



Evaluation of Pathogenic Potentialities and Transmissibility of Aerobic and Anaerobic Bacteria in Sexually Transmitted Diseases

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Authors' contributions

This work was carried out in collaboration between all authors. Author KM managed the literature searches. Authors MM and DM designed the study. Authors MM, DM and MG carried out the experimental studies and the analyses was done by the authors MM and SGD. Author SGD wrote the protocol and also the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Sexually transmitted diseases (STDs) caused by various aerobic and anaerobic bacteria have been reported from many developed and developing countries of the world. However, there is limited data available on the association of these pathogens with STDs on the Indian sub-continent. Therefore, the aim of this is to the presence of anaerobic and aerobic bacteria in sexually transmitted infections.

Study Design: Patients attending the Department of Dermatology, Venereology & Leprology, Calcutta Medical College & Hospital, suspected to be suffering from STDs, were thoroughly examined and those having typical lesions of chancroid were excluded

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from further work. The prevalence of different aerobic and anaerobic bacteria was determined from among the remaining patients after ruling out cases of chancres.

Place and Duration of Study: This work was carried out in the Department of Oral Medicine, R. Ahmed Dental College; Department of Dermatology, Venereology & Leprology, Calcutta Medical College and Hospital; Division of Microbiology, Department of Pharmaceutical Technology, Jadavpur University and Department of Microbiology, Herbicare Healthcare Bio-Herbal Research Foundation, Kolkata, for a period of seven months from April 1 to October 31, 2012.

Methodology: At least two smears were prepared from the infected ulcers of each of the patients who were not diagnosed as of chancroid. One smear was for dark ground microscopy and the other for Gram's staining. Confirmation of chancre was by serological testing, while aerobes/anaerobes were identified following standard procedures.

Results: The organisms isolated were *Staphylococcus aureus*, *Shigella flexneri*, *Shigella sonnei*, *Gardenerella vaginalis*, *Actinomyces* spp, *Veillonella parvula*, *Peptococcus heliotrinreducens*, *Peptostreptococcus magnus* and *Peptostreptococcus hydrogenalis*. These were subjected to tests for their antibiotic sensitivity pattern which was followed by successful specific therapy.

Conclusion: Various Gram positive and Gram negative aerobes and anaerobes were found to be associated with STDs and these were transmissible among homosexual and heterosexual partners.

Keywords: Sexually transmitted diseases; aerobes; anaerobes; peptostreptococcus; peptococcus.

1. INTRODUCTION

Sexually transmitted diseases (STDs) which are also referred to as sexually transmitted infections (STIs) are transmitted by men and women by various sexual behaviours. STDs ranks among the most common of all infectious diseases, with 30 different infections now classified as predominantly sexually transmitted or as frequently sexually transmissible diseases [1-3]. Incidences of STDs have been proved to be considerably high in most of the developed and developing countries [4]. More than 20 pathogens have been known to cause STD and a few of them including *Chlamydia* and viral agents had been reported to be in the process of replacing the classical bacterial diseases of syphilis, gonorrhoea and chancroid with respect to their frequency of transmission among men and women [5]. However, for the past several years many different bacterial pathogens have been reported as causative agents of STD among both heterosexual and homosexual men and women. These include *Shigella* spp, *Gardenerella vaginalis*, *Staphylococcus aureus* and the anaerobic bacteria *Peptostreptococcus* spp [6-13]. The present study describes invasion of genital organs and oral cavities by various aerobic and anaerobic bacteria and the possibilities of transmission of such invaders among sex partners. Further studies revealed that these organisms were often multidrug resistant. Treatment was ensured by administering the selected antibiotics.

2. MATERIALS AND METHODS

2.1 Patients

A total of 117 patients attending the Department of Dermatology, Venerology and Leprology, Calcutta Medical College and Hospital, Calcutta were examined during April 2012 and October 2012. Of these 88 were male patients and 29 were females. Among these 88 male patients 48 had lesions typical of chancroid and 15 out of 29 female patients were found to be suffering from chancroid. All these were excluded from the present study. The remaining 54 patients were found to be suffering from oral ulcers, genital ulcers as well as anal ulcers. At least two smears were prepared from every lesion; one of the smears was observed through dark ground illumination (DGI) microscopy, while the others were stained with Gram's method. Simultaneously blood was drawn from every patient; serum was separated and tested for presence of anti-treponemal antibody with the help of VDRL test as described [14-15]. In 31 out of 54 cases *Treponema pallidum* was found to be the causative agent and these were excluded from the study. The remaining 23 patients were investigated thoroughly for invasion by various aerobic and anaerobic organisms. Specimens were collected from the ulcers and inoculated on to all the test media for isolation of different pathogens. History of infection of the patients revealed that the lesions in many of them were often 15 to 30 days old; however, a few of them mentioned that they had the infection for more than 3 to 5 months. Such patients were mostly female who felt shy of visiting an STD Clinic due to the normal inhibition prevailing among Indian women with rather poor financial background. Seven pairs of patients were investigated to determine sexual behavior practices. Of these 4 pairs were involved in both oral and genital sex, one pair was engaged in oral sex only, while the remaining two pairs were homosexual men.

2.2 Media

Liquid media were nutrient broth (NB, Oxoid) and thioglycollate broth (TGB, Oxoid). Solid media were nutrient agar (NA, Oxoid), Deoxycholate Citrate Agar (DCA, Oxoid) and blood agar (BA) which was prepared by adding 10% defibrinated sheep blood to molten NA.

2.3 Collection of Material and Inoculation

2.3.1 From the oral ulcers

The patient was allowed to wash the mouth with sterile drinking water, to avoid the commensal organisms as much as possible. Sterile gloves were used to obtain the material from the infected region. A sterile swab was rubbed over the surface of the lesion and taken straight away to the adjacent laboratory where they were inoculated into different media as described below.

2.3.2 From the genital and anal ulcers

The lesions in the genital organs as well as the anal region were cleaned with sterile saline, adherent layer was gently removed where necessary without causing bleeding, but allowing outflow of clear fluid by gentle pressure around the ulcer, fluid was collected by a sterile capillary tube or a narrow sterile cotton swab.

2.3.3 Inoculation and incubation

Each swab was first rubbed on one corner of BA or NA and also on DCA plates. The same swab was then inserted into TGB and finally to NB. With the help of a sterile loop, the inoculated material was gently spread over the entire surface of the agar plates in such a manner that numerous isolated colonies could appear after incubation, that would help in eliminating contaminants as well as in further identification process. NB, TGB, NA and DCA plates were incubated at 37°C and BA plates were kept into the anaerobic jar (Gas pack system) at 37°C. After 24h, they were all examined for the appearance of growth. Incubation was extended in the broth or plates, where growth had failed to appear.

2.4 Identification

As the growth appeared on BA or NA or DCA plates, typical isolated colonies were picked up and stained for gram reaction. Gram positive and Gram negative aerobic organisms obtained from NA and DCA plates were identified with the help of various physical and biochemical tests as described by Barrow and Feltham [16]. The causative anaerobic bacteria as observed on BA plates and in TGB were also stained first for gram reaction. All the growths were then tested further with the help of several biochemical parameters, some of which were common with aerobes, while some tests were specific for final identification of anaerobes [17].

2.5 Determination of Minimum Inhibitory Concentration (MIC) of Various Antibiotics

Agar dilution technique was employed to determine the MIC of different antibiotics with respect to different organisms following international standard guidelines [18,19,20]. The MIC of an antibiotic was taken to be its lowest concentration in which there was no visible growth. The antibiotics tested were penicillin (Pc), carbenicillin (Cb), erythromycin (Er), doxycycline (Dx), gentamicin (Gm), ciprofloxacin (Cf), ofloxacin (Of) and cotrimoxazole (Cmx); these were procured from their respective manufacturers in India in pure dry powder form and stored at 4°C. The amounts of an antibiotic ($\mu\text{g/ml}$) were: 0 (control), 2, 5, 10, 25, 50, 100 and 200. Antibiotic sensitivity test was performed on NA plates for aerobic organisms and on BA for anaerobic bacteria. The bacteria that showed MIC between 0 and 10 $\mu\text{g/ml}$ level were considered as sensitive (S). When the bacteria could be inhibited between 25 and 100 $\mu\text{g/ml}$ of an antibiotic, the reaction was considered as intermediate (I), while the growth of an organism above 100 $\mu\text{g/ml}$ was designated as resistant (R).

3. RESULTS

3.1 Transmission of Aerobic and Anaerobic Bacteria among Sex Partners

Out of 23 cases of STD, 7 pairs of patients revealed transmission of bacteria among sex partners Table 1. Of these, the first pair was found to be engaged only in oral sex and subsequent 4 pairs were heterosexual and the remaining 2 pairs were homosexual men. In Group I, where the pair used to perform only oral sex *G. vaginalis* was cultivated from the oral ulcer of the man and genital ulcer of the woman, while pure culture of *S. aureus* was obtained from the oral cavity of the woman and penile ulcer of the man. In Group II, patients were engaged in both oral and genital sex. Of these, the first pair was found to be suffering from invasion by *Veillonella parvula* in their oral cavities as well as the genitalias. The

following pair harboured *Peptococcus heliotrinreducens* in their oral and genital ulcers, while the third pair in this group revealed pure cultures of *Peptostreptococcus hydrogenalis* in either of the affected regions. The fourth pair showed invasion of both the affected regions by *Actinomyces* spp. The last group (Group III) shows two pairs of homosexual men who were regular partners performing both oral and anal sex. The bacteria isolated from them were *Peptostreptococcus magnus* and *Peptostreptococcus hydrogenalis* Table 1.

Table 1. Isolation of various aerobic and anaerobic bacteria from oral and genital ulcers of sex partners

Group	Sex	Age	Bacteria isolated from	
			Oral ulcer	Genital/anal ulcer
I	Performing oral sex only	¹ M 22	<i>G. vaginalis</i>	<i>S. aureus</i>
		¹ F 22	<i>S. aureus</i>	<i>G. vaginalis</i>
II	Performing both oral and genital sex	² M 24	<i>Veillonella parvula</i>	<i>Veillonella parvula</i>
		² F 20	<i>Veillonella parvula</i>	<i>Veillonella parvula</i>
		³ M 18	<i>Peptoco.</i>	<i>Peptoco.</i>
		³ F 18	<i>heliotrinreducens</i>	<i>heliotrinreducens</i>
		⁴ M 19	<i>Peptoco.</i>	<i>Peptoco.</i>
		⁴ F 20	<i>heliotrinreducens</i>	<i>heliotrinreducens</i>
		⁵ M 35	<i>Pepto. hydrogenalis</i>	<i>Pepto. hydrogenalis</i>
III	Performing both oral and anal sex (homosexual)	⁶ M 25	<i>Pepto. magnus</i>	<i>Pepto. magnus</i>
		⁶ M 28	<i>Pepto. magnus</i>	<i>Pepto. magnus</i>
		⁷ M 30	<i>Pepto. hydrogenalis</i>	<i>Pepto. hydrogenalis</i>
		⁷ M 34	<i>Pepto. hydrogenalis</i>	<i>Pepto. hydrogenalis</i>
			<i>Actinomyces spp</i>	<i>Actinomyces spp</i>
			<i>Actinomyces spp</i>	<i>Actinomyces spp</i>
			<i>Actinomyces spp</i>	<i>Actinomyces spp</i>

M, male; F, female; superscript numbers 1-7 denote sex partners

3.2 Distribution of Different Aerobic and Anaerobic Organisms among STD Patients

Nine of the remaining 23 patients were found to be infected with a variety of microorganisms Table 2. Patient 1 had infection by *S. aureus* in both the ulcers. In patient 2, *G. vaginalis* was obtained from the oral ulcer while *G. vaginalis* and *K. pneumoniae* were cultivated from the genital ulcer. The following two patients distinctly revealed invasion of oral and genital organs by the same species of genus *Shigella*. The following 4 patients [5-8] were found to harbour the same anaerobes in both the sites. Patient 9 showed oral ulceration due to one anaerobic bacterium, but genital ulceration by two anaerobes, one being common with the oral invader Table 2.

3.3 Effects of Antibiotics on Various Isolated Pathogens

An analysis of the results presented in Table 3 indicates that *G. vaginalis* was resistant to Pc, Er, Dx and Cmx and *S. aureus* was also resistant to the same antibiotics. *V. parvula* was resistant to only Cmx, while *Actinomyces spp.* from Table 1 was resistant to Pc, Er, Dx, Gm and Cmx. *P. heliotrinreducens* and *P. magnus* were resistant only to Er. *P. hydrogenalis* was resistant to Er and Cmx, *S.aureus* obtained from Table 2 was resistant to Pc, Er, Dx and Cmx, while *G. vaginalis* was resistant only to Pc and Er. Strains of shigellae were

simultaneously resistant to Pc, Er and Cmx. *P. heliotrinreducens* was resistant to Er and Cmx, while *P. hydrogenalis* was resistant only to Cmx. *P. magnus* Table 2 was a rather sensitive bacterium. Since most of the pathogens were sensitive to Cb, Cp and of the patients were treated with either of the flouroquinolones.

Table 2. Detection of different aerobic and anaerobic bacteria from oral and genital lesions of patients suffering from STI

Patient no	Age	Sex	Bacteria isolated from	
			Oral ulcer	Genital ulcer
1	36	M	<i>S. aureus</i>	<i>S. aureus</i>
2	40	F	<i>G. vaginalis</i>	<i>G. vaginalis</i> ; <i>K. pneumoniae</i>
3	52	M	<i>Shigiella flexneri</i>	<i>Shigiella flexneri</i>
4	60	M	<i>Shigiella sonnei</i>	<i>Shigiella sonnei</i>
5	35	M	<i>Pepto. heliotrinreducens</i>	<i>Pepto. heliotrinreducens</i>
6	38	F	<i>Pepto. magnus</i>	<i>Pepto. magnus</i>
7	40	M	<i>Pepto. hydrogenalis</i>	<i>Pepto. hydrogenalis</i>
8	40	M	<i>Pepto. magnus</i>	<i>Pepto. magnus</i>
9	45	M	<i>Peptoco. heliotrinreducens</i>	<i>Peptoco. heliotrinreducens</i> <i>Pepto. hydrogenalis</i>

Table 3. Antibiotic sensitivity pattern of aerobic and anaerobic bacteria isolated from STD patients

Bacterium	Source	Inhibitory reaction to antibiotics							
		Pc	Cb	Er	Dx	Gm	Cp	Of	Cmx
<i>G. vaginalis</i>	Table 1, group I	R	S	R	R	S	S	S	R
<i>S. aureus</i>	Table 1, group I	R	S	R	R	I	S	S	R
<i>V. parvula</i>	Table 1, group II	I	S	I	S	S	S	S	R
<i>P. heliotrinreducens</i>	Table 1, group II	S	S	R	S	I	S	S	I
<i>P. hydrogenalis</i>	Table 1, group II, III	S	S	R	S	S	S	S	R
<i>Actinomyces spp</i>	Table 1, group II	R	I	R	R	R	S	S	R
<i>P. magnus</i>	Table 1, group III	S	S	R	S	I	S	S	I
<i>S. aureus</i>	Table 2, patient 1	R	S	R	I	R	S	S	R
<i>G. vaginalis</i>	Table 2, patient 2	R	S	R	I	I	S	S	I
<i>Sh. flexneri</i>	Table 2, patient 3	R	S	R	I	I	S	S	R
<i>Sh. sonnei</i>	Table 2, patient 4	R	S	R	I	I	S	S	R
<i>P. heliotrinreducens</i>	Table 2, patient 5,9	S	S	R	S	S	S	S	R
<i>P. magnus</i>	Table 2, patient 6,8	S	S	I	S	S	S	S	I
<i>P. hydrogenalis</i>	Table 2, patient 7	I	S	I	S	S	S	S	R

S, sensitive; R, resistance; I, intermediate; Pc, penicillin; Cb, carbenicillin; Er, erythromycin; Dx, doxycycline; Gm, gentamicin; Cp, ciprofloxacin; of, ofloxacin; Cmx, cotrimoxazole

4. DISCUSSION

Many STDs or STIs are transmitted through the mucous membranes of penis, vulva, rectum, urinary tract, mouth, throat, respiratory tract and even eyes [21]. The visible membranes covering the tip of penis or lip in the mouth are mucous membranes, though they do not produce mucous. Mucous membranes differ from skin in that they allow certain pathogens to enter while skin offers its own defense mechanisms [22].

The frequency of contact with the infective sources which can cause an infection varies with each pathogen, but in all cases a disease can result from light contact, like contact of venereal fluids on to the mucous membranes. Since mucous membranes exist in both the genitalia as well as in the mouth STIs are easier to be transmitted through oral sex than through deep kissing.

Although the likelihood of transmitting various diseases by different sexual activities varies a great deal, all sexual activities between two people should be considered as being a two way route for the transmission of the STIs. Many infections not considered primarily to be STDs include shigellosis salmonellosis, campylobacteriosis [11] and gardenellosis [8]. Sexual transmission of shigellosis among homosexual men had been reported from USA, Canada, Australia and Germany since 1970s [9-11].

Apart from Gram negative organisms, multidrug resistant *Staphylococcus aureus* had been a frequent invader in STIs particularly among men who had sex with men [23]. In an intensive study for determining antibacterial activity of a novel drug Pleuromutilin B against STIs, Paukner et al. [13] reported that several anaerobic bacteria including *Peptostreptococcus* spp were isolated quite frequently, and that they were highly sensitive to the novel agent. In this way a large number of studies have been carried out that have evaluated the involvement of many different microorganisms as causative agents of STDs.

Thus it may be summarized that STDs pose a global health problem of a distinctly high magnitude. However, the causative agents of infection differ from one country to another, which may be explained on the differences in the social background of life. The STD clinics in India have been primarily engaged in determining the frequency of HIV infection and the rates of mortality and morbidity in such an infection for the past several years. Hence most of the studies describe the magnitude of STD with respect to HIV. According to Setla et al. [24] there is a strong association of HIV among men and also among transgenders. They concluded that such groups should be as the focus of intensive intervention in order to reduce the increasing incidences of HIV related STDs in India. In a separate study Pal et al [25] observed that apart from HIV several kinds of STDs are prevalent among sex workers in this large city in Eastern India. Along with cases of HIV, chancroid, chancre, gonorrhoea and trichomonas infections, there were 8.77% incidence of candidiasis and 3.0% incidence of *Gardenella* spp. Such an observation revealed that STDs due to different aerobic microorganisms are also common in India. *G. vaginalis* is known to be present in the vaginal secretions of women suffering from bacterial vaginosis and can also be present as commensals in normal women. It is one of the commonest cause of vaginal discharge and can be associated with complications of pregnancy. At this stage *G. vaginalis* becomes a major infective agent. The finding that *G. vaginalis* inhabit the rectum indicates a potential source of sexual transmission among homosexual men [8]. Our observation on STD infection among homosexual men confirms this earlier study Table 1.

Sexual transmission of shigellosis between homosexual men has been reported from various parts of the world [12]. In this study we had observed two cases of shigellosis in two elderly men, who may have acquired the infection through unusual behavior Table 2.

It has been observed in this investigation that numerous Gram positive and Gram negative aerobes and anaerobes were responsible for STDs among young men and women in this city. However, all the patients were treated on the basis of the results on the antibiotic sensitivity tests following standard therapeutic regimens [26] and there was no report on treatment failure. It may be pointed out here that several female patients reported to be

suffering from gonorrhoea simultaneously had vulvovaginitis due to *Candida albicans*. Thus the present study suggests that the transmission of many different organisms is quite frequent among young men and women in this part of India. Further studies are in progress to investigate the occurrence and invasion of genital organs by other aerobic and anaerobic bacteria as observed by others [13].

5. CONCLUSION

Several different gram positive and gram negative aerobic and anaerobic bacteria were found to be frequent invaders in sexually transmitted diseases. Such infections were communicated among both homosexual and heterosexual partners. The infections were observed more frequently in the age group of 18 to 40 years.

CONSENT

Patient approval was obtained from all the individuals that were included in the study.

ETHICAL APPROVAL

The authors received prior approval from the institutional ethical committee of Calcutta Medical College and Hospital, Calcutta.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Williards C. Estimation of the Incidence and prevalence of sexually transmitted Diseases in the United States. *Sex Trans Dis.* 1999;26:S2-S7.
2. European Centre for Disease Prevention and Control. Annual Epidemiological Report. Reporting on 2010 Surveillance Data and 2011 Epidemic Intelligence Data; 2012.
3. World Health Organisation. Global prevalence and incidence of selected curable sexually transmitted infections: Overview and Estimates. Geneva: WHO; 2012
4. Weinstock H, Berman S, Cates W. Sexually transmitted diseases among American youth: Incidence and prevalence estimates, 2000. *Persp Sex Rep Hlth.* 2004;36:6-10.
5. Schryver AD, Meheus A. Epidemiology of sexually transmitted diseases: The global picture. *Bull Wld Hlth Org.* 1990;68:639-654.
6. Bader M, Pedersen AHB, Williams R, Spearman MN, Anderson H. Veneral transmission of shigellosis in Seattle–King County. *Sex Trans Dis.* 1977;4:89-91.
7. Dritz SK, Ainsworth TE, Black A, Boucher LA, Palmer RD, River E. Pattern of sexually transmitted enteric disease in a city. *Lancet.* 1977;2:3-4.
8. Catlin BW. *Gardenerella vaginalis*: Characteristics, clinical considerations and controversies. *Clin Microbiol Rev.* 1992;5:213-237.
9. Walker CK, Workowski KA, Washington AE, Soper D, Sweet RL. Anaerobes in pelvic inflammatory disease: Implications for the center for disease control and prevention's guidelines for treatment of sexually transmitted diseases. *Clin Inf Dis.* 1999;28:S29-S36.
10. O'Sullivan B, Delpech V, Pontivivo G, Karagiannis T, Marriott D, Harness J, et al. Shigellosis linked to sex venues, Australia. *Emerg Inf Dis.* 2002;8:862-864.

11. Marcus U, Zucs P, Bremer V, Hamouda O, Prager R, Tschaepe H, et al. Shigellosis—a re-emerging sexually emerging infection : An outbreak in men having sex with men in Berlin. *Int J STD AIDS*. 2004;15:533-537.
12. Morgan O, Crook P, Cheasty T, Jiggle B, Giraudon I, Hughes H, Jones SM, Maguire H. *Shigella sonnei* outbreak among homosexual men, London. *Emerg Infect Dis*. 2006;12:1458-1460.
13. Paukner S, Gruss A, Fritsche TR, Ivezic-Schoenfield Z, Jones RN. *In vitro* activity of the novel pleuromutilin B-3781 tested against bacterial pathogens causing sexually transmitted diseases. Paper presented at 53rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Denver, CO, September 10-13; 2013.
14. Coles AC. *Spirochaeta pallida*: Methods of examination and detection especially by means of the dark ground illumination. *Brit Med J*. 1909;4:1117-1120.
15. Harris A, Rosenberg AA, Del Vecchio ER. The VDRL slide flocculation test for syphilis: A supplementary report. *J Ven Dis Inform*. 1948;29:72-75.
16. Barrow GI, Feltham RKA, editors. *Cowan and Steel's Manual for the identification of medical bacteria*. 3rd ed. Cambridge, U.K. Cambridge University Press; 2004.
17. Brown R, Collee JG, Poxton IR. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. *Mackie & McCartney's Practical Medical Microbiology*. London and New York: Churchill Livingstone. 1996;501-519.
18. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing*. Twenty-First Informational Supplement. M100-S21. CLSI, Wayne, PA, USA; 2011.
19. Dasgupta A, Dastidar SG, Shirataki Y, Motohashi N. Antibacterial activity of artificial phenothiazines and isoflavones from plants. *Bioactive Heterocycles*. 2007;15:67-132. Springer, Berlin, Heidelberg.
20. Dastidar SG, Kristiansen JE, Molnar J, Amaral L. Role of phenothiazines and structurally similar compounds of plant origin in the fight against infections by drug resistant bacteria. *Antibiotics*. 2013;2:58-71.
21. Naidu KM. *Community health nursing*. Gen Next Publications. 2009;246-248.
22. Kupper TS, Fuhlbrigge RC. Immune surveillance in the skin: Mechanisms and clinical consequences. *Nature Rev Immunol*. 2004;4:211-222.
23. Diep AB, Chambers HF, Graber CJ, Szumowski JD, Miller LG, Han LL, et al. Emergence of multidrug resistant, community-associated, methicillin-resistant *Staphylococcus aureus* clone USA 300 in men who have sex with men. *Ann of Int Med*. 2008;148:249-257.
24. Setla MS, Lindan C, Jerajani HR, Kumta S, Ekstrand M, Mathur M, et al. Men who have sex with men and transgenders in Mumbai, India: An emerging risk group for STIs and HIV. *Indian J Dermatol Venerol Leprol*. 2006;72:425-427.
25. Pal D, Raut DK, Das A. A study of HIV/STD infections amongst commercial sex workers in Kolkata. (India) Part-IV laboratory investigation of STD and HIV infections. *The J Comm Dis*. 2004;36(1):12-16.
26. Brunton L, Chabner B, Knollman B, editors. *Goodman & Gilman's the pharmacological basis of therapeutics*. 12th ed. USA: MacGraw Hill Companies; 2010.

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