**High level of HIV drug resistance and virological non-suppression among female sex workers in Ethiopia: a nation-wide cross-sectional study**

**Supplementary document**

**Study design**

This study was part of a larger cross-sectional study done to assess HIV prevalence and related risk factors among female sex workers in Ethiopia in 2014. The data collection was conducted in eleven cities using respondent driven sampling technique (RDS). RDS is a chain referral sampling technique recommended for hard-to-reach population including FSWs and when conducted according to the guideline it gives a representative sample of the study population. Before conducting the study, formative assessment was conducted to obtain the information needed for RDS sampling including seed selection, study site location, and for the amount of compensation to be paid. Seeds who reported better connection with large social network size were selected to start the data collection process. To avoid duplication of participants a fingerprint scanning device was used, and recruitment pattern was tracked, and network size of each participant was also determined.

**Study area, and population**

The study was conducted in eleven cities including: Addis Ababa, Mekelle, Bahirdar, Adama, Diredawa, Gambela, Hawassa, Metema Kombolcha Semera/Logia and Shashemene. Seven of the study sites (Addis Ababa, Adama, Mekelle, Bahir Dar, Hawassa Gambella and Dire Daw,a) are regional capital cities where many female sex workers reside relative to other regional cities. The other four sites, (Shashemene, Kombolcha, Metema, Logia), were transport corridor cities to Addis-Djibouti, Addis–Metema, Addis-Mekele, and Addis-Moyale, and are known to have more FSWs and long-distance truck drivers (hotspot area). In this survey, long distance truck drivers were also considered to be high risk group and were part of the survey. The source populations were all FSWs living in the selected eleven cities. The operational definition of FSWs was as follows: ‘women who practice sexual activity with the preconditions of financial or in-kind benefits. The inclusion criteria for the survey were: receiving money or other benefits for sex with four or more people within the last 30 days, being 15 and above years old, properly recruited by a peer (presenting with the coupon) and giving consent both for the interview and blood drawing.

Map

Description automatically generated

Figure 1. Ethiopian map showing location and details of the cities and town included in this study. Details of the survey is shown in boxes. The figure was adopted and modified from http://www.nearby.org.uk/google/nolabels-map.php

**Sample size**

A minimum FSW sample size of 400 was calculated for each town using anticipated HIV prevalence of 25%, 6% precision, 95% CI and design effect of two. Due to RDSs sampling requirement, which is equilibrium, the number of FSWs who participated in each town was not exactly 400 and the total number of FSWs who participated in all 11 cities was 4900. For the current analysis only those participants who fulfill the criteria for Virologic failure, PDR and ADR were included.

**Data collection procedure**

Six initial FSWs (seeds) were selected to initiate coupon-based recruitment in each town. Seeds were selected purposively to represent the geographical and occupational (e.g., Bar/hotel, street based etc.) diversity of the target populations. Seeds were identified through key informant interviews and in-depth interviews with key stakeholders and representatives of key population groups.

Before data collection informed consent were collected from each participant, then the interview was conducted in a private room by trained nurse counselor with a structured questionnaire in Amharic language. After completing the interview, every participant provides blood for HIV, CD4, viral load and drug resistance testing. Then, FSWs were given a primary incentive of 100 ETB ($5.0) and an additional 50 ETB ($2.50) for each eligible peer recruited and enrolled into the study. Finally, participants were provided with up to three coupons and instructed to recruit their FSW peers into the study. Fingerprint recognition software was used to create a unique study identification number for each participant to assist in preventing the same participant from enrolling more than once.

**Data and specimen collection:**

Data abstraction tool were used to collect the socio demographic and behavioral data of the study participants This original data abstraction tool was modified into a modified data abstraction tool to contain selected variable from the original data abstraction tool for this study. Data were collected on socio-demographic characteristics, numbers, and types of partners, transactional sex characteristics, use of condoms and lubrication, sexually transmitted infections (STI), health seeking behavior, exposure to sexual risk, HIV and AIDS knowledge and testing, alcohol and substance abuse, and media and HIV intervention exposure. The type of sex worker, which may include those FSWs who are bar- and/or hotel-based, red light houses, local drinking houses, street-based and hidden (cell phone-based) and geographic location of site were also collected. Information about the self-knowledge of HIV status, prior treatment exposure, including PMCT, previous date of HIV diagnosis, the date of first HIV test positive, date of ART initiation was also collected. The participant self-report information was used to classify study participants as treatment naïve or currently on ART or categorize HIVDR as pretreatment (PDR) or acquired HIVDR (ADR). Based on this, those who diagnose HIV positive by the survey and those who know their HIV status before the survey but not exposed to treatment were categorized for the analysis of pre-treatment HIV drug resistance (PDR). Study participant who are currently on ART but with viral load greater than 1000 copies/ml were used to assess the level of acquired HIVDR. Plasma samples collected during the survey were stored at -80 degrees at EPHI National HIV Laboratory and samples of each study participant with viral load ≥ 1000copies/ml shipped to the WHO-designated laboratory at the U.S. Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia for genotyping

**Factor associated to HIV virological failure and HIVDR**

To examine factors which has potential association with the dependent variable; the following independent measures were included: current age, monthly income from selling sex, educational status, sex selling venue, khat chewing, heavy alcohol drinking, violence, ever give birth, number of sexual partners per month, vaginal discharge and genital ulcer, forced in to selling sex and CD4 count.

Current age was a continuous variable and for the purpose of analysis was categorized as ten-year intervals: younger (15-24), middle age (25-34), and older one (35+), with the younger age group used as the reference category. Monthly income from selling sex was an open-ended question and for the analysis was categorized in to two groups, <=$100 and >$100 considering the cost of living during the data collection. Educational status was categorized as no formal education, primary first cycle (grade 1-4), primary second cycle (grade 5-8), and secondary and above for analysis, in accordance with the country education system. In addition, sex workers were categorized based on their sex selling place which includes street based, red light houses, bar/hotel, etc.

Violence was assessed with physical beating during the last one year and all who had reported beating at least once were considered as exposed to violence. Frequency of heavy episodic drinking (HED) was measured using the following question: “How often do you have 6 or more drinks on one occasion?” The response alternatives were: 0 - Never, 1 - less than once a month, 2 - monthly, 3 - weekly, and 4 - daily or almost daily. For the purpose of analysis, those respondents who answered with alternatives 2 through 4 were categorized as engaging in monthly HED. HED is defined as consumption of at least 60 grams (6 standard alcoholic drinks) or more of pure alcohol on at least one occasion in the past 30 days (1)

Khat chewing was assessed with the frequency of days they chewed in a week. Khat (Catha edulis) is a stimulant leave, and after chewing an individual may become, alert, feel excitement, and increased imagination (1).Vaginal discharge and ulcer were measured by asking whether they had the cases in the past 12 month with a Yes/No answer.

**HIV testing**

HIV Screening was done using point of care rapid testing format employed for HIV diagnosis in Ethiopia. This algorithm uses HIV (1 + 2) Antibody Colloidal Gold (KHB, Shanghai Kehua Bio-engineering Co Ltd, China) as a screening test, followed by HIV 1/2 STAT-PAK (Chembio Diagnostics, USA) if positive. In cases with negative STAT-PAK results following a positive KHB test, a third test, Unigold HIV (Trinity Biotech, Ireland), was used as confirmation (2).

Quantification of absolute counts of CD4+ T cells on whole blood specimens were determined using the FACS Calibur and FACs count systems (Becton Dickinson, San Jose, USA) according to the manufacturer’s recommendations. HIV-1 viral load was determined by using Abbott Real Time HIV-1 assay (Abbott Molecular Inc. Des Plaines, IL 60018 USA). The lower detection limit of the assay was 150 RNA copies per ml of plasma for 0.2ml sample volumes(2).

1. Amogne MD, Balcha TT, Agardh A. Prevalence and correlates of physical violence and rape among female sex workers in Ethiopia: a cross-sectional study with respondent-driven sampling from 11 major towns. BMJ Open. 2019;9(7):e028247.

2. Arimide DA, Abebe A, Kebede Y, Adugna F, Tilahun T, Kassa D, et al. HIV-genetic diversity and drug resistance transmission clusters in Gondar, Northern Ethiopia, 2003-2013. PLoS One. 2018;13(10):e0205446.