Research Article

Superadded bacterial and fungal infections in oral and genital herpes simplex lesions

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Received: August 08, 2020; **Accepted:** August 27, 2020

ABSTRACT

Background: Two types of herpes simplex virus (HSV) HSV type 1 (HSV-1) and HSV type 2 (HSV-2) can cause oral and genital infections, respectively. Superadded bacterial and fungal infections of HSV lesions are one of the complications seen in herpes simplex infections. **Objective:** The study aims to identify the super added bacterial and fungal infections in oral and genital herpes simplex infections. **Materials and Methods:** This is an observational study carried out at SRM Medical College Hospital and Research Centre, Tamil Nadu, India, from February 2019 to February 2020 after the Institutional Ethical committee approval. Patients with clinically suspected oral and genital herpes infections in all age groups are included in the study excluding neonates and infants. Under aseptic precautions vesicular fluid, blood and wound swabs were collected. Vesicular fluid examined by Tzanck smear for multinucleated giant cells, and Immunoglobulin M enzyme-linked immunosorbent assay performed in positive cases. Superadded bacterial and fungal infections identified using conventional culture and identification methods. **Results:** In this observational study, a total of 75 patients were screened for oral and genital herpes infection. Thirty-five (46.6%) were clinically positive. Out of the positive cases, Tzanck smear was positive in 17 (48.6%), superadded bacterial and fungal infections are seen in 18 (51.4%). The most common super added infections are *Candida albicans* 9 (50%), *Candida* non-albicans 4 (22.2%), *Escherichia coli* 2 (11%), *Staphylococcus* 1 (5.6%), *Enterococcus* 1 (5.6%), and *Proteus mirabilis* 1 (5.6%). **Conclusion:** Most common superadded infections associated with oral and genital herpes simplex infections are with *Candida* species, *E. coli*, and *Staphylococcus*.

KEY WORDS: Herpes Simplex Virus; Superadded Infections; Tzanck Smear, Multinucleated Giant Cells; Oral and Genital Herpes

INTRODUCTION

Herpes simplex virus (HSV) is a viral infection occurring naturally in human beings. HSV belongs to the Herpesviridae family and its sub family Alpha. It can be classified into two types, namely, HSV type 1 (HSV-1) and HSV type 2 (HSV-2).[1,3]

Access this article online	
Website: http://www.ijmsph.com	Quick Response code
DOI: 10.5455/ijmsph.2020.08135202027082020	

Human herpes virus infections are caused by the eight types of herpesviruses. Most commonly gingivostomatitis, herpes labialis, and herpes keratitis are caused by HSV-1 and genital lesions caused by HSV-2.[5]

In worldwide, HSV infections are occurred without the seasonal distribution. As per the World Health Organization, HSV-1 is prevalent in 3.7 billion people under the age of 50 years (67%) and HSV-2 is prevalent in 417 million people under aged 15–49 years (11%).[10]

Mode of transmission is by direct contact with virus in secretions. The incubation periods usually ranges from 1 to 26 days.[2] HSV-1 transmission by direct oral contact or

infected saliva termed as oral herpes.[6] HSV-2 transmission due to direct sexual contact or vertical transmission termed as genital herpes.[6] HSV is transmission by direct contact with a person actively shedding virus. HSV remains dormant in nerve ganglia and emerges periodically causing symptoms Recurrent herpetic eruptions are precipitated by immunosuppressive conditions, febrile illnesses, stress involving physical or emotional status and prolonged exposure to sunlight.[4,6]

Complications of HSV in immunocompromised patients involve the degree of damage to the immune cell. Extensive tissue damage and necrosis remain the most common complications in HSV infections in immunodeficiency patients, contiguous mucosal spread resulting in systemic dissemination. Both severe and frequent recurrent infections are seen in solid organ transplant recipients, human stem cell transplant recipients, and higher risk groups such as HIV/AIDS infected patients.[21,24]

The human microbiome is a niche of diverse organisms at various sites. Microbiome includes viruses causing chronic and persistent infections.[28] In the oropharynx/nasopharynx and genital tract HSV-1 and HSV-2 are members of the microbiome, respectively, and viral shedding occurs in the presence or absence of symptoms. In immune-competent individuals HSV causes latent infections with chronic and persistent asymptomatic viral shedding.[5,6,17]

Alterations in the herpes surface entry mediators such as heparan sulfate, nectins, and tumor necrosis factor receptors occur after the entry of HSV into the cells. Because of this alteration and the interactions with the colonizers and the opportunistic pathogens, biofilm formation is initiated. The most common colonizers *Staphylococcus* and *Candida* occupy the nasal nares and oral mucosa, respectively, in genital tract co-colonization of *Staphylococcus aureus* causes aerobic vaginitis, *Candida albicans* produces mucosal lesions.[20] The oral and genital microbiome and their interaction and it impacts on the ability to initiate biofilm formation through adherence to the host cell surface has been applied to define co-colonization/infection.[22,28]

The common fungal coinfection in HSV lesions can be caused by variety of *Candida* species, and the most common bacterial coinfection are be caused by organism such as *Streptococcus* spp., *S. aureus*, *Treponema pallidum*, *Chlamydia trachomatis*, and *Mycoplasma* spp.[11,12,23,26,29]

HSV and candida infection of the oral mucosa are very common among humans.,[24] *C. albicans* remains the most common co infective agent.[18,24] HSV-1 infections are most commonly developed during childhood, with most common presentations as oral mucosal lesions, gingivostomatitis, labialis, tonsillitis, keratitis, conjunctivitis, vesicular lesions of the skin, and encephalitis.[1]

About 60–90% of HIV infected persons are coinfected with HSV-2. More serious complications involving central nervous system and multiorgan involvement and dissemination are seen in HSV-2 infections in immunocompromised patients. Infectivity of HSV is more with HIV coinfection.[12,13,23,25]

Polymicrobial infections, coinfection, and cocolonization are more frequently discussed in any lesions. The analyzed data of phylogenetic sequence have revolutionized the myths of microbial interactions with the host. Many multi-factorial mechanisms are proposed, mainly the polymicrobial growth, biofilm formation, tolerance of antimicrobial therapy, property to coinvade host tissue, exacerbate quorum sensing and toxin production, and synergism in infections. All these factors contribute to increased pathogenicity and coinfections.[22,27]

Biofilms are complex structure with one or more microbe in an extracellular matrix polysaccharide. Biofilm can be formed in living tissues as well on surfaces and devices. Biofilms promotes microbial growth, adhesion to body surfaces, protection from the host immune system, and unfavorable conditions resulting in resistance to antimicrobial agents. These factors contribute, biofilm as an important source of infections and virulence factor.[28]

C. albicans is an opportunistic pathogen in the human gut and the mucosa. Immune status of the patients contributes to the transformation from commensal to pathogen, especially following bacterial or viral infections. Mucosal candidiasis associated with inflammation is the result of yeast cell overgrowth.[22]

Bacterial infections most often follow viral infections, and the resulting coinfection causes more complications, the interaction between the viral components and the bacterial agents increases the tissue damage and enhances invasiveness.[19] Production of toxins, manipulation of immune mechanism, avoidance of clearance by immune cells, formation of biofilms, antimicrobial resistance increases the virulence of bacterial agents, and promote the coinfections and superadded infections.[22,27]

Polymicrobial nature, cointeractions among colonizers and the invaders, host immune status together exacerbates pathogenicity and the superadded bacterial infections.

Aim and Objectives

The aim of the study was to identify the super added bacterial and fungal infections in oral and genital herpes simplex lesions.

MATERIALS AND METHODS

Study type: This was a observational study

Study Period: 1 year (February 2019–February 2020)

Study location: This study was conducted in the Department of Microbiology, SRM Medical College Hospital and Research Centre (MCH and RC), Kattankulathur

Study sample size: The sample size was 75

Institutional Ethical Committee approved- Ethics Clearance number:1730/IEC/2019.

Criteria

Inclusion criteria

Clinically suspected oral and genital herpes in all age groups from SRM MCH and RC in Kattankulathur were included in the study.

Exclusion criteria

Neonates and infants were excluded from the study.

Materials and Methodology

Specimen collection

Under aseptic precaution as per the standard protocol, vesical fluid from oral and genital lesions collected using a sterile swab by the clinician with the appropriate consent from patient.

The collected samples observed under the direct microscopy (Tzank smear) for multinucleated giant cell. Immunoglobulin (Ig) M enzyme-linked immunosorbent assay performed in positive cases.

Bacterial and fungal coinfection identified using conventional culture and identification methods.

Microscopic Examination

Tzanck smear

Principle

Tzanck smear is a simple and rapid technique. The sample should be collected from a fresh vesicle, to ensure the production of virus infected cells and not from the crust. The crust removed and the vesicle must be unroofed, the base scraped with a scalpel or with the edge of spatula. Take a clean slide, the material is transferred to glass slide by touching the spatula to glass slide repeatedly with gently.[7-9]

Procedure

Tzanck smear

↓

Preparation of Tzanck smear

↓

Fixation of Smear

↓

Staining of Tzanck smear
(Add cytochrome stain 8–10 drops)

↓

Wait for 1 min and

Add 20 drops of buffer (washed after 6 min)

↓

Dry and examination Cytopathology by wrights or giemsa stain detects
Formation of multinucleated giant cell [Figure 1]

Gram stain

Gram stain helps to identify the morphology and preliminary identification of bacterial and fungal infections in the specimen. This shows the presence of Gram-positive bacteria, Gram-negative bacteria, and Gram-positive budding yeast cells in the specimen.

Principle

A basic crystal violet dye and iodine combine to form an insoluble dark purple compound in the protoplasm and cell wall of bacteria. This compound is dissolvable in decolorizes and removed from the cell. The removal is slower from Gram-positive than from Gram-negative bacteria. As the Gram-positive bacteria have thicker peptidoglycan layer in the cell wall, they are less permeable to the stain.

Procedure

Step 1: Primary Stain

Crystal violet for 1 min (water rinse)

↓

Step 2: Mordant
Gram's iodine for 1 min (water rinse)

↓

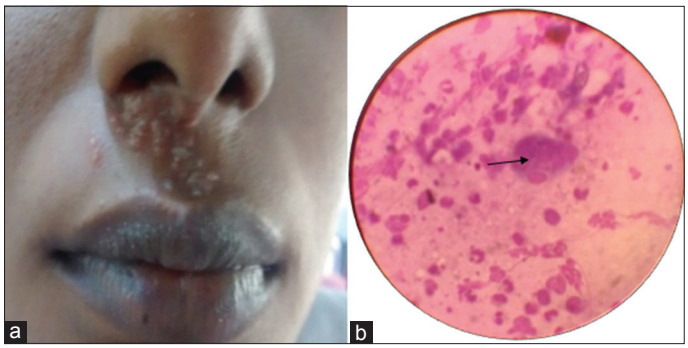

Figure 1: (a) Oral vesicular lesions, (b) Multinucleated cells in Tzanck smear

Step 3: Decolourization
Acetone for 2–3 s (water rinse immediately)

Step 4: Counterstain
Dilute carbol fuchsion or safranin for 3 s
(Water rinse and blot)

Smear is examined under oil immersion objective.

Culture: [Figures 2-7]

The specimen collected is cultured in Nutrient agar, MacConkey agar, and Blood agar for bacterial growth and in Sabouraud's dextrose agar for fungal growth.

After 24 h incubation at 37°C, all the culture plates were observed for the bacterial and fungal growth.

Bacterial Growth

Gram staining and required biochemical reactions performed based on the isolates and identified.

Fungal Growth

After the sufficient incubation temperature and conditions, colony morphology seen. The pure fungal growth further identified by Gram staining, lactophenol cotton blue mount, slide cultures and required identification tests performed based on the fungal isolate.

RESULTS

In this observational study, a total of 75 patients were screened for both oral and genital herpes infection. The samples were collected from Dermatology and Gynaecology Department. All age groups were included in the study except neonates and infants.

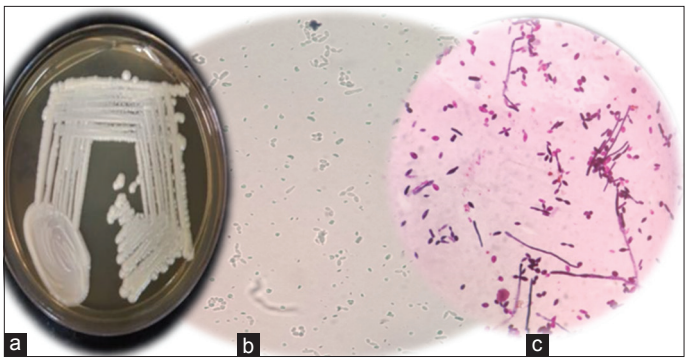

Figure 2: (a) *Candida* colonies in Sabouraud's Dextrose Agar, (b) Positive Germ tube test (c) Gram staining showing Gram-positive budding yeast cells

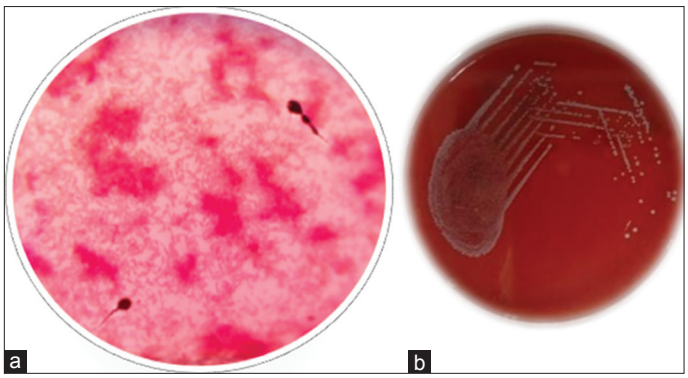

Figure 3: (a) Gram-negative bacilli, (b) Non-hemolytic gray colonies in blood agar plate

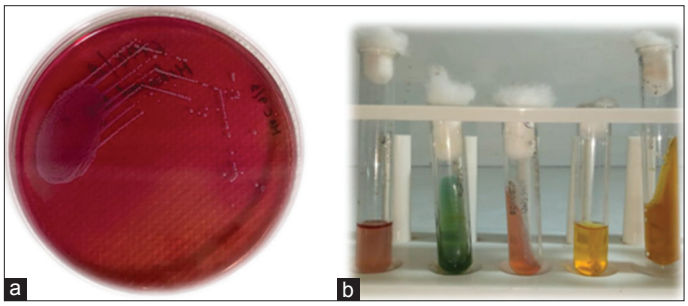

Figure 4: (a) Lactose fermenting colonies in MacConkey agar plate (b) Biochemical reactions of *Escherichia coli*, Indole -positive, Triple sugar iron agar with Acid/Acid with gas, citrate and urease negative, mannitol motility medium showing mannitol fermentation and a motile organism

Out of total 75 patients, 35 (46.7%) patients were positive and 40 (53.3%) patients were negative for herpes simplex infections. The oral lesions are seen in 12 (34.3%) and genital lesions seen in 23 (65.7%) out of 35 positive cases [Chart 1].

Out of the 35 positive cases, Tzanck smear was positive in 17 (48.6%) cases and Superadded bacterial and fungal infections seen in 18 (51.4%) cases [Chart 2].

In the present study, the most common super added infections are *C. albicans* 9 (50%), *Candida* non-albicans 4 (22.2%) *Escherichia coli* 2 (11%), *Staphylococcus* 1 (5.6%), *Enterococcus* 1 (5.6%), and *Proteus mirabilis* 1 (5.6%) [Table 1].

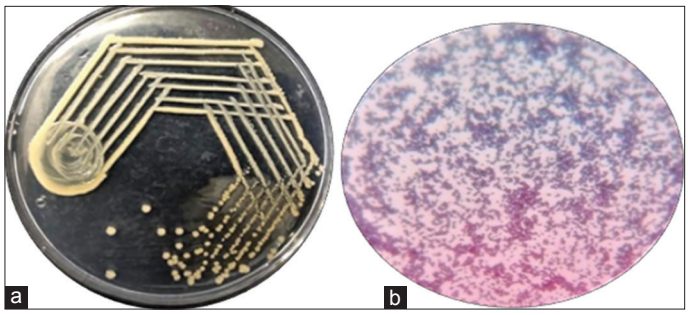

Figure 5: (a) Golden yellow colonies of *Staphylococcus aureus* in nutrient agar plate (b) Gram Stain showing Gram-positive cocci in clusters

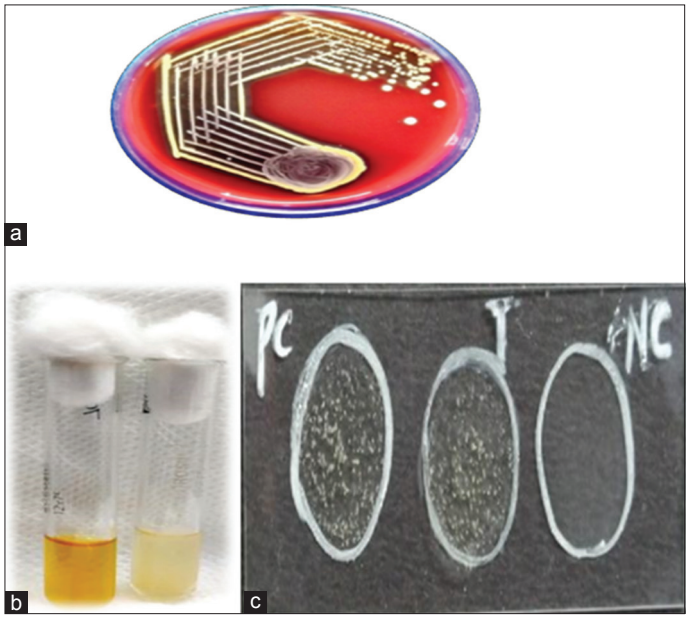

Figure 6: (a) Beta-hemolytic colonies in blood agar plate, (b) Mannitol fermentation (c) positive Slide Coagulase test

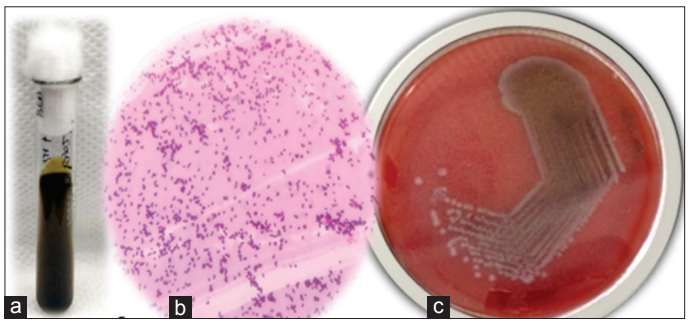

Figure 7: (a) Enterococcus colony grown in, Bile esculin agar, (b) Gram stain showing Gram-positive oval cocci in pairs, (c) Non-hemolytic colonies in blood agar plate

n = 75.35 (46.7%) positive for herpes simplex infections, out of 35 positive cases, superadded bacterial and fungal infections seen in 18 (51.4%) cases and Tzanck smear positive in 17 (48.6%)

n = 35, with 12 (34.3%) oral lesions and 23 (65.7%) genital lesions

n = 18, Most common superadded fungal infections are by *C. albicans* 9 (50%), *Candida* non-albicans 4 (22.2%)

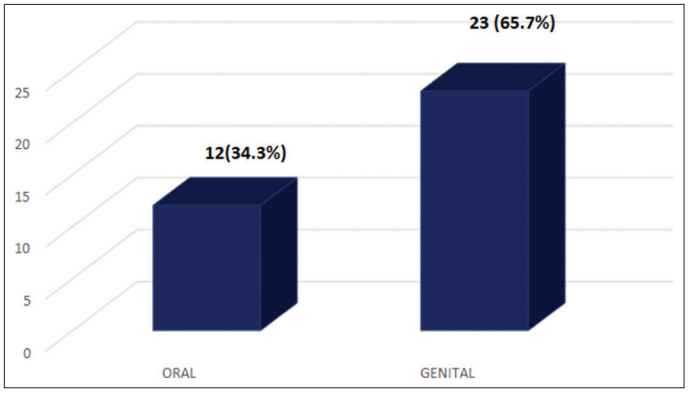

Chart 1: DitributionDistribution of oral and genital lesions

Table 1: Distribution of superadded bacterial and fungal infections in HSV 1 and HSV 2 lesions

Organism	Total no. of patients with superadded infections *n*=18 (%)
Candida albicans	9 (50)
Candida non-albicans	4 (22.2)
Escherichia coli	2 (11)
Staphylococcus aureus	1 (5.6)
Enterococcus	1 (5.6)
Proteus mirabilis	1 (5.6)

Most common bacterial infections are with *E. coli* 2 (11%), *S. aureus*, *Enterococcus* species, and *P. mirabilis* each 1 (5.6 %).

DISCUSSION

HSVs known as HSV belong to the Herpesviridae family. Two types of herpes, namely, HSV-1 associated with oral herpes simplex infections and HSV-2 most commonly associated with genital herpes simplex infections. In the present observational study conducted at SRM MCH and RC after institutional ethical approval, a total number of 75 patients screened for oral and genital herpes simplex infections presented with clinical symptoms of fever, malaise, acute toxicity, vesicular lesions, and ulcerative lesions with a scalloped border/crust. Out of total 75 patients, 35 (46.7%) patients were positive and 40 (53.3%) patients were negative for herpes simplex infections. The oral lesions are seen in 12 (34.3%) and genital lesions seen in 23 (65.7%) out of 35 positive cases. Out of the 35 positive cases, Tzanck smear was positive in 17 (48.6%) cases and superadded bacterial and fungal infections seen in 18 (51.4%) cases

In a study conducted by Sripriya *et al.,* a total of 296 adults were surveyed in various districts of Tamil Nadu. IgG and IgM anti HSV antibodies test performed in the collected samples.[14] The prevalence of HSV-2 in population, 10.2%, 7.14%, and 14.5% in Kanchipuram, Thiruvannamalai, Erode districts, respectively. It showed that women had higher prevalence. Similarly, present study conducted at SRM MCH and RC, also women had higher prevalence.

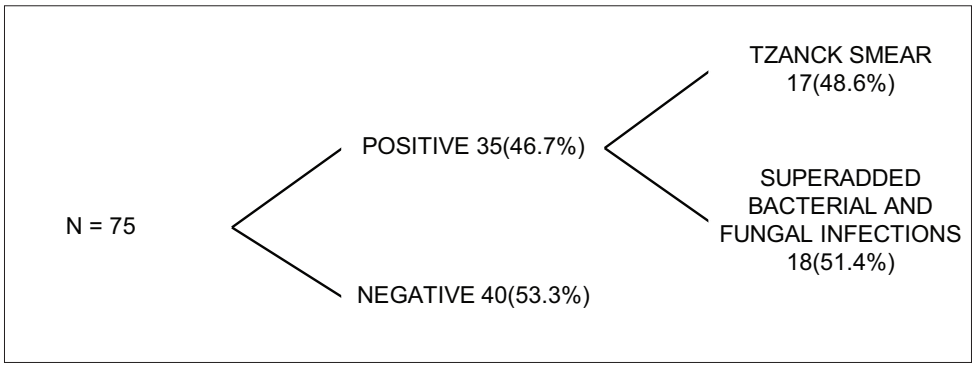

Chart 2: Herpes simplex virus type 1 and 2 lesions with positive Tzanck smear and superadded bacterial and fungal infections

The present study shows 51.4% of superadded bacterial and fungal infections, which has a slight increase in percentage compared with the study conducted by Mehrabani *et al*., where *Candida* spp. and *Coccobacilli* cervical infection were considered as fungal-bacterial infection with a prevalence of 45.3% and non-fungal-bacterial infection with a prevalence of 54.7%. In the present study, the most common super added infections are *C. albicans* 9(50%), *Candida* non-albicans 4 (22.2%) *E. coli* 2 (11%), *Staphylococcus* 1 (5.6%), *Enterococcus* 1 (5.6%), and *P. mirabilis* 1 (5.6%).

The strength of this study is screening of superadded fungal and bacterial infections in the oral and genital herpes lesions, which has not been done previously in this geographical location. This study will help in treatment methods in recurrent herpes infections and to evaluate the coinfections. The limitations of this study are the lesser sample size than the other studies done and also concerns with using more specific culture methods for the bacterial and fungal isolates, which would have identified more isolates and helped in the evaluation of the isolates *C. trachomatis* and *Neisseria gonorrhoeae* were not identified in this study, as more specific culture media were not used.

CONCLUSION

In this study, the most common superadded bacterial and fungal infections associated with oral and genital herpes simplex lesions are with *C. albicans* and Candida non-albicans, *E. coli*, *S. aureus*, *Enterococcus* species, and *P. mirabilis*. The study shows that most common superadded infections in HSV lesions are by fungal agents than the bacterial agents. Among the bacterial agents Gram-negative infections are more prevalent than Gram-positive agents. The present study superadded fungal infections are more common than the bacterial infections. This study will help in identifying the bacterial and fungal coinfections associated with HSV-1 and HSV-2 lesions. Screening for the superadded bacterial and fungal infections will help in the treatment modalities of recurrent herpes simplex infections and in preventing the complications associated with it.

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How to cite this article: Jayaraman M, Leela KV, Rajalakshmi E. Superadded bacterial and fungal infections in oral and genital herpes simplex lesions. Int J Med Sci Public Health 2020;9(8):468-474.

Source of Support: Nil, **Conflicts of Interest:** None declared.