probabilities and outcomes of STDs than other organisms. The most lethal viral STIs are human immunodeficiency virus-1 (HIV), herpes simplex viruses 1 and 2 (HSV-1 and HSV-2), and human papillomavirus (HPV), which are responsible for major sexually transmitted viral infections including AIDS, herpes simplex, and genital warts, respectively. Despite the fact that several prevention strategies such as vaccination, abstinence from sex, limiting sex partners, the use of condoms and a range of therapeutic drugs have drastically reduced the risk of contracting STIs, these three infections continue to spread at an alarming rate. The high incidence and lack of effective vaccine, instigated scientists to look for alternate, cheap, and efficient strategies for controlling these deadly viruses. Microbicide are relatively new approach that may be helpful in preventing STIs transmission when applied inside the genitals before intercourse. Like other interventions, microbicides are used as prophylactic measures against STIs. Therefore, an excellent safety and efficacy profile analysis is mandatory before their approval for human use. Although no safe and efficacious microbicide is yet available, many candidates including nonoxynol-9, Savvy, cellulose sulfate, Carraguard, VivaGel, tenofovir gel, and PRO 2000 have shown promising in vitro activity and many more are under development. However, very few of them have moved to large-scale phase III trials. This chapter aims to provide a brief overview of various microbicides along with their mechanism of actions and recent updates on safety and effectiveness trials.

Keywords: HPV, HIV-1, HSV-2, sexually transmitted infections (STIs), microbicides, prevention of STIs

1. Introduction

Sexually transmitted diseases (STDs) or venereal diseases (VDs) being responsible for millions of deaths worldwide have proven to be a major burden on human health [1]. Approximately 19 million new cases of STDs are reported in the United States every year [2]. More strikingly, according to Centers for Disease Control and Prevention (CDC) recent press release, the largest increase in STD cases was observed from 2014 to 2015. STDs are caused by more than 30 different pathogens including bacteria, viruses, parasites, yeast, and fungus commonly known as sexually transmitted infections (STIs) (**Figure 1**). Among all known STIs, viruses exhibit more serious risks, probabilities, and outcomes of sexually transmitted diseases. Viral STIs include human immunodeficiency virus-1 (HIV), herpes simplex viruses 1 and 2 (HSV-1 and HSV-2), human papillomavirus (HPV), hepatitis B virus (HBV), and molluscum contagiosum virus (MCV) causing acquired immunodeficiency syndrome (AIDS), herpes simplex, genital warts, viral hepatitis, and molluscum contagiosum, respectively. However, HPV, HIV-1, and HSV-2 targeting the mucosa of the penis, vulva, rectum, and urinary tract account for major sexually transmitted viral infections. In order to understand the wreckage caused by these infections, it is imperative to understand the biology and pathogenesis of the abovementioned sexually transmitted viruses.

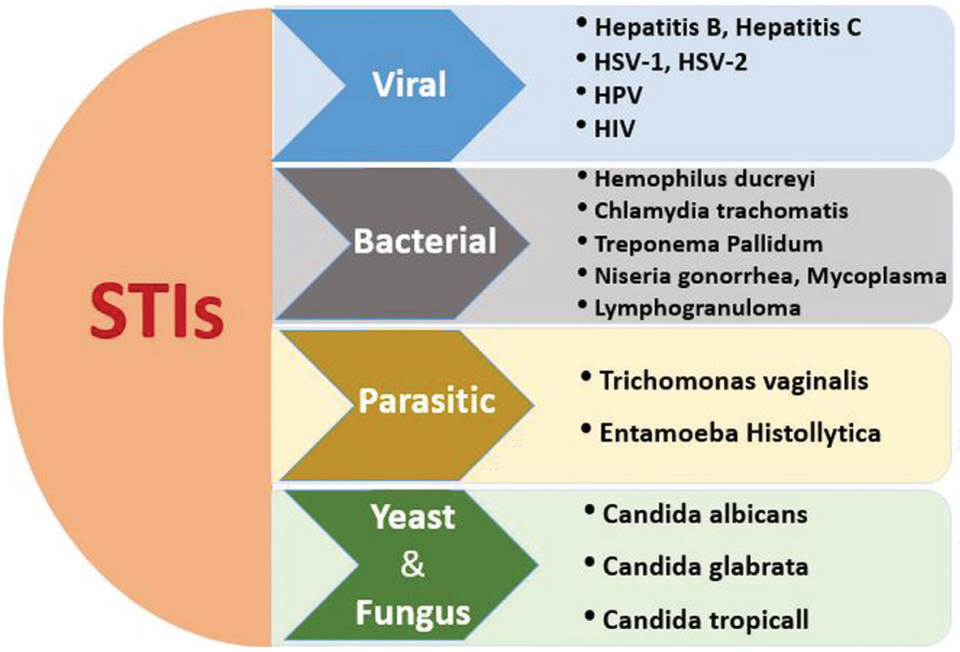

Figure 1. Sexually transmitted infections (STIs).

1.1. Biology of human papillomavirus

Human papillomaviruses (HPVs) named for warts (papillomas) are the most common sexually transmitted infectious agents both in men and women across the globe, particularly in

undeveloped countries. It is believed that nearly all men and women acquire HPV infection at least once at some stage of their lives [2]. However, sexual transmission being the major route of HPV infection, the probability of getting HPV infection in adulthood is high due to increased sexual activity. The HPV prevalence falls with the increasing age probably as a consequence of decreased sexual activity and establishment of immune response against the virus [3].

HPV is a small, nonenveloped, and double-stranded DNA virus having genome size of 8 kbp. The circular genome of HPV encodes six early and two late overlapping open reading frames (ORFs) and a noncoding long control region (LCR) [4]. Upon infection, the virus first transcribes six early proteins (E1, E2, E4, E5, E6, and E7), which are mainly involved in viral DNA replication and HPV-mediated pathogenesis. Late structural proteins L1 and L2, which make up the viral capsid, are transcribed during later phases of virus replication [5]. The early genes are expressed within the basal surface of the epithelium while late genes in supra-basal layer of the epithelium. The LCR located upstream of early and late genes contains various promoter and transcriptional regulatory sequences which act as binding sites for several viral and host transcription factors [6].

Based on L1 gene nucleotide sequence, the HPVs are classified into genera, species, and types. To date, almost 151 types of HPV have been identified and divided into five genera known as *alpha*, *beta*, *gamma*, *mu*, and *nu* [7]. HPV types are further categorized into cutaneous and mucosal types. While cutaneous HPV types target keratinocytes in the hand and feet skin, mucosal types infect the inner lining of the respiratory, digestive (mouth, throat, esophagus), and anogenital tracts. The cutaneous HPVs mostly belong to beta and gamma genera, whereas mucosal types are included in alpha genus [8]. About 30 HPV types have been reported to be transmitted through sexual contact, thereby infecting mucosa of the genitalia [9]. The genital HPV types are further categorized into high risk (HR) and low risk (LR) based on the severity of clinical manifestation. The LR-HPVs such as subtypes 6, 11, 42, 44, 51, 53, and 83 induce warts or hyperproliferative benign lesion on genital. On the other hand, HR-HPV subtypes including 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68,73, and 82 are linked with premalignant and malignant cervical, penile, vulvar, vaginal, anal, and head and neck carcinomas [10]. Notably, majority of the LR-HPV infections are caused by HPV 6 and 11 subtypes, while subtypes 16 and 18 are responsible for most of the HR-HPV infections [11, 12]. In fact, HPV 16 and 18 are the most lethal subtypes, which together account for 70% cases of cervical cancer, the fifth most commonly diagnosed type of cancer and leading cause of cancer deaths [13].

The HPV lesions are believed to commence from the basal keratinocytes, which are exposed to HPV infection as a consequence of microabrasions or trauma during sexual intercourse [9, 14]. The virus then binds to specific cell surface receptors and is subsequently internalized in to the cells where it establishes episomal or integrated persistent infection, a pivotal step in cervical cancer causation. Various viral proteins are expressed during the replication cycle of HPV that control the transcription as well as replication of virus and induce cell proliferation. The E5, E6, and E7 are the essential proteins which help the virus during initiation and progression of cervical cancer [15]. These oncoproteins interfere with cell cycle and other regulatory pathways and induce genome instability mainly by inhibiting key tumor suppressor

proteins such as p53 and pRB [16]. The p53 being the guardian of genome is targeted by HPV E6 protein for proteasomal degradation, while E7 competes with pRB protein releasing the E2F transcription factor, which helps the transcription of genes that drive cell cycle further on. Likewise, HPV oncoproteins maneuver host cell in such a way to keep them in a condition favorable for virus replication. For example, overexpression of E1 and E2 proteins has been evidenced to push the cell in S and G2 phases that stably maintain viral episomes [17]. HPV has also been described to alter numerous cell regulatory pathways; for instance, E6 and E7 are believed to be involved in beta-catenin nuclear accumulation leading to activation of Wnt signaling pathway that is one of the major deregulated signal transduction pathways in cancer [18]. Another salient example elaborating the role of HPV proteins in carcinogenesis was described by Accardi et al. who proved that HPV16 E6 and E7 proteins jointly dissociate Na+/H+ exchange regulatory factor-1 (NHERF-1), which is involved in the regulation of various cellular processes including signaling and transformation. The degradation of NHERF-1 leads to activation of the PI3K/AKT signaling pathway, which is known to be a major player involved in carcinogenesis [19].

1.2. Biology of herpes simplex virus

Herpes, from the ancient Greek meaning to creep or crawl, is the name ascribed to the infections caused by a large family of DNA viruses called *Herpesviridae*. The members of this virus family are known to equally infect human and animals. Among the described human herpes viruses, closely related herpes simplex virus-1 (HSV-1) and herpes simplex virus-2 (HSV-2) that show 70% genomic homology are considered to be most contagious human herpes viruses and are transmitted via sexual contact [20]. HSV-1 may be transmitted by oral to oral or oral to genital contact, thereby causing oral or genital herpes, while HSV-2 is exclusively transmitted by sexual contact and is responsible for genital infections only [21]. Mostly oral and genital herpes are symptomless; however, complications can cause painful blisters or ulcers at the site of infection. Both of these viruses are widespread in human population. In 2012, it was estimated that 67% human population under the age of 50 were living with HSV-1 infection while 11% with HSV-2 [22].

The HSV virion is structurally divided into four parts: an electron dense core containing the viral genome, an icosapentahedral capsid, a tegument comprising a protein cluster, and a glycoprotein-based envelope [23]. The genome of HSV-1 and HSV-2 is a complex large double-stranded DNA molecule which is divided into two unique regions: the long unique (U_L) and the short unique regions (U_s). The U_L transcribes 56 viral genes whereas U_S merely 12 [24]. The translated proteins from these genes are involved in making virus components, controlling virus replication and infectivity. The virus gets entry into the nerve cells in the lower layer of the skin as a consequence of interaction between numerous viral glycoproteins and host cell receptors mainly heparan sulfate [25]. Upon internalization, the virion is dismantled, and capsid is routed to the nuclear pore ejecting its DNA into the nucleus where transcription of viral genes takes place with the help of RNA polymerase II [26]. The HSV replication involves sequential production of different viral proteins. At first, immediate early proteins are synthesized that regulate viral gene expression during replication. The enzymes carrying out viral replication are also products of early gene transcription. The late transcribed genes predominantly encode proteins required for capsid and envelop formation [24].

The primary HSV infection occurs in the epithelial cells from where virus ascends to the sensory nerve terminal at peripheral site. Then by retrograde axonal transport virus enters the trigeminal nerve ganglion and establishes latency resulting in long-time persistence [27]. During the latency phase, virus expresses latency-associated transcript (LAT) which regulates the host cell genome in order to maintain the virus reservoir in the host without any clinical manifestation [28]. Furthermore, HSV evades host immune response either by mimicking the human interleukin 10 (HIL-10) or by downregulation of the major histocompatibility complexes I and II (MHC I and II) in the contaminated cell, thus ensuring virus survival in latency [29]. The HSV encodes a HIL-10 homologous protein that blocks the production of pro-inflammatory cytokines such as IFN-γ, IL-1α, GM-CSF, IL-6, and TNF-α, thereby reducing the natural killer cells and cell-mediated response against virus [30]. Likewise, for the downregulation of MHCI-II, HSV encodes ICP47 protein that blocks the presentation of MHC-class-1 proteins on the cell surface by retaining the newly synthesized MHC molecules in the endoplasmic reticulum [31]. The lack of MHC expression on the surface of infected cells results in the absence of T-cell activation ultimately helping virus to hide from the immune system. In some infected persons, viral reactivation occurs sporadically due to some triggering factors such as physical or emotional stress, fever, ultraviolet light, tissue damage, and other immune-compromising events [32]. Upon activation, virus travels from the dorsal root ganglion in conjunction with sensory nerve cells to the epidermal-dermal junction. During virus activation phase, a transition in the gene expression takes place, and virus expresses multiple lytic genes which direct the elevated viral replication and host cell death on the other hand [28]. The active virus is ultimately transported to the skin again where virus sheds and initiates new cutaneous or mucosal sores.

1.3. Biology of human immunodeficiency virus

Human immunodeficiency viruses (HIV) are members of *Retroviridae* family, which cause disease in both genders of almost all ages. Though two closely related HIV types (HIV-1 and HIV-2) have been described, however, HIV-1 is more virulent and is responsible for majority of HIV-related infections [33]. This virus is known to infect cells of the immune system including macrophages and dendritic and $CD4^{+}$T cells, thereby destroying them and impairing host immune function [34]. If left untreated, HIV infection may lead to a devastating disease called acquired immunodeficiency syndrome (AIDS). It is estimated that during 2015 alone, 36.7 million people got infected with HIV, while 1.1 million died of HIV-related causes worldwide.

The HIV is a tiny enveloped virus consisting of two copies of positive sense RNA molecules, which are accompanied by several nucleocapsid proteins and enzymes, for instance, proteases and integrases [35]. The genome of HIV is complex and for the most part marked as 5′LTR-gag-pol-env-LTR′3 [36]. However, six other genes—*tat*, *rev*, *nef*, *vif*, *vpr*, and *vpu* are also encoded by virus genome. The *gag* (group antigens) and *env* genes encode major nucleocapsid and structural proteins, while pol transcribes enzymes such as reverse transcriptase required for virus replication [35]. In fact, the *gag*, *pol*, and *env* proteins act as precursor and cleaved by proteases to give rise several other proteins. The remaining six genes are considered as accessory genes which are required for efficient virus replication and for regulation of viral gene expression [37]. Among these six accessory proteins, *nef* and *vif* are of extreme importance as they help virus in immune evasion and deal with antiviral activity of host APOBEC3G

protein. The *nef* reduced the antigen presentation on the HIV-infected cells, thereby hiding from the immune system, while *vif* neutralized the infectivity of APOBEC3G protein, which degrades the viral RNA in the infected cell [38].

The HIV targets CD4+ T cells, macrophages, and dendritic and microglial cells for its multiplication. The life cycle of the virus begins with the virus attachment to CCR5 and CXCR4 receptors through its trimeric glycoprotein complex made up of gp120, gp160, and gp41. The surface proteins of HIV fuse with host cell membrane releasing viral genome inside the cells. The virus ssRNA is converted into complementary DNA (cDNA) by utilizing virus enzymes that are the part of mature HIV virion. The complementary part of the cDNA is synthesized and then transported to the nucleus where it integrates into host genome as provirus again with the help of virus-encoded integrase enzyme [39]. The integrated genome is transcribed into mRNA which is utilized simultaneously to produce viral proteins as well as the viral genome. The viral-encoded proteins tet and rev regulate the expression of HIV genes. For the synthesis of HIV virion, structural protein gp 160 is transported to the cell membrane where all virus components are assembled and finally bud off from the cell [40].

The destruction of CD4+ T cell is the mainstay mechanism of HIV-mediated pathogenesis. The HIV reduces the number of CD4+ T cells by several mechanisms. Programmed cell death or apoptosis is among the most prominent mechanisms underlying HIV-mediated destruction of CD4+ T cells [41]. The increased apoptosis of CD4+ T cells in HIV infection could be due to direct viral cytotoxicity or due to signaling events triggered by viral proteins. The apoptosis in HIV-infected patients is not limited to infected T cells only, but uninfected cells are also destroyed by the so-called bystander mechanism. While several viral proteins are believed to play a role in apoptosis of bystander CD4+ T cells, interactions between viral Env glycoprotein expressed on surface of infected T cells and specific receptors and coreceptors on the surface of neighboring uninfected T cells have been proposed as the major mechanism responsible [42]. The bystander apoptosis reduces the number of T cells to an alarming level making the person more likely to get other opportunistic infections including viruses and bacteria that put the life in serious danger [43].

2. Conventional methods for the prevention of STIs

Ever since their discovery, successful prevention and treatment of STIs, including HIV-1, HSV-2, and HPV, have been a high-priority research area. To date, several recommendations with varying effectiveness have been put forward by researchers and healthcare providers in order to limit STIs. The focal point of these described strategies is the prevention, i.e., blocking the acquisition of STIs. The STI prevention approaches are mainly based on reducing the risk factors, deployment of physical barriers, prophylactic immunization against sexually transmitted agents, efficient and timely diagnosis of STIs, and treatment of active infection [44, 45]. There has been remarkable progress in the diagnosis and treatment of STIs; however, the discussion on them would be beyond the scope of this chapter. Nevertheless, other preventive measures against STIs particularly HIV-1, HSV-2, and HPV will be discussed thoroughly in the coming sections of this chapter.

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2.1. Curtailing risk factors for STIs

The act of sexual intercourse in humans is known to create small unnoticeable microabrasions which in turn pave the way for entry of numerous STIs. The epidemiological synergy has also hinted that the presence of some STIs favors the acquisition of other STIs. For instance, the existence of chlamydia, herpes, gonorrhea, and syphilis in an infected person makes him/her more likely to acquire HIV infection [46]. It is also worth mentioning that multiple STI coinfections prove to be more harmful than the single STI [47]. Therefore, the paramount approach in treating STDs would be to combat the transmission of STIs altogether. This could only be achieved by reducing risk factors, such as unprotected sex, early age sex, and multiple sex partners that increase the chances of catching various STIs [48].

The most reliable way of controlling STDs is the complete abstinence from any type of sex particularly during teen ages and comprehensive sex education [49]. Nevertheless, this does not seem to be a practical approach. However, limited number of sex partners and long-term sexual relationship with a single uninfected individual are believed to be most pragmatic ways in this regard [50]. Talking with partners about sexual health prior to sexual activities also mitigate the risk of getting STIs. Some important considerations before, during, or after sexual intercourse such as washing ahead of performing sex, avoiding sex when drunk, and circumvent unharmful sex positions have significantly reduced the STI burden [51]. Recently, male circumcision has been reported as a vital mean of reducing STI risk. Three separate clinical trials have demonstrated that circumcision can reduce the HIV acquisition by 60% [52, 53]. Moreover, male circumcision was also found to be effective against other STIs including HPV and HSV-2 [54].

2.2. Putting the physical barriers to STIs

The use of physical barriers, including male and female condoms, is not only among the most commonly used birth control methods but also serves to curtail the spread of STIs effectively. Male condoms are classified into natural or synthetic categories based on the material they are made of. Natural membrane condoms usually derived from lamb cecum are primarily meant for pregnancy prevention rather stopping STIs. In fact, the pores in the natural condoms are large enough to let the passage of small STI-causing organisms, particularly viruses [55]. On the other hand, the efficacy of synthetic condoms in the prevention of STIs has been proved by various epidemiological and laboratory studies [55]. Synthetic condoms are either made of latex or other nonlatex material such as polyurethane or polyisoprene. Latex condoms are flexible, broadly available, and least expensive among all types of condoms. They are exceedingly effective in preventing the sexual transmission of plethora of STIs, including HPV, HIV-1, and HSV-2 [56–58]. The failure of latex condoms to safeguard STIs or unintended pregnancy is usually due to inconsistent or incorrect use [59]. The nonlatex condoms are particularly suitable for those allergic to latex. Polyurethane condoms are relatively thin and odorless. They provide comparable protection as of latex condoms against various STIs [60]. However, polyurethane condoms are at higher risk of breakage during intercourse. Both latex and nonlatex condoms' efficiency of protecting STIs can be enhanced by using some germicidal spray on them [61].

Female condoms are usually made up of thin plastic polyurethane material and have rings on the both ends. The ring inside the vagina covers up the cervix with a plastic sheet while outer ring is open and resides outside the vagina covering the vulva. Like male condoms, these are designed to avoid pregnancy as well as to prevent the infection spread during sexual process [62]. Female condoms are usually recommended to sex partner when male condoms cannot be used appropriately. Contradictory reports have been presented regarding efficiency of female condoms. One systemic review based on different randomized control trials revealed that female condoms are good in avoiding pregnancy but not in protection from STIs [63]. On the contrary, another randomized control trial concluded that female condoms' efficacy is comparable with male condom [64]. As a matter of fact, the female condom efficiency like male condom varies according to their use. In the nutshell, it has been estimated that female condoms are more efficient if used consistently with accuracy [65].

Another way of protecting pregnancy and STD in females is the use of cervical diaphragms. Diaphragm is a dome-shaped bowl made of thin and flexible rubber that sits over the cervix. In order to use it as a contraceptive, spermicide is placed into the bowl and edges of the diaphragm before inserting into the vagina [66]. After sex, the diaphragms are left inside the vagina at least for 6–24 h. There is ample epidemiological and biological data suggesting that diaphragm use can reduce the risk of acquiring some of the STDs including gonorrhea, chlamydia, and trichomoniasis [57]. However, diaphragm has been proved to be ineffective in reducing the risk of acquiring HIV infection. Moreover, spermicide use along with diaphragm increases the risk of bacterial urinary tract infections [67]. It is therefore recommended that targeted clinical trials must be conducted before approval of diaphragms as a method for STI prevention.

2.3. Immunization against HPV, HIV-1, and HSV-2

Vaccines prime individuals' immune response to build up adaptive immunity, thereby protecting them from subsequent infection. Preexposure vaccination probably is the most effective means of preventing transmissible infection including STIs [51]. Unfortunately, except for HPV, no vaccine is approved for other sexually transmitted viral infection. However, vaccines against HIV-1 and HSV-2 are under developmental phase. **Table 1** enlists and describes characteristics of all proposed vaccines for HPV, HIV-1, and HSV-2.

HPV vaccine being a major public health breakthrough is administered in both males and females before reaching the age where HPV risk is maximum. Up till now, three HPV vaccines under the trade name of Cervarix, Gardasil, and Gardasil 9 have been approved by FDA [68]. The Cervarix is bivalent vaccine designed against HPV types 16 and 18 that are responsible for 70% cervical cancer. In addition to HPV 16 and 18, Gardasil provides protection against HPV 6 and 11, which cause 90% of genital warts [69]. The Gardasil got approved from FDA in 2006 while Cervarix in 2009. Recently in 2014, another vaccine Gardasil 9 was approved and is meant to protect against 9 HPV types: 6, 11, 16, 18, 31, 33, 45, 52, and 58 (FDA press release). All these vaccines are administered in a series of three doses at the ages of 16–26. However, they can be administered up to the age of 45. The effectiveness of all these vaccines has been assessed clinically in many randomized controlled trials, and all these trials

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Virus	Vaccine name/ pharmaceutical company	Vaccine type	For which type	Clinical status
HPV	Gardasil/Merck & Co.	Recombinant	HPV 16, 18, 6, and 11	FDA approved (2006)
	Cervarix/ GlaxoSmithKline	Recombinant	HPV 16, 18	FDA approved (2009)
	Gardasil 9/Merck & Co.	Recombinant	HPV 6, 11, 16, 18, 31, 33,45, 52, and 58	FDA approved (2014)
	GEN-003/Genocea	HSV subunit vaccine	HSV-1 and HSV-2	Phase II
	HerpV/Agenus	Peptide vaccine	HSV-1 and HSV-2	Phase II completed
HSV	Vical HSV-2 (therapeutic vaccine)/ Vical	DNA vaccine	HSV-2	Phase II
	Shingrix/ GlaxoSmithKline	Subunit vaccine	HSV-2	Phase III
	HSV-529/Sanofi	Live attenuated vaccine	HSV-2	Phase I
	gD2t/ GlaxoSmithKline	Synthetic vaccine	HSV-2	Phase II completed
	AGS-004/Argos	Therapeutic vaccine	HIV-1	Phase IIb
HIV	GTU multi-HIV+ Lipo5	DNA+ lipopeptide vaccine	HIV-1	Phase II
	VAC-3S InnaVirVax	Peptide based	HIV-1	Phase II

Table 1. The list and characteristics of all available/proposed vaccines against HPV, HIV-1, and HSV-2.

endorsed the safety of these vaccines [70, 71]. Likewise, HPV vaccine is safe and is associated with no or mild side effects such as fever, nausea, and headache. The available HPV vaccines have noticeably reduced the incidence of genital warts, cervical intraepithelial neoplasia, as well as cervical cancer worldwide [72].

Several preventive and therapeutic antiherpes vaccines are under different developmental stages, but no vaccine has been approved so far. Most of these vaccines target HSV-2 rather than HSV-1. Nonetheless HSV-2 vaccine would also be effective against HSV-1 because of homology between two viruses. Nowadays, several approaches have been used by academic institutes, government agencies, and pharmaceutical companies who are engaged in developing and testing HSV vaccines. Most of the endeavors are based on the concept of using virus glycoproteins to design subunit vaccine. The largest clinical trials were conducted on "Herpevac" vaccine which employs virus glycoprotein D-2 (gD2) as immunogen. These trials showed that vaccine provided significant protection against HSV-1 but not HSV-2 [73]. A replication-defective HSV-2 vaccine (HSV529) has also entered phase I trials [74]. One live attenuated vaccine has produced marvelous results in the mouse model. However serious concerns have been noticed regarding the safety of this vaccine. One of the important points for the

failure of HSV-2 vaccine is the nonavailability of suitable animal model for HSV. Due to spontaneous reactivation in the genital tract, guinea pigs are considered a better model than mice [75]. For the last decade, almost three anti HSV-2 vaccines, namely, GEN-003, HerpV, and gD/UL46, have entered phase I/phase II clinical trials. These vaccines were designed to stimulate T-cell immunity.

It has now been more than a quarter century that researchers are engaged in finding an effective measure that can provide a significant degree of protection against HIV infection. Some promising advances regarding HIV vaccine development have been witnessed during last two decades, but an effective and safe HIV vaccine is still needed. In fact, HIV being an RNA virus exhibit high mutation rate and hence increased genetic variability. This particular aspect of HIV biology is the main reason hindering HIV vaccine development efforts [76]. A range of approaches are being tested for developing an effective HIV vaccine such as protein subunit vaccines, viral vectors encoding for HIV proteins, DNA vaccines, as well as prime-boost strategy that uses a canarypox viral vector encoding HIV Env, Gag, and Pol proteins to prime the immune system followed by a mixture of same protein subunits as booster dose [77]. All these approaches employed one of the three scientific concepts that include induction of neutralizing antibodies, cell-mediated immunity, and exploration of combination approaches [76]. The vaccines being developed for HIV prevention have been subjected to clinical trials for safety and efficacy analysis. The VAX003 and VAX004 were the first efficacy trials of bivalent HIV vaccine, conducted by in men who have sex with men (MSM) and injection drug users [78]. These phase III trials show that vaccine was not effective in preventing HIV disease progression [79]. Similarly, two trials—HVTN 502 and HVTN 503 also called "Step Study" of *Adenovirus* vector-based HIV vaccine—failed to prove the efficacy of vaccine. HVTN 505 vaccination trials were also stopped in 2013 because initial results revealed that the vaccine was ineffective in preventing HIV infections [80]. However, the encouraging results were obtained in RV144 trials of HIV vaccine where it has noticed that vaccine reduced the infection in 31% cases [81]. This was the only trial that showed somewhat positive results.

3. Microbicides for the prevention of STIs

As mentioned in preceding discussion, a range of preventive strategies with varying degrees of efficacy and efficiency have been employed to curtail the transmission of HPV, HIV-1, and HSV-2 infections. However, these three deadly viruses continue to spread at alarming rates. Increasing incidence and failure in the development of effective HIV-1 and HSV-2 vaccines have instigated scientists to explore alternate research avenues. As STI spread is directly correlated with socioeconomic status of subjects, economically disadvantaged population being at high risk, there is also a dire need for cheaper options.

Microbicides are antimicrobial compounds claimed to be helpful in controlling the transmission of STIs upon self-administration inside the vagina and rectum. Some of the microbicides have been witnessed to provide considerable protection against STIs including HIV-1, HPV, and HSV-2 [82]. However, it is not clear whether microbicides render anticontraceptive effect

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too. Ideal microbicides are colorless, odorless, and tasteless compounds available in different biological formulations such as gels, creams, rings, suppositories, pessaries, films, and invisible condoms. Other criteria for microbicides to be ideal are safety and effectiveness in preventing broad range of STIs. Moreover, its repeated use should cause minimal or no symptoms in the vagina or rectum. The microbicides as STI-controlling measure are advantageous in many ways. In comparison to other interventions, microbicides are cheap, offered over the counter, and available in many formulations [83]. They can be easily applied by women themselves and do not create a physical barrier to intimate contact. Similarly sex worker can apply them without prior information to their clients. Moreover they are safe and nonirritating.

Currently, more than 50 potential microbicide agents have been identified. However, very few of them proved to be effective and have gone under advance phase III clinical trials [84]. The coming section of this chapter will provide a comprehensive review of various microbicides, along with their mechanism of action and current status of clinical trials, in order to highlight their strengths and shortcomings.

3.1. Stages in microbicide development

Drug development is a long-term work, which can even be of a decade or more sometimes. The microbicide development would have to follow the same protocols of drug development as any other drugs do [85]. The development of drug starts with the insight of the researchers to a new potential compound or chemical that can hinder or stop the cascade of destruction by the infection or to eliminate the pathogen as well. So once the potential candidate from many compounds is isolated for research and development of drug, then every aspect of it is practically assessed like its absorbance, distribution, destabilization, mechanism of action, dosage and side effects, etc. Then comes the preclinical testing of the drug, which can be in vitro or in vivo. Once the pre-clinical testing is complete, the clinical trials of the drug will show how it works in the real system. There are four phases of the clinical trials from 1 to 4 depending upon the no. of volunteers assigned to it, the duration of the phase, and the purpose of testing. Phase I of clinical trials is to assess safety and dosage, and almost 70% of the drugs pass this phase. Phase II clinical trial is to judge efficacy and side effects of the drug and could take up to 2 years, and 33% of the drugs pass it. In phase III, efficacy and adverse reactions are monitored, and about 25–30% move to the next level [86]. In phase IV, several thousands of volunteers are involved, and safety and efficacy of the drug are observed. If the drug shows the satisfactory results in all phases of trials, then it got reviewed by the drug regulatory authority that then monitors the marketing and follows active surveillance. The developmental phases of microbicides are summarized in **Figure 2**.

3.2. Microbicide mechanism of STI prevention

Microbicides exert their antimicrobial function by a range of mechanisms which can be divided broadly into four categories: vaginal defense enhancers, inactivation of virus in the vagina, virus attachment and fusion inhibiters, and virus replication inhibitors. **Figure 3** enlists some categories of microbicides and their mechanism of action altogether.

	Description	Time Line	No. of Volunteers
Ideas	Hypothesis	-	-
Pre- clinical testing	Lab and Animals	2-6 Years	-
Phase 1	Multiple Studies (Early Safety)	2 – 12 Weeks	25-200
Phase 2	Expanded Safety	6- 12 Months	200-800
Phase 2b	Safety and Effectiveness	Up to 3 Years	1500-3000
Phase 3	Effectiveness	1- 4 Years	3,000-10,000
Regulatory approval	Regulatory Approval	1-2 Years	-
Introduction (Phase 4)	Post Marketing Studies	-	-

Microbicide development phases

Figure 2. Phases of microbicide development.

3.2.1. Vaginal defense enhancers

Naturally, the acidic pH of the vagina provides an established defense against invading infections. Some organisms such as lactobacilli being natural inhabitant of the vagina play a pivotal role in maintaining the low vaginal pH [87]. This organism also releases some antimicrobial compounds, for instance, lactic acid, hydrogen peroxide, bacteriocins, and biosurfactants, which keep the vagina protected from pathogens. However, semen and some bacterial

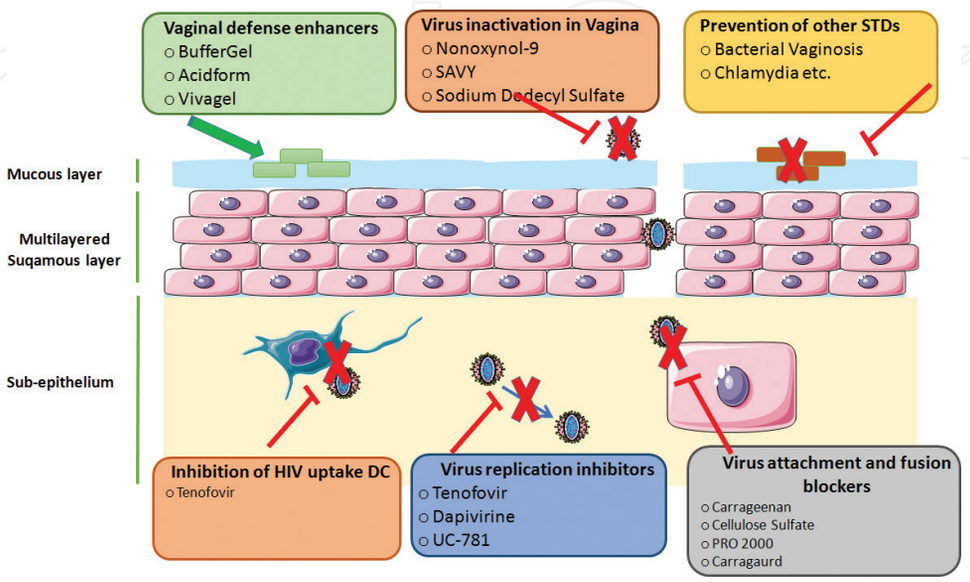

Figure 3. Categories of microbicides along with their mechanism of action.

infections increase the pH, thereby making the vagina more likely to catch infections including STIs [88]. Some acid-buffering microbicides can reduce the pH of the vagina to make it protected again from STIs. The pH-reducing microbicides include AcidForm and BufferGel. The microorganism such as *Lactobacillus crispatus* replacing missing *Lactobacillus* could also be important in enhancing vaginal defense [89].

3.2.1.1. BufferGel

BufferGel is a polymer of buffering Carbopol which is osmotically balanced with some physiological salts. They are not irritating for the genital surfaces and therefore can be used along with condoms or diaphragms. It helps to maintain the acidic pH of the vagina even in the presence of semen, thereby reducing the germ flow inside the female genital tract. The BufferGel has been reported to reduce the bacterial population in the vagina other than *Lactobacillus*, thus maintaining the natural milieu of the vagina [90]. The phase I clinical trials for BufferGel showed satisfactory results, and the agent was tolerated well by subjects [91, 92]. These trials also endorsed the potential of BufferGel to avoid pregnancy and to check the transmission of HIV-1, HPV, HSV-2, and chlamydia infections. However, phase III trial results failed to exhibit required performance level; therefore further production of BufferGel was abandoned [93].

3.2.1.2. AcidForm

AcidForm is another buffering gel used as spermicidal and microbicide. The mode of action of this gel is to maintain the vaginal acidity for a long period of time, hence protecting the vaginal and cervical epithelium from pathogens. This buffering gel is reported to be effective against HSV, chlamydia, gonorrhea, HPV, and HIV-infected leukocytes. Besides the protection from STIs, it can also act as contraceptive [94]. AcidForm trials for assessment of safety and bactericidal activity showed that it may augment mucosal defense. However, AcidForm was associated with more irritation than placebo and lower levels of antimicrobial (lactoferrin) and anti-inflammatory (IL-1ra) [95]. Currently, AcidForm has cleared phase I safety trials, while phase III trials are in progress for this buffering microbicide [96, 97].

3.2.2. Inactivation of virus in the vagina

First-generation detergents and surfactants, for example, Nonoxynol-9, sodium dodecyl sulfate (SDS), and Savvy (1% C31G), kill viral infection by disrupting their outmost coverings, i.e., envelope or capsid, thereby causing their destruction [98]. These types of microbicides are equally dangerous for the normal cells of the genital mucosa. Initial clinical trials showed their ineffectiveness in controlling the transmission of STIs including HIV-1. On these grounds, further trials were abandoned. Nonetheless, broad-spectrum viral inactivating topical microbicides are a promising agent for fight against STIs; therefore, efforts to develop novel drugs as well as combination regimes are ongoing.

3.2.2.1. Nonoxynol-9

Nonoxynol-9 (Nonylphenoxypolyethoxyethanol or N-9) was one of the earliest known spermicidal compounds that have been clinically evaluated as topical microbicides against HIV transmission [99]. Nonoxynol-9 is the active ingredient in most of the spermicidal and is

available over the counter in the form of creams, jellies, foams, gel, film, and suppositories. In various clinical trials, Nonoxynol-9 was proved to be a good spermicidal but not effective against STIs. Two phase III clinical trials in Africa demonstrated that Nonoxynol-9 does confer any protection against HIV in comparison to the placebo. In addition, its use increases the risk of genital diseases and even contracting HIV [100]. It was lately revealed that N-9 induces superficial de-epithelialization and high rate of petechial hemorrhages, thereby making the genital mucosa prone to other infections [101]. On these grounds, the World Health Organization (WHO) recently asked to include the sentence "this product does not block STIs" in the labeling of that compound [84].

3.2.2.2. Savvy (1% C31G)

Savvy or C31G (Cellegy Pharmaceuticals, Quakertown, PA, USA) is another spermicidal and antimicrobial surfactant containing acetyl betaine and myristamine oxide. The mode of action of Savvy is not much different from the N-9 but with less side effects. Moreover it has the ability to be quickly dissolved and spread on the genital mucosa [90]. At very low concentration like 0.001%, it has shown very minimum toxicity in preclinical trials and even no toxicity at 0.003% to mammalian cells as measured by MTT assay [99]. Several in vitro studies have suggested that C31G (Savvy) has the ability to disrupt the outer membrane of HIV [104]. Although, in phase I trials, Savvy proved to be safe in use, its production was stopped several years ago due to ineffective results in phase III clinical trials in Ghana and Nigeria [84].

3.2.2.3. Sodium lauryl sulfate

Because of the limitations of N-9, efforts have been directed toward the development of second-generation microbicidal agents with broader activity and lower toxicity. Sodium lauryl sulfate (SLS) is an anionic surfactant and has recently been tested as novel microbicidal agent that demonstrated significant lethal activity against a broad spectrum of STD pathogens, including HIV-1 [102]. This agent behaves as a liquid at room temperature and converts into gel form at body temperature to protect the STI transmission [103, 104]. Therefore it can be used as invisible condoms. Two phase II trials in Cameron revealed that SLS is safe to use intravaginally for long period of time and can be moved on to phase III trials [105].

3.2.3. Virus attachment and fusion-blocking microbicides

Second-generation microbicides are designed to block the entry of STI-causing pathogens into the susceptible host cells. These agents usually interfere with viral entry process by altering or blocking cellular receptors that are the first attachment sites for the pathogens. Receptor-blocking microbicides act both by nonspecific and specific mechanisms. The former mechanism blocks the attachment of multiple organisms, while latter mechanism hinders the entry of specific organism, for example, targeting CD4 receptors for the blockage of HIV entry [106].

3.2.3.1. Carrageenan

The carrageenan is an unbranched sulfated polysaccharide belonging to polyanoin class and extracted from red algae. It is chemically similar to heparan sulfate, which is used as receptor

by many pathogens to initiate the entry. These entry inhibitors work through electrostatic interaction with the viral surface proteins. The negatively charged polyanoin molecules may neutralize the positively charged surface of virus, for instance, HIV, thereby blocking attachment and entry into the healthy target cells [107].

It is in rife commercial use as a thickener in a number of products including cosmetics, food products, sexual lubricants, and infant feeding formulas. It has also been reported as an extremely potent inhibitor for a broad range of sexually transmitted infections [84]. This tropical microbicide has been proven to block HPV infectivity in vitro, even when diluted a million-fold. Carrageenan prevents the binding of HPV virions to the host cells and is observed to be more potent than heparin, a form of cell-free heparan sulfate that has been regarded as a highly effective HPV inhibitor. Carrageenan can also block HPV infection through a second, post-attachment heparan sulfate-independent effect. Besides HPVs carrageenan can inhibit HSV-2 and some strains of HIV-1 in vitro [108].

Two preparations of carrageenan, polynaphthalene sulfonate (PRO 2000) and Carraguard, have been introduced. Both the products strongly bind to STI pathogens including HIV-1, HPV, and HSV-2. Both of the carrageenan preparations have been proven to be safe in phase I clinical trials albeit in low doses. However, in phase III trials, these products proved to be ineffective; therefore, further trials were abandoned [109].

3.2.3.2. Cellulose sulfate

Polyanion cellulose sulfate is another long-chain sulfated polysaccharide that is being developed as a contraceptive and microbicidal agent. It has manifested in vitro activity against a broad spectrum of sexually transmitted pathogens. Moreover, inhibitory effect was marked up to 8 h after initiation of infection [84]. Cellulose sulfate has antimicrobial activity in vitro against various sexually transmitted pathogens including *Neisseria gonorrhoeae*, *Chlamydia trachomatis,* and HIV-1. It has been shown to bind with HIV-1 gp120 and block its interaction with D4+ receptors [110]. Phase I safety studies revealed that cellulose sulfate is safe and well tolerated [111]. However, in phase III trials, it failed to protect against HIV and tends to increase the risk of infection [111].

3.2.3.3. VivaGel

VivaGel belongs to dendrimers that are new members of polyanoin family and are highly branched nanoscale macromolecules having various surface features. These macromolecules have recently been described as microbicides with considerable safety and effectiveness [112]. Dendrimers utilize polyvalent interactions for binding and initiating biologic activity. VivaGel™ (SPL7013) is a commercial polyanion dendrimer developed by Starpharma (Melbourne, Australia). It is a water-based vaginal product in the form of mucoadhesive gel containing 3% w/w active ingredient and is administered in the vagina alone or mixed in Carbopol buffered gel that is used to maintain the physiological pH of the vagina [113]. It was developed for specific binding to surface proteins of virus, thereby blocking their attachment with the receptors of host cells. For instance, in the case of HIV, it binds with gp120 protein which virus use to attach CD4+ T cells. Several trials have been conducted to evaluate the safety and efficacy of this product. These studies unanimously describe that SPL7013 is nontoxic and safe to use even at very high concentrations of (1000 μg/ml) [112, 114]. Likewise, in

vitro trials showed effectiveness in inhibition of HIV-1 and HSV-2 infections in human cell lines as well as macaque PBMCs. Recognizing its potential, the FDA included VivaGel™ in Fast Track status since January 2006 for the prevention of HIV indication. Recently in 2016, Starpharma announced the completion of enrollment for phase III trial of VivaGel effectiveness in preventing the bacterial vaginosis and several STIs including HIV and HSV-2. Trial results are now expected in the first quarter of 2017.

3.2.4. Virus replication inhibitors

Unlike previously described microbicides, third-generation microbicides work by interfering with a specific step of virus replication cycle when administered locally on the mucosa. The most widely studied examples of virus replication blocker are antiretroviral (ARV) microbicides, which can be divided into nucleoside reverse transcriptase inhibitors (NRTI) and non-nucleoside reverse transcriptase inhibitors (NNRTI). Both of these ARV microbicides block the activity of HIV reverse transcriptase enzyme that is required for the conversion of RNA into DNA, an essential step required for integration of HIV into the host genome [115]. This blockage ultimately reduces the number of HIV virions in the infected cell. These next-generation microbicides are formulated in the form of long-acting vaginal ring, film, or gels. In addition to other benefits, the reverse transcriptase inhibitor class of microbicides is also cost-effective. Some of the ARV examples being tested include tenofovir, dapivirine, and UC-781.

3.2.4.1. Tenofovir

Tenofovir is a highly acclaimed antiretroviral drug which obstructs the virus growth inside the host cells. It is usually taken in the form of pills, and it has become a necessary component of the three-drug cocktail for antiretroviral therapy approved by the FDA [116]. However, it has also been prepared in the form of gels to be used as topical microbicides. The gel preparation of tenofovir is currently under assessment as a tropical microbicide for the prevention of HIV infection in high-risk populations, and several safeties and efficacy-related trials are underway.

A study known as "CAPRISA 004" completed by scientists at the Centre for the AIDS Programme of Research in South Africa (CAPRISA) in 2010 revealed that 1% tenofovir gel application before and after sex was 39% efficient in reducing a woman's risk of getting infected with HIV and 51% effective in preventing genital herpes infections [117]. Moreover in this study, the protective effect against HIV increased with gel adhesiveness and increased use of tenofovir. For example, 54% reduction in HIV infections was observed in women who used gel in more than 80% of their sex acts, whereas those who used the gel in less than half of their sex acts had a 28% reduction in HIV infections. The efficacy of tenofovir gel for the prevention of HIV was also evaluated in another study known as VOICE. Unlike CAPRISA, this study reported that tenofovir preparation does stop the transmission of HIV-1 [118]. However, poor adherence was employed by the participants of this trial.

Another trial on tenofovir gel known as "The FACTS 001" was conducted to evaluate the reproducibility of the CAPRISA 004 trial results. The results of this trial were announced on February 24, 2015. Disappointingly, the study did not confirm the pericoital tenofovir gel

effectiveness. In this trial, the gel only showed a protective effect when used consistently and covered most of the sex acts, but most women in this trial were unable to use it in this manner. However, the gel appeared to be acceptable and easy to apply for most of the women [119]. Very recently, the use of tenofovir on the genital mucosa has been linked with some toxic effects such as suppression of anti-inflammatory mediators, increased T-lymphocyte infiltration of the mucosa, and induced mitochondrial dysfunction, which were noticed [105]. These unexpected results led to tenofovir gels being dropped from a large ongoing clinical trial.

3.2.4.2. UC-781

UC-781 is a hydrophobic thiocarboxanilide nonnucleoside reverse transcriptase inhibitor (NNRTI) developed as antiretroviral drug having high affinity for HIV reverse transcriptase. This agent eagerly crosses membrane barriers and inactivates reverse transcriptase even before entry into the cell, thereby acting as potent antiretroviral agent. However the production of it as antiretroviral drug was abandoned due to poor bioavailability. Later on, this agent was developed in the form of tropical formulation. Phase I safety trials confirmed its safety for vaginal application at lower concentrations [120]. Recently UC-781 in the form of vaginal ring has been reported to provide strong protection against HIV transmission. Moreover no toxicity was observed in this study [121]. Further large-scale clinical trials are required before the UC-781 microbicide gel formulation reported to be successful in the prevention of HIV-1 sexual transmission.

3.2.4.3. Dapivirine

Dapivirine being nonnucleoside reverse transcriptase inhibitor has been reported to act as microbicide against various STIs, particularly HIV-1. It is reported to have dual mode of action against HIV-1; it inhibits both viral entry and reverse transcription stopping the conversion of viral RNA to proviral DNA. Because of dapivirine's tight binding and lipophilic characteristics, it may be active against both cell-free and cell-associated HIV [122]. Dapivirine is the only microbicide used in human in the form of vaginal rings and believed to be nontoxic [123]. The phase III clinical trials (the ASPIRE study) showed a 27% reduction in HIV-1 acquisition upon using dapivirine vaginal rings [124]. The protection effect was more pronounced in the women aged 21 or more; however, no significant protection was observed for women under age 21 [10]. Another phase III trial for efficacy evaluation of dapivirine is in progress.

4. Conclusion and future prospects

Microbicides offer an accessible, easy-to-use, and low-cost option for the control of sexually transmitted infections and hence have been an area of high interest for scientists and researchers around the world. However, progress in this area has been dismal, and very few microbicide drugs have so far been able to enter the market. With ever-growing epidemic of STIs, particularly in less developed areas of the world, there is an immediate need to gear up concerted efforts for the development and discovery of novel microbicide compounds. Moreover,

it is imperative that efforts should be made to develop novel delivery methods to improve the efficacy of existing microbicides. Results from recent trials can guide for more development of more rationally, accurately, behaviorally, and socially accepted and single solution maximum potential STIs.

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