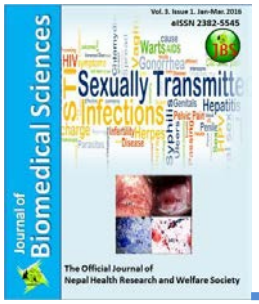

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Multiple acquisitions of STIs: invitation to precision in clinical care and summons to intangibles in laboratory diagnosis.

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ORIGINAL ARTICLE

Multiple acquisitions of STIs: invitation to precision in clinical care and summons to intangibles in laboratory diagnosis.

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ABSTRACT

Background
Co-existence of one sexually transmitted infection (STI) with another viral or bacterial agent may change the classical behavior of the genital infection and may provide the stimulus for reactivation. The objectives of this study were to document and appraise the relevance and importance of co-infection in STIs in their prevailing permutation and combination.

Material and methods
This cross-sectional study included 200 consecutive patients, attended the STI clinic of a tertiary care hospital in East Delhi with one or more complaints related to STI.
Samples were collected, stained, cultured and antibody detection was done.

Results
A number of STIs patients had significant association with multiple co-infecting agents. Dual coinfection was seen in 53.1%, while ≥ 3 STIs pathogens were seen in18% cases. Importance of serology in detecting subclinical cases is also highlighted in the study.

Conclusion
Present study highlights the requirement for periodic need based surveillance of STIs for clinical intervention with challenge to clinicians for diagnosing multiple co-infections. The number of subclinical STIs in the present study underscores the importance of serology for detection of common STIs in all patients presenting to the STI clinic.

Keywords
Sexually transmitted infections; co-infection in STIs, serodiagnosis of STIs.

Introduction:

Sexually transmitted infections (STIs) can co-existence with another STI agent which may change the classical behaviour of the genital infection and, may provide the stimulus for reactivation [1]. Syndromic approaches especially in cases of co-infection may not be sufficient to eliminate multiple organisms from the host with certainty [1, 2]. As the STI control Programme gathers momentum, and the prevalence of easily treatable and curable bacterial STI infections is progressively reduced in the community, the residual cases are likely to be more resistant or difficult to control in the same patient. Literature is scarce on dual or multiple STIs. The increasing presentation of such multiple co-infections amongst STI patients provoked this study which aims to document and appraises the relevance and importance of co-infection in STIs in their prevailing permutation and combination.

Material and methods

This cross-sectional study was conducted among 200 patients with one or more complaints related to STI visiting the tertiary care STI clinic OPD attached to Department of Microbiology, UCMS & GTB Hospital in East Delhi, during the period November 2013 and April 2015.

All patients aged below 18 years, pregnant, menstruating women and patients who received treatment in the preceding four week or those who were on follow up treatment were set up as exclusion criteria. After carrying out risk assessment, all patients were treated according to syndromic approach by National AIDS Control Organization (NACO), India [3]. However, those included in the study were investigated in detail to identify the etiological agents of STI. Urine, urethral swab, vaginal and endocervical swab, tissue biopsy and swab from ulcer, if any, were collected from patients. Blood samples were also collected for serological investigations. Normal saline wet mount examination was done to detect clue cells suggestive of bacterial vaginosis (BV), budding yeast cell in cases of vulvo vaginal candidiasis and to detect trophozoites of *Trichomonas vaginalis*. Giemsa stained Tzanck smear detected multinucleated giant cells (MNGC) of *Herpes simplex virus* (HSV). Gram negative intracellular diplococci (ICDC) in cases of suspected gonorrhea were evident by gram staining of urethral discharge. Budding yeast cells on direct smear prompted culture on saboraud's dextrose agar (SDA), followed by Candida speciation by germ tube test, sugar fermentation and assimilation. Nugent's criteria were used for interpretation of BV. Histopathology from biopsy samples were done for diagnosis of genital warts, donovanosis, and genital molluscum contagiosum. Gonococci (GC) was isolated on chocolate agar base with hemoglobin and vancomycin, colistin, nystatin and trimethoprim (VCNT) supplements (HI-media, India). The isolates were identified by colony morphology on culture plates followed by gram staining, oxidase test, superoxol test and rapid carbohydrate utilization test (RCUT) test. Antimicrobial susceptibility testing of the GC isolates was performed by CDS method with following antibiotics: penicillin (0.5 IU), tetracycline (10µg), ceftriaxone (0.5µg), spectinomycin (100µg), ciprofloxacin (1 µg), naladixic acid (30 µg), azithromycin (15µg), cefpodoxime (10µg) (Oxoid, U.K). MIC of ceftriaxone was estimated by E- strips containing 0.002-32 µg/ml (Hi-Media, India).Penicillinase producing Neisseria gonorrhoea (PPNG) was detected by chromogenic cephalosporin method [4]. In the absence of ICDC, a presumptive diagnosis of non-gonococcal urethritis/cervicitis (NGU/C) was made in respective genders [5].

The following serological tests were also performed. HSV-1 IgM antibody (Maddens Diagnostics HSV-1 Netherlands) and HSV-2 IgM antibody (Ridascreen HSV-2 IgM Germany) were detected by µ capture ELISA, as per manufacturer's instruction. Sera were also tested for HBsAg (HBV: 0003463 Hepalisa Kit) and HCV antibody (HCV; third generation HCV Microlisa Kit, India) by ELISA. For syphilis, VDRL (antigen from Serologist to Government. of India, Kolkata) and *Treponema pallidum* haemagglutination assay (Plasmatic TPHA test kit,Hansard Diagnostics, United Kingdom) in VDRL reactive cases were performed. Antibody (IgM) detection for *Chlamydia trachomatis* (CT) in the serum of all patients were performed by the EIA (XEMA microplate IgM EIA kit, K105, Russia) as per manufacturers instruction. All the patients were tested for HIV by ELISA/Rapid test using approved kits, following NACO guidelines after pretest counseling and written informed consent followed by post-test counseling.

Ethical clearance was obtained form the institution prior to study. Intergroup comparison was done by Chi Square test/ Fischer Exact Test and intragroup comparison were done by Mc Nemar's test. $P<0.05$ was considered as a statistically significant.

Results

Viral STIs were significantly higher (45%) when compared to bacterial & fungal STIs, (Fischer exact test, degree of freedom (df) 1, P0.039). (Fig 1)

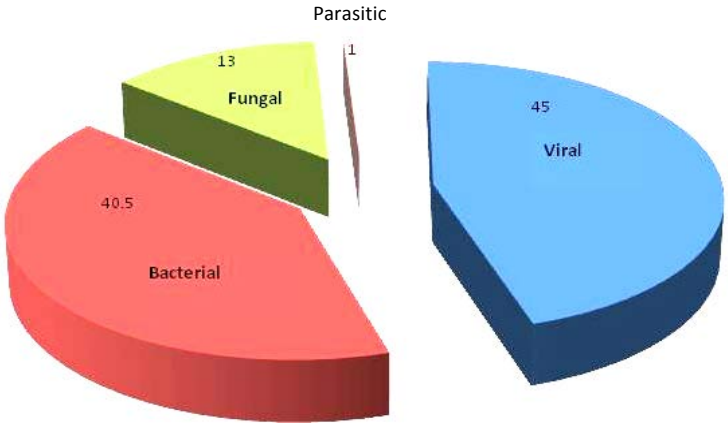

Figure – 1 % Prevalence of STIs

Majority of the patients who were included in the study were women's (M: F=1:7.4). Mostly (64%) belonged to the age group of 20-35 years, although the comparison between age and sex groups was not statistically significant (Fisher's Exact test, df 1 P 0.140).

Table - 1 Age and gender profile of STIs patients

Age group (Years)	Male (n=24)	Female (n=176)	Total (n=200)	M:F
	No (%)	No (%)	No (%)	
<20	3(12.5)	12(6.8)	15(7.5)	1:4
20-25	4(16.6)	28(15.9)	32(11)	1:7
26-30	4(16.6)	58(32)	62(31)	1:14
31-35	6(25)	38(21.5)	44(22)	1:6
36-40	4(16.6)	24(13.6)	28(14.1)	1:6
>40	3(12.5)	16(9.0)	19(9.5)	1:5

Patients were mostly married (74.5%) at the time of presentation and half of them (53 %) were literate. Most common symptoms in male patients was urethral discharge (25%) followed by dysuria (20.8%), genital ulcer (16.6%),genital itching (12.5%) and genital growth (4.1%).Females presented maximally with the genital discharge (31.08%) followed by dysuria (17.04%),lower abdominal pain (16.1%), genital itching (15.3%), genital ulcers(9.09%) and genital growth (3.4%). The difference was not statistically significant between patients' complaints amongst male and female STIs clinic attendees. (**Fig: 2**).

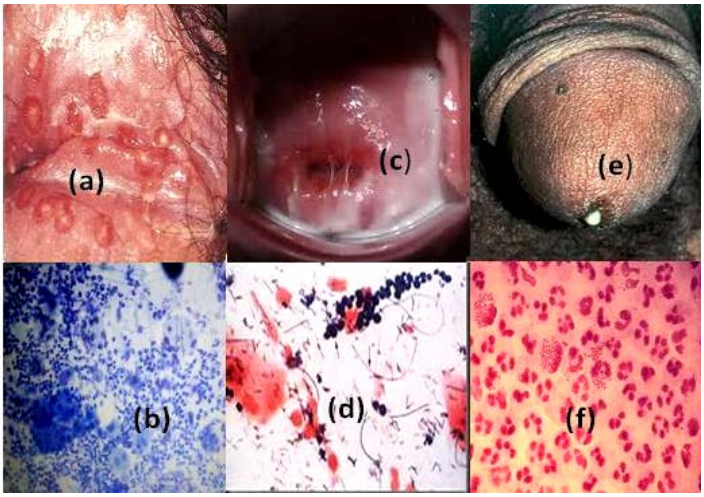

Figure – 2 Clinical picture of STIs cases with their microscopic picture

(a) Genital herpes (b) Tzanck smears showing multinucleated giant cells.100X magnification (c) Genital candidiasis (d) Gram staining showing budding yeast cells.100X magnification (e) gonorrhoea (f) Gram staining showing intracellular gram negative diplococci 100X magnification.

In the serologically diagnosed STIs too, viral STIs predominated, registering a prevalence of 20.5%. Highest seropositivity rate for a single pathogen (11.5%) was seen with *C. trachomatis* (Fig 3).

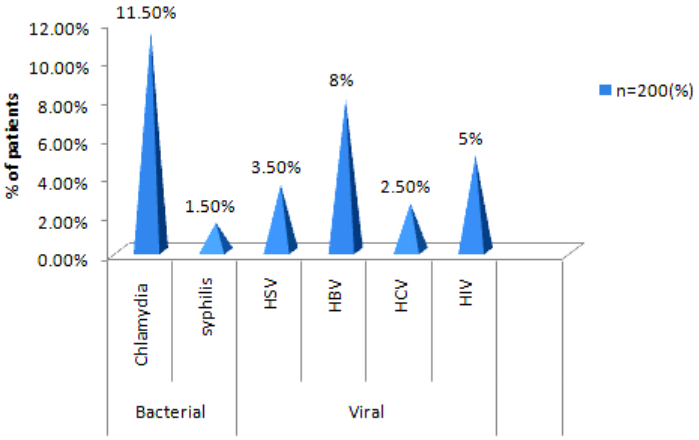

Figure – 3 Seroprevalance of STIs.

Bacterial co-infections were predominantly (60.8%) seen along with genital chlamydiasis (14/23, **Table 2**). The total co-infections in viral STIs (20.5%) were nearly equal as in bacterial STIs (18.5%).

Table - 2 Number of associated co-infections with different laboratory confirmed STIs

Laboratory confirmed STIs	n(%)	Associated co-infections with STIs			
		Bacterial	Viral	Fungal	Parasitic
Gonorrhoea	8(4)	0	2	0	0
Syphilis	6(3)	3	2	1	0
Chlamydiasis	23(11.5)	14	9	10	0
HSV-2	7(3.5)	5	4	1	0
HSV-1	3(1.5)	2	0	0	0
HBV	16(8)	2	3	8	0
HCV	5(2.5)	3	4	1	0
HIV	10(5)	7	4	1	0

Some of the laboratory confirmed STIs were found to have the following associated co-infections:

- Gonorrhoea along with CT, genital herpes and HIV.
- Syphilis in association with BV, non-gonococcal cervicitis/ urethritis (NGC/U) and HIV.
- Genital chlamydiasis together with GC, BV, NGC, Syphillis, HIV, HSV, HBV, HCV and VVC.
- Genital herpes with GC, HBV, HCV and VVC.
- Hepatitis B seropositive cases occurring with CT, NGC/U, HSV, HCV, and VVC.
- Hepatitis C seropositive cases co-infected with CT, HSV, HIV, HBV, and VVC.
- HIV seropositive cases had co-infection with GC, CT NGC/U, BV, syphilis, HCV and HSV.

Table - 3 Ulcerative and non-ulcerative STIs during study period

	STIs	Total cases 200	Male 24(12%)	Female 176 (88%)
Ulcerative STIs n=13	Syphillis	6(3)	2(8.3)	4(2.3)
	Chancroid	0(0)	0(0)	0(0)
	Herpes	7(3.5)	4(3)	3(1.7)
	Donovanosis	0(0)	0(0)	0(0)
Non-ulcerative STIs n=63	Trichomoniasis	0(0)	0(0)	0(0)
	Vulvovaginal candidiasis	32(16)	4(3)	28(15)
	Bacterial vaginosis	8(4)	0(0)	8(4.5)
	Genital chlamydiasis	23(11.5)	0(0)	23(13)
	Genital warts	0(0)	0(0)	0(0)
Other STIs n=31	HIV	10(5)	3(4)	7(3.9)
	HBV	16(8)	1(4.1)	15(8.5)
	HCV	5(2.5)	1(4.1)	4(2.2)

HIV - Human immunodeficiency virus; HBV - Hepatitis B virus; HCV - Hepatitis C virus

Overall, non-ulcerative (NU) STIs predominated over the ulcerative (U) ones (NU: U= 4.8:1) but this difference in males (NU: U= 6:1) and females (NU:U= 7.7:1) was not statistically significant (Fischer's exact test, df 1, P 0.8126).

Amongst ulcerative disease in males, 0.5%, 1% and 0% of patients had HIV, HBV and HCV sero-positivity while the same in females were 1.5%, 2% and 0.5% respectively. The same seropositivity in non-ulcerative disease was 1%, 1% and 0.5% in males and 2%, 4% and 1.5% in females respectively. Although, seropositivity of HIV, HBV and HCV were more in non-ulcerative STIs, there was no significant association of HIV, HBV and HCV with ulcerative and non-ulcerative STIs amongst attendees of our STI clinic. Seropositivity of HIV, HBV and HCV was more commonly associated with females in single pathogen STIs (usually bacterial) (**Table 3**).

Table - 6 Correlation between clinically and serologically diagnosed STIs.

STIs (n)	Clinical diagnosed	Serologically confirmed	Serology test	Mcnemar's Test (P value)
***C. trachomatis* (23)**	17	23	IgM ELISA	0.031*
Syphillis (6)	2	6	VDRL (titre≥16) + TPHA	0.125[x]
HSV (7)	1	7	IgM ELISA	0.035*

[x]P>0.05 statistically not significant
*P<0.05 statistically significant

Table 6 shows the correlation between symptomatic and asymptomatic STIs.

Co-infection with two pathogens was seen in 53.1 % (106 cases); A bacterial pathogen with a viral agent was seen in 21% (42 cases) and a bacterial pathogen with a parasite in 3.1% (6 cases).

Co-infection with three or more pathogens was seen in 18 % (36 cases=bacterial +viral+ fungal pathogen) (**Table 4**)

Table - 4 Clinical presentation associated with co-infection in STIs patients.

Type of STIs	%	Presenting complained			
		Most common		2nd most common	
		Male	Female	Male	Female
Single pathogen	30.5	Genital discharge at urethra	Genital discharge with itching	Itching, dysuria	Lower abdominal pain Backache
Dual Pathogens	53.1				
B+V[1]	21	Genital discharge with itching and burning micturition	Genital discharge, Lower abdominal pain	Dysuria, Painful scrotal swelling	Backache dysuria
B+F[2]	11				
B+P[3]	3				
B+B[4]	6				
V+F[5]	8				
V+V[6]	4.1				
Triple pathogens		Genital discharge with genital lesion and burning micturition	Genital discharge and burning micturition	Genital discharge with genital lesion	Backache Dysuria, itching and genital lesion
B+V+F[7]	18				

[1]bacterial+viral; [2]bacterial+fungal; [3]bacterial+parasitic; [4]bacterial+bacterial; [5]viral+fungal; [6]viral+viral, [7]bacterial+viral+ fungal

Table - 5 Association between co-infecting agents

STIs	Coinfecting agent	P value
Genital chlamydiasis	HCV	0.006[†]
	HSV	0.04*
	Candida	0.687[x]
Gonorrhoea	Bacterial vaginosis	0.029*
	HSV	0.016[†]
Syphilis	Bacterial vaginosis	0.007[†]
	HIV	0.049*
Bacterial vaginosis	Syphilis	0.007[†]
	HBV	0.050[x]
HIV	Syphilis	0.022*
Hepatitis B	Candida	0.000[†]
Hepatitis C	Candida	0.034*
HSV	HBV	0.044*

[†]P<0.01 statistically significant
*P<0.05 statistically significant
[x]P>0.05 statistically not significant

Discussion

Amongst the 200 patients studied, majority were females (88%; M:F =1:7.4), a stark contrast to previous study done in same setting 8 years back (M:F=2:1)[1]. A study by Sharma *et al* also found 10 times higher male preponderance [2]. Higher female turn out in our STI clinic may be a reflection of increasing promiscuity among females and a weakening behavioural inhibition as a result of lack of education. STIs were also more commonly seen in married females, consistent with other studies from north India [2, 6].

The peak age group of 20-35 years in the present study is comparable with other studies [2, 7]. As this is the sexually active age group clustering and high STI attainment risk is there. Most common clinical presentation among STI patients, though not statistically significant, was that of discharge followed by lower abdominal pain in females, while genital ulcer & dysuria were common in males. Studies from Delhi and Trivandrum (India) have documented similar findings [6, 8]. A previous research from our hospital had reported genital ulcer along with genital discharge as the most common presentation [1, 9].

There appears to have been a pathogen shift in the causation of STIs in the community, that is being reflected in the changed burden, found in our study as compared to the predominance of bacterial STIs, a decade ago [10, 11]. Other researchers from different parts of the country also report similar changed predominance [7, 12, 13]. It is due to the generous and prophylactic use of over-the-counter broad spectrum antibiotics and probable up-gradation of quality of care at health facilities at peripheral and private health care levels.

An 11.5% prevalence of *C. trachomatis* in the present study was higher in comparison to 1.1% reported by Manju *et al* [14]. 74% of these patients presented with complains suggestive of pelvic inflammatory diseases (PID) /Lower abdominal pain (LAP). However, the remaining 26% (6 cases) were subclinical cases which were only diagnosed on serological examination alone revealing an overall subclinical *C. trachomatis* infection rate of 3%. Amongst the bacterial co-infections existing with genital chlamydiasis, gonorrhoea was observed in 8.6% cases contrasting with an earlier Delhi study five years back reporting a lower prevalence gonorrhoea positivity of 2% with *C. trachomatis* [14]. A higher co-infection rate of 27.2% was reported from Mumbai [15]. A study from United States documented even higher association, up to 67% [16]. Amongst the viral co-infections, *C. trachomatis* cases occurred with genital herpes in 13.04% cases. A recent study from Saudi Arabia reported similar (12.8%) occurrence of *C. trachomatis* in genital herpes patients [17]. The present study documented a statistical significant correlation of genital chlamydiasis with HCV, HSV and VVC. (Table: 5).

Syphillis was documented in 3% of our study subject (Table 2) contrasting with a higher prevalence of 20-23% reported from East Delhi, Nepal and Ajmer [1, 18, 19]. In this study asymptomatic syphilis cases (4) were more as compared to clinical cases (2), again demonstrating the overall subclinical syphilis infection rate of 2%. Thirty three percent of our syphilis patients were also HIV seropositive and this association was significant (Table 5); this is comparable to 31% HIV seropositivity in syphilis from Nepal [18]. Although the present study did not document any co-infection of syphilis with gonorrhoea, a previous study from Delhi had recorded a 6.3% co-infection rate [14]. Present study also documents a significant association of syphilis with BV.

Overall HIV seropositivity was low (5%) in our STI clinic attendees comparable with reports from Trivandrum (3.2%) and Rohtak (1.7%) [8, 20]. The current HIV seropositivity is less than half in comparison to the study reports from the same setting eight years ago [1]. A 10% co-infection of HIV with gonorrhoea and HCV is seen in the present study. This finding is similar to rate of co-infection of HIV with gonorrhoea from Mumbai (11.7%) and Kenya (17%) [15, 21]. Co-infection of HIV with HCV reported from Kolkata (7.35%) is almost similar to present study (10%) [22]; although a lower rate is documented from South India (2.2%) and China (3.3%) [23, 24]. Present study did not encounter co-infection of HIV with HBV while studies from other parts of India have reported this co-infection ranging from 9 to 15.19% [22, 23]. All our HIV seropositive subjects were co-infected with bacterial, viral or fungal STIs. Maximum co-infection was seen with *C.trachomatis* (30%) although significant association was seen only with syphilis. This again emphasizes the need for assessing HIV status in STIs as it makes the individual prone to acquire and spread the virus through sexual contact by altering tissue integrity. Periodic STIs screening of HIV positive patients attending antiretroviral treatment (ART) clinic is also warranted even if they are asymptomatic and empirical treatment should be given on suspicions of STI.

Prevalence of gonorrhoea was also low (4%) in our setting contrasting with higher prevalence from Delhi (14.4% in 2011; 15.4% in 2002; 19.4% in 2001) [2]. Such a decreasing trend from Delhi has been seen in reports from Pondicherry (1.8% in 2009; 11.9% in 2001) [25] and Kerala (15% in 2005; 60% in 2000) [26]. Gonorrhoea was associated with BV in 25% cases and this was statistically significant (Table 5). Other reports from Delhi (15.8%), Indonesia (3.2%) and United states (2.2%) document lower rate of this co-infection [14, 27, 28]. The United States and Indonesia study population were pregnant females.[27,28]A statistically significant association was also noted between gonorrhoea and genital herpes (25%), comparable to a report from Ajmer (36.6%) but contrasts with the low co-infection rate of the same agents from Saudi Arabia (4.7%) [17, 19].

The HSV sero-prevalence was 5% in the present study. An earlier report from the present institution observed a decreasing trend from 32.7% in 2006 to 14.8% in 2014 culminating in the present 5%. This is comparable to a study from Andhra Pradesh where too a decreasing tendency was noted (6.78% in 2000-2002 to 3.47% in 2003-2005) [28]. However prevalence of HSV over the

years has been documented to rising in reports from studies from Chandigarh, Rohtak, North-eastern India, and even Kerala [8, 13, 29, 30] In the present study 90% of the HSV cases were serologically detected again highlighting the common subclinical existences of this infection.

In the present study VVC cases were frequently associated with HBV (80%) and HCV (60%), both the associations being statistically significant (table 5). More than 50% of our VVC cases grew *C.albicans* followed by *C.tropicalis comparable* to similar reports from Karnataka (66%) and Haryana (51.6%) [31, 32].

In our study 17 cases of genital chlamydiasis and 1 case of genital herpes were diagnosed clinically. However after appropriate serological tests the total number of chlamydiasis detected increased to 23 and genital herpes to 7, indicating thereby the importance of serology in detecting subclinical cases. Applying Mcnemar's test, this data was statistically significant demonstrating the importance of serological tests (Table 6). Two cases of clinically diagnosed syphilis increased to 6 cases after combining with serology though it did not enhance the statistical significance. As syphilis continues to be an important bacterial STI, importance of serological test for its diagnosis need not be over emphasized. The above serological tests in the present study indicated active disease and thus treatment was warranted in such cases. The name of the earlier National STD control programme had been changed to National STI control programme to expand the scope of the programme for the detection and management of both symptomatic and asymptomatic infection.

Present study highlights the requirement for periodic need based surveillance of STIs for clinical intervention with challenge to clinicians for diagnosing multiple co-infections. The rise in number and shift in the type of STIs stress the need for periodic review of the pattern of existence of these infections in the community for effective management through the WHO/NACO syndromic approach. Necessary modifications may be adapted as per the epidemiological pattern of STIs in a given setting or locality. All-inclusive in formation of the various epidemiological aspects is essential to design protective measures and regulate strategies and restrain the spread of the STIs and take an STI control programme to the next level.

As many of the detected STIs in this study were asymptomatic it would be unwise to underestimate the risk for exposure and prevalence in such patients. The number of subclinical STIs in the present study underscores the importance of serology for detection of common STIs in all patients presenting to the STI clinic. WHO/NACO syndromic treatment regimen currently in use would have missed such co-occurring STIs leading to inadequate treatment and perhaps an increasing trend in the prevalence of these sexually active asymptomatic diseases in the community. A delay in diagnosis and treatment can also nevertheless lead to grave consequences for the patient especially if immunosuppressed or in the event of vertical transmission. For women in the reproductive age group this situation is also attendant with long term consequences. Transmission of infection to sexual partners may further aggravate the situation. Thus detection of subclinical STI co-infection by serology not only facilitates initiation of appropriate treatment but also reduces the risk of recurrence and transmission to contacts. Among various STIs, prevalence pattern of syphilis and herpes genitals is pivotal and NACO has already issued guidelines to test for these two diseases in AIDS as well as non-AIDS patients by serology [2]. It is perhaps time for the health policy custodians to resist the current STI control programme strategy and insist revisions to overcome these obvious weakness well illustrated by this study.

Conclusion

Present study highlights the requirement for periodic need based surveillance of STIs for clinical intervention with challenge to clinicians for diagnosing multiple co-infections. The number of subclinical STIs in the present study underscores the importance of serology for detection of common STIs in all patients presenting to the STI clinic.

Competing interests

Authors declared that they do not have any competing interest.

Authors' contribution

All authors have equally contribution for this study, which includes study design, experimentation, manuscript write up, statistical analysis & revision. All authors critically revised and approved the final manuscript.

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Abbreviations

bacterial vaginosis (BV), gram negative intracellular diplococci (ICDC), Hepatitis B virus (HBV), Hepatitis C virus (HCV), Herpes simplex virus (HSV), Herpes simplex virus-1 (HSV-1), Herpes simplex virus-2 (HSV-2), Human immunodeficiency virus (HIV), multinucleated giant cells (MNGC), National AIDS Control Organization (NACO), saboraud's dextrose agar (SDA), sexually transmitted infections (STI), vancomycin, colistin, nystatin and trimethoprim (VCNT)

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