ISOLATES OF STDs CAUSATIVE AGENTS FROM SEX WORKERS ADDIS ABABA (A PRELIMINARY REPORT)

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ABSTRACT: Cervical and vaginal discharge samples, collected from282 randomly selected prostitutes of Addis Ababa, were examined for Sill causative agents. The subjects represented 900 females included in the 1990 sero-survey-for HIV infection. The results of the laboratory tests indicated that 117 (41.5%) of the examined females had a single infection by one of the five organisms tested for, while the others 18.1 % had experienced mixed infections of two organisms, and 4.2% of three organisms. The five organisms included Neisseria gonorrhoea isolated from 78 subjects (28.1 %), Trichomonas vaginalis -from 56 (20.6%), Candida albicans -from 40 (14.7%), and Gardnerella vaginosis -associated clue cells -from 27 individuals (9.9%). The presence of syphilis infection was indicated by the TPHA test in 72 subjects, representing 37.3% of the study samples. Among the N. gonorrhea isolates, PPNG strains comprised 57 (73.0%). Resistant strains were obtained to penicillin in 100.0%, ampicillin in 96.0%, bactrim (sxt) in 80.7%, among the PPNG strains; 19.1% of the NPPNG strains to penicillin, 38.1% to kanamycin, 66.6% of a bactrim and 66.7% of ampicillin. Both groups were, however, sensitive to erythromycin, tetracycline, chloramphenicol, norfloxacin, spectinomycin and ceftriaxone. Significant correlation was observed between these results and the MIC values obtained for each drug.

INTRODUCTION

Sexually transmitted diseases (STDs) are among the major public health problems at large in almost all countries. Before World War II, all laboratories had to be contained primarily to the five classical diseases, namely: gonorrhoea, syphilis, chancroid, lymphogranuloma venereum, and granuloma inguinale (1). The incidence of specific diseases varies markedly and recent advances in microbiological and serology have greatly facilitated the identification of their causative agents (1,2). A significant proportion of infected individuals harbour two or more associated sexually transmitted disease agents (1).

The type of infection and causative agents are influenced by a number of factors, including: sexual activity, contraceptives use (especially, intrauterine devices), genital tract instrumentation, child birth, and prior infections (2). Infections of the female genital tract are caused by a wide variety of microorganisms including bacteria, viruses, fungi, and chlamydiae; the most commonly reported pathogens being Neisseria gonorrhoea, Treponena pa/lidum. Candida a/bicans .Trichomonas vagina is and Ozlamydia trachomatis. These pathogens are, however, isolated from only a minor proportion of females with abnormal vaginal discharges, a common complaint in primary health care. In many instances the bacterial vaginosis has been associated with Gardnerella vagina/is, typically identified by its clue cells formation (3-6).

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Sex workers such as bar girls, have generally been reported to be an important reservoir for many STD pathogens, especially, in the developing countries (7). Moreover, the (3 prevalence of STD agents like N. gonorrhoea. on individually, or in association with the others pathogens was reported to be highest in women ill with multiple sexual partners (8-10).

In Ethiopia no work could be cited to indicate the associative existence of the STD etiologic agents in symptomatic of asymptomatic women of high risk groups for STD infections. The aims of this investigation were to assess the prevalence of the STD pathogens, to establish sero-prevalence rates of syphilitic infections, and to determine chemosensitivity of the bacterial pathogens to the WHO recommended antibiotics.

MATERIALS AND METHODS

Specimens

The study population comprised of 282 multi-partner sexual contact (MPSC) females, randomly selected from 1225 women included in the HIV serosurvey conducted by the Department of AIDS Control from 16 July to 16 August, 1990. in 40 Kebeles of Addis Ababa.

For every subject, questionnaire administration and physical examinations relevant for STD-associated factors, were performed by the collaborating clinical specialists. Vaginal and cervical swabs and blood samples were collected from each participant, irrespective of the presence of signs and symptoms, according to the WHO Bench-level Laboratory Manual for Sexually Transmitted Diseases (11). Genital swabs, inoculated into the Amies transport media, and blood sera were transported immediately to the STD diagnostic unit of Bacteriology Division of NRIH.

Specimen processing

As the collected specimens reached the laboratory , each cervical sample was immediately inoculated into blood agar and Thayer Martin agar plates and smears were prepared for direct Gram-stain examination. The vaginal swabs were used for: (1) the wet-mount microscopy with 10% KOH and saline, (2) smear for direct Gram-staining, and (3) for the inoculation as the cervical swabs. The pH value and its smell in the KOH suspension have also beep recorded. All plates were incubated at 35-37°C in an atmosphere of humidity above 10%, with 3-10% CO_2 tension, generated by a candle-jar or CO_2 gas-jar systems. The incubation continued for at least 48 hrs.

Isolation and identification of the pathogens Each inoculated plate was examined after 18, 24 and 48 hrs of incubation. Typical colonies of 0.5-3mm in diameter, gray to white, transparent to opaque, and of raised or convex to flat structures were presumptively taken as gonococcal isolates, while the other Beta-haemolytic Streptococci-like colonies were pooled for further purification and identification purposes. Gram-stain and oxidase tests were performed on each typical colony. Then all Gram-negative, diplococci and oxidase positive colonies were confirmed as Neisseria gonor- rhoea strains by the carbohydrate fermentation (11). All confirmed isolates were then stocked at -80°C for further tests.

The wet-mount preparations of the vaginal swabs with drops of 10% KOH were used to test for yeast cells and Candida albicans identification (11). The wet-mount was also used for determining Trichomonas vaginalis. All vaginal swabs negative by this test were inoculated into tubes of CPLM trichomonas broth medium. After 7 to 14 days of incubation the samples were examined by direct microscopy for Trichomonas vaginalis .

The KOH-vaginal discharge mixture drops were also examined for the typical fishy odour that is indicative of bacterial vaginosis.

This was later supported by the KOH wet-mount micropsy and direct Gram-stain examination for presence of clue-cells which indicates the presence of Gardnerella vaginalis.

Detection of PPNG strains and testing of chemosensitivity All N. gonorrhoea isolates were screened for penicillinase production by Chromogenic Cephalosporin substrate inetftod (12). All PPNG and non-PPNG strains were also tested for susceptibility to penicillin, tetracycline, erythromycin, ampicillin, kanamycin, sulphametoxazol trimethoprim (bactrim), ceftriaxone, norfloxacine, chloramphenicol, and spectinomycin, by the Kirby-Baur agar-disc diffusion method.

At the end of the study all N. gonorrhoea isolates were reconfirmed by NH4-Gallery biochemical test. Each strain was then examined for chemosensitivity to all above mentioned antimicrobial drugs by the agar-dilution MIC determination technique. For this test, two-fold serial concentrations were prepared, and incorporated into chocolate agar media.

For the MIC determination of the Neisseria gonorrhoea strains of this study, we used the standard interpretive categories for each drug as recommended by the National Committee for clinical laboratory standards (NCCLS).

Thus MIC values like $\geq$1 JLg/ml, $\geq$ 0.25 ILg/ml, $\geq$ 64 JLg/ml, $\geq$16 JLg/ml, $\geq$ 1 JLg/ml, and 1 $\geq$ JLg/ml were used as the cut-of points for resistance of strains against penicillin, ampicillin, spectinomycin, tetracycline, ceftriax one, and norfloxacin, respectively.

Serological tests

Syphilitic infection was diagnosed by non-Treponemal Rapid Plasma Reagin card (RPR) and Treponema! Pallidum Haemaggultination Assay (rPHA) methods, for all subjects with or without genital ulcerative lesions.

RESULTS

272 vaginal and cervical swab samples were submitted for the laboratory tests, that represented 96.5% of 282 subjects selected. Blood sera for serological tests were obtained from 193 (68.4%) of the females. Lesions of genital ulcers sampled from few cases gave no Haemophilus ducreyii isolates. Table 1 shows the results of the tests on six agents, which were identified in 18.5% of the 272 examined samples. Frequency of single or multiple infections is shown in table 2. Single infection was

Table 1. Prevalence of STO causative agents in sallf>les fr~ 272 sex workers in Addis Ababa, 1990

Nu.aer of s~les OrganiSIlW isolated Negative Positive X Positives

Organisms isolated	Negative	Positive	% positives
Neisseria gonorrhoea :			
PPNG strains	215	57	21.0

Non-PPNG strains	251	21	7.7
Trichomonas vaginalis	216	56	20.6
Candida albicans Gardnerella vaginalis	232	40	14.7
associated clue cells	245	27	9.9
Syphilis (TPHA)	121	72	37.31

observed in 117 (41.5%) cases, while multiple infection with two or more pathogens in 64 (22.7%) of the females.

Association of clinical symptoms presented on physical examination with the isolation of pathogens is shown in table 3. The prevalence of all pathogens was significantly higher in the asymptomatic females, (173 -61.4% of all subjects), than in those with a single symptom (64 - 22.7%), and multiple symptoms (75 - 12.4%). As shown in table 4, the isolation of the pathogens and frequency of symptoms are significantly associated with the number of sexual partners per week ($p < 0.1$). N. gonorrhoea strains were most frequently isolated in both symptomatic or asymptomatic females. The results of the petlactamase detection and chemosensitivity tests are shown in tables 5 and 6. The results of Kirby Baur agar-disc tests (13) and MIC determination assay correlated well for each of the examined antimicrobial drugs.

DISCUSSION

Testing of 272 vaginal and cervical specimens resulted in isolation of the four most common sm pathogens:Neisseria gono"hoea Trichomonas vaginalis, Candida albicans , and Gardmerella vaginalis. 72 sera samples, that is 37.3% of the 193 samples tested were positive for syphilitic infection. Significant correlation was found between the qualitative TPHA test results and qualitative RPR tests. These agents were found to cause single infection in 41.5%, in 18.1% as concomitant agents for double, and in 3.7% for multiple infections. Since we could not perform the culture and identification tests for Gardnerella vagina lis, due to the lack of selective media, indirect

Table 2. Single ard nultiple infections in sex workers in Addis Ababa, 1990 Pathogens

Pathogens	Number	Percent
Single infection		
PPNG strains	31	11.4
Non PPNG strains	6	2.2
T. vaginalis	19	6.9
G. vaginalis	10	3.6
C. albicans	13	4.8
Syphilis (TPHA)	28	14.0
Total	117	41.5
Double infections		
T. vaginalis and C. albicans	4	1.5
T. vaginalis and PPNG	7	2.6
T. vaginalis and NPPNG	3	1.1
T. vaginalis and G. vaginalis	1	0.4
T. vaginals and TBHA	10	3.7
C. albicans and PPNG	2	0.7
C. albicans and TPHA	5	1.8
G. vaginalis and TPHA	9	3.3
G. vaginalis and PPNG	3	1.1
PPNG and TPHA	2	0.7
NPPNG and TPHA	3	1.1
Total	51	18.7
Triple Infection		
G. vaginalis, C. albicans and PPNG	5	1.8
T. vaginalis, TPHA and PPNG	3	1.1
T. vaginals, TPHA and NPPNG	1	0.4
C. albicans, TPHA and PPNG	2	0.7
C. albicans, TPHA and NPPNG	1	0.4
G. vaginalis, TPHA and PPNG	1	0.3
Total	13	4.6

criteria were used for the diagnosis: the vaginal pH value greater than 5.0, fishy odour of the grey-white dif'J;harge in 10.0% KOH drop suspension, and the clue cells microscopic observation. The other workers (5,14) proved that these criteria have high (94%) specificity and sensitivity (82%). Epidemiological studies estimated that GardnereIla vaginalis infection prevailes in up to 50% of sexually active women at a time (15-17). The 10.0% prevalence found in our study goes well in line with the above estimation. This infection occurred, in 10 (3.6%) of the 27 Gardnerella infected females, as the sole agent of single infection.

Many epidemiological studies (5,15-17) showed that there is a correlation between Gardnerella-associated vaginitis and promiscuity, marital stability, and previous pregnancy.

In the present study, among 27 females with Gardnerella-associated vaginitis the majority, 25 (92.6%) had over two pregnancies, 15 (55.6%) of the females had on an average over three sexual acts per partner and, the other 14 (51.9%) had on an average 2 or more sexual partners per- week. Although Gardnerella infection is usually associated with abnormal vaginal discharge, Leopord (18) and others (19) have reported frequent isolation of G. vaginalis from asymptomatic women. These agrees with the result indicated in table 2, where 8 out of ten females with this sole agent

were asymptomatic. But Osborne and associates (20) demonstrated that the organism was isolated from 23 %
of symptomatic women, and only from 5% of asymptomatic women that had multiple sexually transmitted agents. In disagreement to the latter report our result showed that in the females with multiple agents, G. vaginalis was one of the concomitant agents more in asymptomatic than in symptomatic cases.

The other agents causing non-specific vaginitis were Trichomcnas vaginalis and Candida albicans, isolated from 20.6% and 14.7% of the females respectively. The isolation rate
of T. vaginalis fits within the range 3% to 88% reported by Rein, et al. (21) which depended upon the population w4diagnostic method used.

Table 3. Association of STD causative agents with clinical symptoms in sex workers, Addis Ababa, 1990

Clinical symptoms	Single infections							Multiple infections		Total
	None	PPNG	Non-PPNG	C.a	T.v	G.v	TPHA	Two path.	Three and more Path.	
None	43	32	2	8	7	8	25	43	5	173
Discharge	14	6	2	1	7	1	3	6	3	43
Rash warts	-	1	-	-	-	-	1	-	-	2
Abdominal	5	1	1	-	1	1	6	4	-	19
Two symptoms	8	3	-	-	2	-	-	7	1	22
Three or more symptoms	3	2	1	3	1	-	-	2	1	13
Total	73	45	6	12	18	10	36	62	10	272

PPNG = Penicillinase Producing N. gonorrhoea.
C.a = Candida albicans
T.v = Trichomonas vaginelis
G.v = Gardnerella vaginelis
Path. = Pathogens

Table 4. Prevalence of STD causative agent's in sex workers with different number of sexual partners Addis Ababa, 1990

	Number of partners and Clinical symptoms					
	1		2-3		More than 3	
	With no symptoms	With symptoms	With no symptoms	With symptoms	With no symptoms	With symptoms
None	6	4	18	12	23	9
PPNG	-	1	17	6	3	5
Non-PPNG	2	-	1	4	2	1
TPHA	2	-	4	5	11	7
G. vaginelis	2	-	5	2	4	-
T. vaginalis	2	-	9	7	5	5
C. albicans	-	1	6	2	2	1
Two pathogens	-	-	24	11	20	9
Three and more pathogens	1	-	2	-	3	5
Total	13	6	86	49	73	42

The prevalence of T. vaginalis was higher among women with multiple agents. It occurred in the subjects with double infections in 25 (44.6%) females and 7.1% of females with multiple

infections. In contrast to these results, Trichomonas vaginalis occurred as a sole agent in only 19 (33.9%) of the females. These results agree with the reports of previous studies (10). This organism associates more freqlently with gonococcal infection.

Therefore it well advised (9,10) that the patients with T: vaginalis should be screened for the other sexually transmitted infections such as gonorrhoea. 7: vagina/is occurs frequently in both asymptomatic (38.8%) and symptomatic (61.1 %) women with the single infection. This agrees with other reporters (10,22) who diagnosed it in 25% of asymptomatic and in 50% to 75% of symptomatic women. Similar to a previous report (10) abnormal discharge and abdominal discomfort or pain were the most common symptoms recorded in women with trichomoniasis. None of the symptoms is indic'\tive to trichomoniasis since in almost all cases dtis agent is accompanied by other genital infections, as it has been shown by us and other workers (10). Both our results and previous works (10,14,21,22) show that the risk of 7: vaginalis infection increases with the number of sexual partners.

Neisseria gonorrhoea was the most highly prevalent agent in the present study; this goes well in line with the results of other investigators (23-33). In Ethiopia, after the first report in 1983 (24) on the emergence of penicillinase producing N. gonorrhea strains, its prevalence has been increasing, as in many other parts of the world (23,34,35). In this study, 73.0% of all Neisseria isolates were PPNG strains; an alarming increase as compared to the report of 1985 work (4).

The PPNG strains were found most frequently in females with single or multiple

Table 5. Frequencyof resistance of penicillinase producing N. Gonorrhoea (PPNG) and non-PPNG strains to antimicrobial drugs

Strain	Drugs tested	Resistant No.	%
PPNG*(n= 57)	Penicillin	57	100.0
	Ampicillin	55	96.0
	Sulphametoxazol trimptoprim (bactrim)	46	80.7
	Kanamycin	13	22.8
Non-PPNG*(n= 21)	Penicillin	4	19.0
	Ampicillin	2	3.5
	Sulphametoxazol trimptoprim (bactrim)	14	66.7
	Kanamycin	2	38.1

*Both PPNG and non-PPNG strains were sensitive in 10DX to each of the following drugs: erythromycin, tetracycline, chloramphenicol, norfloxacin, spectinomycin and ceftricaxone

infections, in both symptomatic and asymptomatic females having more than the sexual: partners per week. The role of the resistance to Imost beta-lactam antibiotics like penicillin in better survival of this agent is evident (24,26).

The results of the test on PPNG strains for sensitivity to antibiotics by two techniques (Kirby Baur agar-disc diffusion and MIC determination assay) were found highly correlated.

Both PPNG and NPPNG strains were sensitive to all drugs except penicillin, ampicillin, sulphametoxazole trimethoprim (Sxt) and kanamycin. The PPNG strains were more resistant ($p > 0.05$) to both penicillin and ampicillin than non-PPNG strains. All of the PPNG strains, were resistant to ~enicilli~ with the majority of them (80%) showIng as high as 32 p.g/ml MIC value. This shows significant difference bet- ween MIC of PPNG and non-PPNG strains, where only 4 (19.1 %) of the latter strains were resistant and all of them with only ~ 1 p.g/ml MIC value. Similarly most of the ampicillin and bactrim resistant PPNG strains show as high MIC as $\geq$ 32 and $\geq$ 64p.g/ml in 75% and 67% respectively. Among the non-PPNG strains the MIC value was as high as $\geq$ 64 p.g/ml for
bactrim only and in almost the same percentage (70%) as the PPNG strains. But for ampicillin this value was only $\geq$ 1 p.g/ml and in a limited number of cases (9.6%). It was a surprise that bactrim, the recently WHO recommended drug for N. gonorrhoea primary treatment has been moderately resisted by both the PPNG and non-PPNG strains.

Resistance of gonococci to multiple drugs has also been observed in this study, as has usually been previously reported by other workers (25-28). The frequency of such multiple drug resistance in this study, 43.4% of PPNG strains and 13.03% of the non-PPNG strains, is in good agreement with the frequency of multiple drug resistant gonococcal strains 47.4% obtained by previous workers (25-26). The results (table 6) are in accord with the studies in Addis Ababa (29,30), and in other countries (28,31). Gedebou et al. (25) have offered various explanations for the occurrence of multiple antibiotic resistance of Neisseria

1- gonorrhoea. The fact that the PPNG strains in our study are all showing only multiple drug resistance (table 6) may support the recent (31,32) explanation that B-Lactamase - producing gonococci can conjugatly transfer the gene for the enzyme to other gonococci and to other bacterial species. Such transfer of R plasmid between gonococci and other species may be a possible mechanism for the development of multiple resistance (25).

Syphilitic infection was found in 38 (13.8%) of the examined blood sera by qualitative tests of RPR and TPHA methods, and the results of these two test have well correlated. Syphilis was the sole agent in some cases. In double and multiple infections positive TPHA values were observed in at least 5 of the twelve pairings and in 5 of the 6 combination of three agents. Interestingly enough, the syphilis diagnostic tests were negative in a number of multi symptomatic females.

Table 6. Frequency of resistance of (PPNG) and non-PPNG strains to antimicrobial drugs and coobination of drugs

Sensitivity Pattern	PPNG strains n=57 Resistance		Non-PPNG n=21 Resistance	
	No.	%	No.	%
Ampicillin	-	-	8	38.1
Sulphametoxazol trimptoprim (bactrim)	-	-	1	4.8
Kanamycin	-	-	6	28.6
Penicillin				
Sulphametoxazol trimptoprim (bactrim)	2	3.51	3	3.5
Penicillin				

Kanamycin	-	-	2	9.5
Penicillin				
Ampicillin	10	17.54	-	-
Pen Amp Sxt Knm	13	22.81	-	-
No resistance	-	-	6	28.6

In conclusion, our results reveal the coexistence of various STD pathogens in the studied group that reportedly increases the risk of HIV transmission. Further studies on larger groups with a broader spectrum of test are recommended.

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